THE SLOW INWARD CURRENT AND CARDIAC ARRHYTHMIAS

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THE SLOW INWARD CURRENT AND CARDIAC ARRHYTHMIAS

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PREFACE

Since Paul Cranefield published his monograph, The Conduction of the Cardiac Impulse, in 1975, much has been learned about the role of the slow inward current in cardiac electrophysiology. Because of this expanse in knowledge, both basic and clinical, it appeared reasonable to review in a monograph once again what was known. When Martinus Nijhoff first approached us to undertake the task of updating this information, we were initially reluctant for several reasons. First, we did not feel that the subject could be adequately and thoroughly reviewed, from the cell to the bedside, by a single person. Second, time constraints on all of us precluded even attempting such a task.

However, we were encouraged by several of our friends ('egged on' one might even say, since they wished the job done but did not want to do it themselves!) who promised faithfully to contribute chapters on time if we accepted the task. So we did, and most of them did also.

In assembling this work, we have used the editorial pen sparingly because we felt that we had invited experts to review their own areas of interest and they needed little help from us. Naturally, such an attitude on our part leads to inconsistencies in style, in points of view, sometimes in data or statements of 'fact' and most certainly results in unavoidable redundancy, as parts of one chapter overlap another. However, from the outset we decided that, although the book might suffer from these failings, it would prosper from a minimum amount of meddling by us.

To all contributors we extend our gratitude. The book in fact belongs to each of them. The adage, if you wish something accomplished, give it to a busy person, is certainly true. The scientific leaders are all very busy, but yet found time to write 'one more manuscript' for this book. As usual, they are paid in the academic currency of self-satisfaction that comes from participating in, what we hope, was a worthy venture. Most assuredly their share of the royalty will not alter their tax bracket.

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Indianapolis, Indiana
January, 1980

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I. OVERVIEW

1. HISTORICAL PERSPECTIVE

SILVIO WEIDMANN

The history of the slow currents as relevant to the content of this book starts around 1950, although several basic facts had been described long before.

In 1879/80 Burdon-Sanderson and Page [1] presented a rheotome analysis of the cardiac action potential and found the long-lasting plateau to be typical for the 'injury action potential.' Direct recording by a capillary electrometer (Figure 1) followed in 1883 [2]. Reversal of polarity in the course of cardiac activity was reported to the Dutch Royal Academy of Sciences in 1873 by Engelmann et al. [3] but was declared to be doubtful by Engelmann himself in 1877 [4]. The minimum ionic requirement for a frog's heart to beat in an artificial salt solution has been known since the work of Sydney Ringer in

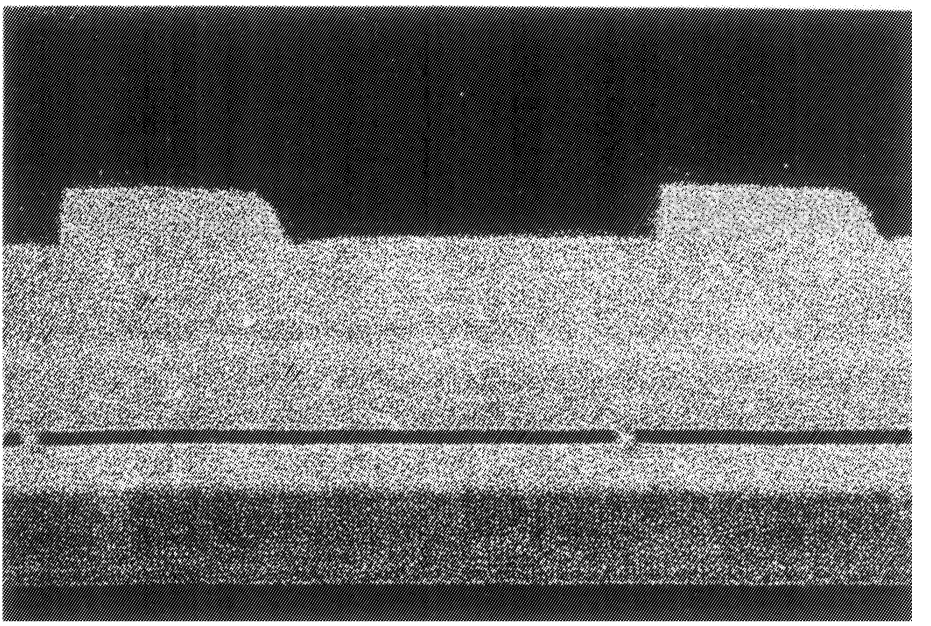

Figure 1. Action potentials of a frog's heart as recorded by a capillary electrometer. One electrode is placed at the base of the ventricle on intact surface. The other electrode is placed near the apex, where the tissue is injured by a hot wire. Breaks in the black line are about 5 sec apart and mark direct ventricular stimulation. From ref. 2, Plate II/6, with permission.

D.P. Zipes, J.C. Bailey, V. Elharrar (eds.), The Slow Inward Current and Cardiac Arrhythmias, 3–9.

1883 [5], and the role of Ca^{++} in 'producing the wave of contraction out of the wave of excitation' was recognized by Locke and Rosenheim in 1907/8 [6].

In 1931 Adrian [7], having reported on slow depolarizations in insect ganglia, presented a record from an isolated sinus node of the frog showing practically no diastolic depolarization (d.c. recording, but diphasic) prior to activity (his Figure 10B). He argued that threshold can be reached in two different ways: (a) by slow depolarization as in the case of insect ganglia or (b) by slowly rising excitability at a practically constant membrane potential. Asher and Scheinfinkel [8] had been educated to believe that cardiac rhythmicity depends on nerve cells and thus did not know what to expect when, in 1936, they set out to re-investigate the 'electrosinogram.' Leading off between the sinus and the right atrium they described 'Formenreichtum' and failed to comment on the slow potential drift preceding the sinus action potential (their Figure 5). In the same year, Goldenberg and Rothberger [9], aware of the autorhythmic properties inherent in the ventricular conduction system of mammals, set out to record monophasically from 'false tendons' of dog hearts in a moist chamber (Figure 2). Two years later, in 1938, Arvanitaki [10] reported on the slow depolarization typical for the pacemaker region of the snail heart.

It was clear in 1938, at least to Cole and Curtis [11], that a change of membrane potential requires the space charge on the double-layer capacity to be altered. But, strangely enough, Cole and his co-workers never attempted to identify the charge carriers. This decisive development in our present concept was left to a former pupil of Kenneth Cole by the name of Alan Hodgkin who, together with Andrew Huxley, extended Bernstein's membrane theory of 1912 [12] to what is known as the ionic theory of the action potential [13].

All through the history of electrophysiology there has been a tendency to take for granted that differences between nerve, skeletal muscle, cardiac muscle and smooth muscle are of a quantitative rather than of a qualitative nature (see [14]). Starting with the early 1950's, interest in cardiac electrophysiology expanded rapidly and there is no indication that this development is nearing its end. The reasons for its growth are twofold. First, the technique of recording by Ling–Gerard microelectrodes [15] could be used with relative ease to give absolute values of transmembrane potentials [16, 17]. Secondly, and perhaps more important, there was the new concept of specific ions carrying net charge across the surface membrane just waiting to be exploited by cardiac electrophysiologists. Identification of Na^+ ions as charge carriers for the upstroke of the action potential [17] required little more than repeating the experiments published by Hodgkin and Katz [18] for the squid giant axon in 1949. To show that the cardiac membrane at rest was mainly K^+-perme-

able [19] required a plot of membrane resting potential against external K^+ concentration and a comparison with the values expected from the Nernst equation.

The real troubles started when efforts had to be made to establish (a) why action potentials of cardiac tissue lasted so much longer than those of nerve, and (b) why slow diastolic depolarization existed in pacemaker regions. The

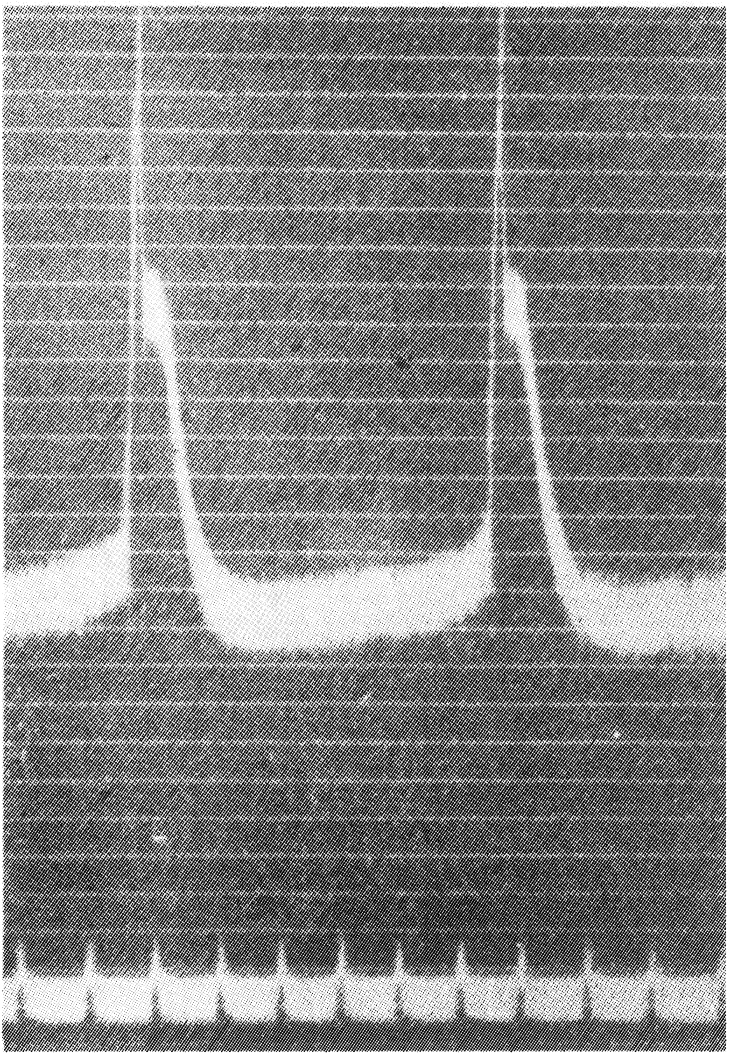

Figure 2. String galvanometer records from a spontaneously beating 'false tendon' of the dog. One end of the preparation has been squeezed by forceps to produce a monophasic record (showing slow diastolic depolarization). Time marks at 200 msec. From ref. 9, Figure 1a, with permission.

appearance of this volume on 'slow currents' in 1980 must be taken to indicate that many problems regarding the slow voltage drifts as seen in heart are still open to discussion.

In my monograph of 1956 ([20], Figure 20) I tried to indicate possible conductance changes to Na^+ and to K^+ which might produce an action potential typical for a Purkinje fiber. Since g_K in the membrane of the squid axon *rises* upon depolarization, it did not occur to me that one might assume g_K to fall. One day in 1959 Edouard Coraboeuf came from Paris with a manuscript in his luggage. It was difficult for him to convince me that there was no way to account for the strongly positive intracellular values of *ventricular* action potentials other than to assume a voltage-dependent *drop* of g_K [21]. I felt somewhat heretical towards Hodgkin and Huxley but had to give in. From that moment, I was ready to re-interpret my findings published in 1955 [22]. We now refer to the phenomenon of a potential-dependent g_K drop as 'anomalous rectification' since the finding is opposite to that observed in the squid giant axon membrane, where K^+ conductance rises upon depolarization.

In 1952 Hodgkin and Huxley had assumed that the passive flow of any ion is dependent on the driving force and conductance for that particular ion (independence principle). A case where the principle does not hold was first published by Hodgkin and Keynes in 1955 [23]: Increasing extracellular K^+ concentration decreased $^{42}K^+$ efflux from squid giant axon by a much larger amount than could be accounted for by the small decrease of driving force; they hypothesized that the interaction might be understood by the help of a 'single file model.' In the heart, the delicate balance between inward current and outward (K^+?) current seemed to be influenced in a rather unexpected way: By increasing extracellular $[K^+]$ the net outward current increased though the outward driving force for K^+ must have decreased. The effect could be demonstrated even if the extracellular $[K^+]$ was raised in the turtle coronary circulation at a time when the action potential had started, thus excluding the idea that depolarization of the resting membrane is the clue to explain an increase of K^+ efflux [24]. A thorough study showing $[K^+]_0$ to influence unidirectional $^{42}K^+$ efflux and unidirectional $^{42}K^+$ influx was presented in 1962 by Carmeliet [25].

On the occasion of the 22nd International Congress of IUPS held at Leyden in 1962 Denis Noble convinced his listeners that the time course of Purkinje fiber action potentials as well as the corresponding changes of conductance could be accounted for by assuming that specific membrane conductances to Na^+ and K^+ change as a function of membrane potential and as a function of time [26]. The report by Otsuka and Coraboeuf [27] and its confirmation by Délèze [28], showing action potentials of normal duration in a solution which contained only 8.3% of the normal $[Na^+]_0$, were taken as nonconformist

information clarifying nothing and perhaps unnecessarily confusing our theoretical concepts. Another source of confusion were the findings by Matsuda et al. [29], Wright and Ogata [30] and Pillat [31], who demonstrated that under special conditions the cardiac action potential could be dissociated into two parts, an initial spike and a later dome, the second sometimes missing or following the spike after a more or less pronounced notch. Since many of us were aware that a certain level of free intracellular Ca^{++} is necessary to activate contraction of skeletal muscle [32], and that the lack of Ca^{++} in the bathing solution uncouples the electrical and mechanical activity [6] of cardiac tissue, it would have been natural to assume that Ca^{++} ions are displaced from the outside to the inside of the cell during the plateau phase. Evidence to this effect was much delayed by our knowledge that the action potential of dog ventricular muscle becomes prolonged rather than shortened in a Ca^{++}-free medium (for example, see [33]). In 1966 Reuter applied depolarizing direct currents to Purkinje fibers in a Na^+-free solution. In the presence of Ca^{++} the potential time course showed two steps, the second one having a 'threshold' in the range of 30–40 mV, inside negative [34]. Niedergerke and Orkand [35], working with frog heart driven at a very low rate (1/min), showed the size of the overshoot to be proportional to $\log [Ca^{++}]_0$ over a considerable range of Ca^{++} concentrations (0.1–5 mM, 18 mV for a 10-fold change) in a solution containing normal $[Na^+]_0$. The compromise that evidently had to be made was that depolarization of the membrane by fast inward current (Na^+) opens a different channel that is activated with some delay and has a poor specificity for cations such as Na^+, Ca^{++} [36] or even Sr^{++} [35, 37]. The evidence for Na^+ still playing some role, at least in Purkinje fibers, was recently emphasized by a pronounced shortening of the plateau following the administration of TTX at such a low concentration that the upstroke of the action potential would not be affected to a major extent [38].

As to the nature of the slow pacemaker depolarization in Purkinje fibers, the situation at present is rather confusing. Three possible hypotheses were advanced in 1956 [20]: 1) a time-dependent increase of inward current carried probably by Na^+, 2) a time-dependent decay of outward K^+ current and 3) a gradual slowing of an electrogenic Na^+ pump. The computations by Noble [26] published in 1962 were based on hypothesis No. 2. Experimental evidence in favor of this hypothesis was seemingly presented in 1966 by Vassalle [39], who voltage-clamped spontaneously active Purkinje fibers at their maximal diastolic potential and noted a decrease of membrane conductivity as a function of time. This interpretation of a slowly decreasing g_K, universally accepted up to 1979, is now given a different interpretation by Noble and co-workers (no full papers available as yet), who argue in favor of a rising inward current being responsible for pacemaker depolarization in Purkinje fibers.

A similar mechanism had been postulated in 1955 by Hutter and Trautwein [40] and by del Castillo and Katz [41] who showed that vagal stimulation could slow down or even temporarily reverse slow depolarization of a frog's sinus venosus.

As to slow currents observed in the AV nodal region, much of our present knowledge is the result of Brian Hoffman's visit in the summer of 1956 to Antonio Paes de Carvalho and Walmor de Mello in Rio de Janeiro. The findings are summarized on slightly more than one page [42]: AV nodal fibers have a low resting potential and prominent diastolic depolarization; the upstroke velocity of their action potentials decreases to very low values in the center part of the node and then increases again as the recording microelectrode is moved to locations near the His bundle.

A hundred years ago, in Volume 2 of the Journal of Physiology (1879/80) Burdon-Sanderson and Page introduced their contribution by stating 'As regards the subject now before us (changes of electromotive forces in heart, S.W.) the labours of Engelmann... leave little to be done.' Let us hope that readers of the present texts will not be left with similar impressions.

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2. THE SLOW RESPONSE AND CARDIAC ARRHYTHMIAS *

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More than twenty years ago Brian Hoffman and I were deeply impressed by the fact that total AV block can occur at a time when fibers within the node are fully excitable. If atrial activity and His bundle activity occur at the right time, a normal action potential may appear within the node at a site which, when either atrial or His bundle activity occurs alone, shows only a low amplitude local response ([1], Figure 6–9F). We thought about this observation from time to time and wondered whether the action potential thus elicited could actually propagate. In 1970, working with Klein, we investigated excitation and conduction in strands of Purkinje fibers, the central part of the strand being exposed to high potassium and epinephrine [2–4]. We found that conduction through the depressed central portion could become very slow, could show 2:1 block at high rates of drive, could be unidirectional, and in many ways resembled conduction through the AV node. Using a 'T-shaped' preparation, in which a branch emerged from the depressed area, we found that at a time of bidirectional block, if the depressed central area were invaded by impulses from both ends of the fiber, an action potential could arise in the central area, just as it can in the AV node, and could propagate throughout the branch that arose in that area. This mutual reinforcement of the action potentials, bringing the depressed fiber to threshold, we called summation. In some preparations excitation of one end could give rise to an action potential in the center which might propagate out the branch, but such excitation could be prevented by excitation of the other end of the bundle. This we called inhibition.

The family of properties elicited by depression of the excitability of a segment of a bundle of Purkinje fibers, i.e., slow conduction, rate-dependent block, summation and inhibition, suggested that a segment with such properties could easily become the locus of reentrant arrhythmias. Our research then turned to the effort to prove that such reentry could actually occur. Studying preparations exposed to high K^+ and epinephrine, Wit and I evoked circus

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movement of excitation around short loops of Purkinje fibers as well as 'reflection' in unbranched bundles of Purkinje fibers [5, 6]. In the course of those studies, Dr. Wit and I were plagued by the appearance in certain preparations of the phenomenon we now call triggering, a phenomenon we regarded as a nuisance since when it was present one could not attribute the sustained rhythmic activity to reentry, ([5], p 21).

The more we studied the electrical activity of fibers exposed to high levels of K^+ and epinephrine, the more we became convinced that we were not dealing with a simple 'depression' of the normal action potential. We therefore decided to test whether conduction could occur through a segment of Purkinje fibers exposed to a wide variety of conditions under which normal fast channel activity should be blocked, and found that it could do so [7].

As Weidmann has pointed out elsewhere in this book, quite a few pieces of evidence had accumulated in the late 1950's and in the 1960's that pointed to the role of some inward current other than fast channel current in the production of at least some propagated action potentials in the heart. As a matter of fact Hoffman and I, in our monograph, which was completed in July, 1959, had expressed considerable caution about the evidence in support of a straightforward 'Hodgkin–Huxley' theory in explaining cardiac action potentials:

> reduction of external Na^+ alters the action potential but it does so to a lesser degree than the theory predicts. This fact suggests the possibility that some other ion participates in the depolarization. It seems extremely likely that an increase in P_{Na} plays a significant role in the process of depolarization in most cardiac fibers; it also seems likely that some additional unknown factor is important ([1], p 272)

and we had devoted the closing pages of that monograph to an interpretation of repolarization in which inward-going rectification was taken as a central feature. Since Hoffman and Paes de Carvalho had, in the 1960's, argued persuasively that the action potential of the AV node might be different in kind from that of the Purkinje fiber [8] and since our preparations of Purkinje fibers exposed to high K^+ and epinephrine had so many properties in common with the AV node, we were quite prepared to entertain the idea that there may be situations 'in which depolarization causes the normal action potential to be replaced by the slow response' ([7], p 242). By then, of course, the articles of Wright and Ogata [9], Hagiwara and Nakajima [10], Niedergerke and Orkand [11] and Rougier et al. [12] had appeared, as had other studies suggesting the existence and importance of the slow inward current (see Weidmann's chapter in the present volume and [13]).

Aronson and I [14–16] pursued the question of whether one could obtain action potentials in Purkinje fibers exposed to Na-free solutions, on the assumption that obtaining action potentials under those circumstances would provide persuasive evidence for the existence of the slow channel. Whether it

did that or not (and I believe that it did), it certainly again directed our attention to the importance of the resting potential [15] and to the observation that Purkinje fibers exposed to Na-free, Ca-rich solutions are triggerable, i.e., can be thrown into a state of rhythmic activity in which each action potential emerges from a delayed afterdepolarization that follows the preceding action potential [16]. The latter observation subsequently received a good deal of study in our laboratory [17–19]; its relationship to the slow inward current, which remains obscure, is discussed further below.

In an attempt to investigate the ionic specificity of the slow channel, Wiggins and I [20] added Na^+ to the perfusate of fibers that were rhythmically active in Na-free, Ca-rich solutions with the unexpected result that the addition of very small amounts of Na^+ caused rather marked hyperpolarization (an effect which we attributed to electrogenic sodium extrusion but which might perhaps be reexamined in terms of recent ideas about electrogenic sodium–calcium exchange). The addition of small amounts of Na^+ seemed to inhibit the slow-channel dependent upstroke more than one might expect from any increase in outward current associated with enhanced sodium extrusion or Na–Ca exchange, raising the possibility that Na^+ may reduce the permeability of the slow channel to Ca^{++} and that Ca^{++} may reduce the permeability of the slow channel to Na^+ ([13], p 84–86; [21]). The same study led to Wiggins noting two stable levels of resting potential in Purkinje fibers exposed to Na-free solutions [22].

The examination of the important implications of that phenomenon and of electrogenic sodium extrusion for excitation and conduction subsequently occupied Gadsby and myself [23–26] and continues to do so. Indeed, a recurrent theme in our laboratory has been the appearance of unexpected interactions between studies of slow conduction, studies of the resting potential, studies of triggered activity and studies of electrogenic sodium extrusion. We first noticed triggering in calf Purkinje fibers exposed to high K^+ and epinephrine [6], began to study it in earnest when we saw it in the course of studying the slow response in Purkinje fibers exposed to Na-free, Ca-rich solutions [16] and became convinced of its clinical significance when we demonstrated that it exists in fibers of the coronary sinus [18]. But the more we studied triggering in the coronary sinus the more we realized that its appearance and cessation are critically dependent not only on resting potential but also on the rate of electrogenic sodium extrusion [27] and we eventually found two levels of resting potential in fibers of the coronary sinus (see Figure 1). Preliminary observations suggest that fibers at the high level of resting potential can show spontaneous depolarization and both early and delayed afterdepolarizations and are triggerable; at the low level of resting potential the fibers can show delayed afterdepolarizations and are triggerable.

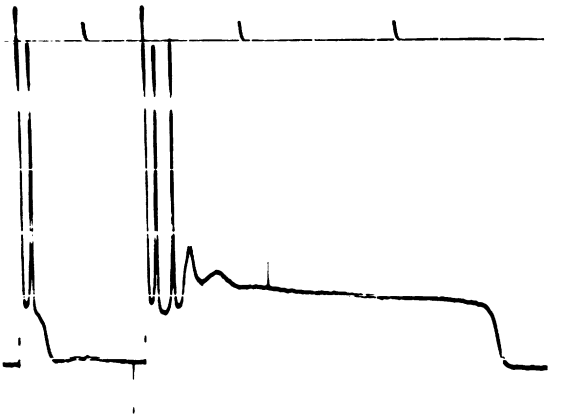

Figure 1. Transmembrane action potentials recorded from a fiber of the canine coronary sinus, showing the presence of two levels of resting potential and the appearance of afterpotentials at each level. The fiber, perfused with 4 mM K^+, Cl^--free solution, had previously been triggered into sustained activity during transient exposure to norepinephrine. This record was obtained while the effect of enhanced electrogenic sodium extrusion, caused by that sustained activity, was wearing off. The record shows the response to two applied stimuli. The initial action potential is driven and is followed by a second spike and, subsequently, by a delayed return to the original resting potential; the second upstroke may be regarded as having arisen in the course of an early afterdepolarization evoked by the driven action potential. A low amplitude delayed afterdepolarization occurring at the high level of resting potential is seen between the first and second driven action potentials. The second driven action potential is followed by two non-driven action potentials which are in turn followed by a large delayed afterdepolarization that occurs at the low level of resting potential. A further subthreshold oscillation leads into what may be regarded as either a second level of resting potential or as a prolonged early afterdepolarization evoked by the driven action potential. The potential is not quite stable, and a slow drift in the direction of increasing negativity brings the membrane to the level at which it abruptly shifts to the high level of resting potential. The time marks occur at 5 sec intervals. The time mark line also marks +20 mV. The resting potential at the beginning of the record is about −83 mV (previously unpublished record obtained by Gadsby and Wit).

By 1973 I became sufficiently convinced of the importance of the slow inward current to begin an exhaustive review of the literature bearing on it and to write a monograph [13], which was completed in July, 1974. In the course of that activity I convinced myself of the reality of the slow channel and of the kinetically slow inward current, of the existence of propagated action potentials in fibers in which normal fast channel activity is absent and of the existence of rhythmic activity in fibers in which normal fast channel activity is absent.

In the introduction to that monograph ([13], p v) I offered the following theses:

(1) Fibers of the heart can produce two fundamentally different kinds of action potential, the fast response and the slow response.

(2) The slow response and the fast response differ profoundly in conduction velocity and in the

nature of their upstrokes and probably depend upon the flow of current through different channels.

(3) The fast response can arise only in fibers that have a high resting potential, whereas the slow response can propagate only in fibers that have a low resting potential.

(4) Most, perhaps all, arrhythmias result either from slow conduction or rhythmic activity in a discrete and localized area of cells that show only slow response activity.

(5) Some foci, such as the SA node and AV node, show slow response activity under normal conditions and are thus always potentially arrhythmogenic.

(6) Other foci, that normally produce the fast response, may become arrhythmogenic when loss of resting potential secondary to injury or disease abolishes their ability to produce a normal action potential and unmasks slow response activity.

I would modify these statements today only by mentioning the possible role of the 'depressed fast response,' by placing more emphasis on the role of inward-going rectification, and by replacing, in (4), the words 'most, perhaps all' by 'many.'

I also pointed out ([13], p 100) that 'channels are defined by the way in which their permeabilities change as a function of time and voltage and by their responses to pharmacological agents. In the heart the terms "fast channel" and "slow channel" are much to be preferred to terms that suggest a specific ionic permeability. It is particularly desirable to avoid thinking of the slow channel as a calcium channel since it is almost certainly permeable to both calcium and sodium and may well carry chiefly sodium under certain conditions' and that ([13], p 115, [16]) 'the differences between the two sorts of propagated activity are so great that it is reasonable to regard them as functionally distinct phenomena even in the absence of a definitive understanding of the current–voltage relationships and permeability changes that cause them; such a definitive understanding presumably must await a resolution of the problems that surround the use of the techniques of voltage–clamping,' and added that ([13], p 348):

One might ask whether the slow channel is a distinct entity or is merely a fast channel that is profoundly modified when the membrane potential is less than -60 mV. This is an important and fundamental question from the point of view of membrane biophysics; it is also an unanswered question. From the point of view of the phenomenology of excitation and conduction in the heart, however, the question does not appear to be as fundamental. If one grants the existence of two profoundly different sorts of inward current it might not make much difference, from the point of view of excitation and conduction, if those currents flow through different sets of channels or if they flow through a single set of channels that has two profoundly different sorts of properties, switching more or less discontinuously from one sort to the other as a function of membrane potential.

Much later, in a talk before the Cardiac Muscle Society in 1978 and in a subsequent review [28] I said:

Little consideration has been given to the possibility that a fiber at a resting potential low enough to inactivate the fast channel almost completely might possess a low g_K so that the few remaining fast channels might sustain regenerative depolarization. Such a regenerative depolarization might give rise to an action potential that could propagate over a fairly long distance (but

with decrement), and these action potentials could not easily be distinguished from propagating slow responses. Such action potentials could be blocked by TTX or lidocaine. Alternatively, the fast channel current, although insufficient to sustain even decremental conduction, might cause a local response sufficient to depolarize the membrane to the threshold of the slow channel, thereby evoking a propagated slow response. The appearance of such a response might be prevented by TTX or lidocaine acting solely on the fast channel dependent local response; but the propagated slow response could, of course, be blocked by verapamil. Under such circumstances relatively small changes in resting potential (for example, of a kind that might be caused by changes in the rate of electrogenic sodium extrusion) might markedly change the character of the action potential by causing an increase or decrease in fast channel current. One can even envision blocking an arrhythmia that arises in such a fiber by use of lidocaine, only to have a subsequent depolarization (caused, for example, by digitalis toxicity) bring the fiber to the potential at which lidocaine-insensitive slow responses would appear and become arrhythmogenic....

One of us has suggested [13] that many arrhythmias result from a loss of resting potential that leads to replacement of the normal action potential by a slow response. To that possibility we would now add the replacement of a normal action potential by an action potential in which the upstroke depends on current carried via the largely inactivated fast channel. We would also emphasize the importance of g_K and again point out that the delayed afterdepolarizations responsible for triggered activity can arise at membrane potentials well negative to the threshold of the slow response.

These last remarks draw attention to the questions that have arisen because of the finding that some of the arrhythmias associated with myocardial infarction appear to be insensitive to verapamil but sensitive to lidocaine. Whether that means that activation of slow channel currents plays no role in those arrhythmias remains, in my opinion, an open question. One of the difficulties in using pharmacological criteria to analyze arrhythmias is that we do not have agents that sharply discriminate one cause of arrhythmia from another. For example, a fiber at the low level of resting potential can be shifted to the high level by adding lidocaine or TTX [23]. This antiarrhythmic effect results from a reduction in a steady-state inward current and points to an action of lidocaine phenomenologically distinct from its ability to block the much larger currents responsible for normal rapid upstrokes [28–32]. Similarly, verapamil can enhance certain repolarizing currents [33–35] and the racemic mixture has a minor but real ability to block fast channel currents by binding to inactivated fast channels [36]. If we concede that loss of resting potential is an essential prerequisite for the appearance of slow conduction and reentry or for the appearance of 'depolarization-induced rhythmicity,' the variety of forms of slow conduction and rhythmic activity that can appear in depolarized fibers, and the multiplicity of effects of antiarrhythmic drugs make it difficult to decide what causes any particular arrhythmia or how any particular antiarrhythmic drug acts on any particular arrhythmia.

A striking paradox appears in connection with triggered activity in the coronary sinus. Such activity is exquisitely sensitive to verapamil but it is the delayed afterdepolarization required to sustain the arrhythmia that is sensitive to verapamil. The upstroke in many such fibers appears to be fairly rapid, and verapamil-insensitive; the paradox arises because the verapamil-sensitive

afterdepolarization is seen at voltages well *negative* to the threshold of the 'conventional' slow channel. The ease with which this arrhythmia can be blocked by verapamil does not show that it is directly caused by slow channel currents; instead it shows that verapamil sensitivity cannot be taken as proof that an arrhythmia is directly caused by slow channel currents.

At this stage in the development of our knowledge of the cause of arrhythmias I think that (1) reentry can occur only in a heart that contains depolarized fibers [35] or in the presence of drastically shortened action potentials [37, 38]; (2) that the slow conduction necessary for reentry can result from slow response activity (and probably does so in supraventricular reentrant arrhythmias) but may perhaps arise in fibers in which a significant part of the excitatory current is fast channel current; (3) that antiarrhythmic drugs presently assumed to act by blocking the regenerative inward currents that cause the upstrokes of action potentials may also act by causing hyperpolarization; (4) that triggered arrhythmias, although verapamil-sensitive, depend on afterdepolarizations the cause of which remains unexplained; and (5) that most 'depolarization-induced' rhythmicity is 'triggerable', i.e., is dependent on afterdepolarizations.

I have several times alluded to the importance of inward-going rectification in the above discussion. A marked fall in g_K is associated with depolarization in Purkinje fibers; Gadsby's study of the phenomenon of two stable levels of resting potential [23] serves to emphasize the fact that fibers brought in certain ways to a low level of resting potential may have a low overall permeability and can thus far more readily develop action potentials, the upstrokes of which result from small and kinetically slow inward currents, than can fibers depolarized by high K^+ (see above and [28]). The time when we could look on the resting potential as being simply a potassium equilibrium potential is long past and I have no hesitation in repeating the closing sentence of my monograph [13]: 'To complete our understanding of the electrophysiology of the heart we must acquire far deeper insights into the nature of the resting potential than we now possess; only a few fragmentary clues are now available to help in that task.'

In general, in the 5 years since I wrote my monograph, we have become increasingly aware of the complexity and importance of the resting potential as a determinant of excitability; of the importance of inward-going rectification (for review see [23, 28]); of the ability of slowly- or non-inactivating fast channel current to contribute to the total inward current in depolarized fibers [23, 39, 40]; of the effect on cable properties of cellular uncoupling [41–43]; of the possibility that Na/Ca exchange is electrogenic [44]; of the fact that depletion and accumulation of K^+ in extracellular spaces not only complicates the interpretation of results obtained by voltage-clamping but also exerts an important influence on the configuration of the action

potential [45–47]; and of the fact that electrogenic sodium extrusion has a far greater effect than previously supposed on resting potential, phase 4 depolarization, triggered activity and the time course of repolarization [25, 27].

As I said in my monograph ([13], p 28):

> If cardiac fibers can, in fact, produce two distinct kinds of action potential in two distinct ranges of resting potential, the complexities of analysis of that fact are enormously increased by our ignorance of the nature of the resting potential. To understand the activity of a given fiber of a given cardiac tissue at a given resting potential we ought to know the extracellular and intracellular ionic concentrations, the permeabilities to each ion, the dependence of those permeabilities on membrane potential, the dependence of those permeabilities on the intracellular and extracellular concentrations of each ion, the cable properties (which are largely, but not entirely, defined by the properties listed above), the time- and voltage-dependent characteristics of any regenerative inward currents and of any outward currents as a function of membrane potential and as a function of the intracellular and extracellular concentrations of each ion, the existence and degree of activity of all neutral and electrogenic ion pumps as a function of membrane potential and as a function of the intracellular and extracellular concentrations, the way in which any of the above factors are influenced by such agents as catecholamines and acetylcholine, and the way in which each of the above factors affects the other.

Until fairly recently many investigators believed that the significant events contributing to the cardiac action potential arose from stable diffusion potentials and from 'gated' currents that had been measured and characterized by voltage-clamp experiments and subsequently reconstructed by mathematical simulation. It is now widely recognized that ion accumulation and depletion may explain current changes once confidently attributed to permeability changes. It is also increasingly obvious that the cardiac action potential is significantly affected by currents that are not only not gated but are not even attributable to diffusion potentials; electrogenic sodium extrusion generates such a current.

Indeed, it seems that we remain very far from being able to make a rational reconstruction of the cardiac action potential even of normal cells. Nevertheless, I *think* that *perhaps* we have now identified all of the factors that do need to be taken into account, in other words that no really startling surprises lie in wait. If so, 10 years work by many investigators may suffice to characterize each of those factors and, with luck, another 10 years might tell us 'the way in which each of the above factors affects the other.' Once that is accomplished for each type of cell in the heart, under normal and abnormal conditions, it may be possible to analyze how various types of cells, under various kinds of conditions, interact to produce normal and abnormal rhythmicity and normal and abnormal conduction. Since the analysis of an arrhythmia requires that we understand the processes of conduction and rhythmicity in normal and abnormal fibers and in the transitional fibers that connect normal fibers to arrhythmogenic foci ([13], p 294–304), only by answering the above questions can we fully understand any arrhythmia. We can now ask and answer questions of a far more fundamental kind than we could 10 years

ago, and I believe that the answers to those questions will slowly begin to fit into place and enable us to understand in full detail the electrical basis of both normal and abnormal rhythm and conduction in the heart.

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II. BASIC PROPERTIES OF THE SLOW INWARD CURRENT

3. VOLTAGE CLAMP STUDIES OF THE SLOW INWARD CURRENT

EDOUARD CORABOEUF

1. INTRODUCTION

As early as 1911, Eiger [1] distinguished two phases in the ECG, the first (QRS complex) being considered as an action current, whereas the second (ST interval and T wave) was interpreted as being the result of biochemical processes. Other authors [2–5] considered that, if QRS was related to excitation, the T wave was related to contraction. Therefore several previous observations suggested that two different mechanisms might be responsible for early and late ECG waves. Moreover the R wave was recognized as being very stable wheras the lability of the T wave was established in many experimental conditions (see [6]).

When cellular action potentials were recorded, it became clear that the spike and the plateau of cardiac action potentials could be separated quite easily. As the very first transmembrane Purkinje fiber action potentials [7] were recorded in preparations in rather poor condition, they did not exhibit spikes. They constituted what is called today 'slow responses,' whereas later the same year, in preparations in better condition, the existence of a large and brief spike preceding the plateau was demonstrated [8]. Therefore, it seems obvious that two different processes, the spike and the plateau, could develop independently in cardiac action potentials. This was confirmed by further observations made independently by Weidmann and myself, of Purkinje fiber action potentials with initial notches of increasing depth until the plateau spontaneously disappeared, leaving only isolated spikes as shown in Figure 1A. Later, conditions were found where it was possible to suppress spikes entirely without affecting the amplitude of the plateau (Figure 1B) [9].

It was shown by Weidmann [10] that the spike of the Purkinje fiber action potential is related to an increase of the membrane conductance to sodium (Na^+) ions. However other observations showed that in the guinea pig ventricular muscle, the amplitude of the overshoot was rather insensitive to the external Na^+ concentration ($[Na^+]_0$) as no change occurred after 15 min of superfusion with a Tyrode solution containing only 5% of the normal $[Na^+]_0$ [11]. It was suggested that: 'these effects are to be put together with those described by Fatt and Katz [12] in crab muscle. The behavior of the

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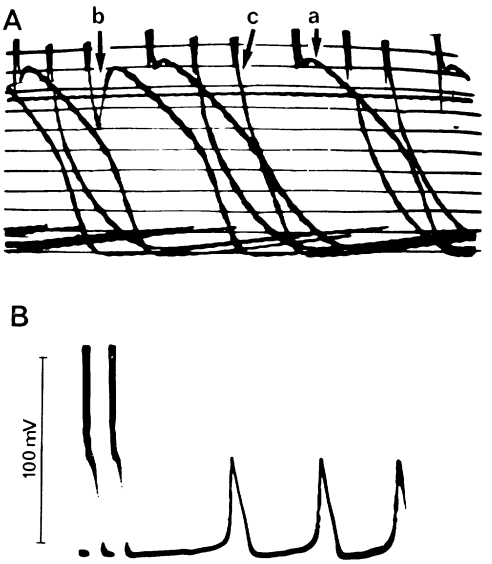

Figure 1. Observations showing the independence of spike and plateau in isolated kid Purkinje fibers. A: successive action potentials exhibit either a normal shape (a) or a marked notch between spike and plateau (b) or only a spike (c). Thick horizontal line: 0mV; between thin lines: 10mV. Weidmann Feb. 2, 1950, unpublished. B: the first two action potentials are triggered by brief stimulations. The last three are spontaneous action potentials; they no longer show spikes but the amplitude of the plateau is the same as in the first two responses although its shape is different. [9, reproduced with permission.]

guinea pig ventricular muscle seems in some way intermediary between that of most of the tissues studied until now and that of the crustacean muscle which is very curious and very different. It is not impossible that this particularity is related to the existence of a long-lasting plateau, normal in cardiac muscles and easy to trigger in crustacean muscles' [11]. Since it was shown that the long-lasting crustacean muscle action potential is due to the entry of divalent cations into the cell [13] it became obvious that the cardiac action potential plateau could have the same origin. However the situation remained rather obscure for several years, because of the well-known fact that in the complete absence of divalent cations or in the presence of a chelating agent, the cardiac plateau not only persists but is markedly prolonged [14, 15]. Nevertheless the possibility for calcium (Ca^{++}) ions to enter the cell during the periods of activity became more and more obvious, following two observations published simultaneously namely (a) in the frog ventricle, periods of potassium-induced depolarization or of electrical stimulation increased the uptake of labelled Ca^{++} [16], (b) in turtle ventricle, a very short period of perfusion with a Ca^{++}-rich solution starting at the beginning of a systole (i.e.

after the onset of the membrane depolarization), increases the amplitude of this systole, indicating that Ca^{++} ions 'mediate between electrical and mechanical activity throughout the period of membrane depolarization' [17]. The first clearly expressed indication that the plateau of the cardiac action potential may be related to an increase in Ca^{++} conductance was given by Orkand and Niedergerke [18]. At the same time, using artificially shortened Purkinje fibers, Deck et al. [19] introduced a voltage clamp technique which allowed direct measurements of membrane ionic currents in cardiac tissues.

2. HODGKIN-HUXLEY CONDUCTANCES

Excitable membranes possess different systems (channels and carriers) allowing ions to enter or leave the cells. In agreement with the Hodgkin-Huxley theory [20], it is generally accepted that time- and voltage-dependent changes in ionic conductances are due to the opening and closing of channels that are controlled by gating mechanisms. Transitory currents which develop when a membrane is depolarized, such as the rapid Na^+ inward current (i_{Na}) and as will be shown later the slow inward current (i_{si}) are controlled by activation and inactivation gates, whereas other currents such as some outward potassium (K^+) currents (i_K) are controlled only by activation gates. The corresponding conductances are labelled g_{Na}, g_{si} and g_K respectively.

The equation of a given membrane current carried by one ion χ crossing the membrane through a specific channel of maximum conductance $\bar{g}_\chi$, controlled by an activation gating system a (or governed by an activation parameter or variable a) and an inactivation gating system b, is the following:

$$i_\chi = g_\chi (E_m - E_\chi) \tag{1}$$

with

$$g_\chi = \bar{g}_\chi a^\gamma b^\delta \tag{2}$$

therefore

$$i_\chi = \bar{g}_\chi a^\gamma b^\delta (E_m - E_\chi) \tag{3}$$

where E_m is the membrane potential and E_χ the equilibrium potential for the ion $\chi \cdot \gamma$ and δ indicate that g_χ is proportional to a given power of variables which obey a first order equation. γ and δ could be the numbers of independent mechanisms controlling activation and inactivation processes. In the case of the Na^+ channel of the squid axon, where the activation and inactivation variables are labelled m and h respectively, $\gamma = 3$ and $\delta = 1$. This can mean that the Na^+ channel is controlled by three independent activation gates, all three having to be open simultaneously for the activation system to conduct

whereas there is only one inactivation gate. It is possible that activation and inactivation processes are not entirely independent. At the resting potential (E_r), all *a* gates are generally closed (the steady-state proportion of *a* gates in an open state is zero, $a_\infty = 0$), whilst almost all *b* gates are open ($b_\infty = 1$). When the resting membrane is suddenly submitted to a maintained depolarization, the channel first activates quickly (*a* gates open, i.e., *a* rapidly reaches a new steady-state value, for example $a_\infty = 1$ with a time constant τ_a) then inactivates more slowly because the *b* gates close (*b* reaching a new steady-state value for example $b_\infty = 0$, with a time constant τ_b). A peak of inward (negative) current flows as a result of the transient opening of the conductance g_χ and the existence of a driving force (E_m–E_χ).

When ions cross an unclamped membrane, they carry charges from one side to the other and therefore change the membrane potential. Inward movements of positive charges (inward currents, i_i) tend to depolarize the membrane whereas outward currents (i_o) tend to repolarize it. For this reason when a membrane possessing Na rapid channels or slow channels is moderately depolarized so that g_{Na} or g_{si} begins to open, the resulting inward current further depolarizes the membrane giving rise normally to an action potential. It is therefore impossible to study the development of a given current at a chosen constant membrane potential (E_m), if the membrane is not clamped.

3. EXISTENCE OF THE SLOW INWARD CURRENT: FIRST VOLTAGE CLAMP EXPERIMENTS

The first attempt to clamp the membrane potential at a constant level in cardiac tissues was made by Weidmann [10, 21]. By means of a feedback circuit the membrane potential of calf and sheep Purkinje fibers was maintained at a chosen level in order to measure the rate of rise of action potentials starting from the pre-established level. The membrane potential could be clamped only during slowly evolving phases, i.e., when the membrane was close to equilibrium (diastole and plateau) and transmembrane currents were not measured. Other voltage or current clamp experiments allowed the measurement of membrane conductance as a function of membrane potential [22–25]. Accurate voltage clamp experiments, allowing the direct measurement of transmembrane ionic currents which develop when the membrane potential is clamped at a control value that is constant, were first performed in artificially shortened Purkinje fibers [19, 26–30].

The rapid inward Na^+ current and outward K^+ currents were analyzed by Deck and Trautwein [28]. In addition to the initial Na^+ current with its rapid

inactivation, they described a prolonged Na^+ current which declined with a time constant of around 100 msec. Vassalle [31] and Giebisch and Weidmann [32] obtained results which do not exclude a similar slow time-dependent fall in g_{Na} during the plateau. In 1965, Trautwein et al. [33] using both rectangular clamp pulses (voltage changes of constant amplitude) and ramps (voltage changes of constant speed), observed current voltage relationships displaying long-lasting negative conductance. Because regions of net inward current still occurred when all ions, except 1 mM K^+ and the buffer, were removed from the external solution the authors suggested that the long-lasting inward current was carried neither by Na^+ nor by Ca^{++} ions flowing down their concentration gradients, but possibly by some intracellular anion or that it was due to the operation of an ionic pump. On the other hand, Reuter [34, 35] using short sheep Purkinje fibers kept in a Na^+-free solution observed that to maintain the membrane at $E_m \simeq 0$ mV, less current was necessary from an external source when the Ca^{++} concentration was high. He concluded from such current clamp experiments that a transitory inward Ca^{++} current was probably triggered by strong depolarizations. McAllister and Noble [36] observing small spikes in a Na^+-free solution, attributed them to Ca^{++} ions flowing possibly through Na^+ channels. A definitive conclusion about the nature of the long-lasting inward current remained however difficult to reach [37, 38]. It was shown that in Purkinje fibers a positive (outward) dynamic current was partially superimposed on the long-lasting inward current, both currents exhibiting rather similar properties [39, 40]. Reuter [41] using the same preparation bathed in a Na^+-free solution observed that when the membrane was submitted to rectangular depolarizing pulses, membrane currents were different in the absence and in the presence of Ca^{++}. In a Ca^{++}-free solution currents were always outward whereas in a Ca^{++}-rich solution (7.2 mM Ca^{++}), the initial outward current decreased and, 50 to 100 msec after the beginning of the pulse, turned into an inward current. This slow inward current was not affected by large concentrations of tetrodotoxin (TTX, 10^{-5} g/ml) whereas it was increased by adrenaline. Extrapolation of the current–voltage relationship obtained in a Ca^{++}-rich medium, suggested a reversal potential around +180 mV which could be considered to represent the Ca^{++} equilibrium potential (E_{Ca}). These results therefore strongly suggested the existence of an inward Ca^{++} current participating in the depolarization of the membrane during the plateau of the Purkinje fiber action potential.

The fact that in cardiac cells a long-lasting plateau develops either in the presence or in the absence of external divalent cations was explained by voltage clamp experiments performed on frog atrial fibers using the double sucrose gap technique [42–44] and by comparing voltage clamp results and standard electrophysiological analysis [45]. Results confirmed the existence of

a rapid Na^+ current inhibited by TTX and responsible for the first part of the ascending phase of the action potential. The existence of another time- and voltage-dependent inward current, normally 10 to 20 times slower than the former and not influenced by TTX but inhibited by manganese (Mn^{++}) ions was also demonstrated. As in the Na^+ current, the secondary inward current underwent activation and inactivation. It appeared that in normal conditions this current was carried by both Na^+ and Ca^{++} ions, whereas in a Na^+-free solution it was carried by Ca^{++} ions only and in a Ca^{++}-free solution by Na^+ ions only. The slow current was claimed to be responsible for the second part of the ascending phase of the cardiac action potential and to play an important role during the plateau and the repolarization of the action potential. The term 'slow channel' was proposed for this pathway for slow current [44]. The existence of a passive slow channel accepting both Ca^{++} and Na^+ ions explains the existence of a plateau even when cardiac fibers were bathed in an entirely Ca^{++}-free solution [45]. A rapid inward Na^+ current and a slower inward Ca^{++} current or Ca^{++}–Na^+ current were also recorded in ventricular trabeculae of sheep [46], dog [47–50], rat [51] and guinea pig [52]. The existence of the slow inward current was also confirmed in Purkinje fibers and its characteristics were analyzed [53].

From the above described investigations it may be deduced that the secondary inward current undergoes both activation and inactivation, and therefore resembles currents obeying Hodgkin–Huxley kinetics [20]. However the kinetics of this current are markedly slower and the level of depolarization necessary to trigger the current (threshold potential) is higher than that corresponding to the rapid inward current. Therefore the existence of a slow inward current, independent of the rapid Na^+ current, carried by Ca^{++} or by Ca^{++} and Na^+ ions and controlled by activation and inactivation processes seemed to be quite well established by the beginning of 1971. However, Johnson and Lieberman [54] in a critical and useful survey of the limitations of the voltage clamp techniques cast some doubt on the existence of this slow inward current, suggesting that it might be in fact carried by Na^+ ions flowing through the rapid channel. The secondary current might be slower than the primary Na^+ current only because of the electrotonic decrement of the command pulse along the fibers whereas its persistance in a Na^+-free solution could be attributed to imperfect washing of deep-lying cells. Such a careful analysis stimulated research in order to prove or disprove the existence of the slow inward channel as a distinct mechanism, independent of the rapid Na^+ channel. None of the subsequent papers failed to recognize the existence of two different ionic mechanisms responsible for the development of the membrane depolarization during the cardiac action potential [55–57]. For reviews concerning this point and analysis of the slow channel and ionic currents in cardiac tissues see [58–71].

4. VOLTAGE CLAMP TECHNIQUES

The voltage clamp technique allows the membrane potential to be rapidly and permanently adjusted at a chosen value E_m, by using a negative feedback system which almost instantaneously through the membrane resistance (R_m) delivers a current i_m, just sufficient to attain this purpose, whatever the variations in R_m may be. In square-pulse experiments, E_m is modified instantaneously when necessary, then maintained at a constant value; in ramp

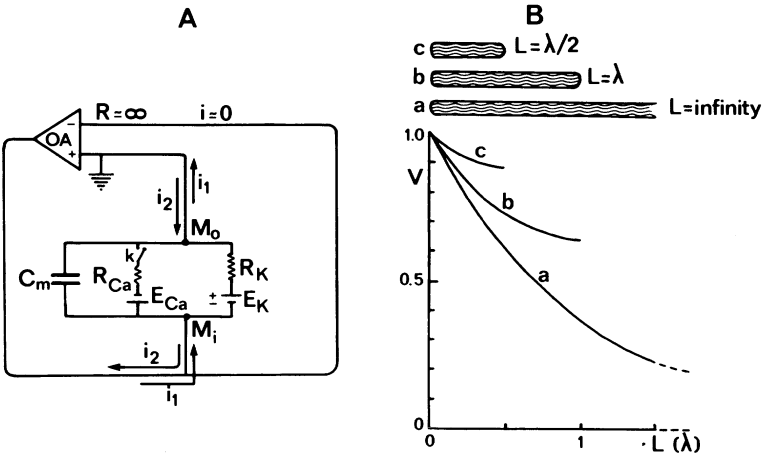

Figure 2. A: Principle of voltage clamp. Let us consider a schematic and simplified membrane with only two conductances g_{Ca} and g_K (the corresponding resistances being R_{Ca} and R_K so that the membrane resistance is given by $1/R_m = 1/R_{Ca} + 1/R_K$) and two equilibrium potentials $E_{Ca} = +80$ mV and $E_K = -100$ mV. At rest (g_{Ca} 'closed', key k open) the potential of M_i is -100 mV with respect to M_o, it is the resting value of E_m. For M_i to be clamped to -40 mV (depolarization step of 60 mV) it is necessary that the operational amplifier (OA) delivers through R_m (i.e., through R_K) an outward 'depolarizing' current (i_1, carried through the membrane by K^+ ions) which will make M_i less negative than before by 60 mV). Let us assume now that the depolarizing step is large enough to open largely g_{Ca} (i.e., to close key k). The potential of M_i would now tend to move towards $+80$ mV (instead of being equal to -100 mV) because R_{Ca} and R_K act as a voltage divider and R_{Ca} becomes transiently smaller than R_K (g_{Ca} transiently larger than g_K). Therefore, to prevent spontaneous depolarization of the membrane from occurring and to maintain the potential of M_i at -40 mV, the operational amplifier must now deliver through R_m (mainly through R_{Ca}) a current in the reverse direction as previously, namely an inward 'repolarizing' current (i_2, mainly carried through the membrane by Ca^{++} ions). It must be noted that closing and opening of the key k is a poor image of activation and inactivation of g_{Ca}. A progressive decrease followed by a progressive increase in R_{Ca} would be the correct representation. B: Spatial distribution of electrotonic potential V along a fiber when current is applied to the interior of the fiber at a sealed end (left end). Such a sealed end may be a natural end (papillary muscle) or a cut end after healing over. In case a, the length (L) of the fiber is assumed to be infinite; in case b, it is equal to the space constant λ, whereas, in case c, it is equal to $\lambda/2$. In case c, the voltage drop along the short fiber (i.e., at a distance of $\lambda/2$) is about 11% whereas in a long fiber (case a) it reaches about 40% at the same distance of $\lambda/2$.

experiments it increases or decreases linearly as a function of time. According to Ohm's law, $E_m = i_m R_m$. The aim of the voltage clamp technique is to measure the transmembrane current i_m. The apparatus, principally an operational amplifier permanently measures the membrane potential E_m and, when R_m varies, it adjusts i_m immediately so that, in square-pulse experiments, $i_m R_m$ (therefore E_m) remains constant (Figure 2A). It is necessary for all the current delivered by the amplifier (i.e., the current measured in voltage clamp experiments) actually to cross the membrane, in order to be equal to i_m. This is generally achieved by passing the current either through an intracellular electrode or through extracellular electrodes across a region of the fiber in which the extra-and-intercellular spaces have been rendered nonconducting by superfusion with sucrose or some other equivalent isotonic solution.

One of the main problems encountered in voltage clamp experiments is that of the nonuniformity of the membrane voltage as a function of space. For example let us assume that at a given point of a long fiber the resting membrane is suddenly depolarized by 40 mV, giving rise to an inward current. Because of the electrical properties of the membrane, the depolarization decreases exponentially as a function of distance according to the space constant λ and falls to a very low value ($\simeq 15$ mV) at a distance as short as 2 mm, so that, at this distance, it gives rise to an outward current. It is therefore impossible to study ionic currents in such a 'long' fiber since currents of different amplitude and even of opposite direction can flow in different parts of the fiber. Because membrane currents change with membrane voltage, one of the basic requirements of the voltage clamp is that the voltage be the same in each part of the preparation. A means of fulfilling this requirement is that the area of membrane in which the current is measured be made as small as possible. This can be achieved in several ways: (a) by cutting or ligating small segments of fibers (short fibers), (b) by using long fibers but artificially limiting the area in which the current crosses the membrane (artificial node), and (c) by using single cardiac cells. It is noteworthy that the spatial distribution of electrotonic potential is different in long and short cable-like fibers (see Figure 2B). The situation is however complicated by the fact that λ is not constant since it depends upon the membrane resistance multiplied by the unit length (r_m, in $\Omega \cdot$ cm) and upon the resistance of the core per unit length (r_i, in Ω/cm). Neglecting the resistance of the external fluid, $\lambda = \sqrt{r_m/r_i}$. When the membrane resistance decreases, as at the peak of the rapid g_{Na}, even short fibers tend to become long compared with λ. If the tissue under study cannot be considered as a cable but rather as a bi- or tridimentional syncytium, the spatial decrement of voltage from a point source gives a curve (Bessel function) which is even steeper than an exponential ([72]; see also [73]). Unless it is extremely thin, a bundle of cardiac cells can exhibit radial voltage nonuniformities, see for example [74–80].

Several techniques (Figure 3) are used in voltage clamp experiments applied to cardiac tissues (see for reviews [63, 64, 66, 70, 81]).

— (a) the double microelectrode technique was first applied to short (1 to 2 mm long) ligated Purkinje fibers [19, 26] then to short cut Purkinje fibers [27, 31, 36, etc.]. Almost all the results obtained with Purkinje fibers were carried out by this technique. Recently the same technique was applied to different preparations, namely small pieces (0.3 mm × 0.3 mm) of sino-atrial nodes [82], embryonic cardiac cell strands [83] and aggregates [84]. In this technique, the membrane potential is recorded by one microelectrode and the current delivered by the other.

— (b) the double sucrose gap technique first applied to cardiac fibers for nonvoltage clamp experiments by Rougier et al. [85] is a development of

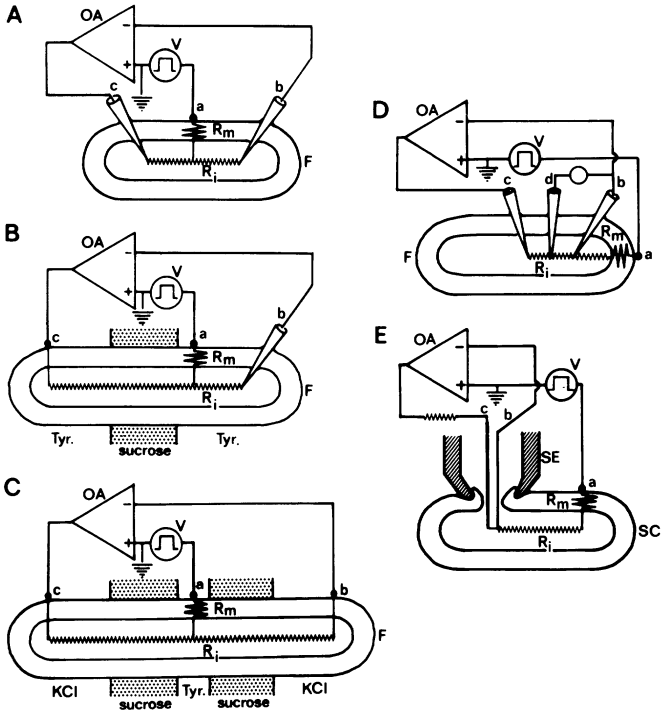

Figure 3. Schematic representation of arrangement for voltage clamp recording using 2 microelectrodes (A), single sucrose gap (B), double sucrose gap (C), 3 microelectrodes (D) and suction electrode applied to single cell (E). The membrane potential is measured between a and b. The current delivered by the operational amplifier (OA) flows through c and a and therefore through the internal resistance (R_i) and the membrane resistance R_m (membrane current). No appreciable current flows through b because the OA input resistance is very high. In D the longitudinal voltage difference between d and b is also a measure of the membrane current. F: multicellular fiber or preparation; SC: single cell; SE: suction electrode; Tyr: Tyrode's solution; V: voltage generator. Partly redrawn from [67], with permission.

Stampfli's method [86]. It allows measurement of the membrane potential across one sucrose gap whereas the current is delivered across the other one. In a variant of this technique [44, 87], all fluid streams are separated by vaseline seals (the physical properties of which must be carefully selected). In a second variant introduced by Jullian et al. [88, 89] a liquid partition system is used without any physical membrane separating the flow of the different solutions [90–92]. In yet a third variant, sucrose is replaced by isotonic glycerol (because of its better diffusion and slower penetration into the cells) and fluids are separated by four rubber membranes [93, 94].

— (c) in the monosucrose gap technique [32, 46, 47, 49] the muscle chamber with three compartments (first used for nonvoltage clamp experiments by Heppner et al., [95]) is a modification of that described by Kavaler [96]. The fiber is pulled through small holes punched into two rubber partitions. In the set up described by Mascher and Peper [46] the diameter of the holes is adjusted by a system of levers and screws. The voltage is measured by means of a microelectrode impaled in the small free end of the fiber superfused with a Tyrode solution whereas the current is delivered across the sucrose gap.

— (d) more recently the double microelectrode technique was improved by impaling Purkinje fibers with three microelectrodes (one to pass the current and two to record the membrane potential) [97, 98]. This disposition is similar to that developed by Adrian [99] for long skeletal muscle fibers, but allows, by using short fibers, simultaneous measurement of the transmembrane current in two different ways, namely as in the normal, double microelectrode technique and by recording the potential difference (Δ V) between the two microelectrodes, ΔV being proportional to the membrane current. This technique was also extended to the single sucrose gap technique (by using one sucrose gap and two microelectrodes, [100]).

— (e) also very recently [101] voltage clamp measurements were applied to entirely isolated cells of adult rat heart by using a suction pipette method, similar to that used in snail neurones [102, 103]. This allowed very good measurement of the sodium current.

The limitations of the voltage clamp techniques applied to complex preparations have been extensively analyzed [49, 54, 64, 66, 77, 80, 91, 104–112]. In addition to the above mentioned problem of the lack of uniformity of potential, one of the most serious limitations comes from the existence of an electrical resistance (R_S) which lies in series with the membrane resistance R_m (Figures 4 and 5). R_S consists of several components, namely the resistance of the electrode (R_{el}), that of the bulk solution (R_{bs}) and that resulting from the structure of the bundle itself. R_{el} may be lower than 50 Ω [113], but is often $\simeq 100\,\Omega$; R_{bs} is generally rather small (74 Ω for a test compartment of 100 μm width, 7.4 Ω for a 1 mm compartment, see [80]) whereas the bundle series resistance may be rather large. This resistance includes that of the

endothelial sheath (R_{es}) which exists around each trabeculum and behaves as a diffusion barrier [114] as well as the resistance (R_C) resulting form the existence of the narrow clefts which connect the cells situated in the depth of

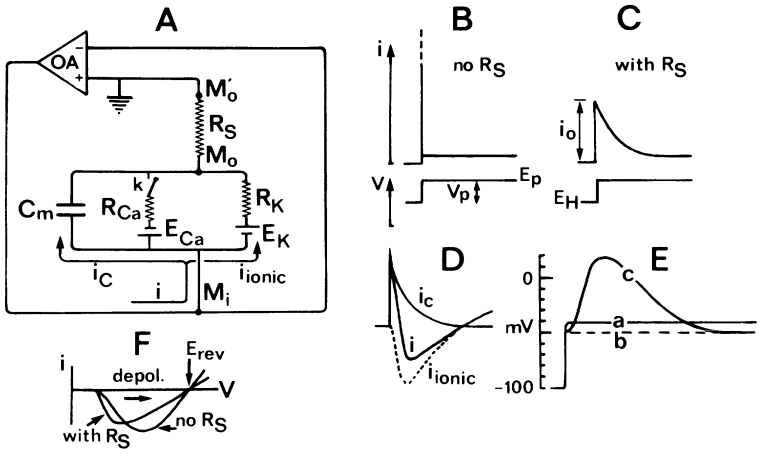

Figure 4. Influence of R_S (resistance in series with the membrane) on the capacity current and on the evolution of the potential immediately across the membrane during a depolarizing pulse. A: schematic drawing of the membrane similar to that of Figure 2 ($E_K = -100$ mV, $E_{Ca} = +80$ mV). At rest the Ca^{++} channel is assumed to be closed (key k open) so that $E_r = E_K$ and $R_m = R_K$. A, B, C: when a rectangular voltage pulse V_p is applied to the membrane from the holding potential E_H (assumed to be equal to E_r) to E_p, the amplifier (OA) generates a current which must first charge C_m (capacity current, i_C). In the absence of R_S the capacity current is initially 'infinitely' large because it flows in a resistance which is infinitely small ($R_S = 0$); it decreases with a time constant ($R_S C_m$) which is, for the same reason, infinitely short, i.e., the charge of the capacity is instantaneous (B). In the presence of R_S, the initial value, i_o, of the capacity current is limited by R_S, because $i_o = V_p / R_S$. Since for a given value of V_p, i_o is inversely proportional to R_S, the amplitude of i_o may be used as an index of R_S, a large i_o corresponding to a low R_S and vice versa. Moreover the charging of the capacity is now far from being instantaneous and the capacity current decreases exponentially with time (C). A, D, E: the capacity current can interfere with the measurement of the time-dependent ionic current, so that the resulting current, i, is not a correct representation of i_{ionic} (D). Moreover, in the presence of R_S, the potential across the membrane (b or c) is different from that, a, corresponding to the clamp pulse (E). Because the electrode in the solution is not situated in M_o but in M'_o, V_p is not applied between M_o and M_i but between M'_o and M_i. Therefore, if g_{Ca} is not activated (key k open), $V_p = i_K(R_K + R_S)$. The potential across the membrane is only $V_{pm} = i_K \cdot R_K$. If $R_S = 0.2\, R_K$ and V_p is a 60 mV depolarizing pulse, $V_{pm} = 50$ mV and E_m is brought to -50 mV instead of -40 mV (the voltage clamp error is -10 mV). If g_{Ca} is markedly activated by the depolarizing pulse (key k closed) so that g_{Ca} reaches $5\, g_K$ (i.e., $R_{Ca} = 0.2\, R_K$), E_m tends now to become close to E_{Ca}. In order to maintain E_m at the same value as before, the amplifier will now deliver a current in the reverse direction (inward current) through R_S, R_{Ca} and to a small extent R_K. If we neglect the current crossing R_K, i_{Ca} will produce across $R_S + R_{Ca}$ a potential drop from $+80$ to -40 mV (-120 mV). However since $R_{Ca} = R_S$, the potential drop across R_{Ca}, i.e., across the membrane, will reach only half this value (-60 mV) so that at the top of g_{Ca} activation E_m is only $+20$ mV instead of the chosen value of -40 mV (the voltage clamp error is $+60$ mV). After inactivation of g_{Ca}, E_m will reach again -50 mV. F: The existence of R_S induces typical alterations of the current-voltage (i–V) relationship. (Redrawn from [111], with permission.)

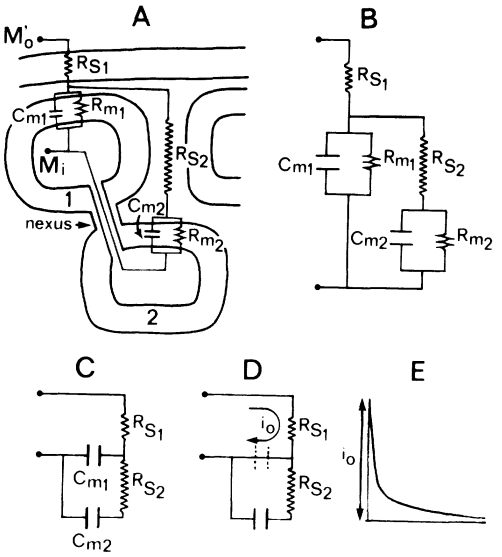

Figure 5. Localization of electrical resistances (R_{S1}, R_{S2}) in series with the membrane resistance in a multicellular preparation and their influence on the capacity current. A: schematic cross section through a bundle of fibers showing two cells, 1 and 2, electrically coupled via a low resistance pathway (nexus). Each cell has a membrane resistance (R_{m1}, R_{m2}) and a membrane capacitance (C_{m1}, C_{m2}). R_{S1} is in series with cell 1 and $R_{S1}+R_{S2}$ in series with cell 2. M'_o and M_i are as in Figure 4. $R_{S1} = R_{bs}+R_{es}$; R_{S2} is a part of R_C (see text). Circuits B and C are equivalent to that shown in A, but in C, R_{m1} and R_{m2} have been neglected. At the beginning of a voltage pulse, V_p, the resulting current, i_o, crosses R_{S1} and C_{m1}. Initially the resistance of C_{m1} is zero so that R_{S2} is short-circuited (D). Since initially no current crosses R_{S2}, the amplitude of i_o, which is therefore equal to V_p/R_{S1}, does not reflect the existence of R_{S2}. Because of the existence of two components of series resistance and membrane capacitance, the decay of the current is not monoexponential but exhibits also two components (E).

a cardiac bundle of fibers, with the periphery. It is clear that such clefts [74, 115, 116] are narrow enough to restrict diffusion markedly, since, in most cases, they allow ionic accumulation and depletion to develop in the course of the cyclic activity (see for example [117]). R_{es} has been estimated by Attwell and Cohen [80] to be 400 Ω or more for a ventricular bundle in a 1 mm test compartment, such as that used by Beeler and Reuter [49], whereas the R_C corresponding to the outer part of the bundle (cells occupying the region between radii r/2 and r) was estimated by the same authors at 1.6 kΩ in series with a membrane resistance of about 4 kΩ in the resting state and only 0.8 kΩ during the Na^+ upstroke.

The existence of R_S causes the amplitude and the time course of pulses immediately through the membrane, to be very different from that recorded by the electrode, mainly when a conductance allowing a strong current to enter the cell is activated. In this case, true transmembrane pulses are no longer rectangular (Figure 4E, c) but exhibit an initial hump which may be

fairly large and follows the kinetics of the conductance. In fact the oversimplified situation described in this figure is worse than that generally occurring when studying g_{Ca}, because R_S is generally much smaller than R_{Ca}. Such a bad situation more frequently tends to occur when the rapid Na^+ channel is under study becase g_{Na} is generally much greater than g_{Ca}. Figure 4E, c shows that depolarizing pulses of moderate amplitude (60 mV) can in fact behave as transiently large pulses (120 mV). This explains the phenomenon illustrated in Figure 4F that the maximum of the current–voltage relationship is shifted towards negative potentials when R_S is present (the current is more fully activated than it should be for moderate depolarizations). According to Figure 4F the reversal potential of the inward current is not altered by the presence of R_S. However this is true only if the conductance of the membrane for other ions is very small [80]. To a certain extent the value of R_S may be deduced from the size and the time course of the capacity current which develops when a rectangular voltage pulse is applied to the membrane (Figure 4B and C). When no R_S is present the charge of the membrane capacity is instantaneous (i.e., the capacity current is infinitely large but infinitely short). When R_S is present the capacity current is no longer infinitely large and, as a rule, decreases exponentially with a time constant $\theta = C_m(R_m \cdot R_S)/(R_m + R_S)$. If θ is large, the capacity current can alter the time course of the ionic membrane current (Figure 4D). However when two membrane circuits with two different R_S exist as in Figure 5, the decay of the capacity current is no longer monoexponential but exhibits two components (Figure 5E). In this case the initial amplitude of the current depends only on the value of the series resistance R_{S1} (Figure 5B, C and D). It may be seen in Figure 5A that R_{S1} is in series with the most peripheral cells. Therefore the initial amplitude of the capacity current does not reflect distributed cleft access resistances, such as R_{S2}.

Since R_S is distributed and has a different value for each cell, it is difficult to compensate. However several attempts have been made to minimize artifacts by electronic compensation of R_S [113, 118, 119]. Maintenance of a virtual ground at the very surface of the preparation has been used to remove from the measurements the voltage gradient across the resistance of the bath and electrodes [56]. Another system to eliminate R_S is that using a chopped clamp [120–125]. In this technique, the applied current is turned on and off at a high frequency (0.5 to 10 kHz) and the membrane potential, sampled during each zero current phase, is fed back through the clamp loop. The voltage clamp error ($i_m \cdot R_s$) disappears because E_m is measured when $i_m = 0$. However the cleft resistance is not compensated [80].

Among the many other sources of errors which can alter the results obtained by voltage clamp technique, it may be useful to indicate the following:

— (a) when polarizing with a microelectrode, all the current is delivered through one cell which is probably more or less altered and whose nexuses are possibly partially blocked. In addition, problems resulting from point polarization are encountered, namely the rapid spatial decay of potential around the electrode [126, 127].

— (b) when passing a current through a sucrose gap, a part of the current crosses the sucrose either when some calcium is added to prevent the uncoupling of cells (10^{-5} M Ca^{++}, [56]) or even in the absence of any added ions, because of imperfect diffusion of sucrose in the intercellular clefts. For this reason a short circuit (or isolation) factor must be taken into account, which is generally higher than 0.8 [87] or 0.9 [110]. From a pratical point of view it may be remembered that the ratio of the action potential amplitude when recorded with a microelectrode (AP_i) to that recorded across a sucrose gap (AP_o) allows the determination of the ratio of inside longitudinal resistance per unit length (r_i) to outside longitudinal resistance per unit length (r_o) in the sucrose gap (see 105, 128, 129) according to the equation $AP_i/AP_o = 1+(r_i/r_o)$. The isolation due to sucrose is generally considered as acceptable when r_o is about 7 to 10 times larger than r_i, i.e., when an action potential recorded across a gap is about 105–110 mV, assuming the maximal amplitude of an intracellular action potential to be around 120 mV. In order to eliminate the extracellular component of the applied current (shunt current) a 'guard gap' has been used as a slightly conducting sucrose gap maintained at earth potential between the normal sucrose gap and the test chamber [125]. In spite of incomplete washing of external ions, cells progressively disconnect [130] and partially uncouple. This is demonstrated by the observation that in a pure sucrose solution, the internal longitudinal resistance increases 10 times during 4 hours of sucrose perfusion [105]. Another point concerns the distribution of sucrose through the bundle: sucrose diffuses along the core of the bundle towards the test compartment, thus diluting ions, whereas the physiological solution diffuses similarly in the reverse direction, making the sucrose more conducting in the core of the bundle. The thicker the bundle the larger this phenomenon is. This situation has been theoretically analyzed by Attwell and Cohen [80]. Moreover, sucrose isolation can induce hyperpolarization [88] as a consequence of liquid junction potential occurring between the sucrose and physiological solutions. This potential generates loop currents which cross the membranes [131]; it can be largely eliminated by the interposition of a vaseline seal [132] or a rubber membrane between the sucrose and the saline. The sucrose gap also induces a shortening of the action potentials elicited in the saline compartment [109]. This effect is due to the electrotonic influence of the area of membrane in the sucrose Na^+-free, Ca^{++}-free medium which remains at the resting potential and therefore acts as a repolarizing anode [133].

In spite of the above mentioned limitations, it may be considered that the voltage clamp technique applied to cardiac tissues allows rather correct and reliable analysis of ionic currents, mainly those with a slow kinetics as the slow inward current is. In fact, even the rapid Na^+ inward current may be fairly well controlled, either in thin frog atrial fibers by using the double sucrose gap technique [44, 87, etc.] or, as more recently demonstrated in mammalian Purkinje fibers, by using the double microelectrode technique provided that thin and structurally convenient fibers are used and provided that the maximum amplitude of the current is not too large [134]. In the latter case, the temperature was also lowered (13–23 °C) to improve temporal resolution of i_{Na} (see also [135]). Transmembrane microelectrode recordings during voltage clamp experiments using the double sucrose gap method have recently yielded direct evidence of the fairly good quality of voltage control during the flow of the peak of rapid Na^+ current in frog atrial fibers [136–139]. Clearly, this control becomes quite satisfactory during the flow of the slow inward current (see for example [136, 137], etc.). Other indications of good voltage control have also been given recently [140, 141].

5. METHODS OF ANALYSIS OF THE SLOW INWARD CURRENT

As already stated, initial voltage clamp experiments which demonstrated the existence of the slow channel suggested that, like the rapid Na^+ channel [20], the slow channel was controlled by activation and inactivation processes. In a resting membrane, the channel is closed but when the membrane is suddenly depolarized, it opens (activates) then closes (inactivates) more slowly. When a resting cardiac fiber, for example a frog atrial fiber (Figure 6), is submitted to depolarizing pulses of increasing amplitude, large and brief peaks of inward current develop. The amplitude of the current which is reached at the top of these peaks depends on the membrane potential during the pulses and the corresponding current–voltage relationship (i–V curve) is shown in Figure 6D,a. This rapid inward current is the Na^+ current (i_{Na}). It results from the brief opening of the rapid Na^+ channel. After suppression of this current by TTX, a slower and smaller peak of inward current (slow inward current, i_{si}) can be observed but only for rather large depolarizing pulses (the threshold of i_{si} being higher than that of i_{Na}). i_{si} is suppressed either by adding Mn^{++} (2–4 mM) to the Ringer solution or by removing both Ca^{++} and Na^+ ions. After its suppression only outward current remains. The i–V curves corresponding to i_{si} and to the outward current measured at two different times (t_1 and t_2) are shown in Figure 6D,b,c and d.

Let us consider a 'statistical' channel (Figure 7A) in which the degree of opening or closing of the channel is indicated by gates *a* and *b* which are

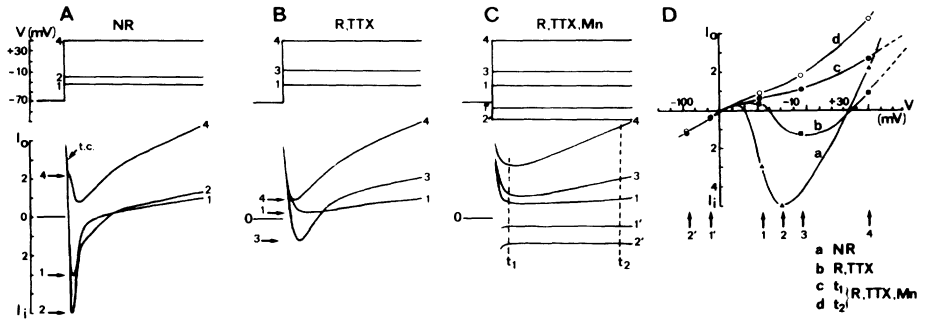

Figure 6. Ionic currents (A, B, C, lower traces) measured in a frog atrial fiber submitted to rectangular voltage pulses of different amplitudes (A, B, C, upper traces). Semischematic representation. Voltage clamp experiment using double sucrose gap technique. NR: normal Ringer solution; R, TTX: tetrodotoxin added to Ringer solution; R, TTX, Mn: manganese and TTX added to Ringer solution. t.c.: transient capacity current. In A and B the fiber is submitted to depolarizing pulses only; in C it is submitted to depolarizing and hyperpolarizing pulses. D: current–voltage (i–V) relationships plotted at times corresponding to the peak of i_{Na} (curve a), peak of i_{si} (curve b) and at times indicated in C, t_1 (curve c) and t_2 (curve d). Pure i_{si} is approximately given by b–c. I_o: outward current; I_i: inward current. Reproduced with permission from [67].

open to varying degrees so that more or fewer ions can cross the membrane (see [67]). The upper gate *a,* which is supposed to be closed at rest, is the activation gate and the lower one *b,* which is open at rest, is the inactivation gate. We shall also assume that the degree of opening of each gate, *a* or *b,* may be quantitated from zero (gate closed) to 1.0 (gate open). Such a statistical channel corresponds in fact to the sum of individual channels of the membrane, so that the degree of opening of the gates actually corresponds to the proportion of individual channels with an open gate (or to the amount of time spent by each gate in the open state). The fact that the channel is controlled by only one gate *a* and one gate *b* corresponds to the assumption that $\gamma = 1$ and $\delta = 1$ (equation 2). We shall see later that such an assumption has been made in the case of the slow channel. According to equation 3, the current passing through the channel is proportional to the *product* of $a \times b$, i.e., if gate *a* is opened by 30% and gate *b* by 60%,

$$i_\chi = \bar{g}_\chi \cdot 0.3 \times 0.6 (E_m - E_\chi) = 0.18 \bar{g}_\chi (E_m - E_\chi).$$

The schematic picture shown in Figure 7 may be of some help in visualizing activation and deactivation (opening and closing of the activation gate) and also inactivation and removal of inactivation (closing and re-opening of the inactivation gate). The term reactivation is sometimes used in place of removal of inactivation although it does not concern the activation gate. The terms repriming and recovery from inactivation are also used to describe the way in which the current recovers after inactivation.

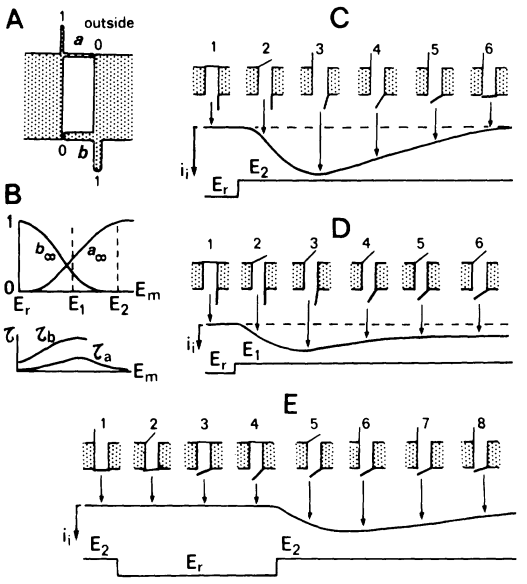

Figure 7. A: schematic picture representing a statistical ionic channel assumed to be controlled by independent voltage sensitive (positively charged) activation (*a*) and inactivation (*b*) gates. *a* and *b* also represent the degree of opening (from 0 to 1) of the corresponding statistical gates. It must be noticed that the current crossing the channel is proportional to the product $a \times b$, a point which cannot be deduced from the figure. B: schematic representation of steady-state values, from 0 to 1, of *a* ($8a_\infty$) and *b* (b_∞) as functions of E_m (upper curves) and time constants of activation (τ_a) and inactivation (τ_b) as functions of E_m (lower curves). C, D and E: development of inward current, i_i, and simultaneous displacement of statistical gates *a* and *b* (steps 1, 2, 3, etc.) when the resting membrane (E_r: resting potential) is submitted to depolarizing pulses bringing E_m to E_1 or E_2 (see B). C: complete activation of the inward current, followed by its complete inactivation when the membrane is depolarized to E_2. D: incomplete activation and inactivation of the inward current when the membrane is depolarized to E_1. In this case a steady-state inward current persists during the depolarizing pulse due to the overlap of $a_\infty - E_m$ and $b_\infty - E_m$ curves. E: deactivation and removal of inactivation of the inward current. When a new depolarization occurs at a time when the removal of inactivation is still incomplete (steps 4 and 5), the inward current resulting from this premature activation is smaller than normal (compare with C).

In order to determine parameters of the activation and inactivation processes it may be sufficient to analyse the amplitude and kinetics of currents triggered by voltage steps of different amplitudes. For example from the inward current shown in Figure 7C, the time constant of activation at $E_m = E_2$ can be determined by plotting on a semi-logarithmic graph the values of the current as a function of time. Measurements should be limited to periods of time when inactivation remains negligible (step 2 and possibly 3 in Figure 7C). Similarly, the time constant of inactivation is obtained by plotting the current after the channel is fully activated ($a = 1$), i.e., between

steps 3 and 6 in Figure 7C. However this is not always easy to do, because the recorded current is generally not a pure current. For example the initial phase of an inward current may be altered by the capacitive transient (see Figure 4D) whereas its inactivation phase may be superimposed on progressively increasing outward currents (Figure 8A). In such a case it is very difficult to determine, for example, the time at which the inward current is totally inactivated, and therefore the time course and also the true amplitude of the inward current As a consequence, the determination of the conductance giving rise to the inward current becomes also highly speculative. Different means of measuring inward currents are reported in Figure 8B. The total current resulting from the simultaneous occurrence of inward and outward currents can be measured at the peak of the slow inward current (maximum net inward current) (Figure 8B, a) or at the minimum of the outward current (a'). This cannot however allow the determination of the true or pure inward current. The i–V relationship obtained in this way (Figure 9, i_T curve) is different from that corresponding to pure inward current and the value of E_m at which the i–V curve crosses the voltage axis (E_3) is necessarily different from the reversal potential (E_{rev}) of the inward current. Determination of pure inward current may be done either after elimination of this current (by removal of the external ion which is thought to carry the current, or after blocking the channel by a selective inhibitor) or by graphical methods. When the delayed outward current is small, the inward current may be measured as in Figure 8B, b. When the delayed outward current is rather large it may be eliminated by assuming its change to be either linear (Figure 8B, c) or exponential (Figure 8B, d or d'). Although exponential extrapolation is in most cases valid, it must be remembered that in some tissues outward currents with a nonexponential time course have been described [142, 143]. In frog atrial septal trabeculae, such a current seems to be rapid enough [143] to possibly interfere with i_{si} measurements. Graphical methods of measuring pure inward currents give acceptable results when the inward current is thought to be totally inactivated. If the current is not totally inactivated or if outward currents of complex kinetics are superimposed on i_{si} (as in Purkinje fibers, see [53]), the determination of its amplitude by graphical methods as, in Figure 8B, b, c or d, is impossible and so is that of the corresponding conductance.

A way of overcoming some of these difficulties is to terminate depolarizing pulses at different times during the decay of the current and to measure the amplitude of the current tail which develops at the time of repolarization. Because g_{si} is time-dependent and therefore cannot vary instantaneously, immediately after repolarization the conductance should still be the same as that which was present just before repolarization. The peak amplitude of the current tail is therefore proportional to g_{si} at the moment of repolarization

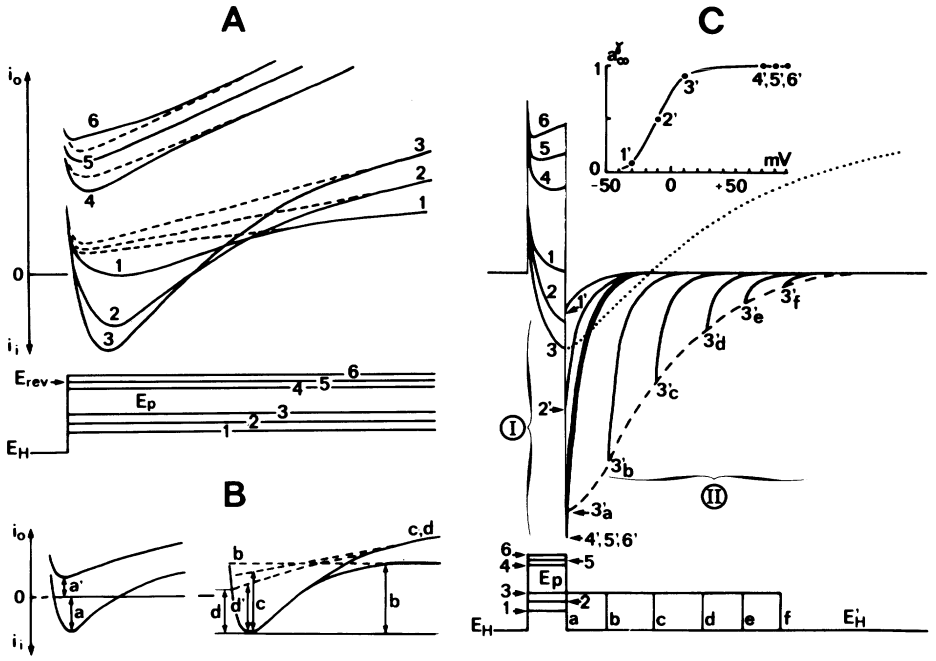

Figure 8. A: schematic drawing of slow inward currents occurring in a frog atrial fiber after inhibition of the rapid sodium channel in response to depolarizing pulses of amplitudes 40 (1), 60 (2), 80 (3) and 140 (4) mV. Dotted lines are corresponding outward currents as they may be obtained after suppression of the inward current by removal of the ion carrying this current or selective inhibition of the channel. True or pure inward currents are therefore given by the area comprised between each continuous trace and the corresponding dotted line. In 5 (150 mV pulse) the inward current becomes zero (reversal potential, E_{rev}) and in 6 (160 mV pulse) it reverses into an outward current. E_H: holding potential; E_p value of E_m during the pulses. B: Different ways of measuring the slow inward current. Total current measured at the maximum of inward current, a, or minimum of outward current, a', (see [44, 50, 56, 189]). Pure slow inward current measured after graphical subtraction of outward current, when the delayed rectification is small, b, [56] or large, c, [161] and d [165]. C: analysis by current tails of kinetic parameters of a conductance governed by activation and inactivation processes. I: determination of steady-state values of the activation parameter as a function of E_m. The fiber is submitted to the same sequence of depolarizing pulses as in A, but pulses are interrupted and the fiber is repolarized to E'_H (in this example $E'_H = E_H$) when the activation gate has reached its new steady-state value and the inactivation gate is still fully open. Because the driving force ($E_H - E_{rev}$) is the same for all tails of inward current, the initial amplitude of the tails is proportional to the steady-state degree of opening of the activation gate. Tails are used to plot steady-state activation curve (inset). II: determination of the time course of inactivation. Pulses of a given amplitude (80 mV in this example) are interrupted at different times (b–f). The decrease in the corresponding tail amplitudes (3'b–3'f) indicates the progressive closing of the inactivation gate. The time constant of inactivation may easily be calculated from the dashed curve. In this example, the slow inward current is assumed to undergo complete inactivation. Current tails following pulses shorter than a may be used to analyze the kinetics of activation of the inward current. The dotted line indicates the time course of the current for a long lasting pulse. Decays indicated by dotted and dashed lines are similar because the time course of i_K is not as complex as it is in Purkinje fibers (compare with Figure 3 from [53]).

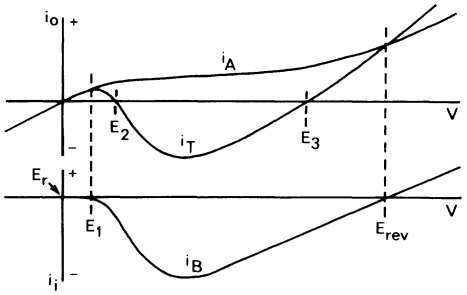

Figure 9. Schematic current–voltage (i–V) relationship of inward current. Curve labelled i_T: i–V relationship of the total current measured at the peak of inward current, i_i (between E_2 and E_3), or at the minimum of outward current, i_o (beyond E_3), as in the left part of Figure 8B. Curve labelled i_A: i–V relationship of the outward current measured (at the same time as for the i_T curve) either after graphical substraction (see Figure 8A and right part of Figure 8B) or after specific inhibition of the inward current. Curve labelled i_B: pure inward current measured as in the right part of Figure 8B or obtained after subtraction of the two upper curves, $i_B = i_T - i_A$. E_1 is the threshold of the i_B inward current. In the absence of conduction (short fiber), E_2 would be the threshold and E_3 the top of a response induced by the inward current. E_{rev}: reversal potential of the i_B current. i_B current as measured between E_1 and E_{rev} is sometimes referred as net inward current. However it is preferable to restrict this term to i_T current as measured between E_2 and E_3.

provided that the contribution of outward currents (mainly K^+ currents) to the total current is small at the holding potential. Since K^+ conductance is high in the potential range corresponding to the resting potential, this can generally be achieved only by repolarizing the membrane at a holding potential (E_H) close to E_K, so that the driving force for K^+ ions is as small as possible. In such a case, and assuming that other currents do not complicate the situation, the envelope enclosing the current tails which follow pulses of a given amplitude and different durations (dashed curve in Figure 8C, for pulses labelled 3) gives a good indication of the time course of the conductance change responsible for i_{si} (see [50, 53, 57, 144]). This envelope can therefore be used to determine the time constants of activation and inactivation of i_{si} at a given potential E_p, corresponding to that of the depolarizing pulses. When the membrane is repolarized at a potential appreciably less negative than E_K, tails of inward current, which develop at the end of short-lasting depolarizing pulses, change progressively from being inward to being outward, when the pulse duration increases because of the time-dependent activation of K^+ conductances. It is however possible to separate graphically i_{si} from i_K in the current tail method (see [129]).

It may be worth noting that the decay of each individual inward current tail corresponds to the rapid closing of the activation gate (deactivation) at a moment when the inactivation gate remains almost completely or partially

open. Since activation and deactivation have in theory the same time constant (when measured at the same potential) such tails may be used to calculate the time constant of activation of i_{si} at E'_H (Figure 8C; in the case of the figure $E'_H = E_H$). It may also be noted that because the time constant of activation is generally much shorter than that of inactivation (compare any of the current tails with the dashed line in Figure 8C), brief depolarizing pulses interrupted at the time of maximum inward current, are sometimes used as an index of a good clamp. A tail much briefer than the time course of inactivation indicates that the membrane has in fact been suddenly repolarized, i.e., has obeyed the command pulse at a time (maximum inward current) when the probability of loss of membrane potential control is the greatest. On the other hand the observation of a tail decaying slowly or with a complex kinetics may suggest that repolarization was not immediately imposed on the membrane.

Another interest of measuring current tails is to allow determination of the steady-state value of the activation parameter of the conductance giving rise to an inward current, as a function of the membrane potential (a_∞–E_m relationship; Figure 8C, inset). This can be done by depolarizing the membrane at different levels from E_H by brief pulses as shown in Figure 8C (pulses interrupted at time t = a, i.e., when the activation gate is supposed to have reached its new steady-state degree of opening and the inactivation gate is still completely open or almost (step 3 in Figure 7C or D). Even when no current crosses the channel during the pulse because the pulse has reached the reversal potential of the current (E_m–E_{rev} = 0, pulse n° 5 in Figure 8A and C), the large subsequent tail due to the sudden establishment of a large driving force after the pulse (in the case of tail n° 5′, the driving force, E_m–E_{rev} = 150 mV), indicates that the activation gate of the channel is fully open. As noted by Gibbons and Fozzard [144] some uncertainties can occur in the current tail analysis, because of the existence of capacitive transients (not shown in Figure 8); the end of the capacity current merges with the tail current so that it is sometimes difficult to judge the points which should be considered as being the maximum tail current. Accumulation–depletion phenomena can also interfere with the time course of currents measured during voltage clamp pulses.

Determination of the relationship giving the steady-state value of the inactivation parameter as a function of membrane potential (Figure 10) is performed in the following way: the membrane is depolarized at different levels by conditioning prepulses, long enough for the inactivation gate to reach its new steady-rate degree of opening, then is submitted to a second depolarizing test pulse reaching a constant membrane potential (for example +10 mV, i.e., a value sufficient to open the activation gate completely or almost and subsequently close the inactivation gate), thus allowing an inward current to cross the membrane (i_i, Figure 10). The amplitude of this inward current is

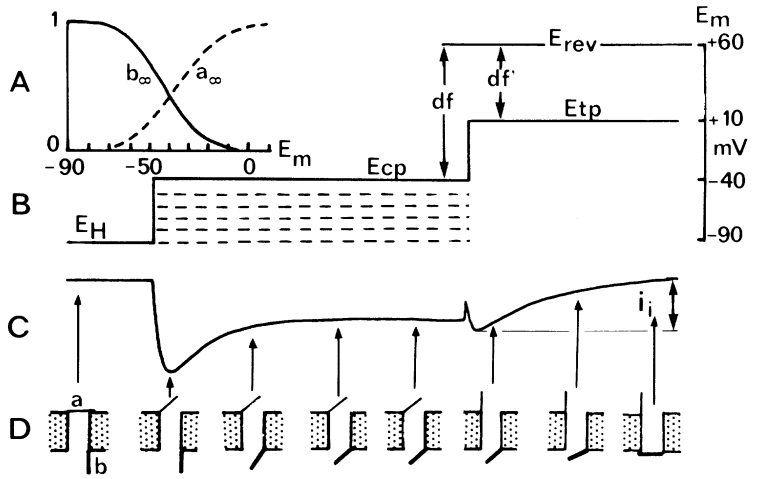

Figure 10. A: steady-state value of the inactivation parameter, b_∞, of inward current as a function of the membrane potential, E_m. B: clamp procedure used to obtained b_∞. E_H: holding potential; E_{cp}: membrane potential during conditioning prepulse; E_{tp}: membrane potential during test pulse; E_{rev}: reversal potential of the current under study; df, df': driving force. The inward current (C) and the schematic channels (D) have been drawn to correspond approximately to the upper conditioning pulse (B, solid line). If the conditioning pulse is long enough, the amplitude of the inward current during the test pulse, i_i, is proportional to the steady-state value, b_∞, of the inactivation process at $E_m = E_{cp}$. When different values are given to E_{cp}, the curve b_∞–E_m can be drawn. For sake of clarity outward currents have been ignored. The curve b_∞–E_m may also be obtained by using another clamp procedure, similar to that shown here, except that $E_H = E_{tp}$.

proportional to the degree of opening reached by the inactivation gate at the end of the prepulse. Removal of inactivation is an important functional parameter because it conditions the amplitude of the inward current during a premature extrasystole. If the extrasystole occurs too early, so that the removal of inactivation is incomplete, the inward current is smaller than normal. Removal of inactivation is generally measured by submitting the fiber to two depolarizing pulses (Figure 7E) separated by periods of repolarization of varying duration (d_r) and by measuring the current i_i, triggered by the second depolarizing pulse. Plotting i_i as a function of d_r for a given membrane potential between the two pulses, allows the determination of the time constant (τ_r) of the removal of inactivation at this membrane potential which is, in this particular case, the time constant of recovery of the inward current from inactivation. A more direct means of measuring the removal of inactivation may be used in the potential range where a_∞ and b_∞ are both different from zero, i.e., in the potential range where the curves $a_\infty - E_m$ and $b_\infty - E_m$ overlap as shown in Figure 10. If a membrane is repolarized in this potential range after having been strongly depolarized, the kinetics of the resulting inward current corresponds mainly to removal of inactivation. In theory,

if first order kinetics is assumed, inactivation (closing of *b* gate) and removal from inactivation (re-opening of *b* gate) should have the same kinetics when measured at the same E_m. This however is not always the case as we shall see later.

6. CHARACTERISTICS OF THE SLOW INWARD CURRENT

In order to analyse the slow inward current it must be separated from i_{Na}. This may be performed in several ways: (a) removal of extracellular Na^+ [41]; (b) addition of TTX [44] — TTX is a well-known inhibitor of i_{Na} in many tissues (see [145]) including heart [146]; (c) use of conditioning pulses large enough to inactivate the Na^+ channel without activating the slow channel [48].

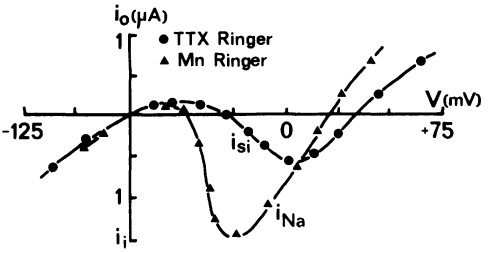

Figure 11. Current–voltage relationships of rapid inward current (i_{Na}) and slow inward current (i_{si}). Currents are measured as in Figure 9 (curve i_T). Frog atrial fiber. Double sucrose gap technique. Redrawn from [44], with permission.

The slow inward current is generally smaller than i_{Na} (Figure 11). In cardiac tissues exhibiting action potentials with a rapid ascending phase, i_{Na} lasts only a few milliseconds and is apparently similar to that in nerves and skeletal muscles. In frog atrial fibers, the maximal value of i_{Na} ranges most often between 1.0 and 5.5 μA and that of i_{si} between 0.1 and 2.5 μA [44, 55, 147]. In mammalian cardiac muscles, values as large as 25–30 μA have been reported for i_{Na} whereas in the same type of preparation i_{si} was $\simeq 3\,\mu$A [56]. Peak amplitudes of i_{si} ranging between 1 and 5 μA have been reported [61]. In Purkinje fibers rather low values (0.05 to 0.35 μA) are generally reported [98, 148]. Such differences reflect, besides possible differences in membrane structure, a difference in size of the preparations and other individual variations such as the degree of steady-state liberation of mediators by nerve terminals existing in the bundle and the level of metabolic activity of the tissue. In entirely isolated adult heart cells, the i_{si} peak was reported to be about 0.15 nA [149] but more recently values as high as 3 nA were obtained [150]. It is difficult to determine with accuracy the value of the peak

current density because of the uncertainties in the estimation of the membrane surface of the preparations. Another uncertainty comes from the fact that whether or not the current density is markedly different in the different parts of the cell membrane (outer surface, intercellular clefts, transverse tubules, sarcolemmal-terminal cisternae appositions) remains unknown up to the present. From the determination of the area enclosed by i_{si} in voltage clamp measurements, it is possible to estimate the Ca^{++} flow into the intracellular volume of the bundle during depolarization. For $[Ca^{++}]_o = 1.8$ mM and depolarizing steps eliciting maximum inward current, values ranging from 5×10^{-7} to 5×10^{-6} mole of Ca^{++}/litre fibre and impulse were reported for the cat ventricle [57]. Somewhat higher values (net gain $\geq 10^{-6}$ mole/litre) were calculated for the dog ventricle [151, 152], and Reuter [60] estimated the increase in Ca^{++} influx between 0.3 and 1.3 pmole/$cm^2 \cdot$ impulse.

The slow inward current is clearly controlled by activation and inactivation processes. This may be observed for example when having been triggered by a depolarizing step, this current is then suppressed by an inhibitor of the slow channel such as Mn^{++} or D–600 (Figure 12). i_{si} can be described according to the Hodgkin–Huxley theory [44, 50, 56, 57, 100, 151, 153–159]. Activation and inactivation variables of i_{si} have been labelled either m' and h' [153, 160] or d and f [59] respectively. As the latter term has been more widely used in the literature, we shall therefore use it in this paper. The problem of deciding

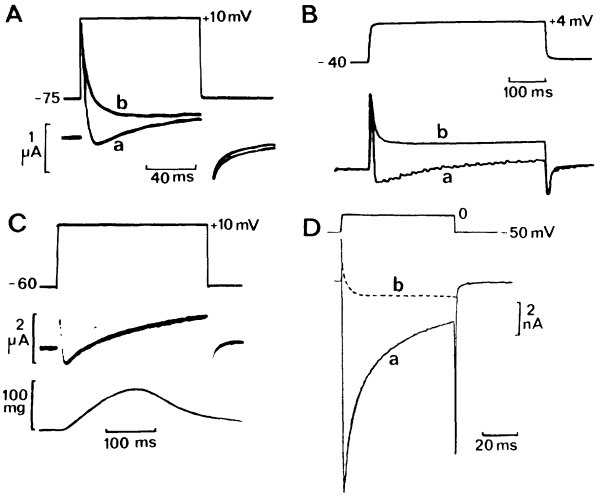

Figure 12. Some examples of slow inward current recorded in cardiac tissues. A: frog atrial fiber; current measured in Na^+-free medium before (a) and after addition of 2 mM Mn^{++} (b), [44]. B: Purkinje fiber; current measured in the absence (a) and presence (b) of 5×10^{-6} g/ml D-600 [148]. C: cat ventricular muscle; simultaneous record of i_{si} and contraction [156]. D: isolated bovine heart cell; current measured in the absence (a) and presence (b) of 2μM D-600 (By courtesy of Dr. G. Isenberg.).

whether or not d has to be raised to a power greater than 1 has not yet been clearly solved. However, it is generally admitted that the power of both d and f may be 1 [59, 71, 157, 158] although some authors [153, 160] have extended to the activation parameter of g_{si}, the power 3 determined in the case of the rapid Na^+ channel of the squid axon [20].

Table 1 gives a certain number of values of τ_d and τ_f reported in the literature. In fact information concerning τ_d remains limited. For example rather large values ($\simeq 40$ msec at $E_m = 0$ mV, [100]) have recently been reported for deactivation time constants in mammalian cardiac muscles (cow ventricular muscle) whereas smaller values have been reported for activation time constants in frog atrial fibers, i.e., $\simeq 3$ msec [153] and 5 msec [147] at $E_m = 0$ mV. Both activation and deactivation time constants should have the same value at a given membrane potential, if they really correspond to the same mechanism operating in one direction and in the reverse. Marked differences could mean that the d gate is in fact composed of two kinetically different systems. Such differences might also result from species or experimental differences. Interestingly recent observations on isolated single bovine heart cells show a very short activation time course, $\tau_d = 0.6$ msec at $E_m = 0$ (Isenberg, personal communication).

Inactivation time constants have been more frequently determined. Results fall roughly into three categories, those showing a large and regular increase in τ_f, as E_m becomes more and more positive (for example Beeler and Reuter, in the dog ventricular muscle [50]), those showing a moderate increase (for example Trautwein et al., in the cat ventricular muscle [156]) and those showing a decrease (for example Horackova and Vassort, in frog atrial fibers [161]). Large values of τ_f observed in the potential range corresponding to i_{si} threshold [56, 155] have been attributed to a distortion of the inactivation process by a rather slow activation process in this potential range [156]. Most authors now agree that τ_f increases when the membrane is depolarized from the i_{si} threshold to $\simeq 0$ mV. More surprising is the difference observed for depolarizations beyond 0 mV. In the mammalian ventricular muscle, it seems clear that τ_f continues to increase when the membrane is depolarized at positive potentials [49, 56, 129, 155, 156], a phenomenon which has been introduced in reconstruction of action potentials of ventricular myocardial fibers by Beeler and Reuter [158]. On the other hand, in frog atrial fibers [153, 161, 162], τ_f is clearly smaller at positive than at negative membrane potentials, a fact which has been observed both by direct analysis of ionic currents and indirect analysis of the phasic tension [161]. Unpublished data by Christé et al. have shown that in both the dog atrium and the human atrium τ_f also decreases for depolarizations beyond 0 mV. It is difficult, in the present state of our knowledge, to give the reasons for such a different behavior.

Table 1. Approximative values of activation, inactivation and reactivation time constants of i_{si}.

Tissues, species	*Temperature* (°C)	E_m (*mV*)	τ_f (*msec*)	τ_d (*msec*)	τ_r (*msec*)	*Authors*	*Ref.*
Atrial fibers							
frog	16–18	−20	25–30			Vassort et al. 1969	(193)
	22–24	−20 to +10	12–24	12–15 (r.t.)		Rougier et al. 1969	(44)
	21	−50	50	10		Besseau 1972	(153)
		0	20	2.8			
		+60	17	1.4			
		−70			τ_{r1} 80 τ_{r2} 350	Mironneau et al. 1975	(175)
	20	−13	55			Horackova & Vassort 1976	(161)
		+50	11				
	18	−75			τ_{r1} 94 τ_{r2} 305	Ducouret 1976	(147)
		−80	15	15			
		0		5			
		+10 to +30	20	3–4			
	17	−25	115			Tsuda 1979	(162)
		0		40 (r.t. ?)			
		+20		20			
		+25	35				
dog	27	−60	25			Christé, Ojeda & Rougier unpublished	
		−30 to −10	40				
		+20	20				
human		−45	10			Christé, Ojeda & Rougier unpublished	
		−35	15				
		0	7				
Purkinje fibers							
sheep	36	−70	40			Vitek & Trautwein 1971	(53)
		−25	90				
	35–37	−77			670	Gibbons &Fozzard 1975	(144)
	37	−56	100			Isenberg 1977	(320)
		−46 to −48	120–165				
		−35 to −38	145–180				

Table 1. Approximative values of activation, inactivation and reactivation time constants of i_{si} (*continued*).

Tissues, species	*Temperature* (°C)	E_m (*mV*)	τ_f (*msec*)	τ_d (*msec*)	τ_r (*msec*)	*Authors*	*Ref.*
Ventricular fibers							
cat		−40	>120	20			
		−10		6–8			
	37	−25 to +10	60–70			New & Trautwein 1972	(56)
		>+30	>120				
		−80			118		
		−60			144		
	37	−40	60	20–40	198		
		>−20		5–10			
		−20	90				
		>+20	150–200			Trautwein et al. 1975	(156)
		−80			230		
	32	−60			250		
		−40			335		
		−40			150–200		
		−35	60–100				
		−30			180–220		
	32	−30 to 0	40–70			Kohlhardt et al. 1975	(155)
		−20			260–320		
		−10			300–380		
		>+30	110–150				
	31	−20 to +40	58–160			Simurda et al. 1976	(332)
	36	0	95			Trautwein & McDonald 1978	(159)
		−60		50–55			
		−40	50				
	36	0	80			McDonald & Trautwein 1978	(129)
		+30	120				

(*continued on page 52*)

Table 1. Approximative values of activation, inactivation and reactivation time constants of i_{si} (*continued*).

Tissues, species	*Temperature* (°C)	E_m (*mV*)	τ_f (*msec*)	τ_d (*msec*)	τ_r (*msec*)	*Authors*	*Ref.*
pig	37	−69 to −75			55–60	Gettes & Reuter 1974	(171)
		−50			75		
		−38	90				
		−17	120		110		
		−15	200				
		−9			250		
pig, sheep		−80	30–50			Reuter 1973	(60)
		−50		20			
		+15		1			
		+20	200–500				
dog	35	−35	40			Beeler & Reuter 1970	(50)
		0	300				
		+50	700				
cow	35	−50		16		Reuter & Scholz 1977	(100)
		−20	60	40	140		
		−10			250		
		0	140	40			
Isolated cells							
cow		0		0.6		Isenberg 1980 unpublished	
Embryonic cell aggregates							
chick	35–37	−30	20			Nathan & DeHaan 1979	(84)
		−20	12.5				
		+10	22				

r.t. = rise time

The gradual development with increasing depolarizations of time-dependent ionic currents, which differ from i_{si} (specially K^+ currents) may certainly be a source of difficulty for the accurate determination of τ_f, in spite of the efforts made to substract i_{si} from other currents. Among currents which are able to interfere with i_{si}, the most obvious are delayed currents labelled i_{x1}, i_{x2} ([163–165] but see [143]), I_1, I_2 [166], i_K, i_x [129]. Another characteristic example of interference is given by the transient outward current [39] mainly attributed previously to chloride (Cl^-) ions (for references see [167]) and which is known today to be carried to a large extent by K^+ ions [168]. This current (Figure 13) which may be called i_{qr} [169] is responsible for the early

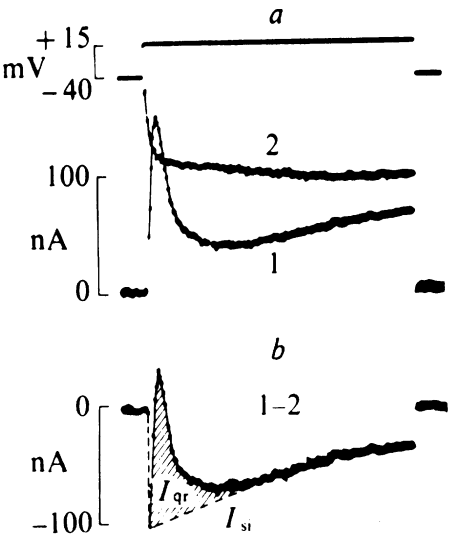

Figure 13. Apparent alteration of the time course of the slow inward current in a calf Purkinje fiber due to the development of the transient outward current (positive dynamic current) labelled i_{qr} · a: current measured before (1) and 30 min after (2) addition of 5×10^{-6} g/ml D-600 · b: i_{si} obtained by subtracting trace 2 from trace 1. [169, reproduced, with permission, from Nature.]

repolarization in cardiac Purkinje fibers and has apparently not been observed in other cardiac tissues. It can distort entirely the apparent kinetics of the slow inward current by superimposing on i_{si} a surge of outward current of variable amplitude and duration. Because this surge of outward current is reduced or suppressed after blocking the slow channel with verapamil or D–600 [169], or when Ca^{++} is replaced by strontium (Sr^{++}) or barium (Ba^{++}), or after ionophoretic injections into the fiber of the Ca^{++} chelator EGTA * [170], it has been suggested that i_{qr} is largely controlled by

* EGTA: Ethyleneglycol-bis-(β-aminoethyl ether) N, N′-tetra-acetic acid.

$[Ca^{++}]_i$ [170]. If this is true, i_{qr} would directly result from the increase in subsarcolemmal Ca^{++} concentration produced by the flow of i_{si}. It cannot be excluded that in most cases the time course of i_{si} inactivation results partly from the development of a transient outward current superimposed on i_{si}.

Another problem concerns the comparison between the kinetics of inactivation (τ_f) and the removal of inactivation (τ_r) of i_{si}. Contradictory results have appeared in the literature on this point. Gettes and Reuter [171] have reported experiments where τ_f and τ_r were very similar, i.e., about 100 msec at $\simeq -35$ mV and 200–250 msec between -15 and -10 mV for both time constants. On the other hand, on a different species, Kohlhardt et al. [155] found very different time constants for both processes when measured at the same membrane potential, for example 39 and 300 msec at $\simeq -10$ mV for t_f and τ_r respectively (Figure 14). Considering that in Kohlhardt et al.'s experiments the temperature was lower than in those of Gettes and Reuter (32 °C instead of 37 °C), values for τ_r are not contradictory since Trautwein et al. [156] have shown that when the temperature is decreased from 37 to 32 °C τ_r increases by 65% at $E_m = -40$ mV. The discrepancy comes from τ_f measurements. The validity of direct measurements has been discussed by Reuter and Scholz [100]. They consider that the method which consists of analyzing the exponential decay of the current (semi-logarithmic plot of the current vs. time) may be subject to considerable error, as it depends on the accuracy by which the steady-state value to which i_{si} declines can be estimated. Using this method, Reuter and Scholz [100] found τ_f which is clearly smaller than τ_r by at least a factor of two. Although such a difference may be

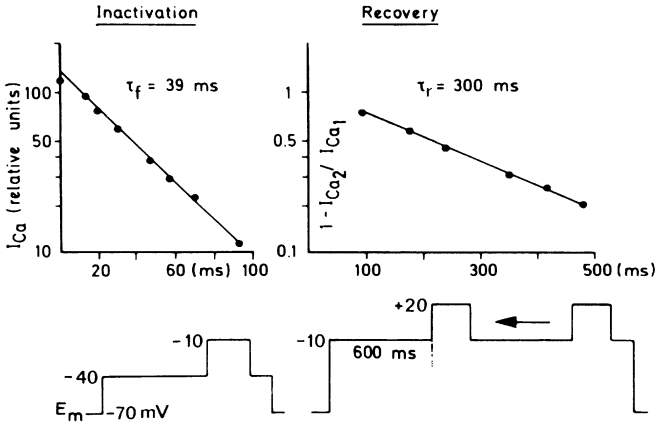

Figure 14. Experiment showing that the time constants of inactivation (τ_f) and removal of inactivation (τ_r) of i_{si} may have different values at the same membrane potential ($\simeq -10$ mV). Semi-logarithmic plot of the inactivation of i_{si} elicited by a test pulse from -40 to -10 mV (left) and the recovery from inactivation when the interval between pulses from -10 to $+20$ mV is progressively shortened (right). Cat ventricular muscle. Modified from [155], with permission.

attributed to the inaccuracies of the method, it is more difficult to accept the assumption that differences by a factor of almost 8 [155] may be due to similar errors. Kohlhardt et al. [155] measured both the activation of the i_{si} elicited when the membrane was depolarized from -40 mV to -20 mV and the removal of inactivation at the same potential, when the f gate partially re-opened by repolarizing the membrane from 0 to -20 mV. In this case a progressively increasing current developed. Time constants were 75 and 290 msec respectively, suggesting that τ_r measured directly from the change in i_{si} really is the same as τ_r measured indirectly from the recovery of inward currents elicited at varying intervals, after a conditioning depolarizing pulse. It may be argued that the progressively increasing inward current observed by Kohlhardt et al. [155] when repolarizing the membrane from 0 to -20 mV may be strongly contaminated by a progressively decreasing outward current of the i_{xl} type, since this current deactivates in the $+20$ to -50 mV potential range with a time constant of a few hundred milliseconds [129, 172]. Nevertheless it seems difficult to escape the conclusion that τ_f and τ_r may have different values at a given potential.

In order to explain that τ_r is greater than τ_f it has been proposed [155] that inactivation is controlled, not by one first order variable f, but by two first order variables f and l, l being similar to but slower than f. In this case $i_{si} = \bar{g}_{si} \cdot d \cdot f \cdot l(E_m - E_{si})$. If it is assumed that f and l correspond to two inactivation gates located in series in the slow channel [155], the inactivation of the channel will be mainly controlled by the closing of the rapid gate (the channel being inactivated as soon as the rapid gate is closed), whereas the removal of inactivation will be mainly controlled by the re-opening of the slower gate (the channel not being fully available until the slow gate is completely opened). The fact that τ_f and τ_r differ is not really a surprising phenomenon, since a similar situation has been described in the case of the rapid Na^+ channel in frog atrial fibers [173], where recovery from inactivation was found to be 50 times slower than inactivation at E_m between -50 and -30 mV. In mammalian cardiac tissues, Gettes and Reuter [171] reported values for τ_r of i_{Na} which increase markedly with depolarization (from less than 25 msec at membrane potentials between -80 and -70 mV to about 100 msec at $E_m = -65$ mV) making it clear that in the case of the rapid channel, inactivation and recovery from inactivation may be quite different, although both apparently follow a single exponential time course. By contrast, other authors [147, 174–177] found two different time constants in the process of recovery from inactivation of i_{Na}.

Two different time constants ($\tau_{r1} = 80$ to 90 msec; $\tau_{r2} = 300$ to 350 msec) have been described by some authors in the process of recovery from inactivation of i_{si} in frog atrial fibers [147, 174, 175]. More surprisingly, in the dog ventricle, Hiraoka and Sano [178] observed a fast ($\tau_{r1} < 100$ msec) and a slow

($\tau_{r2} = 500$ to 700 msec) inactivation process but, in addition, they described a transient increase in i_{si} for an interval of about 200 msec between the depolarizing pulses so that the curve corresponding to the recovery from inactivation of i_{si} is no longer smooth but exhibits a hump. Such a process can explain the paradoxical but well-known increase of the plateau amplitude and duration observed by several authors, when premature action potentials are triggered in cardiac tissues of different species (for references and further analysis of this phenomenon, see [179, 180]). A similar transient increase in i_{si} [181] and in $[Ca^{++}]_i$ [182] has also been observed in some neurones of invertebrates for certain intervals between stimulations. Interestingly, oscillatory repriming of i_{si} (and twitch contraction) has also been observed to occur in calf Purkinje fibers when they are treated with low concentrations of strophantidin [183]. Such a development of oscillatory restitution precedes and may therefore be separated from toxic effects, such as transient depolarizations (TD, [184]) and transient inward currents (TI, [185]). The existence of two inactivation processes is not limited to cardiac tissues but seems rather to be a general property of voltage-dependent channels. A slow inactivation process has been repeatedly demonstrated during the last ten years in the case of the Na^+ channel of nerve fibers ([186]; for details and references, see [187, 188]). Time constants ranging from a few seconds to 100–200 seconds have been observed depending on the preparation and on the experimental conditions.

Several observations show that the slow channel may not inactivate completely during prolonged depolarization. This may be easily seen either by comparing recordings of i_{si} before and after suppression of the inward current by an inhibitor supposed to be reasonably specific (see Figure 12B) or by using the current tail method. In frog atrial fibers the residual part of i_{si} which does not inactivate is generally small at membrane potentials around -10 mV or more positive, at least in the case of a pure Ca^{++} current (see for example Figure 4d, ii in [189]). In the Purkinje fibers [190] a quite large residual inward current was observed upon repolarization from $\simeq 10$ mV to the resting potential. This inward current did not disappear completely even after a 10 sec depolarization. However, because this tail of inward current could not be recorded in a Na^+-free solution and because these results were interpreted by Reuter [190] as suggesting a rather slow time-dependent decrease of g_{Na} at the plateau level of the action potential of Purkinje fibers, it was difficult at that time, to decide whether i_{si} really undergoes incomplete inactivation. In the cat ventricular muscle [57], a sizeable fraction of i_{si} seems to remain after 300 msec when the membrane is depolarized at $E_m = -25$ mV. However a more global estimation which confirmed results obtained by Ochi [52] in the guinea pig myocardium, indicated that after a depolarizing step longer than 200 msec, the inward tail after repolarization

was in fact rather small: if a part of i_{si} was not inactivated, this portion would be smaller than 1/10 of the peak of the tail [61]. Similar conclusions were reached by McDonald and Trautwein [129]. However in sheep Purkinje fibers, Gibbons and Fozzard [144] noticed that about 18% of g_{si} triggered by a depolarizing pulse to -32 mV was still observed after 500 msec.

Whether or not such a persistent i_{si} is due to the existence of the overlap between $d_\infty - E_m$ and $f_\infty - E_m$ relationships deserves some comments. The range of overlap of these relationships extends from about -50 mV to about 0 or $+10$ mV (Figure 15). This means that under steady-state conditions, the f gate does not reach complete closure and therefore that the steady-state i_{si} may flow until the membrane is depolarized to about $+10$ mV. Such steady-state currents should however decrease with increasing depolarizations and entirely disappear for depolarizing pulses beyond $+10$ mV. Observations by Kass et al. [148] in Purkinje fibers and by Reuter [71] in cow ventricular muscle show that very probably this is not the case. In the observation of Kass et al., the residual inward current measured after 500 msec pulses, (Figure 12) reaches a maximum between -15 and 0 mV, instead of disappearing around the latter value. In Reuter's observation (Figure 16), the i–V curves measured 10–20 msec and 500 msec after the beginning of the depolarizing pulses, cross each other at $+40$ mV and the latter demonstrates a net inward current at least up to $+20$ mV. These observations strongly suggest that a part of the residual i_{si} is due to the fact that there is a population of slow channels, which does not possess inactivation gates. This assumption

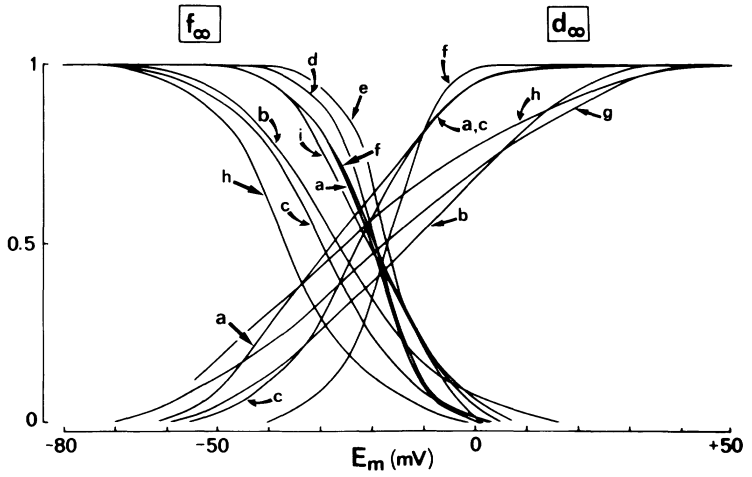

Figure 15. Steady-state activation ($d_\infty - E_m$ relationships) and inactivation ($f_\infty - E_m$ relationships) of slow inward current in different preparations. Frog atrial fibers: a [160]; d [161]; g [203]; h [217]. Dog atrial muscle: i (Christé, Ojeda and Rougier unpublished). Cat ventricular muscle: c [156]; e [195]. Sheep and pig ventricular muscle: b [59]. Cow ventricular muscle: f [100]. In a, g and i, E_m was assumed to be -75 mV.

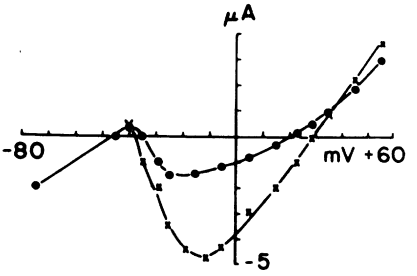

Figure 16. Current–voltage relationships plotted at the peak of i_{si} (crosses) and 500 msec after the beginning of voltage–clamp pulses (filled circles) in a cow ventricular preparation, showing that a large fraction of i_{si} does not inactivate or inactivates very slowly. $E_r = -75$ mV; $E_H = -45$ mV. [71, reproduced, with permission, from the Annual Review of Physiology.]

has been introduced in McAllister et al.'s reconstruction of the electrical activity of cardiac Purkinje fibers and expressed by the following equations [157]:

$$i_{si} = \bar{g}_{si} \cdot d \cdot f \cdot (E_m - E_{si}) + \bar{g}'_{si} \cdot d' \cdot (E_m - E_{si})$$

The population of 'abnormal' channels was assumed to be rather small since $\bar{g}'_{si}$ was taken as 5% of $\bar{g}_{si}$. In Beeler and Reuter's reconstruction of the action potential of ventricular myocardial fibers [158], the existence of slow channels with no inactivation process was not introduced in the equations. It is worth noting that if a fraction of the slow channels had their f_∞–E_m relationship (or their d_∞–E_m and f_∞–E_m relationships) shifted towards positive potentials, most of the experimental results could be explained just as well as by the above mentioned hypothesis. Such a shift was recently observed in Purkinje fibers in the case of the rapid Na^+ channel [191].

The threshold of i_{si} is determined by the position of the d_∞–E_m relationship on the voltage axis (Figure 15). Most reports indicate that i_{si} begins to develop and therefore that the d gate begins to open at membrane potentials around -50 mV. In the mammalian ventricular muscle superfused with a standard Tyrode solution, early measurements indicated a rather high threshold, i.e., -25 mV [48], -35 mV [50, 57], -40 mV [52]. In the rat ventricle [51, 192] i_{si} was observed only for depolarizations larger than 50 mV, i.e., for $E_m \simeq -30$ mV assuming $E_r \simeq -80$ mV. In frog atrial fibers, threshold values between -55 and -45 mV were reported [44, 165, 193], assuming a resting potential close to -75 mV [194]. Similar or somewhat more negative values were also reported (-58 mV) in the bullfrog ventricle [94], whereas in Purkinje fibers i_{si} had a threshold in the voltage range of -60 to -40 mV [41].

Recent reports confirmed that more often than not, the threshold of i_{si} lies between -50 and -35 mV. This has been observed in the cat ventricular muscle [129, 156, 195, 196], rat ventricular muscle [197–199], guinea pig ven-

tricular muscle [199], rabbit sino-atrial node cells [200–202], Purkinje fibers [148] embryonic heart cell aggregates [84] and single bovine heart cells [149]. On some occasions however somewhat lower or higher values have been reported for i_{si} threshold. Low values (−50 to −60 mV) were mainly observed in the case of frog atrial fibers (see [147, 203]) and frog sinus venosus [204], whereas high values (−25 mV) were reported in rabbit sino-atrial node cells [82]. Whatever the precise value of the i_{si} threshold it seems clear that i_{si} activates in a potential range where the rapid i_{Na} is already largely or almost completely inactivated. This is apparent from results obtained in different tissues: sheep and pig ventricular trabeculae [60], frog atrial fibers (compare Figure 5 [160]) and Figure 5 [205]; see also [147]), Purkinje fibers (compare Figure 1 [148] and Figure 4 [134]).

Consequently, when a partially depolarized cardiac fiber no longer shows action potentials with a rapid upstroke, because its rapid channel is partially or totally inactivated, it can still develop full size propagated slow responses (see [206] and Carmeliet, this book), because at its diastolic potential d_∞ remains close to zero and f_∞ close to one. It must be noticed that the threshold for i_{si} (the membrane potential at which i_{si} begins to occur) and the threshold for slow responses (the potential at which the membrane must be brought by a stimulus to trigger a response) are not the same. In the case when the membrane potential is identical at each point of a fiber (conditions of 'membrane response' or 'membrane action potential'), the threshold for the slow response is the membrane potential at which the slow inward current becomes greater than the outward current (see Figure 9). If the outward current is increased, it will be more difficult for a given inward current to trigger a response. As already stated, the overlap of the steady-state activation and inactivation curves appears to be very large in the case of the slow channel, since the f_∞–E_m curve begins to become smaller than one when $E_m = -70$ to -40 mV and reaches zero when $E_m = -20$ to $+15$ mV [56, 59, 60, 71, 100, 156, 160, 161, 203, 205]. One exception to this was reported by Besseau [153] who found that the f_∞–E_m curve shifted towards negative potentials by 35–40 mV and the d_∞–E_m curve by about 20 mV as compared with the majority of other results.

7. IONIC SELECTIVITY OF THE SLOW CHANNEL

7.1. Reversal potential of the slow inward current

Knowing the membrane potential at which a current reverses (E_{rev}, see Figure 9) is an important clue to the determination of the ion (or ions) carrying the current.

If a current is carried by only one ion, E_{rev} is equal to the equilibrium po-

tential for this ion as given by the Nernst equation $E_x = RT/nF \cdot L([x^+]_o/[x^+]_i)$. In this equation, R is the gas constant, T the absolute temperature, F the Faraday and n the ion charge. Therefore at 35 °C (in mV) $E_{Na} = 61 \log_{10}([Na^+]_o/[Na^+]_i)$ and $E_{Ca} = 30.5 \log_{10}([Ca^{++}]_o/[Ca^{++}]_i)$. Values for $[Na^+]_i$ may be obtained from recent estimations using selective electrodes [207, 208]. It must be noticed that activities must be considered instead of concentrations. In sheep Purkinje fibers, the intracellular Na^+ activity has been found to be 7.2 ± 2.0 mM, a value which corresponds to an internal concentration of 9.6 mM if an activity coefficient of 0.75 is used for the intracellular solution. In this case the value obtained for E_{Na} is +71 mV [208], a value higher than that generally chosen for the E_{rev} of i_{Na} (+40 mV, [157]). The discrepancy could be explained by an imperfect selectivity of the rapid channel for Na^+ ions, or by a rather large permeability for K^+ ions at the top of the Na^+ spike of the action potential [208]. Direct measurements of the level of ionized intracellular Ca^{++} using either the photoprotein aequorin, or other Ca^{++}-indicators, show that the resting ionized $[Ca^{++}]_i$ in axons is between 0.3 and 1×10^{-7} M (see [209, 210]). Although precise values of $[Ca^{++}]_i$ are not known in cardiac tissues there is no reason to suppose that in the absence of rhythmical activity $[Ca^{++}]_i$ should be noticeably different from that in other resting excitable tissues. The observations that rat cardiac myofibrils begin to exert a detectable tension when $[Ca^{++}]_i$ is greater than 1.8×10^{-7} M [211] is in quite good agreement with the assumption that the diastolic $[Ca^{++}]_i$ is around 10^{-7} M. It is therefore reasonable to assume that $[Ca^{++}]_o/[Ca^{++}]_i \simeq 10^4$, a ratio which leads to a high positive value for E_{Ca} ($\simeq +120$ mV). Assuming an activity coefficient γ_{Ca} of 0.33 for Ca^{++} concentration in the external solution (calculated according to Blaustein [212]; for the determination of Ca^{++} activity in physiological solutions, see also [213]), a value for E_{Ca} of +115 mV has been recently proposed [100]. If a current is carried by several ions with different equilibrium potentials, the individual components of the ionic current and E_{rev} may be calculated by using the constant field equations [13, 100, 213–215]. It can easily be understood that if a current is carried for example by both Na^+ and Ca^{++} ions, its reversal potential will be between E_{Na} and E_{Ca}; if in addition some K^+ ions cross the channel, E_{rev} will be slightly attracted towards E_K, i.e., towards a strongly negative potential.

Original constant field equations have been modified by Frankenhaeuser [216] to account for the fact that in the squid axon kept in normal $[Na^+]_o$ the i_{Na}–V curve is linear at large depolarizations whereas in some other tissues it is not. It has been assumed that in the squid axon fixed charges on the membrane created a potential step E' which modified locally the Na^+ concentration and the potential gradient in the membrane thus suppressing Goldman rectification [214] when $E' = E_{Na}$. In this case $[Na^+]_i$ and $[Na^+]_o$

become equal in the close vicinity of the membrane. Equations including a voltage step of large value (80 mV) corresponding to fixed negative charges at the inside of the membrane, were also used by Reuter and Scholz [100] in the case of the slow channel of the mammalian cardiac muscle.

In the case of i_{si}, several methods have been used to estimate E_{rev}: (a) Measurement of the peak current, and plotting of the i–V relationships before and after the action of a substance supposed to be a specific inhibitor of i_{si}. In such a case E_{rev} may be considered as being given by the intersection of the two i–V curves (see Figure 9). (b) Depolarization of the membrane by successive steps of increasing amplitude and direct determination of the exact depolarizing step at which i_{si} disappears (i.e., is no longer inward and not yet outward). The determination of this value may be influenced by the method chosen to estimate i_{si}. If for example we consider the current traces shown in Figure 8A, it is not certain that an outwardly directed i_{si} would be recognized in trace 6 unless the delayed outward current was correctly extrapolated by means of the dashed line. Concerning similar uncertainties about E_{rev} (and i–V curves) see also McDonald and Trautwein [129]. (c) Determination of instantaneous i–V relationships. Starting from a very negative E_m so that $h_\infty = 1$, the fiber is submitted to a depolarizing prepulse (+10 to +20 mV) which fully activates the channel ($d = 1$) and is brief enough (15–30 msec) for the inactivation parameter to remain close to one at the end of the pulse. Therefore, the conductance opened by the pulse corresponds to $\bar{g}_{si}$, i.e., to the complete opening of the slow channel. At the end of the pulse, the membrane is suddenlyd brought to another potential by a second pulse (200–500 msec) and the amplitude of the current tail triggered by this pulse is plotted as a function of E_m during the second pulse. If the channel does not undergo instantaneous rectification, the curve is linear; its intersection with the voltage axis corresponds to E_{rev}. Linear instantaneous current–voltage relationships have been obtained in sheep [59] and cow [100] ventricular trabeculae and in frog atrial fibers [217] whereas in other cases [50, 53] the relationship was linear in a restricted range of potentials.

Determination of E_{rev} is far from being simple for several reasons. Inhibitors of the slow channel suffer from lack of specificity. Measurement of i_{si} in the positive range of membrane potentials (i.e., for strong depolarizations) is sometimes made difficult by the development of increasingly large outward currents so that errors of measurements more or less proportional to the amplitudes of the depolarizing pulses may alter the slopes of the i–V relationships. It has been recently suggested that among outward currents able to alter the measurement of E_{rev}, a component resulting from the electrogenic operation of the Na^+–Ca^{++} exchange mechanism may play a large role [218]. Moreover, the existence of series resistance may also cause error in E_{rev} measurements if the membrane, in this potential range, is noticeably perme-

able to ions other than those giving rise to i_{si} [80]. Finally it has recently been demonstrated [219] that when currents are measured by means of the double sucrose gap technique, E_{rev} appears to be underestimated and the conductance overestimated. This phenomenon increases with the ratio: width of the central compartment/space constant. An increase in g_{si} induces an artifactual increase of E_{rev} and viceversa.

Rather different values have been found for the reversal potential of the slow inward current (Table 2). In frog atrial fibers bathed in normal physiological solutions, initial measurements of E_{rev} gave values which were intermediate between that attributed to E_{Na} and E_{Ca}. This was interpreted as indicating that i_{si} was normally carried by both Na^+ and Ca^{++} ions [44, etc.]. On the other hand, in mammalian cardiac fibers, only Ca^{++} ions were assumed to cross the slow channel [50, 59]. Therefore, the rather low value of E_{rev} measured in a normal Tyrode's solution was considered as resulting from an intracellular Ca^{++} concentration higher than that generally assumed ($\simeq 10^{-7}$ M) in the bulk intracellular solution [50, 59]. It was suggested that instead of flowing into the intracellular bulk solution, i_{Ca} flows into a small subsarcolemmal space in which the Ca^{++} concentration become very rapidly much higher than in the cytosol. The assumption that such a restricted space exists was strengthened by the observation made by Bassingthwaighte and Reuter [59] that E_{rev} changed with time as a function of the duration of the depolarizing pulse, i.e., as a function of the quantity of Ca^{++} crossing the membrane. They observed that E_{rev} became less and less positive when the amplitude of the pulse was below the initial value of E_{rev}, i.e., when i_{si} was inward, a situation in which Ca^{++} was supposed to accumulate in the restricted space. On the other hand, E_{rev} became more and more positive when the amplitude of the pulse was beyond the initial value of E_{rev}, i.e., when i_{si} was outward (depletion of Ca^{++} in the restricted space). From the integral of i_{si} and the change in E_{rev} the volume of the restricted space was estimated at about 1% of that of the bundle in which i_{si} was measured [59, 60]. It was suggested that this space is the one separating the subsarcolemmal cisternae of the sarcoplasmic reticulum from the sarcolemma. This hypothesis is not in contradiction with the fact that marked changes in E_{rev} with prepulse duration have not been observed in the frog heart, since cardiac sarcoplasmic reticulum is poorly developed in this species. Because of the measured low value of E_{rev} (an E_{rev} of +42 mV requires $[Ca^{++}]_i$ to be 7.5×10^{-5} M with 1.8 mM $[Ca^{++}]_o$ [60]), this hypothesis implies that the Ca^{++} concentration in the sarcolemma–cisternae interspace 'is normally higher by more than two orders of magnitude than the free calcium ion concentration in the myoplasm of the unstimulated muscle' [60]. Such a high local diastolic $[Ca^{++}]_i$ would mean that the threshold for the release of Ca^{++} induced by Ca^{++} from the sarcoplasmic reticulum [220, 221] may be, in some species,

Table 2. Some determinations of the reversal potential of i_{si} in cardiad tissues.

Tissues, species	$[Ca^{++}]_0$ mM	$[Na^+]_0$	E_{rev} mV	Method	Fig.	Authors	Ref.
Sinus venosus							
frog	1.1	N	+ 30	2,5	3	Brown et al. 1977	(204)
Atrial fibers							
frog	1.8	N	+ 45	1, 5, 9	14	Rougier et al. 1969	(44)
	1.8	O (C)	+ 95				
	1.8	O (C)	+110	1, 9	6	Vassort et al. 1969	(193)
	1.8	N	+100	1, 7, 9	2	Vassort & Rougier 1972	(226)
	1.8	N/10 (C)	+ 58	2, 9	9	Leoty & Raymond 1972	(316)
	7.2	N/10 (C)	+ 63				
	1.8	O (Li)	+110	2, 9	5	Vassort 1973	(336)
	1.8	O (S)	+ 65				
	1.8	N	+ 55	2, 7, 9	4	Vassort 1974	(337)
	1.8	O (C)	+ 95	2, 6, 9	6	Chesnais et al. 1975	(189)
	1.8	N	+ 45	1, 7, 9	5	Mironneau et al. 1975	(175)
	1.8	N	+ 75	1, 7, 9	2	Nargeot 1976	(338)
	1.8	O	+110	1, 5, 9	5	Ducouret 1976	(147)
	1.8	N	+ 60	1, 5, 9	5	Lenfant & Goupil 1977	(205)
	1.8	N	+ 50	1, 7, 9	3	Nargeot et al. 1978	(339)
dog	1.8	N	+ 50	1, 7, 10		Christé, Ojeda & Rougier	unpublished
human	1.8	N	+ 30	1, 7, 9		Christé, Ojeda & Rougier	unpublished
Purkinje fibers							
Sheep, calf	7.2	O (C, S)	+180	2	2	Reuter 1967	(41)
Ventricular fibers							
guinea pig	1.8	N	+60 to +80	4	2	Ochi 1970	(52)
dog	1.8	N	+ 60	2 & 3	9	Beeler & Reuter 1970	(50)
cat	3.6	N	+ 80	2, 7	8	New & Trautwein 1972	(57)
	0.9	N	+ 40			New & Trautwein 1972	(56)
	0.9	N	+ 65	1, 7	3	Trautwein 1973	(225)
	2.2	N	+50 to +65	2	2, 3, 4	Kohlhardt et al. 1973	(246)
	2.2	N	+35 to +52	2	2, 5	Kohlhardt et al. 1973	(224)
	0.55	N	+28 to +40				
	1.8	N	+ 70	2, 8	14	McDonald & Trautwein 1978	(129)
sheep	1.8	N	+ 42	1	4	Bassingthwaighte & Reuter 1972	(59)
cow	1.8	N	+ 33	3	6	Reuter & Scholz 1977	(100)
	0.45	N	+ 15				
	0.2	N	+ 8				

N = normal $[Na^+]_0$; O = Na^+-free medium, Na substituted by choline = C, lithium = Li, or sucrose = S. 1 = i–V relationship; 2 = extrapolated from i–V relationship, 3 = instantaneous i–V relationship; 4 = direct determination (depolarizing pulse at which i_{si} disappears); 5 = currents measured before and after i_{si} inhibition; 6 = currents measured before and after Ca^{++} removal; 7 = i_{si} measured as in Figure 8B, b; 8 = i_{si} measured after semi-logarithmic graphical extrapolation of i_K; 9 = E_r is assumed to be −75 mV; 10 = E_r is assumed to be −80 mV.

higher than assumed, unless the sarcoplasmic reticulum can largely adapt to high Ca^{++} concentrations when they develop progressively [222]. In fact, shifts of E_{rev}, similar to those previously described, are not observed in all mammalian cardiac tissues. For example they apparently do not occur in cow trabeculae [100]. Nevertheless, the value of E_{rev} in this tissue is very low. Therefore, Reuter and Scholz [100] assumed that the low value of E_{rev} is mainly attributable to the fact that ions other than Ca^{++}, can cross the slow channel, namely Na^+ and K^+.

7.2. Ions carrying the slow inward current and inhibitors of the slow channel

The fact that in frog atrial fibers E_{rev} is consistently more positive in the absence than in the presence of extracellular Na^+ ions has been considered by Rougier et al. [44] as a clear indication that i_{si} is normally carried by both Ca^{++} and Na^+ ions which justified the term 'slow $Ca^{++}-Na^+$ current' (i_{Ca-Na} or $i_{si(Ca-Na)}$) and 'slow Ca–Na channel' frequently used in the literature. This does not mean that i_{si} is not principally a Ca^{++} current. It must be remembered that in frog atrial fibers superfused with a normal Ringer solution, the amplitude of the slow response decreases by about 20 mv for a tenfold decrease of $[Ca^{++}]_o$, a value much closer to the theoretical values for a system using Ca^{++} (29 mV) rather than Na^+ (58 mV) [44]. In the mammalian heart, the observation that E_{rev} is much lower than E_{Ca} was also attributed by Ochi [52] to the fact that Ca^{++} and Na^+ ions flow during the slow inward current. In Purkinje fibers, tail experiments (not E_{rev} measurements) favored the conclusion that both Ca^{++} and Na^+ ions seem to carry the inward tail current, each of them roughly one half of the total current [53]. The situation however was not really so clear, since in the cat myocardium [57] the existence of a Ca^{++}–Na^+ channel although suggested by some experiments was excluded by the observation that i_{si} frequently disappeared in a nominally 'zero' Ca^{++} solution. Assuming that Ca^{++}, Na^+ and K^+ ions are the only charge carriers of i_{si} and using the constant field equations for i_{Ca}, i_{Na} and i_K (which allowed the estimation of E_{rev}) Reuter and Scholz [100] found that the permeability ratios ($P_{Ca}/P_{Na} = P_{Ca}/P_K = 100$) fitted well their experimental data on E_{rev}. They also observed that changes in $[Ca^{++}]_o$ from 0.2 to 5.4 mM, induced a shift in E_{rev} (from +10 to +53 mV) which corresponded to the theoretical expectations. The existence of P_K means that while Ca^{++} and Na^+ ions carry the slow inward current, K^+ ions are responsible for a far from negligible outward current which flows through the slow channel at the same time as the inward Ca^{++}–Na^+ current and contributes in the shift of E_{rev} towards less positive values of E_m.

The fact that P_{Na} is low compared with P_{Ca} may seem surprising in view of the obviously noticeable participation of Na^+ ions in i_{si}. However it must be

remembered that for a given ionic permeability, the intensity of the current carried by one ion changes not only with the transmembrane gradient of concentrations (more precisely, activities) but also with the absolute value of activities. For example, if the permeability for one ion is constant the current carried by this ion decreases to 1% of its initial values when both internal and external activities for this ion are divided by 100. For this reason, in spite of the small permeability of the slow channel for Na^+ ions, compared with its permeability for Ca^{++} ions, the percentage of i_{si} carried by Na^+ may be very large, simply because Na^+ is much more concentrated than Ca^{++} in biological fluids. On the other hand, because Ca^{++} activities are so low, the participation of Ca^{++} in i_{si} (i.e., its electrophysiological role), would remain negligible if the slow channel were not so highly selective for Ca. Reuter and Scholz [100] calculated that when $[Ca^{++}]_o = 1.8$ mM, about one third of i_{si} at $E_m = 0$ mV, is carried by Na^+ ions. This proportion increases to 2/3 and 4/5 when $[Ca^{++}]_o$ is reduced to 0.45 mM and 0.2 mM respectively.

The ratio P_{Ca}/P_{Na} is probably not fixed but may differ somewhat according to the tissue under study and the experimental conditions. Using the same theoretical approach as Reuter and Scholz [100], but a different technique (action potential recording) and assuming that the overshoot of the slow action potential of the atrio–ventricular (AV) node is a quasi-steady state in which inward and outward ionic currents are equal, Akiyama and Fozzard [223] found that $P_{Ca}/P_{Na} \simeq 70$ and $P_K/P_{Na} = 1$. Relative contributions of these ions to i_{si} were estimated to be: $i_{Ca} \simeq 17\%$, $i_{Na} \simeq 33\%$ (inward currents) and $i_K \simeq 50\%$ (outward current). It is likely that in the sinus node, the P_{Ca}/P_{Na} ratio is normally smaller than in the AV node, since in the former tissue i_{si} is extremely sensitive to Na_o^+ removal and decreases by 68% when $[Na^+]_o$ is reduced to 30% of the normal control [202]. In many other tissues, mainly in the mammalian ventricular muscle, i_{si} is probably mainly carried by Ca^{++} ions since it is very sensitive to removal of external calcium, Ca_o^{++}, in the presence of a normal concentration of Na_o^+ [50, 57, 100, 224, 225]. According to New and Trautwein [57] in the cat ventricle i_{si} is not affected by lowering Na_o^+ to 10% of the normal concentration. A similar observation was made in guinea pig ventricle by Ochi [52] who in fact observed a small increase of i_{si} in a Na^+-free solution. In frog atrial fibers, i_{si} is also markedly depressed by a Ca_o^{++} reduction in the presence of a normal $[Na^+]_o$ [55, 136, 160, 226]. Reducing Na_o^+ by half was reported to alter i_{si} only slightly [136], whereas in the bullfrog ventricle a similar reduction produced a marked increase of i_{si}, a phenomenon that was attributed to Ca^{++}–Na^+ antagonism in the slow channel [227]. The fact reported by Benninger et al. [136], that i_{si} is almost insensitive to changes in $[Na^+]_o$, was not observed by Lenfant et al. [160]. These authors show that either in Na^+-free (Figure 17B) or in Ca^{++}-free media, i_{si} is strongly depressed in comparison with its value in a

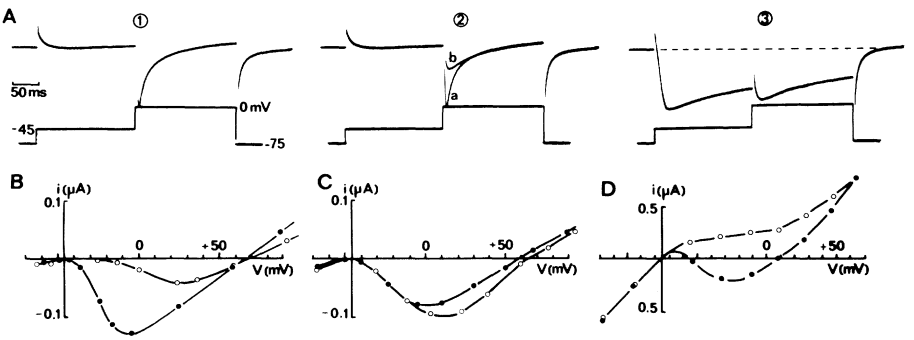

Figure 17. Effect of removal of $[Ca^{++}]_o$ in the presence of EGTA on i_{si} in a frog atrial fiber and development of the slow inward Na^+ current, $i_{si(Na)}$. Upper traces: currents; lower traces: voltage pulses. 1: TTX, Mg^{++}-free Ringer solution. 2 and 3: TTX, Ca^{++}-free, Mg^{++}free Ringer solution (EGTA, 1 mM). 10 sec after Ca^{++} removal (2) i_{si} decreases from a to b, then 20 sec later (3), a large $i_{si(Na)}$ develops at $E_m = 0$ mV and -45 mV (Chesnais, Coraboeuf, and Sauviat unpublished). B: effect of Na^+ removal on i–V relationship of i_{si} (frog atrial fiber bathed in TTX, Mg^{++}-free Ringer solution). Filled circles: control; open circles: Na^+-free (choline) medium. C: effect of increasing $[Ca^{++}]_o$ on i–V relationship. Same conditions as in B. Filled circles: control; open circles: $Ca^{++} \times 2$. Redrawn from [160]. D: the substitution of Li^+ for Na^+ does not allow the development of i_{si} in the absence of Ca_o^{++}. Frog atrial fiber in the presence of TTX. Filled circles: Na^+-Ringer solution; open circles: Li^+-Ringer solution. Redrawn from [189], with permission, from Academic Press.

normal Ringer solution. Increasing either Na_o^+ or Ca_o^{++} (Figure 17C) also increases i_{si}. Such differences may very well be due to differences in the type of atrial fibers used by the different authors, since it has been clearly observed that fibers with different electrophysiological characteristics exist in the frog atrium [143, 166] and since fibers of the pacemaker type are very likely more permeable to Na^+ than true contractile fibers. Strengthening this conclusion is the observation by Tarr [55] that in most of the fibers of the frog atrium i_{si} was only moderately sensitive to Na_o^+ removal, whereas in other preparations, i_{si} was clearly carried mainly by Na^+ ions, since it was abolished by Na_o^+ removal and persisted in Ca^{++}-free solution.

The question of knowing whether or not the current $i_{si(Na)}$ which develops in the complete absence of divalent cations [44, 45, 189, 228, etc.] is of the same origin as the Na^+ component of the normal i_{si} may deserve some comments. In frog atrial fibers bathed in a Mg^{++}-free Ringer solution, the complete removal of Ca_o^{++} in the presence of TTX produces a biphasic effect, i.e., a shortening of the action potential followed by a lengthening. This lengthening does not occur when small amounts of Ca^{++} remain in the nominally free Ca^{++} solution, but addition of 0.05–0.2 mM EDTA or EGTA always induces the development of action potentials lasting from 1 to several seconds [45, 189]. Similar results have been described in the perfused rat heart [45] where long-lasting plateaus are generally obtained easily without the

addition of a chelating agent. In the frog atrial fibers, the evolution of i_{si} after removal of divalent cations is also biphasic, i.e., i_{si} undergoes a transient decrease followed by a secondary increase (Figure 17A) then it stabilizes. This current which is carried by Na^+ ions but not by lithium (Li^+) ions (Figure 17D) is suppressed after Na^+ removal or the addition of divalent ions such as Mn^{++} and magnesium (Mg^{++}), whereas Mg^{++} exerts only small inhibitory effect on $i_{si(Ca)}$ [189]. It is rather surprising that Li^+, which is known to pass easily through the rapid Na^+ channel, since $P_{Li}/P_{Na} = 0.93$ in the frog myelinated fiber [229] and 1.1 in the squid axon [230], cannot carry any appreciable current through the slow channel [189] as it is confirmed by the observation that Li^+ cannot generate action potentials in cultured heart cells of chick embryo (see [231]). The fact that $i_{si(Na)}$ apparently does not develop immediately in the absence of Mg^{++} and Ca^{++} may suggest that this current occurs only when the membrane is altered by divalent cation deprivation and therefore that it does not exist in normal conditions. If this were the case, the Na^+ current normally crossing the slow channel would be different from the Na^+ current measured in Mg^{++}-free, Ca^{++}-free solutions. Two arguments seem to strengthen this hypothesis: (a) the part of the i–V curve which exhibits a negative slope is generally (but not always) negatively shifted by 10–25 mV in the case of $i_{si(Na)}$ as compared with $i_{si(Ca-Na)}$. This could mean that two systems exist which do not activate in the same potential range. However, the shift may simply result from the suppression of Ca_o^{++}, i.e., from a decrease of the binding–screening process (see § 8). (b) the value of τ_f of $i_{si(Na)}$ is much larger than that of $i_{si(Ca)}$ (by 3–4 times, according to Rougier et al. [44]) and sometimes even more. However, because τ_f decreases with $[Ca^{++}]_o$ as will be discussed later, the difference may also result from the Ca^{++}-removal. Interestingly, very large $i_{si(Na)}$ have been recently observed in the absence of divalent cations in mollusk neurones when EDTA or EGTA was applied either intra- or extra-cellularly [232]. The authors did not attribute the development of this current to the simple removal of Ca^{++} but to the binding of the chelating molecule to the channel, a binding which was assumed to induce a deep rearrangement in the channel kinetics and selectivity. This special role of chelating agents may be discarded in cardiac tissues since $i_{si(Na)}$ frequently occurs in the absence of EDTA or EGTA.

In frog atrial fibers bathed in a normal (Mg^{++}-free) Ringer solution containing TTX (no rapid i_{Na}), the removal of Na_o^+ (Na^+ being replaced by choline) depresses i_{si} in a potential range which is more negative than that corresponding to $i_{si(Ca)}$ [160] (Figure 17B). The same result is obtained when Na^+ is replaced by Li^+, or when the Na^+ component of i_{si} is inhibited by Mg^{++}. If in the case of the addition of Mg^{++}, the result may be partly, but not entirely explained by a positive shift of the d_∞–E_m curve due to a screening effect, this interpretation can hardly be retained when Na^+ is

replaced by Li^+, if one eliminates the effect of a possible increase in i_{K1} mediated by Ca_i^{++}. Therefore it seems that a Na^+ component of i_{si}, activating in a potential range more negative than $i_{si(Ca)}$ can exist even in the presence of Ca_o^{++}. If this is true, channels corresponding to $i_{si(Ca)}$ and $i_{si(Na)}$ could in fact correspond to somewhat different membrane structures [189]. Some indication that two different types of slow channels may coexist in the same cell has recently been obtained in snail neurons [233]. It is also worth noting that some drugs act more efficiently on $i_{si(Na)}$ than on $i_{si(Ca)}$, some of them increasing (tryptamine [234]) whereas some others decrease (ervatamine [139]) the Na^+ component of i_{si}.

Several ions other than those previously indicated, can pass through the slow channel. This is the case for divalent cations such as strontium (Sr^{++}) and barium (Ba^{++}) which resemble Ca^{++} in many respects. In the frog atrial fiber superfused with a Ca^{++}-free or Ca^{++}- and Na^+-free Ringer solution the addition of Sr^{++} allows the development of an i_{si} of the same amplitude as or somewhat larger than the normal one and gives rise to long-lasting action potentials [58, 235–238]. A similar result has been obtained in the cat ventricular muscle [224]. Ba^{++} ions are also able to carry i_{si} in both species [224, 237]. Sr^{++} and Ba^{++} increase the membrane resistance and at the same time Ba^{++} decreases E_r [237, 239]. The capacity of Sr^{++} and Ba^{++} to cross the slow channel explains their efficiency in triggering action potentials in several other tissues, for example in cultured chick heart cells [231, 240] or cow Purkinje fibers [241]. When both Ca^{++} and Sr^{++} are present, Ca^{++} acts as a competitive inhibitor of the Sr^{++} current in Purkinje fibers [242]. A similar competition between Ca^{++} and other divalent cations has also been described [224, 243, 244].

Other ions carry a sizable current through the slow channel only in rare cases, whereas in other conditions, tissues or species, they exert essentially an inhibitory effect. Several divalent or trivalent cations are frequently used as g_{si} blockers, in spite of additional effects which prevent them from being really specific for the slow channel. The capacity of Mg^{++} ions to carry i_{si} is much more limited than that of Sr^{++} and Ba^{++}. In sheep and calf Purkinje fibers [41] and frog atrial fibers [189, 238] Mg^{++} does not pass through the slow channel, whereas in the cat ventricular muscle a clear contribution of Mg^{++} to i_{si} has been described [224]. By contrast, in frog atrial fibers, Mg^{++} exerts a moderate inhibitory effect on $i_{si(Ca)}$ and a strong inhibitory effect on $i_{si(Na)}$ [189]. A somewhat similar situation exists with Mn^{++} ions: Mn^{++} acts in many cases as a strong inhibitor of the slow channel, since in the frog heart it suppresses both $i_{si(Ca)}$ and $i_{si(Na)}$ [44] and consequently the global slow inward current [44, 55]. Such an effect on i_{si} has also been observed in other species, such as rat ventricles [51, 245], sheep Purkinje fibers [53], cat papillary muscles [246], calf Purkinje fibers [247] and rabbit sino–atrial

cells [202]. However Mn^{++} may also pass through the slow channel, and generates i_{si} in the guinea pig ventricular muscle [52, 248, 249] a phenomenon which is apparently not limited to cardiac tissues [250]. Other divalent cations such as cobalt (Co^{++}) [58, 246], nickel (Ni^{++}) [244, 246] or trivalent cations such as lanthanum (La^{+++}) [251, 252] inhibit the slow channel and have not been reported as being able to carry some i_{si}. Mn^{++} and La^{+++} ions have also been reported to exert some inhibitory effect on the delayed outward current i_x whereas they increase the net outward time-independent current in the plateau range [247]. It is unclear whether a part of the latter effect might result from the inhibition of the incompletely inactivated i_{si} component described by Kass et al. [148]. Vitek and Trauwein [53] did not observe any alteration of the outward current–voltage relation under the effect of Mn^{++} ions.

Organic compounds such as verapamil or D–600 have also been found capable of blocking i_{si} [253] and are commonly used as tools for pharmacological dissection of cardiac currents. However because the inhibitory effect of both drugs is very small when responses or currents are elicited at a very low frequency [254–256], it may be assumed that the action of these drugs consists mainly in a marked slowing down of the time course of reactivation of i_{si}. In the rat ventricle driven at a frequency of 6/min the maximum inhibitory effect of D–600 or verapamil on g_{si} does not exceed 70–80% [245]. In the rat, concentrations of drug inducing a 50% inhibition of i_{si} are 1.4×10^{-6} M for verapamil and 3.6×10^{-6} M for D–600, i.e., values much lower than that of Mn^{++} giving a similar inhibition (0.6×10^{-3} M [245]). In the guinea pig heart, the affinity of verapamil to its membrane receptor has been reported to be 10^4 times larger than the affinity of Ni^{++} ions [243]. It has been reported that verapamil blocks slow responses induced by $i_{si(Na)}$ [257, 258] although an opposite effect was observed with D–600 [259]. The activation of i_{si} is markedly slowed down by D–600 whereas τ_f remains almost unchanged [260]. Another organic compound, nifedipine, reduces i_{si} without altering the time course of reactivation of i_{si} [196]. Clearly, organic compounds are not specific blockers of the slow channel. In Purkinje fibers, D–600 exerts an inhibitory effect on i_x whereas it increases the time-independent outward current at the plateau level [247]. A reduction of the delayed outward current was also observed in the cat papillary muscle [260].

8. STRUCTURAL CHARACTERISTICS OF IONIC CHANNELS

As other ionic channels, the slow channel is probably a protein macromolecule inserted in the membrane and forming a pore through which from one to as much as several thousand ions can pass in one millisecond, jumping from

site to site across a series of energy barriers. The concept of pores as ionic channels was reinforced, in the case of the Na^+ or K^+ channels, by measurement of the density of channels in the membrane. Three different methods, namely titration of channels with a specific blocker (for example tritiated saxitoxin in the case of the Na^+ channel, see [261]), measurement of the charge displacement of gating currents (see [262]) and analysis of current fluctuations [263], allowed the determination of the density of the channels ($\simeq 300$ Na^+ channels/μm^2 in the squid axon) and of the conductance of one individual channel. Values of 4 pS (1 pico Siemens $= 10^{-12}$ mho) and 12 pS have been found for the Na^+ and K^+ channels respectively [263]. The resulting number of ions crossing each channel per millisecond (several thousand) is higher by several orders of magnitude than the turnover rates for carriers and constitutes the strongest argument that Na^+ or K^+ channels are pores instead of mobile carriers [264]. Although such arguments cannot at present be extended with certainty to the slow channel, similarities between the kinetics properties of the rapid and the slow channels strongly suggest that the latter is also a pore.

Ionic channels are generally considered as being composed of a selective filter and of a gating mechanism. In the model of Na^+ channel proposed by Hille [265] the selectivity filter has been assumed to be a short narrowing of the pore (the dimensions of the hole being $\simeq 3 \times 5$ Å) with atoms of oxygen of a carboxylic acid group constituting the binding site. In this system ions, which are normally fully hydrated in the bulk solution, first begin to lose water molecules when approching the carboxylic acid oxygen, then have to shed more water molecules in order to enter the narrow pore (high energy barrier) then jump to the second oxygen on the other side of the filter, while regaining some water molecules and finally jump into the bulk solution as fully hydrated ions [266, see 267]. The fact that several ions probably cannot enter the filter abreast, suggests the existence of single file transport (2 or more ions being simultaneously present in the channel, one after the other) first assumed by Hodgkin and Keynes [268] to explain that $[K^+]_o$ influences the outflux of radiolabelled K^+. In the simplest model of a single file pore, the conductance of the pore strongly increases (until it reaches a maximum, then decreases) with the concentration of the permeant ions on both sides of the membrane. This is because each ion which is in the pore exerts a strong repulsion on the others, slowing down their entrance and speeding their exit. Therefore at low concentrations (i.e., in singly occupied channels), the exit rate is much lower than at high concentrations (i.e., in doubly occupied channels), whereas at very high concentration there are no vacancies left, so that the conductance decreases [269]. In such a system, ionic movements through the channel no longer fit with the classical concept of free diffusion and the independence principle, since complex interactions between ions

clearly exist. In the independence principle [270] the probability that one ion of a given type crosses the membrane, must be independent of the presence of other ions of the same type and of all other types of ions [271]. Therefore no saturation of the channel can exist. In fact, even in the Na^+ channel, a small systematic deviation from independence was observed which is indicative of saturation [271]. It is worth noting that the flow of ionic current through a pore can induce by itself changes in ion concentration near the mouth of the pore, increasing the concentration at the exit side of the membrane and decreasing it at the entrance side. If such a discrete accumulation–depletion effect likely remains negligible in the case of channels working with large ionic concentrations (Na^+ or K^+ channels) it may become significant in the case of Ca^{++} channels, thus inducing fairly large and rapid changes in 'transchannel' E_{Ca}, so that this effect could contribute to the observed inactivation of $i_{si(Ca)}$ [272]. This effect is different from the accumulation-depletion effect resulting from the restriction of the diffusion between the membrane and the bulk solution as a consequence of the existence of narrow clefts between cardiac cells [117, 273–275].

The gating mechanisms responsible for the activation and inactivation of the channels are not clearly understood from a molecular point of view. From experiments showing for example that in the Myxicola giant axon the density of fixed negative charges appears much greater near the gating machinery than near the ionic pores [276], it may be concluded that the charged structure (voltage sensor) which triggers gates is not in the immediate vicinity of the pore but may be immersed in a fluid region of the lipid bilayer [277]. Gates may correspond to mobile fragments of a macromolecular structure of the channel. For example, Armstrong et al. [278] described the selective destruction of the Na^+ inactivation mechanism after internal perfusion of squid axons with pronase, whereas the subsequent addition of tetraethylammonium derivative reinstates inactivation-like behavior [279]. It has been proposed that the inactivation process results from the plugging of open Na^+ channels by an internally located mobile protein [280, 281] which may bear a positive charge on an aminoacid residue of arginine or lysine [279], whereas other results have suggested that a tyrosine residue is essential for Na^+ inactivation [282, 283]. Among other possible systems, the one proposed by Mullins [284] consists of several successive variable-size rings forming the pore, each of them having to adjust themselves to the ion diameter in order for the channel to be conducting. Much recent information concerning the Na^+ channel, came from the measurement of very small 'gating' currents [285] which are assumed to result from the movement of charged gating structures (corresponding mainly to the most rapid gate, i.e., to the activation gate) in response to a change of membrane potential. Gating currents are also known as asymmetry or displacement currents. Several observations, for example

that of the immediate rise of charge displacement compared with the delayed increase of the opening of the Na^+ channels during a depolarizing pulse (meaning that the gating particles are already displaced before the opening of the channel, see [264] suggest a multistate model for the activation mechanism [264, 281, 286]. It has also been observed in nerves that no component of gating currents has the time course of inactivation, therefore apparently little charge movement is associated with this step although inactivation affects gating currents. This suggests that inactivation is not necessarily a process independent of activation but rather that it may be coupled in some way to activation and may derive much of its voltage dependence from the activation process [287]. Although most of the gating current measurements in excitable tissues concern the Na^+ channel, two types of gating currents associated with Na^+ and Ca^{++} currents respectively have been described in an Aplysia neuron [288, 289]. The observation of two distinct gating currents in a neuron which is known to have separate rapid Na^+ and slow Ca^{++} currents, confirms that the slow channel is controlled by mechanisms similar to those controlling the rapid Na^+ channel. A gating current associated with g_{Ca} has been also described in snail neurones [290].

Like any structure bearing electrical charges, gating systems are directly sensitive to the electrical field around them, i.e., to the potential profile across the membrane. However an important point is that this electrical field does not result only from the existence of E_m, as it is measured between the external and internal bulk solutions, but also from the local influence of fixed (essentially negative) charges on both sides of the membrane [291, see also 292, 293]. Fixed negative charges may have several origins, for example glycoproteins with sialic acid residues (see [294]), lipids of the bilayer with charged polar head groups (see [295]) or exposed free carboxyls of glutamic and aspartic acids of proteins such as those forming the channel itself. For this reason, the bulk and the actual transmembrane potentials differ (Figure 18). Very likely fixed charges are not distributed uniformly along the membrane, but rather they are discretely localized [293]. Their density in the vicinity of a given membrane structure, for example a given type of ionic channel, is characteristic of this structure. As a consequence, for a given E_m, the actual transmembrane potential in the vicinity of Na^+ channels is very probably different from that in the vicinity of slow channels, or of K^+ channels. If one assumes that a gate opens or closes when the electrical field within the membrane in the vicinity of the gate, changes its direction and if we call 'threshold' the change in E_m (from E_r) necessary for this situation to be reached (Figure 18), it is clear that the thresholds of, for example, the rapid and slow channels, will not be identical. For the same reason the corresponding activation and inactivation curves will be situated differently on the voltage axis. Another important point is that cations are able to alter consid-

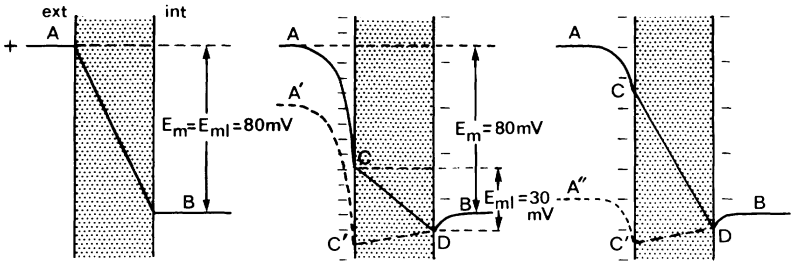

Figure 18. Left: potential profile in the resting membrane in the absence of fixed negative charges. The local membrane potential, E_{ml} (i.e., the potential just across the membrane), is the same as that, E_m, measured in the bulk solution. Middle: if in the vicinity of a channel the membrane bears fixed negative charges at its external (ext) and internal (int) surface the potential profile near the membrane is modified, for example from A to C and from B to D depending on the charge density, so that at rest E_{ml} may become much smaller than E_m. Therefore a moderate depolarization (from A to A') is sufficient to reverse the electric field within the membrane in the vicinity of the channel, a situation which may induce gate movements and activation–inactivation process. Right: Ca^{++}-rich media, by screening the surface charge or binding to individual fixed charges, increase the depolarization (AA'' instead of AA') necessary to reverse the electric field within the membrane (see [292, 296, 340, 341]).

erably the influence of fixed negative charges either by simply screening them or by binding to them (Gouy–Chapman–Stern theory, see [292, 296]). As a result, the increase of cations at the outside of the membrane shifts activation and inactivation curves towards positive potentials (positive shift) whereas the increase of cations at the inside of the membrane shifts curves towards negative potentials (negative shift). Anions probably exert opposite effects [297]. It has been shown by Hille et al. [296] in the case of the Na^+ channel of myelinated nerve fibers, that a tenfold increase in concentration of divalent cations above $\simeq 1$ mM causes a positive shift of 20 to 25 mV of the Na^+ activation curve, either with cations that bind ($Zn^{++} > Ni^{++} > Mn^{++} \simeq Co^{++} > Ca^{++}$) and cations that do not (Mg^{++}). The difference is that binding reduces the concentration of divalent ions needed to reach a given surface potential (or produce a given shift). In the absence of Ca^{++}, monovalent ions are also very efficient in shifting activation curves, whereas in the presence of Ca^{++}, their influence is much smaller. The increase of the external concentration of H^+ ions (i.e. the decrease in pH of the solution), also produces a positive shift [296], in addition to other more specific effects.

It must be remembered that at the level of individual channels, a given channel opens and closes very rapidly (in microseconds). It also opens and closes on a random basis. Because of collisions between the gates and surrounding molecules, 'it is as if each channel repeatedly flipped a coin to decide whether or not to remain open' [298]. According to this probabilistic concept, saying that the degree of opening of a channel increases with depo-

larization, does in fact mean that the average amount of time each individual channel spends in the open state, increases with depolarization. Saying that a time-dependent conductance is fast or slow means that the time required to force a large number of these channels to modify the average open time is either short or long [299]. Such random opening and closing give rise to very small potential and current fluctuations known as 'membrane noise.' Several reviews have summarized the principles and results of membrane noise analysis applied to excitable tissues (see [300–302]).

The slow channel so far described in cardiac membranes, closely resembles that existing in other tissues such as invertebrate muscles, where Ca^{++}-dependent action potentials are observed either when K^+ currents are reduced by quaternary ammonium [12, 13] or when Ca_i^{++} is lowered by Ca^{++} chelating agents [303], or in the absence of any treatment ([304], for details and other references see [305, 306]). As previously stated, the selectivity of the cardiac slow channel is rather modest, since Ca^{++}, Sr^{++}, Ba^{++}, Na^+ and K^+ ions (and in some rare cases, Mg^{++} and Mn^{++}) can cross the channel. However, it must be remembered that in other tissues, for example in the barnacle muscle fiber [307], the slow channel is also permeable to Ca^{++}, Sr^{++} and Ba^{++} and possibly to some other ions, whereas even the most selective channels, such as the Na^+ and the K^+ channels, are permeable to several ions (more than 10 different ions for the Na^+ channel in nerves and at least 4 ions for the K^+ channel, see [271]). The moderate selectivity of ionic channels has been attributed by Mullins [284] to the fact that ions probably do not enter the channel as totally unhydrated ions, but rather as partially hydrated ions. For example the fact that the Na^+ channels are about 10 times more permeable to Li^+ and Na^+ than to K^+, may conveniently be explained by assuming that these ions enter the channel with a single hydration shell. Proposing a model of ionic channels somewhat different from that of Hille [265], Mullins [284] assumed that because of thermal fluctuations, the size of a given type of channels may vary around a mean value. For example, at a given instant, the distribution of pore sizes is such that most of the individual Na^+ channels have a radius of 3.6 to 3.7 Å (equal to the singly hydrated radius of Na^+ and Li^+ ions), whereas the remaining Na^+ channels have somewhat smaller or larger pores. According to this distribution, only a small number of individual Na^+ channels have pores with radius of 4.0 Å (equal to the singly hydrated radius of K^+ ions) and are therefore, at this instant, permeable to K^+ and not to Na^+. Another point which may be worth noting is that in barnacle muscle fibers, the selectivity of the slow channel may vary greatly after partial inhibition of g_{si}, for example by Co^{++} ions. Normally, the Ba^{++} and the Sr^{++} currents are greater than the Ca^{++} current ($i_{Ba}/i_{Sr}/i_{Ca} = 1.3/1.05/1.0$) whereas in the presence of Co^{++} ions, the reverse situation occurs; i_{Ca} is much less inhibited than i_{Sr} and i_{Ba} so that for 20 mM

Co, $i_{Ba}/i_{Sr}/i_{Ca}$ becomes 0.3/0.5/1.0 [306]. This is interpreted by saying that Ba^{++} ions, which are more mobile than Ca^{++} ions through the channel, have a lower affinity for the site, thus making the Ba^{++} current more sensitive to the blocking agent than the Ca^{++} current is.

According to Mullin's concept [284] a moderately selective channel such as the slow channel, cannot distinguish between ions when their singly hydrated sizes are almost identical. Because singly hydrated Na^+ and Li^+ ions have very similar sizes, the slow channel should be approximately equally permeable to Na^+ and Li^+ as the rapid channel is. However this is not the case since in the frog atrial fibers replacing Na^+ by Li^+ ions readily supresses i_{si} measured in the absence of divalent cations. In Mullin's concept [284] this suggests that $g_{si(Na)}$ is a much more selective system than $g_{si(Ca)}$ and therefore that $g_{si(Ca)}$ and $g_{si(Na)}$, although being similarly inhibited by several drugs or agents, may be in fact different mechanisms. The observation of a strict selectivity for Na^+ might indicate that the mechanism which is directly or indirectly responsible for the Na^+ component of i_{si} is much more like that of a carrier than that of a channel. For example it is well known that the sodium pump extrudes Li^+ much more slowly than Na^+ [308]. Similarly Li^+ ions cannot replace Na^+ ions in the functionning of the Na^+–Ca^{++} exchange mechanism in cardiac tissues [309].

The origin of Ca^{++} ions entering the cell through the slow channel deserves some comments. Langer and his group have repeatedly drawn attention during the last few years to the important role that may be played by the cell coat or glycocalyx as an extracellular store for Ca^{++} ions [310–312]. The glycocalyx may be divided into two parts, the external lamina and the surface coat, both containing large quantities of sialic acid, i.e., of negative charges. The surface coat, which is close to the sarcolemma, is an integral part of it because it contains oligosaccharide chains attached to proteins embedded in the plasma membrane (see [312]). The ability of glycocalyx to bind cations, particularly Ca^{++} and La^+, has been clearly established [313] and it has been shown that the removal of sialic acid residues by neuraminidase increases markedly the sarcolemmal Ca^{++} permeability without increasing the permeability to K^+ ions [314]. For these reasons it may be assumed that the Ca^{++} contained in the external bulk solution or in the blood, has to bind to the glycocalyx before reaching the plasma membrane and before entering the slow channel. It is therefore possible that the intensity of i_{si}, in addition to being dependent on other previously studied parameters, is partly controlled by the binding capacity of the glycocalyx. However, very recently, Isenberg and Klöckner [150] observed that i_{si} is quite normal in entirely isolated cardiac cells in which the glycocalyx has been destroyed by isolation procedures. Langer [312] suggests that the glycocalyx, with its binding sites for Ca^{++}, may also play a role in the prevention of Ca^{++} overloading. It is possible that,

when a cell is submitted to progressively increasing Ca^{++} concentrations, the influx of Ca^{++} is prevented from rising in a linear fashion when the negative binding (and presumably transfer) sites for Ca^{++} become saturated. Saturation seems to be a characteristic of the slow channel. In the barnacle muscle fiber in which artifacts resulting from fixed charges have been suppressed by 100 mM Mg_o^{++}, the maximum rate of rise of the Ca^{++}-dependent action potential increases steeply as Ca_o^{++} increases in the 0–15 mM range, then saturates [315]. It is therefore possible that this effect is due partly to the above mentioned protective effect of the glycocalyx. If this is true, saturation should decrease or disappear after disruption of the glycocalyx, for example by Ca^{++} removal. Some indication that such an effect can indeed occur is given by the observation that when a frog atrial fiber is submitted to a Na^+-free, Ca^{++}-rich solution (18 mM Ca^{++}) after having been washed for 10 min in low-Ca^{++} then Ca^{++}-free media, the slope of the i–V curve indicates a large increase in both g_{si} and $[Ca^{++}]_i$ (g_{si} increases fivefold as compared to its value in 1.8 mM Ca^{++} [58]), i.e. the opposite of saturation. Such an increase in g_{si} as $[Ca^{++}]_o$ increases is not a priori surprising. It has been observed to a limited extent (in agreement with calculations based on the constant field theory) in the mammalian ventricular muscle [100]. In Purkinje fibers bathed in a Ca^{++}-free solution, Vereecke and Carmeliet [242] observed that g_{Sr} increases about threefold when $[Sr^{++}]_o$ is increased from 2 to 15 mM. Such a behavior, as well as saturation phenomena, can result, to a certain extent, from ionic interactions inside the pore giving rise either to increases or decreases of conductance according to the concentrations, as already mentioned [269].

9. EFFECT OF IONS AND SOME PHYSICAL FACTORS ON THE SLOW INWARD CURRENT

The effects of several monovalent and divalent ions on the cardiac slow channel, have been reported in the literature. Information about the effect of Ca^{++} on the activation of i_{si} may be deduced from the analysis of i–V relationships. The observation that Ca^{++} shifts the curve towards positive potentials, could be in line with the assumption that negative charges exist in the vicinity of the slow channel and could result from screening and binding phenomena. Surprisingly, in many cases, the threshold of i_{si} seems to be shifted negatively instead of positively [48, 50, 57, 61, 100, 316], whereas the value of E_m at which i_{si} reaches its maximal amplitude ($E_{m(i_{si}\,max)}$), does not seem to be significantly altered. However in other cases [58, 160, 224] $E_{m(i_{si}\,max)}$ is clearly shifted towards positive potentials when $[Ca^{++}]_o$ increases. Shifts are however of different values: +18 mV for an increase from 1.8 to

18 mM and -12 mV for a decrease from 1.8 to 0.18 mM in Na^+-free Ringer solution [58], about $+10$ mV for an increase from 1.8 to 3.6 mM [160], -5 to -7 mV for a decrease from 2.2 to 0.55 mM [224] and about -20 mV for a decrease from 1.8 to 0.13 mM [317]. In spite of these differences such shifts indicate that negative charges very likely are present in the vicinity of the slow channel at the external surface of cardiac membranes. Although Reuter and Scholtz [100] did not observe any shift in the activation curve of i_{si} when $[Ca^{++}]_o$ was reduced from 1.8 to 0.45 and then to 0.2 mM, however, in two experiments in which $[Ca^{++}]_o$ was increased from 1.8 to 5.4 and then 7.2 mM, they saw shifts of 4 and 7 mV towards more positive potentials. The reason for discrepancies observed is not clear. They may result partly from the presence or the absence of Mg^{++} ions. It has been observed in the Helix neuron [318] that i–V curves corresponding to $i_{si(Ca)}$ are shifted in the foreseen direction by $[Ca^{++}]_o$ when $[Mg^{++}]_o$ is zero but are little affected in the presence of Mg^{++}. All the above mentioned authors who have observed significant shifts in the i–V curves of the cardiac i_{si}, used Mg^{++}-free normal solutions [58, 160, 224, 317]. Among other explanations, one might result from the existence of fixed charges at the inside of the membrane. If $[Ca^{++}]_i$ is not strictly independent of $[Ca^{++}]_o$ but increases more or less in a Ca^{++}-rich solution (as observed in frog atrial fibers submitted to Na^+-free media [58]), the resulting decrease of the negative internal surface potential should shift the I–V curves towards negative potentials, thus partially masking the positive shift induced by the increase in $[Ca^{++}]_o$. It has indeed been observed by Akaike et al. [318] that in Helix neurons, increasing the intracellular Ca^{++} activity (from 1.2×10^{-8} M to 10^{-5} M) shifts $E_{m(i_{si}\,max)}$ by about 35 mV and the threshold of i_{si} by 15–20 mV towards negative potentials.

The effect of Ca^{++} is not limited to the above-mentioned parameters. In the cat ventricular muscle an increase of $[Ca^{++}]_o$ from 2.2 to 8.8 mM produces a decrease of τ_f from 35 to 24 msec [155], whereas in the rat ventricular muscle, τ_f decreases from $\simeq 30$ to $\simeq 7$ msec when $[Ca^{++}]_o$ increases from 0.5 to 7 mM [319]. τ_f has also been reported to decrease in sheep Purkinje fibers during Ca^{++} injections, whereas the amplitude of i_{si} increases [320]. The latter result, which may seem surprising, has been attributed by Isenberg to an accelerated extrusion of Ca^{++} and therefore to a larger concentration of Ca^{++} in the intercellular clefts. For this reason, the decrease in τ_f may also be due to an increase in $[Ca^{++}]_o$. In the same way as τ_f, τ_r is also modified by changes in $[Ca^{++}]_o$, since in the cat ventricular muscle it decreases from 250 to 140 msec, at $E_m \simeq -30$ mV, when $[Ca^{++}]_o$ is increased from 2.2 to 8.8 mM [155].

Divalent cations other than Ca^{++} also influence the functioning of the slow channel, in addition to their already described ability to act, for some of them as charge carriers and for others as channel blockers. For example, in the

presence of 5 mM Mn^{++}, the i–V curve of i_{si} is shifted by about 20 mV towards positive potentials, either in the absence or in the presence of 1.8 mM Ca^{++} [52]. This observation was made possible because the guinea pig ventricular muscle was used. Indeed in this species the slow channel is permeable to Mn^{++} whereas in other species i_{si} is entirely suppressed by 5 mM Mn^{++}. This result shows that like Ca^{++}, Mn^{++} acts at the level of membrane fixed charges. Mg^{++} ions seem also to produce a positive shift of the i–V curve of i_{si} [224]. In frog atrial fibers, the positive shift of the i–V curve produced by an increase of $[Mg^{++}]_o$ from 0 to 1.8 mM is of 5 to 7 mV in a Na^+-free medium [189]. The shift becomes much greater ($\simeq$ 15 mV) in the presence of a normal $[Na^+]_o$, a phenomenon which seems related to the blocking of a part of i_{si} rather than to a screening effect. No significant shift occurs with Co^{++} 2 mM [246], and Ni^{++} 1 mM [244] whereas Sr^{++} ions apparently do not shift the i–V curves of i_{si} to the same extent as Ca^{++} ions do [224, 236, 237]. Another difference between Ca^{++} and other divalent cations is that the time course of i_{si} differs when the current is carried either by Ca^{++} ions or by other divalent ions. The time course of the rise and the decay of i_{si} is slower when the current is carried by Mn^{++} than by Ca^{++} [52]. The decay of i_{si} is also slowed down in frog atrial fibers when Sr^{++} or Ba^{++} are subtituted for Ca^{++} [237] whereas in the cat ventricular muscle, kinetics of the current (activation and inactivation) are not markedly altered [224]. In Purkinje fibers the substitution of Ca^{++} by Sr^{++} or Ba^{++} strongly reduces the transient outward current, revealing a long lasting i_{si} [170], but it remains unclear whether or not i_{si} inactivation is altered by Sr^{++} (or Ba^{++}) substitution.

Among monovalent ions, H^+ ions are well known to give a positive shift of Na^+ activation and inactivation curves in nerves by screening and binding and, in addition they reversibly block the Na^+ channel by a different mechanism: they are supposed to bind to a single negative charge which is assumed to control the permeability of each Na^+ channel; if the group bearing the charge is no longer ionized, the channel ceases to function [296, 321]. Shifts of activation have also been described in cardiac tissues for i_{Na} [322] and i_{K2} [322–324]. However a shift of activation or inactivation is not clearly detectable in the case of the slow channel of cardiac tissues, either in frog atrial fibers (the i–V curve is not shifted along the voltage axis by lowering pH from 7.8 to 5.6 [189]) or in the mammalian heart (possibly the i–V curve is shifted towards positive E_m by a few mV when the pH is decreased from 7.4 to 5.5 but the f_∞–E_m curve is not [195]). On the other hand the amplitude of i_{si} is reduced at low pH [189, 195, 325] in agreement with the observation that in embryonic chick ventricle slow responses are blocked at pH 6 [326]. Moreover both τ_f and τ_r seem to be appreciably increased [195]. When the pH is lowered from 7.8 to 5.6, $i_{si(Ca-Na)}$ measured in a normal Ringer solution is reduced by about 50%, $i_{si(Ca)}$ mesured in Na^+-free

solution is reduced by $\simeq 20\%$ whereas $i_{si(Na)}$ measured in Ca^{++}-free solution is entirely suppressed [189].

K^+ ions seem to exert a strong influence on i_{si}, since it has been shown in the rabbit sinus node that i_{si} is depressed by excess K^+ (an eightfold increase in $[K^+]_o$ reduces i_{si} by 30% when depolarization and Na^+ reduction are compensated for [200]), whereas in the atrial fibers i_{si} undergoes a transient increase in low-K^+ media [162, 327]. In addition the rising time of i_{si} appears to be shortened and the decay time lengthened after K^+ depletions [162].

The influence of Na^+ ions on i_{si} is rather complex. The idea that Na^+ and Ca^{++} ions can compete for some site at the level of the cardiac slow channel has been put forward [44, 226, 227] in order to explain the effect of varying $[Na^+]_o$ on the mechanical tension, as well as some aspects of the Ca^{++}–Na^+ antagonism described by Lüttgau and Niedergerke [328]. It was observed that when $[Ca^{++}]_o$ was increased or decreased, i_{si} was correspondingly modified, whereas when $[Ca^{++}]_o$ was altered, keeping constant the ratio $[Ca^{++}]_o/[Na^+]_o^2$, i_{si} appeared unchanged [226]. However, more recent results [317] show that in low $[Ca^{++}]_o$ and low $[Na^+]_o$ with a ratio $[Ca^{++}]_o/[Na^+]_o^2$ constant, i_{si} in fact decreases. Because there is a simultaneous shift towards negative potentials of the i–V relationship, this decrease is small (and may be undetectable) for depolarizing pulses of moderate amplitude.

The effect on i_{si} of modifying $[Cl^-]_o$ has not been extensively studied. However, in frog atrial fibers, it has been shown that the replacement of Cl^- by an impermeant anion, methylsulphate, leaves the amplitude of i_{si} almost unchanged as well as i–V and $f_\infty - E_m$ relationships [205]. This is at variance with the suggestion that in the guinea pig ventricle, i_{si} may be increased by Cl_o^- removal, because the amplitude and duration of the slow response and accompanying contraction are enhanced in Cl^--free media [329]. It is worth noting that the replacement of Cl_o^- by an impermeant anion may alter the density of internal charges by decreasing $[Cl^-]_i$ and therefore changes the availability of several ionic systems [297].

Besides above mentioned ionic factors, many other factors influence i_{si}. This is the case of physical and metabolic factors. Because other contributors (Mirro, Bailey and Watanabe, this book) will analyze the mechanisms involved in the intracellular regulation of i_{si}, we shall not consider here relations between i_{si} and metabolism. Moreover a review concerning this problem appeared recently [330]. Among the physical factors influencing i_{si}, frequency is an important one because of its influence on contractility and action potential configuration. The influence of changes in frequency on repolarization in cardiac cells was analyzed in a recent review [331]. When depolarizing pulses are suddenly applied to dog ventricular fibers at a frequency of 0.3 Hz, i_{si} is fully activated during the first pulse, then does not change during the following repetitive depolarizations [48, 151]. A similar phenome-

non occurs in the cat [57] and the rat ventricular muscle [197] when the interval between pulses is sufficiently long (2.5 and 10 sec respectively). On the other hand, in sheep Purkinje fibers submitted to depolarizing pulses at a frequency of 1 Hz, i_{si} is larger for the first clamp than it is for succeeding clamps [144]. This difference may be due to the fact than in the first three cases, enough time is allowed for i_{si} to fully recover, whereas in the last case the time of recovery from inactivation is longer than the interval between pulses. However in the cat papillary muscle driven at a rate of 30/min it has been observed that i_{si} gradually diminishes in amplitude and is more slowly activated and inactivated during trains of identical depolarizing pulses [332]. These progressive changes cannot be attributed to an incomplete recovery, since a 20 sec rest period after the last pulse does not allow the current to recover its initial characteristics. The region of the i–V curve corresponding to positive membrane potentials appears to be shifted in the upper direction and towards less positive potentials, so that E_{rev} decreases, suggesting a progressive increase in $[Ca^{++}]_i$ [332]. Opposite results have been observed: (a) in frog atrial fibers, where a continuous stimulation at frequencies of 0.3 to 1 Hz, given after a rest period, leads to an increase in i_{si} mainly when Ca^{++} is subtituted by Sr^{++} [333]; (b) in the rat ventricular muscle, in which an increase in stimulation frequency produces an increase in the amplitude of i_{si} and a marked reduction of τ_f [245]. Opposite results may be attributed to differences in species but, possibly also, to the fact that i_{si} can increase at certain stimulus intervals [178].

The effect of temperature on i_{si} does not seem to have been extensively studied. In the cat ventricular muscle [155] both τ_f and τ_r increase with the same Q_{10} (2.3) when the temperature is decreased from 32 to 22 °C, whereas the amplitude of i_{si} does not react so sensitively ($Q_{10} = 1.56$, whether i_{si} increases or decreases is not indicated). In the same preparation, an increase of τ_r (by about 90% at $E_m = -80$ mV and by about 65% at $E_m = -40$ mV) was also observed upon lowering the temperature from 37 to 32 °C [156]. In the bullfrog atrial fiber, both $i_{si(Ca-Na)}$ measured in a Ringer solution containing TTX and $i_{si(Ca)}$ measured in a Na^+-free solution, appear enhanced at low temperature (9 °C) when the i–V curves are compared with the control curves (17 °C); by contrast, $i_{si(Na)}$ measured in a Ca^{++}-free solution containing EDTA and TTX is depressed at low temperature [334]. On the other hand, Brown and DiFrancesco observed in the rabbit sinus node cell that i_{si} increases with temperature. This increase is linearly correlated with an increase in i_K, which suggests that a part of i_K may be controlled by $[Ca^{++}]_i$ [335].

10. SUMMARY

In spite of its limitations, the voltage clamp technique has allowed a clear demonstration of the existence in cardiac tissues of a slow channel separate from those carrying the rapid inward sodium current and outward potassium currents. The slow channel which gives rise to a slow inward current, i_{si}, has been analyzed in terms of Hodgkin–Huxley conductances. It is controlled by activation and inactivation mechanisms. Steady-state activation increases from 0 to 1 and steady-state inactivation decreases from 1 to 0 when the membrane is depolarized from $\simeq 50$ mV to $\simeq 0$ mV. Information about the kinetics of i_{si} remain rather contradictory and may reflect both technique and species or tissue differences. i_{si} overlaps with and may be partially obscured by several outward currents, some of them being partly dependent on the entry of Ca^{++} into the cell through the slow channel. As in many other cases, fixed charges probably exist on both sides of the membrane in the vicinity of the slow channel and therefore influence gating mechanisms controlling i_{si}. The slow channel is moderately selective since it is permeable to Ca^{++}, Sr^{++}, Ba^{++}, Na^{+}, K^{+} and in some rare cases Mg^{++} and Mn^{++} ions. In myocardial fibers i_{si} is mainly carried by Ca^{++} ions whereas in other tissues (sinus node cells, some types of frog atrial fibers, etc.) the participation of Na^{+} to i_{si} may be very large. In the absence of divalent ions, a large and long-lasting slow inward Na^{+} current develops. The amplitude and kinetics of i_{si} is modulated by many ionic, physical and metabolic agents as well as by transmitters of the autonomic nervous systems and many substances. Only the first two types of agents have been considered in the present chapter.

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4. THE SLOW INWARD CURRENT: NON-VOLTAGE-CLAMP STUDIES

EDWARD CARMELIET

1. INTRODUCTION

Although Na^+ is the primary ion carrier responsible for the greatest part of the upstroke in myocardial fibers, it has become clear that Ca^{++} ions play an important role in the depolarization process, especially in determining the late part of the upstroke and the total height of the action potential. As early as 1956, Coraboeuf and Otsuka [1] made an observation that was difficult to reconcile with the simple Na^+ hypothesis. In the guinea-pig ventricle, they found that, although $\dot{V}_{max}$ of the upstroke was reduced in low Na^+ media, the amplitude and the plateau height were not. In the frog myocardium the amplitude of the action potential also did not behave as a Na^+ electrode when the external Na^+ concentration was lowered [2, 3].

Other observations also suggest that the depolarization phase is of complex nature. In contrast to the smooth upstroke in cardiac Purkinje fibers, the depolarization in myocardial fibers was found to consist of two successive components [4, 5]. Sometimes the two components were separated by a dip [6]. During the first phase the membrane is depolarized at a high rate, but, during the second phase, the rate of depolarization falls to one-tenth or one-hundredth of the initial value. Both phases are differently affected by changes in membrane potential, ions and drugs: $\dot{V}_{max}$ and amplitude of the first phase, for instance, are sensitive to small deviations from the resting potential, whereas the second phase is not [7]; the first phase is inhibited by Na^+ removal or addition of TTX, the second phase by Ca^{++} removal or addition of Mn^{++} ions [8, 9].

2. Ca^{++}-MEDIATED ACTION POTENTIALS. SENSITIVITY TO K_0^+ AND REFRACTORINESS

Following the original observation of Fatt and Ginsborg [10] in crayfish muscle fibers that an inflow of Ca^{++} ions was responsible for the upstroke of the action potential, information has been accumulating to support the existence of an inward current system, distinct from the fast Na^+ current. This inward

D.P. Zipes. J.C. Bailey. V. Elharrar (eds.). The Slow Inward Current and Cardiac Arrhythmias. 97–110.

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current is mainly responsible for the amplitude and the plateau height of the atrial and ventricular action potential; it is also the main current responsible for the upstroke of the action potential in the sinoatrial and auriculoventricular node [11, 12]. The importance of this current is easily demonstrated by the existence of regenerative depolarizations and conducted action potentials in conditions in which the fast inward current is blocked or absent. Such conditions are: elevated external K^+ concentration, passage of a depolarizing current, presence of TTX, or absence of Na^+ in the external medium [13–15]. The regenerative depolarizations and conducted action potentials are favored by measures that [1] increase the inward current, such as elevation of extracellular Ca^{++}, stimulation of the slow channel conductance by addition of catecholamines, histamine, xanthine derivatives, fluoride [16–20] and (2) decrease outward current, such as addition of tetraethylammonium chloride (TEA) [21] or use of Cl^--free media [22, 23].

Compared to the fast Na^+ inward current, the slow inward current is characterized by a distinct *ion transfer mechanism* and specific *kinetic behavior.* The channel is not very selective with respect to ion species; it is permeable to bivalent ions (Ca^{++}, Sr^{++}, Ba^{++}) as well as to monovalent ions (Na^+ and K^+) and can be blocked by Ni^{++}, Co^{++}, La^{++} and Mn^{++} [9, 24–31]. Although the channel is quite permeable to K^+ and can carry an appreciable proportion of the current (one-fifth at zero membrane potential; [31]) the amplitude as well as the duration of Ca^{++}-mediated action potentials are insensitive to changes in K_0^+ between wide limits. This insensitivity is in contrast to the marked shortening of the plateau phase which can occur in the normal action potential. In cow Purkinje fibers for instance, an increase in K_0^+ elicits a shortening of the normal action potential but has no effect on the epinephrine induced plateau-like depolarization [17] (Figure 1A–D). This remarkable difference is related to the effect of K_0^+ on the steady-state current–voltage relation and the level of the plateau. In the normal Purkinje fiber the plateau occurs at a level at which K_0^+ strongly modifies and increases i_{K_1}; in the presence of epinephrine the inward current is increased, and the plateau is moved to a positive level. At this level large changes in K_0^+ do not affect the current–voltage relation and therefore the action potential

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Figure 1 A–D Increase of K_0^+ in the presence of adrenaline (5.5×10^{-6} M) in successive steps: 2.7, 5.4, 10.8 and 16.2 mM K_0. Plateau height and duration are practically unchanged by a 6-fold increase in K_0. Cow Purkinje fiber. E: Stimulation at a frequency of 1/sec elicits a decrease in amplitude of the fast depolarization with no change in amplitude of the secondary depolarization. Cow Purkinje fiber; K_0^+ was 16.2 mM, Ca_0^{++} 15 mM, adrenaline (5.5×10^{-6} M). F: Spontaneous alternation of two types of action potentials, without and with fast depolarization phase. Cow Purkinje fiber; K_0 10.8 mM, adrenaline 5.5×10^{-6} M. Vertical bars: 50 mV; horizontal bars: 1 sec. (Modified from 17.)

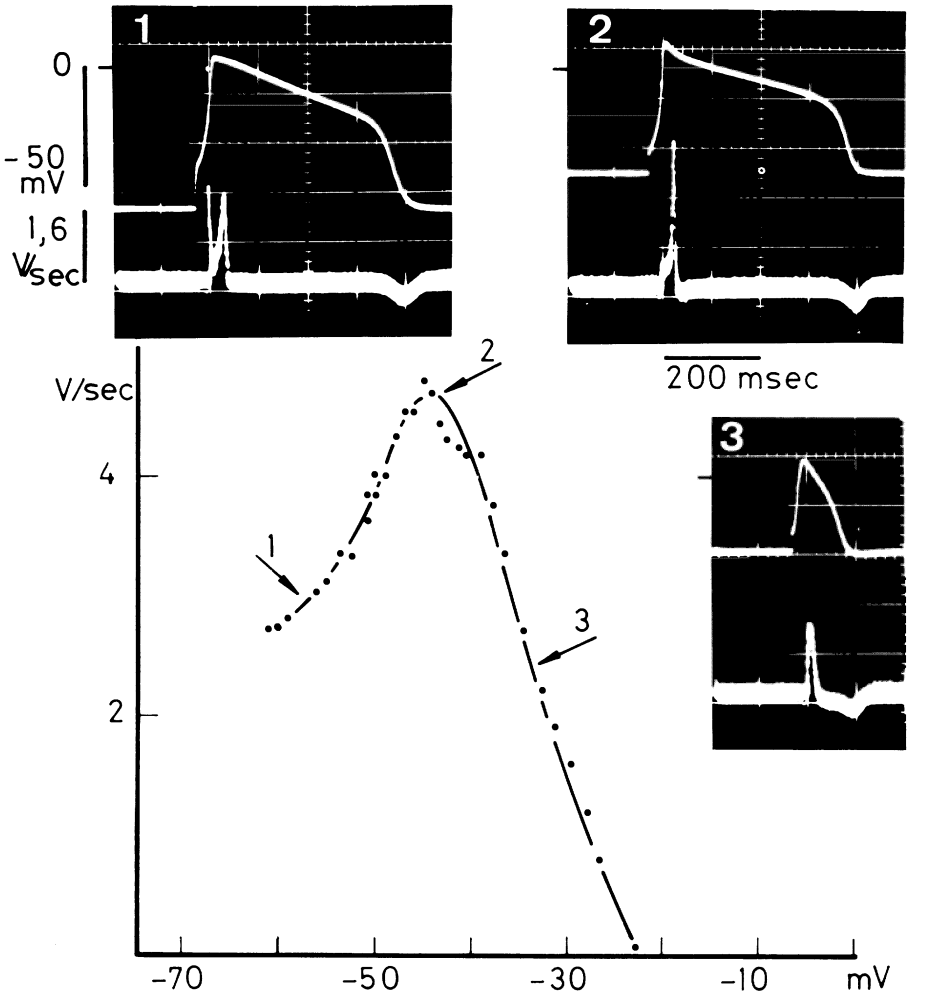


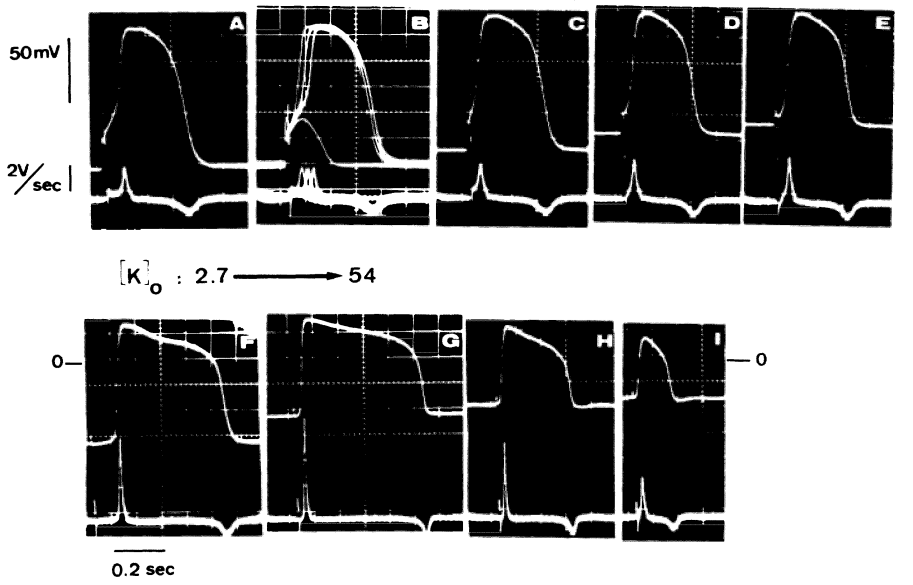
Figure 2. Change in $\dot{V}_{max}$ of Ca^{++}-mediated action potentials when K_0^+ is increased from 10.8 mM to 54 mM. Cow Purkinje fiber. Isoproterenol 0.4 mg/liter. Examples of action potentials at different levels of membrane potential, indicated by arrows in the graph. $\dot{V}_{max}$ as a function of membrane potential does not show a plateau but increases for potentials between −60 and −40 mV (Carmeliet, unpublished).

duration becomes insensitive to external K^+. The Purkinje fiber behaves under those conditions similar to muscle; in many mammalian cardiac muscle preparations the shortening at elevated K^+ concentrations is small or absent [32–34]. Under special conditions (i.e., Cl^--free media) the action potential may even become prolonged at elevated K^+ concentrations [22, 23] (see next section and Figure 3).

The slow channel is further characterized by its kinetic behavior. In comparison with the Na^+ channel, the steady-state inactivation and activation are displaced to less negative potentials [11]; the time constants for both processes are in general longer, which justifies the name of 'slow' channel. Except for the frog, time constants for inactivation increase between -40 mV and $+40$ mV, with extreme values of 50 msec near threshold to 300–400 msec at potentials more positive than the zero level (for a comparison between different species see [12, 22]. It is not yet clear whether time constants for recovery from inactivation are identical to those for inactivation; differences seem to exist among species. In general, however, the time constants for recovery increase at less negative potentials. Comparing the time constants for removal of inactivation for the 'fast' Na^+ and 'slow' Na^+-Ca^{++} channel, it is clear that recovery is faster for the Na^+ system at high levels of membrane potentials; at reduced levels of membrane potentials (-50 mV), however, the reverse situation may occur. Such a comparison predicts that frequency effects on both systems will depend on the level of membrane potential; at reduced levels of membrane potential an increase in frequency should have greater effects on the first phase of the action potential. An illustration of such behavior is shown in Figure 1E. Stimulation at 1/sec of a cow Purkinje fiber bathed in a solution of 16 mM K_0^+, 15 mM Ca_e^{++} and epinephrine (5.10^{-6} M) reveals a gradual decline in the amplitude of the first phase, without change of the amplitude or configuration of the secondary depolarization. At first glance this result may appear to contrast with the general finding that full recovery of excitability in fibers that show the slow response takes a long time following full repolarization and that such preparations do not remain in a steady-state when driven at any but a low rate. In this respect the following remarks should be made: (1) The above given example only illustrates that at depolarized levels the 'fast' Na^+ system shows a slower recovery than the 'slow' response; recovery in both systems however remains slow. (2) Recovery of slow responses is very dependent, not only on the membrane potential level, but also on the presence or absence of other ions. It is clear that recovery of Sr^{++}-action potentials in Na^+-free media is very slow; rates of stimulation as low as 1/min are required to remain in a steady-state [28]. This aspect may be specific for Sr^{++} or related to the absence of Na^+ [22].

3. Existence of an optimum membrane potential for the generation of Ca^{++}-mediated action potentials

It is generally known that a cardiac Purkinje or muscle fiber becomes inexcitable when the external K_0^+ is raised above 15 mM. This phenomenon is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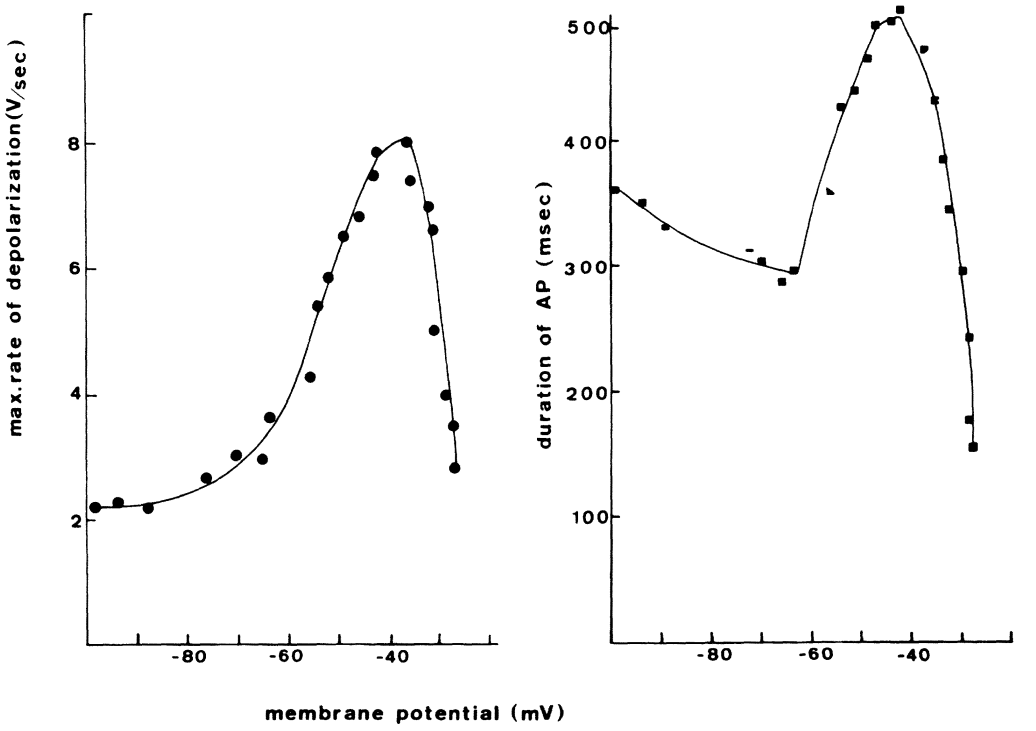
max.rate of depolarization(V/sec)
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-80
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duration of AP (msec)
500
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200
100
-80
-60
-40
membrane potential (mV)

due to a gradual inactivation of the fast Na^+ current when the membrane is depolarized from -80 mV to -50 mV [35]. In conditions in which the genesis of Ca^{++}-mediated action potentials is favored (addition of isoproterenol 0.4 mg/liter or Cl^--free medium) the preparation remains excitable for K_0^+ concentrations up to 54 mM. This can be explained by the fact that inactivation of the Ca^{++} conductance occurs at more depolarized levels compared to the inactivation of the Na^+ conductance. During an increase in K_0^+ and gradual depolarization from -80 mV to -20 mV it is thus expected that $\dot{V}_{max}$ of the Ca^{++}-mediated action potentials, after full inactivation of the Na^+ conductance, stays constant between certain limits of membrane potentials (-60 to -40 mV) before decreasing towards zero. Figure 2, which shows the results obtained in a cow Purkinje fiber, demonstrates however that $\dot{V}_{max}$ for the Ca^{++}-mediated action potentials does not show a plateau but *increases* for potentials between -60 and -40 mV. At more depolarized levels $\dot{V}_{max}$ decreases again and reaches half-maximum value at -33 mV. The increase in $\dot{V}_{max}$ between -60 and -40 mV is accompanied by an increase in overshoot, while the action potential duration stays constant or may even lengthen above control values. In the potential range where $\dot{V}_{max}$ is minimal, excitability is reduced and strong currents are needed to excite the preparation; for more positive potentials excitability increases and less current is required to reach threshold.

Similar results can be obtained in a cat papillary muscle preparation. Figure 3 shows an example of the results obtained when a cat ventricular preparation is bathed in a Cl^--free solution with 10 mg/liter TTX added to block the Na^+-mediated activity [23]. $\dot{V}_{max}$ is very low for negative potentials, but increases about 4-fold between -60 and -40 mV. In muscle preparations this rise in $\dot{V}_{max}$ is accompanied by a clearcut lengthening of the action potential duration.

In the case of propagated action potentials, one could argue that the dip between the two 'inactivation' curves is due to electrotonic interaction between excited and non-excited regions of the preparation. At potentials far from the threshold of the slow channel more current is needed to depolarize the membrane to a critical level at which regenerative conductance changes

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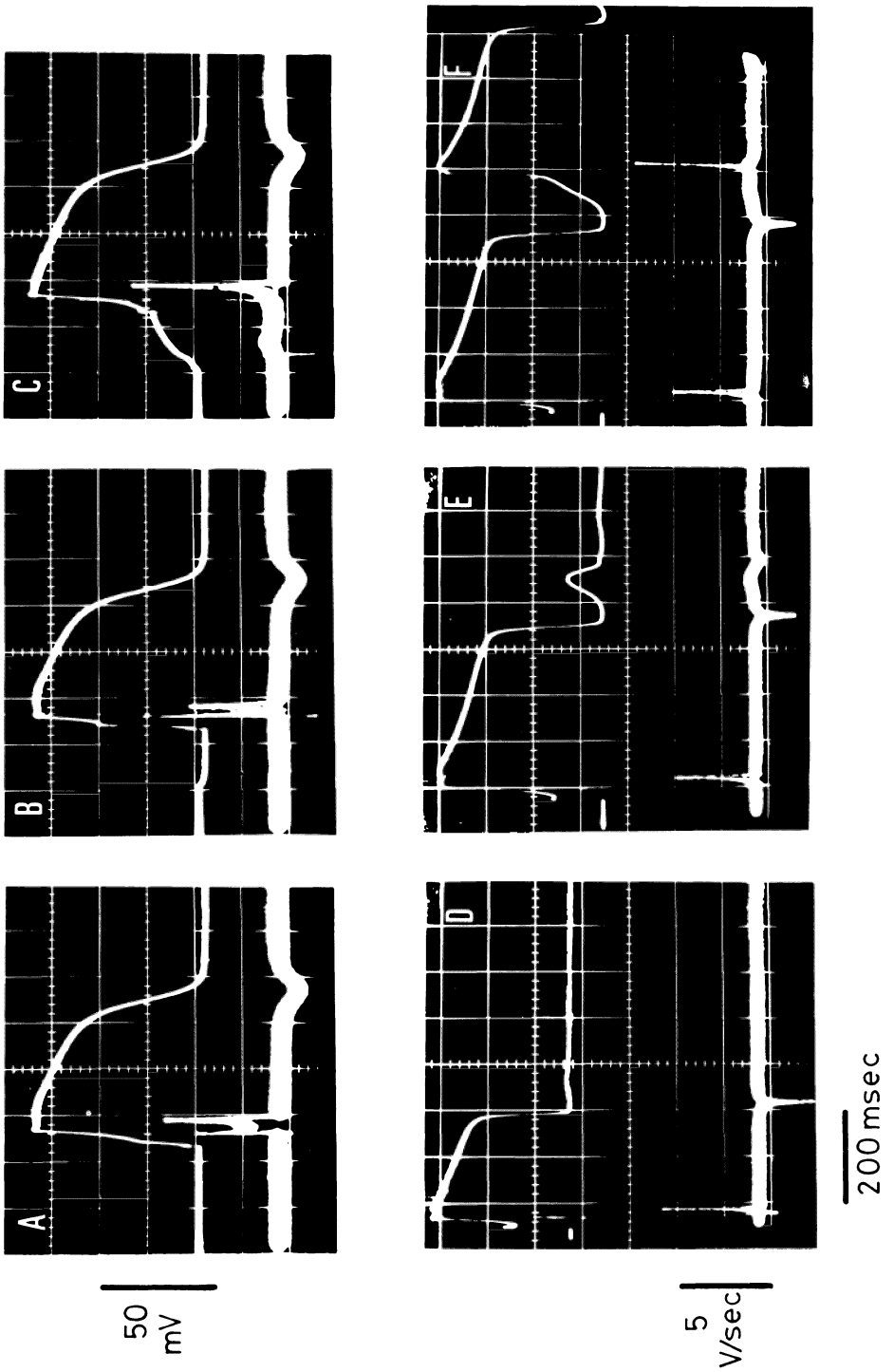
Figure 3. Upper part: Changes in action potential and $\dot{V}_{max}$ of a cat papillary muscle when K_0^+ is increased from 2.7 to 54 mM in the presence of tetrodotoxin (10 mg/liter). Cl-free, acetylglycinate solution. Lower part: Graphical representation of the evolution of $\dot{V}_{max}$ and action potential duration as a function of membrane potential. At a membrane potential of about -40 mV, $\dot{V}_{max}$ and duration are maximal; both variables are decreased at more negative or more positive membrane potentials (from [23]).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50 mV
5 V/sec
200 msec

occur. This current drain may be responsible for the lower $\dot{V}_{max}$ and action potential amplitude, and also for the shorter duration. For potentials closer to the threshold the current drain will be smaller and $\dot{V}_{max}$, amplitude and duration will increase.

Electrotonic interaction, however, cannot provide the full explanation, since the same phenomena are still observed for 'membrane action potentials' in a single sucrose-gap set-up (muscle preparation) or in a short Purkinje fiber, where requirements for conduction are absent. Other explanations can also be proposed: (1) The existence of a optimum membrane potential for $\dot{V}_{max}$ could be due to a specific effect of K^+ ions (the membrane was depolarized by varying K_0^+). The same changes in $\dot{V}_{max}$ however can be obtained when, in the presence of a fixed K_0^+, the membrane potential is changed by application of a transmembrane current (Figure 4A–C) or when the membrane spontaneously depolarizes during a transient afterdepolarization (Figure 4F). (2) The observed phenomena could further be due to an interaction between the fast Na^+ and slow Ca^{++}-Na^+ channel. This explanation is ruled out by experiments performed in the presence of TTX in a concentration sufficient to block the Na^+-mediated activity. Figure 3 [23] shows that under those conditions an increase in K_0^+ still elicits an increase in $\dot{V}_{max}$, overshoot and duration for membrane potentials between -90 mV and -40 mV. Other results also confirm the independence of Ca^{++}-mediated activity on the presence or absence of a preceding Na^+-spike. In cow Purkinje fibers (high K^+, high Ca^{++} perfusion) we have occasionally observed spontaneous alternation of action potentials with or without a fast Na^+-spike (Figure 1F); the height of the plateau phase was identical in both cases [17].

Although the existence of an optimum membrane potential in the generation of the slow response remains thus unexplained, the phenomenon may be of practical importance, and should be taken into account when comparing results obtained in different K^+ concentrations or when studying the effect of ions and drugs on the slow response.

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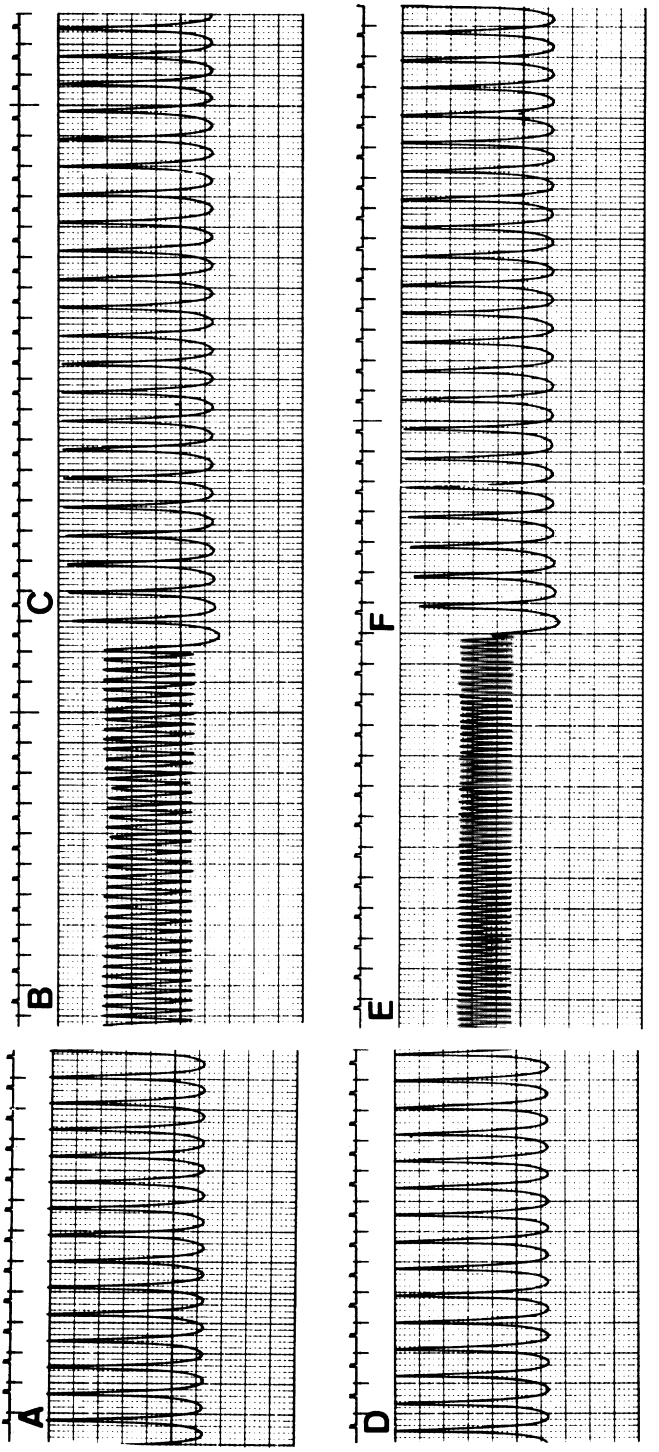
Figure 4A–C. Effect of a preceding hyperpolarization (B) and depolarization (C) by transmembrane current on $\dot{V}_{max}$ of the Ca-mediated action potential. $\dot{V}_{max}$ is increased by previous depolarization. Cow Purkinje fiber; K_0^+ 10.8 mM, isoproterenol 0.4 mg/liter. D–F: Ca^{++}-mediated action potentials in 27 (D) and 10.8 mM K_0^+ (E, F). In 10.8 mM K_0^+ (higher negative membrane potential) $\dot{V}_{max}$ is smaller; the action potential is followed by a transient depolarization, which eventually attains threshold and results in a conducted action potential with a higher $\dot{V}_{max}$. Cow Purkinje fiber (Carmeliet, unpublished).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4. THE SLOW RESPONSE AND PACEMAKER ACTIVITY

Activation of the slow response plays an essential role in the normal as well as abnormal pacemaker activity of the heart. In the case of the sinoatrial pacemaker evidence has been presented that it is due to the simultaneous activation of the slow inward current and an outward K^+ current [36, 37].

The flow of Ca^{++} through the slow channel is also involved, directly or indirectly, in the genesis of pacemaker activity in depolarized Purkinje or cardiac muscle preparations. The depolarization can be brought about by different procedures, of which some are relevant to clinical situations: application of external current [38–41], metabolic inhibition or blocking of the Na^+ electrogenic pump [42, 43], perfusion with low external K^+ [44, 45] or low pH [46, 47], addition of Ba [48], TEA [21] or Cs [49]. The induced pacemaker activity can be modified by different experimental manipulations that affect either the slow diastolic depolarization or the regenerative upstrokes. Oscillations or action potentials at low levels of membrane potential are well maintained in TTX [38, 39], are enhanced in elevated Ca_0^{++} [38, 40] and in the presence of catecholamines [50–52] and are blocked by verapamil [53] and Mn^{++} [38]. In comparison with the pacemaker activity at high levels of membrane potential in Purkinje fibers, which undergoes overdrive suppression [54], stimulation at high frequency had no effect on the poststimulatory spontaneous activity at reduced levels (Figure 5).

The mechanism underlying the depolarization-induced pacemaker activity has been studied with the voltage clamp technique [39, 52, 55–57]. In all cases the depolarization following the repolarization can be explained by deactivation of an outward current and simultaneous activation of the slow inward current; how far activation of the inward current determines the speed of diastolic depolarization however is less clear (see 55). In Purkinje fibers the increase in frequency of oscillations is due to an enhanced activation of both inward and outward currents [52].

During recent years it has become apparent that Ca^{++} ions play an important role in the genesis of afterdepolarizations. These afterdepolarizations may reach threshold and result in pacemaker activity. The amplitude of the afterdepolarizations and the probability to reach threshold is dependent on the

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Figure 5. Spontaneous activity in 20 mM cesium, K^+-free solution in a sheep Purkinje fiber. Electrical stimulation during 1 min at frequencies of 180/min (B) and 300/min (E) did not affect the inherent frequency (compare C with A and F with D). A and D: controls before electrical stimulation; in B and E only the final part of the electrical stimulation periods is shown. Time scale: 1 sec. Voltage scale: one large division: 10 mV (Carmeliet, unpublished).

frequency of stimulation (poststimulatory enhancement; overdrive excitation) [58–60]. The involvement of Ca^{++} is indirect: the current responsible for the transient depolarizations seems to be carried mostly by Na^{+} ions, but the conductance of the channel is modulated by intracellular Ca^{++} [61, 62].

Triggered pacemaker has been recorded in Purkinje fibers in the presence of toxic doses of glycosides, but is also seen without addition of ouabain in atrial muscle fibers [63] and fibers of the sinus coronarius and mitral valve [64]. Catecholamines exert a stimulatory effect on the transient depolarizations and spontaneous activity may be seen even in the presence of elevated 10.8 mM K_0^{+} concentrations (Figure 4E and F; see also [17]). Since extracellular K^{+} as well as catecholamines are increased in myocardial infarction, this observation may have some clinical relevance.

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5. THE ROLE OF CYCLIC AMP IN REGULATION OF THE SLOW INWARD CURRENT *

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1. INTRODUCTION

Two distinct inward membrane currents underly the electrical excitation of atrial and ventricular myocardium. When a stimulus is applied to quiescent cardiac muscle or specialized conducting tissues regenerative membrane depolarization occurs which, when recorded by means of an intracellular electrode, produces characteristic time-dependent changes in transmembrane voltage. The initial rapid phase of this membrane depolarization is due to a sudden passive increase in membrane permeability to Na^+ ions [1–10]. This initial inward current can be abolished by removal of Na^+ ions or addition of appropriate concentrations of tetrodotoxin [1–10]. Because of its rapid activation and inactivation kinetics this current has been designated the fast inward current and appears to be due to the flow of Na^+ ions through specific membrane channels (fast Na^+ channels) [2–10]. This fast inward current is responsible for the rapid upstroke velocity of the transmembrane action potential and influences the rate of propagation of electrical impulses through specialized cardiac conducting tissues [6–10]. Autonomic neurotransmitters do not appear to modify the magnitude or kinetics of this fast inward current in normal fibers [11, 12].

In addition to the fast Na^+ current, atrial myocardium and ventricular myocardium possess a second distinct inward current which is quantitatively smaller than the fast Na^+ current and is carried primarily but not exclusively by Ca^{++} ions. This secondary inward current has slow activation and inactivation kinetics and therefore has been termed the slow inward current [2–10]. The magnitude of this slow inward current is modified by alterations in extracellular Ca^{++} concentration and the current is thought to flow

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through specific membrane ionic channels (slow Ca^{++} channels)[2–10]. Certain pharmacologic compounds (verapamil, D-600, nifedipine) and cations (La^{+++}, Mn^{++}) have been identified which attenuate the slow inward current presumably by blocking slow Ca^{++} channels[7, 9, 10].

The slow Ca^{++} current appears to be responsible for the plateau phase of the cardiac action potential and its inactivation facilitates the repolarization process[3]. The slow Ca^{++} current is believed to be involved in the process of excitation-contraction coupling both directly by increasing Ca^{++} entry into the cell and indirectly by inducing Ca^{++} release from intracellular stores[13–16]. In the SA and AV nodal regions, the slow Ca^{++} current appears to be the primary depolarizing current. This accounts for the relatively slow upstroke velocity of the membrane potentials recorded from these cells[10–17]. In contrast to the fast Na^{+} current, the magnitude of the slow Ca^{++} current is regulated importantly by the autonomic nervous system. A variety of studies have documented that one effect of the interaction of sympathetic neurotransmitters with beta-adrenergic receptors is an increase in the magnitude of the slow Ca^{++} current[18, 19]. Analysis of the effects of beta-adrenergic receptor activation on transmembrane Ca^{++} conductance indicates that whereas the activation/inactivation kinetics and the ion selectivity are unaffected, the density (or number) of functional slow Ca^{++} channels is increased[20–22].

Current theory suggests that the effects of beta-adrenergic receptor stimulation on the slow inward current are mediated through the adenylate cyclase–cyclic AMP system. This hypothesis has been supported by experiments in which lipid soluble derivatives of cyclic AMP or iontophoretically applied cyclic AMP have been shown to increase the slow Ca^{++} current[23–25] and studies with calcium-dependent action potentials ('slow responses') in which pharmacologic agents (e.g., catecholamines, histamine, theophylline) known to increase cyclic AMP content also increase the slow inward current (measured indirectly)[26–29]. It has been postulated that the availability of functional slow Ca^{++} channels is regulated by cyclic AMP by means of cyclic AMP-dependent protein kinase-directed phosphorylation of membrane proteins[7, 10, 16, 30, 31]. This scheme proposes the following sequence of events for catecholamine regulation of the slow inward current: (1) catecholamines stimulate beta-adrenergic receptors; (2) this stimulation of beta-adrenergic receptors leads to activation of adenylate cyclase; (3) adenylate cyclase activation increases intracellular cyclic AMP levels; (4) the elevation of intracellular cyclic AMP levels leads to activation of cyclic AMP-dependent protein kinase; and (5) activated cyclic AMP-dependent protein kinases phosphorylate protein constituents of the membrane-bound slow Ca^{++} channels, thereby making these Ca^{++} channels available for voltage activation during subsequent membrane depolarizations. Following activation, the slow Ca^{++}

channels may be inactivated by dephosphorylation which is mediated by the action of intracellular phosphatases. Although this postulated sequence of events appears plausible, much experimental work still must be completed to establish the detailed biochemical mechanisms responsible for catecholamine regulation of the slow Ca^{++} current.

Recent data suggest that stimulation of muscarinic cholinergic receptors is associated with a reduction in the magnitude of the slow Ca^{++} current. Voltage clamp studies performed in atrial tissues have demonstrated that acetylcholine directly reduces the inward Ca^{++} current [32, 33]. In ventricular myocardium, muscarinic receptor stimulation does not directly affect the slow Ca^{++} current [34]. However, when the slow Ca^{++} current has been augmented, presumably by elevation of intracellular cyclic AMP levels, muscarinic agonists appear to produce a reduction in the number of slow Ca^{++} channels [31, 34, 36]. Thus, cholinergic regulation of the slow Ca^{++} current may be different in atria and ventricles. This notion is consistent with the well recognized fact that muscarinic receptor activation produces direct negative inotropic effects in atria but requires the co-existence of increased sympathetic stimulation to produce these effects in ventricles ('accentuated antagonism') [37–39].

The purpose of this paper is to review the known relationships between autonomic control of the slow Ca^{++} current and cyclic nucleotides. Specifically, the role of cyclic AMP in regulating the slow inward current will be reviewed as it pertains to sympathetic and parasympathetic control of transmembrane Ca^{++} flux.

2. ROLE OF CYCLIC AMP IN MEDIATING THE EFFECTS OF BETA-ADRENERGIC RECEPTOR STIMULATION ON THE SLOW INWARD CURRENT

Numerous investigators have explored the relationship between stimulation of beta-adrenergic receptors, cyclic AMP levels and changes in the functional properties of cardiac tissues [16, 40, 41, 44, 56, 74]. It is well established that the metabolic effects (activation of glycogen phosphorylase) of beta-adrenergic receptor stimulation are mediated by increases in myocardial cyclic AMP content [41–44]. A large body of data have linked also the contractile and electrophysiologic effects of beta-adrenergic agonists to changes in intracellular cyclic AMP content [16], although the causal role of this nucleotide in mediating these physiological effects of catecholamines is less well established than its role in the metabolic effects [56, 74]. These physiological effects of beta-adrenergic receptor activation are associated with increases in the slow Ca^{++} current. Thus, it has been suggested that the contractile and electrophy-

siologic effects of catecholamines are, in part, related to increases in transmembrane calcium flux through slow calcium channels and that the alteration in calcium flux is regulated by cyclic AMP.

Evidence that beta-adrenergic receptor stimulation increases the slow Ca^{++} current was first provided by Engstfeld et al. [45]. This early study demonstrated that catecholamines restored excitability in potassium depolarized cardiac tissues and that this alteration in membrane excitability was related to increased calcium flux. Grossman and Furchgott [46] demonstrated by calcium tracer studies that epinephrine increased transmembrane calcium flux in isolated atrial tissues. In 1967, Reuter [18] first demonstrated directly with voltage clamp studies that beta-adrenergic receptor stimulation increased the magnitude of the slow inward current. These studies were soon verified by several other laboratories [22–25, 47]. Carmeliet and Vereecke suggested that the increase in the plateau phase of the cardiac action potential was due to the enhancement of the slow calcium current produced by catecholamines [47]. This latter study also demonstrated that the effects of catecholamines on the slow Ca^{++} current could be mimicked by caffeine which, among other things, acts as a phosphodiesterase inhibitor. Since it was generally accepted that both stimulation of beta-adrenergic receptors and inhibition of phosphodiesterase are associated with increases in cyclic AMP content in cardiac tissues, these data suggested that cyclic AMP might mediate the beta-receptor induced increase in the magnitude of the slow Ca^{++} current.

Tsien et al. first demonstrated directly that cyclic AMP per se could increase the slow Ca^{++} current [23, 24]. These investigators showed that exposure of calf Purkinje fibers to lipid soluble derivatives of cyclic AMP increased the magnitude of the slow Ca^{++} current. In a separate study, these same investigators demonstrated that direct iontophoretic application of cyclic AMP into cardiac cells mimicked the effects of catecholamines on the plateau phase of the action potential. These observations were supported by the work of Meinertz et al. who demonstrated that dibutyryl cyclic AMP increased $^{45}Ca^{++}$ uptake which was associated with increases in contractile force [48]. Under voltage clamp conditions, Reuter subsequently demonstrated that the increases in the slow Ca^{++} current induced by both catecholamines and dibutyryl cyclic AMP were due to an increase in the number of functional slow Ca^{++} channels rather than an alteration of the ion selectivity or kinetics of the slow Ca^{++} channels [20].

The notion that the enhancement of the slow Ca^{++} current produced by beta-adrenergic receptor stimulation was related to increases in cyclic AMP content was further supported by studies with potassium depolarized tissues in which the action potentials were primarily dependent upon calcium influx. Pappano [26] and Thyrum [49] demonstrated that beta-adrenergic receptor stimulation increased excitability in potassium depolarized atria, similar to the

observations of Engstfeld et al. [45]. This effect was postulated to be related to an increase in the number of functional slow Ca^{++} channels. Shigenobu and Sperelakis [27] confirmed the observations of Pappano [26] and demonstrated further that phosphodiesterase inhibition and dibutyryl cyclic AMP administration produced calcium-dependent action potentials in chick hearts. Using this same model, Watanabe and Besch further established the relationship between beta-receptor stimulation, cyclic AMP levels and the slow Ca^{++} current by directly measuring cyclic AMP content in tissues exposed to catecholamines following inactivation of fast Na^{+} channels [28]. In these studies, increases in the slow Ca^{++} current produced by isoproterenol, histamine, theophylline and papaverine were accompanied by increases in cyclic AMP content. Positive inotropic agents, such as ouabain and glucagon, which did not increase cyclic AMP levels, did not restore excitability to these depolarized (22 mM K^{+}) or tetrodotoxin-blocked hearts.

3. AUGMENTATION OF THE SLOW INWARD CURRENT DUE TO INCREASES IN INTRACELLULAR CYCLIC AMP INDEPENDENT OF BETA-ADRENERGIC RECEPTOR ACTIVATION

As alluded to previously, the evidence for a role of cyclic AMP in mediating the effects of catecholamines on the slow inward current includes many studies which demonstrated that a variety of methods of increasing intracellular cyclic AMP levels, independent of stimulating beta-receptors, increased the slow inward current. These methods, which will be discussed in more detail in this section, included: (1) increasing cyclic AMP content by iontophoretic injection or by applying lipid soluble derivatives of cyclic AMP to isolated cardiac tissues; (2) activating adenylate cyclase independent of beta-adrenergic receptor stimulation; and (3) inhibiting phosphodiesterases.

Voltage clamp studies performed by Tsien et al. [25] in calf Purkinje fibers demonstrated that bathing these tissues in buffer containing lipid soluble analogues of cyclic AMP increased I_{SI} and I_{XI}. These studies were supported by the observation that lipid soluble derivatives of cyclic AMP restored excitability in potassium depolarized tissues [27, 28, 50]. Shigenobu and Sperelakis demonstrated that dibutyryl cyclic AMP (2 mM) restored excitability in tetrodotoxin-blocked cultured embryonic chick hearts [27]. Watanabe and Besch showed that dibutyryl cyclic AMP (3 mM) restored excitability in isolated perfused guinea pig ventricles depolarized with 22 mM K^{+} [28]. In addition, Tsien demonstrated that iontophoretic injection of cyclic AMP increased the plateau phase of the cardiac action potential thus suggesting an enhancement of the slow Ca^{++} current [23]. Thus, these studies provided evidence

that cyclic AMP may mediate the effects of catecholamines on the slow Ca^{++} current by satisfying one of Sutherland's original criteria for establishing the role of cyclic AMP as a 'second messenger' for hormones [41]. The results must, however, be interpreted with caution. The assumption must be made that iontophoretic application or administration of lipid soluble derivatives of cyclic AMP to cardiac tissues exerts the same intracellular effects as endogenously generated cyclic AMP. Reasons for concern with this assumption include the fact that relatively large concentrations of the exogenous nucleotide are required to mimick the effects of catecholamines and that the time course of the effects of the nucleotide is quite different from that of catecholamines.

Another method employed to study the effects of cyclic AMP on the slow Ca^{++} current has been to activate the enzyme adenylate cyclase without stimulating beta-adrenergic receptors. Histamine receptor stimulation has long been known to activate adenylate cyclase, increase cyclic AMP levels and produce positive inotropic effects in cardiac tissues. Watanabe and Besch demonstrated that histamine produced concentration-dependent increases in the slow Ca^{++} current which were paralleled by increases in intracellular cyclic AMP content [28]. Inui and Imamura subsequently demonstrated that the increases in the slow Ca^{++} current produced by histamine receptor stimulation were augmented by simultaneous phosphodiesterase inhibition (papaverine) and attenuated by phosphodiesterase stimulation (N-methylimidazole) [29]. Another method of hormonally activating adenylate cyclase via a non-beta-receptor mechanism is with glucagon. Glucagon has been shown to activate adenylate cyclase and to elicit positive inotropic effects in cardiac tissues [41, 53–56]. However, Watanabe and Besch were unable to detect any increase in cyclic AMP content or the slow Ca^{++} current following administration of glucagon to guinea pig hearts [28]. This observation is consistent with the fact that glucagon does not activate adenylate cyclase in guinea pig myocardium [57].

In addition to hormonal activation of adenylate cyclase, direct activation of the enzyme by a variety of agents has provided another approach to elevating intracellular cyclic AMP levels while bypassing beta-adrenergic receptors. It is well known that the enzyme adenylate cyclase requires guanine nucleotides (GTP) for maximal enzyme activity in vitro [58]. Based on the knowledge that the non-hydrolyzable analogue of GTP, Gpp(NH)p, can produce sustained and marked activation of adenylate cyclase in cardiac membrane preparations [59], Josephson and Sperelakis have recently examined the effects of Gpp(NH)p on the slow Ca^{++} current [60]. These investigators observed that Gpp(NH)p increased the maximum rate of depolarization and overshoot of calcium-dependent action potentials recorded from cultured embryonic chick myocardial cells. Presumably, these effects were due to direct activation of adenylate

cyclase, leading to increased levels of intracellular cyclic AMP and augmentation of the slow Ca^{++} current through cyclic AMP directed membrane protein phosphorylation. However, in these experiments cyclic AMP content was not determined and therefore these results can only be interpreted in a speculative fashion.

A second method of directly stimulating adenylate cyclase is with cholera toxin. The mechanism by which cholera toxin activates adenylate cyclase is presumably by inhibition of adenylate cyclase-associated GTPases which thereby leads to increased amounts of endogenous GTP available for interaction with the regulatory site of adenylate cyclase [61]. Cholera toxin has been shown to produce positive inotropic effects in cultured embryonic heart cells; however, its effects on the slow Ca^{++} current have not been explored yet [62]. This method of increasing intracellular cyclic AMP levels possibly could be used as an additional tool to study the relationships between cyclic AMP and the slow Ca^{++} current.

Another widely employed method of increasing intracellular cyclic AMP levels, independent of beta-adrenergic receptor activation, has been to inhibit the breakdown of cyclic AMP to 5'-AMP. Phosphodiesterase inhibitors have been used by numerous investigators to study the relationship between cyclic AMP and the slow Ca^{++} current [25, 27–30, 47, 50]. Methylxanthines were the first group of phosphodiesterase inhibitors to be used for this purpose. Carmeliet and Vereecke [47] demonstrated in isolated Purkinje fibers that caffeine increased the plateau phase of the cardiac action potential similar to the effects of catecholamines. These investigators postulated that the electrophysiologic effects of phosphodiesterase inhibition could be due to an increase in the slow Ca^{++} current mediated by cyclic AMP. Tsien et al. [25] demonstrated directly with voltage clamp studies in calf Purkinje fibers that theophylline produced an augmentation of the slow inward current similar to that observed with catecholamines and N^6-mono-butyryl-cyclic AMP. Since these early observations, numerous investigators have demonstrated directly (voltage clamp) or indirectly (calcium-dependent action potentials) that phosphodiesterase inhibition with methylxanthines could augment the slow Ca^{++} current [25, 27–30, 47, 50]. Watanabe and Besch showed that phosphodiesterase inhibition (theophylline) produced concentration related increases in cyclic AMP levels which were paralleled by increases in the slow Ca^{++} current (developed tension of Ca^{++}-dependent action potentials). The methylxanthines, unfortunately, also produce a number of nonspecific contractile effects on cardiac tissues and therefore their usefulness as tools for studying the relationship between cyclic AMP and the slow Ca^{++} current is limited [63, 64].

Another type of phosphodiesterase inhibitor that has been used to elevate intracellular cyclic AMP levels in order to study the slow Ca^{++} current is

papaverine. This isoquinoline derivative is a potent phosphodiesterase inhibitor which markedly elevates intracellular cyclic AMP levels [27, 28, 65–67]. Watanabe and Besch demonstrated that papaverine produced concentration-dependent increases in the slow Ca^{++} current (measured indirectly) and cyclic AMP content of guinea pig ventricles[28]. Inui and Imamura demonstrated that papaverine augmented histamine-induced increase in the slow Ca^{++} current. At high concentrations, however, papaverine produces negative inotropic effects which have been attributed to blockade of slow Ca^{++} channels [67]. This latter pharmacologic effect of papaverine limits the usefulness of this compound as a tool for studying te relationship between cyclic AMP and the slow Ca^{++} current.

A third type of phosphodiesterase inhibitor that has been used to study the relationship between cyclic AMP and the slow Ca^{++} current is RO 7-2956. This compound does not appear to produce many of the cyclic AMP-independent pharmacologic effects in cardiac tissues associated with methylxanthines or papaverine [25, 50, 68, 69]. This drug closely mimicks the electrical and mechanical effects of catecholamines on isolated cardiac tissues. Tsien et al. demonstrated that this compound elevated the plateau phase of the cardiac action potential and augmented the slow inward current [25]. Tritthart et al. demonstrated that RO 7–2956 increased the slow Ca^{++} current and simultaneously increased cyclic AMP content [50]. Of the phosphodiesterase inhibitors available, RO 7–2956 appears to possess the fewest disadvantages due to nonspecific effects and provides the most reliable tool to study the effects of phosphodiesterase inhibition on cyclic AMP levels and the slow Ca^{++} current.

The studies reviewed in this and the preceding section provide evidence that cyclic AMP plays a role in regulating slow Ca^{++} channels. When cyclic AMP levels are increased by a variety of different means, slow Ca^{++} current is also increased. The various approaches of increasing cyclic AMP levels include hormonal activation of the enzyme adenylate cyclase, direct activation of the enzyme (by-passing hormone receptors), administration of cyclic AMP analogues, iontophoretic injection of cyclic AMP into cardiac cells and inhibition of phosphodiesterase. Although in many cases the evidence is correlative and a causal relationship between total tissue cyclic AMP levels and the slow current has not yet been unequivocally established, the evidence strongly suggests that cyclic AMP is involved in regulating the magnitude of the slow current. The detailed biochemical mechanism by which increases in cyclic AMP levels augment the slow current is considerably less well defined. However, it is reasonable to assume that the mechanism involves phosphorylation of specific membrane proteins by cyclic AMP dependent protein kinases.

4. AUGMENTATION OF THE SLOW INWARD CURRENT INDEPENDENT OF CYCLIC AMP

Evidence that the slow Ca^{++} current is influenced importantly by alterations in intracellular cyclic AMP levels has been reviewed in the preceding sections. It is well recognized that transsarcolemmal Ca^{++} flux through slow Ca^{++} channels also can be modified experimentally, independent of the adenylate cyclase–cyclic AMP system. The most straightforward method employed to augment the slow inward current was to elevate extracellular calcium concentration and thus increase the chemical gradient for Ca^{++} across the membrane [18]. This experimental approach provided important evidence in voltage clamped preparations that the slow inward current was carried primarily by calcium ions.

Reuter, in 1967, first explored the relationship between the slow inward current and calcium in cardiac tissues [18]. This early work demonstrated that the magnitude of the slow inward current was very sensitive to variations in the external calcium concentration. Subsequently, a number of laboratories confirmed and extended these observations [2, 9, 21–30]. Systematic ion-substitution experiments have shown that the slow inward current is carried primarily, but not exclusively, by calcium ions. Studies performed by Reuter and Scholz indicated that the membrane channels carrying the slow inward current are at least 100 times more selective for calcium than for sodium or potassium [20]. However, at very low extracellular calcium concentrations, sodium does appear to become the predominant charge carrier for the slow inward current. The slow Ca^{++} current possesses additional unique features compared to other membrane currents in that the Ca^{++} channels appear to saturate at high extracellular calcium concentrations [10]. Thus, transmembrane Ca^{++} conductance through slow Ca^{++} channels appears to deviate from the independence principal (typical for other membrane ionic currents) and at high extracellular Ca^{++} concentrations this inward current reaches a plateau. At this point further increases in extracellular Ca^{++} do not increase the slow inward current. Recent data provided by Lee et al. suggest that this effect may be due to accumulation of intracellular Ca^{++} which limits Ca^{++} conductance through slow Ca^{++} channels [70].

Studies performed with calcium-dependent action potentials ('slow responses') have also demonstrated the dependence of the slow inward current on extracellular Ca^{++}. Pappano showed that restoration of excitability in potassium (22 mM) depolarized atrial tissues was dependent on extracellular Ca^{++} ions [26]. This study documented that the overshoot of the calcium-dependent action potential varied directly with extracellular calcium, thus the membrane behaved as a calcium electrode. This early study also demonstrated that other divalent cations could substitute for calcium and restore

electrical excitability. The relative ability of these divalent cations to restore excitability was inversely related to the size of the hydrated radii of each divalent cation studied ($Ba^{++} > Sr^{++} > Ca^{++} > > Mg^{++}$). These early studies were later confirmed by Shigenobu and Sperelakis [27]. Studies performed by Watanabe and Besch demonstrated that restoration of excitability in potassium depolarized tissues by elevation of extracellular Ca^{++} was not associated with any change in intracellular cyclic AMP content [28]. Thus, these studies as well as the voltage clamp work indicate that the magnitude of the slow Ca^{++} current can be directly increased by elevating extracellular Ca^{++}. Elevation of extracellular Ca^{++} directly increases the driving force for Ca^{++} across the membrane. The increase in slow Ca^{++} current that occurs under this circumstance appears to be independent of changes in cyclic AMP levels.

It has recently been shown that stimulation of alpha-adrenergic receptors could increase the slow Ca^{++} current independent of changing cyclic AMP levels. Miura demonstrated that phenylephrine could restore excitability to potassium-depolarized rabbit papillary muscles [71]. This effect was blocked by phentolamine. The production of calcium-dependent action potentials with phenylephrine ($\leq 10^{-6}$ M) was not associated with cyclic AMP accumulation. These observations are consistent with work of other investigators who have shown that alpha-adrenergic receptor stimulation produces positive inotropic effects but do not increase cyclic AMP content of cardiac tissues [72–74]. The mechanism by which this occurs remains to be elucidated.

Recently, the polypeptide angiotensin II has been shown to restore electrical excitability in cardiac tissues in which fast Na^+ channels were inactivated (tetrodotoxin or 22 mM K^+) [75]. This study demonstrated that these effects of angiotensin II were mediated through specific angiotensin II receptors (the effects were blocked with angiotensin II receptor antagonists). Although cyclic AMP levels were not reported in this study, Sperelakis and co-workers subsequently reported that cyclic AMP levels are not elevated by angiotensin II receptor stimulation [30]. Presumably, the positive inotropic effects of angiotensin II on cardiac muscle could be explained by an enhancement of the slow Ca^{++} current. The mechanism by which angiotensin II receptor stimulation augments the slow Ca^{++} current is also unclear.

Vogel and coworkers [76] have reported recently another method of directly increasing the slow Ca^{++} current independent of cyclic AMP. Their study demonstrated that NaF restored excitability in embryonic chick hearts in which fast Na^+ channels had been blocked. NaF is known to activate adenylate cyclase in broken cell preparations [77, 78]. However, this compound did not elevate cyclic AMP levels in this study. The authors speculated that since NaF is known to be a phosphatase inhibitor [79], the slow Ca^{++} current could have been increased by inhibition of dephosphorylation of slow Ca^{++} chan-

nels by NaF. Thus, the density of slow Ca^{++} channels would have been increased since the usual inactivation mechanisms (dephosphorylation of slow Ca^{++} channels) would have been inhibited.

5. CHOLINGERGIC REGULATION OF THE SLOW INWARD CURRENT

Experiments have recently been performed which suggest that muscarinic receptor stimulation can diminish the magnitude of the slow Ca^{++} current in both atrial and ventricular myocardium [31–36, 80, 81]. Giles and Tsien demonstrated in voltage clamped frog trabeculae that acetylcholine directly decreased the magnitude of the slow inward current [80]. These studies were later confirmed in mammalian atria by Ikemoto and Goto [81] and by TenEick et al. [32]. The studies performed by TenEick and coworkers in a variety of mammalian atria (dog, cat, guinea pig, sheep) demonstrated that at low concentrations (0.5 μM) acetylcholine increased outward currents, shortened action potential duration and decreased twitch tension. At higher concentrations (2 μM) acetylcholine reduced the slow Ca^{++} current in addition to the increased outward currents. Thus, these studies suggested two mechanisms for the negative inotropic effects of acetylcholine in mammalian atria. First, at low concentrations, acetylcholine 'indirectly' affected the slow Ca^{++} current by reducing action potential duration, thus decreasing the time during which this inward current can flow. Secondly, at higher concentrations, acetylcholine appeared to inhibit 'directly' the slow Ca^{++} current. The above observations provide a plausible mechanism for the negative inotropic effects of muscarinic receptor stimulation in mammalian atrial tissues.

In mammalian ventricular myocardium, studies performed by Bailey et al. [34] and Inui and Imamura [35] suggest that acetylcholine does not directly inhibit slow Ca^{++} channels, but rather interferes with cyclic AMP-facilitated increases in the slow Ca^{++} current. Both of these studies demonstrated that acetylcholine abolished calcium-dependent action potentials produced by isoproterenol or phosphodiesterase inhibition without affecting calcium-dependent action potentials produced by elevation of extracellular Ca^{++}. Although cyclic AMP levels were not measured, the results of both of these studies suggested that muscarinic receptor activation impaired transsarcolemmal Ca^{++} flux through slow channels primarily by an effect on the adenylate cyclase–cyclic AMP system. Recent studies performed by Biegon and Pappano [31] support the conclusion that muscarinic receptor inhibition of the slow Ca^{++}current is mediated by alterations in the adenylate cyclase–cyclic AMP system. This study demonstrated that cholinergic agonists inhibited calcium-dependent action potentials in avian ventricular muscle by two mechanisms. First, in the embryonic heart, acetylcholine had no direct effect on calcium-

dependent action potentials but, if these responses were augmented by isoproterenol, acetylcholine produced a striking inhibitory effect. In these same embryonic hearts, acetylcholine had no effect on basal cyclic AMP content but attenuated isoproterenol-induced increases in cyclic AMP levels. Second, in the post-embryonic avian heart, acetylcholine depressed calcium-dependent action potentials both in the presence and absence of isoproterenol. In this ventricular muscle preparation, cholinergic agonists produced a direct reduction in cyclic AMP content as well as an attenuation of the isoproterenol-induced increase in cyclic AMP.

6. PHARMACOLOGIC METHODS OF BLOCKING THE SLOW INWARD CURRENT

That the slow Ca^{++} current is present during a particular voltage or current recording is supported by experiments in which the addition of compounds or ions which block slow Ca^{++} channels attenuate or abolish the observed recordings. Inhibitors of slow Ca^{++} channels provide important tools to study the slow Ca^{++} current. Unfortunately, no specific inhibitors of slow Ca^{++} channels are available and interpretation of data generated with compounds that are available should be tempered with the knowledge that they produce other membrane effects.

The cations, La^{+++}, Mn^{++}, and Ni^{+++} all possess the ability to block slow Ca^{++} channels. However, all three of these ions permeate the sarcolemmal membrane and also effect outward currents [82]. The pharmacologic compounds verapamil and D–600 (methoxyverapamil) block slow Ca^{++} channels yet these drugs also decrease the fast Na^{+} current and outward K^{+} currents. Thus, changes in membrane excitability induced by any of these compounds could occur independent of slow Ca^{++} channel blockade. The ability of verapamil and D–600 to block slow Ca^{++} channels is determined in part by the optical stereochemistry of these drugs [83–85]. The negative optical isomer of verapamil is 10 times more potent than the positive isomer at blocking slow Ca^{++} channels. In contrast, the positive optical isomer of verapamil appears to be much more potent than the negative isomer at blocking fast Na^{+} channels. Both optical isomers appear to be equipotent at depressing transmembrane K^{+} conductance.

Other pharmacologic compounds for blocking slow Ca^{++} channels have been developed. Nifedipine and diltiazem appear to possess slow Ca^{++} channel blocking properties [29, 86]. However, rigorous analysis of the effects of these drugs on membrane ionic currents under voltage clamp conditions have not been done.

In addition to direct blockade of slow Ca^{++} channels, pharmacologic agents may also impair the slow Ca^{++} current indirectly through their effects on the

cyclic AMP system. Propranolol is an example of a drug which may produce such effects. Under conditions in which the slow Ca^{++} current is dependent on beta-adrenergic receptor stimulation, blockade of beta-adrenergic receptors by propranolol will reduce the slow Ca^{++} current [28]. Similarly, under conditions in which the slow Ca^{++} current has been augmented by histamine receptor stimulation resulting in increases in cyclic AMP levels, the slow Ca^{++} current may be diminished by histamine receptor blockade [29].

Drugs which uncouple adenylate cyclase from the beta-receptor or directly reduce the activity of the enzyme adenylate cyclase would also be predicted to reduce the slow Ca^{++} current under conditions in which it is dependent on cyclic AMP. Inui and Imamura [29] demonstrated that phosphodiesterase stimulation with imadazole derivatives reduced the slow Ca^{++} current which had been increased by histamine (cyclic AMP-dependent mechanism). Similar evidence that the slow Ca^{++} current can be attenuated via cyclic AMP dependent mechanisms has been provided by studies with the metabolic inhibitor dinitrophenol (DNP). DNP depletes myocardial ATP by uncoupling oxidative phosphorylation. This depletion of ATP is paralleled by a reduction in cyclic AMP directed increases in the slow Ca^{++} current [30]. Thus, by depleting the substrate for adenylate cyclase, intracellular cyclic AMP content may be reduced, thus decreasing the slow Ca^{++} current.

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6. THE ROLE OF THE SLOW INWARD CURRENT IN IMPULSE FORMATION *

MARIO VASSALLE

1. THE ROLE OF THE SLOW INWARD CURRENT IN IMPULSE FORMATION

Under normal conditions, impulse formation originates in the sinus node. However, the ability to initiate impulses is shared by subsidiary pacemakers in the atria and in the ventricles. In examining the role of the slow inward current in impulse formation, it is necessary first to consider the characteristics of the slow inward current and then the events underlying diastolic depolarization and the upstroke in different pacemaker tissues of the heart. Impulse formation may be normal or abnormal and the slow inward current can be important in both situations. Finally, slow inward currents, which are different from that flowing during the plateau, may be important in determining impulse formation at least under abnormal conditions. I shall present evidence for the role of the slow inward current in impulse formation under normal conditions and under some abnormal conditions.

2. THE SLOW INWARD CURRENT

The characteristics of the slow inward current are described in detail in other chapters in this book. Here, a brief survey of these characteristics is made necessary for an appreciation of the topics to be discussed. This current was first described in cardiac Purkinje fiber [1] and it was found later on in several cardiac tissues (see [2] and [3]). It can be distinguished from the fast inward sodium current for it persists in the absence of sodium and in the presence of the fast sodium channel blocker tetrodotoxin. Also, the slow inward current can be activated by depolarizations from a potential at which the sodium carriers are inactivated. Therefore, it is apparent that different channels are involved in the production of the fast inward and slow inward currents. The slow current is largest at about -10 mV and may not fully inactivate during

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the plateau. It appears that repriming the slow inward current is relatively slow for it has been observed some time ago that the sinus node action potential grows in amplitude when elicited later on during diastole in spite of the lower take-off potential [4]. The channel is not specific for it may admit both Na^+ and Ca^{++}, although preferentially the latter. Because both ions can enter the slow channel, the current is not abolished when one of the two ions is omitted from the solution (see [2] and [3]).

The development of slow channel blockers (see [5]) has provided pharmacological means to analyze some of the features of the slow inward current. These blockers (verapamil, D600, nifedipine, etc.) block the slow channel, shorten the plateau and abolish the force of contraction. Nickel, cobalt and manganese also compete with calcium for the slow channel. While undoubtedly useful, these agents have proved not to be highly selective in that they affect other currents as well (see [2] and [3]).

The slow inward current plays a role in the initiation of contraction in all cardiac tissues. However, it plays a role in the electrical phenomena which are different in different tissues. In some tissues, the slow current flows only during the plateau, in others during the upstroke and yet in others during the last part of the upstroke (see [6]). In fact, in some tissues it can flow during part of diastolic depolarization.

3. THE PACEMAKER POTENTIAL

The process of impulse formation requires the presence of a diastolic depolarization. During diastole in all cardiac pacemakers (whether dominant or subsidiary), the membrane potential is not maintained but becomes progressively less negative. The difference between the dominant and subsidiary pacemakers in this regard is that in the dominant pacemaker, the slope of diastolic depolarization is steeper and it attains threshold. In the subsidiary pacemakers, attainment of threshold is brought about by the process of conduction at a time when the local diastolic depolarization is still negative to threshold.

The ionic events underlying diastolic depolarization have been studied in different tissues of the heart and differences and similarities have been found in the pacemaker current of atrial and ventricular pacemakers.

4. THE PACEMAKER CURRENT IN PURKINJE FIBERS

Diastolic depolarization must be brought about by a net inward current. This could be accomplished either by a time-dependent increase in an inward sodium current or a decrease in an outward potassium current or by a

decrease in an electrogenic sodium extrusion [7]. The evidence available to date indicates that a time-dependent decrease in a potassium current during diastole is a basic mechanism underlying diastolic depolarization. But, we shall see that under certain conditions the other two factors just mentioned do modify diastolic depolarization.

Deck and Trautwein [8] showed that clamping the membrane potential from a depolarized potential back to the holding potential resulted in a brief inward current followed by an outward current tail which was attributed to a delayed increase in K conductance. The outward tail appeared only with repolarizations negative to -50 mV which was taken as the threshold for the activation of the pacemaker current. The reversal of the current at potential negative to E_K showed that the pacemaker current was a potassium current. In retrospect, it is clear that the 'delayed' onset of potassium conductance appears only due to an interposed tail of slow inward current. Vassalle [9] clamped the diastolic depolarization of spontaneously active Purkinje fibers and could compare the potential change in the absence of clamp with the current change in the presence of clamp. During the clamp, an inward current flowed as a function of time which would have caused diastolic depolarization in the absence of clamp. The reversal potential for this current and the increase in membrane resistance during the clamp allowed the conclusion that diastolic depolarization is due to a time-dependent fall in potassium conductance. Interestingly, a deactivation of the current occurred also when the resting potential was clamped to a more negative value. This finding showed that the pacemaker current was (partially) activated at the resting potential independently of a previous depolarization. Noble and Tsien [10] found that in the steady state the pacemaker current (labelled I_{K_2}) is fully activated at -50 mV and deactivated at -90 mV and that it shows anomalous rectification [10] with a negative slope [11]. At the maximum diastolic potential, the current is fully activated and begins to deactivate toward the steady state values appropriate to the negative potentials during diastole. Thus, the pacemaker current in the Purkinje fibers is generally agreed to be carried selectively by potassium ions.

5. THE PACEMAKER CURRENT IN THE SINUS NODE

In the sinus node, Irisawa [12] reported that clamping the membrane potential at the maximum diastolic value resulted in an inward current which increased with time. As seen in Purkinje fibers, clamping at more negative values resulted in a decline of the inward current as a function of time. Thus, Irisawa concluded that a decrease in potassium conductance is responsible for diastolic depolarization in the sinus node (as it is in Purkinje fibers). However,

it was not clear whether the current decreasing in diastole was the outward current i_{x_1}. This current is activated during the plateau and, if the maximum diastolic potential is sufficiently low, its decay results in diastolic depolarization. Alternatively, the pacemaker current could be specific for diastolic depolarization, as in Purkinje fibers. Subsequent experiments by Noma and Irisawa [13] showed that during clamps from -40 to 0 mV the slow inward current is followed by a slowly increasing outward current. On returning the potential to -40 mV, a current tail flows which decays exponentially, indicating that only one current underlies the pacemaker potential. The reversal potential for such pacemaker current shifted by about 60 mV with a 10-fold change in $[K^+]_0$. Therefore, the current appears to be selectively carried by potassium ions. In the steady state, the current is deactivated at -60 mV and fully activated at about $+10$ mV as judged from the tails recorded at the holding potential. Its deactivation is slower at a more negative potential and it shows anomalous rectification but not a negative slope.

Brown et al. [14, 15] studied a trabecula in the frog atrium which shows pacemaker activity even in the presence of TTX (as the sinus venosus does). As in the sinus node, a depolarizing clamp resulted in a slow inward current followed by an outward current. And, on clamping back to the holding potential, a tail of outward current was recorded. The tail could be dissected into a fast and a slow component (time constant of the decay at -40 mV is 2–3 and 0.4–0.6 sec, respectively). The slowly decaying current resembles the pacemaker current in Purkinje fibers in that its reversal potential is close to that determined with a potassium-sensitive microelectrode. Therefore, it should be carried only by potassium ions. Also, its activation curve spans from -70 to about -10 mV (which is close to the values reported by Noma and Irisawa [13] for the sinus node). The fast decaying current resembles the plateau current i_{x_1} in that its reversal potential is positive to that of the slowly decaying component (but apparently negative to E_{max}) which would indicate that the current is predominantly (but not solely) carried by K ions (as i_{x_1}). Also, the activation curve for the fast decaying component is shifted to a less negative range of potentials (-40 to 0 mV). Both fast and slow decaying currents participate in the process of diastolic depolarization as the pacemaker current can be dissected into faster and slower exponentially decreasing current components, with the contribution of the fast component decreasing more rapidly during diastole.

Recently, Isenberg [16] has shown that in Purkinje fibers cesium (20 mM) abolishes the pacemaker current but not the plateau current i_{x_1}. At a concentration of 1 mM, cesium selectively suppresses diastolic depolarization in canine Purkinje fibers [17]. For this reason it was of interest to test whether cesium also suppresses diastolic depolarization of the sinus node. In the presence of cesium, diastolic depolarization should become flatter only if it is

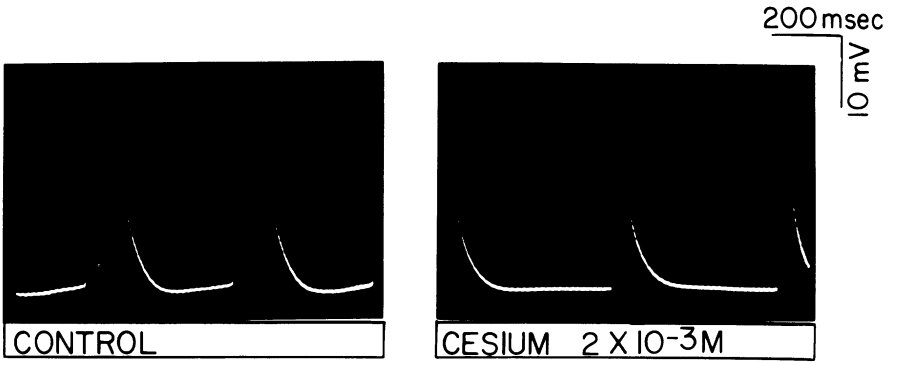

Figure 1. Effect of cesium on the diastolic depolarization of a cell in the sinus node of the guinea pig. The first panel shows the lower part of the action potential of a subsidiary pacemaker. The right panel shows the potential recorded from the same cell in the presence of 2 mM cesium. It is apparent that cesium flattened diastolic depolarization, slowed the rate and also shortened the action potential. Voltage and time calibrations are shown in the right upper corner (M Bhattacharyya and M Vassalle, Unpublished experiments.)

due to a pacemaker current similar to that in Purkinje fibers; but it should be unaffected if it is due to the deactivation of the plateau current i_{x_1}. In Figure 1, the first panel shows the lower part of an action potential recorded from the sinus node of a guinea pig. The second panel was recorded in the presence of cesium (2 mM) and shows that there is no longer a diastolic depolarization: the potential is flat during diastole.

Therefore, it appears that in cardiac pacemakers a time-dependent decrease of a specific potassium conductance underlies diastolic depolarization, although the characteristics of the pacemaker current are different in different tissues.

6. THE INWARD BACKGROUND CURRENTS

In the absence of an inward background current, the membrane potential at the end of an action potential would be close to the potassium equilibrium potential. Therefore, a time-dependent decrease in potassium conductance would have little effect on the membrane potential during diastole. In the presence of an inward background current, the maximum diastolic potential is less negative than E_K and the membrane potential decreases as potassium conductance decreases.

As to the ions responsible for the background current there is no general agreement. For the sinus node, Dudel and Trautwein [18] and Trautwein and Kassebaum [19] proposed that a relatively large background current responsi-

ble for the low E_{max} in the dominant pacemakers is carried by sodium. This is supported by the finding that lowering $[Na^+]_0$ [19, 20, 21] causes hyperpolarization as often does the administration of TTX [15]. If activity ceases, the resting potential of the sinus node is found to be rather low (about -40 mV [20]). Still the membrane responds to changes in $[K^+]_0$, at least at higher concentrations. The fact that the reduction in $[Na^+]_0$ results in hyperpolarization and in an enhanced response to changes in $[K^+]_0$ suggests that sodium conductance at rest is higher than in other tissues.

However, it has been known for a while that a reduction of $[Na^+]_0$ up to a certain value not only does not decrease but may even increase the rate of discharge of the sinus node [19]. This may indicate that sodium either is not the carrier of the background current or can be substituted by another ion when its concentration is decreased. Calcium is an interesting candidate for the background current [22] since an increase in $[Ca^{++}]_0$ increases the rate of discharge of the sinus node [23, 24, 25]. Of course, a reduction of $[Ca^{++}]_0$ may also increase the rate and a very high $[Ca^{++}]_0$ decrease it [24]. But this does not deny a role for calcium in the background current for the discrepancies can be explained by other effects of calcium, such as a shift in the threshold. It has been postulated that calcium enters into the fiber during diastole [22] and this is consistent with the lack of effect of TTX on dominant pacemakers [26] and the depressant effect of manganese on sinus rate [22, 27].

It is interesting that Bouman et al. [28] found that an increase in $[Ca^{++}]_0$ increased the slope of diastolic depolarization and the rate of discharge while decreasing the maximum diastolic potential in the dominant pacemaker of the sinus node. However, when the temperature was lowered to 30 °C, increasing calcium induced a decrease in rate (instead of an acceleration). The explanation for this finding was that at a lower temperature the pacemaker shifts to a new site which has a different calcium sensitivity. It was concluded that the cells which usually act as dominant pacemakers in the head of the sinus node have a substantial influx of calcium during diastole and such influx would be sensitive to temperature changes.In the cells of the sinus node tail, the influx of calcium would play a minor role and, on increasing calcium, the effect on the threshold would predominate.

In Purkinje fibers, the importance of sodium for the leakage current is stressed by the fact that the slope of diastolic depolarization flattens when $[Na^+]_0$ is decreased [7]. And spontaneously active Purkinje fibers discharge at a faster rate when $[Na^+]_0$ is increased and at a slower rate when $[Na^+]_0$ is decreased [29]. Furthermore, the increase in $[Ca^{++}]_0$ decreases the rate of discharge by shifting the threshold to more positive values [30].

The role of chloride ions as contributors to the background current has been studied in frog atrial trabeculae in which pacemaker activity was induced by depolarizing pulses [31]. Substitution of chloride with methylsulfate results

in a transient depolarization of the resting potential. The induced repetitive discharge is accelerated at the time the resting potential is decreased but the activity is slower when the resting potential has recovered. Voltage clamp experiments in the same preparation show that the slow inward current is not affected by chloride substitution but the tail of the outward current appears decreased. This reduction is due to a reduction of the background current and to some extent also of the time-dependent potassium current. Because the equilibrium potential for chloride is negative to the resting potential, the efflux of chloride would contribute to the inward background current. Thus, an increase of this current (due to an enhanced efflux of chloride) could account for the initial increase in discharge. The late slowing may be accounted for by the fact that the reduction of the time-dependent outward current is outweighed by a reduction in the steady-state current. The authors propose that a time-independent chloride current modifies pacemaker activity in the atria. On the other hand, Seyama [32] found that sodium and potassium are far more important than chloride in determining the resting potential, as chloride conductance is small at the resting potential.

In conclusion it appears that sodium is the ion carrying the background current in Purkinje fibers and sodium and calcium (perhaps interchangeably) in the sinus node, with chloride playing a lesser role.

7. THE SLOW INWARD CURRENT DURING DIASTOLIC DEPOLARIZATION

Diastolic depolarization eventually attains threshold and an action potential ensues. The events leading to attainment of threshold are determined by several variables. As the membrane potential becomes less negative, the pacemaker current decreases as a function of a time-dependent fall in conductance but the extent of the fall in conductance is also a function of the potential values attained in diastole. This may lead to a steady potential short of threshold. Also, the outward potassium current tends to increase as the outward driving force increases with depolarization. These changes tend to be opposed by the inward-going rectification of the background and the pacemaker potassium currents. These factors apply to all cardiac pacemakers. However, there is evidence that ahead of the threshold an inward current flows which may be all-important in determining whether the threshold is attained or not.

In the sinus node, this inward current appears to be the slow inward current. Brown et al. [14] clamped the potential at the level of the maximum diastolic potential and found that in the presence of TTX a small (4 mV) depolarizing clamp already elicited a slow inward current which became net inward with a depolarizing clamp of 6 mV. Therefore, it appears that the slow

inward current can be activated at potentials negative to the onset of the upstroke and that it is responsible for the attainment of the threshold.

It is unlikely that similar findings should apply to Purkinje fibers for an increase in $[Ca^{++}]_0$ shifts the threshold to more positive values and slows the rate of spontaneous discharge [30]. Furthermore, Purkinje fibers are usually driven by the sinus node at a rate faster than their own intrinsic rate. As long as the Purkinje fibers are driven at a constant rate the diastolic depolarization is constant and its slope is frequency-dependent. When the drive is interrupted there is a period of quiescence (overdrive suppression, see [33]) during which the membrane potential slowly declines to the threshold. If, instead of interrupting the drive, the rate is decreased, the diastolic depolarization becomes progressively steeper until the threshold is attained (Valenzuela and Vassalle, unpublished experiments). A result of this kind is illustrated in Figure 2. In the top strip, the drive of a Purkinje fiber was decreased from 30 to 6/min. It is apparent that the latter part of the diastolic depolarization attained progressively less negative values until the last action potential was spontaneous. The reciprocal finding is illustrated in the bottom strip. In the quiescent fiber, the initiation of a slow drive led to a progressive flattening of the latter part of diastolic depolarization. And at the end of the drive, it took a relatively long time before the membrane potential attained its original value. Because of the process underlying overdrive suppression (see [33]), the slope of diastolic depolarization in Purkinje fibers is a function of the driving rate and it is flatter when the rate is faster. This seems to contribute importantly to maintain the subsidiary pacemakers in abeyance. When the drive is slow or is interrupted altogether (as with the sudden onset of AV block), overdrive suppression is removed and Purkinje fibers become spontaneously active. However, recent experiments show that the spontaneous activity does not involve only a steepening of diastolic depolarization in that a TTX-sensitive component may be detected in the late diastolic depolarization of spontaneous

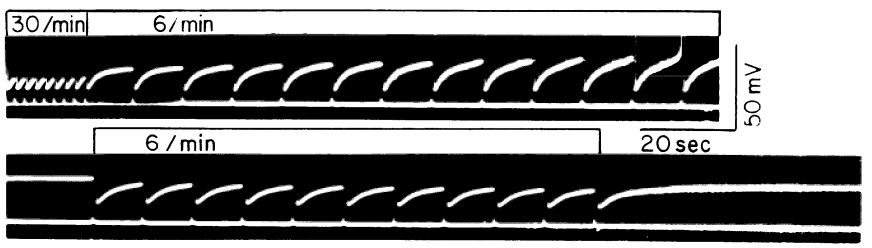

Figure 2. The effect of drive at different rates in canine Purkinje fibers. The fiber had been driven for 4 min at 30/min and the last few beats at that frequency are shown at the beginning of the top strip. Shortly thereafter, the rate was reduced to 6/min and this led to an enhancement of diastolic depolarization and spontaneous discharge. In the bottom strip, the preparation was initially quiescent and was driven at 6/min for a while. $[K^+]_0 = 2.7$ mM (recorded by Vassalle and Scidá.)

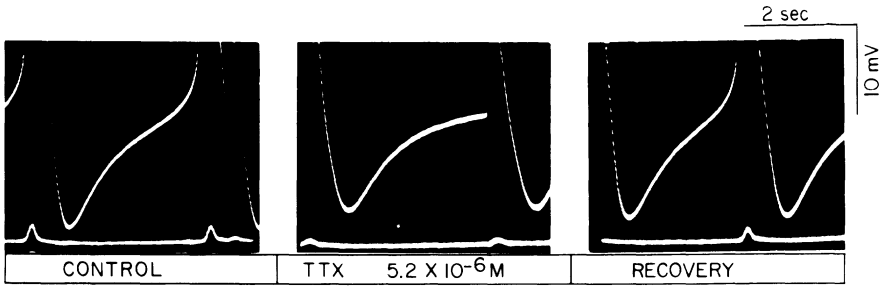

Figure 3. Effect of TTX on the late diastolic depolarization. In the first panel, the lower part of an action potential of a canine Purkinje fiber is shown. The fiber was driven at 18/min but at that slow rate spontaneous beats intermingled with driven beats. The middle panel was recorded in the presence of TTX (5.2×10^{-6} M). Spontaneous activity had ceased and the fiber was following the slow drive: the flattening of the last part of diastolic depolarization is apparent. The last panel shows the recovery in Tyrode's solution. $[K^+]_0 = 1.35$ mM (Vassalle and Scidá, unpublished experiments.)

fibers [17]. In other words, as diastolic depolarization proceeds a potential range is entered in which the inward sodium current begins to increase. When this happens the membrane potential should decline more rapidly and the time-course imposed by the decay of the pacemaker current is abandoned. This is demonstrated in Figure 3. In the first panel, the diastolic depolarization and the force curves of a Purkinje preparation driven at 18/min are shown. At this slow rate the fiber was spontaneously firing and driven impulses caused 'extra beats.' As one would expect for a dominant pacemaker, the latter part of diastolic depolarization smoothly merged into the upstroke of the action potential. In the second panel, tetrodotoxin was added and it is apparent that the late part of the diastolic depolarization flattened considerably and the fiber responded to each stimulus. The resumption of the spontaneous activity when TTX was discontinued is shown in the third panel. The picture shows that there is a TTX-sensitive component in the diastolic depolarization which appears in spontaneously discharging fibers well ahead of the threshold. It must be pointed out that TTX shortened the action potential duration, decreased somewhat the maximum diastolic potential (E_{max}) but had little effect on the early part of diastolic depolarization. Therefore, it seems unlikely that TTX decreased a background sodium current. In spontaneously active fibers in the absence of electrical drive, TTX induces quiescence by abolishing the late component of diastolic depolarization so that the threshold is missed [17].

Therefore, it appears that spontaneous activity requires a time-dependent decay of potassium current, a background current and a slow inward component. In the sinus node the background current may be carried by sodium and calcium and the slow component prior to the upstroke by the slow inward

current. In Purkinje fibers, the background current is carried by sodium and so is the slow component prior to the upstroke. When Purkinje fibers are driven by the sinus node (as they usually are), the rate is much faster than when the fibers are spontaneously active and therefore the diastolic depolarization is cut short of the 'sodium zone'. This mechanism (in addition to overdrive suppression) helps to keep subsidiary pacemakers under sinus control.

8. THE EXCITATORY PROCESS

Once the events in diastole allow the attainment of threshold, the upstroke of the action potential begins and excitation is brought about. The maximal velocity of the upstroke varies in different tissues in relation to the activation of different channels (fast or slow). In the present context, the question arises as to the role of the slow current in the excitatory process in different tissues. The fact that TTX has little effect on the action potential and diastolic depolarization of dominant pacemaker cells in the sinus node [26] suggests that the activation of the fast channel is unimportant in the excitation of these cells. This finding, in turn, elicits the question as to whether a fast sodium channel is absent in the dominant pacemaker cells or is simply inactivated because of the low take-off potential. The answer to this question is provided by the work of Noma and Irisawa [34]. These authors held the sinus node potential clamped at -40 mV and then applied a clamp to 0 potential: this resulted in the flow of a slow inward current. If instead the holding potential was at values negative to -40 mV, on depolarization a fast inward current preceded the slow inward current. The concept that a fast channel is present (but inactivated) in the dominant sinus node cells was suggested also by the findings of Kreitner [35]. When the take-off potential was shifted to a more negative value by increasing the membrane conductance to potassium with carbamylcholine, the rate of rise of the upstroke increased and this increase was abolished by TTX.

Not all the sinus node cells behave as dominant pacemakers and pacemaker shifts in the sinus node are known to occur in several situations (see [22]). In subsidiary pacemaker cells, the excitatory process is due to electrotonic interactions which depolarize the potential to the threshold before diastolic depolarization attains it. Of necessity, the take-off potential in subsidiary pacemakers is more negative than in the dominant and this implies the possibility that the fast sodium channel is not completely inactivated at the moment of excitation. That this is so is shown by several findings [36]. In subsidiary pacemakers, the upstroke is made up of two components (a fast followed by a slow one). Tetrodotoxin makes the fast component disappear altogether: as a

consequence, diastolic depolarization proceeds to the threshold for the slow current and the upstroke is made up only of the slow component. In this manner, the action potential of the subsidiary pacemakers becomes that of a dominant pacemaker. That TTX does indeed eliminate a fast component is shown by the fact that eliciting extrasystoles at more negative potentials during diastole results in the enhancement of the fast component and this enhancement does not occur if TTX is present. Similarly, the fast component is enhanced by acetylcholine-induced hyperpolarization (but not if TTX is present).

The slow inward current is also wholly or in part responsible for the excitation of the cells in the AV node [37, 38]. In the other cells of the heart under normal conditions, the slow inward current flows after the end of the upstroke. In abnormal conditions (decrease of the resting potential), even in these other tissues the slow inward current can bring about excitation (see [39]).

9. THE SLOW CHANNEL BLOCKERS AND IMPULSE FORMATION

The distinction between fast and slow channels has been helped by the use of TTX and slow channel blockers (verapamil, D600, nifedipine, manganese, etc.). The latter compounds were first studied in the laboratory of Fleckenstein (see [6]) and have been shown to reduce or abolish the slow inward current under voltage clamp procedures (see [2, 3 and 6]). Even if the specificity of their action is not absolute, these slow channel antagonists have proved rather useful in the analysis of the events underlying the activity of different tissues of the heart. If under normal conditions, the slow inward current is responsible for the activation of the sinoatrial and atrioventricular nodes, these tissues ought to be sensitive to the slow channel blockers. This has been repeatedly found.

Lenfant et al. [27] used both TTX and manganese on the sinus node and on the atrial tissues of the rabbit. While TTX had little effect on the sinus node, it abolished the action potential of the atrial tissue. In contrast, manganese accelerated the initial repolarization in the atrial fibers but had more marked effects on the action potential of the sinus node: the amplitude and the rate of rise of the upstroke decreased, the repolarization proceeded more slowly to the maximum membrane potential, the rate of discharge decreased and eventually all activity ceased. However, the slope of diastolic depolarization was not affected: 'Seule, la pente de dépolarization diastolique lente n'est pas modifiée'. The slowing of the rate should have been brought about by a shift in the threshold. It was concluded that excitation of the sinus node was brought about by the activation of a slow channel.

At the same time, Lu and Brooks [40] reported in a meeting of the New York Heart Association (the abstract was printed in January 1969) that manganese suppressed both diastolic depolarization and the action potential of dominant pacemakers in the sinus node and that this effect was partially counteracted by increasing $[Ca^{++}]_0$. Subsidiary pacemakers and atrial cells were still excitable at concentrations of manganese sufficient to suppress dominant pacemakers but became inexcitable when TTX was added. It was concluded that calcium flux played an important role in the genesis of the action potential and diastolic depolarization in the sinus node. This was in agreement with the findings of others who showed that an increase in $[Ca^{++}]_0$ increases the rate of discharge of the sinus node [23, 24].

Wit and Cranefield [41] confirmed the results with manganese and extended them to verapamil. Verapamil was found to decrease the rate of rise of the upstroke of the sinus node cells and the rate of discharge. The amplitude of the action potential decreased but the maximum diastolic potential did not. The slope of diastolic depolarization was depressed but this was not correlated necessarily with the slowing in rate, probably because of a shift in threshold. At the highest concentrations, activity ceased and only membrane oscillations remained. These effects were not modified by autonomic blockade. The depression of the sinus rate was overcome by epinephrine. In atrial fibers, the only pronounced effect was an acceleration of early repolarization. In upper and middle AV node, verapamil had actions similar to that in the sinus node (decrease of action potential amplitude but not of E_{max}). This drug also impaired AV conduction (an effect reversed by epinephrine) and lengthened the refractory period. Little effect of verapamil was found of the fibers of the lower AV node and of the His bundle.

The conclusions drawn by Wit and Cranefield were that the slow inward current is responsible for excitation in the sinus and upper and middle AV node. The depression of the rate was due to either a decreased slope of diastolic depolarization or a shift in threshold to less negative values. Shifts in pacemaker site made it difficult to decide one way or the other.

Essentially similar effects were obtained by Kohlhardt et al. [42] in the sinus node: verapamil reduced the rate of rise of the upstroke, the overshoot, the discharge frequency and the slope of diastolic depolarization. E_{max} remained unchanged and the threshold shifted to a less negative value by a few mV. When quiescence ensued, sometimes there were slight oscillations which increased in amplitude and frequency on addition of isoproterenol. The effects of a derivative of verapamil, D600, were similar. These effects were not antagonized by increasing calcium. A reduction of the rate of rise and overshoot could also be obtained with nickel, cobalt and manganese. The action of nickel (but not that of manganese) was antagonized by catecholamines.

In an in vivo study, Zipes and Fischer [38] showed that perfusion of the

sinus node via the SA nodal artery with verapamil, D600, Mn^{++} and La^{+++} decreased the rate of discharge. This effect was not affected by autonomic blockade (including alpha receptor blockade) and was reversed by isoproterenol but neither by high calcium nor by phenylephrine. Perfusion of the posterior septal artery to the AV node with the same agents slowed or blocked AV conduction and increased the duration of the AV node refractory period. Again, these effects were not influenced by autonomic blockade and were reversed by isoproterenol. These authors concluded that the slow channel is important for the excitation of the atrial pacemakers. In a microelectrode study of the AV node, Zipes and Méndez [37] found that the action potential magnitude decreased, the cell became inexcitable and conduction failed with manganese. In contrast, TTX abolished the activity of the His bundle but not that of the nodal cells where spontaneous activity continued. TTX did not affect the magnitude of the resting potential, the overshoot or the maximum rate of rise of the N cells of the AV node. Therefore, it is quite apparent that the AV nodal cells behave as those of the sinus node with respect to their response to slow channel blockers.

10. THE ION(S) CARRYING THE SLOW INWARD CURRENT

The results reported in the previous section show that the activation of a slow channel is responsible for excitation of the sinus node and AV node. This contrasts with the response of other tissues of the heart where excitation is brought about by the activation of a fast sodium channel. However, agreement ceases when it comes to the ion carrying the slow inward current. Already, it has been mentioned that TTX has little effect on the action potential of dominant pacemaker cells of the sinoatrial node. While this excludes a major role for the fast sodium channel in the excitation of those cells, it does not rule out sodium ions as current carriers since TTX does not block the slow current [43]. If it is generally believed that the slow channel can admit both sodium and calcium ions, little is known as to which ion carries the current (or to what extent) under normal conditions. Furthermore, the situation is complicated by the fact that calcium can substitute for sodium (and vice versa).

On the basis of response of the sinus node to TTX, sodium, calcium, potassium and manganese, Brooks and Lu [22] suggested that in the sinus node a calcium current participates in the generation of the action potential and a constant calcium inward current in the development of the diastolic depolarization. In contrast, Noma et al. [44] found that decreasing $[Ca^{++}]_0$ reduced the amplitude of both the fast and slow current in the sinus node and induced a shift in the reversal of the slow current to more negative

values. These authors proposed that the current carried through the slow channel is predominantly a sodium current, in agreement with the finding of a reduced rate of rise of the upstroke when $[Na^+]_0$ (but not $[Ca^{++}]_0$ was reduced [45].

In the experiments of Kohlhardt et al. [42] the upstroke and rate of rise decreased on decreasing $[Ca^{++}]_0$ while the rate of discharge sometimes slightly increased. Increasing $[Ca^{++}]_0$ from 2 to 4 mM augmented the overshoot and upstroke velocity although at 8 and 10 mM Ca these values actually decreased. It is of interest that Figure 1 of Kohlhardt et al. shows that the slope of diastolic depolarization increased steeply with higher $[Ca^{++}]_0$ even when the upstroke and rate of rise decreased. When $[Na^+]_0$ was decreased to 35 mM, the upstroke velocity decreased by 80% and the peak potential by 34 mV so that rate of rise and overshoot were more sensitive to sodium than calcium changes. The authors conclude that the slow inward current during the upstroke is carried by both Ca and Na ions. This conclusion is supported by the findings of Haastert and Fleckenstein [46] who reported that pacemaker activity was depressed not only by a lower $[Ca^{++}]_0$ but also by local anesthetics and by Na reduction.

Such a conclusion appears to apply also to subsidiary pacemakers of the sinus node [36]. When the fast component is eliminated by TTX, the slow component accounts for the entire upstroke. Under these conditions, the rate of rise of the slow component increased when either $[Ca^{++}]_0$ was decreased or $[Na^+]_0$ was increased. This suggests that more sodium enters the cell through the slow channel when either the competition by calcium is decreased or the driving force for sodium is increased. Increasing $[Na^+]_0$ resulted in an increase in the magnitude and the rate of rise of the slow component also when the fast component was abolished by increasing the $[K^+]_0$ (and therefore decreasing the take-off potential). Thus, the events underlying the slow response are sensitive to both Na and Ca in the subsidiary as well as in the dominant pacemakers.

11. CONTROL OF THE SLOW CURRENT ON IMPULSE FORMATION

The sinus node rate is enhanced by sympathetic stimulation and is depressed by vagal stimulation [47]. Sympathetic stimulation increases the overshoot, the maximum diastolic potential and the slope of diastolic depolarization. Since catecholamines increase the slow inward current in cardiac fibers [48] it is not too surprising that the overshoot and the rate of diastolic depolarization should increase.

The inhibitory effect of vagal stimulation has been attributed to the well-known effect of acetylcholine on potassium conductance. However, it has

been shown that acetylcholine reduces the slow inward current either at concentrations that have little effect on g_K [49] or at concentrations larger that needed to increase g_K [50]. In support of the former finding is the fact that carbachol reduced the amplitude and rate of rise of spontaneous TTX-resistant trabecular tissue of the frog at a concentration which did not increase E_{max}, suggesting that the slow inward current was decreased but g_K was not increased [15].

12. ABNORMAL AUTOMATICITY

The role of the slow current in abnormal automaticity is considered in other chapters of the book. Here, the discussion will be limited to some arrhythmias induced by cardiac glycosides and to overdrive excitation.

12.1. Arrhythmias caused by cardiac glycosides

Cardiac glycosides induce several types of arrhythmias by mechanisms which are not well understood. In Purkinje fibers, arrhythmias are brought about by oscillatory potentials superimposed on diastolic depolarization (see [51]). These oscillatory potentials are sensitive to agents which modify calcium movements and therefore the possibility should be considered [38] that the oscillatory potentials are brought about by an inward current flowing through the slow channel. In fact, it has been proposed that the oscillatory potentials could be caused by an inward movement of calcium (see [52]). Recent experiments, however, have shown that the oscillatory potentials are rather sensitive to interventions which affect Na movements. Administration of TTX suppresses the abnormal rhythms caused by strophanthidin in spontaneous [53] and driven Purkinje fibers [17]. Also lowering $[Na^+]_0$ prevents or abolishes strophanthidin induced arrhythmias [54] and no arrhythmias are induced in a sodium-free solution [55]. Furthermore, strophanthidin induces arrhythmias sooner in a low calcium solution and the onset of arrhythmias is generally delayed in a high calcium solution [56]. An example of the dissociation between aftercontractions and oscillatory potentials is shown in Figure 4. In the first panel, strophanthidin had induced both aftercontractions and oscillatory potentials. When the NaCl was decreased by 52%, the calcium influx should have increased as in nonintoxicated fibers wherein the contractile force increases markedly [54]. In an intoxicated fiber, the force does not increase on exposure to low Na solution, but the increased calcium load is revealed by the shorter period and increased number of the aftercontractions. Yet, the oscillatory potential decreased markedly.

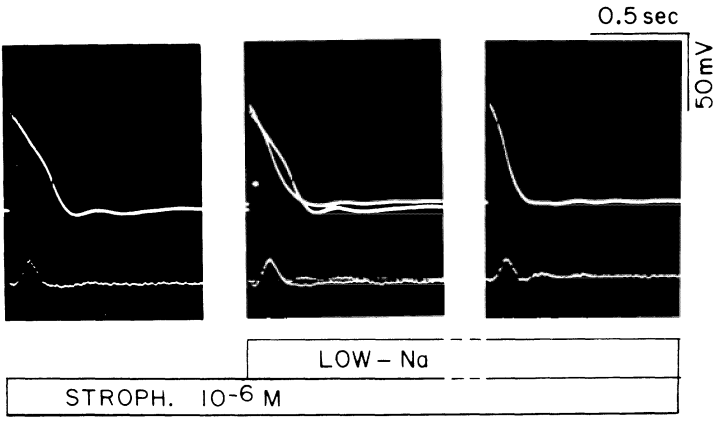

Figure 4. Effect of low sodium on aftercontractions and oscillatory potentials in canine Purkinje fibers exposed to strophanthidin. The first panel shows typical changes induced by a toxic amount of strophanthidin: oscillatory potential in diastole and aftercontractions. The middle panel shows the superimposed traces recorded in Tyrode's solution and in the presence of low (−52%) sodium; and the third panel the traces in the low sodium solution. It is apparent that in the presence of low Na the force decreased and the period and number of aftercontractions increased (which indicates a progression in strophanthidin toxicity) while the oscillatory potential decreased (recorded by Lin.)

A role for sodium in the genesis of the oscillatory potentials in Purkinje fibers is suggested also by the use of local anesthetics [57]. As shown in Figure 5, the first panel shows the control action potential and contractile force. In the second panel, strophanthidin had caused the usual changes [54, 56], namely an increase in force of contraction, a steepening of diastolic depolarization and a decrease in E_{max}. In the third panel, adding procaine to the strophanthidin solution markedly shortened the action potential (as it does in the absence of strophanthidin) and abolished the steepening of the diastolic depolarization due to an oscillatory potential. These changes

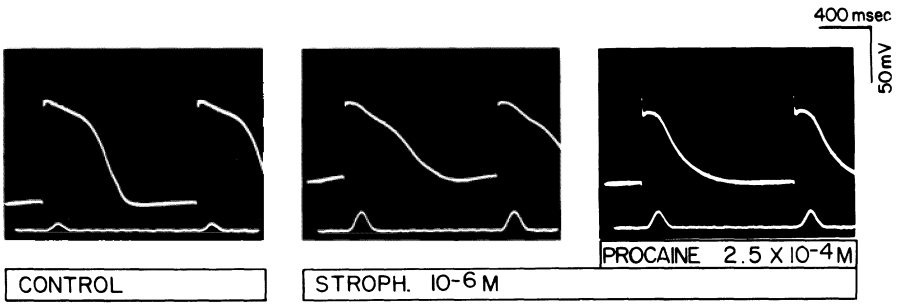

Figure 5. The effect of local anesthetics on the toxic manifestations of digitalis. The first panel shows the control recordings in a canine Purkinje fiber. The second panel shows the changes induced by strophanthidin. The last panel shows the shortening of the action potential and the flattening of the diastolic depolarization induced by procaine in the continued presence of strophanthidin (Bhattacharyya and Vassalle, unpublished experiments.)

were brought about in spite of a continuing perfusion of strophanthidin. The concept that sodium is important in the genesis of the oscillatory potentials is supported by the finding that the underlying current is carried mostly by sodium ions [52]. However, this would not exclude the possibility that a slow channel is involved in the oscillatory potentials since such channel admits also sodium ions but experimental evidence is against such interpretation [52].

Voltage clamp experiments in sheep Purkinje fibers [58] have shown that the oscillatory current may be present even in the absence of cardiac glycosides and that it is enhanced by conditions which enhance calcium influx. However, the slow inward current is only indirectly related to the oscillatory current as the oscillatory current can be elicited without a preceding slow inward current and vice versa. The slow inward current is important in the genesis of the oscillatory current in that it determines how much calcium is stored intracellularly. If the slow inward current decreases (e.g., by decreasing $[Ca^{++}]_0$), the intracellular calcium also decreases, as reflected by the fall in force of contraction. It is the change in intracellular calcium which is important for the oscillatory current. However, the force of contraction is not always a reliable index of intracellular calcium. In Purkinje fibers, when the calcium load becomes excessive under the influence of strophanthidin, oscillatory potentials and aftercontractions are likely to appear but the force of contraction decreases [56]. Under this condition of calcium overload, decreasing $[Ca^{++}]_0$ actually increases the force transiently ('rebound phenomenon', [56]). The force of contraction increases because the internal calcium falls from an excessive to an optimal value. Because the internal calcium decreases, the oscillatory potentials disappear at the same time as the force increases. This is illustrated in Figure 6. The first panel shows the electrical and mechanical events in a Purkinje fiber in Tyrode's solution. The second panel shows the same events in high (16.2 mM) calcium: an oscillatory potential (easily demonstrated by interrupting the stimulation) is present and the force is higher than control. However, the force was less than at 8.1 mM calcium as the fiber was calcium-overloaded. In the last panel, it is shown that when the calcium overload is relieved by perfusion in a low calcium solution, the force actually increased but the oscillatory potential was no longer present. Eventually, the force fell to a value appropriate to the low calcium but the experiment shows that the oscillatory potential and force of contraction can be made to vary in opposite directions.

The finding that an oscillatory current and oscillatory potentials can be present in the absence of cardiac glycosides is of interest for other types of arrhythmias, such as overdrive excitation.

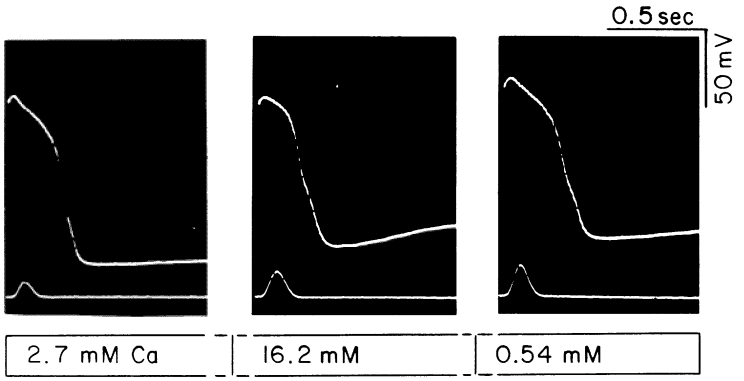

Figure 6. The rebound phenomenon in canine Purkinje fiber. The first panel shows the action potential and the contractile force curve in Tyrode's solution. The second panel shows the same traces recorded in high 16.2 mM $[Ca^{++}]_0$. The usual changes induced by high Ca are apparent. The last panel shows the prolongation of the action potential and the flattening of diastolic depolarization when $[Ca^{++}]_0$ was decreased to 0.54 mM: at the same time the force increased ('rebound phenomenon'.) The force eventually decreased to a value appropriate to the low $[Ca^{++}]_0$ (recorded by Lin.)

12.2. Overdrive excitation

It is well known that overdrive of a pacemaker is followed by a period of temporary quiescence (overdrive suppression, see [33]). However, this is not universally the case. In sheep Purkinje fibers perfused in a solution containing 5.4 mM potassium and norepinephrine, it was found that fast drive of a quiescent fiber induced a spontaneous rhythm ('overdrive excitation,' [59]). A short drive applied during the induced rhythm was followed by an acceleration (not suppression). The mechanism of the overdrive excitation is a steepening of the diastolic depolarization which at the end of the drive may attain the threshold and thus initiate repetitive activity. This phenomenon can be obtained also in canine Purkinje fibers [60] although not as easily as in the sheep, as high calcium or phosphodiesterase inhibitors may be needed. The steepening of diastolic depolarization after drive is due to an oscillatory potential which is responsible for the initiation of the spontaneous activity. When the induced rhythm ceases, this is due to the failure of an oscillatory potential to reach threshold. Overdrive excitation can be shown also in vivo [61] and is favored by adrenergic enhancement [62]. Fast rhythms may occur which are initiated by normal pacemaker activity and one example of this is the fast idioventricular rhythm [63]. The mechanism by which overdrive causes spontaneous rhythms may be an enhanced entry of calcium through the slow channel in the unit of time due to the more numerous action potentials. This concept is supported by the fact that other agents

which enhance the slow inward current (catecholamines, high calcium) are also effective in favoring overdrive excitation [63].

Thus, the slow inward current (by enhancing cellular calcium stores) may be responsible for arrhythmias induced by oscillatory potentials in the absence of cardiac glycosides.

12.3. The antiarrhythmic effects of slow channel blockers

It is interesting that verapamil was shown to have antiarrhythmic properties [64, 65] when the slow inward current or the oscillatory potentials were still to be discovered. The experimental work discussed above has clarified many aspects of the slow inward current and its modification by slow channel blockers. Because of the role of the slow inward current in diastolic depolarization and action potential of the sinus node, it is not surprising that the sinus rate should be slowed or sinus tachycardia reduced by these blockers [66]. A flattening of diastolic depolarization and a shift in the threshold would do just that. The other actions of the slow channel blockers are also important in other types of arrhythmias. The prolongation of the refractory period and the decreased conduction velocity in the AV node [38] would explain the decrease in ventricular rate in the presence of atrial flutter or fibrillation [64, 65, 66] or the elimination of AV nodal reentrant tachycardia [41]. The other effect which may be quite important in the suppression of arrhythmias is the decrease in the magnitude of the slow inward current. This can be a direct or indirect effect. The direct effect may result in the elimination of the slow responses in partially depolarized tissues. The indirect effect may be the decrease in $[Ca^{++}]_i$ (as reflected in the weakening of the force of contraction) and therefore of the oscillatory potentials in overdrive excitation.

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7. SLOW CONDUCTION IN THE HEART *

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Although the cardiac impulse can travel as rapidly as 4 m/sec in parts of the ventricular conducting system, conduction as slow as 0.02–0.05 m/sec underlies important features of normal cardiac excitation, such as AV nodal delay [1, 2]. Slow conduction can also be important in reentry secondary to circus movement of excitation, which can occur if the conduction time around the circuitous pathway exceeds the refractory period; for example, nodal tachycardia is probably caused by circus movement of excitation and the slow conduction necessary to sustain that circus movement presumably occurs in the AV node [2–4]. Reentry can also occur if, in an otherwise normal heart, some fibers that ordinarily conduct rapidly undergo a change that causes them to conduct slowly [5]. Such a change occurs when Purkinje fibers are depolarized by high K^+ and exposed to epinephrine; the conduction velocity falls from 2 m/sec to 0.05 m/sec [5–8]. Many studies suggest that certain cardiac cells may be able to display two fundamentally different kinds of action potential, the 'normal' rapidly conducting action potential that arises from a high resting potential and the 'slow response,' a slowly conducting action potential that arises in depolarized fibers [2, 5, 9–13]. Other studies have demonstrated the existence of the so-called 'slow channel,' or, to use Fozzard's more correct term, the 'kinetically slow channel' [14] through which the inward current responsible for the upstroke of the slow response flows [2, 15–20].

We propose to examine various ways in which slow conduction might arise; these include an increase in intracellular longitudinal resistance, replacement of the normal rapid upstroke by the slow response, the upstroke of which depends on current flowing through the slow channel and the replacement of the normal upstroke by an upstroke in which the excitatory inward current is carried via largely inactivated fast channels. The level of membrane potential is a critical determinant of the excitability of both fast and slow channels and the conductance of the membrane to potassium ions near the resting level of membrane potential is important in determining whether a

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given small and slow increase in inward current can initiate regenerative depolarization and conduction. We therefore begin with a brief resume of some determinants of the resting level of membrane potential.

Cells of the SA and AV nodes exhibit low resting potentials that are relatively little affected by changes in K_0 [1, 2]. At a normal physiological K_0 of about 5 mM, working myocardial cells of the atrium and ventricle and cells of the ventricular conducting system have a 'high' (i.e., large, negative) resting potential of -80 to -90 mV which varies with moderate changes in K_0 approximately as predicted by the Nernst equation, i.e., those cells behave more or less like potassium electrodes. It has, however, long been known that lowering the concentration of K^+ in the fluid bathing Purkinje fibers below about 1 mM causes them to exhibit a depolarization which is paradoxical in the sense that lowering K_0 might be expected to cause hyperpolarization. That paradoxical depolarization has been attributed to a fall in potassium permeability at low levels of K_0 [1, 21–23]. Such a fall in potassium permeability is characteristic of inwardly-rectifying potassium channels, that is channels whose permeability varies inversely with the difference between the membrane potential and the equilibrium potential for K^+ [24–29]. The presence of inward-going rectification contributes to the appearance, under certain conditions, of two distinct levels of resting potential in cardiac Purkinje fibers [30, 31]. Over a range of K_0 of 1–5 mM (which includes the presumed normal plasma level) Purkinje fibers may have either a 'high' resting potential associated with a high permeability to K^+ or a 'low' resting potential associated with a low permeability to K^+. If the membrane potential is lowered to, say, -50 mV by raising K_0, then the permeability of the membrane to K^+ near the resting potential is expected to be relatively large, and the conductance therefore high. Under those conditions the small, slow increase in inward current caused by opening the slow channels must be able to 'overcome' a large outward K^+ current in order for propagated action potentials to arise. On the other hand, when the membrane is depolarized to near the 'low' level of resting potential (e.g. -50 mV) in the presence of a 'normal' K_0 of 4–5 mM, the permeability of the membrane to K^+ is relatively small and the membrane K^+ conductance is therefore low. Activation of the slow inward current can more readily give rise to propagated action potentials with slow upstrokes when the opposing outward K^+ currents are small. The excitability of a fiber that has a membrane potential of -50 mV because it is exposed to high K_0 thus differs in an important way from that of a fiber that shows the 'low' (-50 mV) level of resting potential when exposed to 4 mM K_0 [31, 32].

The fast upstroke of the action potential of atrial and ventricular myocardial cells and cells of the ventricular conducting system appears to result from a regenerative increase in the permeability to Na^+, caused by opening of the

so-called 'fast channels' [33]. The permeability to Na is low when the cell is at its normal resting potential and increases greatly when the cell is depolarized beyond a threshold potential of about −68 mV, causing the membrane potential to change rapidly from about −90 mV to about +30 mV. That depolarization leads to a subsequent *decrease* in permeability to Na ('inactivation').

Repolarization somehow 'removes' inactivation, so that, after a significant period of repolarization, the fast Na^+ channels are once again able to show an increase in permeability if the membrane is again depolarized. Each fast channel thus has three states: (1) at a high (i.e., negative) membrane potential it is 'resting,' i.e., closed but excitable; at less negative membrane potentials it is initially (2) 'open,' but becoming 'closed' by inactivation with a time course determined by the membrane potential; and (3) 'inactivated,' i.e., closed and inexcitable. Inactivated channels return to the resting state if the membrane potential is made more negative. The transitions from each state to the other depend on membrane potential and require time to occur. It is helpful to think in terms of the very large number of such channels in an excitable membrane as constituting a population which is distributed between the three states according to the membrane potential. After a long time at a high resting potential nearly all of the channels are in the resting state. At the other extreme, at a steady membrane potential of say +30 mV, nearly all of the channels will be inactivated. At −50 mV, about 99% of the fast channels are inactivated [34] and essentially all the remainder are open. At other membrane potentials there may be a substantial number of channels in each of the three states, resting, open, and inactivated. The probability that a particular channel will be in a particular state depends on the membrane potential and, on the average, on the length of time the channel has been in its present state, so that the kinetics of opening, inactivating and undergoing removal of inactivation may be thought of as the net result of the probabilities (determined by the membrane potential) that a given channel in a given state will undergo a transition to the next state. (The phrase 'to the next state' is used to indicate that a channel usually makes transitions from resting to open to inactivated to resting and rarely makes those transitions in the opposite direction.)

If the resting potential of cells of the atrial or ventricular conducting system is 'lowered,' to say −60 mV, the action potential becomes 'smaller' and 'slower,' i.e., it loses amplitude, shows a slower upstroke and propagates more slowly. These changes can be evoked simply by raising K_0 and are readily explained by a reduction in the number of sodium channels in the resting state (i.e., available to open if the membrane is further depolarized by an applied stimulus or by a propagating action potential). There is a corresponding increase in the number of inactivated channels and a slowing of the removal of inactivation. It is the relative 'unavailability' of excitable fast

channels that causes the slowing of the upstroke and loss of amplitude of the action potential and the consequent lowering of its conduction velocity.

For many years interpretation of action potentials in cardiac cells rested on efforts to relate the upstrokes to the time- and voltage-dependent characteristics of fast sodium channels. It might be noted that the fast channel is so-called not only because it opens relatively rapidly but also because it is rapidly closed by inactivation; if both opening and inactivation occur on a millisecond time scale, the fast channel can carry inward current for only a short period. In recent years it has been recognized that there is, in many, and perhaps all, cardiac cells, a second excitable channel capable of carrying inward current, the so-called slow channel. Although the specific ionic permeabilities of that channel and the details of its time- and voltage-dependence have not been fully established, it clearly has pharmacological sensitivities quite different to those of the fast channel, being sensitive to D–600, verapamil and Mn while being insensitive to tetrodotoxin [2, 35, 36]. It is also clear that the slow channel is closed at membrane potentials in the range of -90 to -60 mV and that it carries inward current chiefly in the voltage range -40 to $+20$ mV [2, 35, 36]. The slow channel has two characteristics important for the purpose of this chapter: it carries far less current than the fast channel and the kinetics of its opening and closing are much slower than those of the fast channel. Important factors in determining conduction velocity are the magnitude and duration of the inward excitatory current. We will see that slow conduction with low upstroke velocities demands that the inward current be small but long-lasting. The kinetics of the opening, and especially the closing, of the fast and slow channels thus become important in any consideration of conduction velocity; particularly since one possible explanation for slow conduction of action potentials evoked at low resting potentials is that an action potential dependent on fast channel activity has been replaced by one dependent on slow channel activity.

The velocity of the propagating action potential depends both on the magnitude and duration of the inward current generated by excited channels and on how the cable-like properties of the tissue determine the distribution of that current in space and time. The physical laws that relate these several factors to one another in a quantitative way have been worked out in considerable detail for axons and for skeletal muscle fibers. We now review some of the principles derived from those studies and will then examine their applicability to propagation in various parts of the heart [14, 37–44].

Let us first consider the simplest case, exemplified by the unmyelinated axon, which is described by a cable equation of the form:

$$\frac{1}{r_i + r_e} \cdot \frac{\partial^2 V}{\partial x^2} = c_m \cdot \frac{\partial V}{\partial t} + i_i \qquad (1)$$

where V is the difference between the membrane potential and the resting potential; r_e is the longitudinal resistance (ohm/cm) of the external pathway; r_i is the longitudinal resistance (ohm/cm) of the cytoplasm; c_m is the membrane capacitance per unit length; and i_i is the ionic current that flows across the membrane.

The cable properties are specified in equation 1 by r_i, r_e and c_m, which are constants during excitation and propagation of the impulse. Their values are usually derived from electrical measurements made on quiescent fibers by use of current pulses that are kept so small that they do not excite a nonlinear response of the ionic channels, i.e., under conditions where $i_i = V/r_m$ (where r_m is the membrane resistance of a unit length of the resting cable, in ohm·cm). The measured quantities include the resting time constant, τ_m, defined by:

$$\tau_m = r_m c_m \tag{2}$$

and the characteristic length, λ, defined by:

$$\lambda^2 = r_m/(r_i + r_e). \tag{3}$$

In order to derive r_i and c_m, the input resistance and fiber geometry must also be measured, but even in the absence of this extra information, λ and τ_m can be used by noting that:

$$(r_i + r_e) c_m = \tau_m/\lambda^2. \tag{4}$$

To use equation 1 to predict the characteristics of a propagating impulse requires that we know in full detail how i_i varies as a function of membrane potential and of time, i.e., that we possess a complete mathematical description of the ionic current of the sort that was obtained for the giant axon of the squid by Hodgkin and Huxley [37]. However, if the fiber diameter is constant and the density of ionic channels is uniform along the fiber, we would expect equation 1 to predict that an impulse that propagates along the axon would do so at a constant conduction velocity, θ. Assuming that such a uniformly propagating wave does exist, equation 1 can be simplified [37, 38] to a second order ordinary differential equation:

$$\frac{d^2V}{dt^2} = K\left(\frac{dV}{dt} + \frac{i_i}{c_m}\right) \tag{5}$$

where

$$K = (r_i + r_e) c_m \theta^2. \tag{6}$$

Although the simplifying assumption that there exists a solution of equation 1 for a constant θ enables us to use the far simpler equation 5, that equation cannot be solved in the absence of information about i_i. However,

we do not need a *complete* mathematical model (and, in particular, we do not need a complete knowledge of the dependence of i_i on time and membrane potential) to be able to estimate the value of K under a particular set of conditions. This is because certain other features of the propagating action potential are determined by the same factors that determine K and we can therefore use those features to estimate K. With this in mind we may rewrite equation 6 as follows:

$$\theta^2 = K/(r_i + r_e)c_m \tag{7}$$

and a simple substitution from equation 4 permits us to write equation 7 as:

$$\theta^2 = K(\lambda^2/\tau_m) \tag{8}$$

Our overall strategy is to show how one can obtain estimates of K from the waveform of the action potential and then use those estimates in conjunction with estimates of λ and τ_m obtained from the resting fiber to show how the conduction velocity can be predicted from the waveform. In other words, we propose to show how the speed with which the action potential moves in *space,* i.e., along the fiber, is related by the cable properties to the speed with which the membrane potential changes as a function of *time* at a single site.

It is, in fact, found that there is a value of K for which equation 5 gives a physically realistic wave if, and only if, the ionic channels generate a phase of net inward current whose magnitude and duration exceed certain minimum values, in other words if the safety factor for propagation exceeds unity. That value of K depends on the magnitude of i_i. When the duration of the inward current is brief, only relatively large values of i_i and K are possible, but in order to obtain a low value of K, i_i must be small and the duration of the inward current must be correspondingly longer [38].

The action potential is generated by a net inward ionic current at the excited region and, as equation 1 shows, the circuit is completed by current flowing *out* of the fiber via two paths, through one of which it charges the local capacitance and through the other of which it depolarizes the quiescent region ahead of the impulse. The greater the inward current the faster is the upstroke of the action potential and the quicker is the depolarization of the adjacent quiescent region, hence the greater is the conduction velocity.

We can make this idea more quantitative by examining the shape of the rising phase of the action potential. The toe of the action potential results from the spread of current from the excited region into the still quiescent region, where $i_i = V/r_m$. Substituting this linear current–voltage relationship into equation 5 permits an analytical solution which shows that the toe grows

exponentially with a rate constant, μ, which is related to K by the quadratic equation:

$$\mu^2 - K\mu - K/\tau_m = 0. \tag{9}$$

This equation can be solved for K as follows:

$$K = \mu/(1+1/\mu\tau_m). \tag{10}$$

We see from equation 10 that K differs from μ by a correction factor that depends on $\mu\tau_m$. Where $\mu\tau_m$ is large, as is the case for the squid axon [37] and for rapid propagation in the heart, K is about equal to μ. But even when μ is very small, as is often the case for slow conduction in the heart, $\mu\tau_m$ has a value near unity and K is about $0.5\,\mu$.

Uniform propagation in a simple cable requires that the longitudinal current, which is the stimulus that excites the adjacent quiescent fiber, be maximal at the same point in time and space that the upstroke velocity is maximal. Thus we can expect a very direct dependence of μ on maximum upstroke velocity, $\dot{V}_{max}$. Although the complicated nonlinear voltage dependence of the channel kinetics precludes an analytical expression for the relationship between μ and $\dot{V}_{max}$ [43] we have found a very good approximation by examining the rising phase of propagating action potentials. By examining the solution of equation 5 over a wide range of safety factors and ionic channel kinetics, for both fast and slow conduction, we have found (Dodge, unpublished) that the empirical relation:

$$\mu = \dot{V}_{max}/0.4\,V_p \tag{11}$$

where V_p is the peak amplitude of the action potential, is generally accurate, to within an error of less than 20%.

From these several relationships we see how we can, in principle, interpret the mechanism for alterations of the conduction velocity by distinguishing between, on the one hand, changes in the cable properties and, on the other hand, changes in the safety factor and kinetics, which are intrinsic properties of the ionic channels. This can be done simply by careful examination of the waveform of the propagating action potential. Our strategy is to estimate K either from the maximum upstroke velocity and peak amplitude of the action potential, using equations 10 and 11, or from the measured rate constant of the exponential toe of the action potential, using equation 10, and then applying equation 7, which may be used in either of two equivalent forms:

$$\theta^2 = K/(r_i + r_e)c_m \tag{7}$$

or

$$\theta^2 = K(\lambda^2/\tau_m). \tag{8}$$

depending upon what information we have about the cable properties.

Table 1 shows values of θ calculated from equation 8 for three values of λ, three values of τ_m and three values of $\dot{V}_{max}/V_p$. The first column uses $\lambda = 0.5$ mm and $\tau_m = 5$ msec, values not very different from those found in the SA and AV nodes. The second column uses $\lambda = 1$ mm and $\tau_m = 10$ msec, values not very different from those found in ventricular fibers; column 3 uses $\lambda = 2$ mm and $\tau_m = 20$ msec, values very close to those found in Purkinje fibers. The values for $\dot{V}_{max}/V_p$ roughly correspond to those found in the SA and AV nodes, in ventricular fibers, and in Purkinje fibers. The numbers along the diagonal, in bold-face, may thus be taken as the conduction velocities predicted for the nodes, ventricular fibers and Purkinje fibers by using equation 8 in connection with measured values of the cable constants, measured values of the maximum upstroke velocity, measured values of the amplitude of the action potential, and values of K calculated using equations 10 and 11.

Table 1. Calculated values of conduction velocity.

	$\lambda = 0.5$	$\lambda = 1$	$\lambda = 2$
$\dot{V}_{max}/V_p$	$\tau_m = 5$	$\tau_m = 10$	$\tau_m = 20$
0.05	**0.049**	0.083	0.13
1	0.34	**0.49**	0.7
4	0.7	1	**1.4**

The first column shows the value of $\dot{V}_{max}/V_p$ used to calculate θ for each of the three sets of values of λ and τ_m in the corresponding row. The calculations were made by estimating μ from $\dot{V}_{max}/V_p$, using equation 11; calculating K from μ and τ_m, using equation 10; and calculating Θ from K, λ and τ_m, using equation 8. The values of $\dot{V}_{max}/V_p$ are based on rough approximations of those found in the SA and AV nodes (4 V/sec, 80 mV; ratio = 0.05), the ventricle (100 V/sec, 100 mV; ratio = 1.0) and Purkinje fibers (500 V/sec, 125 mV; ratio = 4).

The values given in Table 1 show, in a general way, that conduction is slow when $\dot{V}_{max}$ is low; they also show that a high $\dot{V}_{max}$ will lead to rather rapid conduction even in fibers that have the cable characteristics associated with fibers of the SA and AV nodes. Before we apply equation 8 we must examine certain questions and problems that arise concerning the applicability of the assumption of uniform conduction in a simple cable to conduction in real cardiac tissues.

For example, since there are discontinuities in the cytoplasm of cardiac fibers [45–47], we must consider whether those discontinuities may lead to nonuniform conduction, or, at any rate, to nonuniformity so great that equation 5 (and hence equation 8) no longer provide a good approximation to the relation between the waveform and the conduction velocity. The application of our strategy to cardiac fibers thus requires that we carefully consider the implications of the fact that such fibers are not simple cables but instead consist of many small 'cells' connected, mainly at each end, by junctions

which normally have a negligibly low resistance [45]. In interpreting measurements of the cable properties of cardiac fibers it is generally assumed that a decrease in the finite conductance of the junctions is equivalent to an increase of r_i above the value determined by the specific resistance of the cytoplasm. This assumption is certainly reasonable when the cells are much shorter than the characteristic length. But, under certain conditions, the resistance of the intercellular connections can increase, even to the point at which the cells become completely uncoupled electrically [47]. In order to determine the range over which the simple cable approximation is valid, we simulated a 'cable' made up of cells connected end to end. We did this by interrupting the axoplasm of the theoretical squid axon by leaky septa at intervals of $0.1\,\lambda$. Solving the resulting nonlinear partial differential equations by the methods of Dodge and Cooley [48] (for room temperature, at which the normal safety factor is about 4) we found that conduction failed only when the junctional resistance increased so much that the effective r_i was 400 times the normal value. Furthermore, over much of the range we found that the equivalent uniform cable assumption accurately predicted the conduction velocity. A 100-fold increase in r_i reduces the characteristic length 10-fold, i.e., to the length of a single 'cell.' Use of the uniform cable equation predicts a 10-fold fall in conduction velocity for a 100-fold increase in r_i; if that 100-fold increase in r_i is obtained solely by an increase in the coupling resistance, the conduction velocity calculated for our discrete coupled cell model is only 20% less than that predicted by the uniform cable equation.

The multicellular structure of cardiac fibers also complicates the interpretation of c_m. Bundles of cardiac fibers can have a much larger surface of cell membrane per unit length of bundle than is calculated from the bundle diameter, and the external electrical path for current flow through the membranes of cells deep inside the bundle can be via very narrow and tortuous clefts between more superficial cells. Fozzard [49] showed that a large fraction of the total c_m of a bundle of Purkinje fibers appeared to be decoupled from the toe of the propagating action potential. His measurement of the capacity current in a voltage-clamp of the Purkinje fiber and of the transient response to small current pulses was shown to be consistent with an equivalent circuit in which about 20% of the total capacitance (of about $12.5\ \mu F/cm^2$) could be attributed to surface cells while the remaining $10\ \mu F/cm^2$ was decoupled by a series resistance of about $300\ \Omega cm^2$. Virtually all of this series resistance could reasonably be ascribed to narrow extracellular clefts, at least in the sheep Purkinje fiber; although some part of it might arise in the resistance of intercellular connections. Carmeliet and Willems [50] showed that, if the upstroke velocity is low enough, all the membrane capacitance is charged during the rising phase of the action potential. Thus to make a more accurate mathematical model of the propagation of the cardiac action potential we

should modify equation 5 by putting in the additional terms required by the equivalent circuit given by Fozzard [49]. However, solutions of the modified equation for the cases of present interest justify the use of simpler approximations: for the values of K typical of fast propagation we can neglect the capacitance of the interior cells and use a value of c_m corresponding to the capacitance of the surface cells; for the cases of slow propagation caused by a slow upstroke we can neglect the series resistance but we must use the total capacitance.

We must also consider a fundamental theoretical limit on the applicability of a simple cable model to a multicellular Purkinje fiber bundle, and, perhaps, to other bundles of closely packed cells. Schoenberg, Dominguez and Fozzard [51] have studied how the cable constants and the conduction velocity depend on fiber (bundle) size, in sheep Purkinje fibers. In spite of the rather large variability from fiber to fiber, their data on the cable constants were clearly consistent with the prediction of those constants based on the number of cells of a similar size in a cross section of the bundle. But their data seemed to show, rather unexpectedly, that conduction velocity is independent of bundle diameter. This point is most convincingly established if one looks at the data points for which μ had a value within 20% of the mean value, which selects the population with about the same safety factor. We suggest that the observed independence of conduction velocity and bundle size can be explained by assuming that the velocity is determined by propagation of the impulse along the surface cells, excitation of the interior cells lagging because of the relatively higher resistance of their external current pathway through the clefts. In effect, we would expect appreciable voltage gradients in the radial direction during the upstroke and hence that a simple cable model becomes progressively less accurate the larger the bundle. We have tested this hypothesis by a simulation involving several interconnected fibers, one corresponding to the surface cells, the others showing the lumped equivalent of the current pulses through the clefts. We found that as we assigned a larger and larger fraction of the total series resistance to the interfiber connections, the more nearly the velocity became independent of the number of fibers: when about half the series resistance is in the interconnections, velocity becomes independent of size (Dodge, unpublished). On the basis of these results we conclude that a simple cable model is sufficiently accurate for a bundle of diameter less than about 4 times the diameter of its cells.

The cells of the heart are, in general, arranged in a complex 3-dimensional network. Even in reasonably linear trabeculae the internal resistance is greater in a transverse than in a longitudinal direction [41]. In addition, values for λ and τ_m are not always available, and it is rare to find a study of any cardiac tissue in which the cable properties, the rate constant of the foot, $\dot{V}_{max}$, V_p and conduction velocity were all measured in the same preparation, or even under

comparable conditions in the same set of experiments or studies. Finally, in the two situations in which slow conduction is normally found in the heart, the SA and AV nodes, the condition of uniform conduction in a linear cable seems least likely to be met. For example, it seems very probable that the relative density of fast and slow channels changes virtually continuously as one moves from the center to the edge of the SA node; the cells of the SA node are short and spindle-shaped and the spontaneous depolarization, which occurs at varying rates in front of any propagating impulse, leads to variable degrees of inactivation and variable changes in g_K. The slowest conduction in the AV node occurs in the mid-nodal or N region where there is, at any rate, no spontaneous depolarization, but it is doubtful that membrane properties and conduction are uniform over a distance of even 1 mm since the impulse almost certainly slows continuously as it approaches the center of the mid-nodal region and speeds up continuously as it propagates from the mid-nodal region into the bundle of His. On the other hand, there are conditions in which fast conduction can be replaced by slow conduction, e.g., in ventricular fibers, working fibers of the atrium, and Purkinje fibers, to which equation 8 may be applied with somewhat greater confidence. In any event, it seems worthwhile to examine some specific examples.

Sinoatrial node: Bonke [52] reports a λ of 0.465 mm for rabbit SA node but gives no value for τ_m; Seyama [53] found a λ of 0.83 mm. Noma and Irisawa [54] report a τ_m of 12 msec in short strands of rabbit SA node, a $\dot{V}_{max}$ of 5.5 V/sec, a V_p of 77 mV (from maximum diastolic potential to peak) and a conduction velocity of 0.037 m/sec. In strands too short to permit true conduction, $\dot{V}_{max}$ was 3.6 V/sec and V_p was 69 mV [54]. Although the SA node is singularly unsuited to treatment under our assumptions of uniform propagation in a simple cable, we have made the following calculations: for $\lambda = 0.465$ mm, $\tau_m = 12$ msec, $V_p = 77$ mV and $\dot{V}_{max} = 5.5$ V/sec, $\theta = 0.047$ m/sec. The same values taken with $\lambda = 0.8$ mm give $\theta = 0.08$ m/sec. Using $\dot{V}_{max}/V_p = 3.6/69$ and λ as either 0.5 mm or 0.8 mm yields $\theta = 0.04$ m/sec or $\theta = 0.07$ m/sec.

Atrioventricular node: Values of $\lambda = 0.43$ mm and $\tau_m = 3.4$ msec were obtained in AN cells of the AV node by de Mello [55]; a τ_m of 9 msec was found in N cells. A wide range of values has been reported for $\dot{V}_{max}$ in N cells, but 5 V/sec seems reasonable, as does a V_p of 70 mV. Using $\lambda = 0.43$ mm, $\tau_m = 9$ msec, $\dot{V}_{max} = 5$ V/sec and $V_p = 70$ mV, we obtain $\theta = 0.048$ m/sec, which compares favorably with reported apparent conduction velocities of 0.02–0.05 m/sec [1, 2].

Calculations of the kind just made for the SA and AV nodes are admittedly subject to many errors but it may be instructive to calculate the conduction velocity in a fiber with the cable characteristics of the AV node and the action potential characteristics of a Purkinje fiber, i.e., $\lambda = 0.43$ mm, $\tau_m = 9$ msec,

$\dot{V}_{max}/V_p = 4$. This yields $\theta = 0.45$ m/sec, a result which certainly suggests that reduction of $\dot{V}_{max}$ plays a greater role in causing slow conduction in the AV node than do changes in the cable characteristics.

Atrium: Bonke [56] has reported $\lambda = 1$ mm and $\tau_m = 3$ msec in rabbit crista terminalis and $\lambda = 0.66$ mm, $\tau_m = 2.66$ msec in rabbit atrial trabeculae carnae. His measurements of the time constant of the foot of the action potential (the reciprocal of our μ) yield values of 4.4/msec and 4.6/msec for μ in the crista and trabecula, respectively. Values of θ calculated from these cable characteristics and the measured μ are 1.17 m/sec and 0.83 m/sec for crista and trabecula, which exceed Bonke's measured values of 0.665 m/sec and 0.553 m/sec, respectively. Paes de Carvalho et al. [11] reported a μ of 7.5/msec in atrial fibers; that value taken together with the cable characteristics found by Bonke leads to $\theta = 1.5$ m/sec.

Ventricle: Weidmann [40] found, in ventricular trabeculae of the sheep or calf heart, $\lambda = 0.88$ mm, $\tau_m = 4.4$ msec and the time constant of the foot = 0.38 msec which corresponds to $\mu = 2.63$/msec. These values lead to a calculated θ of 0.65 m/sec which may be compared with the measured value in the same preparation of 0.75 m/sec. In the same preparation Clerc [41] studied the difference in r_i and r_0 along the length of the bundle and transversely to it and found that those differences accurately explained the differences in conduction velocity in the two directions. Conduction velocity in the transverse direction was almost exactly one-third of that in the longitudinal direction and the difference could be accurately predicted from the differences in the effective length constants.

Weingart, in a study of the effects of ouabain on ventricular trabeculae of sheep and calf hearts [42], found that 90 min exposure to 2×10^{-6} M ouabain increased the specific internal longitudinal resistance, r_i, by about 2.5-fold. A concomitant decrease in θ from 0.5 to 0.29 m/sec (at 25 °C) could be explained in part by a decrease in $\dot{V}_{max}$ and in part by the increase in r_i which was attributed to an increase in nexal resistance, i.e., to uncoupling.

Wojtczak [57] found that a 1 h exposure of sheep ventricular trabeculae to an oxygen-free perfusate caused r_i to increase about 3-fold. That increase was attributed chiefly to an increase in r_i caused by 'an increase in nexal resistance.' If the hypoxic preparation was exposed to epinephrine, r_i increased further, for an overall increase of 21-fold. The marked further increase in r_i seen upon exposure to epinephrine was associated with marked slowing of conduction which was soon followed by block that could not be reversed by re-exposure to oxygen-containing solutions. In two experiments the action potential split into a spike and slow wave before the development of irreversible block.

At the stage when r_i was markedly increased the preparations showed reduced $\dot{V}_{max}$, developed contractures, showed abnormal action potentials,

presumably showed loss of resting potential and were on the verge of irreversible block. Yet the increase in r_i was only 21-fold, an increase that might, in the absence of other changes, be expected to lower conduction velocity to about 22% of its normal value, i.e., to about 0.17 m/sec (at 37 °C). Conduction that slow might indeed predispose to reentry, but the preparations in which Wojtczak found that great an increase in r_i were profoundly abnormal in so many ways and so nearly on the verge of irreversible deterioration that we agree with his conclusion that it is premature to draw clear-cut conclusions concerning the importance of the increase in r_i for the development of cardiac arrhythmias during hypoxia and ischemia, the more so since the reversible 3-fold increase in r_i seen under less extreme conditions would be expected to reduce conduction velocity only to about 60% of its normal value.

Purkinje fibers: Table 1 shows a value for θ based on Purkinje fiber cable characteristics of $\lambda = 2$ mm, $\tau_m = 20$ msec and $\dot{V}_{max}/V_p = 4$. The calculated θ is only 1.4 m/sec, which is low compared to measured values, which range up to 4 m/sec. This discrepancy can be explained, in part, by the fact that during rapid conduction in bundles of Purkinje fibers only some 20% of the capacitance is charged during the foot [49], which means that one can (perhaps somewhat circularly) compute a conduction velocity by using $\tau_m = 4$ msec. If one does that, one obtains $\theta = 3.1$ m/sec.

In Purkinje fibers exposed to Na^+-free solutions containing 10 mM Sr^{++}, Carmeliet and Willems [50] have shown that c_m is fully charged during propagation. Under those circumstances, $\lambda = 2.63$ mm, $\tau_m = 20.1$ msec, $\dot{V}_{max} = 5$ V/sec, $V_p = 100$ mV and the calculated $\theta = 0.175$ m/sec, which may be compared with the measured value of 0.15 m/sec. When the same preparation was exposed to normal Tyrode's solution, λ was 2.23 mm, τ_m was 18.6 msec and the measured value of θ was 3.17 m/sec, which shows clearly that the slow conduction of the Sr^{++} spike is essentially wholly attributable to the change in $\dot{V}_{max}$.

Values are not available for the cable properties of canine Purkinje fibers exposed to solutions containing high K and epinephrine (5–7) or to Na^+-free, Ca^{++}-rich solutions [20, 58] but we have calculated θ by using the cable properties found by Carmeliet and Willems in fibers exposed to Na^+-free, Sr^{++}-rich solutions, i.e., $\lambda = 2.63$ mm, $\tau_m = 20.1$ msec, and the upstroke velocities observed in the other solutions. For high K, epinephrine-containing solutions, a $\dot{V}_{max}$ as low as 2 V/sec has been reported, which, with $V_p = 70$ mV, yields $\theta = 0.12$ m/sec, somewhat higher than the values observed. In fibers exposed to Na^+-free, Ca^{++}-rich solutions, $\dot{V}_{max}$ has been reported as 3.7 V/sec and V_p as 80 mV [20, 58] which leads to a predicted θ of 0.17 m/sec, as against the reported value of 0.1 m/sec.

The many uncertainties involved make the above calculations at best rough

approximations. On the other hand, they show clearly that low values of θ are invariably associated with low values of $\dot{V}_{max}$ (see also Table 1). Before turning to a consideration of how low values of $\dot{V}_{max}$ may come about, we will briefly consider the argument that slow conduction may be attributed chiefly to high values of r_i which are, in turn, to be explained by a paucity of low resistance cell-to-cell connections or by uncoupling of such connections. It should be noted that high values of r_i are necessarily reflected in low values of λ, and that the low values of λ found in fibers of the SA node and AV node must be interpreted, in the first instance, in terms of the small diameter of the fibers. Only values of r_i higher than can be explained by the small diameter of the cells can be attributed to a paucity of cell-to-cell connections. In addition we would place some emphasis on the fact that there are fibers in which either a low value of θ or a high value of θ can be obtained by comparatively simple and rapid changes in the extracellular environment, changes that certainly do not seem likely to change the cell size or the specific resistivity of the cytoplasm (we refer, for example, to changes in K_0). We do not doubt that such changes may lead to uncoupling but point out that to explain a 10-fold reduction in conduction velocity by uncoupling demands a 100-fold increase in r_i and that a 100-fold reduction in conduction velocity demands a 10,000-fold increase in r_i. Conduction block occurs when r_i is increased far less than 10,000-fold; moreover even a 100-fold increase in r_i, if entirely attributable to uncoupling, demands more than a 100-fold rise in the coupling resistance. Reduction of conduction velocity to one-third of its usual value may be attributed to changes in r_i [41, 42] but, in all situations known to us in which there is evidence for marked uncoupling and marked reduction in conduction velocity, the cells also show lowered resting potentials and reduced upstroke velocities, and we are not aware of any way in which uncoupling can in itself directly cause marked reduction in $\dot{V}_{max}$.

We therefore argue that markedly slow conduction is never seen in the absence of marked reduction in $\dot{V}_{max}$, i.e., in the absence of an inward current whose magnitude is small but whose duration is long. We now turn to the question of how such changes in kinetics may arise.

A possible source for a small, long-lasting inward current is a component of the fast channel current that is incompletely inactivated, or perhaps even non-inactivating. When the potassium channels of a squid axon are blocked by injection of tetraethylammonium ions the fast spike is followed by a very long-lasting plateau, rather like that of a cardiac action potential [59]. If the potassium conductance is held constant, the Hodgkin-Huxley equations predict such a plateau [60], explainable by the voltage dependence of the fast channel. There is overlap of the two S-shaped curves which relate the activation and inactivation of the fast channels so that, over a certain range of depolarization, those channels admit a steady-state sodium current that is

about 3% of the peak transient current [60]. This is sufficient to cause the steady-state current–voltage relationship to have three intersections with the zero-current axis. Those intersections represent the stable resting state, the stable plateau state, and an unstable state between the two stable ones. When we examine the theoretical effect on propagation of blocking the potassium channels we discover that we can attenuate the ionic current below the point where fast propagation fails but can then find a much slower, uniformly propagating action potential which has a slow upstroke to the plateau level. Thus squid-like fast channels could generate slow conduction under the special condition of very low g_K, as was shown by Lieberman et al. [61].

On the other hand, the fast sodium channels of vertebrate nerve have much less overlap of the activation and inactivation curves [62]. In those cells the steady-state sodium current is less than 0.1% of the peak value, and blocking the potassium channels prolongs the action potential only slightly either experimentally or theoretically [63–66]. The voltage dependence of the fast channels of the heart has not been worked out in detail by voltage-clamp analysis, although important advances have been made recently [34, 67, 68]. On the assumption that the tetrodotoxin-sensitive component of the steady-state current, which is about 0.3% of the peak current required for fast propagation [31, 69], measures the steady-state fast channel current, the available evidence suggests that the voltage-dependence of the fast channels of the heart more nearly resembles that of vertebrate nerve than that of the squid giant axon. Although this TTX-sensitive current is very small, it can be an appreciable fraction of the ionic current during the plateau [70] and it is certainly a very important component of the inward current needed to produce bistability in the resting potential of Purkinje fibers exposed to moderately low K_0 [31]. Since this steady-state component of fast channel current decreases as the membrane is depolarized beyond the low stable resting potential it cannot generate the inward current required for the slow conduction that can be seen in fibers at the low level of resting potential. That slow conduction requires that there be slow channels whose activation potential is positive to the low level of resting potential.

To be sure, fibers at the low level of resting potential are most readily excited at the break of an anode (Gadsby and Cranefield, unpublished observations), strongly suggesting that partial removal of inactivation permits fast channels to cause a transient depolarization that reaches the threshold of the slow channels. This kind of complicated interaction between fast and slow channel activity is undoubtedly of great significance in sustained rhythmic activity in the vicinity of the low level of resting potential since during such activity each action potential is followed by an after-hyperpolarization which acts as an anode.

Undoubtedly the major source of the inward current for slow conduction is

the current carried by the kinetically slow channels, often described as calcium channels. Although these channels have been investigated by several different laboratories (for reviews see above and [2, 35, 36]) there is as yet no general consensus about their kinetics, voltage dependence, relative ionic specificity, and sensitivity to specific blocking agents. This lack of precise knowledge does not result simply from the technical limitations which the multicellular structure of cardiac tissues imposes on voltage-clamp measurements but mainly from the fact that the resolution of this current requires that it be distinguished from several other currents each of which, though small, may be comparable in magnitude to the kinetically slow inward current. Among those currents are the outward currents of the inward-going rectifier and the delayed rectifier, current generated by the electrogenic sodium pump and changes in each of those currents caused by the accumulation or depletion of ions in the clefts, changes which in turn depend on the past history of activity of the fiber. Moreover, this list may well be incomplete. But the fact that all tissues of the heart can be made to conduct a slow response action potential even when the fast channels are blocked by TTX [2] indicates that there is generally a sufficiently high density of slow channels to permit generation of such an action potential. Because those channels are activated at membrane potentials positive to -50 mV, some tissues might be able to generate a slow response only if the resting potential were lowered or the density of the slow inward current were enhanced, or both. In other words, mere abolition of the fast upstroke will not necessarily unmask a slow response, but the fact that any fiber in which the fast upstroke has been abolished by TTX can, by suitable maneuvers, be induced to generate a conducted slow response indicates that the slow channel is present in such fibers.

Indirect evidence that a second kind of TTX-sensitive channel contributes to the plateau has been reported recently [70] and one of us has suggested that such channels might play a role in slow conduction [32]. If there are such channels, the upstroke velocity observed during slow conduction puts rather severe restrictions on their density and kinetic properties. The magnitude of the current must be small at all times, for any transient phase of higher current density will cause at least some part of the rising phase to be more rapid even if the safety factor for fast propagation is much less than unity. Although these channels might open very quickly they must inactivate very slowly, or perhaps not at all, in order to inject sufficient charge. Finally, on the basis of the discussion above, they must be activated only when the membrane is depolarized beyond -50 mV, in order that they will not contribute to the observed bistability of the resting potential.

The above discussion suggests a broad classification of antiarrhythmic agents as follows: (1) agents that 'directly' block excitable channels capable of

carrying inward current; (2) agents that 'indirectly' block excitable channels capable of carrying inward current (e.g., by retarding removal of inactivation); (3) agents that change outward currents, thereby changing the resting potential or altering regenerative repolarizing currents; (4) agents that change inward currents, thereby altering the resting potential or altering regenerative depolarizing current; (5) agents that may act in other ways, by affecting, for example, depolarizing afterpotentials, the threshold potential, electrogenic Na^+ extrusion, or Na^+/Ca^{++} exchange.

Some agents are known that directly block excitable fast channels but none of them is useful as an antiarrhythmic agent; the best known example of such an agent is tetrodotoxin. On the other hand, several agents appear to block excitable slow channels, among them Mn, D–600, verapamil and papaverine. It might be noted that all of the latter agents are capable of slowing the rate of activity of the SA node and of slowing AV conduction.

Local anesthetics appear to bind preferentially to inactivated fast channels and to slow the removal of inactivation, so that, in the presence of these drugs, whenever fast channels move from the resting to the open to the inactivated state they will tend to remain inactivated [71–75]. As a result, fewer channels will return to the resting state. Certain drugs shift the relationship between the recovery of excitability and completion of repolarization of a single action potential which causes the upstroke velocity of a premature action potential to be diminished and causes prolongation of the refractory period. This effect of quinidine and cocaine was noted by Weidmann [71] as was the opposite effect of increasing Ca^{++}. Repetitive activity in the presence of local anesthetics can cause so many fast channels to 'accumulate' in the inactivated state that the upstroke velocity is slowed even when the upstroke arises from a normal resting potential. Finally, a local anesthetic effect is expected to exert a particularly potent action on upstrokes that depend on fast channel current in fibers in which inactivation is already an important factor, i.e., in partially depolarized fibers.

It is not known with certainty what factors determine the recovery of excitability of excited slow channels nor whether they assume a state comparable to inactivation. It may be that such channels, after opening, undergo time-dependent, voltage-dependent, or current-dependent changes that cause their permeability to decline and that their recovery of excitability requires reversal of one or all of those changes but it is not known whether drugs affect that recovery.

Changes in the permeability of channels capable of carrying inward current can, of course, be arrhythmogenic. Arrhythmogenic agents such aconitine and veratrine appear to keep fast channels open; if some fast channels are permanently open while others can undergo their normal cycle of time- and voltage-dependent changes in permeability, the permanently open fraction provides a

steady inward excitatory current that may evoke a new action potential whenever the rest of the channels have returned to their resting state. Catecholamines appear to increase the permeability of the slow channel, thus facilitating conduction of the slow response or increasing the rate of slow response dependent rhythmic activity. Catecholamines also increase the amplitude of delayed afterdepolarizations, thus facilitating the appearance of triggered arrhythmias [76].

An increase in outward current can shorten the action potential, make either the low or the higher resting potential more negative, actually shift a fiber from the low to the high level of resting potential, diminish the extent of diastolic depolarization at any level of membrane potential, diminish the amplitude of depolarizing afterpotentials, and abolish any upstroke that arises because of a relatively small increase in inward current (including the slow response and any upstroke sustained by a small current carried via slowly inactivating fast channels). Enhancement of electrogenic sodium extrusion is expected to have these effects as is any agent that increases membrane K conductance such as acetylcholine or an increase in K_0. It is obvious that most of the effects just described are potentially antiarrhythmic; the opposite effects, which can result, for example, from lowering K_0 or from inhibition of electrogenic sodium extrusion, are potentially arrhythmogenic. In addition, there may well be agents that alter the shape of the plateau and of phase 3 by altering the time- and/or voltage-dependence of any outward or repolarizing current that may be activated by depolarization; it has been suggested that verapamil may have such an effect [77]. On the other hand, it now seems possible that some of the characteristics of repolarization once attributed to the activation of outward currents may be determined by properties of slowly inactivating fast channel currents, by decay of the slow inward current, by accumulation of K in extracellular spaces and by changes in the rate of electrogenic sodium extrusion. Considerable caution is thus indicated at present in interpreting the effects of drugs on the time course of repolarization.

Changes in 'passive' inward currents also change the resting potential. It is well known, for example, that the failure of the resting potential to reach the potassium equilibrium potential reflects the presence of inward current [78]. A sufficient increase in a steady-state inward current will cause depolarization; the arrhythmias caused by aconitine or veratrine are the result of such an increase. Small variations in inward current are of particular importance in fibers at the low level of resting potential since the membrane conductance is low in those fibers. In such fibers, application of tetrodotoxin or reduction of Na_0 can cause the membrane potential to shift from the low to the high level, as can the application of lidocaine [31]. The action of lidocaine can be interpreted in terms of its ability to retard the removal of inactivation; such an

interpretation rests on the assumption that part of the inward current is carried via fast channels and that the progressive shift of fast channels to the inactivated state under the influence of lidocaine reduces that fraction of the inward current. It is probable that other agents with 'local anesthetic' properties, such as quinidine, may have the same effect.

Space does not permit an extended review of the known effects of antiarrhythmic drugs on well-characterized arrhythmias but it should be noted that slow channel blockers slow the rate of impulse generation by the SA node, slow AV conduction, suppress the upstroke of fibers exposed to high K^+ and epinephrine or to Na^+-free, Ca^{++}-rich solution and block nodal reentrant tachycardia in experimental preparations [79]. Each of those effects may well result from 'direct' block of the slow channel. Slow channel blockers also diminish the amplitude of the delayed afterdepolarization seen in triggerable fibers of the coronary sinus [80] but that effect might be indirect.

A number of studies of the effects of verapamil and lidocaine on the arrhythmias associated with myocardial infarction find that lidocaine is effective in preventing those arrhythmias whereas verapamil is not. From those and similar studies the conclusion has been drawn that reentry caused by slow response activity is not the cause of those arrhythmias (see, for example, [81, 82]). On the one hand, if those arrhythmias arise from reentry, the slow conduction necessary to sustain that reentry may depend on the mechanism we have described above in which extremely slow conduction can arise from the activation of very slowly inactivating fast channels. On the other hand, the lidocaine sensitivity of those arrhythmias may result from the necessity for conduction to enter the infarct via partially depolarized 'transitional' fibers on the border between normal and profoundly ischemic tissue [2]. Moreover, the hyperpolarizing effects of lidocaine cannot be ruled out as an explanation of its effects on such arrhythmias.

In summary, we agree with Weidmann [83], Fozzard [14] and Tsien and Siegelbaum [35] that slow conduction is far more likely to arise from the replacement of the 'fast' upstroke by an upstroke that depends on kinetically slow inward currents than from changes in longitudinal resistance. We cite evidence that slow conduction can arise when the only excitable channel capable of carrying inward current is the slow channel. We show that the incompletely inactivated component of the fast channel current probably cannot sustain slow conduction. We show that the presence of a small fraction of fast channel current that is very slowly inactivated can lead to almost arbitrarily slow conduction under special conditions. We derive certain limits on the kinetic properties of any other kind of channel that might carry inward current to generate slow conduction. Slow conduction, dependent as it is on the presence of kinetically slow channels, may arise by various interactions between currents carried via the slow channel and currents carried via

fast channels that are very slowly inactivating. Since both of those channels can display their special characteristics only in depolarized fibers we argue that depolarization is a potent cause of arrhythmias. Moreover, since neither of those channels possesses a high conductance, we argue that the lower the potassium conductance in depolarized fibers, the more likely it is that such fibers can sustain an action potential dependent on kinetically slow inward currents of low density. In consequence, arrhythmias may be abolished not only by agents that abolish excitable channels for inward current but also by agents that alter the resting potential by increasing outward currents or decreasing inward currents. Among such agents, those that increase K^+ conductance, enhance the activity of the sodium pump, or diminish inward currents by retarding the removal of inactivation seem of special interest for future research.

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8. SLOW INWARD CURRENT AND CONTRACTION

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1. INTRODUCTION

The purpose of this discussion is to relate slow inward current to the activation of contraction in heart. The important role of the slow inward current in excitation and conduction is considered in some detail in other chapters. However, one should remember that heart muscle does not simply generate an electrical signal, but it also contracts. Force development and shortening of heart muscle impart energy to the blood to cause it to circulate. This force development can be varied over an order of magnitude, allowing the heart to adjust its contraction to meet the various demands of the circulation. The slow inward current is an important factor in triggering contraction and controlling its strength.

The cellular contraction cycle can be divided into three stages: genesis of the action potential, excitation-contraction coupling and contraction. The action potential functions as the physiological trigger for contraction, causing an increase in intracellular calcium (Ca^{++}) to activate the contractile proteins. While a great deal remains to be learned about excitation and conduction of the action potential, and about the contractile process itself, we know less about the excitation-contraction coupling process than we do about the other two stages. The action potential at the cell membrane, or its ionic components, leads to a massive interaction of actin and myosin. We do know that contraction is a chemical reaction that is activated by a rise in intracellular Ca^{++}. Consequently, the problem of excitation-contraction coupling becomes the problem of how the action potential causes the intracellular Ca^{++} to increase.

Transmembrane flux of Ca^{++} ions is a major component of the slow inward current. The slow inward current is activated during the action potential, and results in entry of Ca^{++} from the extracellular space into the cardiac cell. The discovery of this current [1] immediately raised the possibility that it supplied directly the Ca^{++} needed for activation of contraction. However, control of contraction is quite complex in heart muscle, and this has made it difficult to determine the exact role of the slow inward current.

This discussion of the relationship of the slow inward current to contraction

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of heart muscle will be divided into 4 parts. First, I will describe briefly the characteristics of the slow inward current and the nature of the contractile process. With this as background, I will discuss the relationship of contraction to action potentials, to membrane voltage, and to the slow inward current measured by voltage clamp. In the third part I will estimate the amount of Ca^{++} needed for contraction and review the evidence that the current acts as a trigger for release of Ca^{++} from an intracellular store. Finally, I will describe a synthesis of our understanding of the excitation-contraction process.

The evidence to date shows a strong relationship of the slow inward current to contraction. In myocardium the current is activated during the action potential. Some of the current is carried by Ca^{++}, which triggers release of Ca^{++} from the terminal cisternae of the sarcoplasmic reticulum. The Ca^{++} binds to troponin, and contraction proceeds. Relaxation occurs on reuptake of the Ca^{++} by the sarcoplasmic reticulum. The net entry of Ca^{++} via the slow inward current increases Ca^{++} in the cell, and consequently the amount of Ca^{++} in the intracellular store. A separate membrane process, probably the Na–Ca exchange system, results in efflux of some Ca^{++} from the cell, reducing the amount of Ca^{++} in the intracellular store. The balance between Ca^{++} entry via the slow inward current and Ca^{++} exit via the Na–Ca exchange or a Ca^{++} pump determines the amount of Ca^{++} available for release on the next excitation. The slow inward current therefore plays two roles: it triggers release of Ca^{++} for each contraction and it adds Ca^{++} to the intracellular store available for release by a subsequent excitation.

The reader is warned that this is a dynamic and changing area of cardiac research. Much will be learned in the next few years, which may compel modification of this discussion. I will not attempt to suppress conflicting evidence in order to provide a coherent and pleasing picture, but instead I will risk occasional confusion by identifying what I feel are the problems remaining for future solution.

2. THE SLOW INWARD CURRENT

Uncertainties in the characterization of the slow inward current are apparent to the reader of this monograph. However, to provide a logical discussion of the relationship between the current and the development of tension, I must define certain properties of the current itself. To the extent that future study shows these presumed properties to be in error, the relationships to them that are discussed here must be modified. There are 4 presumed properties:

(1) The slow inward current (I_{SI}) is considered to flow passively through a sarcolemmal channel in a fashion analogous to the flow of sodium and potassium currents through their channels.

(2) The slow inward current channel is voltage- and time-dependent, similar to the better-studied sodium and potassium channels.

(3) The current is mixed, being composed of both Na^+ and Ca^{++} ions, and possibly of other ions.

(4) Finally, the current flow into the cytoplasm, and not into a confined space such as the terminal cisternae.

While the assumptions underlying these statements will be discussed at various stages of the presentation, they represent a basis for relating the slow inward current to contraction.

The current is considered to flow through a channel. This permits the time- and voltage-dependent behavior of the current to be studied in terms of channel gating. The assumption that ion movement is passive implies that Ohm's law may apply in the typical fashion:

$$I_{SI} = g_{SI}(V_m - E_{SI}) \tag{1}$$

where g_{SI} is conductance of the channel, V_m is membrane potential, and E_{SI} is the electrochemical equilibrium potential of the slow inward current. The term g_{SI} is analogous to the sodium and potassium conductances described by Hodgkin and Huxley for channels in the squid giant axon membrane, and is a function of voltage (V) and time (t):

$$g_{SI} = f(V, t) \tag{2}$$

This formula ignores the possibility that the current is produced by a carrier complex in the membrane. Most of the behavior of the current can be explained or modelled by a carrier system, and the usual voltage clamp studies do not directly rule out the carrier model. The best evidence against a carrier is the large single channel conductances that can be calculated from studies of membrane noise. The properties identified above are usually described as H-H properties.

The current is carried substantially by Ca^{++}. The evidence for this conclusion is presented elsewhere in the monograph. However, evidence that the current is not carried by Ca^{++} alone is also compelling. Both Ca^{++} and Na^+ appear to cross the sarcolemma into the cell via this current.

$$\begin{aligned} I_S^{Na} &= g_S^{Na}(V_m - E_{Na}) \\ I_S^{Ca} &= g_S^{Ca}(V_m - E_{Ca}) \\ I_{SI} &= I_S^{Ca} + I_S^{Na} \end{aligned} \tag{3}$$

where I_S^{Na} is Na^+ current via the slow channel and g_S^{Na} is its conductance; I_S^{Na} is Ca^{++} current via the slow channel and g_S^{Ca} is its conductance; and E_{Na} and E_{Ca} are the electrochemical equilibrium potential of Na^+ and Ca^{++}. This simple statement of the mixed current is misleading because it implies that

the movements of Na^+ and Ca^{++} through the channel are independent of each other. There is no a priori reason to argue that Na^+ and Ca^{++} would not interefere with each other during transit, although the rather large single channel conductance suggests that an individual ion may be resident in the channel only briefly.

While the selectivity of the channel may be quite high for Ca^{++} relative to other ions, the extracellular concentration of Ca^{++} is low relative to Na^+. Consequently, the current is also carried to a large degree by Na^+. This may be recognized by considering that current is defined as charge per unit time. The concentration of the ion determines the charge density. The conductance term, g, includes the concentration of the ion, and represents the extent to which that ion may carry current across the membrane in response to a unit electrochemical gradient. Reuter and Scholtz [2] estimated in studies of ventricular muscle that the permeability ratio of the ions (P_{Ca}/P_{Na}) $\simeq 100$, but that $I_S^{Ca} \simeq I_S^{Na}$, because of the much larger amount of Na^+ normally present. Akiyama and Fozzard [3] found similar estimates of P_{Ca}/P_{Na} and I_S^{Ca} and I_S^{Na} for I_{SI} in rabbit AV node cells.

Reuter and Scholtz obtained evidence that K^+ probably also could cross the membrane through the slow inward current channel. There is ample precedent for this mixed selectivity of ionic channels. For example, Baker et al. [4] found that the sodium channel of the squid giant axon had a finite selectivity for Ca^{++}, such that $P_{Ca}/P_{Na} \simeq 0.01$.

The mixed nature of the slow inward current poses serious problems for us in our effort to correlate the Ca^{++} current with contraction. Because the fraction carried by each ion depends on the relative amount of the ion present and on the driving force on that ion, it is not fixed. Consequently, it is impossible to determine in a single voltage clamp recording of I_{SI} how much Ca^{++} has passed through the channel. We can assume only that the upper limit of the Ca^{++} current is close to the total current. In addition, we cannot determine the reversal voltage for the Ca^{++} component simply by measuring the reversal voltage for the entire current, E_{SI}. These constraints to the quantitative interpretation of the current do not prevent its correlation with contraction, but they reduce the certainty of some of the conclusions.

The final assumption about the slow inward current is that the current flows directly into the cytoplasm, rather than into some limited space. This implies that any Ca^{++} in the current would have access to the contractile proteins, and could contribute directly to activation of contraction. Alternatives to this assumption are that the current flows directly into the terminal cisternae, where either it produces a release of cisternae Ca^{++} or it contributes to replenishment of the cisternae store of Ca^{++}. If that were true, then the presumptive channels would need to exist only at the sarcolemmal-terminal cisternae junctions, rather than being generally distributed in the sarcolemma.

The best interpretation of voltage clamp data of the slow inward current favors the conclusion that the current flows directly into the cytoplasm. The voltage dependence of the current argues against the idea that the current flows int the terminal cisternae space, if we presume that there is a significant resistance across the cisternal membrane. The implication that the current flows directly into the cytoplasm also means that if Ca^{++} is released from some intracellular source and its concentration rises in the cytoplasm during contraction, then this release alters E_{Ca} dramatically. Alternatively, the slow inward current could flow into a limited subsarcolemmal space. The only suggestive evidence in favor of such a limited space is reported by Bassingthwaighte and Reuter [5], who found large apparent changes of E_{SI} in response to its current flow. The assumption that slow inward current flows directly into the cytoplasm is an important one for correlation of the current with contraction. More work is needed to resolve any doubts as to the validity of the assumption, perhaps by direct monitoring of intracellular Ca^{++}.

3. THE PROCESS OF CONTRACTION

Consideration of the role of the slow inward current in activating contraction requires a certain understanding of the contraction process itself. The overall function of heart muscle is divided into excitation, excitation-contraction coupling, and contraction. The contraction process is a complex chemical-mechanical transduction that is accomplished by a highly organized protein system. For more thorough reviews, the reader is referred to Huxley [6], Ebashi and Endo [7] Winegrad [8] and Katz [9]. This description will sketch the key features that are needed for the discussion of excitation-contraction coupling.

The sequence of events that eventually results in force development or shortening can be divided into many steps. The general scheme begins with a rise in cytoplasmic Ca^{++} by some unknown mechanism. The Ca^{++} diffuses from its release site to the troponin molecules, which are associated with tropomyosin and actin in the thin filament. When Ca^{++} binds to troponin it causes a change in the tropomyosin. The tropomyosin, which had been inhibiting the actin–myosin interaction, releases its inhibition, and cross-bridges form between the thin (actin) and the thick (myosin filaments. Motion of the cross-bridges generates the force that is obtained. If the muscle is to shorten, the initial set of cross-bridges must be broken and new ones formed, as the actin and myosin filaments are pulled past each other. Eventually, Ca^{++} dissociates from troponin and is sequestered outside the cytoplasm into the sarcoplasmic reticulum. This releases tropomyosin to inhibit the actin–myosin interaction, thus ending contraction.

Evidence that the concentration of Ca^{++} rises in the cytoplasm as the initial step in contraction comes from many studies, mostly performed in skeletal muscle. Recently, measurement of a transient Ca^{++} increase has been made by Allen and Blinks [10] using the Ca^{++} indicator aequorin. They were able to demonstrate that Ca^{++} rose prior to contraction, and that contraction was scaled to some degree by the amount of Ca^{++}. Exact studies of the Ca^{++} contraction relatonship in intact fibers are not possible yet, because of problems with the time constants of the Ca^{++}-aequorin reaction and because of the uncertain stoichiometry of the process. For purposes of our discussion of the slow inward current, we will assume that the end result of excitation-contraction coupling is this rise of cytoplasmic Ca^{++}.

Troponin is a complex of three molecules. One attaches the complex to tropomyosin; one acts on tropomyosin to lead to inhibition of contraction, and one binds Ca^{++}. Cardiac muscle troponin is not identical to skeletal muscle troponin, but it acts similarly. Once the Ca^{++} is bound to troponin C, tropomyosin releases its inhibition of active sites of 7 actin molecules in the thin filament. The active sites of actin are now capable of binding to the heads of the myosin molecules that constitute the cross-bridges. There is some evidence that the actin–myosin interaction (cross-bridge formation) may modify the affinity of troponin for Ca^{++}, acting as a feedback system. The cross-bridges themselves are formed by an activated myosin site, which is a complex of myosin, ADP and P, and requires Mg^{++}. Movement of the cross-bridge pulls the myosin thick filament relative to the actin thin filament, resulting in either force development or shortening. Detachment of the cross-bridge for relaxation or for further shortening requires ATP, so that depletion of ATP results in a tonic contraction (contracture).

Removal of Ca^{++} from troponin is the event responsible for relaxation. This is accomplished by simple competition between the Ca^{++} site on troponin and the Ca^{++} site on the Ca transport system of the sarcoplasmic reticulum. Metabolic changes that alter the affinity of the sarcoplasmic reticulum for Ca^{++}, or change its storage capacity would have a large effect on contraction [11]. For example, an increase in affinity for Ca^{++} would lead to increased competition with troponin for the Ca^{++} initially released from the terminal cisternae, and would reduce contraction. Under some conditions mitochondria may become a part of the Ca^{++} sink for long-term maintenance of low diastolic Ca^{++}, particularly in circumstances where the cell is heavily loaded with Ca^{++}. The role of a sarcolemmal Ca pump in relaxation is uncertain at this time.

The two important points in this discussion of contraction are that contraction is activated by an increase in cytoplasmic Ca^{++}, and that the sequence of events that must occur between Ca^{++} increase and measurable tension includes a large number of complex biochemical reactions. In almost every

study of the relation between membrane voltage, current, and contraction, the rise in cytoplasmic Ca^{++} is monitored indirectly by measurement of tension. It should be apparent that this is an imperfect indicator that makes these studies difficult to interpret. The relaitonship between Ca^{++} and tension is not linear, and there are numerous ways in which the contraction process can feed back on cytoplasmic Ca^{++} or on excitation itself. Consequently, the use of tension as a monitor of cytoplasmic Ca^{++} is a crude tool, which leads to many uncertainties in the study of excitation-contraction coupling. The direct measurement of cytoplasmic Ca^{++} by Allen and Blinks [10] is the first encouraging sign that this severe problem may be resolved in the near future.

4. THE ACTION POTENTIAL AND CONTRACTION

Contraction is normally caused by an action potential (for review, see [12]). The action potential is a triggered sequence of depolarization and repolarization. It is generated by some 8 kinetically separate ionic currents. There are three questions to consider. What property of the action potential causes contraction? Does the action potential simply trigger a contraction, or does it control the size of the contraction? What role does the action potential play in recovery after a contraction? While complete answers are unavailable, consideration of these questions will provide the basis for the main question of how the slow inward current is related to contraction.

The cardiac action potential is a very complex process [13, 14]. It is generated by currents carried by Na^+, K^+, Cl^-, and Ca^{++}. The most obvious feature of the action potential is the sudden depolarization. The first consideration is what is the role of the depolarization itself. Two methods of depolarization other than that occurring in an action potential have been used, potassium contracture and voltage clamp.

4.1. Potassium contracture

The first direct attempt to examine the role of membrane voltage in causing contraction was to produce a potassium depolarization. The muscle cell has a resting potential that is close to potassium equilibrium potential [15], because the membrane is more permeable to K^+ than to any other ion. This potential is negative intracellularly relative to the outside potential, because K^+ is low in the extracellular solution and high in the intracellular solution. Consequently, raising the extracellular K^+ would decrease the K^+ concentration gradient and depolarize the cell. This experiment was systematically performed with skeletal muscle by Kuffler [16] and he found that it produced a sustained contraction called a contracture.

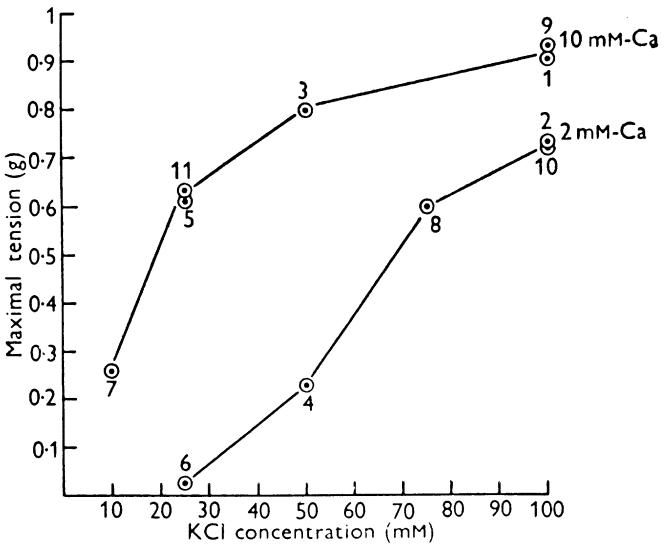

Figure 1. Relation between tension and KCl concentration in frog ventricular muscle. Maximal contraction tension is plotted for two different Ca^{++} concentrations against varying KCl concentrations. During the period immediately prior to the KCl exposure, the strip of ventricle was stimulated at 4 times/min. The order of experiments is indicated by the numbers. From Niedergerke (17) by permission of the Journal of Physiology.

Potassium depolarizations were used by Niedergerke[17] and Luttgau and Niedergerke[18] to demonstrate a clear graded relation between membrane potential and tension in frog ventricle (Figure 1). A threshold for tension was found with 30 mK $[K^+]$ (about -45 mV), when outside Ca^{++} was 2 mM. With further depolarization the tension increased markedly, reaching a plateau as the membrane potential approached 0 mV. Their studies of potassium contractures have been extended by others in recent years[19–21], but these reports do not provide much insight into the relation of the slow inward current to normal contraction. The main conclusion that we can draw from potassium contracture studies is that tension appears to be a graded function of membrane potential.

4.2. Action potential change

The shape of the normal action potential can be modified, and the effects on contraction monitored. A direct change in the action potential was first achieved by Kavaler[22], who greatly prolonged it in a piece of ventricular muscle by passage of current. The muscle showed a twitch, followed by a variable prolonged tension, suggesting that tension was directly controlled by membrane potential. Morad and Trautwein[23] shortened action potentials of

sheep ventricular muscle by passage of current at various times after onset. They found that the contraction was a graded function of action potential duration up to 200 msec at 25 °C. The contraction that was caused by a very short (e.g., 40 msec) action potential was low in amplitude but normal in shape, rising to a peak value in about 200 msec. With longer action potentials the time to peak of contraction lengthened slightly.

Steady current passage was used by Wood et al. [24] to modify the action potential of sheep ventricular muscle. The depolarizing current had several effects: it depolarized the resting potential a small amount and it caused a higher (more depolarized) and a prolonged plateau. Modest depolarizing currents, which prolonged the plateau without much other effects, caused a gradual increase in twitch tension that reach a maximum in 8–10 beats. When the current was halted, the action potentials returned immediately to their control length, but the twitch tensions declined gradually back to control values over 8–10 beats. Larger depolarizations produced large steady tensions like those seen by Kavaler, and they were followed by a potentiation of the twitch.

These studies of action potential changes suggest that the shape of the action potential plays an important role in the control of tension, but the effects were very complex. In the experiments of Morad and Trautwein [23] it appeared that the first few milliseconds of depolarization during the action potential triggers contraction, which can follow a time course greatly exceeding the triggering depolarization. However, the length of depolarization controlled the magnitude of the contraction up to some length that was shorter than the natural action potential. Wood et al. [24] found that the effects of action potential alteration were cumulative over 8–10 beats, and the effects decayed slowly.

4.3. The voltage clamp

The third way to examine the relation between membrane potential and contraction is with the voltage clamp. This voltage clamp technique allows the potential difference across the membrane to be set arbitrarily by the investigator through introduction of current into the cell under feedback control. The two main techniques used are sucrose-gap and micropipette clamps. Methods of voltage clamp are considered elsewhere in this monograph. There are important differences between them, but these differences will be ignored in this discussion. For our purposes, the voltage clamp is simply used to free us from dependence on the naturally occurring action potential and to allow measurement of the slow inward current.

The first voltage clamp studies of contraction were by Fozzard and Hellam [25] and Morad and Trautwein [23]. The initial question to be asked in a

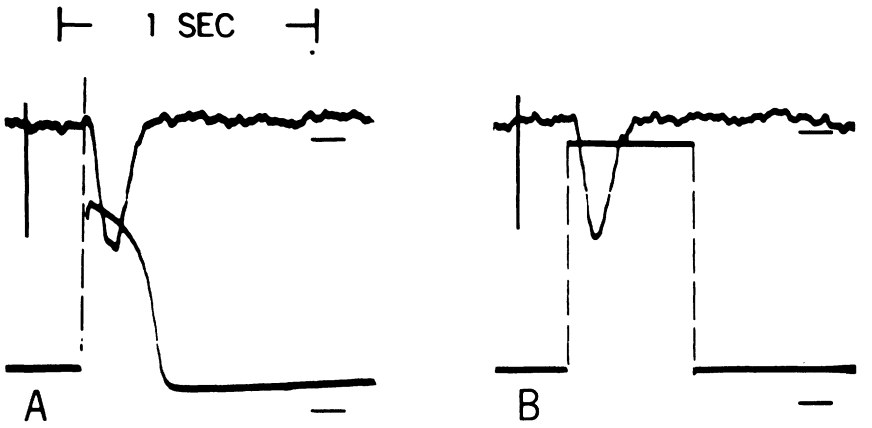

Figure 2. Comparison of the contractions related to an action potential and to a voltage-clamp step. A: cardiac Purkinje fiber action potential (lower trace). The resting potential was −70 mV. The upper right horizontal line is 0 mV and the lower one is −80 mV. Contraction (upper trace) is recorded downward. The vertical bar to the left is 5 mg weight. B: the same fiber as in A, with the same calibrations. A voltage step was imposed from −70 mV to −4 mV for 500 msec. Note that the contraction is almost the same as that provoked by the action potential. From Fozzard and Gibbons (1973) with permission of the American Journal of Cardiology.

voltage clamp study was if the voltage step would provoke a contraction that was similar (or identical) to the one caused by an action potential. Figure 2 illustrates the two events, showing that the contraction in response to a voltage step was substantially the same as that caused by an action potential. Presumably the same process that occurred in a normal action potential also occurred with a voltage clamp. This result was a necessary precondition to use of the voltage clamp in studies of excitation-contraction coupling. Note also that the contraction relaxed before the membrane was repolarized. If tension were simply dependent on the change in transmembrane potential, then the contraction would have lasted as long as the depolarization was maintained. This is best explained by assuming that the depolarization step triggers some secondary process, which then causes the contraction. Once triggered, the secondary activation process runs a biphasic course, turning contraction on, and then turning it off. From these simple experiments one can recognize that contraction is temporally related to depolarization and that it may be induced by some voltage-dependent process[1].

[1] This discussion applies to mammalian heart muscle. In frog heart the tension continues to rise, or is sustained, as long as the depolarization is maintained. Morad and Goldman [26] make a strong case that excitation-contraction coupling is fundamentally different in frog heart. I accept this argument, and choose to direct this discussion to excitation-contraction coupling in mammalian heart muscle. For careful discussion of frog heart the reader is referred to Morad and Goldman [26] and Winegrad [8].

Further insight into the voltage-dependent contraction can be gained from simple voltage clamp experiments. In a previously rested fiber the magnitude of the contraction is determined by the duration and the magnitude of depolarization. When long depolarizing steps (relative to the usual action potential duration) are made, tension is just noticed at -56 mV, and thereafter it is a graded function of voltage (Figure 3). If the depolarizing step is made to a voltage typical of the action potential plateau (-20 mV to $+10$ mV), but of varying duration, the contraction is a function of the clamp duration, approaching a maximal value with steps of 100 msec duration at 37 °C (Figure 4).

Recall that Morad and Trautwein [23] found that the time course of contraction could greatly exceed the shortened action potential. This was also demonstrated in the voltage clamp experiments. For example, a 5 msec depolarization could produce substantial tension (although only a fraction of the

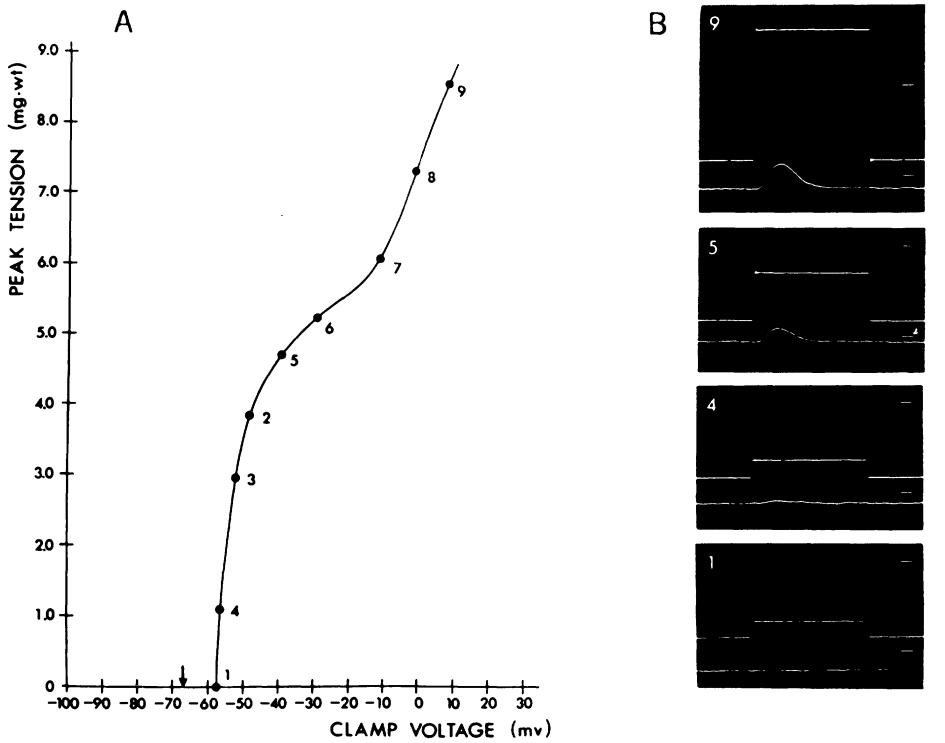

Figure 3. Dependence of contraction on clamp voltage. A: holding potential of the cardiac Purkinje fiber was -67 mV (arrow). Numbers indicate the order of experimental observations. B: tension is recorded in the lower traces and voltage in the upper traces. The panels are numbered to correspond with the experimental points in panel A. The horizontal lines are at -25 mV and -75 mV. The voltage steps were made after long rest periods. From Gibbons and Fozzard (32) with permission of the American Heart Association.

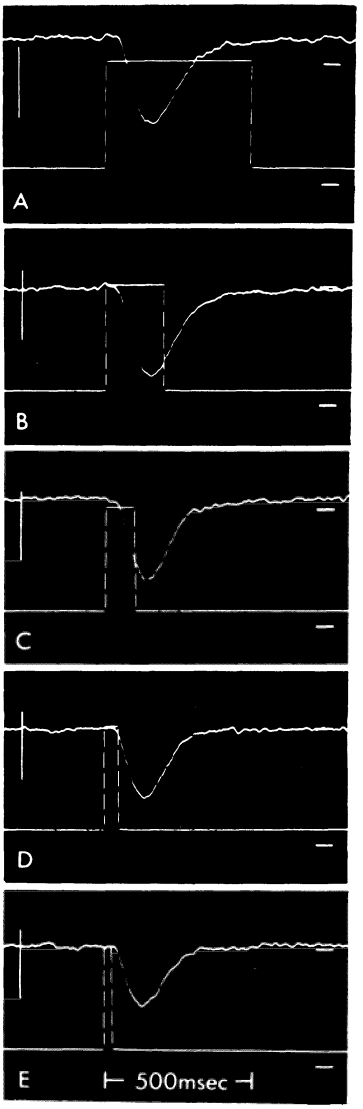

Figure 4. Contraction in response to voltage clamps of various durations in cardiac Purkinje fibers. The upper trace in each panel is tension, with contraction recorded downward. The vertical bars are 5 mg weight. The lower trace in each panel is voltage. The horizontal lines are at 0 mV and −75 mV. The holding potential was −65 mV, and the voltage step was to +3 mV for 490 msec (A), 195 msec (B), 100 msec (C), 50 msec (D), and 25 msec (E). Upstrokes and downstrokes are retouched with dashed lines for visual convenience. From Gibbons and Fozzard (32) with permission of the American Heart Association.

maximal contraction). Yet the tension began after a 5–6 msec latency, so that it began after repolarization, and it followed an approximately normal time course. This initially surprising finding is easily explained by the idea that the time course of contraction is determined by a tertiary process, which has time-dependent steps that exceed the time dependence of activator release. If we accept the idea that depolarization triggers and partially controls a secondary process, then the 5 msec is sufficient to activate this secondary process. While the magnitude of contraction is reduced by early repolarization, the time course of the resultant contraction is little affected. The simplest way to explain this is that the brief depolarization leads to a release of a small amount of Ca^{++}. The magnitude of the contraction depends on how much Ca^{++} is released. It binds to troponin C, initiating the biochemical events of contraction. From that point the contraction follows a time course determined by the rate constants of the cross-bridge reactions and the rate constants of the Ca^{++} pump in the sarcoplasmic reticulum, which are independent of both membrane voltage and intracellular Ca^{++} activity.

More complex voltage clamp experiments, such as the behavior of trains of clamps and recovery of contraction after a clamp, are valuable in understanding the excitation-contraction process. They will be discussed in detail later, as we compare contractile behavior to the slow inward current. But from the simple voltage clamp or constant current experiments reported in this section we can draw certain tentative conclusions. The action potential, by analogy with voltage clamp, triggers contraction. Its magnitude and duration determines the magnitude of the contraction. Furthermore, the effects of action potential change are cumulative over 8–10 beats. It appears that the action potential initiates a secondary process that activates contraction. The magnitude of this secondary process is a very complex function of membrane voltage and time.

5. RELATIONSHIP BETWEEN THRESHOLD FOR I_{SI} AND CONTRACTION THRESHOLD

One of the reasons why we [27] began to think that the slow inward current was related to contraction was that, in very complex voltage clamp experiments, we never saw contraction without also seeing the current. This statement has also been made by New and Trautwein [28]. The apparent correlation of occurrence for the two events is an important piece of evidence to be used in considering causality. There are three ways in which the thresholds of the two events could be closely related. First, the current may trigger contraction, so that the current is necessary for activation, but contraction is not always proportional. Second, the current may activate contraction directly, so

that contraction is proportional to the current. Third, the current may not play any role in activating contraction. The release of intracellular Ca^{++} may secondarily produce a change in membrane permeability, causing the slow inward current. Ca^{++}-activated membrane currents can be seen in such different tissues as invertebrate neurones [29] red blood cells [30] and cardiac Purkinje fibers [31].

Evidence for the correlation of the thresholds for current and contraction will be reviewed. Because of the complex way in which cardiac contraction depends on membrane potential, stimulation pattern, and external ion concentrations, a wide variety of conditions is available for comparison. One warning should be given about these experiments. Threshold is a qualitative point — one where an event is just seen. Therefore, the ability of the investigator to see it depends on the sensitivity of the monitoring apparatus. In general, the methods used so far are sufficiently sensitive to detect tension or current that is perhaps 5% of maximal. This represents a limitation on studies of threshold.

We have previously seen that depolarization steps began to produce tension at about -56 mV in sheep Purkinje fibers [32]. It was obtained in the first depolarization step after a long rest, and with normal $[Na]_0$ and 2.7 mM $[Ca]_0$ at 37 °C. Furthermore, threshold was sensitive to the prior resting membrane potential. The threshold for contraction could be changed systematically by altering the holding potential. When this was done, the thresholds for contraction and for slow inward current remained identical, changing in parallel.

The studies of Beeler and Reuter [33] were performed differently, because of different behavior of their ventricular muscle preparations. In their tissue the first clamp step after a long period without stimulation produced no tension. If the steps were continued in a train, then tension developed, reaching a steady level after about 8 steps. At that steady level the steady-state relations of voltage to tension could be studied. The voltage level of the trains at which tension began to rise was about -30 mV and was considered threshold for contraction. It corresponded to threshold for the slow inward current [1]. This voltage level became more negative when $[Ca]_0$ was raised from 1.8 mM to 7.2 mM, and current threshold became more negative by the same amount.

[1] In their experiments a small ($\simeq 5\%$ of maximal) tension could be first seen upon steps to -60 mV. This small contraction did not increase on further depolarization and produced a 'foot' on the voltage–tension plot. The threshold of the 'foot' corresponded to the threshold for sodium current. They suggested that this was due to a small loss of voltage control by their clamp, so that some part of the muscle was above mechanical threshold. When they removed Na^+ from the superfusing solution, this foot disappeared. Their explanation is no doubt correct. While it is unfortunate that their clamp control was not better, this does not interfere significantly with interpretation of their results.

In these two rather different experiments – rest contractions in Purkinje fibers and steady state contractions in ventricular muscle – mechanical threshold and current threshold were identical.

Beeler and Reuter [33] removed $[Na]_0$ in some experiments and found that thresholds for current and tension remained identical. New and Trautwein [28] also studied the changes in current and mechanical threshold in solutions with various Ca^{++} and Na^{+} concentrations. They report that they never saw current without tension, and vice versa.

More complex experiments have not always confirmed this coincidence of thresholds. As we described, the ventricular muscle studied by Beeler and Reuter [33] failed to develop tension on the first depolarizing step after a prolonged pause. However, the slow inward current associated with the first voltage step was large. With successive voltage steps the contraction gradually rose to its steady level while the slow inward current remained unchanged in size. Therefore, under conditions of recovery from a rest, they could see the current without contraction. Gibbons and Fozzard [27] examined the effect of a train of depolarizing steps in Purkinje fibers to voltages just above threshold. As already noted, the thresholds corresponded for the first step of the train. However, with continuation of the train, the twitch responses became smaller and disappeared, but the current remained visible.

These two experiments raise doubts that under all conditions the current and contraction thresholds are the same. Rather, it appears that under some conditions there may be current without tension. The results are still compatible with the idea that the current plays an essential role in excitation-contraction coupling, but they require the additional condition that other steps are involved after the current step. In summary, it seems that contraction cannot occur without some slow inward current, but under some conditions the current can occur without contraction.

6. Relationship between magnitude of I_{SI} and magnitude of contraction

The first careful studies of the proportionality between the slow inward current and contraction were made by Beeler and Reuter [33] in ventricular muscle. They found different results in Na-containing and in Na-free solutions. In Na-containing solutions the steady-state magnitude of tension with depolarizing steps of various duration continued to increase with steps of from 100 msec to 400 msec, whereas the transient slow inward current was maximal with steps of 100 msec. In Na-free solutions, the times of depolarization necessary for proportional amounts of current and contraction were almost the same. They suggested that in normal solutions the slow inward

current provides a trigger for contraction and a source of Ca^{++} for filling an intracellular releasable Ca^{++} store. However, in Na-free solutions the store is maintained at its maximal level, and under those conditions the current directly determines the amount of contraction.

Gibbons and Fozzard [27] measured the magnitude of the current and of tension with long depolarizing steps just above threshold. They found a nonlinear proportionality between current and tension over a small voltage range for rest depolarizations (Figure 5). Their studies in Purkinje fibers were limited because an outward current develops in this tissue at potentials above -30 mV, making quantitative measurement of slow inward current unreliable. Gibbons and Fozzard [27] also studied the time course of recovery of current and contraction in Purkinje fibers after a depolarizing step by use of a pair of steps with variable coupling. They found that the current (measured either by peak magnitude or area under the current cuver) recovered faster than did contraction (Figure 6). Others have reported coincident recovery of current and contraction in a similar protocol using ventricular muscle [34, 35]. Quantitative measurements of the slow inward current are difficult to make, since it must be separated in certain arbitrary ways from other coexistent currents. In addition, there are reasons to believe that the slow inward current

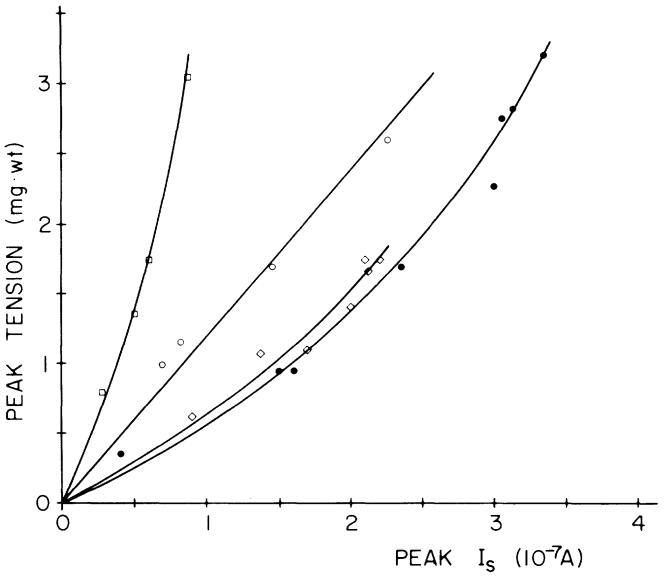

Figure 5. Peak tension responses to voltage steps somewhat above contractile threshold compared to peak magnitude of the slow inward current (I_s). The experiments were done in 4 short Purkinje fibers, with voltage clamp. In each fiber larger tension was associated with larger current, although the relationship is probably not linear. From Gibbons and Fozzard (27) with permission of the Rockefeller University Press.

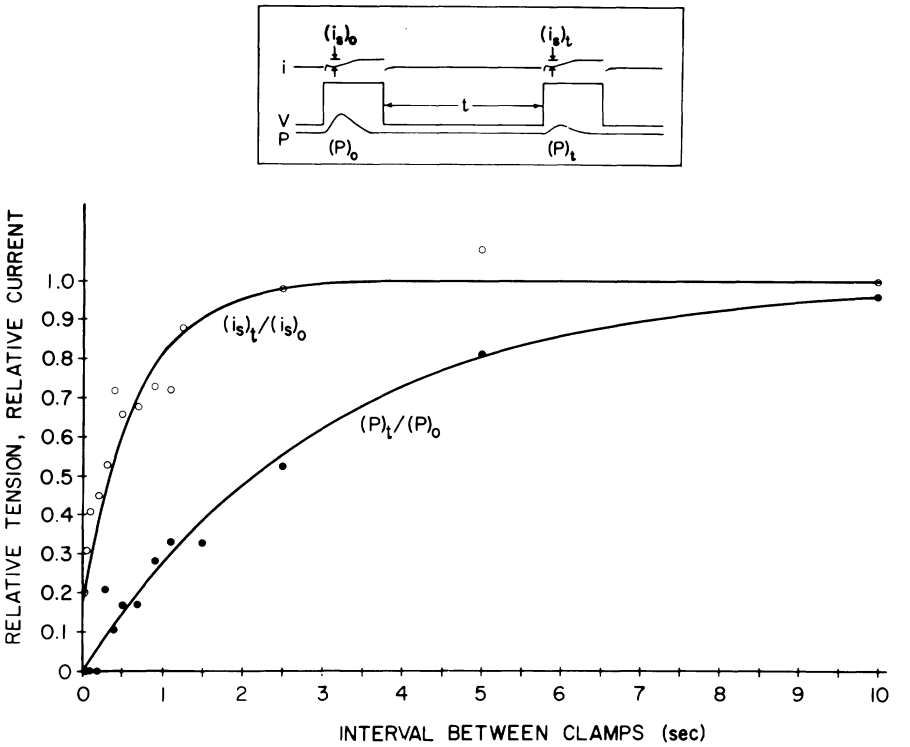

Figure 6. Recovery of slow inward current (i_s) and recovery of twitch tension (P). The experimental protocol is illustrated in the panel. A pair of voltage steps is made with a 2-micropipette voltage clamp in a short sheep Purkinje fiber. With the first voltage step after a rest the magnitude of the slow inward current $(i_s)_0$ was measured and the magnitude of the tension $(P)_0$ measured. Then a second step is made at various times (t) an the current $(i_s)_t$ and tension $(P)_t$ measured. The extent of recovery of the current and tension is estimated by the ratios of the second measurement to the first one. Current magnitude recovers more rapidly than the tension in this experiment. From Gibbons and Fozzard (27) with permission of the Rockefeller University Press.

is not completely inactivated during a long depolarizing step [27, 36, 37]. Such a steady current is difficult to measure, but it would contribute significantly to calcium entry during the depolarization. Consequently, these crucial recovery experiments need to be repeated under other conditions.

A thorough study of the similarity between the conductance of the slow inward current channel and contraction was reported by Trautwein et al. [36]. They used the relation (Bassingthwaighte and Reuter, [5]):

$$g_{SI} = \bar{g}_{SI}\, df$$

where $\bar{g}_{SI}$ is the maximal conductance, d is the probability variable for activation and f is the probability variable for inactivation. The studies were made

in cat ventricular muscle at 37 °C, superfused with solutions containing 3 mM K^+ and 3.6 mM Ca^{++}. Activation (d_∞) was determined by tail currents after 40 msec depolarizations from a −55 mV holding potential to different voltages. This time period was felt to permit full activation, but little inactivation. Inactivation (f_∞) was measured by a hyperpolarizing step of 600 msec from +5 or +10 mV to −80 mV or less, and back to the depolarized level. The peak magnitude of the current provoked upon depolarization after hyperpolarization to different levels was used to measure steady-state inactivation. The conditions were chosen to maintain intracellular calcium stores at their maximal level. The analysis gave surprisingly consistent results, considering the difficulty of accurate measurement of the current and its mixed nature. When plotted against membrane potential, the extracted activation and inactivation variables showed symmetrical sigmoid curves with cross-over at d_∞ and f_∞ of 0.4 at −25 mV. In these experiments peak magnitude of contrac-

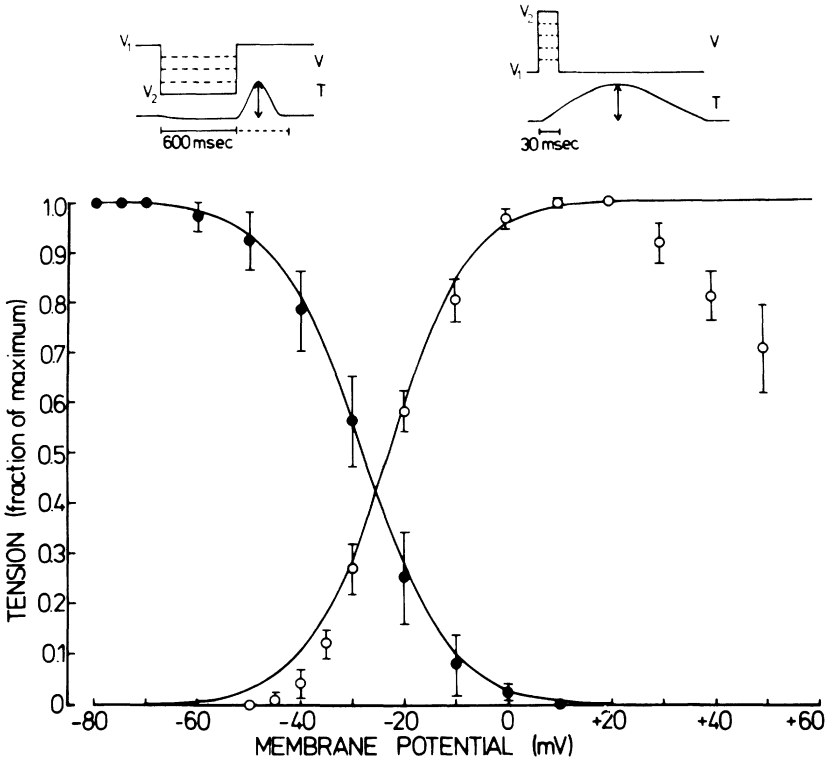

Figure 7. Relation between activation and inactivation variables of g_{SI} and the voltage dependence of tension. The solid lines are best fit measurements of the conductance variables d_∞ and f_∞. The points are the activation and inactivation variables for contraction, measured according to the protocols shown above the graph. The voltage dependence of contraction agrees well with the voltage dependence of g_{SI} except at potentials positive to +20 mV. From Trautwein et al. (36) with permission of Springer Verlag.

tion was measured, and it showed that the steady-state voltage dependence of contraction and activation of g_{SI} agreed very well, except for activation steps to inside-positive values (Figure 7). Above +20 mV, tension was reduced, but activation of the conductance was constant.

The surprisingly good correlation between contraction and conductance of the slow inward current channel must be contrasted with the previous efforts to correlate the magnitude of the current with contraction. Current is the product of the conductance and the driving force (equation 1). Therefore, at each voltage the driving force is altered. The manner of measurement of the inactivation variable f_∞ was such that the driving force was constant during contractile activation, so the correlation with tension is equally good for current or for conductance. For the study of activation, depolarizing steps were made to different levels, but the current was not measured at those levels. Instead, the tail current on repolarization was measured. Therefore, the activation measurement does not give the same results for conductance and current. The lack of apparent agreement between contraction and conductance therefore implies that contraction did not correlate with current over those voltage ranges. It is difficult to estimate what the current should have been because, among other problems, the value of the calcium electrochemical equilibrium potential (E_{Ca}) is unknown. Note that reversal of I_{SI} is not necessarily the value of E_{Ca}. Trautwein et al. [36] were certainly puzzled by this result. They suggested that perhaps contraction is proportional to conductance of the slow inward current channel because this channel represents a shunt across the sarcolemmal–terminal cisternae junction. The shunt would cause a depolarization of the terminal cisternal membrane and lead to Ca release from this internal store.

A decline in tension when depolarizations were to markedly inside-positive values was in conflict with this Ca^{++}-conductance idea. Early studies [20, 25, 33] failed to show this behavior, probably because voltage steps were not made to sufficiently large inside-positive values. Subsequent this decline of tension at inside-positive values of membrane potential has been clearly shown by New and Trautwein [28], Morad and Goldman [26], Reuter [1], and Gibbons and Fozzard [38]. The decline in tension at voltage values of +30 to +60 mV is consistent with an important direct role of Ca^{++} current in causing contraction. The study of this phenomenon has been complicated by different behavior when different experimental protocols were used. Gibbons and Fozzard [38] found the decline when studying the first contraction after a long pause, but did not see it when examining the steady-state behavior after a train of voltage steps.

In summary, efforts to determine proportionality between the slow inward current and contraction have been disappointing. Some methods of study show excellent correlation with magnitude of current and contraction, some

with conductance and contraction. However, under other circumstances the correlations fail. Whether this is because of difficulties in measuring the current or because the association between the variables is not a direct one, remains to be settled by future experiments.

7. HOW MUCH CALCIUM IS NEEDED FOR CARDIAC CONTRACTION?

In order to test the possibility that the calcium current is sufficient to activate contraction directly, it is necessary to know the amount of Ca^{++} entering with each depolarization and the amount of Ca^{++} needed for a contraction. Both quantities are difficult to estimate, but most attempts have led to the conclusion that the Ca^{++} entering with the slow inward current is not sufficient to activate contraction alone.

The amount of isotopically labelled Ca^{++} entering the fiber during quiescence is very small [39], but it increases to about 1 μM/impulse on stimulation. Estimates of Ca^{++} entry from measurements of the slow inward current are difficult, both because of uncertainty as to the current measurement and because of the mixed nature of the current [20]. I have calculated this from the published records of Beeler and Reuter [33], and obtain estimates between 1 and 10 μM/impulse, depending on the conditions of external Ca^{++}, resting potential, and step potential. Beeler and Reuter reported that the voltage steps gave contractions that were usually 2–3 times larger than those resulting from stimulated action potentials, so that typical Ca^{++} entry from an action potential may be smaller. Estimates from Purkinje fibers give comparable values when recordings from Gibbons and Fozzard [27] are used. The best combined estimate from isotopic flux and voltage clamp experiments is that Ca^{++} entry is 2–5 μM for each impulse that generates half-maximal contraction.

The problem of determining the amount of calcium needed to produce a contraction is even more complicated. Troponin has multiple (3–4) binding sites for Ca^{++}, and it is not certain how these sites cooperate in terms of Ca^{++} interaction [40]. The relationship between intracellular Ca^{++} and tension is a complex one, as shown by the studies in skinned fibers by Fabiato and Fabiato [41] (Figure 8). Estimates range from about 20 μM to 50 μM for half-maximal activation, and 100 μM for full activation.

The sarcoplasmic reticulum is able to take up Ca^{++} in significant quantitites. Efforts to determine its capacity are limited by problems of purification. The best studies so far are by Solaro and Briggs [42]. They estimated that cardiac sarcoplasmic reticulum could accumulate 0.1–0.2 mM Ca^{++} from a solution of 10^{-6} M $[Ca^{++}]$. As noted above, maximal tension in heart muscle is achieved with about 0.1 mM. If these estimates are correct, they demonstrate a major difference between heart muscle and fast twitch skeletal mus-

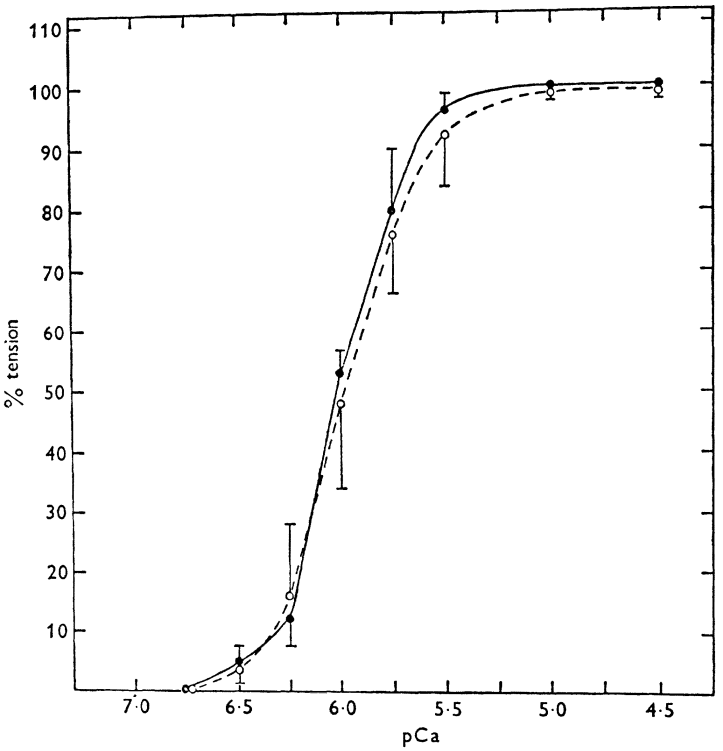

Figure 8. Tension developed by skinned adult rat ventricular cells as a function of pCa of the bathing medium ($-\log[Ca^{++}]$). The experiments were done with 4 mM EGTA with pMg 3.5 and pMg ATP 2.5. The filled circles were from experiments without detergent to destroy the sarcoplasmic reticulum and the open circles were after exposure for one-half hour to Brij 58. Tension developed in a given cell is expressed as a fraction of the tension developed at pCa = 5.0. The tension at 0% is that at pCa = 9.0. The mean and standard deviation for 20 or more observations is indicated. From Fabiato and Fabiato (41) with permission of the Journal of Physiology.

cle. Skeletal sarcoplasmic reticulum can accumulate far more Ca^{++} than is needed for a contraction. In heart, the sarcoplasmic reticulum may have a capacity just large enough for maximal contraction, so that partial loading could play an important role in contractile regulation.

Another problem is whether entering Ca^{++} reaches the contractile proteins. The sarcoplasmic reticulum Ca pump has an affinity for Ca^{++} that is several times greater than that of the troponin. Consequently, if Ca^{++} enters from the outside, there is no certainty that it would reach the troponin sites.

In summary, efforts to compare Ca^{++} entry via the slow inward current show that the current appears to supply directly only about 10–15% of the Ca^{++} needed for contraction. On the other hand, the sarcoplasmic reticulum has the capacity for storage of more Ca^{++} than is needed in a typical single

contraction. The calculations all depend on assumptions, and one should not consider them to be absolutely correct. However, they are the best we can estimate at this time, and they are unlikely to bring us to the wrong conclusions. To the extent that the calculations are accurate, we can conclude that insufficient Ca enters via the slow inward current to activate contraction directly. Consequently, the necessary Ca^{++} must come from another source. While the best evidence favors the sarcoplasmic reticulum as the principal source, electroneutral Ca^{++} entry across the sarcolemma or Ca^{++} efflux from mitochondria represent alternative possibilities of part of the Ca^{++}. If the sarcoplasmic reticulum is the source, then its partial filling could play a role in contractile regulation.

8. TRIGGERED RELEASE OF CALCIUM

If the amount of Ca^{++} that enters the cell via the slow inward current during a single action potential is insufficient to activate the contractile process directly, then how can the current correlate so well with contraction under many circumstances? The two possibilities that have been suggested as a basis for this correlation are (1) that sarcolemmal depolarization somehow directly causes release of stored Ca^{++}, but that the slow inward current determines the size of the store, or (2) that the slow inward current directly causes release of the stored Ca^{++}, and it secondarily maintains the store. The surprisingly good correlation between threshold and magnitude of both current and contraction argues against the depolarization mechanism alone. This section will explore the concept of the slow inward current as a trigger for release of Ca^{++} stored intracellularly.

The concept of triggered release received support by studies of skinned skeletal muscle fibers by Hellam and Podolsky [43], Ford and Podolsky [44, 45] and Endo et al. [46, 47]. They performed elegant studies on single fibers from fast twitch muscles of the frog, from which the sarcolemma had been stripped over a substantial distance. With this preparation they could expose the interior of the cell to an artificial sarcoplasmic solution where $[Ca^{++}]$ was controlled with EDTA or EGTA buffers. The experiments of Hellam and Podolsky [43] demonstrated that the relation between log $[Ca^{++}]$ and steady tension was sigmoidal over the range of 10^{-7} to 10^{-5} M. In their experiments the results differed very little when the sarcoplasmic reticulum was destroyed by detergent. They used heavily buffered Ca^{++} solutions in these experiments, and the tension was developed slowly over many seconds.

Ford and Podolsky [44, 45] and Endo et al. [46] subsequently saw a complex phenomenon that they named Ca-induced Ca^{++} release. If the skinned fiber

was allowed to load its sarcoplasmic reticulum with Ca^{++}, and then it was exposed to weakly buffered or free Ca^{++}, it demonstrated a rapid contraction and relaxation (e.g., 2–3 sec to maximal tension and 20–30 sec to relaxation). They were able to show that this phenomenon was caused by a release of Ca^{++} from the sarcoplasmic reticulum and its subsequent reuptake. Two characteristics of the phenomenon have led to the impression that this Ca-induced Ca^{++} release is not physiologically important in skeletal muscle. First, the level of free Ca^{++} needed to trigger release is about 10^{-4} M, far higher than one could imagine being achieved by any sarcolemmal Ca^{++} current in skeletal muscle. Second, the process was inhibited by $[Mg^{++}]$ greater than 0.5 mM [47]. However, the addition of caffeine to the solution exposed to the skinned fiber favors the triggered release at lower free Ca^{++} and higher Mg^{++}. Regardless of its possible role in fast twitch skeletal muscle, it represents a model for Ca^{++} release that can be considered for heart muscle.

The method of skinning skeletal fibers cannot be applied directly to cardiac fibers, because the cardiac fibers are so much smaller (10 μm in diameter versus 100 μm; 100 μm in length versus 1 cm). However, the need for such a preparation has led to the development of three useful methods for skinning cardiac fibers. Fabiato and Fabiato [48] have developed the most elegant method, that is continuing to be improved in their hands. Ventricular muscle is homogenized in a blender, and fragments of cells are further relieved of surface membrane by teasing with a micropipette. After substantial disruption of the surface membrane, the cell fragment is impaled at both ends with micropipettes, which function as arms of a transducer system for tension recording. With this method they have examined a large number of questions related to excitation-contraction coupling [41]. The second method is similar. Kerrick and Best [49] used larger multicell fragments of disrupted cardiac muscle without teasing. The third approach is to destroy the semipermeable properties of the surface membrane with low Ca^{++} solutions [50], so that large ions can equilibrate freely with the interior of the cell bundle. The most extensive studies of skinned fibers have been made with the Fabiatos' technique.

Fabiato and Fabiato [41] demonstrated the dependence of steady tension on $[Ca^{++}]$, showing that it is very much like skeletal muscle in that regard (Figure 8). They further showed that Ca-induced Ca^{++} release occurred with $[Ca^{++}]$ as low as 4×10^{-8} M and with $[Mg^{++}]$ levels to be expected intracellularly. In ventricular muscle they found that cyclic contraction would occur with a time course very much like normal twitches, as if the sarcoplasmic reticulum suddenly released its Ca^{++} and then gradually reaccumulated it (Figure 9).

One of the major criticisms of the concept of Ca-induced Ca^{++} release as

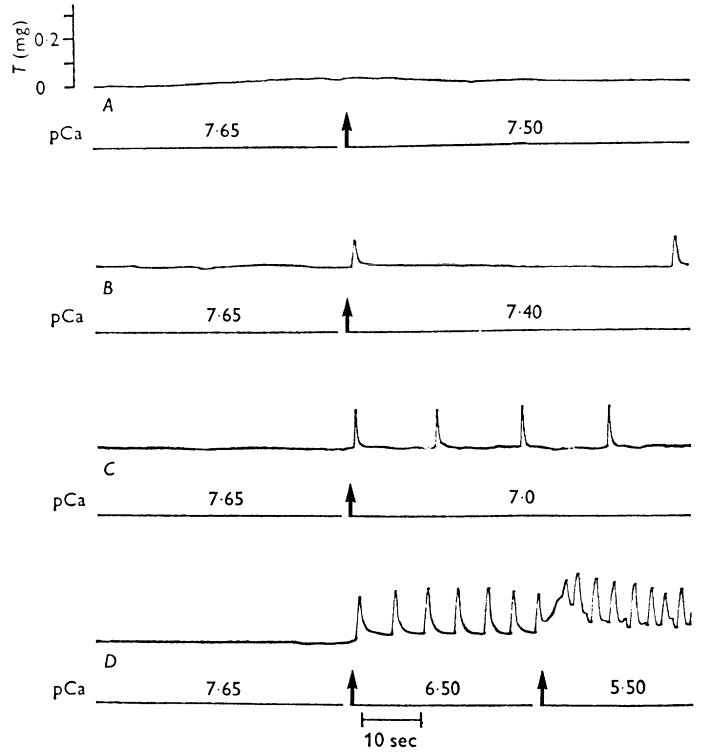

Figure 9. Effect of decreasing the trigger pCa on the amplitude and frequency of cyclic contractions in a skinned cardiac cell of 12 μM width and 42 μM length. Total EGTA was 0.050 mM, pMg was 3.50, pMgATP was 2.50 and pATP was 3.0. The arrows indicate the time of change of solutions containing different pCa. From Fabiato and Fabiato (41) with permission of the Journal of Physiology.

the physiological mechanism for cardiac contraction has been that the triggered release was presumably all-or-none, while contraction is graded as a function of the slow inward current. This all-or-nothing behavior would be expected for the following reason. If a small amount of Ca^{++} entering via the slow inward current were to cause some release of Ca^{++} from the sarcoplasmic reticulum, then this release would raise intracellular [Ca^{++}]. This increase in endogenous [Ca^{++}] would promote further Ca^{++} release, in a regenerative fashion. However, Fabiato and Fabiato [41] have shown that the size of the phasic contraction in the skinned fiber is directly dependent on the [Ca^{++}] to which the fiber is exposed (Figure 9). This finding suggests that the Ca-induced Ca^{++} release in cardiac muscle is a multiplier system, rather than a regenerative system. The way in which such a multiplier system would function remains obscure.

These studies in skinned fibers show three crucial characteristics necessary for the trigger concept of slow inward current in cardiac contraction. First, the tension is a sigmoid function of log $[Ca^{++}]$ in the concentration range that one could expect to achieve by Ca^{++} release from the sarcoplasmic reticulum. Second, triggered release of sarcoplasmic reticulum can be induced by levels of $[Ca^{++}]$ that could be achieved by entry of Ca^{++} via the slow inward current, and in the presence of normal $[Mg^{++}]$. Third, the triggered contraction is scaled as a function of the triggering Ca^{++}, so that a proportionality could exist between the current and contraction. These three properties of the Ca-multiplier system make it a prime candidate for the mechanism of excitation-contraction coupling in heart muscle.

This impressive argument should not lead the reader to the conclusion that the excitaion-contraction coupling problem has been resolved for heart muscle. The methods and conditions of cardiac skinned fiber studies are very unnatural [51]. Triggered release of Ca^{++} is an attractive concept, but every effort must be made to test it thoroughly before it is accepted. For example, there exists no convincing evidence in isolated cardiac sarcoplasmic reticulum vesicles that this Ca-induced release property exists.

9. EVIDENCE THAT I_{SI} REPLENISHES STORES

The concept of an intracellular store of calcium is derived by analogy with skeletal muscle, but for heart muscle the store is thought to be variable. Although fast twitch skeletal muscle is resistant to removal of extracellular Ca^{++}, heart muscle is quite sensitive. Removal of Ca^{++} results in rapid disappearance of contractions, even though action potentials persist. Magnitude of the contraction is closely related to $[Ca^{++}]_o$. Although Ca^{++} could be involved in some indirect way with contractile regulation, the prevailing view is that the extracellular Ca^{++} is directly involved in activating contraction.

If some Ca^{++} enters the fiber with each beat [39], then to maintain a steady-state, the cell must extrude the same amount of Ca^{++} during that time. Beat-dependent entry of Ca^{++} is presumably via the slow inward current, although other mechanisms could participate. Ca^{++} efflux must be via some mechanism that can move Ca^{++} against a large electrochemical gradient. We have already considered the evidence that changes in the contraction usually take 8–10 beats to reach some new steady-state, suggesting that influx and efflux come into a new balance after that time. The concept of a variable store is based on these two factors: the sensitivity to extracellular Ca^{++} and the time (or number of beats) required to come to a new steady-state of contraction.

When ventricular muscle is stimulated after a long pause, the response to

the first stimulus is a very weak contraction [33, 52]. With repetitive stimulation using either action potentials or voltage steps, the contraction will increase over the usual 8–10 beats (a positive tension staircase) to its steady-state value for those conditions. Beeler and Reuter [33] found that the first beat of the train at 0.3 Hz showed a slow inward current, and this current did not change during the positive tension staircase. The same phenomenon occurred if they suddenly changed the magnitude of the depolarization. The current would change with the first step of the new train, but the contraction required 8–10 beats to reach its steady-state level. This behavior of ventricular muscle has been amply confirmed by New and Trautwein [28] and by Morad and Goldman [26]. The interpretation of this phenomenon by these investigators involves a series of steps. First, the process maintaining low intracellular Ca^{++} (presumably the sarcoplasmic reticulum, but perhaps the Na–Ca exchange system) reduces Ca^{++} to a low value. With the onset of depolarization steps, Ca^{++} entry gradually replenishes the intracellular store. Finally, a balance is achieved between entry and exit of Ca^{++}. The behavior of staircase events in ventricular muscle is variable, according to this view, and it depends on the balance between entry and exit of Ca^{++}.

Purkinje fibers are somewhat different in their staircase properties [38]. The first contraction after a rest is larger than the second one. Thereafter, the contractions become smaller or larger, depending on the condition of the experiment. Note that others have described similar behavior in kitten atrial-muscle [53] and rabbit ventricular muscle [54]. The important point in this case is not that different tissues show different behavior, but that during a voltage-clamp train there are identical voltage conditions. Under these conditions Gibbons and Fozzard [27] found that the slow inward current was somewhat less in the second step of a train (the frequency of steps was faster than that of Beeler and Reuter), and the current remained constant thereafter. In spite of the constancy of voltage and of current, the contraction changed over 8–10 beats to a new steady-state level. The most straightforward explanation for this is that the balance between influx of Ca^{++} and its exit was such that the amount in the intracellular store was altered. Whatever the process, it led to progressive enhancement of the steady state size of contraction up to depolarizing steps of $+40$ mV, even though the initial contraction with a $+40$ mV step was less than those for a less inside-positive step. In addition, the phenomenon was dependent on the prior holding potential. When trains were made from more depolarized holding potentials, the staircase phenomenon in Purkinje fibers disappeared.

The interpretation of these experiments as a change in the quantity of calcium in an intracellular store is only an inference, since it assumes that the other steps in excitation-contraction coupling and contraction are not changed. Several alternative possible explanations for the frequency depen-

dence of contraction will be mentioned. Repetitive stimulation could alter events in the contractile sequence beyond Ca^{++} release, so that contraction changes. There could be a change in other intracellular Ca^{++} buffers, such as the mitochondria, which might take up more or less of the released Ca^{++} with subsequent beats [55]. The affinity of troponin C for Ca^{++} could be altered, so that the same amount of Ca^{++} release would result in more or less Ca^{++} binding. Or the effect of stimulation could directly influence the contractile proteins themselves. Many metabolic changes accompany a contraction. ATP is used, so that its concentration falls concomitant with a rise in ADP. Anerobic glycolysis leads to a change in pH, which is known to affect the contractile proteins. Various phosphorylation reactions take place in muscle on stimulation, and the role of these reactions in the staircase phenomenon is unknown.

The repetitive stimulation could affect the Ca^{++} release mechanism itself, so that the changes are not related to the size of the store but to a fractional release of the store. The sarcoplasmic reticulum could have an altered affinity for Ca^{++}, binding it before it can reach the contractile proteins. Other intracellular ions could be altered by repetitive stimulation, and secondarily influence contraction. For example, the concentration of Mg^{++} determines the rate of many of the crucial chemical reactions. Repetitive depolarization causes a loss of intracellular K^+ and a gain of intracellular Na^+.

If these other mechanisms are important for staircase, then they must be very sensitive to the conditions. Voltage factors influencing staircase include the resting potential, the magnitude and the duration of depolarization, the frequency of stimulation, and a host of other factors. Resolution of this problem will await the development of techniques to monitor intracellular free Ca^{++}, the Ca–troponin complex, and other known steps beyond the trigger for contraction.

10. SYNTHESIS

The two important questions about excitation-contraction coupling are: *How is a contraction triggered?* and *How is the size of the contraction controlled?* The steps in each will be summarized and the best explanation for the step described. The evidence supporting or challenging these explanations has already been presented. While this picture of excitation-contraction coupling is unlikely to be completely correct, it provides a coherent hypothesis for further testing.

Triggering of a normal contraction requires membrane depolarization, either with an action potential, a voltage clamp, or a high-K^+ depolarization. Vol-

tage threshold is between -55 and -30 mV, depending on the tissue and the conditions. High external Ca^{++} and negative resting potential favors a more negative threshold. Above threshold the contraction in mammalian cells follows a rather constant time course.

Threshold for contraction is also threshold for slow inward current. All investigators agree that development of the slow inward current is essential for contraction to occur. The slow inward current allows a little Ca^{++} to enter the cell. The amount of Ca^{++} entering is about 10% of the amount needed to produce the observed contraction. The slow inward current can occur without observable contraction under special circumstances.

Contraction is triggered by the slow inward current via a Ca-generated Ca^{++} release from the sarcoplasmic reticulum. The mechanism of this phenomenon is not understood, and it has not been reproduced in preparation of isolated sarcoplasmic reticulum vesicles. The process may be to permit a change in permeability of the sarcolemmal–terminal cisternae junction. In intact preparations this would lead to a depolarization of the sarcoplasmic reticulum, and that event could not be reproduced in isolated vesicles. In summary, the action potential triggers a slow inward current. This allows Ca^{++} entry, which triggers release of Ca^{++} from the terminal cisternae of the sarcoplasmic reticulum. This Ca^{++} initiates the chemical events of contraction, which follow a more-or-less constant time course.

The size of contraction is determined by the amount of Ca^{++} in the terminal cisternae and the fraction of this amount that is released. The fraction released is related to the size of the slow inward current in a nonlinear way, so that more current tends to release more Ca^{++}. Therefore, the Ca^{++}-triggered Ca release is a nonlinear multiplier, rather than a regenerative process.

The amount of Ca^{++} in the terminal cisternae (or in a releasable form) is the result of a balance between influx via the slow inward current and efflux via the Na–Ca exchange mechanism. Loading of the Ca^{++} store is determined by the size of the slow inward current, the fraction that is carried by Ca^{++}, and the electrochemical driving force on Ca^{++}. Efflux of Ca^{++} via the Na–Ca exchange system depends on the Na^{+} gradient. It is not yet clear if the Na–Ca exchange also is voltage-dependent, but membrane potential may be important.

In resting ventricular muscle the Na–Ca exchange appears to be effective in lowering the Ca^{++} store to a small value. Consequently, the first depolarization after a rest produces a normal slow inward current, but little Ca^{++} is available for release. Typically the positive staircase requires 8–10 depolarizations before the store is in a steady-state. In resting Purkinje fibers the Ca^{++} store is well-filled, so that the first depolarization produces a large contraction. The second contraction is less because of partial recovery of the slow inward

current. Subsequently there is a positive or a negative staircase that depends on the size of the slow inward current.

In summary the action potential provides a standard trigger for Ca^{++} release, but the contraction depends on the size of the Ca^{++} store. This is dependent on the resting potential, the frequency of stimulation, the duration of depolarization, and the transmembrane Na^{+} gradient. The steady-state cardiac contraction is the result of the balance between these factors. The slow inward current triggers contraction, and loads the cellular Ca^{++} store for future contractions.

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9. EFFECTS OF NEUROTRANSMITTERS ON THE SLOW INWARD CURRENT *

HARALD REUTER

1. INTRODUCTION

Although beating of the heart is in principle independent of nervous inputs, stimulation of sympathetic and parasympathetic nerves modifies cardiac function. Release of noradrenaline from sympathetic nerve endings causes acceleration and increase in force of the heartbeat, while the parasympathetic neurotransmitter, acetylcholine, has opposite effects. Much evidence has accumulated that a major site of these neurotransmitter actions is the plasma membrane. Not only are the respective receptors located on the membrane surface [1–4], but neurotransmitter–receptor interactions also cause changes in the ion permeabilities of the membrane. These membrane effects may not be a *direct* consequence of the neurotransmitter–receptor reaction, but are thought to be linked to changes of the cyclic nucleotide content of the cell [1, 3, 5–7]. Prominent, possibly cyclic nucleotide mediated, effects of catecholamines and of acetylcholine concern alterations of the membrane permeability to calcium ions. An increase in the calcium permeability by catecholamines and a reduction by acetylcholine has been measured with tracer methods as well as with electrophysiological techniques. The regulation of the calcium permeability of the cardiac cell membrane by neurotransmitters is of primary importance for the function of the heart [6, 8, 9]. Therefore, it is not surprising that considerable attention has been paid to this problem. This article reviews the main experimental evidence concerning the regulation of voltage-dependent calcium channels by β-adrenergic and cholinergic agonists. Voltage clamp results will be emphasized, since they permit the most straightforward interpretation of neurotransmitter actions on cardiac cell membranes.

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2. ^{45}Ca FLUX MEASUREMENTS

2.1. Catecholamines

First evidence for an increase in the calcium permeability of cardiac cell membranes by catecholamines came from ^{45}Ca flux measurements in isolated cardiac preparations [10–16]. Several important points have been established by these studies (see Figure 1):

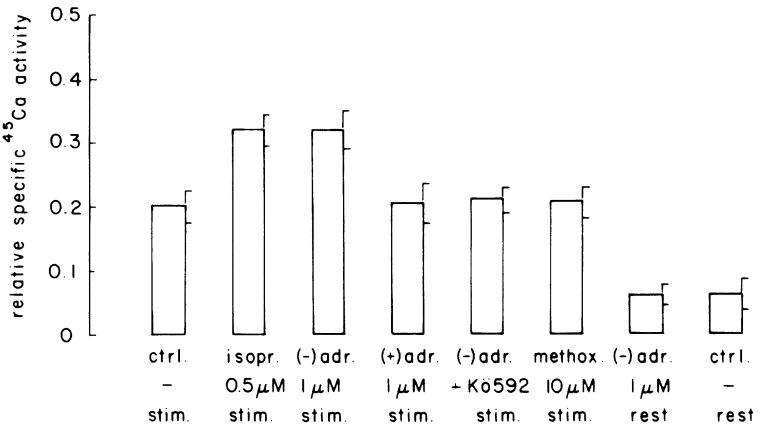

Figure 1. Effects of isoproterenol, (−)-adrenaline, (+)-adrenaline, (−)-adrenaline + β-blocker Kö 592 (1-(3-Methylphenoxy)-2-hydroxy-3-isopropylamino-propan), methoxamine on ^{45}Ca uptake (relative specific ^{45}Ca activity in muscle after 5 min incubation periods) in stimulated (stimulation 3 Hz) and resting (rest) guinea pig auricles; ctrl = controls. (Data from [13] and [14].)

(a) Catecholamines increase ^{45}Ca influx and efflux in beating, but not in resting preparations [11, 12, 16] indicating that calcium exchange is increased only during excitation.
(b) β-Adrenergic, but not α-adrenergic (methoxamine) agonists increase ^{45}Ca influx [14]. The effect is stereospecific for (−)-isomers [14]. β-Adrenergic antagonists inhibit the catecholamine effect on ^{45}Ca uptake [13]. These studies show that an increase in calcium permeability by catecholamines is a specific β-adrenergic effect.
(c) Dibutyryl cyclic AMP [16] and the phosphodiesterase inhibitor theophylline [17], though less potent, mimic the catecholamine effects on ^{45}Ca uptake.

These results are in agreement with the hypothesis that the β-adrenergic effect on the calcium permeability of cardiac sarcolemma may be somehow mediated by an increase in intracellular cyclic AMP levels [14, 18, 19]. How-

ever, the tracer flux studies have several shortcomings. They are difficult to quantitate and give no information as to during which phase of the cardiac action potential the enhanced calcium influx occurs. They give no answer to the question of *how* β-adrenergic agonists regulate the calcium permeability of the cell membrane. Finally, they present little evidence concerning the relation between the change in the free calcium ion concentration in the myoplasm and the increase in force of contraction.

2.2. Cholinergic agonists

Acetylcholine greatly diminishes ^{45}Ca uptake in beating but not in resting guinea pig auricles [12, 20]. This inhibition is antagonized by atropine [12] indicating the involvement of muscarinic receptors. However, the interpretation of this acetylcholine effect is not straightforward. It has been known for a long time that acetylcholine greatly shortens the action potential duration [21–23] (Figure 3) as a result of an increase in the potassium permeability. The reduced ^{45}Ca uptake has been attributed to the shortening of the action potential, rather than to a genuine decrease of the calcium permeability of the cardiac cell membrane by this neurotransmitter [12, 20]. However, voltage clamp analysis (see below) has shown that acetylcholine, in addition to causing a large increase in the potassium permeability [22, 23–25], inhibits calcium influx. Both effects probably contribute to the shortening of the action potential. It is of interest to note that 8-bromo-cyclic GMP seems to mimic the effect of acetylcholine on ^{45}Ca uptake, but not on ^{42}K efflux [7].

3. ACTION POTENTIALS AND 'SLOW RESPONSES'

3.1. Catecholamines

A prominent and reproducable effect of β-adrenergic agonists on cardiac action potentials is the elevation of the plateau height (Figure 2) [1, 8, 26–28]. Whether this is accompanied by a prolongation or a shortening of the action potential duration depends on experimental conditions such as external potassium and calcium concentrations [29, 30] and rate of stimulation [31].

Another important electrophysiological effect of catecholamines is the increase in steepness of pacemaker potentials in spontaneously active cardiac tissue (sinus node, atrioventricular node, Purkinje fibers; for review see [32]). Both are β-adrenergic effects. Extracellularly applied dibutyryl or monobutyryl cyclic AMP [1, 19] or intracellularly applied cyclic AMP [33, 34] mimic the catecholamine effects on the action potential plateau.

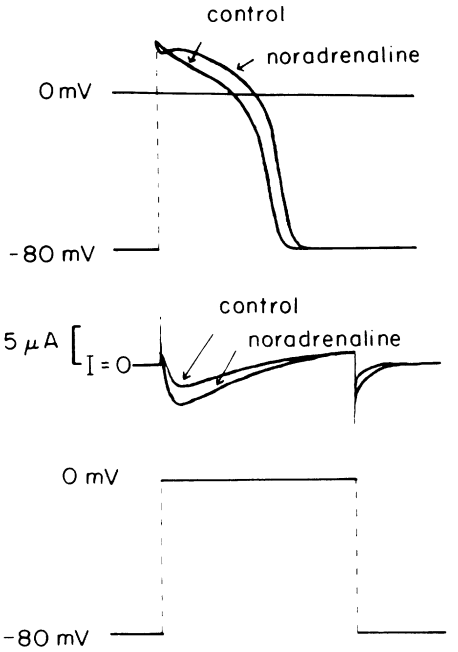

Figure 2. Two superimposed action potentials (top traces) recorded from a calf ventricular trabecula under control conditions and in the presence of noradrenaline (10^{-6} M), and two corresponding membrane currents (middle traces) without and with noradrenaline measured in the same experiment during voltage clamp steps (bottom trace) from a holding potential −80 mV to 0 mV. The initial sodium current was blocked in the voltage clamp experiment by 5×10^{-5} M tetrodotoxin. Note increase in I_{SI} (downward deflection of current traces from I = 0) and corresponding increase in plateau height in presence of noradrenaline. (For method see [58].)

Until 1966 a common explanation for the effect of catecholamines on the plateau of the action potential was a possible increase in driving force for inward sodium current by activation of the sodium pump [26, 29, 35, 36]. The large increase in amplitude, particularly of attenuated action potentials, by catecholamines was explained by the same mechanism [23, 26, 29, 36]. It was puzzling, however, why the prominent effect on the action potential amplitude was accompanied by only little change in the maximal rate of rise ($\dot{V}_{max}$) of the upstroke [35]. In other words, it seemed as if the action potential *plateau* was rather selectively influenced by β-adrenergic drugs. This effect was demonstrated most clearly in partially depolarized cardiac tissue which had a resting potential around −50 mV, e.g., during potassium depolarization [29]. Later on these slow, catecholamine induced action potentials were often called 'slow responses.'

In 1966 three independent studies [37–39] suggested that Ca^{++} ions may carry an appreciable fraction of the total inward membrane current during the

plateau of the cardiac action potential. It was also shown in one of these papers [38] that, in the absence of external sodium, adrenaline greatly increased calcium-dependent regenerative responses in Purkinje fibers. This effect has been confirmed for a variety of cardiac tissues [40–44]. It was explained by an adrenaline induced increase in the calcium permeability of the cell membrane, an interpretation that was supported by the ^{45}Ca flux studies and, later on, by voltage clamp experiments.

It must be noted, however, that the interpretation of such calcium-dependent 'slow responses,' and particularly of drug effects on these action potentials, is far from being straightforward. Both an increase in inward current and a decrease of outward current could lead to a larger regenerative response. A definite answer to this question can only be provided by voltage clamp analysis.

3.2. Cholinergic agonists

The most prominent effects (see Figure 3) of acetylcholine or carbachol on action and pacemaker potentials of various cardiac tissues include shortening of the action potentials [21, 22, 32, 45], hyperpolarization [22, 32, 46] and flattening of pacemaker potentials [32]. These are muscarinic effects, and in

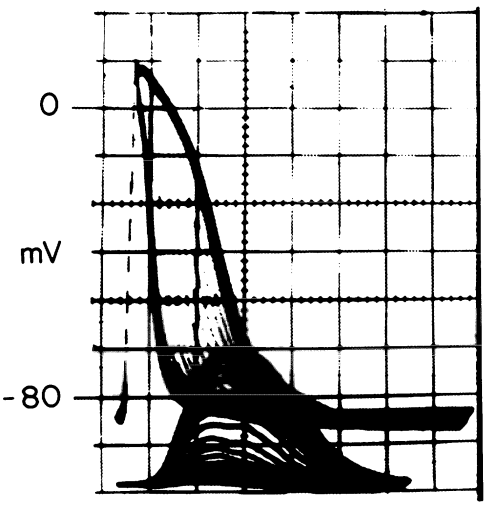

Figure 3. Effects of acetylcholine (0.5 μM) on a series of subsequent action potentials and on force of contraction recorded from a guinea pig auricle. Longest action potential and largest force of contraction are controls, while shortening of action potentials and reduction in force occur during application of acetylcholine. Note hyperpolarization (thickening of trace at resting potential) in presence of acetylcholine. (Modified from [45].)

mammalian hearts they are much more pronounced in atrial than in ventricular tissue [32]. The cholinergic actions on electrical properties of the heart were conveniently explained by the clear demonstration of an increase in the potassium permeability [32, 47]. Therefore, the suggestion that acetylcholine could reduce the slow inward calcium current [48] was somewhat surprising, and clear evidence for this effect came only from voltage clamp results. Although calcium-dependent slow responses can be suppressed by acetylcholine or carbachol [49], this effect can be explained either by an increase in outward potassium currents, a decrease in slow inward current, or both.

Some cholinergic effects could be mimicked by extracellularly applied 8-bromo-cyclic GMP [7] or after intracellular application of cyclic GMP [50].

4. VOLTAGE CLAMP RESULTS

4.1. Catecholamines

In the first paper that demonstrated a calcium-dependent slow inward current [27], adrenaline was shown to increase the size of this current component. Moreover, a substantial increase in the plateau height of cardiac Purkinje fibers by adrenaline, first observed by Otsuka [26], was related to the increase in the slow inward current [27]. Thereafter this relation has been confirmed under a variety of experimental conditions ([1, 19, 51, 52]; Figure 2). What is the underlying mechanism for the observed increase in the calcium-dependent slow inward current? In principle changes of ionic currents of cell membranes can occur by 4 mechanisms: (a) the driving force, i.e., the difference between membrane potential and equilibrium potential for a given ion species ($E_m - E_R$), may be altered; (b) the gating properties of the ion channels, and hence the kinetics of the ionic current, could be changed; (c) the number of functional ion channels may be variable; (d) the conductance of single ion channels may be changed. The only possible way to distinguish between these mechanisms is the use of the voltage clamp technique. (For various voltage clamp techniques applied to cardiac muscle see reviews in [53–55]). The voltage clamp technique permits rapid and controlled changes of membrane potential while the ionic currents flowing across the membrane as a result of this potential change are being recorded.

According to the Hodgkin-Huxley formulation of membrane ionic currents [56] the slow inward current (I_{SI}) can be described (see [55]) as:

$$I_{SI} = \bar{g}_{SI} \cdot d(V, t) \cdot f(V, t) \cdot (E_m - E_R). \tag{1}$$

$\bar{g}_{SI}$ is the average limiting conductance of membrane ionic channels responsible for carrying I_{SI}. Provided the conductance of a single ion channel is

constant, $\bar{g}_{SI}$ is a measure of the number of channels that open upon strong depolarization. The dimensionless kinetic parameters, d and f, reflect the voltage- and time-dependent opening, closing and inactivation properties, i.e., gating, of the channels. d_∞ determines the fractional number of open channels in the steady state, while f_∞ indicates the steady-state inactivation of these channels at any given voltage. Finally, the driving force for ions moving through these channels is given by the potential difference $(E_m - E_R)$, where E_m is any membrane potential, while E_R is the reversal potential of I_{SI}. Measurements of E_R can be used to determine the ion selectivity of the membrane channels carrying I_{SI} [56]. Reuter and Scholz [58] have estimated that these ion channels are approximately 100 times more selective for calcium ions than for sodium or potassium ions and therefore can be named 'calcium channels.' It is worth mentioning that in some cardiac tissues (frog atrial trabeculae, guinea pig or cat papillary muscles, Purkinje fibers), where outward currents are prominent and are also affected by β-adrenergic drugs, the kinetic analysis of I_{SI} may be complicated.

Figure 2 shows the increase of I_{SI} by noradrenaline in a mammalian ventricular myocardial fiber. This effect is β-adrenergic, since it can be blocked competitively by β-adrenergic blocking agents, and can also be

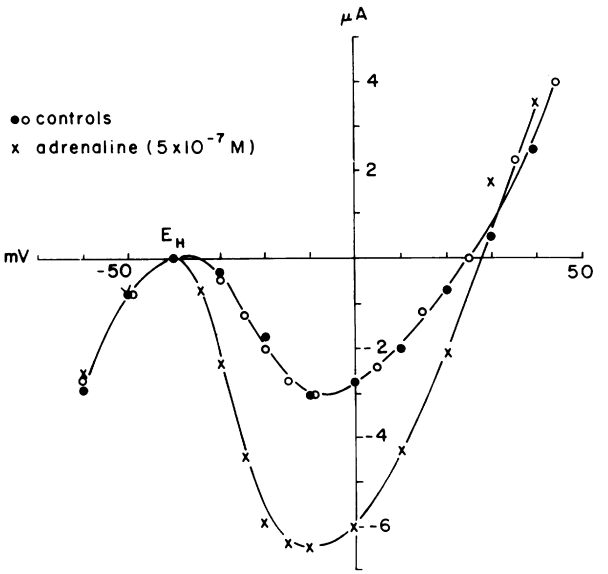

Figure 4. Current–voltage relations of peak inward or initial (10 msec after beginning of clamp steps) outward current in absence (controls) and presence of adrenaline (0.5 μM). Peak I_{SI} (negative range of current scale on the ordinate) is reversibly increased by adrenaline. Holding potential, E_H, was set to -40 mV in order to inactivate initial sodium current. (Data obtained from cat papillary muscle; For method see [58].)

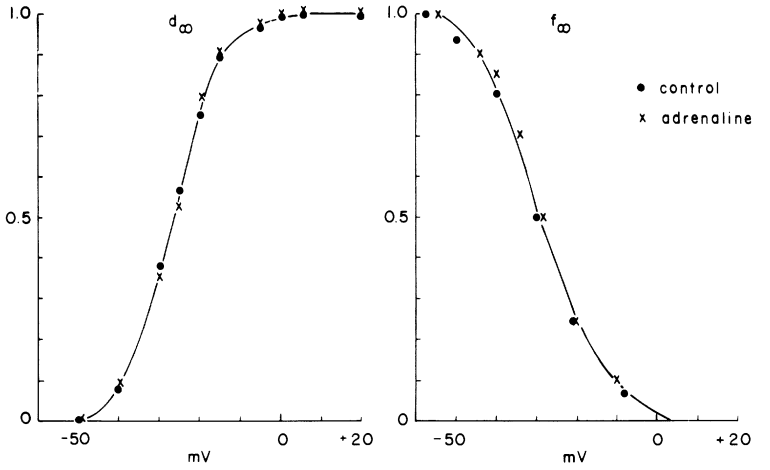

Figure 5. Voltage ranges (abscissa) of steady state activation (d_∞) and inactivation (f_∞) of I_{SI} in the absence (control) and presence of adrenaline (0.5 μM) (Data obtained from cat papillary muscle; for method see [58].)

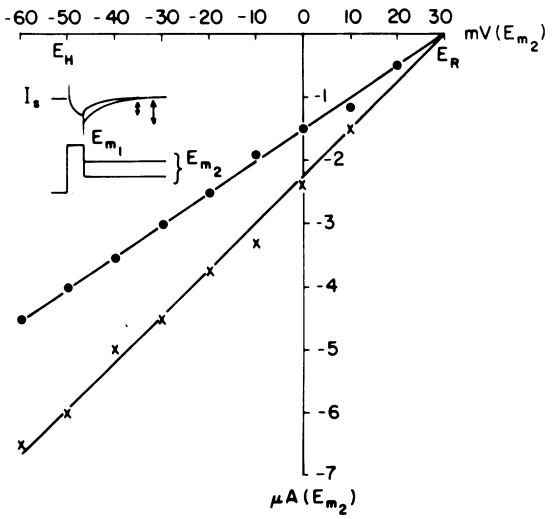

Figure 6. Instantaneous current–voltage relations of I_{SI} measured in a cow ventricular trabecula in the absence (filled circles) and presence (crosses) of adrenaline (10^{-6} M). Experimental protocol (see insert): in a first voltage clamp step the membrane potential was always depolarized from the holding potential ($E_H = -50$ mV) to the same membrane potential E_m (+10 mV); in a second step the potential was clamped to various membrane potentials E_{m2} (plotted on the abscissa); the amplitudes of the tail currents (I_{SI}) at various values of E_{m2} are plotted on the ordinate. Note that the reversal potential E_R is identical in the absence and presence of adrenaline, although the conductance (slope of the lines) is increased by adrenaline. (For method see [58].)

obtained with the pure β-adrenergic agonist isoproterenol. A plot of current–voltage relations in the absence and presence of adrenaline is shown in Figure 4. The holding potential, E_H, indicates the membrane potential from which voltage clamp steps were applied to different levels (abscissa). Peak inward currents (negative current on the ordinate) or minimal outward currents (positive current) at each potential before (open circles), during (crosses) and after (closed circles) adrenaline have been plotted on the ordinate. It is clear from Figure 4 that I_{SI} is greatly and reversibly increased by adrenaline in the voltage range -40 to $+30$ mV. Effects on I_{SI} similar to that illustrated in Figure 4 have been obtained with the phosphodiesterase inhibitor theophylline and butyrate derivatives of cyclic AMP [1, 19, 59]. Therefore, the voltage clamp experiments are qualitatively in good agreement with the tracer experiments discussed before, in showing a marked increase in calcium influx by substances which enhance intracellular cyclic AMP levels.

However, the voltage clamp experiments also provide a clue regarding the possible mechanism of regulation of the calcium permeability of cardiac cell membranes by catecholamines. A kinetic analysis of I_{SI} [1, 60] revealed that neither the steady-state kinetics of d and f (Figure 5) nor their voltage-dependent time constants [1, 60] are altered by β-adrenergic agonists or by dibutyryl cyclic AMP [1]. In addition, the reversal potential, E_R, is unaffected by the drugs ([60], Figure 6). This indicates that neither the selectivity of the calcium channels nor the driving force are altered. In conclusion then, the only factor in equation 1 that is affected by catecholamines is $\bar{g}_{SI}$. The increase is steepness of the slope of the instantaneous current–voltage relation by adrenaline in Figure 6 is a direct measure of this increase in $\bar{g}_{SI}$.

As discussed before, $\bar{g}_{SI}$ is interpreted as reflecting the maximal number of calcium channels that open upon strong depolarization, provided the conductance of a single channel stays constant. Therefore, the most likely explanation of the β-adrenergic neurotransmitter effect on the calcium permeability of cardiac cell membranes is an increase in the number of functional calcium channels. The possibility that the conductances of individual calcium channels are increased by these neurotransmitters, though it cannot at present be totally excluded, is highly unlikely. It is difficult to see how the single channel conductance could be increased up to 3- or 4-fold without loss of the ion selectivity of the channels. A change in ion selectivity, however, should have been observed by a shift in E_R.

4.2. Cholinergic agonists

Acetylcholine or carbachol decrease I_{SI} in frog [61, 62] and mammalian [63] atrial trabeculae. This is a muscarinic cholinergic effect since it can be inhibited by atropine. However, while the reduction in I_{SI} seems to be particularly

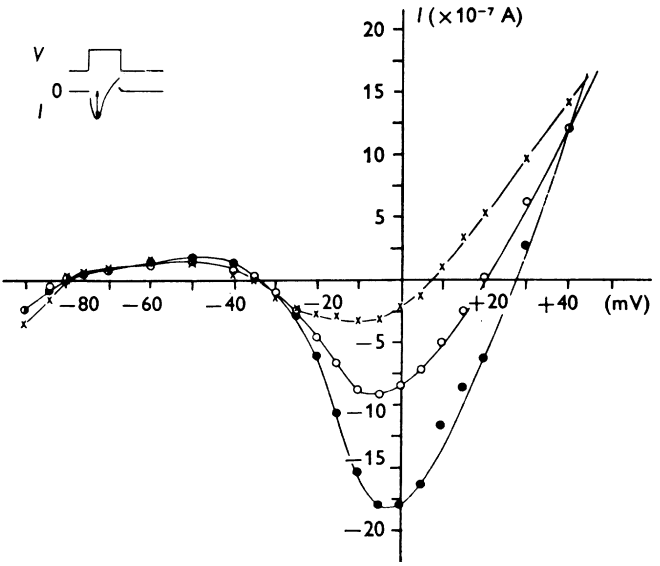

Figure 7. Current–voltage relations of peak inward or initial outward current in absence (filled circles) and presence of acetylcholine (open circles 0.03 μM; crosses 0.12 μM). Peak I_{SI} (negative range of currents on the ordinate) is greatly reduced by acetylcholine. The initial sodium current was blocked by tetrodotoxin. Data obtained from bullfrog atrial trabecula by Giles and Noble (reproduced with permission from [61].)

prominent and occurs with very low concentrations of the cholinergic agonists in frog trabeculae [61, 64], it can be seen only with higher concentrations in mammalian atrial tissue [63]. In the latter tissue the most prominent effect of muscarinic agonists is on outward potassium currents [24–46]. This probably also accounts to a large extent for the shortening of the atcion potential in Figure 3. Nevertheless, the decrease of I_{SI} by the cholinergic neurotransmitter is of great interest and probably of functional importance, since it is exactly opposite to the effect seen with adrenergic neurotransmitters. Figure 7 shows an experiment by Giles and Noble [61] on a bullfrog atrial trabecula. The slow inward current was measured in the presence of tetrodotoxin which eliminated the fast initial inward sodium current. Acetylcholine was applied at concentrations of 0.03 and 0.12 μM. The current–voltage plot in Figure 7 of peak inward or initial outward current (see insert) indicates that, compared to the controls (filled circles), the lower and higher acetylcholine concentrations (open circles and crosses) greatly reduce I_{SI} over the voltage range -40 to $+40$ mV while the outward current over the voltage range -90 to -40 mV is remarkably constant. The reversal potential, E_R, of I_{SI} can be estimated from the crossover of the current–voltage relations around $+40$ mV. There may be

a minor (3–4 mV) shift of E_R towards more positive potentials with the higher acetylcholine concentration. This small effect is certainly within the error of the resolution of E_R by the voltage clamp technique used (double sucrose gap technique). Moreover, it is in the wrong direction to account for the reduction of I_{SI}. A likely explanation for the muscarinic cholinergic effect on I_{SI} is a reduction in $\bar{g}_{SI}$ which would be opposite to the β-adrenergic effect. However, a detailed kinetic analysis of I_{SI} under the influence of cholinergic drugs needs still to be done.

5. CONCLUSIONS

The analyses of β-adrenergic and muscarinic cholinergic neurotransmitter actions on cardiac cell membranes revealed prominent effects on the voltage-dependent calcium permeability of these membranes. Qualitatively there is satisfactory agreement between ^{45}Ca flux measurements and electrophysiological, notably voltage clamp, results.

The latter results indicate that it is the number of functional calcium channels that is regulated by neurotransmitters. A third piece of information, that fits into the picture of β-adrenergic drug actions on the calcium permeability of cardiac cell membranes, was obtained with aequorin measurements by Allen and Blinks [65]. Aequorin is a bioluminescent protein from the jelly-fish *Aequorea forskålea*. An increase in luminescence can be measured with small increases in cytoplasmic free calcium ion concentrations when the protein is injected into cells. Such an effect was observed by Allen and Blinks when frog atrial trabeculae, injected with aequorin, were electrically stimulated in the presence of isoproterenol. The increase in luminescence by isoproterenol, reflecting an increase in the free calcium ion concentration during stimulation, was accompanied by an increase in force of contraction. The extra calcium required for the positive inotropic effect of isoproterenol in frog cardiac muscle flows into the cell during the cardiac action potential [66]. Therefore, the aequorin result agrees with the ^{45}Ca flux measurements and with the electrophysiological data. In addition, it provides the information that the rise in the free cytoplasmic calcium concentration by isoproterenol may cause the positive inotropic effect. In the mammalian heart the situation is complicated by the presence of sarcoplasmic reticulum (SR) as a calcium store. Calcium uptake into the SR is accelerated by cyclic AMP [67–69]. Therefore, the increase in calcium influx during the cardiac action potential by catecholamines together with the accelerated uptake provides for a better filling of the sarcoplasmic reticular calcium stores. More calcium ions can be released from the SR during subsequent beats and thus cause an increase in force of contraction (see reviews in [6, 69]).

Finally one may ask the question, which molecular events are involved in the regulation of calcium channels of cardiac cell membranes by neurotransmitters? The following hypothesis (Figure 8) would be in agreement with the

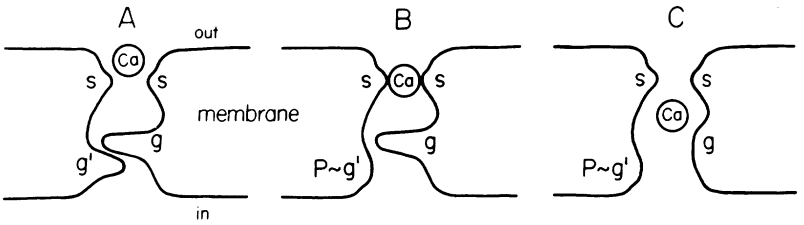

Figure 8. Hypothetical scheme for regulation of calcium channels in cardiac muscle. The channel contains a filter determining the calcium selectivity (s), and two gates (g and g'); g is the voltage-dependent gate governing voltage-dependent activation and inactivation of the channels; g' is a phosphorylation-dependent voltage-independent gate. Phosphorylation of g' may result from a cyclic AMP-dependent protein-kinase reaction; dephosphorylation may depend on a phosphatase. A: without phosphorylation g' is closed and channels are not available for voltage-dependant calcium flow. B: channels are available for voltage-dependent gating, but non-conducting when g' is phosphorylated (P~g') and g is closed. C: phosphorylated channels conduct (i.e., calcium ion flow occurs) when g opens during depolarization of the membrane. (Reproduced with permission from [55].)

experimental results. Calcium channels, like other ion channels [56, 57, 70], could have a selectivity filter (s) and a voltage dependent gate (g). In addition it may have another gate, g'. The gate g' could be phosphorylated via a cyclic AMP dependent protein kinase [71, 72]. Only in the phosphorylated state is the calcium channel available for voltage dependent ion flow. Hence the number of functional calcium channels depends on the cyclic AMP level in the cell. β-Adrenergic agonists, phosphodiesterase inhibitors and cyclic nucleotide derivatives increase cyclic AMP in the cell. Therefore, these drugs, acting via cyclic AMP dependent protein kinase, could shift some additional fraction of the total number of calcium channels in the membrane from state A to state B in Figure 8. Consequently more channels could be opened by voltage dependent gating, and $\bar{g}_{SI}$ should increase. Cholinergic muscarinic agonists are known to decrease cyclic AMP and to increase cyclic GMP in cardiac cells [69]. This could cause a shift of calcium channels from state B to state A in Figure 8, and hence the observed reduction in $\bar{g}_{SI}$ would be expected. It will be interesting to see whether in the future a combination of biochemical and biophysical methods will confirm or refute this hypothesis. One also wonder whether a similar regulation of calcium channels by neurotransmitters occurs in other excitable membranes [73].

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10. CHANGES IN MEMBRANE ELECTRICAL PROPERTIES DURING DEVELOPMENT OF THE HEART *

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1. INTRODUCTION

Important changes occur in myocardial cells during embryonic development, including ultrastructural, metabolical, pharmacological, and electrophysiological changes. For example, striking changes occur in the electrical properties of ventricular myocardial cells during embryonic development of chick heart. The electrical properties at each stage of development determine many of the functional properties of the heart at that stage. Studies on the electrophysiological properties of embryonic heart cells are useful, not only to elucidate the changes during differentiation, but also to obtain clues for understanding the complex electrophysiology of adult hearts.

In this chapter, many of the important facts concerning changes in the properties of the heart during development will be reviewed. Most of the data presented are for the chick, although some data are presented for developing mammalian hearts. Some findings on organ-cultured hearts and cultured heart cells will be presented to compare and illustrate related phenomena. Since the electrical properties may be affected by morphological, biochemical and pharmacological properties, relevant changes in these properties will also be discussed.

2. INTACT HEARTS DEVELOPING IN SITU

2.1. Precardiac areas of the blastoderm

The early embryo (up to 1 somite) possesses cells which are destined to form the heart. These cells migrate through the early embryo and congregate bilaterally into the so-called precardiac areas (mesoderm) of the anterior half

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of the flattened 16–17 h (head process) blastoderm [1]. Explants of these areas develop spontaneous electrical activity after several days in culture, which include spontaneous action potentials of about 50 mV amplitude [2]. In culture, a tubular heart develops within a vesicle and it beats spontaneously for several days, but further differentiation does not proceed *in vitro*. The precardiac area can be treated with trypsin to facilitate mechanical separation of the three germ layers, and culture of the precardiac mesoderm gives rise to a solid mass of cells which fire spontaneous action potentials and contract [3]. If the postnodal piece (posterior third of the blastoderm) is dissected from the 19-h chick blastoderm and placed into culture, it does not normally give rise to heart tissue. If, however, the postnodal piece is cultured in the presence of an RNA-enriched fraction obtained from adult chicken hearts, a typical spontaneously beating tubular heart forms within a vesicle, as in the case of the precardiac areas described above [4]. The beating cells in the induced hearts were shown to possess some myofilaments. In addition, the induced tubular hearts exhibit spontaneous action potentials [5]. Thus, it appears that either RNA or some other material within the extract obtained from the adult heart can induce cells in the postnodal piece, normally not destined to form the heart, to take on many of the properties of cardiac myoblasts. Subsequently in development, the twin tubular primordia formed bilaterally from the precardiac mesoderm fuse to form a single tubular heart. This fusion begins from the head end and proceeds posteriorly by a zipper-like process, such that the first region fused (ventricle) begins contracting first; the atria are added posteriorly later. The tubular heart begins contracting spontaneously at 30–40 h (9–19 somite stage). Cutting the 2-day heart into bulbus, ventricle and sinoatrium regions shows that each region has its characteristic automaticity, the sinoatrium being the fastest.

The tubular heart begins to propel blood shortly after it starts to beat. The blood pressure is very low at this stage (1–2 mm Hg) and it increases progressively during embryonic development (approximately 30 mm Hg by day 18) and increases further during postembryonic life [6, 7]. The velocity of propagation of the peristaltic contraction wave in 3-day hearts is approximately 1 cm/sec [7].

The heart rate of the chick embryo increases from about 50 beats/min at day 1.5 to the maximal value of about 220 beats/min by day 8; it has been suggested that the increase in beating rate may result from the concomitant rise in blood pressure [7]. The influence of temperature on heart rate decreases markedly during development [7]. The Q_{10} decreases from about 3.6 on day 3 to about 2.0 on day 18 for the same temperature range (McLean and Sperelakis, unpublished observations). Breaks in the Arrhenius plots also occur at different temperatures in young versus old hearts [7].

2.2. Resting membrane and K^+ permeability

2.2.1. Resting potentials

The transmembrane resting potential (E_m), measured by intracellular microelectrodes, of the ventricular portion of the chick and rat hearts increases during embryonic development [8–14]. In the case of the embryonic chick heart (Table 1), the greatest changes occur between days 2 and 7, and thereafter the increase is smaller. For example, in a 2-day-old heart, the mean resting potential is about −40 mV, and this increases to about −51 mV on day 3. The resting potential is close to −80 mV by day 12, nearly the final adult value. As discussed below, the large increase in resting E_m during the first few days may be due mainly to an increase in K^+ permeability (P_K) and not in K^+ equilibrium potential (E_K) (or $[K]_i$). However, some investigators [2] have reported larger values of resting E_m for the young hearts, i.e., less of a change during development. It is possible that low recorded potentials in young hearts are partly caused by current leakage around the electrode tip due to improper sealing of the microelectrode; this effect would be most prominent in cells having a high input resistance.

Table 1. Summary of data obtained from E_m vs. log $[K]_0$ curves and from input resistance (r_{in}) measurements for chick embryonic hearts (ventricular cells) at various stages of development.

Embryonic Age (days)	E_m *(mV)*	*Slope (mV/decade)*	*Extrapolated* $[K]_i$ *(mM)*	E_K *(mV)*	P_{Na}/P_K *ratio*	r_{in} *(mΩ)*
2	−40	30	125	−100	0.21	13.0
3	−51	40	130	−101	0.17	8.5
4	−57	46	140	−103	0.08	6.5
5–6	−58	50	130	−101	0.08	5.5
7–9	−71	51	145	−104	0.07	5.5
11–13	−80	53	145	−104	0.07	4.7
14–20	−78	52	155	−106	0.05	4.5

All values given are approximate. The resting potential (E_m) values are given for a $[K]_0$ of 2.7 mM. The slope is the average at $[K]_0$ levels between 10 and 100 mM. $[K]_i$ was estimated from the extrapolation of fitted curves to zero potential. The P_{Na}/P_K ratios were calculated from the Goldman constant-field equation at every $[K]_0$ level for which E_m was measured, and an average value was calculated for each heart; some individual values for hearts in the 14–20-day age group were as low as 0.02. Data were taken from Sperelakis and Shigenobu, 1972, and from Sperelakis et al., 1975.

Similar values for slope and $[K]_i$ were obtained by Pappano (1972) for embryonic chick atrium at 4 days, 6 days, and 12 days; the 18-day values were −59 mV/decade and 125 mM $[K]_i$, respectively.

2.2.2. Resting potential vs. log $[K]_0$ curves

The relationship between resting potential and external K^+ concentration ($[K]_0$) was determined for embryonic hearts of different ages [12, 13]. Data for 3-day, 5-day, and 15-day-old embryonic chick hearts are shown in Figure 1. $[K]_i$ was estimated by extrapolation of the curves connecting the data points

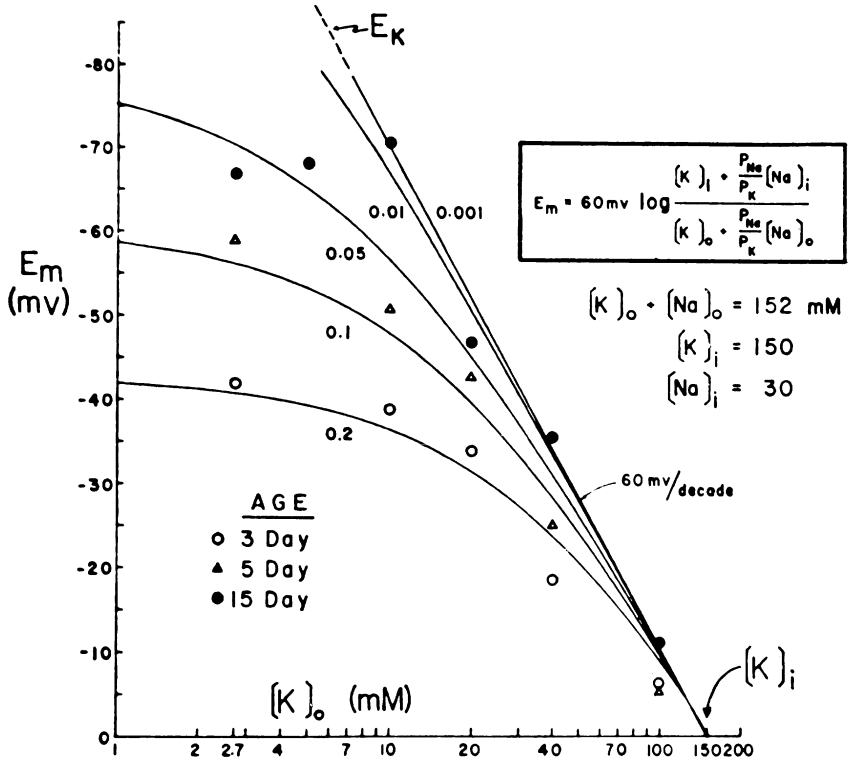


Figure. 1. Resting potential (E_m) plotted as a function of $[K]_0$ on a logarithmic scale for three representative hearts of different ages. $[K]_0$ was elevated by substitution of K^+ for equimolar amounts of Na^+. Continuous lines give theoretical calculations from the constant-field equation (inset) for P_{Na}/P_K ratios of 0.001, 0.01, 0.05, 0.1, and 0.2. Calculations were made assuming $[K]_i$ and $[Na]_i$ values shown. For a P_{Na}/P_K ratio of 0.001, the curve is linear over the entire range with a slope of 60 mV/decade, i.e., it closely follows E_K. Symbols give representative data obtained from embryonic chick hearts at days 3 (○), 5 (△) and 15 (●). The data for the 3-day heart follow the curve for a P_{NA}/P_K ratio of 0.2, those for the 5-day heart follow the curve for 0.1, and those for the 15-day heart fall between the curves for 0.01 to 0.05. The estimated intracellular K^+ activities ($[K]_i$) obtained by extrapolation to zero potential are nearly the same for all ages. (Taken from Sperelakis and Shigenobu, 1972.)

to zero potential, and the values varied between 125 mM (for 2-day hearts) and 155 mM (for 14–20-day hearts) (Table 1). Also plotted in Figure 1 are the theoretical curves (calculated from the Goldman constant-field equation given in the inset) for 5 different ratios of P_{Na}/P_K: 0.001, 0.01, 0.05, 0.1 and 0.2. As indicated in the figure, for these calculations, it was assumed that $[Na]_i$ was 30 mM and $[K]_i$ was 150 mM. It is seen that the data points for the 3-day heart most closely fit the theoretical curve for a P_{Na}/P_K ratio of 0.2; that for the 5-day heart fits the curve for a P_{Na}/P_K of 0.1, and that for the 15-day heart most closely fits between the 0.05 and 0.01 curves. These data suggest that the P_{Na}/P_K ratio is very high in young hearts, and that this accounts for

the low measured resting potential. That is, the low resting potential is not due to a greatly lower $[K]_i$ and E_K. As shown in Table 1, only a very small increase in the calculated E_K occurs during development: from about -100 mV on day 2 to -106 mV on days 14–20. Thus, in the young hearts, the resting potential is far from E_K due to the high P_{Na}/P_K ratio. In this respect, the myocardial cells in young embryonic hearts resemble SA nodal cells in adult hearts.

In old embryonic chick or adult hearts, the E_m vs log $[K]_0$ curve is nearly linear above 10 mM K^+, with a slope approaching the theoretical 60 mV/decade (from the Nernst equation). If the slope were exactly 60 mV/decade, then E_m would be equal to E_K, and the membrane would be completely K^+-selective in high $[K]_0$. The data in Figure 1 and Table 1 show that the slope for hearts 7–20 days old is 51–53 mV/decade, whereas the average slopes (curves continually bend) for 4-day, 3-day and 2-day hearts are 46, 40, and 30 mV/decade, respectively. Similar values for $[K]_i$ and slope were found for embryonic chick atrial cells at various stages of development [15].

2.2.3. Membrane resistance

In order to help determine whether the P_{Na}/P_K ratio is high in the young embryonic chick hearts because of a high Na^+ permeability or because of a low K^+ permeability, input resistance was determined from steady-state voltage–current curves. The input resistance (r_{in}) of the ventricular cells is high (13 MΩ) in young 2-day-old hearts, and rapidly declines over the next few days, reaching the final adult value of about 4.5 MΩ by day 14 (Table 1). If the average cell size and the degree of electrical coupling between the cells remains unchanged during this period, the high r_{in} of the young hearts would suggest that membrane resistivity (R_m) is very high. The latter would be consistent with a low K^+ conductance and K^+ permeability in the young hearts. These results would suggest that the P_{Na}/P_K ratio is high in young hearts because P_K is low, and not because P_{Na} is high.

Consistent with our interpretation that P_K is low in young hearts is the finding that the chronaxie of young hearts (2-day-old) is about 4-fold higher than that of 9–16-day hearts [8]. This indicates that the membrane time constant is about 4-fold higher in young hearts, and if membrane capacitance remains constant, membrane resistivity must be about 4-fold higher.

Carmeliet and coworkers [16] have reported, on the basis of ^{42}K flux measurements, that P_K is about 2–3-fold lower in 6–8-day hearts than in 18–20-day hearts, consistent with the conclusions from the electrical studies described above. (Although they reported that the calculated P_K for 3–5-day hearts was nearly as high as that for the 18–20-day hearts, which disagrees with the electrical measurements, they did not control for spontaneous beating and action potentials, hence increased K^+ efflux, which occurs in the 3–5-day

hearts, thus causing them to calculate an erroneously high P_K.) The values for the P_K coefficients were 13.2×10^{-8} cm/sec for 7-day hearts and about 27.5×10^{-8} cm/sec for 19-day hearts (at a $[K]_0$ of 2.5–5 mM). P_K was greatly reduced in 0 mM external K^+. The P_{Na}/P_K ratios calculated from the constant-field equation were 0.018 for the 19-day hearts and 0.037 for the 7-day hearts. The P_{Na} coefficient, as calculated from P_K and the P_{Na}/P_K ratio, did not change during development (constant at about 0.50×10^{-8} cm/sec).

2.2.4. Sensitivity to elevated $[K]_0$

Since flattening of the resting potential vs. log $[K]_0$ curve at lower $[K]_0$ levels is much more prominent for young hearts, i.e., they are depolarized less by a given increment in $[K]_0$ (see Figure 1), young hearts should be less affected by elevation of $[K]_0$. Young hearts are indeed less affected by elevation of $[K]_0$ than are older hearts [12, 17]. This is true for both inhibition of automaticity of the whole heart as well as for loss of excitability of the ventricle to electrical stimulation [12]. Automaticity and excitability of 2–3-day hearts fail at about 25 mM K^+, whereas failure occurs at about 15 mM (automaticity) and 20 mM (excitability) for the 14–20-day hearts. Depression of automaticity was evident by 12 mM in hearts of all ages.

2.2.5. Lack of effect of acetylcholine on P_K

The young ventricular cells are completely insensitive to acetylcholine (ACh), even though a large hyperpolarization is theoretically possible because the resting potential is much below E_K [12]. Therefore, it is likely that ACh does not significantly increase P_K in ventricular cells. In old ventriculr muscle also, ACh has little or no effect on shortening the action potential, whereas the old embryonic chick atrial action potential is markedly shortened. The atrial cells of young hearts are slightly depolarized by ACh in normal medium, and slightly hyperpolarized in Na^+-free medium, suggesting that ACh increases both Na^+ conductance and K^+ conductance in young hearts [15, 18, 19]. Pappano [15] interpreted the small hyperpolarization produced by ACh in Na^+-free solution to be consistent with a low P_K – namely, that few K^+ channels are available to be opened by ACh.

2.3. (Na, K)-ATPase activity

The specific activity of the (Na, K)-ATPase is low in young embryonic chick hearts and rises during development [20]. The average value on day 4 is about 35% of that on day 16 (7.4 ± 0.7 μmol P_i/h/mg protein), that on day 6 is about 42%, that on day 9 is about 57%, and that on day 13 is about 77%. The ATPase activity is highest on day 20 (about 144% of that on day 16). The adult level is about equal to that on embryonic day 16.

Thus, while P_K is increasing during development, and hence the outward passive leak of K^+ and inward leak of Na^+ (due to the increased electrochemical driving force), the capability of the Na^+–K^+ pump is increasing correspondingly. That is, the increased cation pump capability tends to compensate for the increased demand on the pump due to increased cation leak. However, the pumping capacity of the very young hearts must be sufficient to maintain the relatively high $[K]_i$ and low $(Na]_i$ already present in the young cells.

When the ventricular myocardial cells from 16-day hearts are placed into monolayer cell culture, the specific activity of the (Na, K)-ATPase decreases by more than 3-fold [21]. The lower Na^+–K^+ pumping capability of the cultured cells is consistent with the lower K^+ permeability and somewhat lower $[K]_i$ generally observed in these cells.

2.4. Ion content

2.4.1. Sodium

Early studies of tissue electrolyte analyses in chick embryonic hearts (ventricles) reported that the total tissue content of Na^+ in young hearts is very high, and that it decreases gradually until about day 13, after which the level remains constant [9, 22]. Sodium ion exchangability was reported to be low in young hearts and to rise gradually during development to about 70% near hatching. Thus, there may be a great amount of bound Na^+ in young hearts. Much of the Na^+ may be bound in the nucleus [23] and in the extracellular (subendocardial) mucopolysaccharide cardiac jelly (which serves a valve-like function) in the young heart [24]. More recently, it was reported by McDonald and DeHaan [13] that the Na^+ content of the chick heart did not change during development. The data from electrophysiological studies [12] indicated that the thermodynamically active free intracellular Na^+ must not be too high because the Na^+-dependent action potentials overshoot to +11 mV in day 2 hearts; the overshoot increases rapidly, reaching +28 mV by day 7 (see below). The latter value is the same as the adult value. Carmeliet et al. [16] measured $[Na]_i$ values of 16 mM and 15 mM for 7-day and 19-day embryonic chick hearts, respectively. Harsch and Green [25] reported that the $[Na]_i$ levels remained relatively constant (23–38 mM) between days 8 and 18. Thus, there seems little doubt that the free $[Na]_i$ is already low in young hearts.

2.4.2. Extracellular space; Chloride

There is a decrease in extracellular space during development of embryonic chick heart. The inulin space was reported to decrease from 39% at day 8 to 19% at day 18 [25]. The extracellular space determined by radioactive chromium-EDTA decreased from 34% on days 6–8 to 27% on days 18–20 [16].

The estimates of intracellular Cl^- for days 8–18 were 36–45 mM [25]. These values are too high to be consistent with the measured resting potentials (of -70 to -80 mV) if Cl^- were passively distributed.

2.4.3. Potassium

The K^+ content of chick hearts gradually increases during development, from about 68 mmol/kg on days 2–3 to a plateau level of about 86 mmol/kg on day 13 [22]. Another report gave the calculated $[K]_i$ levels as 145 mM on day 8 and 91 mM on day 18 [25]. McDonald and DeHaan [13] reported that $[K]_i$ changed from 167 mM during day 2 to 118 mM for days 14–18. Similarly, Carmeliet et al. [16] calculated $[K]_i$ values of 151 mM and 122 mM for chick hearts on days 6–8 and days 18–20, respectively. The electrophysiological data from resting potential vs. log $[K]_0$ curves [12] indicate that $[K]_i$ is about 125 mM on day 2, and that it increases gradually to about 155 mM on day 14–20; the calculated E_K increases from about -100 mV at day 2 to -106 mV at day 14–20. Pappano [15] also reported high values of $[K]_i$ (145 mM) on day 4 for chick atrial cells. Thus, $[K]_i$ is already high in young hearts. Hence, the cardiac cells must actively transport cations before day 2, and even during the precardiac blastoderm stage.

2.5. Electrogenic pump potential

The energy-dependent pump located in the cell membrane, which maintains the ionic gradients for Na^+ and K^+ ions across the cell membrane, becomes electrogenic when the ratio of Na^+ ions pumped out to K^+ ions pumped in is greater than one. That is, the pump directly produces a potential which contributes to the measured resting potential, usually by between 2 and 15 mV depending on the type of cell and the physiological conditions. An electrogenic Na^+ pump potential has been demonstrated in nerve cells and in skeletal and smooth muscle cells (see reviews in Thomas [26] and Sperelakis [27]) as well as in various tissues of the heart [28, 29, 30]. For example, a contribution of an electrogenic Na^+ pump to the membrane potential under normal physiological conditions has been suggested for Purkinje fibers [31, 32], atrial fibers [30] and sinoatrial nodal cells [33]. Using flux studies, Lieberman et al. [34] found evidence for an electrogenic pump in cultured embryonic chick (11-day-old) myocardial cells. McLean et al. [35] found that cultured smooth muscle cells from guinea pig vas deferens had a mean electrogenic pump potential of 10 mV (average resting potential of -58 mV in the absence of ouabain and -48 mV shortly after the addition of 10^{-4} M ouabain) (see also Urquilla et al. [36]). Pelleg et al. [37] observed an electrogenic pump potential of a few millivolts in cultured embryonic heart cell reaggregates derived from early (3-day-old) and late (16-day-old) stages of

development that were subjected to overdrive. These studies indicate that electrogenic transport is present in early stages of ontogenesis, and that this ability is retained in vitro. If $[Na]_i$ is somewhat higher in young hearts and if the pump coupling ratio (Na:K) is a function of $[Na]_i$, V_{pump} might be expected to be higher in young hearts, especially in view of the higher membrane resistance, but there is no evidence that the electrogenic pump potential contribution to the resting potential is larger. This might be explained on the basis of a lower density of pump sites in the young hearts.

2.6. Automaticity: Overdrive suppression

The major requirements for automaticity appear to be (a) a low chloride conductance, as is generally true for myocardial cells, and (b) a low K^+ conductance (g_K). A low g_K enhances membrane inductance in series with one type of K^+ channel (the inward-rectifying or anomalous rectification channel having a negative slope conductance region), and tends to cause oscillations in membrane potential. The low g_K also produces some depolarization (moves the resting potential farther from E_K) and places the membrane potential in the region that can support pacemaker oscillations.

Pronounced changes in automaticity of the ventricular cells also occur during development, as would be predicted from the changes in P_K. The incidence of hyperpolarizing afterpotentials and pacemaker potentials is very high (80–100%) in the young hearts, and this incidence decreases to 0% in the old embryonic hearts (Table 2)[12]. If a portion of the ventricle is cut and isolated to remove drive from the nodal cells, the incidence of pacemaker

Table 2. Incidence (%) of pacemaker potentials and hyperpolarizing afterpotentials found in ventricular myocardial cells during embryonic development of chick hearts.

Embryonic age (days)	*Hyperpolarizing afterpotentials*	*Pacemaker potentials*	
	Intact hearts[a]	*Intact hearts*[a]	*Cut ventricles*[b]
2–3	81	80–100	100
4	63	60–100	100
7	38	20–40	100
10	0	0	100
12	0	0	0
17	0	0	0
20	0	0	0
27	0	0	0

[a] Cells which exhibit pacemaker potentials usually also possess hyperpolarizing afterpotentials, but the converse is not always true. Data taken from Sperelakis and Shigenobu, 1972.
[b] Data taken from Sperelakis and McLean, 1978.

potentials observed in the impaled cells is 100% for embryos up through day 10, whereas the incidence is 0% in embryos day 12 or older (Table 2, Figure 2). These results indicate that the ventricular myocardial cells possess automaticity capability when they are young, but that this capability diminishes as the cells become older.

However, old ventricular cells again become automatic when trypsin-dispersed and placed into monolayer cell culture. For example, ventricular myocardial cells dispersed from 16-day-old chick embryos and cultured as monolayers usually revert back towards the young embryonic state with respect to their electrical properties, including gain of automaticity [12], as discussed below. When the cells are allowed to reaggregate into small spheres, however, they often retain their highly differentiated electrical properties,

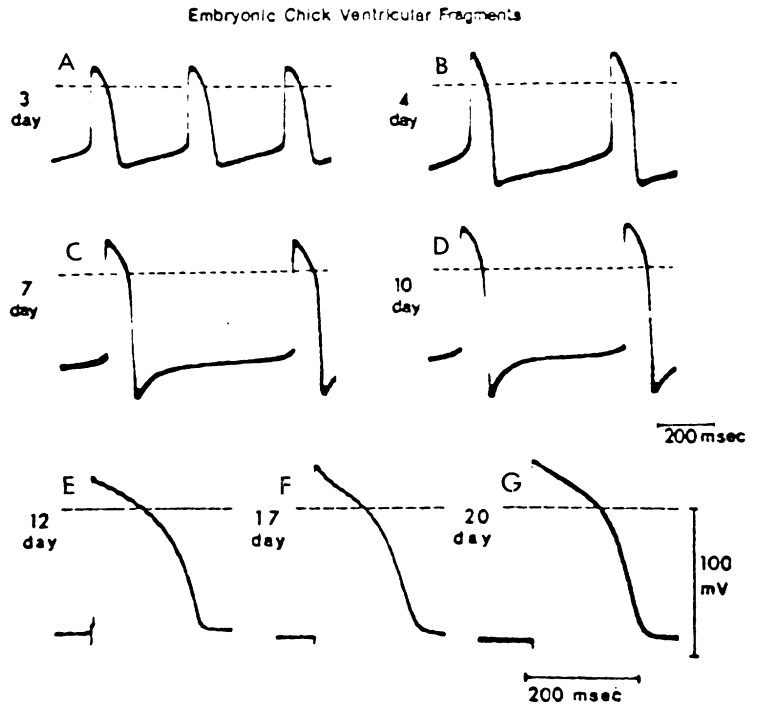


Figure 2. Electrophysiological recordings from ventricular fragments cut from chick embryonic hearts of various ages. A–D: pacemaker potentials preceded the action potentials in impaled cells within fragments from hearts aged 3, 4, 7, and 10 days. Maximal rate of rise and maximal diastolic potentials in the fragments were the same as in the intact heart. The fragments contracted spontaneously. E–G: cut ventricular fragments from hearts 12 days old and older did not exhibit automaticity. Action potentials with fast rates of rise (equal to those recorded from cells in the intact hearts of the respective ages) were elicited by field stimulation (shock artifacts visible) from fragments of 12, 17, and 20-day ventricles. Time calibration in the middle row applies to A–D.

including lack of automaticity [38–40]. In some cases, the reaggregates may exhibit automaticity, even though the action potentials are relatively fast-rising and tetrodotoxin (TTX)-sensitive. The gain in automaticity of cultured cells appears to reflect a decrease in P_K [41–43]. In some cases, isolated single ventricular cells in culture have such a low P_K that they are depolarized too far and do not normally exhibit automaticity or excitability [44]. However, if these cells are hyperpolarized by the intracellular application of current, then spontaneous action potentials and contractions occur [44].

It has been suggested that mechanical stretch of the wall of the tubular heart of the young chick embryo, by the gradual buildup of the blood volume and blood pressure, may play a role in the initiation of the heart beat and in the gradual increase in the heart rate during development [45]. However, it is difficult to reconcile the view that stretch is responsible for initiation of automaticity with other facts, such as (a) the demonstration that the precardiac tissue of the chick blastoderm can develop in vitro into spontaneously contracting heart-like tubes, vesicles or cell clusters [2, 4, 5, 46, 47, 48], with no evidence of an intraluminal buildup of pressure, and (b) the retention of automaticity of cultured isolated single heart cells or monolayers.

When automatic heart cells are driven at a faster rate than their intrinsic rate, upon termination of the drive there is a transient pause followed by a gradual recovery to the predrive firing rate [37, 49, 50]. This phenomenon of overdrive suppression of automaticity is usually accompanied by a small hyperpolarization of a few millivolts; the transient hyperpolarization presumably is the cause of the suppression of the automaticity. Vassalle [50] presented evidence that the hyperpolarization was due to stimulation of an electrogenic Na^+ pump potential, presumably resulting from an increase in $[Na]_i$ and in $[K]_0$ during the drive. Overdrive suppression was observed in intact young 3-day-old hearts (prior to innervation of the heart), and was attributed to the release of an ACh-like substance from within the heart cells [51]. It was recently demonstrated by Pelleg et al. [37] that cultured heart cells (ventricular and atrial) from both young and old embryonic chick hearts that are automatic in vitro exhibit the phenomena of postdrive hyperpolarization and overdrive suppression of automaticity that are blocked by ouabain but not by atropine, thus supporting the view that stimulation of an electrogenic pump is the cause of this behavior. These results also indicate that young embryonic heart cells are capable of exhibiting electrogenic Na–K pumping.

2.7. Action potentials

The action potentials of the cells of intact chick hearts undergo sequential changes during development in situ [12, 52, 53], as shown in Figure 3. There

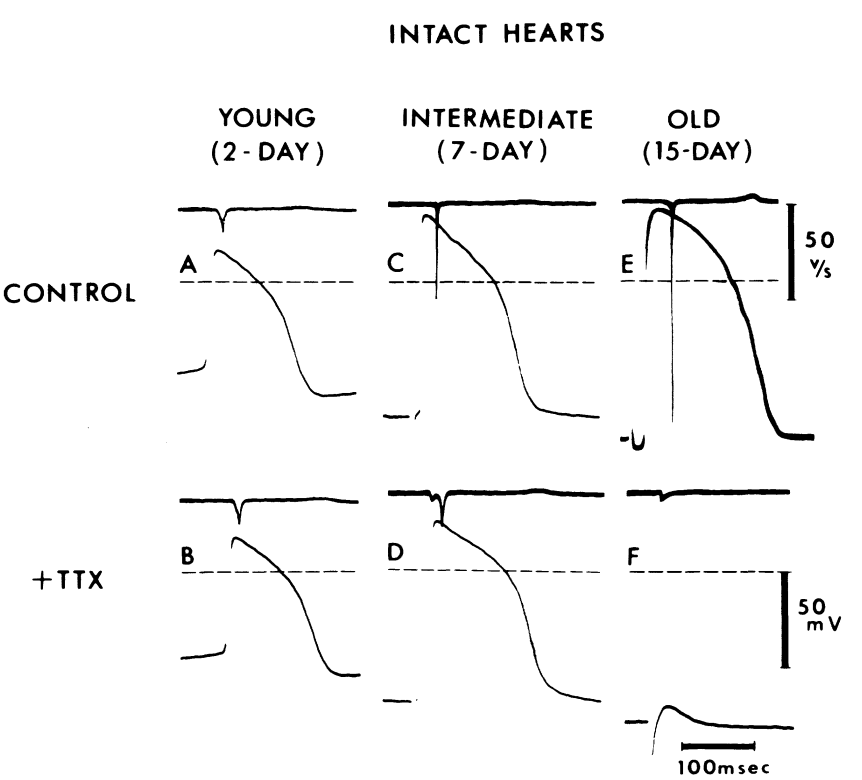


Figure 3. Characteristics of the action potentials in intact embryonic chick hearts at different stages of development. A–B: intracellular recordings from a 2-day-old heart before (A) and 20 min after (B) the addition of tetrodotoxin (TTX) (20 μg/ml). C–D:recordings from a 7-day-old heart before (C) and 2 min after (D) the addition of TTX (2 μg/ml). Note depression of the rate of rise in D. E–F: records from a 15-day-old heart prior to (E) and 2 min after (F) the addition of TTX (1 μg/ml). The action potentials were abolished and excitability was not restored by strong field stimulation in F. Thus, the hearts became progressively more sensitive to TTX during development; i.e., the effect of TTX increased with increasing embryonic age. The upper traces give dV/dt; this trace has been shifted relative to the V–t trace to prevent obscuring dV/dt. The horizontal broken line in each panel represents zero potential. dV/dt calibration (in E) and voltage and time calibrations (in F) pertain to all panels. (Taken from Sperelakis and Shigenobu, 1972.)

is a progressive increase in maximal rate of rise ($+\dot{V}_{max}$) (Figures 3 and 4) and overshoot of the action potential (Figure 3), as well as the increase in resting potential described above. The overshoot averaged +11 mV on day 2, and increased progressively over the next few days, reaching the maximal value of about +28 mV by day 8. The duration (at 50% repolarization) was hardly changed during development, the average value being 110 msec. The time course of the increase in $+\dot{V}_{max}$ usually was not parallel to the increase in

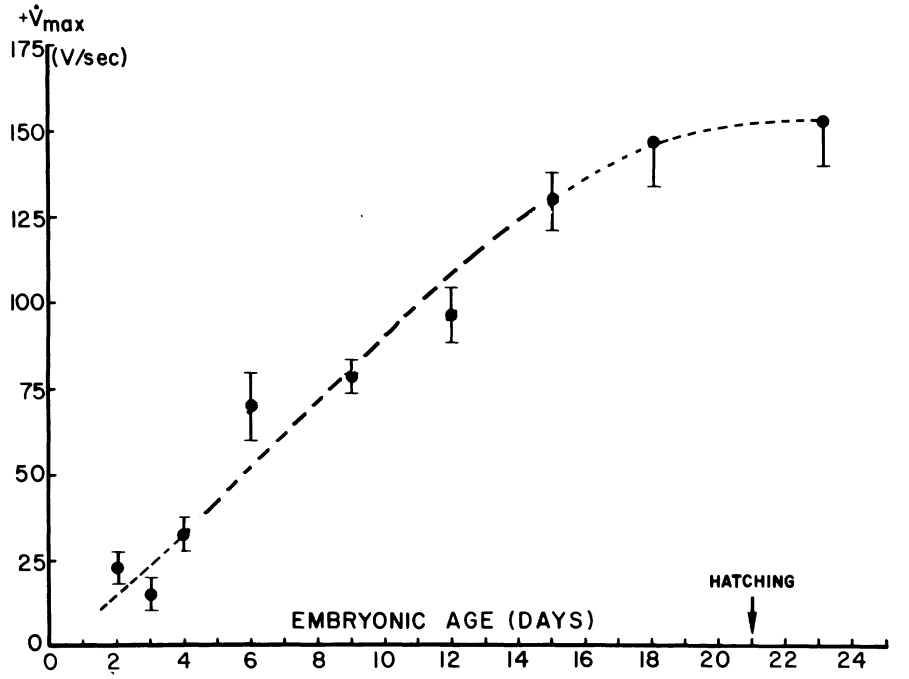


Figure 4. Changes in the maximum rate of rise of the cardiac action potential ($+\dot{V}_{max}$) during embryonic development of chicks. Some of the data were grouped into 3-day periods. There is a progressive increase in $+\dot{V}_{max}$ during development, with a plateau level being reached by about day 18. Vertical bars = ±1 SE. (Data taken from Sperelakis and Shigenobu, 1972, and from Jourdon et al., unpublished observations.)

resting E_m, the increase in resting E_m usually preceding the increase in $+\dot{V}_{max}$ by several days. That is, in young hearts, it was not unusual to find a cell with a large resting potential but with a low $+\dot{V}_{max}$.

Young (2–3 days in ovo) myocardial cells possess slowly rising (10–40 V/sec) (Figure 4) action potentials preceded by pacemaker potentials (Figure 3A). Tetrodotoxin (TTX), a specific blocker of fast Na^+ channels, has no effect or only little effect on the action potential rate of rise or overshoot (Figure 3B) (Table 3). Hyperpolarization does not greatly increase the rate of rise of the action potential (Figure 5), thus indicating that the low $+\dot{V}_{max}$ is not due to inactivation of fast Na^+ channels at the low resting potential, but rather to a low density of fast Na^+ channels. Excitability is not lost until the membrane is depolarized to less than −20 mV (Figure 5), thus indicating the preponderance of slow channels. The action potential upstroke in young hearts is generated by Na^+ influx through TTX-insensitive slow Na^+ channels, as indicated by the dependence of the action potential overshoot (Figure 6A) and

Table 3. Effect of tetrodotoxin (TTX) on the action potential maximal rate of rise ($+\dot{V}_{max}$) of chick embryonic hearts (ventricular muscle) as a function of development age.

Embryonic age (days)	$+\dot{V}_{max}$ *(V/sec)*		*TTX sensitivity*
	Control	*+TTX*	
2–3	15–35	10–30	Little or none
5–6	50–70	10–30	Partial
8–10	75–90	0	Complete
12–16	90–140	0	Complete
17–21	140–170	0	Complete

The $+\dot{V}_{max}$ values are the approximate ranges.
Data taken from Sperelakis and Shigenobu, 1972, and from Jourdon et al., unpublished observations.

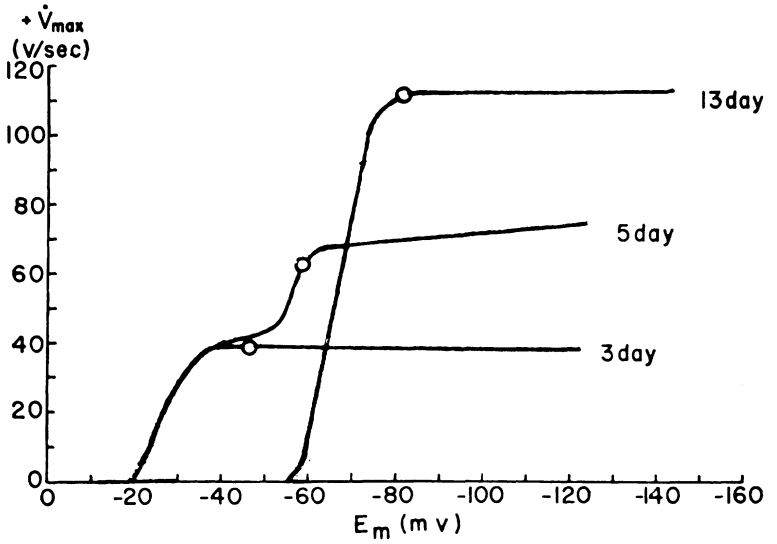


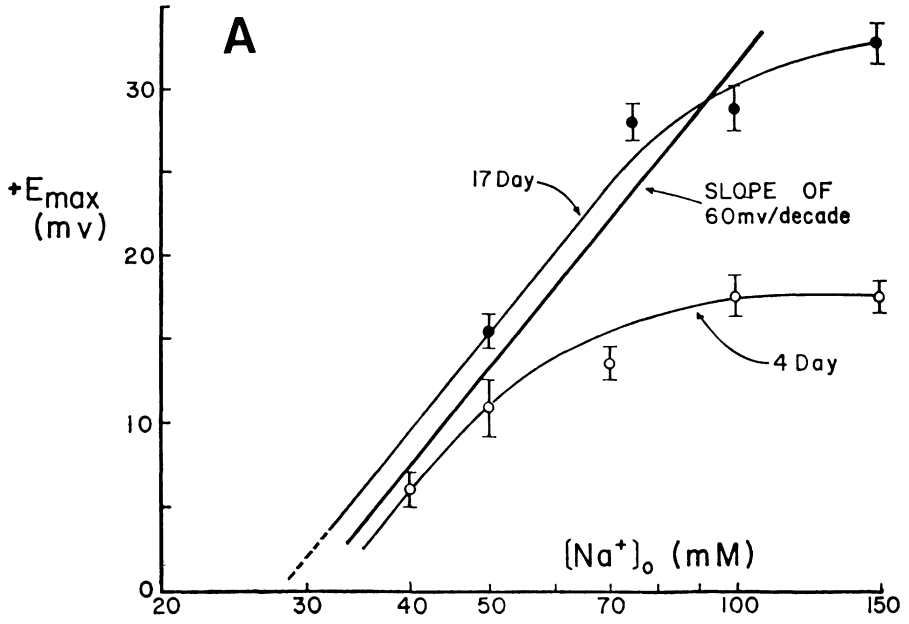
Figure 5. Evidence for two types of Na^+ channels. Data illustrating changes in the maximal rate of rise of the action potential ($+\dot{V}_{max}$) as a function of transmembrane potential (E_m) for three representative hearts of different embryonic ages (3, 5, and 13 days). The membrane potential was changed by applying rectangular polarizing current pulses of long duration (several seconds). The circles give the mean resting potential (polarizing pulses not applied). Note that complete inactivation for the 13-day heart occurs at about −55 mV, whereas complete inactivation does not occur until about −25 mV for the 3-day and 5-day hearts. For the 3-day and 13-day hearts, there appears to be only one type of channel: slow and fast, respectively. The data for the 5-day heart (transition period) suggest than there are two sets of channels, one set inactivating at about −55 mV and the other set at −25 mV. (Modified from Sperelakis and Shigenobu, 1972.)

rate of rise (Figure 6B) on the external Na^+ concentration. The slope of overshoot as a function of $[Na]_0$ approaches the theoretical 60 mV/decade from the Nernst equation at the lower $[Na]_0$ concentrations (Figure 6A).

Kinetically fast Na^+ channels, which are sensitive to TTX, are substantial in number by day 5. This is about the time that innervation reaches the heart and circulation is established to the chorioallantoic membrane for gas exchange. At this time, $+\dot{V}_{max}$ is about 50–70 V/sec (Figure 4; Table 3). During this intermediate stage of development (from about day 5 to day 8), a large number of slow channels still coexist with the fast Na^+ channels in the membrane. TTX causes a reduction in $+\dot{V}_{max}$ to about 10–20 V/sec, but the action potentials and accompanying contractions persist (Figure 3C and D, Table 3).

After about day 8, the action potentials are completely abolished by TTX despite increased stimulus intensity (Figure 3E and F, Table 3). Depolarization to less than -50 mV now abolishes excitability (Figure 5). This indicates that the action potential-generating Na^+ channels now consist predominantly of fast Na^+ channels. The dependence of the inward current on $[Na]_0$ during the action potential of old embryonic hearts is also shown in Figures 6A and B. The density of fast Na^+ channels continues to increase until about day 18, when the adult maximal rate of rise of about 150 V/sec is achieved (Figure 4). A large fraction of the slow Na^+ channels appear to have been lost functionally, and insufficient numbers remain to support regenerative excitation. However, it is not clear to what extent the density of functional slow channels is reduced in old hearts, because the simultaneous increase in resting potential might render propagation more difficult for any given density of slow channels. Addition of some positive inotropic agents increases the number of slow Ca^{++}–Na^+ channels available in the membrane, and leads to the regaining of excitability in cells whose fast Na^+ channels have been voltage inactivated or blocked [5, 12, 54, 55], as discussed below.

The Ca^{++} antagonist drugs, verapamil and D–600, block the action potentials of the young embryonic hearts [56, 57]. This indicates that these agents block slow Na^+ channels as well as slow Ca^{++} and slow Ca^{++}–Na^+ channels, and so are not specific for blockade of Ca^{++} current. In contrast, Mn^{++} at 1 mM does not depress the action potentials of young hearts, although it does block the contractions, indicating a greater specificity for slow Ca^{++} channels [12]. Galper and Catterall [58] reported that the early embryonic chick heart was insensitive to TTX (with regard to contractions), but was sensitive to D–600. During subsequent development, the sensitivity to TTX increased and the sensitivity to D–600 decreased in a reciprocal manner. Kasuya et al. [59] reported that the slowly rising action potentials of 3-day-old embryonic chick hearts involved cation channels that were pharmacologically different from those of old embryonic hearts.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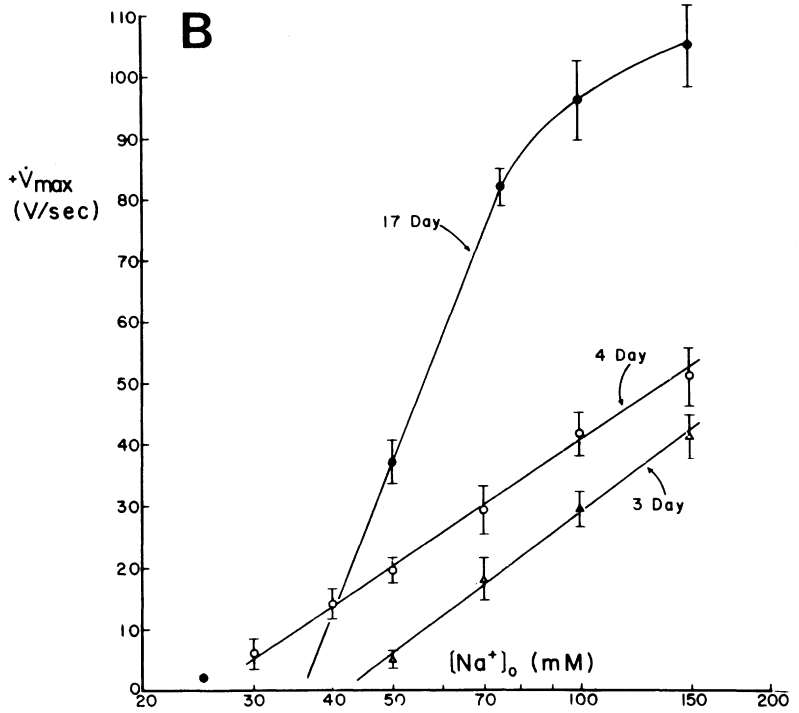
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In summary, young 2–3-day-old embryonic chick hearts have action potentials with relatively low rates of rise and which are only little depressed by TTX [12, 52, 60–63]. Hyperpolarization does not greatly increase the rate of rise [12]. Therefore, the density of functional fast Na^+ channels is low. In contrast, the density of slow Na^+ channels is high in the young hearts, and verapamil-type agents block the slow Na^+ channels, whereas Mn^{++} does not [12, 56]. During development, the number of functional fast Na^+ channels increases progressively, reaching the final adult level by about day 18. In contrast, the number of available slow channels, in the absence of positive inotropic agents, decreases during development, reaching the adult level by about day 8. At this time, TTX completely blocks the action potential, indicating that the number of available slow channels is not enough to support regenerative excitability. However, addition of some positive inotropic agents, such as beta-adrenergic agonists, histamine or methylxanthines, rapidly increases the number of available slow channels, and thus allow regenerative slow action potentials to be generated in the presence of TTX blockade of the fast Na^+ channels. During an intermediate stage of development, days 5–7, a sizable number of functional fast Na^+ channels coexist with the originally high density of functional slow channels, so that blockade of the fast Na^+ channels by TTX unmasks slow action potentials, resembling those present in young (2–3-day-old) hearts.

Consistent with the above, Nathan and DeHaan [64] found that TTX-sensitive fast Na^+ conductance channels were absent or non-functional in cultured cell reaggregates derived from 3-day-old embryonic chick hearts. Ishima [65] reported that the contractions of 3–5-day-old embryonic chick hearts were not affected by TTX.

←

Figure 6. Evidence that Na^+ is the predominant carrier of inward current in both young and old embryonic chick hearts. A: the mean overshoot values ($+E_{max}$) for one representative 4-day heart and one representative 17-day-old heart plotted as a function of external Na^+ concentration on a logarithmic scale. A line having a slope of 60 mV/decade has been arbitrarily superimposed. The curves are nearly linear with a slope approaching 60 mV/decade at lower $[Na]_0$, but flatten at high $[Na]_0$ levels. B: the effect of variation in $[Na]_0$ on maximum rate of rise of the action potential ($+\dot{V}_{max}$) is illustrated. $[Na]_0$ was lowered by replacing the NaCl with equimolar amounts of choline-Cl. Plotted are the mean $+\dot{V}_{max}$ (± 1 SE) values of multiple penetrations into two 3-day-old hearts, three 4-day-old hearts, and one 17-day-old heart. The curve for the old heart is linear at lower $[Na]_0$ levels, and flattens at higher levels; for the young hearts, the curves are linear over the entire range. (Taken from Sperelakis and Shigenobu, 1972.)

2.8. Biochemical changes

2.8.1. Cyclic AMP levels

Pronounced changes in cyclic AMP content occur during embryonic development of the chick heart [53, 66]. The cyclic AMP level is highest in young hearts and it decreases during development. In one set of experiments, the cyclic AMP level was 116 pmol/mg protein on day 4, and this decreased sharply by day 5 to about 41 [66]. There was a gradual further decline to a plateau level of 9.4 pmol/mg protein, which is about the adult level, by day 16. In a second set of experiments, the cyclic AMP level was 33.6 pmol/mg protein on day 4 compared to 11.7 on day 16 [67].

The relationship, if any, between changes in membrane properties and changes in cyclic AMP levels during development of heart remains to be clarified. However, in cultured skeletal muscle, the cyclic AMP level decreases sharply as the myoblasts fuse into myotubes, i.e., when the cells further differentiate [68]. In addition, as discussed below, increase in cyclic AMP level is associated with increase in the number of available slow channels. Therefore, it is tempting to speculate that the decrease in number of available slow channels during development of chick heart, as described above, results from the concomitant drop in cyclic AMP level. In essence, this allows positive inotropic agents that increase the cyclic AMP level to increase the number of available slow channels transiently back towards the density present in young embryonic hearts. In other words, the developmental change in steady-state level of cyclic AMP allows the fraction of slow channels that are available for activation to be modulated by inotropic agents.

Cultured heart cells isolated from 16-day hearts had a slightly higher cyclic AMP level (16 pmol/mg protein) in one set of experiments [66], but had a significantly lower level (4.5 pmol/mg protein) in another set of experiments [69]. Thus, it appears that the cyclic AMP level is reduced in cell culture. A similar reduction in cyclic AMP was observed in cell cultures prepared from 4-day-old chick hearts.

The cyclic AMP level of young 4-day hearts placed into organ culture for 2 weeks also declines (to 5.6 pmol/mg protein), even though the cells do not further differentiate electrically or ultrastructurally [69]. A similar decline in cyclic AMP level (to 5.3 pmol/mg protein) was observed in organ cultured 16-day-old chick hearts.

Isoproterenol was capable of markedly elevating the cyclic AMP level in all cases: young or old, cultured or non-cultured, thus demonstrating the presence of functional beta-adrenergic receptors. For example, 10^{-6} M isoproterenol raised the cyclic AMP level of 7–8-day hearts from a control level of 35 to 80 pmol/mg protein at 3 min (time of peak effect) [66]. Isoproterenol ele-

vated the cyclic AMP level of 4-day-old hearts from 34 to 119 pmol/mg protein, thus clearly showing that young hearts prior to innervation have functional beta-adrenergic receptors. The organ cultures and cell cultures of 4-day-old and 16-day-old hearts also responded markedly to isoproterenol.

In contrast to the above findings for chick heart, it was reported that in rat heart, the cyclic AMP level increases during the period from just before birth to postnatal day 10 [70]. In rat skeletal muscle, developmental changes in cyclic AMP were similar to those for the heart [70]. Likewise, in embryonic chick skeletal muscle, there was an increase in cyclic AMP level between the 7th and 15th day [71].

2.8.2. Glucose uptake; Amino acid uptake; Membrane fluidity

Changes in other membrane properties also occur during development. For example, glucose uptake is very high in young hearts, and decreases during development. A carrier-mediated saturable glucose transport system appears on about day 7, and an enhancement of glucose uptake by insulin can be first demonstrated shortly thereafter [72]. For example, insulin had no effect on glucose uptake of 5-day-old embryonic chick hearts, but did on 7-day-old hearts [72]. Glucose uptake by hearts 5 days old and younger seems to be by simple diffusion across the membrane [73]. A similar conclusion was made in a more recent study [74]. Ouabain stimulates glucose uptake in 10-day-old hearts but not in 5-day hearts [75]. As development proceeds, the heart myocytes also become progressively less permeable to sorbitol [75], presumably due to an increase in the apparent K_m [76].

Amino acids were shown to be actively transported against concentration gradients in 5-day-old embryonic chick hearts, and insulin enhanced the rate of amino acid transport [77]. It was also shown that amino acid uptake decreases with development [77].

There are also changes in membrane fluidity (microviscosity) during development of chick hearts [74]. In general, there was a trend towards increase in fluidity as development proceeded. The cholesterol/phospholipid ratio of the sarcolemma increases during development, concomitant with an increase in the number of unsaturated fatty acid residues. In general, the changes seem to be too complex to correlate with changes in the electrical properties, as, for example, with changes in K^+ permeability. It is of interest that the membrane fluidity of cultured chick skeletal myoblasts increases concomitant with fusion and myotube formation [78].

2.8.3. Metabolic changes

Young 2–3-day-old embryonic chick hearts have large pools of glycogen, and their metabolism appears to be mainly by anaerobic glycolysis. The circulation to the chorioallantoic membrane for gas exchange is not establish-

ed until day 5. Following this event, there is a shift toward aerobic metabolism, accompanied by changes in various enzymes. For example, there is an increase in pyruvate kinase activity [79, 80].

Hearts of young chick embryos utilize the phosphogluconate pathway to a greater extent, relative to the tricarboxylic acid cycle pathway, than do hearts of older embryos or adults [81]. Proliferating cells in general are characterized by high activity of pentose cycle enzymes [82]. Enzymes of the pentose shunt pathway, such as glucose-6-P dehydrogenase and 6-P-gluconic dehydrogenase, decrease from day 4 to day 20, whereas enzymes of the Krebs cycle, such as isocitric dehydrogenase and α-ketoglutaric dehydrogenase, increase during development [83]. In addition, hexokinase activity increases severalfold [83]. In general, the capacity to metabolize long-chain fatty acids appears late in development (after day 12) [84].

In rat hearts, lactic dehydrogenase (LDH) shifts from the embryonic M-form isoenzyme (which catalyzes the reduction of pyruvate to lactate) to the adult-like H-form (which facilitates pyruvate oxidation to CO_2 and H_2O) [85]. Similar changes were shown to occur in embryonic chick skeletal muscle [86]. When old embryonic chick myocardial cells are cultured in monolayers, they begin to synthesize the early embryonic M-LDH [87]. Isoenzyme transformation also occurs with respect to creatine phosphokinase during the development of rat heart [88].

Many of the biochemical changes that occur in hearts during development have been summarized in a recent review by Harary [89].

Consistent with the fact that young embryonic hearts have a low rate of aerobic metabolism, being mainly dependent on glycolysis, the young hearts are relatively resistant to metabolic interventions. For example, hypoxia does not block the slow action potentials of young embryonic chick hearts [90]. Similarly, monolayer cultures of chick embryonic heart cells, that are reverted in morphology, metabolism, and many electrical properties, are relatively insensitive to a variety of metabolic poisons [91]. In mammalian atrial muscle, which has a high glycogen content, the isoproterenol-induced slow action potential was only slightly depressed by hypoxia [92].

2.9. Ultrastructural changes

Thin myofilaments are clearly present at 30 h (they may actually appear by 18 h – the head fold stage) [2], and they begin to collect into groups at about 36 h [93]. Thin filaments are found without thick filaments, but thick filaments usually occur in association with thin filaments. The myofibrils in the 2–3-day hearts are relatively sparse and in various stages of formation (Figure 7A). The myofibrils are not aligned, and they run in all directions, including perpendicular to one another. Bundles of myofilaments attached to one Z line

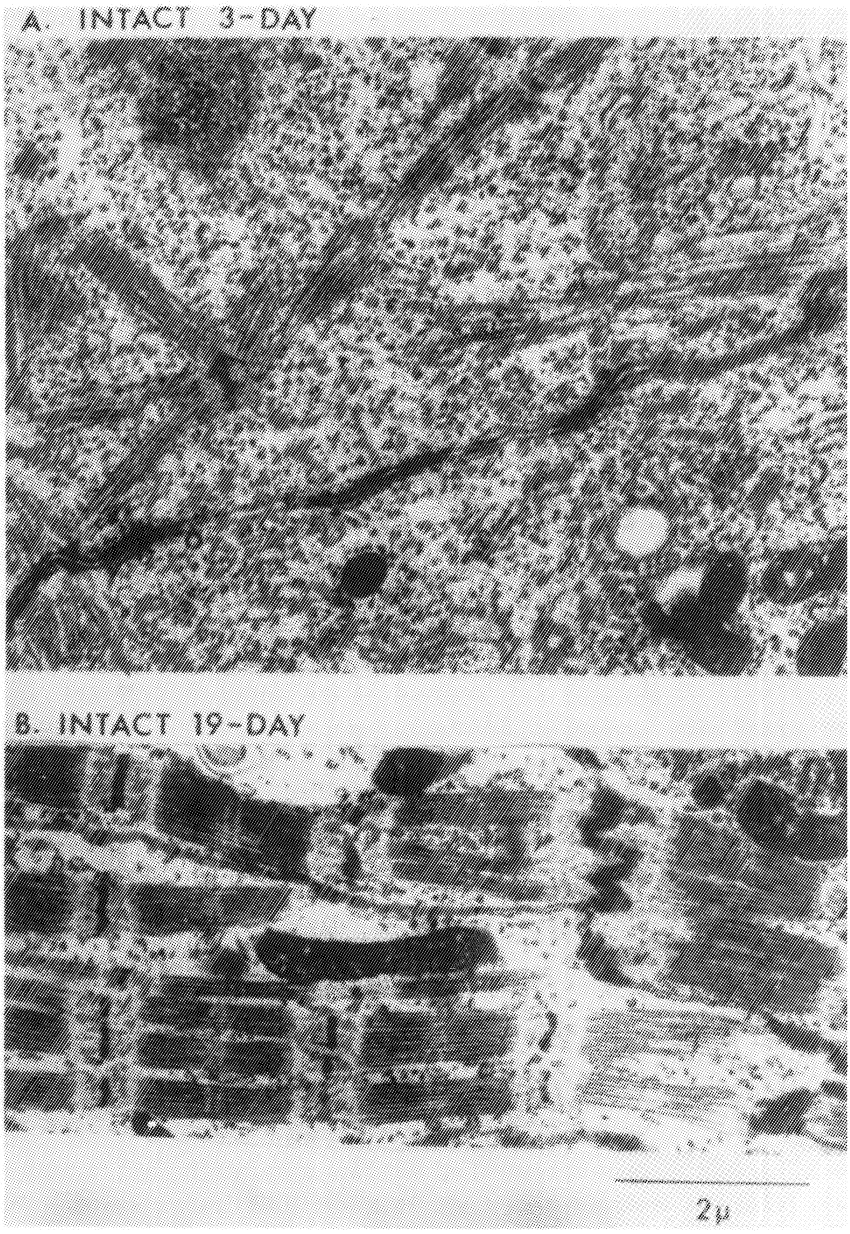

Figure 7. Cell ultrastructure of young (3 days in ovo) and old (19 days in ovo) intact embryonic chick hearts in situ. A: 3-day ventricular cell demonstrating paucity and non-alignment of myofibrils. Ribosomes are abundant in the cytoplasm. The contiguous cells are held in close apposition by desmosomes. B: 19-day ventricular cells with abundant and aligned myofibrils. A convoluted intercalated disc appears between contiguous cells (Taken from Sperelakis and McLean, 1976.)

often radiate in several directions (Figure 7A). Free cytoplasmic polyribosomes are abundant and rough ER tubules lined with ribosomes are prominent in young hearts; the ribosomes are frequently observed in close association with the devleoping myofibrils. The sarcomeres are usually incomplete early in their formation, and the myofibrils are short. H zones first become obvious at 8 days, and M lines do not appear until about day 18.

By day 18, the embryonic myocardial cell closely resembles the adult cell, and has a close packing of completed myofibrils with rows of mitochondria in between (Figure 7B). The myofibrils have the typical hexagonal array of 6 thin actin filaments surrounding each thick myosin filament. The adult level of the tropomyosin–troponin complex is reached by day 16 in embryonic chick skeletal muscle [94].

Sarcoplasmic reticulum (SR) is found in the young hearts, and it increases with development, forming a network of SR around each myofibril. Subsarcolemmal cisterns ('diads,' 'junctional couplings,' or 'junctional complexes'), regions in which elements of the SR come in close apposition to the surface sarcolemma, are observed in young hearts; the junctional cistern becomes flatter and denser with age [95]. The junctional SR is continuous with the network SR. A transverse (T) tubular system is not found in chicken hearts.

Large pools of glycogen are observed in the young 2–3-day-old hearts. Their metabolism appears to be mainly anaerobic glycolysis, because the circulation to the chorioallantoic membrane under the eggshell for gas exchange is not established until about day 5.

3. ORGAN-CULTURED EMBRYONIC HEARTS

3.1. Young embryonic hearts

Cultivation of embryonic hearts in vitro provides a powerful means of analyzing the changes which occur during normal development in situ. When young (3-day-old) embryonic chick hearts, which have not yet become innervated by either cholinergic or adrenergic fibers, are placed into organ culture, they fail to gain tetrodotoxin-sensitive fast Na^+ channels [12, 96]. Instead, the action potentials continue to be generated by TTX-insensitive slow Na^+ channels, the rates of rise remain slow, and pacemaker potentials precede the action potentials (Figure 8). These action potentials resemble those recorded from 3-day-old hearts in situ. Similar findings were obtained when the young hearts were grafted on to the chorioallantoic membrane of host chicks for blood perfusion [97]. Thus, organ-cultured young hearts do not differentiate further in vitro, but appear to be arrested in the young embryonic state.

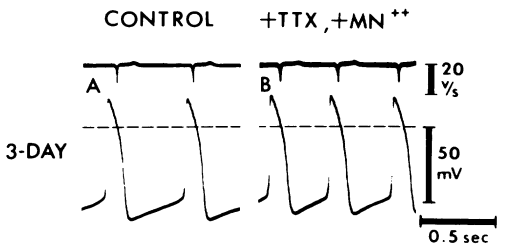

Figure 8. Failure of appearance of fast Na^+ channels in a 3-day-old heart organ-cultured for 6 days. The action potentials recorded from one cell prior to (A) and another cell after (B) the addition of tetrodotoxin (TTX) are almost identical; dV/dt values (upper traces) are similar for the two cells. Subsequent addition of Mn^{++} (1 mM; panel B) in the presence of TTX had no effect on the maximal rate of the rise of the action potential, thus indicating that the inward current during the action potentials is not predominantly carried by Ca^{++} ion. (Modified from Sperelakis and Shigenobu, 1972.)

However, DeHaan et al. [63] have reported that differentiation can be made to proceed in vitro.

When young hearts which have been arrested in the early developmental state are treated with RNA-enriched fractions obtained from adult chicken hearts, they gain fast Na^+ channels and become completely sensitive to TTX [98]. That is, young hearts in vitro can be induced to undergo further membrane differentiation. Cultured young hearts thus provide a useful model for studying the regulation of membrane differentiation.

3.2. Intermediate and old embryonic hearts

Organ-cultured intermediate (5- to 7-day-old) hearts have both fast and slow Na^+ channels, just as in situ [97, 99, 100]. The action potentials have moderate rates of rise (40–70 V/sec), and TTX reduces the maximal rate of rise of the action potential to about 10 V/sec. Cultured 17-day hearts tend to retain their fast-rising action potentials and complete sensitivity to TTX, but survival is limited to a few days. Thus, in organ-culture, the embryonic hearts tend to retian the state of differentiation achieved at the time of placement into culture.

4. CULTURED HEART CELLS

Various stages of cardiac electrical differentiation can be simulated in vitro using cell culture techniques, and their study may facilitate elucidation of the mechanisms operating during normal cardiogenesis. The electrophysiological properties observed depend to a large extent on the age of the hearts from which the cells are isolated, and on the method of cell culture.

4.1. *Old embryonic hearts*

4.1.1. *Monolayer cultures*

When cells are dispersed from old embryonic hearts using trypsin and standard monolayer cultures are prepared, the cells are found to possess slowly rising TTX-insensitive action potentials with pacemaker potentials

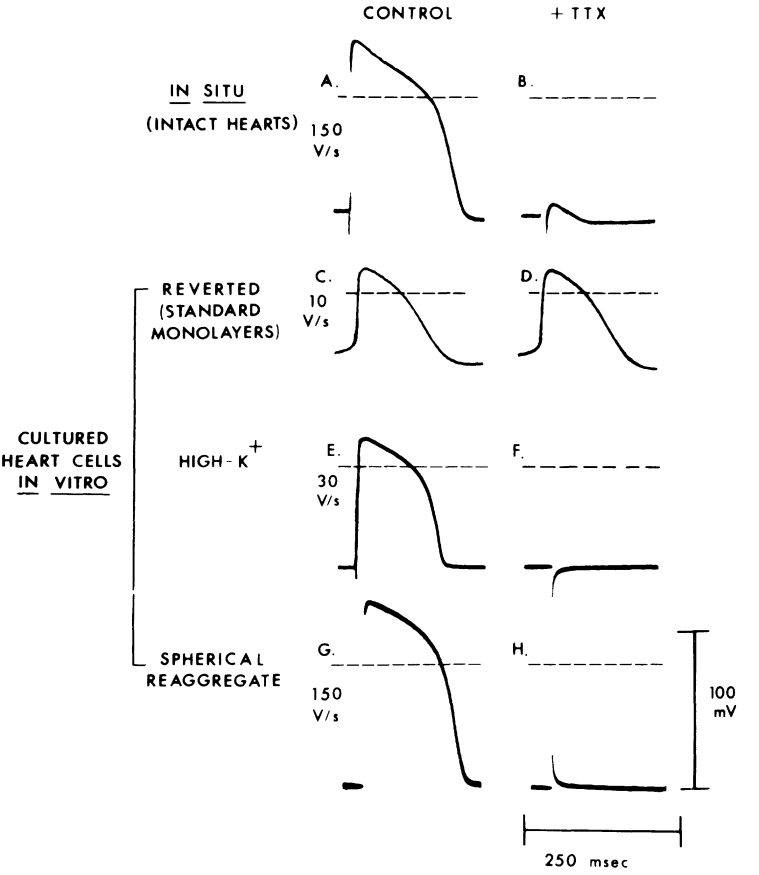

Figure 9. Comparison of the electrophysiological properties of the intact old (16-day) embryonic chick heart in situ (A–B) with those of trypsin-dispersed old ventricular myocardial cells in cultures prepared by three different methods (C–H). A–B: intact heart; control action potential (A) was rapidly rising (150 V/sec), had a high stable resting potential (about −80 mV), and was completely abolished by tetrodotoxin (TTX; 0.1 μg/ml) (B). C–D: standard reverted monolayers; control action potential was slowly rising (10 V/sec) and was preceded by a pacemaker potential, the resting potential was low (about −50 mV) (C), and TTX did not alter the action potential (D). E–F: partially reverted cells cultured as monolayers in media containing elevated K^+ concentration (25 mM); control action potential had a rate of rise of 30 V/sec (E), lacked a pacemaker potential, had a moderately high resting potential (−60 mV), and was completely abolished by TTX (F). G–H: highly differentiated cells in spherical reaggregate culture; control action potentials were rapidly rising (150 V/sec), the resting potentials were high (−80 mV) (G), and TTX abolished the action potentials (H). (Taken from Sperelakis and McLean, 1976.)

(Figure 9C and D), similar to those recorded from young (2- to 3-day-old) hearts, rather than the old hearts from which the cells were taken (Figure 9A and B). It thus appears that cell separation results in a rapid reversion toward the young embryonic state [3, 38, 101–102].

This reversion can be partially prevented (or reversed) by separating the cells in trypsinizing solutions containing elevated K^+ concentraions (12–60 mM; by isosmolar substitution of K^+ for Na^+) [38]. Action potentials recorded from cells prepared in this manner fire from moderately high stable resting potentials of about −60 mV, and they are completely sensitive to TTX; however, the maximal rate of rise of the action potential is still rather slow, averaging about 30 V/sec (Figure 9E and F). The mechanism whereby elevated K^+ helps to prevent reversion of the cultured cels is not known, although $[K]_i$ is reduced in the reverted cells to about 90–100 mM and this does not occur in highly differentiated cultured cells.

4.1.2. Spherical reaggregate cultures

Cells with highly differentiated electrophysiological properties can be obtained following trypsinization (in solutions containing elevated K^+ and 5 mM ATP) and subsequent reaggregation to form small spheres (0.1–0.5 mm in diameter) in vitro [39]. (Reaggregation is achieved either by gyrotation for 24–48 h or by plating the cells onto cellophane – on which the cells adhere poorly.) Action potentials recorded from cells in such reaggregates possess rapid rates of rise (up to 200 V/sec) and fire from high stable resting potentials (about −80 mV), and TTX completely abolishes all excitability (Figure 9G and H). The intracellular records are indistinguishable from those made from the intact 16-day ventricle (Figure 9A and B). As in the case of the intact old embryonic hearts, positive inotropic agents induce slowly rising action potentials in highly differentiated cultured cells following blockade of the fast Na^+ channels with TTX [39, 103, 104, 105]. Acetylcholine was without effect on cultured ventricular cells, both reverted [106] and highly differentiated [39, 107].

4.2. Young embryonic hearts

When young (2- to 3-day-old) embryonic hearts are trypsin-dispersed and the cells are allowed to reaggregate in vitro, the cells retain electrical activity characteristic of the intact young heart, i.e., the cells have low resting potentials, and they fire spontaneous slowly rising action potentials which are unaffected by addition of TTX. (DeHaan and co-workers [62] reported that they can achieve further differentiation of young cells in reaggregate culture without the addition of special extracts.)

However, as observed in the case of the young organ-cultured intact hearts, the addition of RNA-enriched extracts from adult chicken hearts to the reaggregated young myocardial cells induces the appearance of rapidly rising (e.g., 100 V/sec) action potentials which fire from high stable resting potentials (−70 to −80 mV) and are completely sensitive to TTX [98]. This further differentiation results in the achievement of adult-like electrophysiological properties, as occurs in situ. The mechanism of action of the active principal in the extract remains to be elucidated.

Jones and co-workers [108] recently reported that multilayer-cultured ventricular cells can be made to exhibit highly differentiated morphology (e.g., densely packed and aligned myofibrils with mitochondria between the fibrils), as well as advanced electrical properties, if the fetal calf serum concentration is lowered from 10% to 0.1% after the first few days, and the cells are subsequently allowed to age in vitro for several weeks.

5. INDUCTION AND PROPERTIES OF MYOCARDIAL SLOW CHANNELS

5.1. Action of positive inotropic agents

A number of positive inotropic agents exert an effect on the myocardial cell membrane to increase the number of available slow channels, and this action may at least partly explain their effect on increasing cardiac contractility[1]. It is through the slow channels that Ca^{++} influx occurs during the cardiac action potential, and the amount of Ca^{++} ion entering the myocardial cell controls the force of contraction. The Ca^{++} entering directly helps to elevate $[Ca]_i$ involved in activating the myofilaments, and indirectly does so by bringing about the further release of Ca^{++} from the intracellular stores in the sarcoplasmic reticulum (SR). It is well known that blockade of the slow channels, and hence Ca^{++} influx, by Ca^{++}-antagonistic agents, such as verapamil, D600, nifedipine, Mn^{++}, Co^{++}, and La^{+++}, depresses or abolishes the contractions without greatly affecting the normal fast action potential, i.e., contraction is uncoupled from excitation.

The positive inotropic agents which have been shown to affect the number of available slow channels include: catecholamines (such as isoproterenol and norepinephrine and other beta-adrenergic receptor agonists), histamine (H_2

[1] However, this is not meant to imply that the effect of all positive inotropic agents is mediated only via the slow channels of the membrane.

receptor), methylxanthines (such as caffeine, theophylline, and methylisobutyl-xanthine), angiotensin II, fluoride ion, and exogenous cyclic AMP and dibutyryl-cyclic AMP. The action of most of these agents is very rapid, the peak effect often occurring within 1–3 min. The action of the exogenous cyclic AMP compounds is relatively slow, the peak effect occurring in 15–30 min.

One simple method of detecting the effect of positive inotropic agents on the myocardial slow channels is to first block the fast Na^+ channels and excitability by TTX or to voltage inactivate the fast Na^+ channels by partially depolarizing the cells (e.g., to -40 mV) in elevated $[K]_0$ (e.g., 25 mM). Then, addition of agents, such as catecholamines and methylxanthines, which rapidly increase the number of slow channels available for activation upon stimulation, causes the appearance of slowly rising overshooting action potentials (the 'slow responses') which resemble the plateau component of the normal action potential (Figure 10C, F). The slow action potentials are accompanied by contractions, and it has been shown that both Ca^{++} and Na^+ inward currents participate in the slow response [109, 110, 111]. The slow action potentials are blocked by agents which block inward slow Ca^{++} current, including Mn^{++}, La^{+++}, verapamil, and D–600 [57, 110, 112]. The effect of the catecholamines, such as isoproterenol (but not that of methylxanthines, such as caffeine), is blocked by beta-adrenergic blocking agents [111]. Histamine also induces the slow action potentials, and its action is blocked by histamine H_2-receptor blocking agents (but not by beta-adrenergic antagonists or by H_1-receptor antagonists) [104, 111]. Finally, angiotensin II also induces the slow response, and its action is blocked by specific angiotensin receptor blocking agents (P-113 and sar^1-ile^8-Ang II) [103].

5.2. Relationship to cyclic AMP

Histamine and beta-adrenergic agonists, subsequent to binding to their specific receptors, are known to lead to rapid stimulation of adenylate cyclase with resultant elevation of cyclic AMP levels [111]. The methylxanthines are known to enter into the myocardial cells and to inhibit phosphodiesterase, the enzyme that destroys cyclic AMP, thus causing an elevation of cyclic AMP [111]. These positive inotropic agents also rapidly induce the slow action potentials, presumably by making more slow channels available in the membrane. Hence, it would appear that cyclic AMP is somehow involved with the slow channels [109–111, 113].

Consistent with this view, we found that cyclic AMP itself and its dibutyryl derivative also induce the slow action potentials, but only after a much longer lag period (peak effect in 15–30 min), as would be expected from slow penetration through the membrane. Another test of the cyclic AMP hypothesis

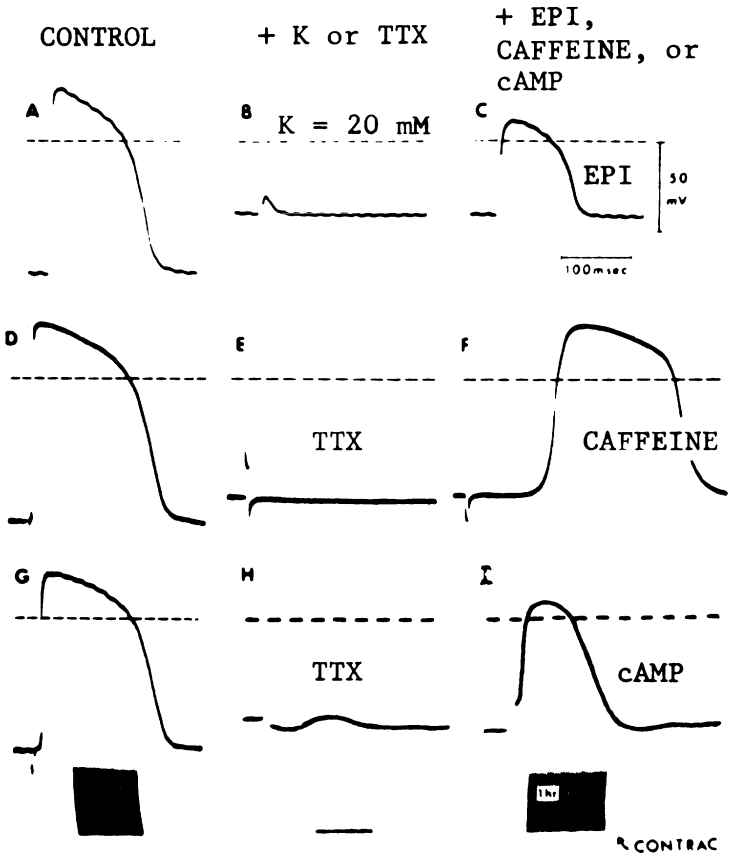

Figure 10. Induction of slow action potentials by positive inotropic agents in chick embryonic hearts (16-day-old) whose fast Na^+ channels have been inactivated by elevated $[K]_0$ (A–C) or tetrodotoxin (TTX) (D–F, G–I). A–C: K^+ inactivation of the fast Na^+ channels, and induction by catecholamine. **A**: control fast action potential. B: abolition of excitability by elevation of $[K]_0$ to 20 mM, despite a 10-fold increase in stimulus intensity. C: slowly rising electrical response produced rapidly (1 min) after addition of epinephrine (Epi, 1×10^{-5} M); contractions (not shown) accompanied the electrical responses. Addition of Mn^{++} or La^{+++} (1 mM) blocked the slow action potentials within 5 min (not shown). D–F: TTX inactivation of the fast Na^+ channels. D: control fast action potential. E: abolition of action potentials 2 min after exposure to TTX (4 μg/ml). F: restoration of electrical (and contractile) activity by addition of caffeine (1 mM). G–I: induction of the slow channels by cyclic AMP. G: control fast action potential. H: abolition of excitability in TTX. I: restoration of slowly rising electrical responses 20–60 min after addition of cyclic AMP (3 mM). The insets in G–I give the contractions recorded on a penwriter at slow speed. Note that the contractile force in I is about equal to thet in G. (Modified from Shigenobu and Sperelakis, 1972.)

was done by using a GTP analog (5′-guanylimidodiphosphate (GPP(NH)P)), an agent known to directly activate adenylate cyclase in a variety of broken cell preparations. The addition of GPP(NH)P (10^{-5} to 10^{-3} M) induced the slow action potentials in cultured reaggregates of chick heart cells within 5–30 min [114]. These results support the hypothesis that the intracellular level of cyclic AMP controls the availability of the slow channels in the myocardial sarcolemma.

Also consistent with the cyclic AMP hypothesis are recent experiments in which cyclic AMP was microinjected intracellularly into dog Purkinje fibers and guinea pig ventricular muscle (Vogel and Sperelakis, unpublished observations). It was found that cyclic AMP injections induced the slow action potentials in the injected cell for a transient period of 1–3 min; the amplitude and duration of the induced slow response was a function of the amount of cyclic AMP injected. In cases in which a slow action potential was induced by theophylline, cyclic AMP injections increased the rate of rise and amplitude of the slow responses for a transient period.

5.3. Metabolic dependence

The induced slow action potentials are blocked by hypoxia, ischemia, and metabolic poisons (including cyanide, dinitrophenol and valinomycin), accompanied by a lowering of the cellular ATP level [115]. This suggests that interference with metabolism somehow leads to blockade of the slow channels. This effect is relatively rapid; for example, the blockade occurs within 5–15 min, depending on the metabolic intervention used or the dose of metabolic poison. Under conditions in which the slow action potentials are blocked, the fast action potentials are unaffected, thus indicating that the fast Na^+ channels are essentially unaffected. Thus, there is a differential dependence of the slow channels on metabolic energy. However, although the normal fast action potential is hardly affected, the contractions are depressed or abolished, indicating that contraction is uncoupled from excitation, as would be expected if the slow channels were blocked.

The slow action potentials blocked by valinomycin are restored by elevation of the glucose concentration [116]. The same is true for slow action potentials blocked by hypoxia (Vogel and Sperelakis, unpublished observations). This suggests that the effect of metabolic poisons or hypoxia is indeed mediated by metabolic interference.

In addition to the effect of metabolism on the slow channels, with prolonged metabolic intervention, e.g., 60–120 min of hypoxia, there is a gradual shortening of the duration of the normal fast action potential, until a relatively brief spike-life component only remains, but which is still rapidly rising. Thus, metabolic interference exerts a second, but much slower, effect on the

membrane, namely to increase the kinetics of g_K turn-on, thereby shortening the action potential. The mechanism of this effect is not known, but it could be mediated by a gradual rise in $[Ca]_i$. It is known that a steady-state elevation in $[Ca]_i$ can cause an increase in g_K, the so-called Meech effect [117, 118].

5.4. Phosphorylation hypothesis

Because of the relationship between cyclic AMP and the number of available slow channels, as discussed above, and because of the demonstrated dependence of the functioning of the slow channels on metabolic energy, as described above, Sperelakis and co-workers postulated that a membrane protein must be phosphorylated in order for the slow channel to become available for voltage activation [52, 115, 119, 120]. A similar hypothesis has also been proposed by Reuter and Scholz [121] and by Tsien, Giles and Greengard [122]. The only well-documented effect of cyclic AMP is the activation of a cyclic AMP-dependent protein kinase (dimer split into two active monomers), and the latter enzyme, in the presence of ATP, phosphorylates a variety of proteins. Greengard [123] has shown that several membrane proteins are phosphorylated under appropriate conditions, and similar results, namely phosphorylation of four membrane proteins, have been shown for a cardiac membrane preparation (Economou and Sperelakis, unpublished observations).

The protein that is phosphorylated might be a protein constituent of the slow channel itself. The mechanism whereby phosphorylation would make the slow channel available for activation is not known, but could result from a conformational change that either allowed the activation gate to be opened upon depolarization or effectively increased the diameter of the water-filled pore so that Ca^{++} and Na^{+} could pass through.

Regardless of mechanism, the phosphorylated form of the slow channel would be the active (operational) form, and th dephosphorylated form would be the inactive (inoperative) form. That is, only the phosphorylated form would be available to become activated upon depolarization to threshold. The dephosphorylated channels would be electrically silent. An equilibrium would probably exist between the phosphorylated and dephosphorylated forms of the slow channels for a given set of conditions, including the level of cyclic AMP. Thus, agents that act to elevate the cyclic AMP level, either by stimulating the adenylate cyclase or by inhibiting the phosphodiesterase, would increase the fraction of the slow channels that are in the phosphorylated form, and hence available for voltage activation. Such agents would increase the force of contraction of the myocardium.

The role, if any, of cyclic GMP in the functioning of the slow channels is

not fully known [124]. However, acetylcholine is known to depress contractility and lead to blockade of the slow action potential. The interpretation is complicated somewhat because ACh is known to increase g_K, and the latter would tend to suppress the slow action potentials. It is not clear whether the effect of ACh on g_K is mediated through changes in cyclic GMP level or by some more direct mechanism. It is also not settled yet whether activation of the muscarinic receptor by ACh leads to stimulation of the guanylate cyclase.

However, there are some positive inotropic agents that do not elevate cyclic AMP. For example, angiotensin II does not elevate cyclic AMP (Vogel et al., unpublished observations), but it does induce the slow channels [103]. It is possible that angiotensin may directly activate protein kinase ([125]; see discussion in Freer et al. [103] and Vogel et al. [105]). As another example, fluoride ion, in low concentrations (<1 mM), induces the slow response and acts as a positive inotropic agent, but does not elevate cyclic AMP [105]. It is possible that fluoride ion acts by inhibiting the phosphatase which dephosphorylates the slow channel protein, thereby resulting in a larger fraction of phosphorylated channels (see Discussion and appropriate references in Vogel et al. [105]). Thus, the results with angiotensin and fluoride can be fitted within the framework of the phosphorylation hypothesis.

5.5. Selective blockade by acidosis

The myocardial slow channels induced by the positive inotropic agents are selectively blocked by acidosis. That is, the slow action potentials induced by isoproterenol (in 25 mM $[K]_0$), for example, are depressed in rate of rise, amplitude and duration as the pH of the perfusing solution is lowered below 7.0 [126]. The slow action potential is completely abolished at about pH 6.1, and 50% inhibition occurs at about pH 6.6. The contractions are depressed in parallel with the slow action potentials.

In the normal fast action potential situation, acidosis has little or no effect on the action potential, i.e., the rate of rise remains fast and the overshoot and duration are only slightly affected. However, the contractions become depressed and abolished as a function of the degree of acidosis [126]. That is, excitation-contraction uncoupling occurs, as has been well-known, and as would be expected from a rather selective blockade of the slow channels.

The mechanism of the pH blockade of the slow channels is not known. Two different buffer systems, HCO_3^-–CO_2 buffer and PIPES buffer, gave essentially the same results and about equally fast. Since the PIPES buffer system should only slowly change the intracellular pH, it appears that the blockade of the slow channels occurs with acidification of the outer surface of the cell membrane. This could change the surface of the membrane

and/or change the conformation of the protein components of the slow channels.

Similar results of acidosis were shown in voltage clamp experiments on cardiac muscle, namely that acid pH depressed the inward slow current (I_{SI})[127]. However, complete blockade did not occur until a considerably lower pH was reached, about 5.5. The difference in results as compared with the slow action potential could be explained by the fact that the slow action potentials would be abolished before all the slow channels were blocked because of the requirement of a minimum density of slow channels for regenerative and propagating responses.

Since the myocardium becomes acidotic during hypoxia and ischemia[128, 129, 130], it is likely that some of the effects of these metabolic interventions on the slow channels are mediated by the accompanying acidosis, and not solely by a decrease in ATP level. During hypoxia and ischemia, the rate of glycolysis is increased greatly, and lactic acid diffuses into the interstitial fluid space. Consistent wih this possibility, it was shown by Belardinelli et al.[131] that the effects of hypoxia on the slow action potential could be almost immediately reversed, but only partially and only transiently, by changing the pH of the perfusing solution to 8.0; even at the alkaline pH, the responses gradually further diminished during the hypoxic period.

5.6. Protection hypothesis

The Ca^{++} influx of the myocardial cell can be controlled by extrinsic factors, as discussed above. For example, stimulation of the sympathetic nerves to the heart or circulating catecholamines or other hormones can have a positive inotropic action, whereas stimulation of the parasympathetic neurons can have a negative inotropic effect. At least part of the mechanism for these effects must be mediated by changes in the levels of the cyclic nucleotides. This extrinsic control of the Ca^{++} influx is enabled by the peculiar properties of the slow channels as, for example, the postulated requirement for phosphorylation.

However, in addition, the myocardial cell itself can exercise control over its Ca^{++} influx, i.e., there is intrinsic control. For example, under conditions of transient regional ischemia, many of the slow channels through which Ca^{++} passes become unavailable (or silent). This effect may be mediated by lowering the ATP level of the affected cells and by the accompanying acidosis. The speed of slow channel blockade in hypoxia is a function of pH, being faster in acid pH than in alkaline pH. As discussed above, acidosis blocks the slow channels directly (presumably), and metabolc interference causes inactivation of the slow channels; both effects are relatively selective for the slow channels.

Thus, the Ca^{++} influx can be partially or completely suppressed by the myocardial cell under adverse conditions. This causes the affected cells to contract weakly or not at all, and since most of the work done by the cell is mechanical, this serves to conserve ATP. Such a mechanism may serve to protect the myocardial cells under adverse conditions such as transient regional ischemia. That is, if the myocardial cell could not control its Ca^{++} influx, then, under such conditions, the ATP level might drop so low that irreversible damage would be done, i.e., the cells would die. Because of the peculiar and special properties of the slow channels, the slow channels become inactivated, thus uncoupling contraction from excitation and thus conserving ATP. The cells might then be more able to recover fully when the blood flow returns to normal. The almost normal resting potential and fast action potential would allow propagation through the ischemic area to be normal, thus minimizing the chances of the induction of arrhythmias.

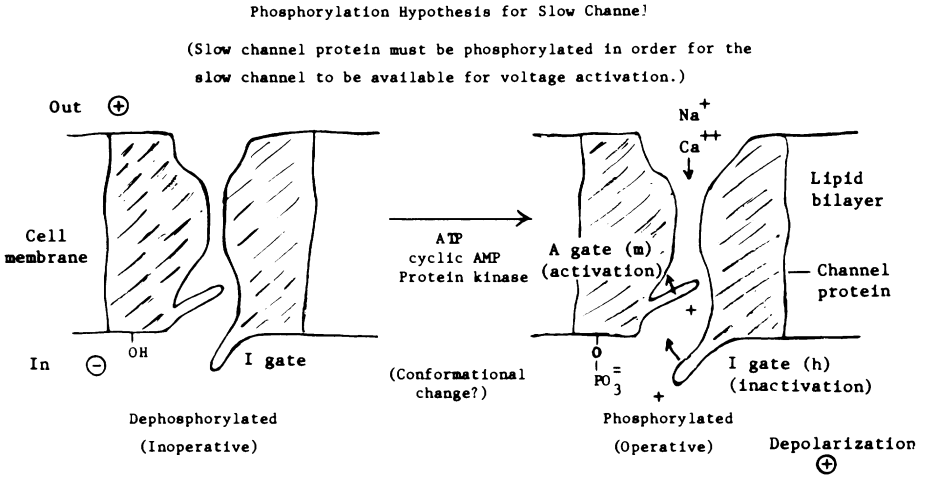

Figure 11. Cartoon model for a slow channel in myocardial cell membrane. Two hypothetical forms of the channel are depicted: dephosphorylated form (left diagram) and phosphorylated form (right diagram). As shown, there are two gates associated with each channel: an activation (A) gate, and an inactivation (I) gate. The gates are presumably charged positively so that they can sense the membrane potential. The I gate moves more slowly than the A gate. The gates of the slow channel are kinetically much slower than those of the fast Na^+ channel. The slow channel in the left diagram is depicted in the resting state (A gate closed, I gate open), whereas in the right diagram, the channel is just beginning the process of activation. In the active state, both gates are open. Depolarization causes the A gate to open relatively quickly, so that the channel becomes conducting (the active state). However, the I gate slowly closes during depolarization and inactivates the channel (the inactive state). During recovery upon repolarization, the A gate closes and the I gate opens (returns to the resting state). Also illustrated is the hypothesis that a protein constituent of the slow channel must be phosphorylated in order for the channel to be in a functional state available for voltage activation. Verapamil and Mn^{++} block the slow channel, perhaps by binding to the mouth of the channel. Tetrodotoxin (TTX) blocks fast Na^+ channels from the outside, presumably by lodging in the channel mouth.

Althougn there is still some debate, it appears that the action potential in a prolonged ischemic region is not a slow-channel action potential, as was originally suggested [132], but rather a depressed fast-channel action potential. The depression of the rate of rise is presumably due to a partial depolarization of the cells, presumably due to K^+ accumulation in the interstitial space and perhaps to depression of electrogenic Na^+ pumping. (Depolarization leads to voltage inactivation of a fraction of the fast Na^+ channels (due to closing of the inactivation (I) gates), as determined by the Hodgkin–Huxley h_∞ vs. E_m curve.) One would expect that the slow channels would be nearly completely blocked because of the acidosis and lowered ATP level, and hence the action potential could not be dependent on an inward slow current (I_{SI}).

The slower effect of metabolic interference on markedly shortening the cardiac action potential, due to enhanced kinetics for g_K turn-on that terminates the action potential and presumably mediated by an elevated $[Ca]_i$, would also help to shut off I_{SI} more quickly, thereby reducing the total Ca^{++} influx per impulse and so helping to conserve ATP.

6. SUMMARY AND CONCLUSIONS

Striking changes occur in the electrical properties of the myocardial cell membrane during embryonic development of chick hearts. The young tubular hearts (2–3 days old) have a low resting potential of about -40–50 mV, even though $[K]_i$ is nearly as high as the adult value. Analysis shows that the low resting potential is caused by a low potassium permeability (P_K). The low P_K can also account for the high degree of automaticity observed in the ventricular cells of the young embryonic heart. P_K increases rapidly during development, nearly attaining the final adult value by day 12; the resting potential increases to about -80 mV, and automaticity of the ventricular cells is suppressed. The young heart has a low (Na, K)-ATPase activity, and this enzyme activity increases during development along with P_K.

The young 2–3-day-old hearts have slow-rising action potentials (10–30 V/sec) which are dependent mainly on $[Na]_0$ and which are not affected by tetrodotoxin (TTX); hyperpolarization does not greatly increase the action potential rate of rise. Thus, it appears that fast Na^+ channels are absent or relatively few in number in young hearts, the inward current during the action potential being carried predominantly through TTX-insensitive slow Na^+ channels. The slow Na^+ channels are blocked by verapamil, but not by 1 mM Mn^{++}. Mn^{++} does block the contractions, presumably by blockade of the Ca^{++} influx during the action potential. In many respects, the young tubular hearts resemble pulsating blood vessels, and their electrical properties are somewhat similar to those of vascular smooth muscle. There is a progres-

sive increase in the maximal rate of rise of the action potential ($+\dot{V}_{max}$) during development. By day 5, $+\dot{V}_{max}$ is about 50–80 V/sec, and TTX now reduces $+\dot{V}_{max}$ to 10–30 V/sec, i.e., to the value observed in 2–3-day-old hearts. Thus, the TTX-sensitive fast Na^+ channels progressively increase in number until they attain the final adult level by day 18. Between day 5 and day 8, fast Na^+ channels and a high density of slow Na^+ channels coexist. After day 8, TTX usually completely abolishes excitability, suggestive that the slow channels have decreased in number sufficiently so as not to support regenerative excitation. The cyclic AMP level is very high in young hearts and this also decreases during development. The fact that isoproterenol can elevate the cyclic AMP level in even young 4-day-old hearts (that already have high levels of cyclic AMP) indicates that functional beta-adrenergic receptors are present prior to innervation. The slow channels in young hearts are predominantly of the slow Na^+ type, whereas those slow channels induced by some positive inotropic agents, e.g., isoproterenol, in older hearts are of the slow Ca–Na type.

Young embryonic rat hearts also have been shown to fire slowly rising TTX-insensitive action potentials; the major difference from the embryonic chick is that the slow channels that pass the inward current for the action potential appear to be predominantly of the slow Ca^{++} type rather than of the slow Na^+ type.

Young embryonic chick hearts (3 days old), removed prior to innervation and placed into organ culture for 2 weeks or grafted on the chorioallantoic membrane of a host chick for blood perfusion, do not gain fast Na^+ channels or otherwise differentiate. This suggests that something in the in situ condition, such as neurotrophic factors, is required for triggering further differentiation. If, however, these young organ-cultured hearts are exposed for several days to an RNA-enriched fraction obtained from adult chicken hearts, they do gain TTX-sensitive fast Na^+ channels. This induction is blocked by inhibitors of protein synthesis, such as cycloheximide. Thus, the synthesis of specific membrane proteins controls the appearance of fast Na^+ channels. Hence, cardiac myoblasts whose development, with respect to some membrane electrical properties, has been arrested in vitro can be induced to differentiate further.

Trypsin-dispersed myocardial cells obtained from young embryonic chick hearts and placed in cell culture for several weeks also do not proceed with differentiation in vitro, unless exposed to the RNA extract from adult heart. Cultured heart cells prepared from old embryonic heart (ventricles) rapidly revert back to the young embryonic state. That is, they lose most or all of their fast Na^+ channels, gain slow Na^+ channels, and gain automaticity because of a low P_K; they also lose many of their myofibrils. If, however, the cells are allowed to reaggregate into small spheres and incubate for a period of

1–3 weeks, they often will regain highly differentiated electrical properties. These cells also retain their membrane receptors for catecholamines, histamine, and angiotensin. Cultured atrial cells possess receptors for acetylcholine. Thus, highly differentiated myocardial cells can be maintained in cell culture under appropriate conditions.

Positive inotropic agents, such as norepinephrine, histamine, and methylxanthines, rapidly induce slow Ca–Na channels in old embryonic myocardial cells. Following blockade of the fast Na^+ channels with TTX, these agents rapidly allow the production of slowly rising action potentials by increasing the number of slow channels available for voltage activation. Since norepinephrine, histamine, and methylxanthines rapidly elevate intracellular cyclic AMP levels, these results suggest that cyclic AMP is somehow related to the number of operational slow channels. Exogenous cyclic AMP produces the same effect, but much more slowly.

The myocardial slow channels induced by the positive inotropic agents are very sensitive to blockade by metabolic poisons, hypoxia and ischemia. This slow action potential is blocked at a time when the rate of rise and duration of the normal fast action potential is essentially unaffected. However, the contraction accompanying the fast action potential is depressed or abolished, i.e., contraction is uncoupled from excitation, as would be expected if the slow channels were blocked. Therefore, the slow channels are metabolically dependent, whereas the fast Na^+ channels are not. A second, but much slower, effect of metabolism is to increase the kinetics of g_K turn-on, perhaps mediated by an increase in steady-state $[Ca]_i$.

The slow channels are also selectively sensitive to blockade by acid pH. That is, at pH 6.8–6.1, the slow action potential is depressed or blocked, whereas the fast action potential is essentially unaffected (but excitation-contraction uncoupling occurs). Part of the rapid effect of ischemia and hypoxia in blocking the slow channels appears to be mediated by the concomitant acidosis.

Because of the dependence of the myocardial slow channels on cyclic AMP level and on metabolism, we have postulated that phosphorylation of a membrane protein constituent of the slow channel, by a cyclic AMP-dependent protein kinase and ATP, makes it available for voltage activation.

By these special properties of the slow channels, the myocardial cell's Ca^{++} influx can be controlled by extrinsic factors, such as by autonomic nerve stimulation or circulating hormones, or by intrinsic factors, such as intracellular pH or ATP level. In the case of regional transient ischemia, the selective blockade of the slow channels, which results in depression of the contraction, hence work, of these afflicted cells, might serve to protect the cells against irreversible damage by helping to conserve their ATP content.

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III. MODELS OF THE SLOW INWARD CURRENT

11. THE SLOW INWARD CURRENT OF THE RABBIT SINO-ATRIAL NODAL CELLS *

HIROSHI IRISAWA and KAORU YANAGIHARA

1. CHARACTERISTICS OF THE SINO-ATRIAL NODAL ACTION POTENTIAL

Action potentials of the sino-atrial nodal cell are characterized by the presence of slow spontaneous phase 4 depolarization with smooth transition to the rapid upstroke of depolarization. The upstroke velocity of the sinus node action potential is slow, resistant to the effects of TTX and sensitive to the manganous ion and D–600.

From these characteristics of the sino-atrial node, most text books of cardiac physiology state that the slow inward current, presumably carried by Ca^{++} ion, may be responsible for the upstroke of the sino-atrial nodal action potential.

In the Purkinje fiber, it is well established that the upstroke of the action potential is mainly caused by the time- and voltage-dependent fast inward current which is carried by Na^{+} ion. The main physiological function of the fast inward current is excitation of the membrane and rapid conduction of electrical impulses. The kinetics of the fast inward current are similar in many excitable tissues, including giant squid axon, frog myelinated nerve fiber and rabbit cardiac Purkinje cells. Those cells which show the slow inward current, on the other hand, have many different physiological functions other than excitation of the membrane. The slow inward current plays an essential role in contraction and plateau formation of the working myocardium, in pacemaker activity of sino-atrial nodal cells, in secretion of gland cells, as well as in the postsynaptic membrane activities of muscle. Filtering action and kinetics of the gating mechanisms of the slow inward current channel are also varied from species to species and from specimen to specimen. In the following discussion, we will review recent findings on the slow inward current of the rabbit sino-atrial cells.

The most conclusive information regarding the slow inward current of cardiac muscle should be obtained by an appropriate voltage clamp method.

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At the present stage, available methods of voltage clamping sino-atrial nodal cells are not ideal, although much progress has been made since Irisawa initially tried the double sucrose gap voltage clamp method of the sino-atrial nodal tissue [1]. The concept of the slow inward current in the sino-atrial nodal cell is derived primarily from data obtained by the classical measurement of the membrane potential through the microelectrode [2]. Microelectrode studies on sino-atrial node have many difficulties as has already been pointed out [3, 4]. Cells of the pacemaker region are usually small in dimension, and coexisting contraction of the muscle cells makes impalement by the microelectrode extremely difficult for a long time. Two kinds of cells exist in the pacemaker region, pacemaker cells and atrial cells. Intermediate cells are in between these two kinds. Recently, Taylor et al. [5] injected lanthanum ions into the pacemaker cells of the rabbit sino-atrial node. They found that the cells exhibiting phase 4 depolarization were the P-cells [6].

From measurements of the membrane potential, it is difficult to differentiate pacemaker cells from intermediate cells. Pacemaker sites often shift either microscopically or macroscopically. An early study by Eyster and Meek [7] described shift of the pacemaker from cranial to caudal sites after local cooling. This information indicated that shifting of the pacemaker site over 5 mm had occurred. However, shifting also can be observed within a small specimen of approximately 0.5 mm in diameter. Even in such a small specimen, one can frequently observe a change of action potential pattern from a smooth transition from phase 4 to phase 0, to an abrupt upstroke accompanying a sudden increase in the magnitude of the maximum diastolic potential.

Maximum rate of rise of depolarization is a measure of the magnitude of the transient inward current. In Purkinje fibers, the value has been used widely since the classic study of Weidmann [8]. Theoretically, this measurement can be applied under the condition that action potentials were conducted with an equal velocity through the muscle strand or that the action potential was measured in a single leaky membrane model. The small sino-atrial nodal specimen approximates the latter condition. However, in the measurement of the rate of rise of upstroke, another condition must be taken into account. The time course of the delayed increase of the outward current is slow compared to the entire time course of the transient inward current. The outward current, activated before the peak of the transient inward current, might act to depress the maximum rate of rise of depolarization and the peak of an action potential. In Purkinje or ventricular muscle fibers, the maximum rate of rise is about 300–500 V/sec, and the time constant of inactivation is on the order of milliseconds at a membrane potential of 0 mV. Therefore, the presence of an outward current will not significantly alter rising velocity or action potential amplitude in these fibers. However, in sino-atrial

nodal cells, the maximum rate of rise of depolarization is always under 10 V/sec. If the outward current is activated early during the transient inward current, the maximum rate of rise will not be a simple measure of the inward current. No such precautions have been observed regarding sino-atrial nodal action potentials in the past.

Action potentials of the sino-atrial node, when recorded from a large specimen, often show notches and summation. These phenomena could be due to unsynchronized cell activity. When these irregular patterns appear in the action potential, the action potential may no longer be regarded as being recorded from a simple cable model or from a simple leaky cell model. Therefore, one cannot use the maximum rate of rise as a measure of the inward current in these cases. In spite of these difficulties, the maximum rate of rise might be used cautiously and with reservation used as a qualitative indicator of the transient inward current.

2. OBSERVATIONS FROM MEMBRANE POTENTIAL MEASUREMENTS

Amplitude of the action potential of the sino-atrial node varies from 50 mV to 100 mV [9–13]. This variability may not be an artifact. In 100 penetrations, Noma and Irisawa [14] confirmed the variation in action potential amplitude and maximum rate of upstroke velocity in various parts of the sino-atrial node (Figure 1). These different amplitudes and maximum rates of rise of action potentials may be due to impalements of different type of cells, some of which may be sinus nodal cells with low amplitudes and rates of rise, compared to nonpacemaker sites.

Different amplitudes may also be due to various cell activities. For example, if certain cells are under the influence of a higher level of Na–K pump activity, their membrane must be more hyperpolarized because of the decreased intracellular Na^+ ion concentration. Within 3 min after penetration of the microelectrode, both the amplitude and the maximum rate of rise of upstroke velocity increase. This phenomenon is always observed in a small specimen, but not often observed in a large specimen. Recovery of the membrane after microscopically induced injury due to electrode penetration may be one of the reasons for this phenomenon.

Cells of the sino-atrial node undergo pulsatile stretch due to arterial pulsation [15, 16]. Stretch of the sino-atrial node usually causes an increased frequency of spontaneous discharge, due perhaps to depolarization of the membrane [17, 18]. Changes in arterial pressure or the local blood supply will immediately vary the membrane potential of the sino-atrial nodal region. Though this is a problem in an intact preparation, some caution must be applied when interpreting the effects of stretch of the muscle in an excised

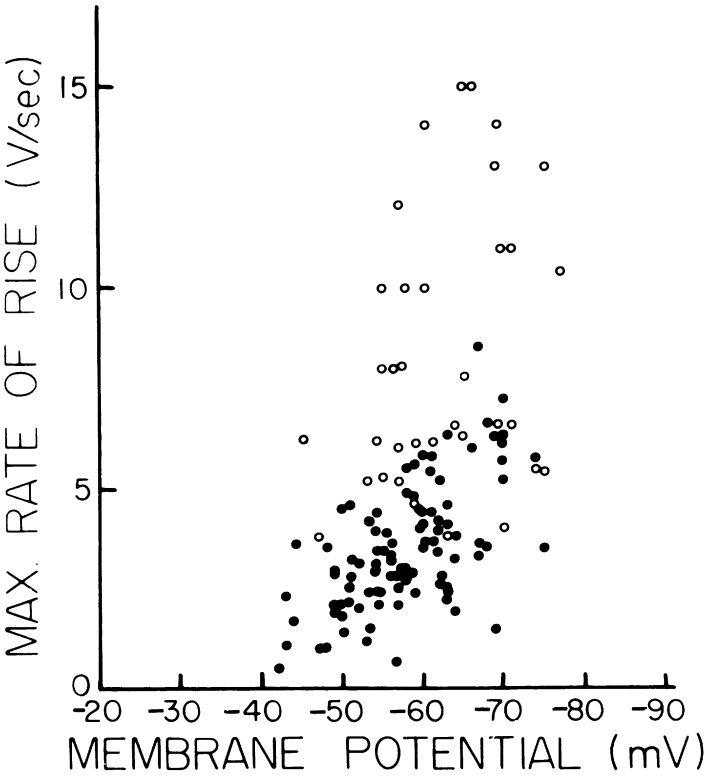

Figure 1. Maximum rate of rise of action potential of S-A node cell. Action potentials were recorded from S–A node region. Closed circles, central part of the S–A node region; open circles, perinodal region of the S–A node cell. (From Noma and Irisawa, 1974, ref 14.)

specimen. In spite of these different conditions, it can safely be stated that the action potentials in the sino-atrial nodal region show low amplitudes and small maximum rates of rise of depolarization compared to these parameters measured in the working muscles.

3. FINDINGS OBTAINED FROM THE TRANSIENT INWARD CURRENTS IN VOLTAGE CLAMPED SINO-ATRIAL NODAL CELLS

A slow maximum rate of rise of depolarization suggests that the transient inward current of the sino-atrial node is the slow inward current. One must ask whether or not ionic channels of the slow inward current of the sino-atrial node show the same filtering action and similar gating kinetics as has been described previously. Voltage clamp methods in sino-atrial nodal cells are still unsatisfactory, especially during the initial 10 msec after the clamp when the

slow inward current occurs. Nevertheless, we are able to describe qualitatively some of the ionic characteristics of the transient inward current in a small sino-atrial nodal specimen. The preparation of this specimen has been reported elsewhere [19, 20]. Figure 2 shows the voltage clamp records at test pulses of −20, 0 and +27 mV, respectively, at the holding potential of −40 mV. Semilogarithmic plots in Figure 2B were obtained from the deviation of the change of the total current during the test pulse from the estimated steady-state value which was given by the extrapolation of the time-dependent outward current. The difference between this line and the continuous curve indicates the time-dependent component of the slow inward current and the maximum value of this component. As shown in this figure, the time course of the current at +27 mV depolarization was almost fitted by a straight line suggesting that the reversal potential of the slow inward current may be close to this potential.

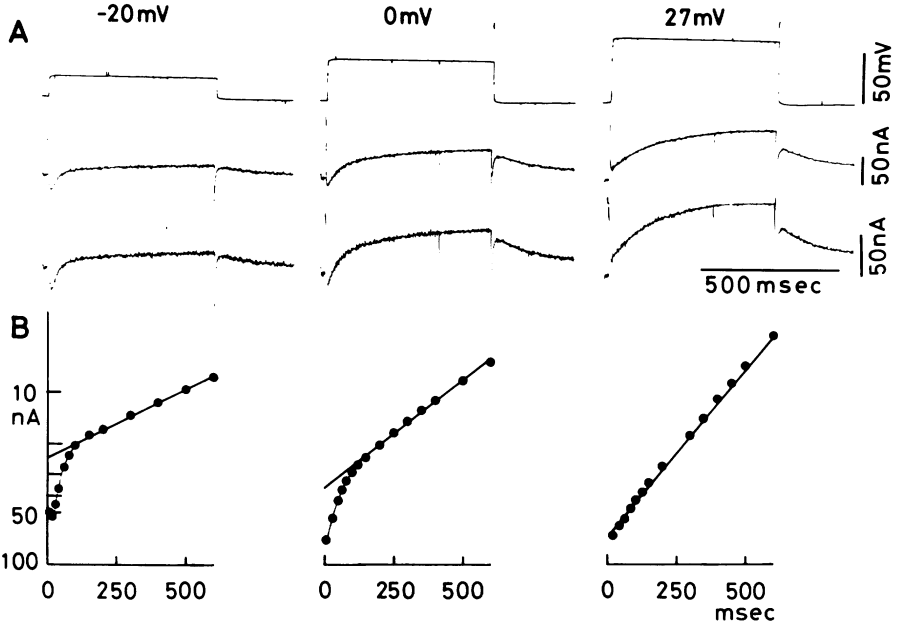

Figure 2. Measurement of the slow inward current in S–A node cell. A: voltage clamp record at 3 step test pulses from the holding potential of −40 mV. B: amplitude of the slow inward current was measured from the semilog plot of the current recordings.

4. RELATION BETWEEN TRANSIENT INWARD CURRENT OF SINO-ATRIAL NODAL CELLS AND TETRODOTOXIN (TTX)

It has been shown that the action potential of the sino-atrial nodal cell is highly resistant to TTX [4, 21–23]. The upstroke velocity of the sino-atrial

nodal action potential is much less sensitive to changes in extracellular Na^+ concentration ($[Na^+]_0$) than is the upstroke velocity of Purkinje fibers. Also, sino-atrial nodal cells are very sensitive to Mn^{++} and D600. From these observations, investigators have assumed that the rising phase of the sino-atrial nodal action potential is due to the inflow of Ca^{++} ions through the slow channel. It was found that the rising phase of the sino-atrial nodal action potential was reduced, although to a very slight degree, by administration of TTX. Kreitner [24] and later Irisawa [23] found that the sino-atrial nodal cell membrane is insensitive to TTX at the control membrane potential, but when the membrane was hyperpolarized by an application of carbamyl choline or by anodal current the rate of rise of depolarization increased. They suggested that the fast inward current channel might exist in sino-atrial nodal cell membranes, but that it is almost completely inactivated at -40 mV. However, Seyama [25] recently showed dose–response curves of TTX on the sino-atrial nodal transient inward current. He measured the membrane current at the test pulse of 0 mV from the holding potential of -40 mV, holding as long as 100 msec. Tetrodotoxin reduced the transient inward current by 5% of the control inward current at a concentration of 1×10^{-7} M, by 10–20% at a concentration of 1×10^{-6} M and by 30–40% at a concentration of 1×10^{-5} M, (Figure 3). Within this range of TTX concentrations, outward current did not change appreciably. Assuming that TTX interacted 1 : 1 with the channel, Seyama [25] computed the dissociation constant as $k = 9.2\times10^{-9}$ M accord-

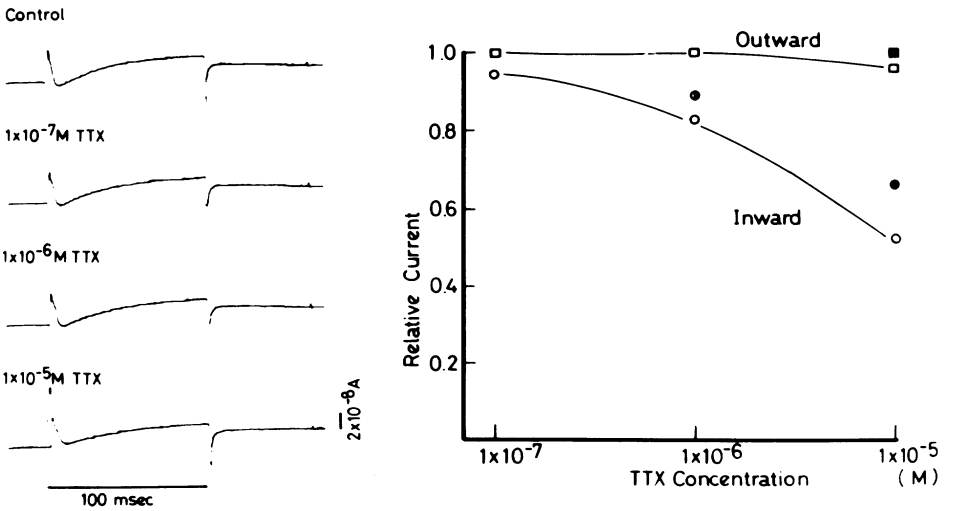

Figure 3. Effect of TTX on the inward and the outward current of the S–A node at various TTX concentrations. Holding potential was -40 mV and test pulse was 0 mV. (Seyama, 1978, ref. 25.)

ing to the studies of Cuervo and Adelman [26]. Therefore, the sensitivity of sino-atrial nodal cells to TTX is approximately 1/3000 that found in squid giant axons.

The presence of the fast and the slow inward current channels in the sino-atrial nodal cell has been suggested by Noma et al. [20]. They showed the presence of an initial fast inward current at the holding potential of -46 mV before the occurrence of the secondary slow inward current. The initial fast inward current disappeared at the holding potential of -41 mV or -38 mV. The spontaneous action potential after the voltage clamp is shown in Figure 4, tracing 3. In 4 examples which showed such a fast inward current, the maximum rate of upstroke was 5.0, 6.0, 14.7 and 15.6 V/sec, respectively. However, in many other experiments, the capacitive current often persisted for more than 5 msec and made it impossible to record the early part of the transient inward current. The fast inward current is sensitively blocked by an application of 1×10^{-7} g/ml TTX. In Figure 5A are shown voltage clamp records before, during and after the administration of TTX. After each voltage clamp experiment, spontaneous potentials were recorded (Figure 5B). The maximum rate of rise was 5 V/sec in the control, but it was reduced to 3.9 V/sec after TTX. In 20 specimens, the rate of upstroke velocity was reduced from 7.6 ± 2.3 V/sec to 5.8 ± 1.9 V/sec (mean$\pm$SD) by TTX. Since TTX is also effective against the slow inward current, the reduction of rate of rise may not be proof of the presence of the fast inward current channel in sino-atrial nodal cells. In some specimens, we were unable to find the fast

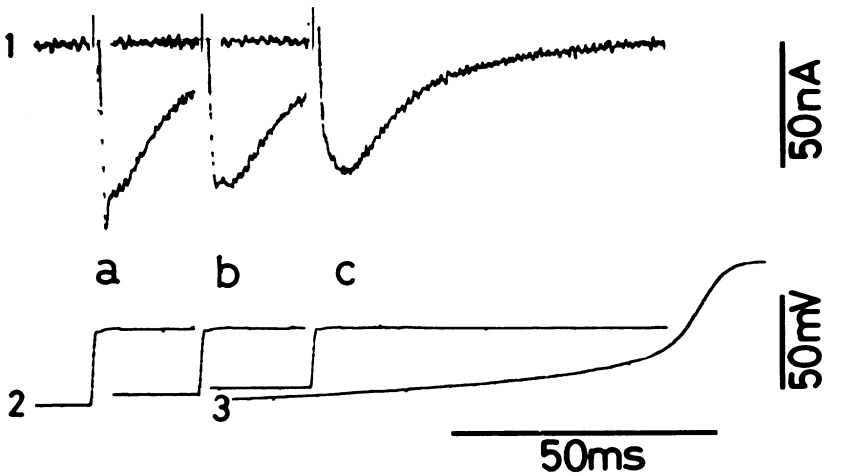

Figure 4. The membrane current (1) and voltage (2) records. In 3, the action potential after the voltage clamp experiment was shown. (Noma, Yanagihara and Irisawa, 1977, ref. 20.) Holding potentials were -46 mV in a, -41 mV in b, and -38 mV in c, and the test pulses were all -8 mV.

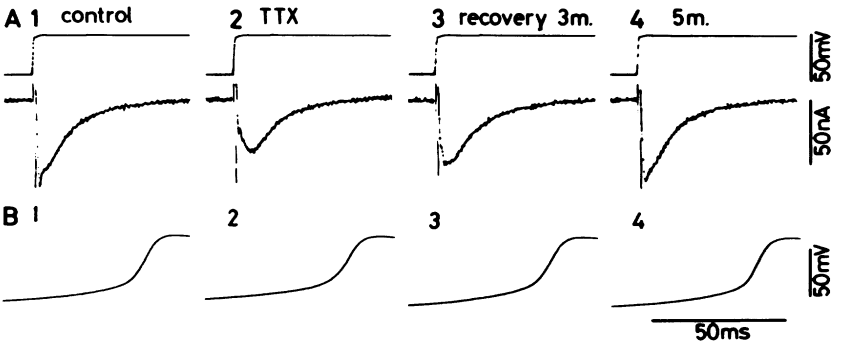

Figure 5. Records of the voltage clamp experiments (A) and the rising phase of the spontaneous action potential (B). 1 is the control; 2, 2 min after application of 10^{-7} g/ml TTX; 3, 3 min recovery in Tyrode solution; 4, after 5 min recovery in Tyrode's solution. (Noma, Yanagihara and Irisawa, 1977.)

inward current prior to the secondary slow inward current. However, this finding cannot rule out the possibility of the presence of the primary fast inward current in the sino-atrial nodal cell. At present, it can be stated that, in some limited number of cells in the sino-atrial node, there appears to exist the fast inward current. We must, however, entertain one alternative possibility – that is, the possibility of artifact due to incomplete spatial clamp. In many preparations, escape of the membrane potential from the control during the large inward current and/or oscillation of the feedback system occurred. When the size of the preparation, the electrical resistance of the current-feeding electrode and series resistance are relatively large, these oscillations are apt to occur. However, we have observed fast inward current where the size of the preparation is reasonably small. Bezanilla et al. [27], using a mathematical model, simulated the fast and the slow inward currents, in which only one Na^+ current component or the rising phase of the action potential was observed. It is likely that the fast inward current in the sino-atrial nodal cell is more sensitive to TTX than the slow inward current. The selective action of TTX on the initial phase of the transient inward current (Figure 5) suggests the presence of the fast inward current in at least some sino-atrial nodal cells. The mathematical simulation of the sino-atrial nodal action potential, however, indicates that most of the fast inward current is inactivated at the membrane potential where phase 4 depolarization occurred, and therefore there might be little physiological significance to the fast inward current in the sino-atrial nodal cell.

Contrary to the high resistance of sino-atrial nodal cell membrane to TTX, the membrane is highly sensitive to Mn^{++} ion and D–600. Mn^{++} effectively depressed both rate of rise of the action potential and the slow inward current. Even in a small concentration of 0.5 mM Mn^{++} the maximum rate

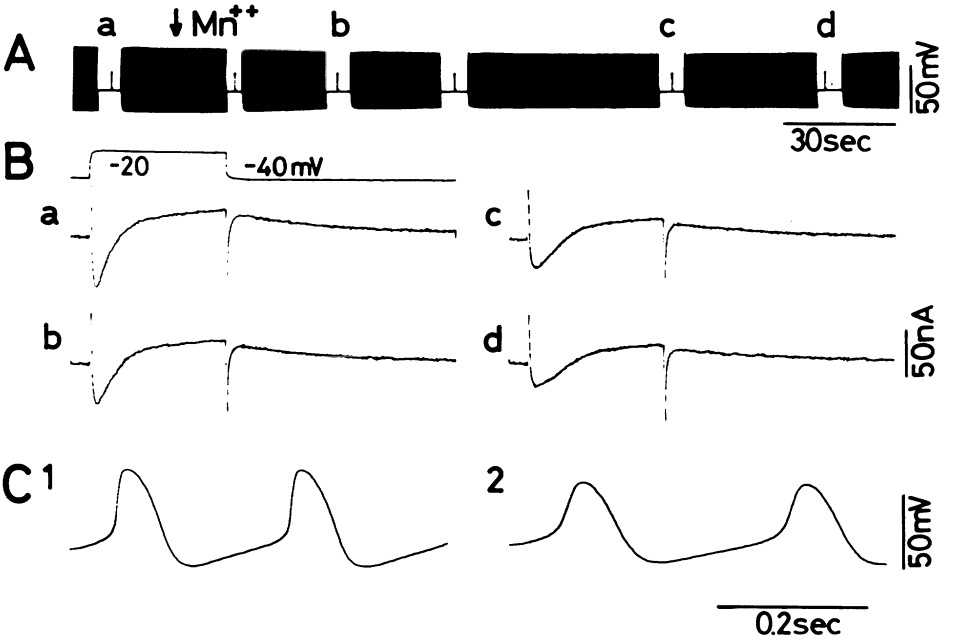

Figure 6. Effects of 0.5 mM Mn on the spontaneous action potential (A, C) and the membrane currents (B). The membrane potential was clamped intermittently. The membrane current at a, b, c and d in record A were shown in B at the higher time resolution. Even in a small concentration of Mn, the slow inward current of the S–A node cell was inhibited. (Noma, Yanagihara and Irisawa, 1977, ref. 20.)

of rise of action potentials declined from 4.4 to 1.6 V/sec (Figure 6, C-2). Concomitantly, the spontaneous frequency decreased to 75% of the control value. The amplitude of the transient inward current, in response to clamp depolarization from -40 mV to -20 mV, decreased by about one-half (Figure 6, Bb). When the concentration of Mn^{++} was raised to 1 mM, the spontaneous activity ceased within 1 min of perfusion. This effect of Mn^{++} was completely reversible when Mn^{++} was washed out of the bath. These results confirm the high sensitivity of the sino-atrial nodal cells to Mn^{++}, observed in other laboratories [28–30]. D–600 also effectively depressed the slow inward current and, as a result, reduced the spontaneous activity of the sino-atrial node. Figure 7 is one example of D–600 application. D–600, 1×10^{-7} g/ml, was applied as long as 5 min. In the inset graph, the steady-state current–voltage relationship is given. D–600 at this concentration relatively selectively blocked the slow inward current, without affecting the outward current. However, when higher doses of D–600 were applied, both inward as well as outward currents were depressed. It is known that D–600 at high concentrations blocks the ionic channels nonselectively [20, 31, 32].

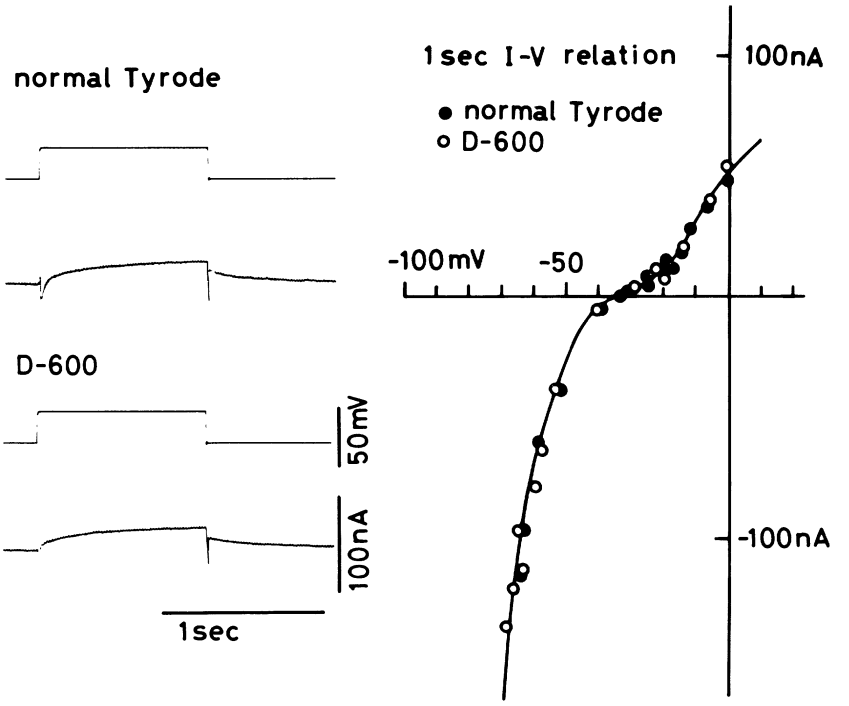

Figure 7. Effect of D-600 on the slow inward current of the S–A anode cell. Voltage clamp record in normal Tyrode (top) and in D-600 solution (bottom) were compared. Holding potential was −30 mV and the test pulse was 0 mV. Graph illustrates the steady-state current–voltage relationship after 1 sec of test pulse. The slow inward current was abolished in D-600 solution, but there was no appreciable change in outward current within 10^{-7} g/ml D-600 solution.

5. EFFECT OF Na^+ ION ON THE TRANSIENT INWARD CURRENT OF SINO-ATRIAL NODAL CELLS

Sino-atrial nodal cells are relatively insensitive to changes of $[Na^+]_0$. Thus, excitation continues even in a reduced Na^+ solution of 68.5 mM, 50% of normal Tyrode's solution, where the spontaneous activity of the Purkinje fiber tends to be abolished completely [1]. However, Noma and Irisawa [14] and Noma et al. [20] showed a high sensitivity of sino-atrial nodal cells to $[Na^+]_0$. Figure 8 shows voltage clamp records obtained at various test pulses from the holding potential of −40 mV. The preparation was perfused with normal Tyrode's solution containing 1×10^{-7} g/ml TTX. Time course of the transient inward current was slow. In 25% $[Na^+]_0$ Tyrode's solution, the slow inward current was reduced noticeably. Peak of the inward current was plotted in relation to the test pulses and the instantaneous current–voltage relationship was obtained. Marked negative slope conductance between −40

and 0 mV was observed. This range of the negative slope was similar to that observed previously [19, 33]. Although the instantaneous current–voltage curve intersects the potential axis at +20 mV, this potential is far below the E_{Na}, because of the presence of the relatively large outward current.

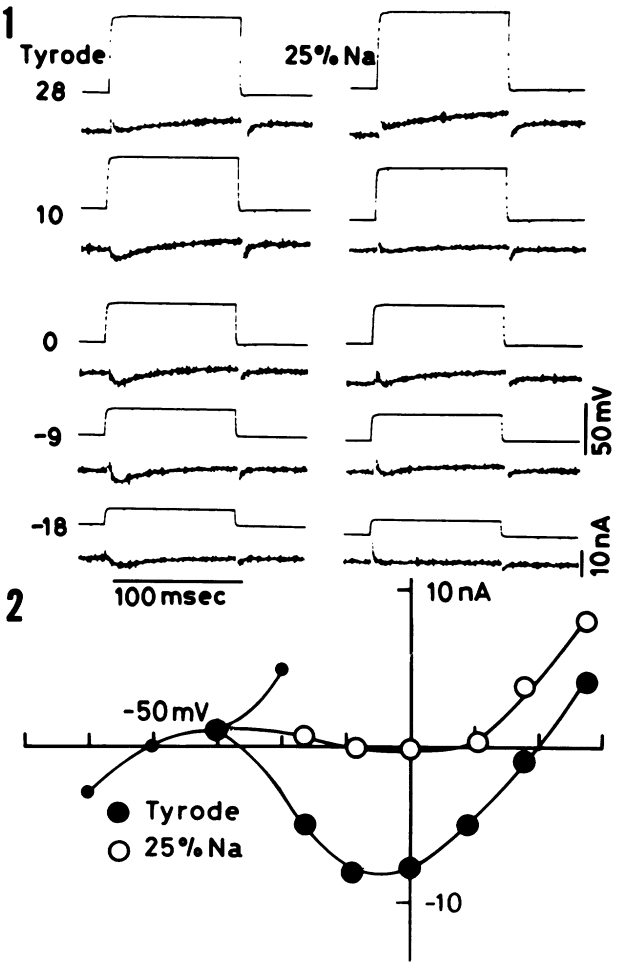

Figure 8. Effect of Na^+-depletion on the slow inward current. Holding potential was −40 mV. Numerals at the left side give the magnitude of the test pulse. The current voltage relationship indicates that, in 25% $[Na]_0$ solution, the slow inward current was markedly reduced. In this figure, the control slow inward current was relatively small. This might be due to the preperfusion of 10^{-7} g/ml TTX. This fact suggests that the TTX resistive slow inward current is sensitive to $[Na]_0$. Small closed circle indicates the current at 0.1 sec after the test pulse. Large closed and open circles as indicated in the figure.

6. EFFECT OF CALCIUM IONS ON THE INWARD CURRENT OF THE SINO-ATRIAL NODAL CELLS

The above findings suggest that Ca^{++} ion may be one of the charge carriers of the inward current of the sino-atrial nodal cell. There are indeed, several lines of evidence indicating that Ca^{++} ion participates in the inward current of the sino-atrial nodal cells: (1) It is not yet clear whether the spontaneous action potential becomes quiescent in Ca^{++} free solution or not but, in several instances, we found cessation of spontaneous activity when the specimen was extremely small. (2) Transient inward current was reduced in 0.18 mM Ca^{++} solution (Figure 9–1, 2). After replacement of Tyrode's solution, 5 mM Ca^{++} was superfused (Figure 9–3, 4). The slow inward current increased noticeably. On replacing 5 mM Ca^{++} with Tyrode's solution (Figure 9–5), the slow inward current increased further. As Figure 9–6 shows, when 5 mM Ca^{++} was reapplied, the slow inward current was reduced. These data suggest that transient inward current increased as the extracellular Ca^{++}

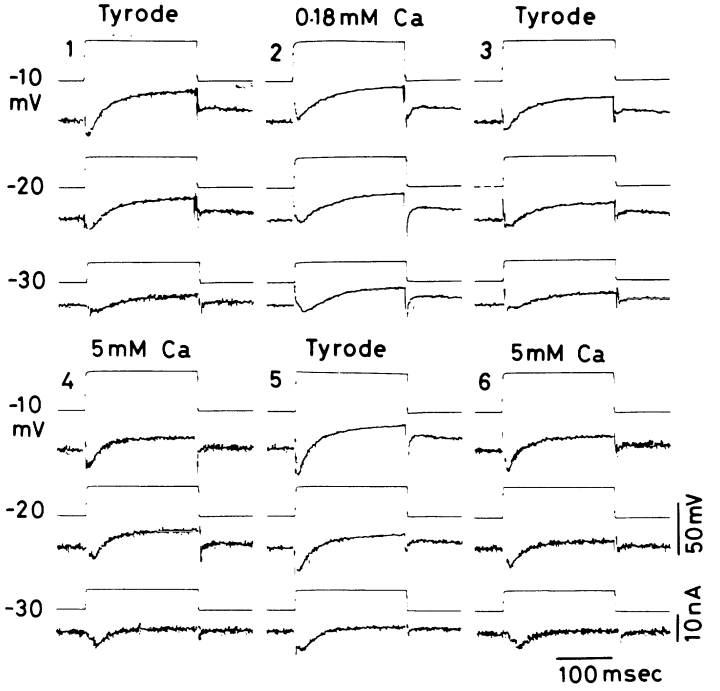

Figure 9. Effect of Ca^{++} ion on the membrane currents of the S–A node cell. Changes of the concentration of Ca^{++} were performed in a sequence of 1, 2, 3, 4, 5 and 6. Concentration of Ca^{++} in normal Tyrode's was 1.8 mM. It can be seen, when $[Ca^{++}]_0$ was reduced from 1 to 2, inward current was reduced at all test pulses, but when it was reduced from 4 to 5, inward current was increased. This apparent discrepancy may come from the hysteresis effect of high Ca^{++} ion on the S–A node cell membrane.

concentration increased within a certain range. After high concentrations of Ca^{++} were applied, there were hysteresis phenomena with regard to the action of Ca^{++} ion. (3) Overshoot of the action potential increased when extracellular Ca^{++} concentration was increased from 0.1 to 1.8 mM [34]. (4) Sensitivity of the sino-atrial nodal membrane to Mn^{++} and D–600 is the additional evidence supporting the role of Ca^{++} ion in the slow inward current.

7. EFFECT OF K^+ ION ON THE TRANSIENT INWARD CURRENT

Increasing the extracellular potassium ion concentration ($[K^+]_0$) suppresses spontaneous activity of the sinus node. The mechanism for this negative chronotropic effect of potassium is partly due to the inactivation of the transient inward current by depolarization of the membrane. The importance

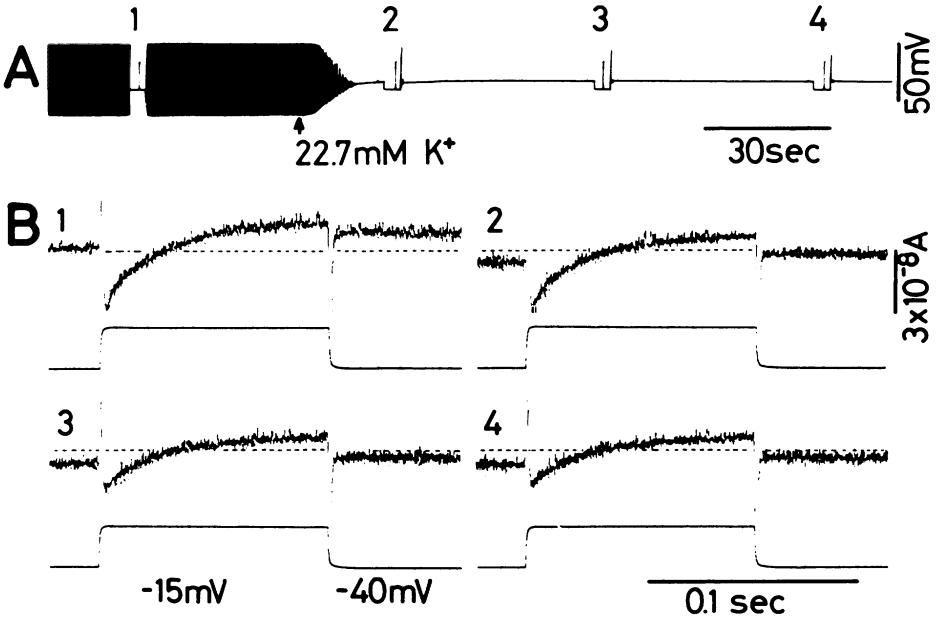

Figure 10. Effect of K^+ ion on the membrane current of S–A node cells. Time course of the membrane current change generated by increasing $[K^+]_0$. A shows the continuous record of the membrane potential before and after changing the perfusate from 2.7 to 22.7 mM $[K^+]_0$ solution. At different times, shown by numerals 1 to 4, the membrane potential was clamped. B shows the membrane current (upper curve) and the test pulse (lower curve). Test pulse was −15 mV from the holding potential of −40 mV. Number of the record corresponds to that in A. The dotted line indicates the zero current. Marked reduction of the inward current as well as the outward tail current was noted. (Noma, 1976, ref. [35].)

of the reduction of the transient inward current is lessened, however, when the effect of membrane depolarization is removed by holding the membrane potential at a constant level while exposing the membrane to various levels of $[K^+]_0$. Noma [35] measured membrane current change before and after application of 22.7 mM K^+ solution (Figure 10). At the holding potential of -40 mV, apparently no net inward current flowed in 2.7 mM $[K^+]_I$, but after changing to 22.7 mM $[K^+]_0$ solution the membrane was depolarized and an inward current of 6×10^{-9} A flowed at -40 mV. The amplitude of the transient inward current was significantly reduced after 70 sec perfusion in 22.7 mM solution. The amplitude of the outward current tail was also reduced 20 sec after the perfusion. All of these current changes were reversible. This experiment clearly showed that the transient inward current was depressed by increasing $[K^+]_0$. The large reduction of the transient inward current at 22.7 mM $[K^+]_0$ (Figure 10) cannot be explained by the neutralizing effect of the time-independent outward current. The magnitude of the outward current at the end of the test pulse was too small to counterbalance the inward current. Mechanisms for the depressing effect of $[K^+]_0$ on the inward current are still unknown.

8. KINETICS OF THE TRANSIENT INWARD CURRENT IN SINO-ATRIAL NODAL CELLS

In order to fully understand the kinetics of the slow inward current, accuracy of the voltage clamp method should be improved further. However, we have tried qualitative approximation of the inward current kinetics in the sino-atrial node. The activation process is not yet fully described. We have assumed that the activation process is governed by a variable, d, and the inactivation process by a variable, f, according to Beeler and Reuter [36], and McAllister et al. [37]. The slow inward current (I_{SI}) can be expressed by the following equation, where $\overline{I_{SI}}$ is the fully activated value of I_{SI}.

$$I_{SI} = d \cdot f \cdot \overline{I_{SI}}.$$

To obtain the voltage dependent degree of activation (d_∞) we held the membrane potential at -50 mV, and test pulses of 5 msec duration and of various intensities were applied. By plotting the magnitude of the inward current tail against the various membrane potentials, Figure 11 was obtained. The measurement of τ_d was difficult experimentally since the time constant of d is rapid compared to those of other kinetic variables. The time from the beginning of the test pulse to the peak of the inward current was measured. From this value, together with the d_∞ and other kinetic values, we have estimated τ_d (Figure 12).

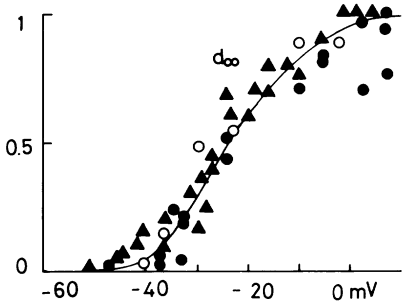

Figure 11. Steady-state activation curve of the slow inward current. Different symbols indicate different specimens.

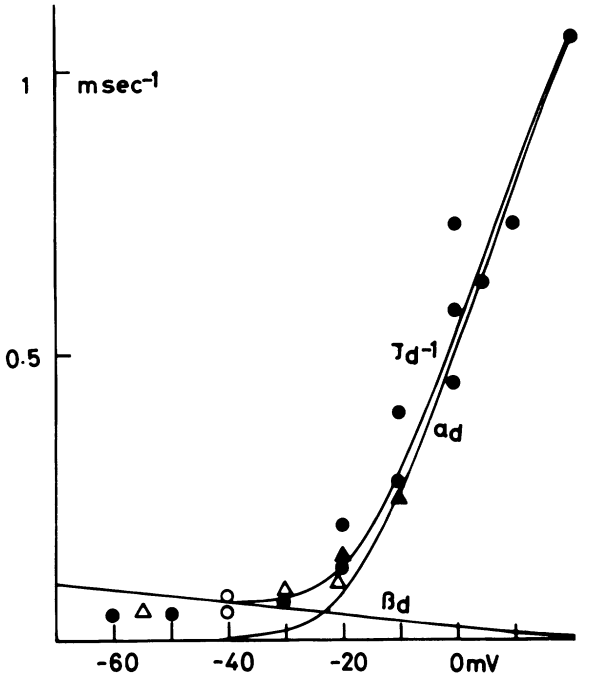

Figure 12. Rate constant of d at various membrane potentials. α_d, β_d were expressed in the following simulational equations:

$$\alpha_d = \frac{0.0278\,(E+18.5)}{1-\exp\left(-\dfrac{E+18.5}{20}\right)};$$

$$\beta_d = \frac{1.125\times10^{-3}\,(E-20)}{\exp\left(\dfrac{E-20}{5}\right)-1}.$$

To obtain τ_f, the time course of the change of maximum inward current tail following the depolarization step pulse with different durations was measured in ventricle muscle [38]. In sino-atrial node, this method was not applicable, because of the large outward tail on repolarization. Therefore analysis of the time constant of the inward current was made directly from the voltage clamp record. τ_f at the depolarizing potential was measured by measuring the slope of the recovery of the inward current transient. τ_f at the hyperpolarizing potential was measured from the two pulse experiment (Figure 13). In Figure 13, membrane potential was held at -50 mV and the test pulse of 0 mV was applied, followed by the second test pulse at various time intervals. As the interval was prolonged, the inward current increased as is shown in the picture. The time constant of the inactivation process (τ_f) at -40 mV was approximately 80 msec and at 20 mV it was 30 msec. Figure 14 is the rate constant and membrane potential relationship of f. With regard to the f_∞ curve, peak of the inward current is assumed to be proportional to f_∞ value at the holding potential (Figure 15).

Compared to the rate constants of recovery from inactivation of ventricular fibers [38–40], the f_∞ curve of the sino-atrial nodal cell shifted to a negative potential by about 20 mV. Similarly, the d_∞ curve shifted toward a positive

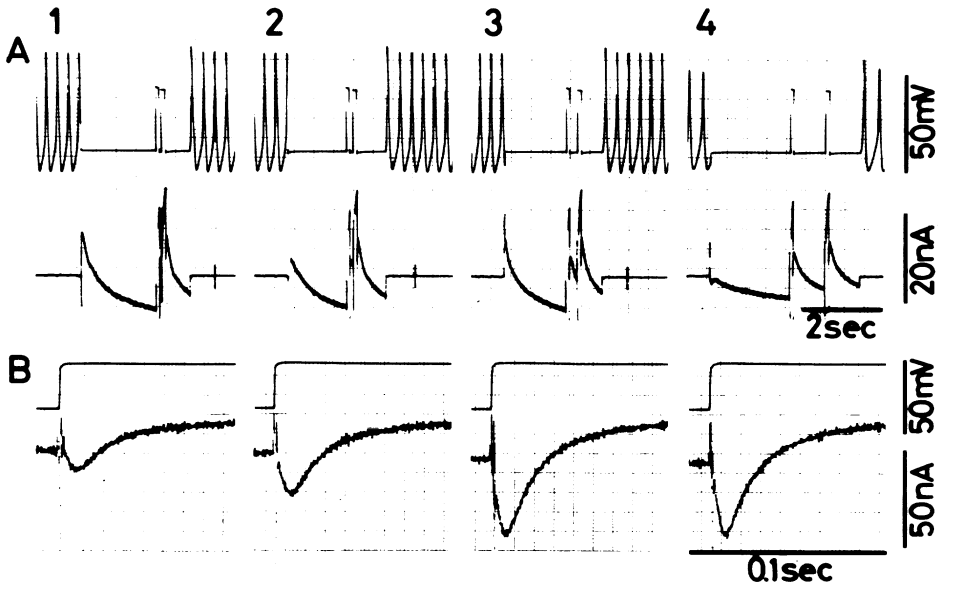

Figure 13. Two pulse experiments showing the recovery from inactivation of the slow inward current. In A 4 voltage clamp records in a slow time speed are shown, While in B voltage and current curves of the second pulse are shown in the fast time resolution. (Noma A; unpublished data.)

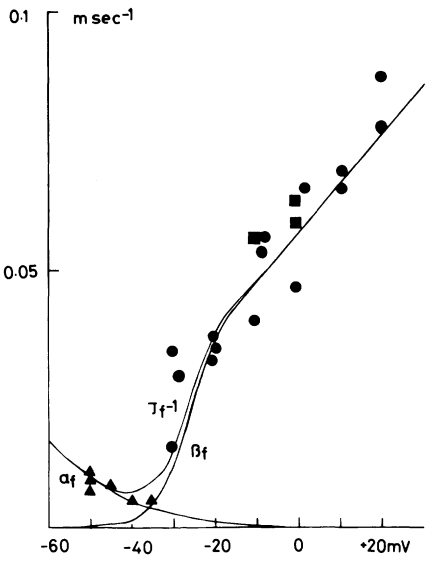

Figure 14. Rate constant of f at various membrane potentials. Different symbol shows different samples. α_f and β_f are given by the following simulational equations:

$$\alpha_f = \frac{7.16\times10^{-4}(E+36.5)}{\exp\left(\frac{E+36.5}{2.23}\right)-1};$$

$$\beta_f = \frac{1.336\times10^{-3}(E+38.5)}{1-\exp\left(\frac{-E+38.5}{3}\right)}.$$

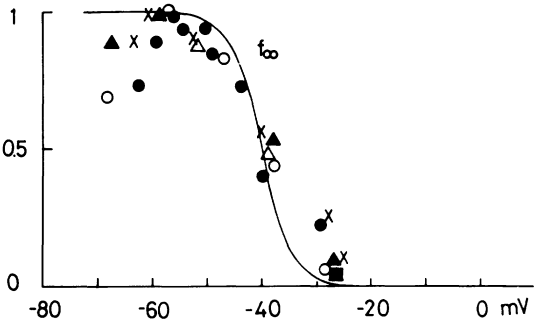

Figure 15. Steady-state inactivation curve of the slow inward current of S–A node cell. Different symbols indicate different samples.

potential by about 20 mV. As a result, the crossover of two curves became smaller than the ventricular muscle. Using these kinetics together with the kinetics of the outward current, the inward current flowing at the hyperpolarization potential, and the background current, we have reconstructed the action potential of the sino-atrial nodal cell. Figure 16 shows the computed action potential (A) and the changes of I_{SI} and I_{fI} (fast inward current). It can be seen that the maximum diastolic phase is mainly caused by the termination of the slow inward current as well as the activation of the outward current.

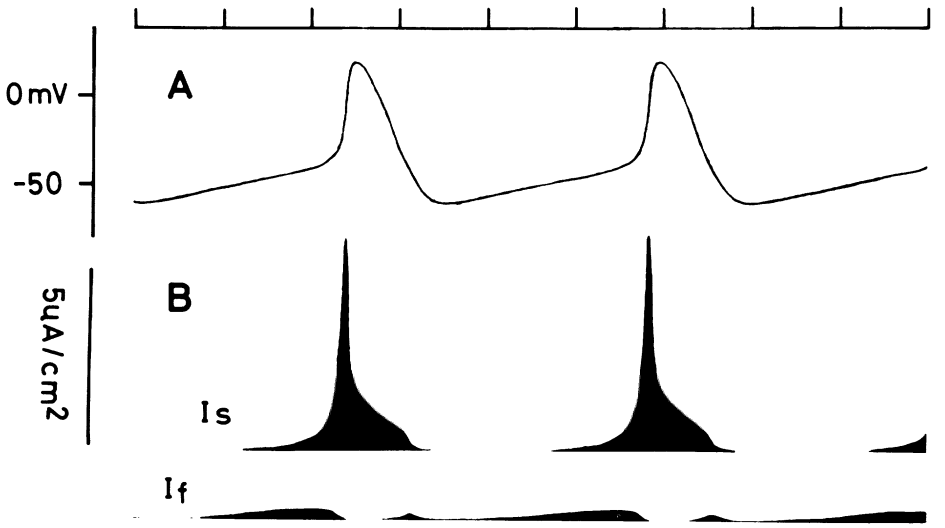

Figure 16. Reconstructed action potential (A) and the participation of slow inward current and the fast inward current in that potential tracing. Time is 100 msec. Slow inward current (I_s) and fast inward current (B) are shown upside down.

In the Purkinje fiber[37], I_{k2} plays an essential role in the production of phase 4 depolarization. In the sino-atrial nodal cell, membrane depolarization in the latter half of phase 4 is mainly caused by an activation of the slow inward current. This also coincides with the previous results of Noma and Irisawa[41], where membrane potential was held at the resting potential for a long time, after which the voltage clamp circuit was switched off. Under this condition, there is no appreciable outward current tail; rather the spontaneous action potential is generated due either to the small increase in slow inward current or to the occurrence of incremental oscillations.

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12. THE SLOW INWARD CURRENT AND AV NODAL PROPAGATION *

CARLOS MÉNDEZ

1. INTRODUCTION

The early work of Erlanger [1], Eyster and Meek [2], Gaskell [3], Hering [4] and Lewis [5], to mention only a few milestones of the past, showed that propagation through AV nodal tissue is rather slow.

More recently, the extracellular records obtained from the region of the bundle of His [6–10] clearly indicate that approximately half of the normal PR interval is due to slow conduction across the AV node. This permits better filling of the ventricles and therefore more efficient ejection of blood into the arteries.

It has also been known for a long time that the margin of safety for propagation across the AV node is relatively low. An important function of the node of Tawara is therefore to impose a limit to the rate of impulses that can reach the ventricles. Thus, during atrial fibrillation, a large number of impulses are blocked in the AV node. This filter property is essential for the survival of individuals who have rapid atrial arrhythmias.

Transmembrane potentials obtained from cells of the mammalian AV node are characterized by their low resting (diastolic) potential and by the slow rate of rise (dV/dt max.) of the depolarization phase [11–14].

At the time of writing this chapter it is not yet possible to use the voltage clamp technique to dissect and analyze the ionic currents responsible for AV nodal action potentials. Accordingly, the conclusions reached in this essay are based on indirect evidence and therefore susceptible to error. For this reason it would be superfluous to write a long and tedious chapter. A short summary of the present knowledge of the nature of AV nodal action potentials seems to be the safest route to follow.

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D.P. Zipes, J.C. Bailey, V. Elharrar (eds.), The Slow Inward Current and Cardiac Arrhythmias, 285–294.

2. THE POSSIBLE IONIC BASIS OF AV NODAL ACTION POTENTIALS

Paes de Carvalho [15] and Paes de Carvalho et al. [16] were the first to propose that typical cardiac action potentials have two distinct phases, each one the result of a different ionic mechanism. The first is a rapid phase, provided by Na^+ channels. The second is a slow depolarizing phase, resulting from a marked decrease in K^+ conductance and the presence of a small inward current. This 'slow' response would be responsible for typical AV nodal action potentials. Figure 1 summarizes the hypothesis in schematic form.

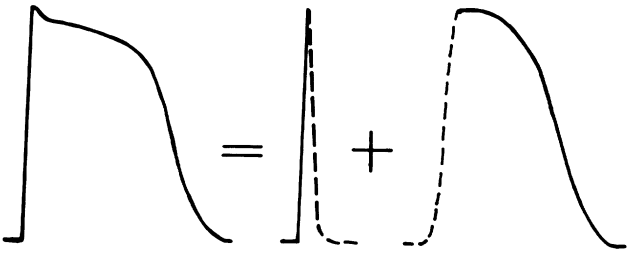

Figure 1. Components of the cardiac action potential. Hypothetical contribution of fast and slow components to a common action potential. The initial fast component marks the onset of the slow component. The sustained depolarization of the slow component prevents membrane repolarization with the termination of the fast component. Modified from Paes de Carvalho, 1965. According to the same author the slow component would be responsible for typical Av nodal action potentials.

The above-mentioned suggestion received partial support by the work of Aceves and Erlij [17]. These authors showed that, in frog atrial muscle, tetrodotoxin (TTX) abolished propagated action potentials but in th presence of the toxin the addition of norepinephrine permitted impulse conduction once again. Yet, the recorded action potentials showed a rather small dV/dt max. Therefore, TTX abolished the rapid component (the fast Na^+ channels) but norepinephrine permitted the propagation of a 'slow' response.

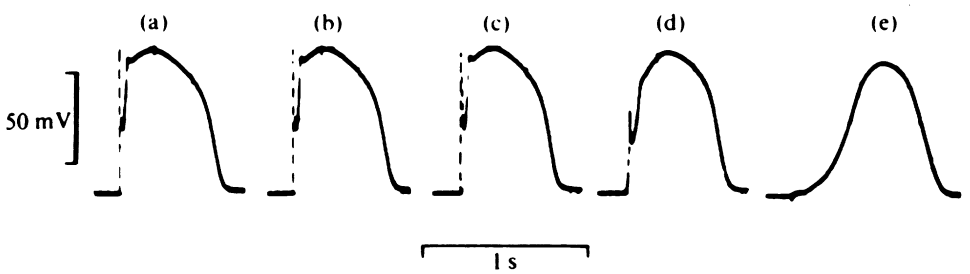

Figure 2. Action potentials recorded from calf Purkinje Fibers in the presence of epinephrine (5.5×10^{-6} M). The Na^+ current was progressively blocked with increasing concentrations of TTX: (a) control; (b) 3×10^{-8} M; (c) 3×10^{-7} M; (d) and (e) 3×10^{-6} M. (Carmeliet and Vereecke [18].)

Essentially the same results were obtained in calf Purkinje fibers by Carmeliet and Vereecke [18]. Figure 2 shows the result obtained by these authors. In presence of epinephrine, when the fast Na^+ current was blocked with progressively larger concentrations of TTX, the depolarization rate was greatly reduced and the 'spike' of the action potentials gradually disappeared until a slow propagated response remained.

Using the voltage clamp technique, Reuter [19] was the first to show, in sheep Purkinje fibers, that a net slow inward current can flow through the cell membranes in Na^+-free solutions containing Ca^{++} ions. Since then the slow inward current insensitive to TTX and carried by Ca^{++} and Na^+ ions has been shown to occur in all cardiac tissues where the clamp technique has been feasible (for references, consult the other chapters of this book).

From the point of view of this chapter, the most incisive studies were performed in frog atrial tissue by the French group [20, 21]. This group suggested that AV nodal action potentials might be originated by the exclusive

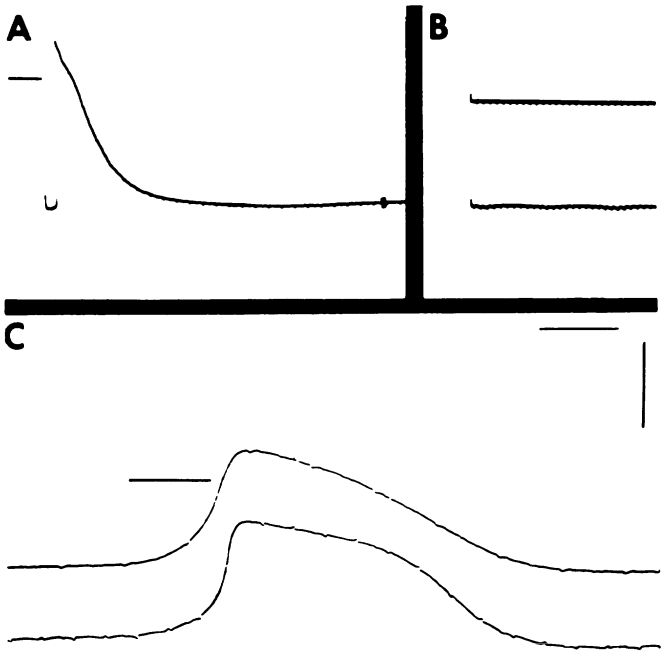

Figure 3. Action of TTX on atrial and AV nodal cells from the rabbit heart. A: control atrial action potential. B: zero potential (top) and resting potential (bottom) of an atrial cell after administration of TTX (5×10^{-6} g/ml). C: action potentials of AV nodal cells after administration of TTX; the top trace was recorded from a N cell and the bottom trace from an NH cell. The spontaneous activity originated in nodal tissue propagated through the AV node and actively engaged the impaled cells. Horizontal calibration: 100 msec for A, 200 msec for B and 20 msec for C. Vertical calibration: 50 mV. Horizontal lines preceding the action potentials in A and C indicate zero potential for the atrial fiber and the N cell, respectively. (Zipes and Méndez, [23].)

activation of the 'slow' channel. Furthermore, Lenfant et al. [22] found in the mammalian SA node, which to a degree resembles the AV node, that TTX had no effects while Mn^{++} ions suppressed the activity of that structure.

Following the suggestion of Rougier et al. [20] and Vassort et al. [21], Zipes and Méndez [23] showed that AV nodal propagation was not affected by TTX. Figure 3 illustrates, in an isolated preparation of the rabbit heart, that TTX suppressed atrial activity; yet the same concentration of the toxin did not interfere with the automatic initiation of AV nodal responses, nor with their propagation.

Figure 4 shows that TTX (5×10^{-6} g/ml) did not modify the configuration nor the dV/dt of middle nodal cells (N cells).

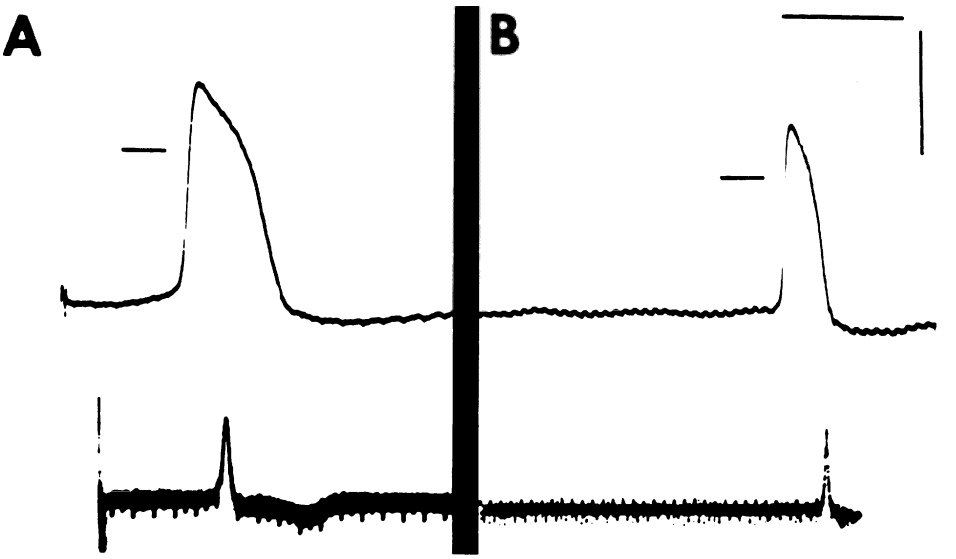

Figure 4. Effect of TTX on action potentials of rabbit N cells (top) and their corresponding values of dV/dt which are displaced to the right (lower trace). A: control. B: after the administration of TTX (5×10^{-6} g/ml of Tyrode's solution). In B the sweep speed was decreased in order to increase the chances of capturing a nodal action potential of automatic origin. Horizontal lines preceding action potentials indicate zero potential. Horizontal calibration: 100 msec for A and 200 msec for B. Vertical calibration: 50 mV and 20 V/sec. (Zipes and Méndez [23].)

The same authors reproduced in rabbit atrial tissue the results obtained by Aceves and Erlij [17] in frog atrial tissue; namely that propagation can take place in the presence of TTX *and norepinephrine*. Cranefield [24] made the point that a crucial experiment would have been to test if, under these conditions, the slow rising atrial action potentials would have propagated along the AV node. In my opinion, the suggested experiment deserves a trial, but the results obtained whether positive or negative would not be crucial. It

has been established [15] that the input to the AV node is provided under normal conditions by transitional cells whose action potentials have an important fast Na^+ component. Whether or not this is essential to have successful propagation in the upper portion of the node is an interesting question but, whatever the results, the information obtained would add little to the subject under discussion.

Zipes and Méndez [23] also showed that Mn^{++} ions, in concentrations ranging from 1 to 3 mM, did not affect the upstroke of atrial action potentials, yet caused complete block at all levels in the AV node (AN, N and NH regions of Paes de Carvalho and de Almeida, [14]). Figure 5 illustrates the case for an N cell.

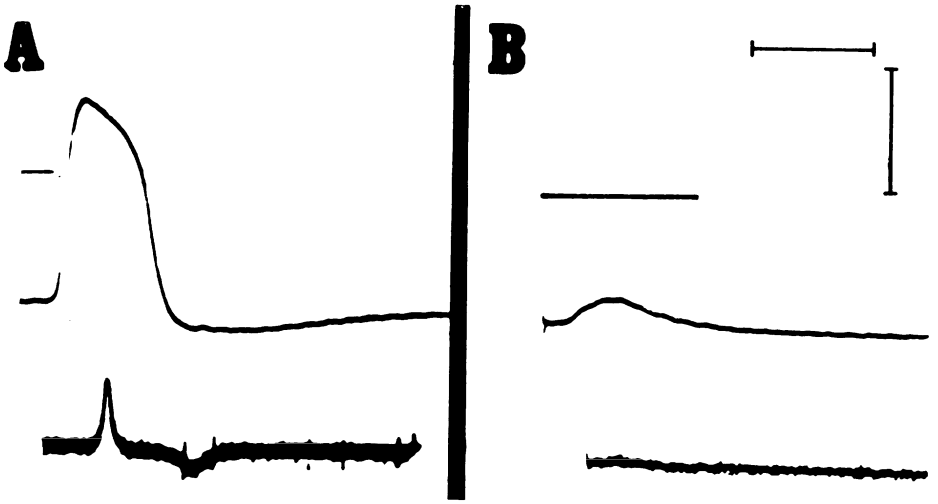

Figure 5. Effect of Mn^{++} ions on action potentials of N cells. A: control. B: after the administration of 3 mM of $MnCl_2$. Upper trace: transmembrane potential of an N cell. Lower trace: corresponding dV/dt displaced to the right. Horizontal lines: zero potential. Horizontal calibration: 100 msec. Verical calibration: 50 mV and 20 V/sec (modified from Zipes and Méndez [23].)

From the above-mentioned results, it was suggested that nodal action potentials are caused exclusively by the activation of the slow inward current. Although it is perhaps tempting to blame the unhealthy state of the preparation for the results obtained, Zipes and Fischer [25] showed in intact hearts, perfused with blood, that the injection into the lumen of the artery of the AV region, of agents that block the 'slow' channel, such as Mn^{++} ions, lanthanum, verapamil and D–600, also caused complete AV nodal block.

Recently, Akiyama and Fozzard [26] have confirmed the observations of Zipes and Méndez [23] and extended them considerably. By means of the constant field equation and a number of reasonable assumptions, these

authors have estimated that at the time of maximal overshoot the relative permeability (P) of the membrane would be $P_{Ca}/P_{Na} \simeq 60$–100 and $P_K/P_{Na} \simeq 1$. The relative contributions of these ions to the currents were estimated as $I_{Ca} \simeq 17\%$, $I_{Na} \simeq 33\%$ (inward currents), and $I_K \simeq 50\%$ (outward current). These authors concluded that AV nodal cells have 'slow' inward-current channels, insensitive to TTX, that are selective for Ca^{++} over Na^+ ions. It goes without saying that an important point is to prove whether or not the assumptions made by Akiyama and Fozzard are truly valid.

The interpretation of results made by Zipes and Méndez [23] are in contradiction with the results obtained by de Ceretti et al. [27]. These authors, with improved perfusion techniques, have found an initial fast Na^+ dependent component in the depolarization limb of AV nodal action potentials. Unfortunately their records, obtained with floating microelectrodes, do not show a convincing sequence of activation and they may very well correspond to transitional cells, which of course have rapid Na^+ ionic channels.

In isolated superfused preparations, the resting (diastolic) potential of rabbit AV nodal cells is low (-60 mV approx.). It might be possible that nodal cells have rapid Na^+ channels which are partially inactivated. Shigeto and Irisawa [28] have shown that prolonged hyperpolarizations caused an increase in the dV/dt of AV nodal action potentials. These results provide strong evidence that the cells explored had fast Na^+ channels partially inactivated.

Very recently we have repeated the experiments of Shigeto and Irisawa [28] in the same species (unpublished observations). Instead of using suction electrodes we employed a micropipette (100–200 μM internal diameter) filled with Tyrode's solution and located over the region of the bundle of His. A suitable polarizing system permitted us to hyperpolarize or depolarize at will the N region and its neighboring NH cells. The preparation was driven at a regular rate by stimulating atrial tissue. Under these conditions the results were conclusive. Prolonged hyperpolarizations of different magnitude caused, in *typical* nodal cells, a *decrease* in the dV/dt max. Figure 6 illustrates one of these experiments.

On the other hand, small depolarizations (up to 10 mV) did not decrease the dV/dt max.; only stronger depolarizations caused a clear effect (see Zipes et al. [29]). These results are in agreement with the data reported by Hoffman [30]. In this study the polarizing electrodes were intracellular micropipettes and, because of the difficulty of maintaining two good impalements simultaneously, there was some doubt as to the validity of the resting potentials recorded during the period of polarization.

The decrease in dV/dt caused by hyperpolarizing currents and the lack of effects of small depolarizations provide strong evidence which indicate that true AV nodal cells are devoid of a rapid Na channel, and that the latter is not just partially inactivated by the low resting potential.

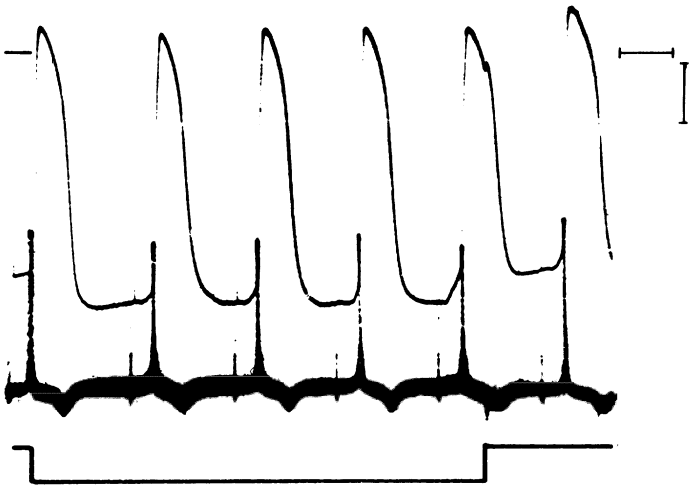

Figure 6. Effect of hyperpolarizing currents on an N cell. The upper record shows the transmembrane potentials of an N cell and the middle trace the corresponding values of dV/dt. The application of a hyperpolarizing pulse is shown by the lower rectangle. Its initiation coincided with the upstroke of the first action and the corresponding dV/dt was slightly reduced. The diastolic potential was hyperpolarized by 8 mV and during the pulse the dV/dt was smaller than that of the last response, once the polarizing current had ceased to flow. Horizontal line preceding action potentials: zero potential. Horizontal calibration: 200 msec. Vertical calibration: 20 mV and 5 V/sec. (Méndez and Hernández, unpublished results.)

It must be emphasized that in *transitional* cells, characterized by a relatively large dV/dt, which suggest the presence of partially inactivated rapid Na^+ channels, we obtained the same results reported by Shigeto and Irisawa [28].

In some preparations it was not uncommon to have, from the beginning of the experiment, pronounced conduction block between the N and NH regions. Under such conditions, small depolarizing currents permitted full propagation. Figure 7 illustrates this point.

In addition, under the same conditions, strong depolarizations where the diastolic potential was more positive than -40 mV, propagation was still facilitated. It is important to recall that in atrial muscle, Purkinje fibers, and in ventricular working muscle, resting potentials of the above mentioned magnitude inactivate completely the fast Na^+ channel.

In most preparations, successful propagation across the AV node took place. Yet, small hyperpolarizing currents caused complete AV block. Figure 8 illustrates typical results.

Anodal block is a well-known phenomenon. Yet, when propagation in excitable tissue depends on the presence of rapid Na^+ channels and conduction is critical because of inactivation caused by low resting potentials, then a

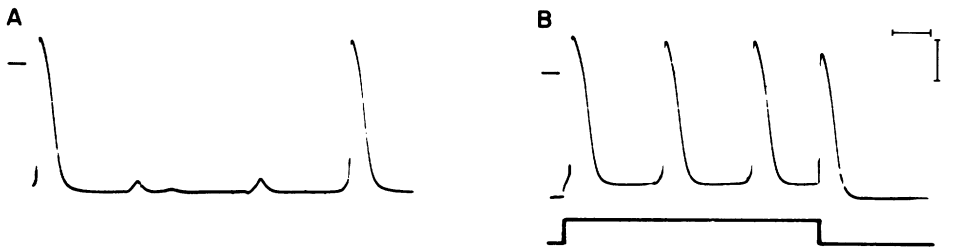

Figure 7. Facilitation of propagation caused by depolarizing currents. A: control. Notice the subthreshold depolarizations recorded from an NH cell. The lower rectangle in B shows the initiation and termination of a depolarizing pulse. Observe in B 1:1 propagation. Horizontal line preceding action potentials: zero potential. Calibrations: 200 msec and 20 mV. (Méndez and Hernández, Unpublished observations.)

small hyperpolarization should improve conduction. In Figure 8, propagation through the AV node was critical, but a small hyperpolarization caused complete block.

As already mentioned, in the AV node, maintained depolarizations facilitate propagation while small hyperpolarizations can cause conduction block. These effects can be easily explained if it is accepted that the initial part of AV nodal potentials are exclusively caused by the activation of the slow inward current. Thus depolarizations should bring the take-off potential closer to the threshold potential of the slow channel (-35 mV approx.). On the other hand, hyperpolarizations should cause the opposite effect.

The same hypothesis explains the positive dromotropic effects of catecholamines on atrioventricular propagation. In heart–lung preparations, Krayer et al. [31] showed that epinephrine caused a definite improvement of atrioventricular conduction. This effect can only be restricted to the region of the

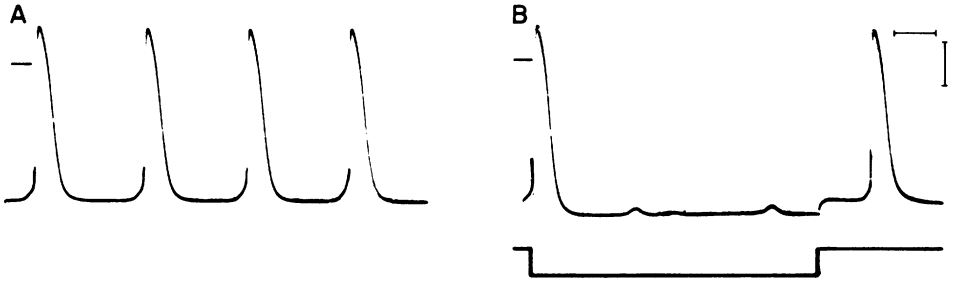

Figure 8. Complete AV block caused by hyperpolarizing currents. A: control. B: same cell during the passage of a hyperpolarizing pulse, lower rectangle. Observe that the pulse started at the peak of the first action potential and, while present, two subthreshold depolarizations were recorded. Horizontal lines preceding action potential: zero potential. Calibrations: 200 msec and 20 mV. (Méndez and Hernández, unpublished observations.)

AV node, since Méndez et al. [32] provided convincing evidence that epinephrine has no direct action on intraventricular propagation, yet the same agent improves AV nodal propagation (Méndez et al., [10]). On the other hand, Reuter [19] and Vassort et al. [21] have established that epinephrine considerably increases the current flow through the slow inward channel. Accordingly the results of Krayer et al. [31] and Méndez et al. [10, 32] are in complete agreement with the above-mentioned hypothesis.

3. CONCLUSIONS

At present, the data obtained strongly suggest that the genesis of action potentials of 'pure' AV nodal cells (not transitional cells) lies on the exclusive activation of the slow inward channel.

In the overall discussion of this chapter it is implicit that propagation through the AV node is electrical in nature. Although we do not have rigorous proof that this is the case, the hypothesis is strongly supported by experimental data. Thus, electrotonic interactions between neighboring nodal cells is a fact [33, 34] and it has been demonstrated that nodal cells are electrically excitable [35]. The slow conduction through nodal tissues must then be the result of passive properties such as a high internal resistance [34] and the intrinsic properties of the slow action potentials.

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13. THE SLOW RESPONSE IN HUMAN ATRIUM *

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There is little doubt that under appropriate conditions 'slow response' action potentials occur in human atrium. However, despite attempts to relate clinical cardiac arrhythmias and conduction disturbances to the slow response as an etiologic factor, no firm association as yet can be made between cellular electrophysiologic and clinical events. This is not to indicate that no associations at all can be made: we [1] and others [2, 3] have demonstrated that when sufficient numbers of atria are studied under carefully controlled conditions there is a consistent relationship between the extent of atrial disease and the level of resting membrane potential. A decrease in resting potential, in turn, is associated with decreases in action potential amplitude and upstroke velocity until, at resting potentials ≤ -50 to -60 mV, 'slow responses' appear.

There are a number of difficulties inherent in assessing the pathophysiologic importance of these action potentials. These stem mainly from difficulties in obtaining for laboratory study bits of atrial tissue that are representative of the electrophysiology of the in situ human atrium. This problem was alluded to in the very first report of human atrial cellular electrophysiology: Trautwein et al. [4] noted that it was '... difficult to obtain recovery and survival of excised atrial tissues' following cardiac surgery. Ventricular tissues, on the other hand, fared better. For Trautwein et al. [4] and for subsequent investigators, an important question to be considered was: when do abnormal atrial action potentials reflect a pathophysiologic process occurring in the atrium and when do they reflect artifacts induced by removing atrial tissues from the heart, placing them in a physiologic salt solution and studying them over several hours? Trautwein et al. [4] studied 5 preparations of human atrium. Two had resting potentials of -30 to -40 mV, one -55 to -60 mV. These three preparations showed oscillatory spontaneous activity, were inconsistently excitable by an external electrode, and showed variable propagation of electrically stimulated action potentials. However, in atrial tissues from two children with atrial septal defects resting potentials ranged

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from −60 to −80 mV, and action potentials having configurations similar to those of other mammalian species were noted. Moreover, two types of action potentials were described (Figure 1): one similar to that seen in normal myocardium, had a rapid depolarization phase, a brief plateau and a short action potential duration. The second type had a very prominent spike, a broad plateau and a long action potential duration. This type of action potential was seen quite commonly, and very closely resembles the type of action potential seen in the ventricular specialized conducting system. In commenting on the plateau of this action potential, Trautwein et al. considered it to be 'unusually prominent' and wondered if it might reflect a decreased K^+ conductance or increased Na^+ conductance in these fibers. Trautwein et al. [4] commented on the fact that the more normal action potentials came from tissues from the hearts of children who had a relatively brief history of cardiac disease, whereas the depolarized tissues were from the hearts of adults with long-standing disease. Although they mentioned the possibility that the presence of disease might have been the deciding factor in generating the different types of resting and action potentials, they also remarked that, in dog atria that are not stimulated, depolarization occurs [5]. This raises the possibility that normal human atrium, if unstimulated, might depolarize to low levels of resting potential and, in such an instance, oscillatory activity might occur. That such depolarization of unstimulated but otherwise normal human atrium does, in fact, occur was shown subsequently by Mary-Rabine et al. [6].

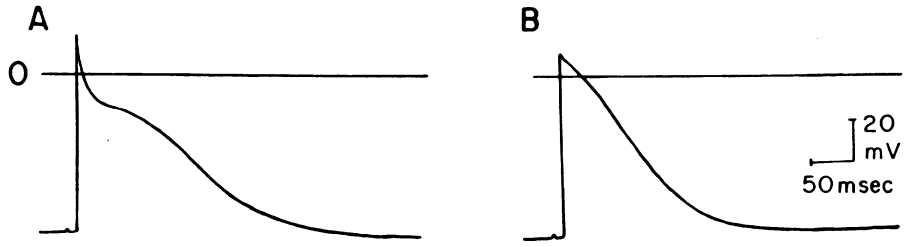

Figure 1. A: action potential from human atrial fiber showing prominent spike and plateau and long action potential duration. B: action potential from human atrial fiber showing small spike and short plateau and action potential duration. The former is consistent with records from mammalian specialized conducting fibers; the latter, with records from mammalian atrial fibers. (Reprinted from Gelband et al., ref. [9], p 295, by permission of the American Heart Association, Inc.).

Shortly after the study by Trautwein et al., Sleator and deGubareff [7] reported the action potential characteristics of 220 atrial strips from 144 patients. The age range of the patients was from 11 months to 66 years. Most of the unsuccessful attempts at study were of tissues from patients in the oldest age group. One hundred and twenty-four successful experiments were

done, of which 44 were on tissues from patients with ventricular septal defects; 38 atrial septal defects; 12 tetralogy of Fallot, and 12 'mitral valve disease' (type not specified). A wide range of resting and action potentials was recorded, but the most persistent pattern was a relatively rapid upstroke followed by a second slower upstroke, with a variable delay between the first and second slower components (e.g., Figure 2; beat indicated by arrow). These investigators were unable to find any differences in resting or action potential characteristics that they could attribute to a specific disease entity or to the drugs the patients were taking. However, it is important to note that their experiments were performed at 27 °C, to maintain contractile strength of the preparations. They noted that, with appropriate concurrence of two upstrokes of the action potential, contractile strength could increase. They also found that acetylcholine would reduce both contractile strength and the second upstroke – an action that was counteracted by atropine. The occurrence of action potentials with early and delayed components of the upstroke was confirmed by Fabiato and Fabiato [8]. They also demonstrated that, whereas such action potential configurations were not routinely seen at temperatures ≥ 32 °C, as the temperature was reduced to 27 °C two components of the action potential were clearly demonstrable. Moreover, they showed the initial spike to be tetrodotoxin sensitive, and hence, to result from the rapid inward (Na^+) current, and the second to be $MnCl_2$ sensitive and, presumably, the result of Ca^{++} and Na^+ entry through the slow channel. Experiments in low Na^+ and low Ca^{++} solutions indicated that some Na^+ was necessary for the slow component to occur; however, its upstroke velocity was related to the Ca^{++} concentration. Fabiato and Fabiato attributed the splitting of the action potential into two components as resulting from inhomogeneous propagation.

Summarizing the studies of Trautwein et al., Sleator and deGubareff and Fabiato and Fabiato, at the time of their completion it appeared that both

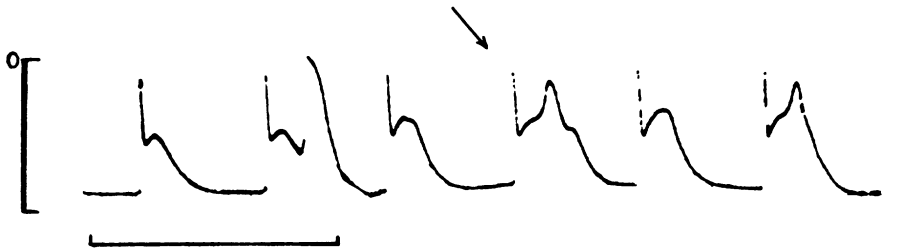

Figure 2. Two components of human atrial action potential. In the initial cycle, the action potential has a prominent spike and relatively short repolarization phase. The subsequent cycles show varying degrees of change in plateau amplitude, and in cycles 2, 4 and 6 a second depolarization occurring during (cycles 4 and 6) and shortly after (cycle 2) the plateau. See text for further discussion. Vertical calibration, 100 mV; horizontal, 1 msec. (modified and reprinted from Fabiato and Fabiato, ref. [8], p 298, by permission of the American Heart Association Inc.).

rapid Na^+ and slow Na^+ and Ca^{++} currents contributed to the human atrial action potential. It further appeared, from the observations of Trautwein et al., that tissues from more seriously or chronically diseased patients might have lower resting potentials and more ectopic impulse initiation than tissues from younger or less seriously ill patients. Although the observations of Sleator and deGubareff might be cited as arguing that action potential configurations and resting potentials were not influenced by disease processes, the fact that the tissues they studied were kept at 27 °C, which Fabiato and Fabiato showed to reduce resting membrane potential and to induce the abnormal action potentials described by Sleator and deGubareff, mitigates against this argument.

In 1972, Gelband et al. [9] reported the cellular electrophysiologic properties of 'normal' human atrium. To ensure the greatest likelihood of obtaining normal fibers that would be relatively easy to study, they selected tissues from patients under 16 years of age. Fourteen of these had ventricular septal defects, 8 congenital aortic stenosis. None had been in congestive failure, had received cardioactive drugs, or had a preoperative history or ECG evidence of arrhythmias. Cardiac catheterization had shown normal right atrial pressures and no shunts at the atrial level; the ECG showed normal P waves. Because only 85 successful microelectrode impalements were made in the 22 atria, it cannot be stated with certainty that the action potentials recorded were 'representative' of each entire preparation. However, it is important to note that the characteristics of the action potentials from the 85 impalements were nearly identical to those which have been described for the normal canine atrium [10]. Both typical contractile fibers, having a short plateau, and atrial specialized fibers, having a more prominent phase 0 upstroke, longer plateau, and the capability of depolarizing during phase 4, were identified. The resting potentials were in the range of -85 mV and the relationship of $[K^+]_0$ to resting potential was that which would be expected from the Nernst equation, with a decrease of 58 mV in membrane potential per 10-fold increase in $[K^+]_0$ (Figure 3). This report, which provided the first compilation of electrophysiologic data from normal human atrium, provides the standard for 'normal' human atrial action potentials referred to in the remainder of this chapter.

Electrophysiologic properties of diseased human atria had been reported earlier by Prasad [11] and were reported subsequently by Singer et al. [2]. Prasad studied tissues from patients over 50 years of age with rheumatic heart disease. In this series, 15 atrial specimens had resting potentials averaging -83 mV and action potential amplitudes of 104.4 mV; not different from the series of Gelband et al. [9]. In 7 specimens, in which spontaneous rhythm was occurring resting potential was -43.5 and amplitude, 44 mV. In the series of Singer et al. [2] over 100 patients with congenital and acquired heart disease were studied, 80% of whom had intermittent or chronic atrial fibrillation.

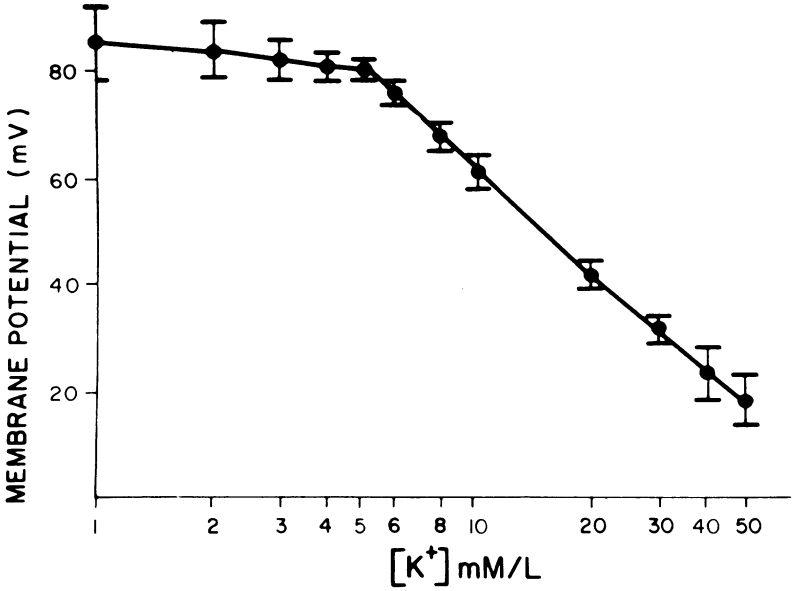

Figure 3. Relationship of resting membrane potential (vertical axis) to extracellular potassium concentration (horizontal axis) in 6 human atrial fibers. Data presented as M ± SD. (Reprinted from Gelband et al. ref. [9], p 298, by permission of the American Heart Association Inc.).

Although normal action potentials were recorded in some atria, the majority had resting potentials of −40 to −55 mV. Moreover, much variability in resting potentials was seen in each preparation. Fibers with low levels of resting potential showed reduced action potential amplitude and upstroke velocity and, from their configurations in the experimental records shown, were not unlike the slow response.

To our knowledge, only one study has thus far attempted to relate slow response activity in human atrium to the electrical activity of the in situ heart [1]. Here, as in the study by Gelband et al. [9], atria were selected from patients under 16 years of age with congenital heart disease. However, no attempt was made to exclude patients with atrial disease. Ten to 20 technically satisfactory microelectrode impalements were made in the subendocardium of each preparation, and the atria were divided into 2 groups based on their maximum diastolic potential. A maximum diastolic potential of −60 mV was used to separate the two groups, since it is usually at −60 mV or less that the fast Na^+ upstroke of the action potential is inactivated and slow response activity becomes more prominent [12]. For 12 patients (identified as Group B) with low maximum diastolic potential (MDP), the mean MDP was −50.3 mV; action potential amplitude was 63.3 mV and maximum upstroke velocity of phase 0 was 5–20 V/sec. This is in sharp contrast to

Group A (10 patients) in whose atria MDP was −71.4 mV, amplitude, 91.7 and maximum upstroke velocity of phase 0, 208 V/sec (Table 1). To test for the presence of 'slow response' action potentials, preparations from both groups were superfused with the slow channel blocker, verapamil 0.1 mg/liter [1]. This concentration rapidly diminished action potential amplitude and then abolished action potentials that were recorded from Group B atria (with 'slow response' activity) while having no effect on tissues from Group A. Procaine amide, which acts primarily to depress rapid Na^+ entry, depressed but did not abolish the action potentials in Groups A and B. This result is entirely consistent with the slow channel assuming importance in the genesis of action potentials in human atrial fibers having low levels of resting membrane potential. Because identification of abnormal electrical activity in human atria might be the result of experimental artifact, as described earlier, it was deemed important to attempt to relate the cellular events described above with the clinical condition of the patients. Within an admittedly small series of 22 patients, the following correlations were noted: for the Group A patients, the atria were of normal size or were only slightly dilated (as estimated by the surgeons at the time of operation); for the Group B patients, atria were moderately to markedly dilated. None of the patients in Group A had a history or ECG evidence of atrial arrhythmias; 4 of the 12 Group B patients had paroxysmal atrial arrhythmias. Most importantly, however, on analyzing the preoperative P waves of all patients (when all were in sinus rhythm and had comparable heart rates) P wave duration was 89 ± 3 msec for Group A and 111 ± 6 msec for Group B, a highly significant difference ($P < 0.005$). This observation suggests that the atria having fibers with low levels of membrane potential and slow response action potentials had ECG evidence of significantly slower conduction than the atria of patients with more normal resting and action potentials.

There are several problems with this interpretation; the first is inherent in the difficulty of identifying accurately the beginning and end of the P wave; the second is that the change in P wave duration might just as well reflect a change in the pathway of atrial activation as a change in conduction velocity; and the third is that the velocity of the slow conduction normally attributed to the 'slow response' is such that were slow responses occurring uniformly in the atria, as appears from this study, the P waves would be expected to be far longer than they actually were. The first objection is relatively unimportant here, as the random error in reading P wave duration should be distributed between both groups and, although it might result in all values reported being somewhat inaccurate, it would not produce the difference in P wave duration reported here. The second objection is a valid one, answerable only by careful mapping of atrial activation and relating conduction velocity in the atria to P wave duration and cellular electrophysiologic events. Such a study

Table 1. Electrocardiographic and cellular electrophysiologic data from right atria.

Group	*Age* (*years*)	*Transmembrane potentials*			*ECG*			
		MDP (*−mV*)	*AP amp* (*mV*)	$\dot{V}_{max}$ (*V/sec*)	*Heart rate* (*beats/min*)	*P–R* (*msec*)	*P amp* (*mV*)	P duration (*msec*)
A (n = 10)	5.8 ± 3.8	71.4 ± 5.1	91.7 ± 8.3	208 ± 38	102 ± 6	156 ± 5	2.8 ± .3	89 ± 3
B (n = 12)	7.2 ± 4.6	50.3 ± 5.7*	63.3 ± 11.3*	< 20	111 ± 5	164 ± 11	2.4 ± .3	111 ± 6**

Results expressed as M ± SD: * $P < .001$ compared to Group A; ** $P < .005$ compared to Group A. Modified after Hordof et al. [1].

currently is being performed by Untereker and Boxer. Their preliminary results suggest there is a good correlation between a decrease in resting potential and attendent slow response activity with slowed conduction in the in situ atrium and a long P wave duration.

The final objection is the most important: if slow responses occurred uniformly in a human atrium, either no electrocardiographically identifiable P wave or one of extremely long duration would be expected. That this has not been shown to occur suggests that although slow response action potentials are recorded from isolated human atrial tissue their identification in subendocardial fibers does not imply that they represent electrical activity in the entire in situ atrium. Even when the majority of depressed action potentials recorded subendocardially in diseased human atrium appear to be slow responses there is a great variability in their amplitude. For example, for Group B of the study of Hordof et al. [1], action potential amplitude was 63.3 ± 11.3 mV, (M $\pm$ SD), and resting potential -50.3 ± 5.7 mV. Although these values are significantly lower than those from fibers with the fast response, they do include a population of fibers which probably are 'intermediate,' i.e., have both fast and slow components. Singer et al. [2] refer as well to this marked variability. Moreover, nothing is known of the electrophysiologic characteristics of the fibers within the atrial wall itself. Because these are poorly supplied with superfusate, they would tend to have abnormal action potential characteristics. It is possible that when they are normally perfused with blood, they are less depressed, and conduct more rapidly. Attempts to study these fibers by cutting into the myocardium with a scalpel have shown markedly depressed action potentials, but this could be the result of cellular injury induced by the scalpel itself. Until we have a greater knowledge of the extent of uniformity of action potential characteristics in the diseased atrium, it will be difficult to provide a firm interpretation of the contribution to atrial conduction and arrhythmias of subendocardial fibers with the slow response. However, although it is still difficult to attribute a specific role to the human atrial slow response with respect to the electrocardiogram, there nonetheless is an association between atrial dilation secondary to congenital heart disease, fibers with low levels of resting potential and slow response action potentials, and abnormal P waves.

Recently Ten Eick and Singer studied the mechanisms responsible for the low maximum diastolic potential in diseased human atria [3]. They reported data from 138 adult patients with atrial disease and 7 with normal atria. Their results were consistent with those of Hordof et al. [1] obtained in children: fibers from the diseased atria had maximum diastolic potentials of -56 mV, those from normal atria -74.4 mV. For normal fibers, they found the relationship of resting potential to $[K^+]_0$ changed by about -50 mV for a 10-fold increase in $[K^+]_0$; this is similar to the relationship described by Gelband et

al. [9]. For diseased atrial fibers, however, there was no depolarization in response to increasing $[K^+]_0$ until the latter was ≥ 10 mM. This suggested to them that the K^+ conductance of depolarized cells in human atria is not the sole determinant of their resting potential. Removing Na^+ from the superfusate in one experiment had little or no effect on the relationship between K^+ and resting potential; neither did changing $[Ca^{++}]_0$. These observations suggested that an inward current 'leak' carried by Na^+ or Ca^{++} was not responsible for the low resting potential. Addition of acetylcholine to the superfusate markedly, although transiently, increased resting potential of depolarized cells, as had been described by Trautwein et al. [4] and Hordof et al. [13]. These observations suggest that an increase in K^+ conductance can increase the membrane potential of diseased human atrial cells to more normal levels. Ten Eick and Singer [3] reached the conclusion that 'a major reduction in the K^+ equilibrium potential and therefore in intracellular K^+ activity is not responsible for the low level of membrane potential seen in the depressed fibers.' They also suggested that the 'K^+ conductance relative to other... significant membrane conductances may be substantially reduced, and that the absolute level of the K^+ conductance may be reduced in cells of diseased atria.'

Thus far we have discussed the characteristics of the human atrial resting and action potentials and their relationship to impulse propagation. The mechanisms for impulse initiation in human atrium also have been the subject of study. Trautwein et al. [14] referred to the oscillatory activity and rapid impulse initiation that occurred in human atrial fibers having low levels of membrane potential. These investigators and, later, Hordof et al. [13] demonstrated that acetylcholine, in hyperpolarizing the cardiac cell membrane, suppressed this type of impulse initiation. Gelband et al. [9] in their study of normal human atrium show a record of phase 4 depolarization and automaticity in an atrial specialized fiber at a high level of membrane potential, after the fiber has been stretched. Our own experience has been that automaticity occurring in normal or diseased human atrial fibers at high levels of membrane potential is infrequent. More usually, when normal human atrial fibers are not stimulated electrically, they tend to depolarize [6]. This is consistent with Trautwein and Dudel's observations of canine atrium [5]. Mary-Rabine et al. [6] found that whether human fibers were initially normal (MDP -79.5 mV) or markedly depressed (MDP -55.1 mV) discontinuation of the drive stimulus resulted in attainment of a mean maximum diastolic potential of -55 to -58 mV and with this, spontaneous impulse initiation. The implication of this observation is that, with infrequent exceptions, if human atrial specialized fibers are to induce a spontaneous rhythm they must depolarize significantly. Having depolarized, they are in the range of potentials that is (a) associated with the slow response and (b) is associated with 'abnormal' mechanisms for impulse initiation (i.e., impulse initiation at low

levels of membrane potential). The latter two considerations may in part explain why atrial tachyarrhythmias in the in situ heart are relatively resistant to the effects of antiarrhythmic drugs that are not predominantly slow channel blockers, and are sensitive to verapamil [14] and to digitalis, which has an acetylcholine-like effect [13]. Another implication of the fact that a decrease in membrane potential results in spontaneous impulse initiation is that for ectopic automatic foci to occur in normal human atrium, all that is required is a site of conduction block which prevents excitation and therefore permits depolarization of the tissues. A final observation is that the range of membrane potentials at which impulse initiation occurs in depolarized human atrial fibers is one in which depolarized mammalian ventricular muscle fibers can also generate spontaneous impulses [15, 16].

When fibers are depolarized and initiating spontaneous impulses their activity can be modified by both acetylcholine and catecholamines. Acetylcholine [4, 13, 17] hyperpolarizes the fibers and, for as long as a high level of membrane potential is attained, they remain quiescent. Trautwein et al. [4] demonstrated that epinephrine initially depresses and then enhances the rate of impulse initiation. Sixteen years later, Mary-Rabine et al. [6] demonstrated that the ability of epinephrine in low concentrations to decrease the spontaneous rate probably was mediated via an action on α-adrenergic receptors, and its ability in higher concentrations to hyperpolarize fibers and increase rate probably was a result of β-adrenergic stimulation. Finally, Gelband et al. [18] demonstrated that, as for other cardiac tissues, superfusion of diseased human atrial fibers with catecholamine could hyperpolarize the fibers, increase action potential amplitude and maximum upstroke velocity and improve conduction.

The mechanisms for impulse initiation in depolarized human atrium are not clear. At least two well-defined types of impulse initiation appear to occur at low levels of membrane potential (Figure 4). One is associated with a gradual slope of diastolic depolarization and a smooth transition to the action potential upstroke. The second is associated with delayed after depolarizations and the paroxysmal occurrence of 'triggered' arrhythmias as described by Cranefield [19]. Both mechanisms frequently occur in the same preparation, and even the same fiber. That which is associated with a gradual slope of phase 4 depolarization is enhanced by catecholamines (in β-adrenergic stimulating concentrations) and depressed by acetylcholine, verapamil, lidocaine and tetrodotoxin. It is not altered by procaine amide. These results suggest that it can be modified by increasing membrane potential and/or potassium conductance (acetylcholine), by decreasing the slow inward current (verapamil), by decreasing an inward Na^+ current (tetrodotoxin, lidocaine) or by increasing background outward current (lidocaine). The second type of impulse initiation described, which is associated with delayed afterdepolariza-

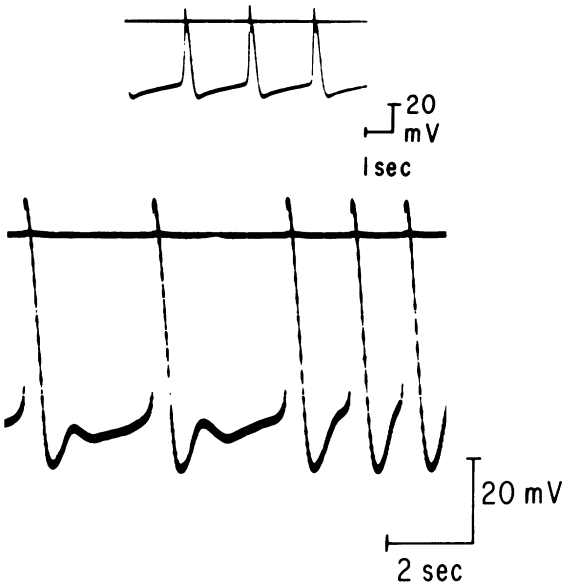

Figure 4. Upper panel: spontaneous impulse initiation as result of gradual diastolic depolarization in human atrial fibers. Lower panel: occurrence of delayed afterdepolarizations superimposed upon gradual diastolic depolarization (first 2 cycles). After the third action potential, the afterdepolarization attains threshold, resulting in a tachyarrhythmia. (Modified after Mary-Rabine et al., ref. [17].)

tions, is enhanced by catecholamines [17] or toxic concentrations of digitalis [12] and depressed by acetylcholine, verapamil and the putative Ca^{++} blocker AHR-2666 [17]. This suggests that a current carried by Ca^{++} or another ion through the slow channel is important in initiation of the afterdepolarizations. The suppression of afterdepolarizations by acetylcholine is consistent with a Ca^{++} blocking effect [20] or with hyperpolarization secondary to increased K^+ conductance. When the afterdepolarizations are enhanced by epinephrine, verapamil, AHR-2666 and acetylcholine continue to depress them; lidocaine and TTX do not [17]. It is interesting to note that the actions of TTX and lidocaine on spontaneously occurring or epinephrine-induced delayed afterdepolarizations in human atrium differ from their effects on digitalis-induced delayed afterdepolarizations in canine Purkinje fibers (see Chapter 20). This difference in drug response is consistent with differences in the mechanisms responsible for the two types of delayed afterdepolarizations or differences in the tissues and/or levels of membrane potential.

A third mechanism for impulse initiation in diseased human atrium was described by Singer et al. [2]. This is sustained low level oscillatory activity in depolarized preparations (Figure 5). Under appropriate circumstances, these

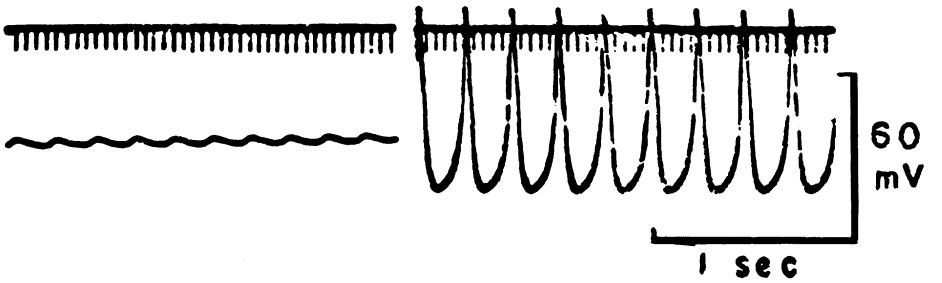

Figure 5. Oscillatory activity in a quiescent human atrial fiber. Right panel: rhythm subsequently generated from the same preparation. Whether the rhythm that occurs on the right was the result of the oscillatory activity *or* some other mechanism cannot be stated with certainty from this record (Reprinted from Singer et al., ref. [2], p 105, by permission of Grune and Stratton).

oscillations can reach threshold potential and initiate spontaneous action potentials. The mechanisms underlying these oscillations, however, have not as yet been studied. Whether they represent the electrotonic effects of nearby cells that are firing spontaneously and initiating action potentials but failing to bring the cell impaled to threshold potential, or whether they represent the spontaneous oscillations of the type described in single cells in tissue culture [21] remains to be determined.

From the multiplicity of drug effects on impulse initiation by human atrium it appears that identification of a single mechanism for the occurrence of such impulse initiation should not be expected. Nonetheless, it appears that the mechanism(s) that awaits identification differs considerably from the i_{K2} current that is held responsible for normal impulse initiation in the Purkinje system.

A variety of arrhythmias have been postulated as possibly arising from the ectopic impulse initiation and abnormal propagation that occurs in diseased or depolarized human atrium. These have been reviewed by Singer et al. [2] and by Rosen and Hordof [22]. Both papers present figures that would be consistent with paroxysmal tachycardia, atrial fibrillation, parasystole and varying degrees of conduction block and reentry. However, it has been impossible to relate these events directly to abnormal conduction or arrhythmias in patients whose atrial tissues have been studied.

In closing, much remains to be done before we understand fully the mechanisms responsible for abnormal human atrial action potentials. It is reasonable to say, however, that in the diseased human heart, depolarization of atrial fibers occurs that is associated with action potentials that appear to be slow responses and with abnormal impulse initiation and propagation. There appears, as well, to be an association between these cellular events and the electrical activity that can be recorded by the electrocardiogram. What is required now are means to better identify the importance of these cellular

events with respect to clinical arrhythmology, as well as the mechanisms responsible for them. This information should further our abilities to predict, diagnose and understand human atrial arrhythmias, as well as improve our abilities to prevent and treat them.

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14. THE SLOW RESPONSE IN HUMAN VENTRICLE

JOSEPH F. SPEAR, LEONARD N. HOROWITZ and E. NEIL MOORE

1. INTRODUCTION

There have been few in vitro studies on the electrophysiologic characteristics of spontaneously diseased ventricular myocardium. The majority of the electrophysiologic data on diseased ventricular tissues have been derived from experimental animal models. These include studies on hypertrophy and failing muscle induced by pulmonary banding [1–3]. In one study, transmembrane potentials were recorded from ventricular papillary muscles, removed from patients with varying degrees of ventricular failure [4]. However, this study did not report abnormal electrophysiology in these tissues. There are numerous experimental studies on the electrophysiology of myocardial infarction induced by coronary artery ligation and these have been reviewed recently [5, 6]. Several laboratories have recently reported abnormal electrophysiologic responses in infarcted human ventricular tissues removed from patients at the time of operation and suggest that these may play a role in arrhythmias [7–9].

The present chapter will be restricted to a consideration of the electrophysiologic characteristics of ventricular tissues obtained from patients at the time of operation for aneurysmectomy or mitral valve replacement. In the tissues which showed evidence of myocardial infarction, there were grossly heterogeneous electrophysiologic characteristics. Action potentials ranged from relatively normal to very abnormal. In the following discussion we will consider aspects of these tissues which suggest that slow response potentials occur spontaneously in diseased ventricular myocardium and the implications of this in arrhythmogenesis.

2. INFARCTED HUMAN TISSUE

Specimens of human ventricular tissues were obtained from 23 patients at the time of cardiac surgery. The characteristics of the ventricular tissues examined are summarized in Table 1. Prior to the time of surgery 18 of the 23 patients exhibited some degree of chronic congestive heart failure. The rela-

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Table 1. Characteristics of ventricular tissues.

Patient	*ECG*	*Months post-infarct*	*CHF*	*VT*	*Tissue*	*Electrophysiology*					
						Spontaneous basic cycle length (sec)	*Normal cells*	*Slow response*	*Variable amplitude response*	*Inexcitable normal resting potentials*	*Inexcitable abnormal resting potentials*
1	AMI	9	++++	0	AN	1.39–1.48		+			
2	ALMI	60	+	+	AN					+	+
3	ASMI	6	++	0	AN			+	+	+	+
4	ASMI	25	+++	0	AN		+	+			
5	IMI	6	++	+	AN						+
6	AMI	9	++++	0	AN	1.25–1.40		+			
7	IMI	12	++++	0	PM	11.11–11.92		+			
					PM*		+				
8	MVP	—	++++	0	PM*		+				
9	AMI	1	++++	0	PM		+	+	+		
10	AMI	18	++++	0	AN		+				
11	AMI	24	++++	0	AN	1.78–2.93		+			
12	AMI	54	+	+	AN						+
13	AMI	11	0	+	AN	0.92–3.75		+			+
14	AMI	5	0	+	AN						+
15	AMI	28	+++	0	AN			+			+
16	AMI	0.25	+++	+	AN	1.36–1.80					+
17	AMI	2	+	+	AN	1.60–4.60		+			+
18	AMI	38	++++	+	AN			+			
19	Pacemaker	26	++++	+	AN	1.76–1.96		+			
					PM			+			
20	IMI	21	0	+	AN	3.10–4.40		+			
21	AMI	4	0	+	AN			+			
22	RBBB–LAHB	6	0	+	AN	2.80–3.40	+	+			
23	AMI	26	++++	0	AN			+			+

* Noninfarcted tissue; AMI, anterior myocardial infarction; ALMI, anteriolateral myocardial infarction; ASMI, anterioseptal myocardial infarction; IMI, inferior myocardial infarction; MVP, mitral valve prolapse; CHF, chronic heart failure; VT, ventricular tachycardia; AN, aneurysm; PM, papillary muscle; RBBB, right bundle branch block; LAHB, left anterior hemiblock.

tive magnitude of heart failure is indicated by 1 to 4 crosses. Cardioactive drugs were discontinued at least 24-h before operation in 11 of the 23 patients and continued in 12 patients with arrhythmias to the time of surgery. The anesthetic agents were morphine and halothane. The techniques for transport of the tissue from the operating room and superfusion in the tissue bath have been previously published [8]. The tissues were studied using standard microelectrode recording techniques [8]. Of the 25 tissues obtained, two of these were noninfarcted papillary muscles from patients undergoing mitral valve replacement. Recordings from these tissues served as a control to which recordings from infarcted tissues were compared. It can be seen in Table 1 that the characteristics of the infarcted tissues ranged from cells with relatively normal action potentials and resting potentials to inexcitable cells with depressed resting potentials. In 10 of the tissues spontaneous automaticity was recorded.

A view of the endocardial surface of one of the resected aneurysms is shown in Figure 1. Action potentials were recorded from the free running trabeculae seen in the photograph. In addition, there were areas at the insertions of the trabeculae where potentials were also recorded. The extensive scar on the endocardium of the aneurysm is apparent in this photograph

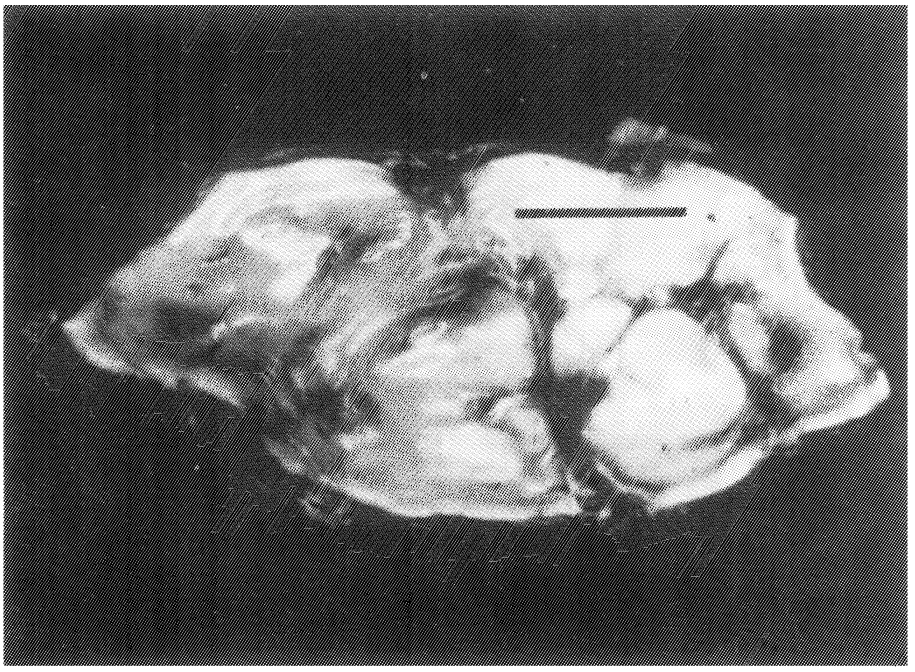

Figure 1. The endocardial surface of a resected human ventricular aneurysm. The bar in the upper righthand quadrant of the tissue indicates 1 cm.

and this made impalement of surviving cells very difficult. In the 23 infarcted tissues studied, we obtained action potential recordings in a total of 87 separate impalements. The electrophysiologic properties described in the subsequent sections are based on these recordings. Because the number of samples in a given tissue were no small it is unlikely that we were able to completely characterize the electrophysiologic properties of each tissue. However, we found similarities in all of the tissues.

The majority of the potentials recorded in 17 of the 23 infarcted tissues appeared to be of the slow response variety. In 4 infarcted tissues (patients 4, 9, 10, and 22) normal action potentials resembling either Purkinje fibers or ventricular muscle cells were recorded. In comparing these normal muscle action potentials with those from noninfarcted papillary muscles the resting potentials, action potential amplitudes and rates of depolarization were similar but the action potential durations of the infarcted tissues were significantly larger [8].

In Figure 2 are presented selected action potentials recorded in the infarcted tissues. In the top row of records are relatively normal action potentials recorded in areas histologically verified to be viable Purkinje fibers (left) and ventricular muscle (right). The second row of records were obtained from an

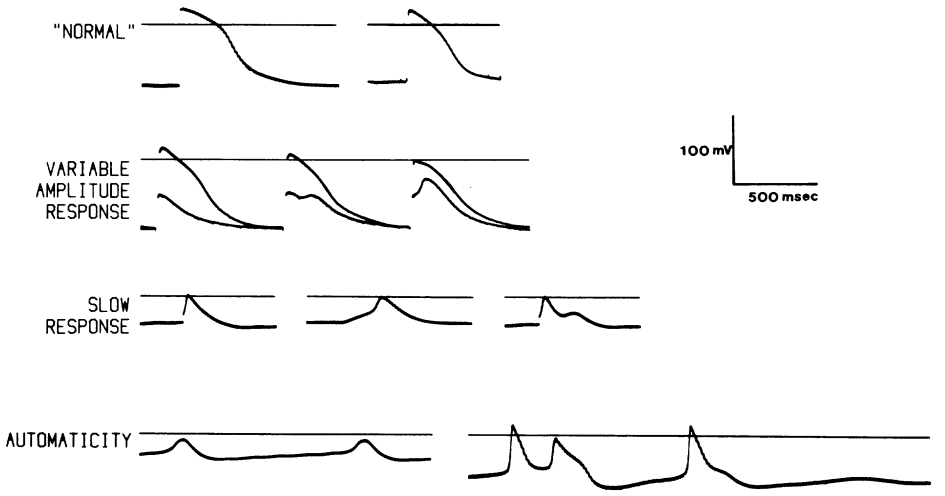

Figure 2. Selected examples of transmembrane potentials recorded from human infarcted myocardium. The records in the upper row show a relatively normal Purkinje-type potential (left), and ventricular muscle cell potential (right). The second row shows a variable amplitude response. The records are two superimposed tracings at two different intensities of stimulation in the same cell impalement (3.0 and 2.8 mA). The third row shows typical slow response potentials. The left record is a slow response potential without a prepotential or postpotential. The middle record shows a prepotential and the right record shows a postpotential. The bottom row shows two examples of spontaneous automaticity. The horizontal lines in each of the records indicate zero potential.

area within a tissue that exhibited normal resting potentials and a variable action potential amplitude depending upon the intensity of stimulation. In the superimposed records at two stimulation intensities it can be seen that at the higher intensity stimulation the action potentials showed a relatively normal action potential amplitude and overshoot. However, at lower intensity stimulation the action potential amplitude was reduced and has a variable configuration. The third row of records in Figure 2 shows typical configurations of the slow response potentials that we observed. The resting potentials were low, averaging −48 mV. The action potentials rarely overshot zero potential and the maximum rate of depolarization was low. The conduction velocity in the area of tissues exhibiting these potentials was also very slow and the action potentials often exhibited multiple components with either prepotentials, shown in the middle record, or post potentials, shown in the far right record. Two examples of the type of spontaneous automaticity recorded in these tissues are shown in the bottom row of records in Figure 2. The data presented in Table 2 contrast the electrophysiologic characteristics of the various excitable cells recorded in the infarcted ventricular myocardium.

3. VARIABLE AMPLITUDE RESPONSE

In two tissues (from patients 3 and 9) there were areas in which the action potentials were strongly dependent on the intensity of stimulation. These action potentials arose from resting potentials that were relatively normal (Table 2). The amplitude and rate of depolarization of the action potential in a given impalement could be modified by changing the location of the stimulating electrode, the polarity of the stimulation, or the intensity of stimulation. In Figure 3A and B, action potentials recorded from one of these tissues at two different intensities of stimulation are shown. In A the transmembrane potential recordings were made adjacent to the point of stimulation through surface bipolar electrodes. The intensity was 3 mA. The resting potential of this cell was −86 mV and the amplitude of the first action potential shown in A was 104 mV. There was, however, complex to complex variation in the amplitude of the action potential. In B, the action potentials were recorded from the same cell impalement as shown in A. The intensity of stimulation in this case was reduced to 2.8 mA. At this intensity there was a considerable variation in action potential amplitude, configuration and duration. While the resting potential remained at −86 mV, the action potential amplitude varied between 42 and 60 mV. The low amplitude and multicomponent nature of this response is reminiscent of the slow current dependent slow response. However, a characteristic of the variable amplitude response, as opposed to the slow response, was that it was relatively insensitive to verapamil. The

Table 2. Characteristics of excitable cells in infarcted myocardium.

	Number of tissues	*Number of cells*	*Resting potential (mV)*	*Action potential amplitude (mV)*	*Maximum rate of depolarization (V/sec)*	*Duration (msec)*
"Normal" muscle	2	5	-81.2 ± 8.2	96.8 ± 7.9	150.0 ± 35.4	427.4 ± 47.4
"Normal" Purkinje	3	17	-82.9 ± 6.2	102.9 ± 6.8	159.8 ± 76.4	602.9 ± 105.7
Variable amplitude response	2	8	-78.2 ± 7.4	(8.0 to 110.0)*	(0.1 to 15.3)*	258.4 ± 77.8
Slow response	17	57	-47.6 ± 9.4	49.8 ± 14.4	1.8 ± 1.7	428.5 ± 162.8

* Data presented as range since amplitude and maximum rate of depolarization varied in a given cell impalement. Other values are means and standard deviations.

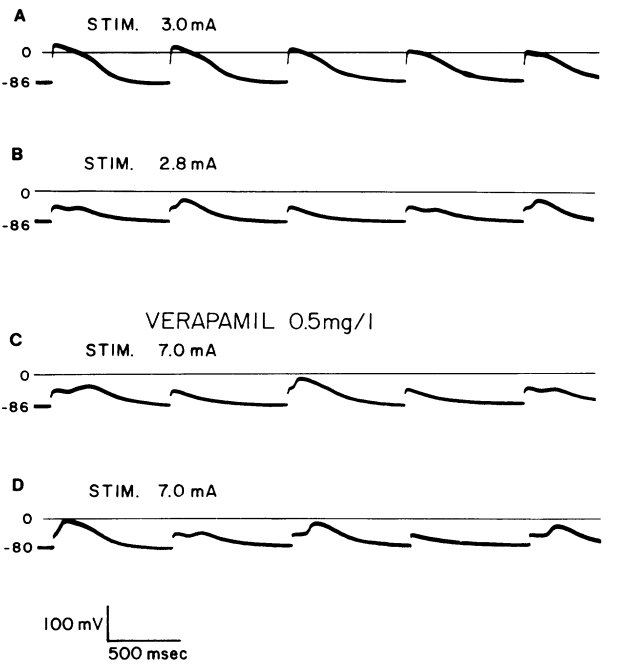

Figure 3. Analog records demonstrating the effects of stimulus intensity and verapamil on the variable amplitude response. Each record is from the same cell impalement. The records in A were obtained adjacent to the stimulating electrode which was being used to pace the tissue with a stimulus intensity of 3 mA. The records in B were obtained when the stimulus intensity was reduced to 2.8 mA. C and D were recorded after the addition of 0.5 mg/liter verapamil to the tissue bath. The pacing stimulus intensity was 7 mA (reproduced by permission of the American Heart Association, Inc. from Spear et al., Circulation 59:247-256, 1979).

transmembrane potential recordings presented in Figure 3C and D were obtained 20 min after the addition of verapamil to the superfusate at a concentration of 0.5 mg/liter. The variable amplitude responses persisted; however, surface stimulation with a higher current intensity (7.0 mA) was required to produce responses which were comparable to those described in B. The durations of the action potentials in these cases tended to be longer, and there was a greater separation of the components of the response. The properties of the slow response and variable amplitude response recorded in our experiments are contrasted in Table 2. The amplitudes and rates of depolarization are given as ranges, since they were stimulus intensity dependent. In a given cell impalement, however, they were observed to range between 8 and 110 mV.

Areas of tissues exhibiting variable amplitude responses also showed very slow conduction velocities with evidence of dissociated conduction of the wavefronts. The mechanism for the variable amplitude response is not entire-

ly clear. Certainly the presence of local block and inexcitability contribute to the variable amplitude response. Whether or not there is a defect in the sodium conductance is unknown. The basis for the local block and inexcitability may be purely morphologic. The extensive fibrosis of these tissues may act to insulate islands of excitable tissue. Variable amplitude responses have also been observed in isolated rat papillary muscles subjected to stretch [10] and also in tissue cultured myocardial cell [11]. In these preparations, disruption of cell-to-cell activation causing local slowing of conduction and block produced responses similar to those of Figure 3. The studies of conduction across Purkinje fiber, papillary muscle junctions [12] and in the AV node [13] indicate that in cases of local conduction block the distal to the site of block exert a strong electrotonic repolarizing influence on the action potentials of active cells proximal to the block. The variable amplitude response observed in these tissues probably reflects electrotonic depression of the action potentials of active cells by a varying sized population of adjacent inactive cells. The stimulus dependent characteristics of the variable amplitude response support this hypothesis. In our tissues we found areas exhibiting variable amplitude responses adjacent to areas exhibiting slow response activity. It is therefore reasonable to expect that electrotonic interactions due to the asynchronously activated tissue may contribute to the properties of the slow response as well as the variable amplitude response. This is also undoubtedly the mechanism of the multicomponent configuration of the slow response action potentials shown in Figure 2. In these tissues, therefore, electrotonic interactions apparently play an important role in their electrophysiologic responses.

4. SLOW RESPONSE

The majority of the action potentials recorded in the infarcted tissues had characteristics resembling what has been referred to as the slow response [14, 15]. In the following discussion we will consider several properties which verify that these potentials are slow inward current dependent slow responses.

As was mentioned previously, the slow response action potentials were of small amplitude and slow rate of rise and arose from low resting potentials (Table 2). An additional property which suggested that they were slow responses was that refractoriness outlasted the action potential duration. Figure 4 shows records obtained in a ventricular aneurysm during surface bipolar stimulation. The stimulation and recording arrangement is shown schematically in A. Transmembrane potentials were recorded along a ridge of viable ventricular muscle (ME_1 and ME_2). The ME_1 recording was obtained 1 cm

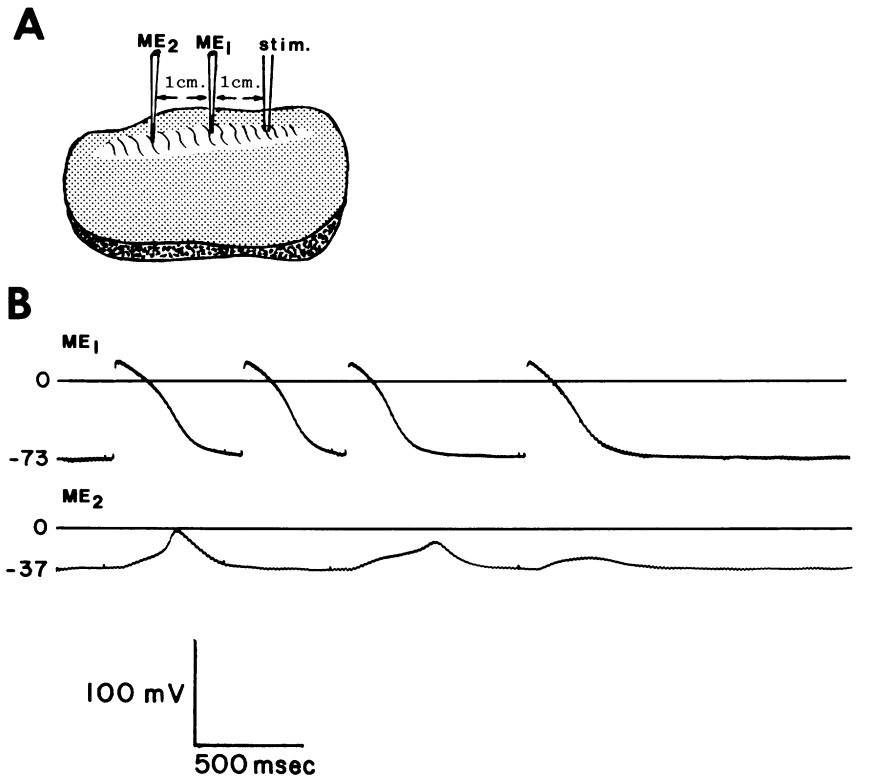

Figure 4. Analog records demonstrating conduction characteristics between relatively normal ventricular tissue and an area exhibiting slow responses. In A is shown the schematic representation of the experimental arrangement. A stimulating bipolar electrode (stim) was positioned at one end of a ridge of muscle. Two microelectrodes (ME_1 and ME_2) were used to record transmembrane potentials at 1 and 2 cm distances from the stimulating electrode. The ME_1 and ME_2 potential records are shown in B (reproduced by permission of the American Heart Association, Inc. from Spear et al., Circulation 59:247-256, 1979).

from the site of stimulation. The analog records in B show relatively normal appearing ventricular muscle cell action potentials at this recording site. Distal to the ME_1 recording, transmembrane potential ME_2 was obtained, and in this case the recording exhibited an abnormal resting potential, as well as low amplitude and multicomponent action potentials. The first stimulated action potential in the sequence conducted from the proximal to distal recording sites with considerable delay. The ME_2 action potential exhibited an initial prepotential followed by a secondary peak response. The second action potential was evoked by a premature stimulus with a coupling interval of 550 msec. In this case, the ME_1 action potential did not engage the distal recording site. However, it can be seen from the ME_2 recording that the transmembrane

potential had returned to its normal resting value at this time. Thus, membrane refractoriness outlasted the action potential duration in the conducting pathway. The third action potential in the records was evoked by stimulation at a coupling interval of 500 msec following the second action potential. Activity conducted from the ME_1 to ME_2 recording site. However, the prepotential is of longer duration and the action potential peak is depressed compared to the initially conducted response. The fourth action potential evoked at a coupling interval of 650 msec following the third response engaged the distal ME_2 recording site whith only a subthreshold response which appears to consist only of the prepotential component of the other responses. In other tissues we observed areas of slow response activity which displayed intermittent entrance block of spontaneous activity originating in adjacent areas and also exit block of spontaneous activity originating in the region of the slow response.

Areas of the infarcted tissues exhibiting slow response potentials were also sensitive to verapamil. This is additional evidence for the slow current playing a role in the generation of these potentials. In Figure 5 are presented transmembrane potential recordings in an aneurysm in a region which histologically was verified to be ventricular muscle [8]. The first two action potentials in panel A were unpaced and occurred at a spontaneous coupling interval of 1.25 sec. The maximum diastolic potential was -40 mV and slow diastolic depolarization was apparent. At the time indicated by S, pacing at a cycle length of 450 msec was commenced. The multicomponent and variable configuration responses to this pacing are displayed in the righthand portion of the records in A. Fifteen minutes following the addition of verapamil to the

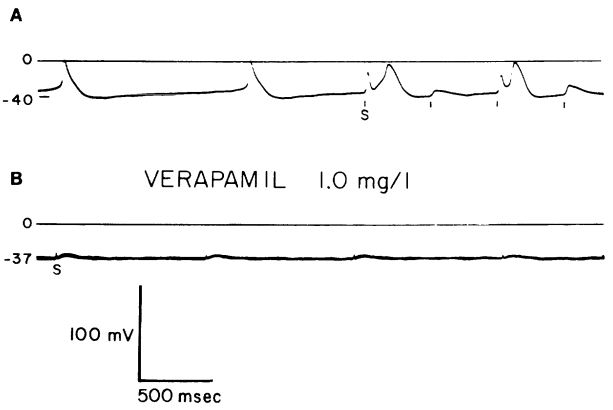

Figure 5. Analog records demonstrating the effect of 1 mg/liter verapamil on spontaneous and paced slow response potentials. In A, S indicates the commencement of pacing at a basic cycle length of 450 msec. In B, the same cell impalement is shown after the addition of verapamil to the tissue bath. S indicates pacing at a basic cycle length of 1000 msec (reproduced by permission of the American heart Association, inc. from Spear et al., Circulation 59:247-256, 1979).

superfusate at a concentration of 1 mg/liter, spontaneous automaticity was eliminated. Pacing at a cycle length of 1 000 msec failed to evoke an action potential. These data support the conclusion that the transmembrane action potentials were of the slow response type and were probably mediated by the slow inward current.

The automaticity shown in Figure 5 was of the slow response variety. The majority of cells in our experiments in which impalements were made showing automaticity were in areas of low resting potential and low amplitude. As seen in Table 1, in a given tissue the rate of automaticity was quite variable, and in all of the tissues ranged from a basic cycle length of 0.92 to 11.9 sec. In Figure 2 the records in the bottom row show two additional examples of spontaneous automaticity recorded in these preparations. The records at the left are of slow response automaticity which was typical in these tissues. The records to the right, however, show a more complex type of automaticity. In this case, the spontaneous action potentials were followed by a postpotential which occasionally reached threshold to produce a secondary spike response. The multicomponent nature of the action potentials again re-emphasize the large degree of asynchrony of activation that we observe in these tissues. The maximum diastolic potential in this cell, however, was relatively polarized at -75 mV. Since we were unable to obtain data on the verapamil sensitivity of this area, we could not determine whether the slow inward current played a role in this automaticity as well. It is interesting that in this tissue, obtained from patient 13, the spontaneous automaticity showed the greatest range from 0.9 to 3.8 sec. One reason for this variability can be seen in the bottom right record of Figure 2. Intermittently in this tissue, automaticity failed to reach threshold. The third spontaneous response shows only a subthreshold depolarization.

In all the cases of automaticity that we recorded in the infarcted tissues, the spontaneous rates were considerably slower than the rates of the ventricular tachycardias that we observed in these patients. In addition, we recorded spontaneous automaticity in three tissues obtained from patients which did not have ventricular tachycardia (Table 1). In none of our tissues were we able to demonstrate triggered automaticity [16].

An observation which suggests that the slow response potentials were calcium dependent was their sensitivity to the concentration of calcium in the superfusate. Figure 6 presents data showing the effects of reducing the concentration of calcium in the superfusate on the configuration of a slow response action potential. In the upper record is an impalement in an area of an infarcted tissue which had spontaneous diastolic depolarization. The concentration of calcium in the superfusate was normal at 1.6 mM. The maximum diastolic potential was -56.5 mV and the action potential amplitude was 56.5 mV. In the lower records, 15 min following superfusion of the

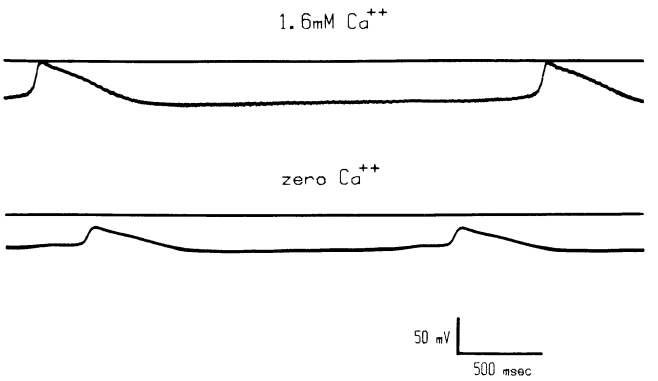

Figure 6. Analog records demonstrating the effect of reducing the concentration of calcium in the superfusate from 1.6 mM to o mM. The upper and lower records are from the same cell impalement. The horizontal line indicates 0 potential.

tissues with Tyrode's solution in which calcium had been eliminated, the resting potential decreased to 47.8 mV and the action potential amplitude was reduced to 31.4 mV. As reported by others for experimentally induced slow response potentials [15, 17, 18], a reduction of calcium in the superfusate reduced the action potential amplitude maximum rate of depolarization and resting potential.

In Figure 7 the upper records show an impalement in a different region of the tissue which also had slow response activity. In this case the maximum diastolic potential was −43.5 mV and the action potential amplitude was 48.0 mV. The concentration of calcium in the superfusate was normal at 1.6 mM. In the records below, following the addition of calcium to bring the concentration to 12.0 mM, the maximum diastolic potential increased to −48 mV and the action potential amplitude increased to 54.5 mV. In this case, increasing the concentration of calcium in the superfusate increased the

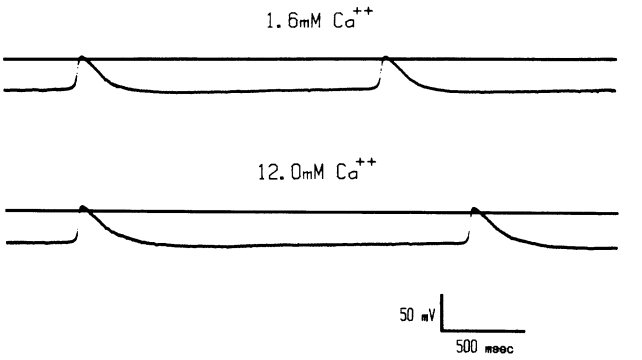

Figure 7. Analog records demonstrating the effect of increasing the concentration of calcium in the superfusate from 1.6 mM to 12.0 mM. The upper and lower records are from the same cell impalement. The horizontal line indicates 0 potential.

resting potential, action potential amplitude and maximum rate of depolarization.

Figures 6 and 7 suggest that these abnormal action potentials recorded in regions of the resected human aneurysms do rely on the slow inward calcium current. However, changing calcium in the superfusate did not produce the expected effect on the rate of spontaneous automaticity. In Figures 6 and 7 it appears that a reduction in calcium increased the rate and an increase in calcium decreased the rate. In both normal canine Purkinje fibers [19] and in canine tissues in which slow response automaticity has been experimentally induced [15, 20], a reduction in concentration of calcium slows the spontaneous rate, while an increase in calcium concentration increases the spontaneous rate. Because of the large variation in the rate of spontaneous automaticity in a given tissue in these experiments (Table 1), it is difficult to evaluate subtle effects of an agent on atomaticity. Because of the difficulty in maintaining impalements in these tissues, we were not able to determine whether the changes in automaticity induced by varying calcium concentration in the superfusate depicted in Figures 6 and 7 represent the characteristic response of these tissues.

It is interesting that these infarcted ventricular tissues showed slow response activity in the presence of normal concentrations of calcium in the superfusate and in the absence of the addition of exogenous catecholamines. In most experimental systems, slow responses are induced by depolarization of the membrane potential by voltage clamp or a high concentration of extracellular potassium in the presence of catecholamines or elevated calcium concentration [14, 15, 17–19]. The slow response potentials observed in the infarcted human ventricular myocardium do have similarities to those occurring in diseased human atria [21, 22] and resected mitral valve leaflets [23].

As in experimental models of the slow response [15] tetrodotoxin did not eliminate the slow response potentials in our infarcted human tissues. In Figure 8 the potential at the far left was recorded in an area exhibiting slow

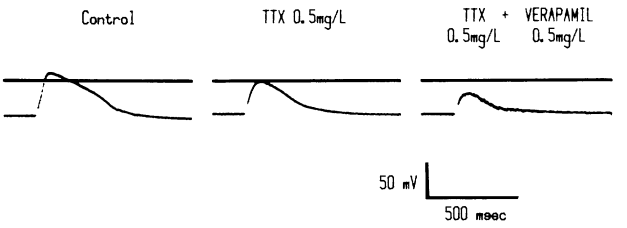

Figure 8. Analog records demonstrating the effect of tetrodotoxin and verapamil on a slow response potential. The left panel indicates the potential in the absence of drugs; the middle panel with the addition of tetrodotoxin (TTX) and the right panel with the addition of both tetrodotoxin and verapamil. All recordings were from the same cell impalement. The horizontal lines indicate 0 mV.

responses. The resting potential was 48 mV and the action potential amplitude was 58 mV. Fifteen minutes following the addition of tetrodotoxin to the superfusate at a concentration of 0.5 mg/liter the maximum rate of depolarization of the maximum diastolic potential and the plateau was depressed. This is shown in the middle panel of Figure 8. The effect of also adding verapamil at a concentration of 0.5 mg/liter is shown in the far right panel. The action potential was reduced in amplitude to 29 mV. In this experiment, while tetrodotoxin did not eliminate the slow response potential, it did decrease the action potential amplitude and maximum rate of depolarization. This may indicate that the action potential in the control state was generated by a tetrodotoxin-sensitive fast channel as well as a verapamil-sensitive slow channel. Alternatively the effect of tetrodotoxin could have been due to the elimination of an electrotonic contribution from adjacent more normal cells which were tetrodotoxin-sensitive. In our experiments, we were not able to determine whether the tetrodotoxin sensitivity of the depolarization phase of the slow response was due to mixed ionic mechanisms in a given cell or due to mixed populations of cells near the recording site. A similar tetrodotoxin sensitivity has been reported for abnormal potentials recorded in canine tissues in experimental myocardial infarction [24].

5. DRUG SENSITIVITY

In studies on normal canine muscle and Purkinje fibers, lidocaine has been shown to have little effect on the depolarization phase of the action potential if the concentration of potassium in the superfusate is low [25] but at normal or high concentrations of postassium lidocaine depresses action potential depolarization [26]. In the isolated human infarcted ventricular myocardium lidocaine influences action potential amplitude and the rate of depolarization in areas exhibiting slow response potentials. Figure 9 shows the effect of

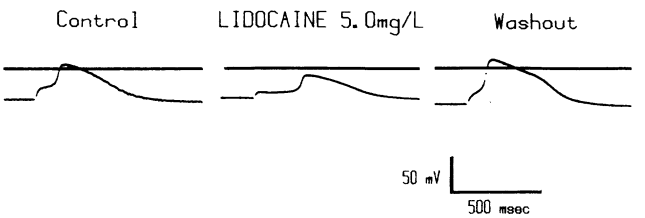

Figure 9. Analog records demonstrating the effect of lidocaine on a slow response potential. The left panel is a recording in the absence of lidocaine. The middle panel was recorded 15 min following the addition of lidocaine to the superfusate and the right panel was recorded 15 min following the washout of the lidocaine from the superfusate. All records are from the same cell impalement. The horizontal line indicates 0 potential.

lidocaine at a concentration of 5 mg/liter on a slow response potential. In the panel at the left before the addition of lidocaine, the resting potential was −45 mV and the action potential amplitude was 50 mV. There was a prominent prepotential seen in this impalement. Fifteen minutes after the addition of lidocaine the maximum rate of depolarization was depressed and action potential amplitude was reduced to 33 mV. Also, the duration of the prepotential was increased and there was a 4 mV decrease in the restig potential. As shown in the right panel, the effects were reversible 10 min after the washout of lidocaine. As compared to the control state, the potential now had an improved resting potential amplitude and a reduced duration of the prepotential.

The depression of abnormal action potentials by lidocaine has previously been reported in hypoxic and ischemic canine cardiac cells [24, 27]. It is interesting that in these depressed human cardiac cells, not only lidocaine, but also tetrodotoxin (Figure 8) influenced the depolarization of the action potential. These studies suggest, as in the canine experimental model [24], that a tetrodotoxin- and lidocaine-sensitive fast channel, although depressed, may be contributing to the depolarization phase of the slow response action potentials observed in this tissue.

The recordings made in the infarcted human ventricular myocardium demonstrate that the tissue has considerable and pharmacologic heterogeneity. There are areas showing abnormal activation and conduction which are insensitive to verapamil (Figure 3) and other areas showing more typical slow response potentials which are influenced by verapamil (Figure 5). Normal action potentials (top row of Figure 2) in the infarcted myocardium are not eliminated by verapamil and exhibit the expected response to lidocaine. Therefore, a given drug influences different regions of these infarcted tissues in different ways.

6. ISOLATED HUMAN INFARCTED VENTRICULAR MYOCARDIUM AS A MODEL OF ARRHYTHMOGENESIS

The electrophysiologic properties of the surviving cells of resected human infarcted ventricular tissues are potentially arrhythmogenic. The tissues were characterized by a large degree of electrophysiologic heterogeneity. Both normal and abnormal action potentials were recorded (Figure 2 and Table 2). There were regions showing spontaneous automaticity (Figure 2), very slow conduction and local conduction block (Figure 4). The mixed electrophysiologic properties of these tissues may in part be due to the morphologic substrate of the tissues. Surviving cells were scattered throughout areas of dense connective tissue. This in itself would serve to establish circuitous

conduction pathways and might contribute to a reduction of cell to cell electrical coupling.

As can be seen in Table 1, the potentials recorded in these tissues were predominantly slow responses. Table 2 shows that their electrophysiologic properties fall within the range for slow responses reported by other investigators using experimental preparations [14, 15]. Our studies support the slow inward current as the primary mechanism involved in generating these potentials since they are sensitive to variations in the calcium concentration in the superfusate (Figures 6 and 7) and they are not eliminated by tetrodotoxin (Figure 8) but are eliminated by verapamil (Figure 5). In addition, these impulses conduct very slowly and their refractoriness outlasts the action potential duration (Figure 4).

Areas exhibiting slow response potentials, however, do appear to have other ionic mechanisms contributing to their depolarization phase. Figure 8 shows that tetrodotoxin does depress the amplitude and rate of rise of the action potential, and these potentials are also sensitive to lidocaine (Figure 9). It is also possible that these mixed properties may be due to the heterogeneous nature of these tissues. The action potentials recorded with a given cell impalement may be strongly influenced electrotonically by adjacent active cells. The multicomponent nature of the action potentials for the variable amplitude response and slow response almost certainly has an electrotonic basis (Figures 2–5, 9). The pharmacologic response in a given cell impalement therefore may partially be due to its effect on adjacent cells which is reflected electrotonically on the action potential of the impaled cells.

While the electrophysiologic properties of the resected ventricular tissues are potentially arrhythmogenic, the nature of these tissues does not allow us to determine whether these properties contributed to the ventricular arrhythmias present in many of these patients at the time of surgery. Routinely, when the aneurysms are resected, a rim of aneurysmal tissue is left attached to the normal myocardium to facilitate suturing the ventriculotomy. We therefore cannot determine whether activity within the isolated tissue was in communication with normal ventricular myocardium. The lack of success of a routine aneurysmectomy in eliminating ventricular arrhythmias in some patients [28, 29] suggests that the central regions of the aneurysm may not be required for the arrhythmia. If the arrhythmias are generated in the border tissues of the aneurysm which are routinely left in the patients our studies may provide a basis for the arrhythmias since these tissues may have similar electrophysiologic properties to those resected portions which we studied in the tissue bath.

Our studies suggest that resected human infarcted ventricular tissues are a model of the slow response. In addition, the properties were found to be quite heterogeneous, both in terms of the electrophysiologic mechanisms and their

responses to pharmacologic agents. These tissues, therefore, may provide a clinically relevant in vitro model for the study of mechanisms of arrhythmias and the effects of therapeutic interventions in myocardial infarction.

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15. THE ROLE OF ELECTROTONUS IN SLOW POTENTIAL DEVELOPMENT AND CONDUCTION IN CANINE PURKINJE TISSUE

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The mechanisms responsible for the conduction of the cardiac impulse have commanded the attention of many noted investigators. Their diligent efforts have added much to our knowledge about the subject, though a uniform and consolidated concept of impulse generation, and especially conduction, has yet to be expounded that might offer an explanation of every electrophysiologic observation.

Cell-to-cell transmission in the normal myocardium is generally assumed to occur by electrotonic means [1, 2]; that is, current from a depolarized cell will affect adjacent cells by a direct form of electrical coupling, thereby depolarizing them. Under less than normal conditions, when a rapid Na^+ dependent depolarization is not elicitable, what mechanism(s) might be responsible for the generation of those reported slow wave potentials, and under what conditions might these potentials be evaluated?

A number of approaches to this problem have been attempted, some with notable success. Early studies evaluated basic membrane parameters and characterized the components of the transmembrane action potential [3–6]. Furthermore, the rapid inward Na^+ current was demonstrated to be a principle determinant of conduction velocity, acting as the very source of the depolarization current, and thereby affecting the passive membrane properties of that cell, its intercellular connections, and all coupled neighboring cells [7]. Environmental manipulations involving both electrical and chemical aberrations were undertaken in a variety of myocardial tissues to evaluate their respective conduction properties [5, 8, 9]. In some instances, tissues were so adversely affected by perturbations, which may be considered extreme, that low amplitude potentials developed [8, 9]. It was subsequently assumed that such low amplitude potentials recorded from depressed tissue resulted from an attenuated form of a rapid Na^+ current [7–9].

With the demonstration and characterization of a slow 'Ca^{++} current' in myocardial tissue [10, 11] re-evaluation of these potentials and their relationship to conduction was necessary. Numerous experiments were performed to demonstrate the existence of slow Ca^{++} dependent and, later, slow Na^+ dependent, potentials [12-15]. The Ca^{++} dependent transmembrane potentials

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differed from the electrical activity of the 'normal' cell by demonstrating a decreased resting membrane potential, depressed total amplitude, an increased effective refractory period and greatly reduced upstroke velocity [16]. These responses were generally associated with a reduced conduction velocity. Some of these parameters (most notably upstroke velocity) were used to infer the character of a concomitantly depressed conduction. Recordings similar to those elicited from 'abnormal' cells displaying slow potential activity were also recorded from the 'normal' sinoatrial and atrioventricular nodes [9]. These data were drawn upon to conclude that the intrinsically slow conduction velocity existing physiologically in nodal tissues may be linked to a slow channel phenomenon [16, 17]. 'Slow, Ca^{++} dependent' transmembrane potentials have been demonstrated in many types of cardiac cells; hence, it is reasonable to propose that these potentials might be associated with conduction delay in depressed cardiac tissue.

It is an interesting exercize to analyze data recorded from AV nodal preparations [9, 18–21], as well as blocked preparations with proximal and distal active regions in light of the above proposal [14, 22, 23]. The AV nodal tissues act as natural 'block' preparations in that a zone of tissue with depressed transmembrane potentials is juxtaposed to areas with more normal action potentials. This physiologic model might therefore be used as a control against which to correlate results obtained from the models to be described. Multiple component transmembrane potentials are readily recorded from the isolated AV nodal preparations. Based on simple wave-form analysis and the correlation between proximal and distal electrical activity, it may be assumed that these waveforms behave electrotonically. Contrary to previous reports, reduction or abolition of these responses by Ca^{++} inhibition does not really allow for the determination of the mechanism by which they commonly develop. Segmental block produced in complex preparations often results in transmembrane potentials with a typical two component waveform [23]. These may be associated with the fusion of electrotonic wave fronts from adjacent active areas. Studies utilizing focal cooling and crushing of isolated Purkinje fibers support an electrotonic origin of these recorded waveforms [24]. Other authors publishing these potentials have commented on their electrotonic origin, but they have rarely detailed an hypothesis concerning the role of electrotonus in delayed conduction. In general, investigators have opted for an active membrane mechanism to explain these waveforms. While this might be a valid approach, we would propose that electrotonus be given much more serious consideration. The data presented herein brings into question the role of Ca^{++} or Na^{+} in slow potential development. Perhaps we should be analyzing electrophysiologic changes in terms of passive membrane parameters rather than those of an active nature with respect to arrhythmogenesis.

Some differences in experimental results and the interpretation of such results may stem from variation among experimental prototypes. For example, studies in which the entire preparation was uniformly exposed to a blocking solution containing no sodium and abundant calcium invalidates the concept of a *zone* of abnormal tissue. Even pathophysiologically, an entire heart would never be confronted with a Na^+ free, high Ca^{++} environment. Under such conditions an *effective* myocardial response would be unlikely. Our previous studies, as well as more recent experiments, have persuaded the author to conclude that slow Ca^{++} dependent responses play little, if any, role in delayed propagation.

Three types of mechanisms have been theorized to explain the development of the 'abnormal' low amplitude potential, and its relationship to conduction: (1) alteration of active membrane properties, (2) alteration of passive membrane properties or the passive properties of the intercellular junctions, and (3) a combination of (1) and (2). It is likely that category 3 will eventually assume the role for unifying the mechanism of conduction. However, the contribution of category 2 must be more fully investigated to insure our complete understanding of its functional physiologic and pathologic importance, especially in those circumstances where low amplitude potentials may predominate.

Since it was initially assumed that electrotonus played a significant role in normal intra- and transcellular conduction, it seemed logical to evaluate its activity under conditions of block. Utilizing current depolarized segments of the canine right bundle branch, Wennemark et al. demonstrated electrotonic interactions throughout the depolarized region [25]. They concluded that transmission through a current blocked segment was purely electrotonic. While there were objections to the use of a depolarizing current as a blocking source, it afforded the variability of block length and rapid return to control that no other manipulations could. Short periods of current block (less than 30 sec) could be produced 40–50 times without any apparent alteration of the transmembrane action potential. These qualities permitted multiple sampling of transmembrane activity among cells within and outside the blocked region during normal transmission, delay and block. Utilizing a current-induced blocked model (to be described below), Wennemark and Bandura [26] extensively evaluated transmembrane potentials from blocked segments of canine Purkinje tissue. We demonstrated that electrotonus played a significant role in transmission across such current-blocked segments. The following discussion reviews our earlier work with current-induced depolarization block, and presents data from more recent studies using chemically induced segmental block. These data permit consideration of an alternative approach to the analysis of these most interesting electrophysiologic waveforms.

For the former type of experiments, Purkinje fibers were taken from adult

canines weighing 10–15 kg. Following anesthetization with sodium pentobarbital (30 mg/kg), the hearts were rapidly excised through right lateral thoracotomies. The posterior division of the left bundle branch, along with its distal Purkinje bundle (usually a single bundle), was carefully dissected from each heart, while submerged in warmed, oxygenated Tyrode's solution. The preparation was placed in a special perfusion chamber bisected by a thin latex membrane containing a small centrally located perforation (about 5 mm in diameter). The Purkinje bundle was then threaded carefully through the hole in the latex membrane (see Figure 1). This was accomplished by placing a polyethylene tube (PE160), with a tapered tip, through the hole in the dividing membrane and applying gentle suction to one end of the tube with the bundle nudged into the other end, thereby pulling the fiber into the fluid-filled PE tubing which provided a rigid channel through the rubber partition. The PE tubing was then carefully retracted, allowing the hole in the membrane to assume a smaller diameter, and providing a seal around the perimeter of the bundle. Each half of the chamber was perfused with warmed (37 °C), oxygenated (95% O_2, 5% CO_2) Tyrode solution at a flow rate of 3 ml/min.

The anatomically proximal portion of the preparation, from which the microelectrode recordings were generally obtained, is shown in the lower half of the chamber depicted in Figure 1. The Purkinje fiber preparations were stimulated at a rate of 1–2 cycles/sec from the free end of the bundle with a small, bipolar electrode (E_s, Figure 1). The stimulus consisted of a 4–10 V square wave of 1 msec duration delivered by Grass stimulator and isolation units. An electrogram was obtained by a small, bipolar electrode (E_r, Figure 1) positioned at the functionally distal portion of the preparation. An electrical blocking current was supplied by a 280 V battery source. The positive polarizing electrode (B+, Figure 1) was placed in one side of the chamber and the negative polarizing electrode (B−, Figure 1) in the other side of the chamber. Current strength was regulated by a rheostat, so that the blocking current could be adjusted to produce differential lengths of blocked segments (1–5 mm). Usually, second or third degree block would occur in the range of 3–4 mm.

Transmembrane action potentials were obtained with glass capillary microelectrodes (ME) filled with 3 M KCl. Electrodes with a resistance of 5–10 MΩ were used in these experiments. Electrode resistance was checked periodically throughout every experiment. All experiments were taped on a 4-channel analog tape recorder and filmed directly from a 4-trace oscilloscope with a Polaroid camera at various gains and sweep speeds for detailed measurements. Procedures were performed under a dissecting microscope with 12.5 times magnification, and anatomical distances were determined with a grid-containing eyepiece.

A variable length of block (0–4 mm) was readily produced by the applica-

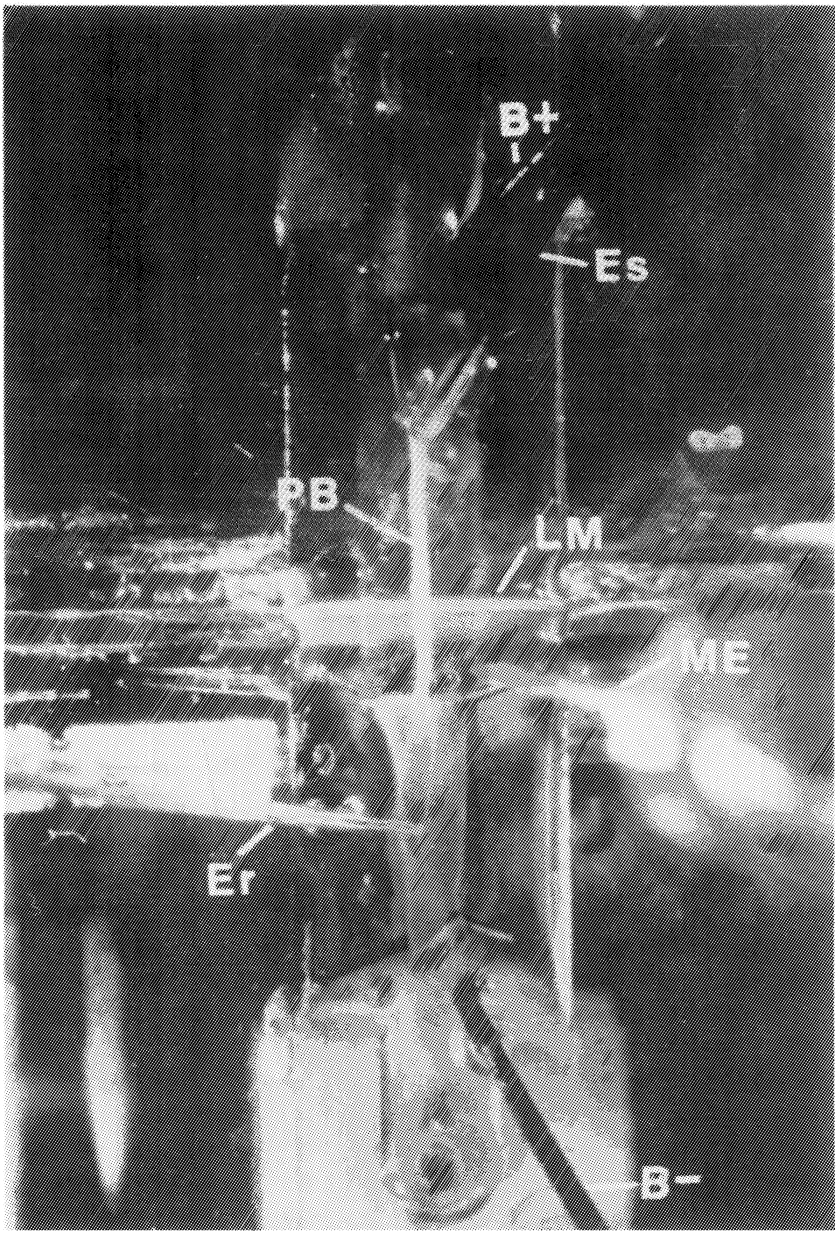

Figure 1. Photograph of the preparation in the divided chamber. E_s and E_r represent the proximal stimulating and distal recording electrodes, respectively. PB is the Purkinje bundle. B+ and B− are the positive and negative blocking electrodes. ME represents the intracellular microelectrode. LM is the latex membrane separating the chambers.

tion of the blocking current. The depolarized zone of the fibers was located in the chamber containing the negative electrode (B−, Figure 2). While the functionally proximal block boundary (FPBB) was situated near the dividing partition, the exact delineation of the distal boundary was difficult to determine, but was accepted to correspond to the site of the earliest regenerative response. Any delay between the distal electrogram and the onset of the second component of the deflection from the blocked zone was considered 'apparent' delay. This resulted from the loss of high frequency components along the cable [25]. Those potentials recorded from the blocked region always

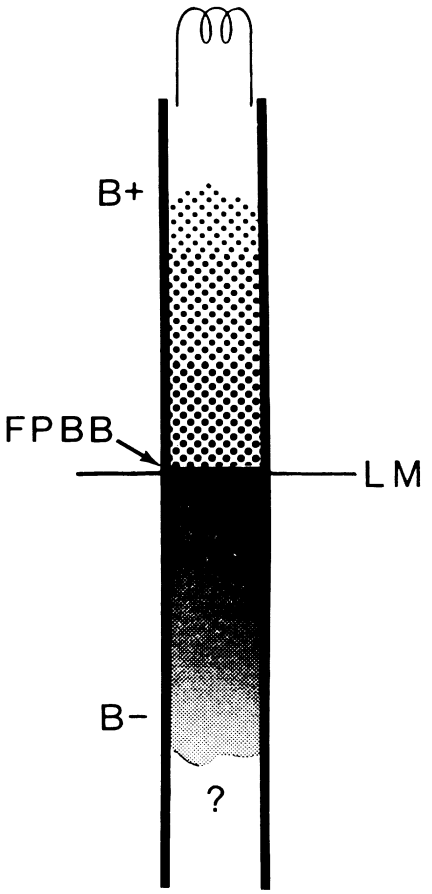

Figure 2. Diagrammatic representation of both hyperpolarized and depolarized zones of the preparation. B+ and B− represent the blocking electrodes. The stippled zone (top) identifies the hyperpolarized region and the gray zone (bottom) represents the depolarized region produced by the application of the blocking current. The question mark stands for the uncertainty of the exact location of the distal block boundary. LM is the latex membrane and FPBB is the functional proximal block boundary. Coil represents the proximal stimulating electrode and serves for anatomic orientation.

demonstrated passive behavior, with distance dependent attenuation of upstroke and magnitude. A corresponding zone of progressive hyperpolarization was located in the chamber containing the positive, B+ electrode. The amount of conduction block was readily controlled in this procedure by regulating the current strength. Those variations in blocking effects produced in different preparations were usually quite apparent (26].

The transmembrane potential recordings of conducted impulses exhibited a characteristic two-component waveform associated with delay. The initial component represented an electrotonic potential originating from the proximal boundary of the blocked zone. The second, delayed component represented a reflected, electrotonic potential which emanated from a regenerative response at the distal block boundary. Nonconducted impulses consisted solely of an initial electrotonic component, without a second component, since no regenerative response occurred at the distal block boundary. Slow, diastolic depolarization was always enhanced during the application of the blocking current and was usually most marked near the functionally distal block boundary.

Figure 3 is an example of first degree block induced in this preparation. Notice the development of the leading hump in the upstroke of the transmembrane potential recorded by ME_2 in the blocked zone. ME_1 was taken

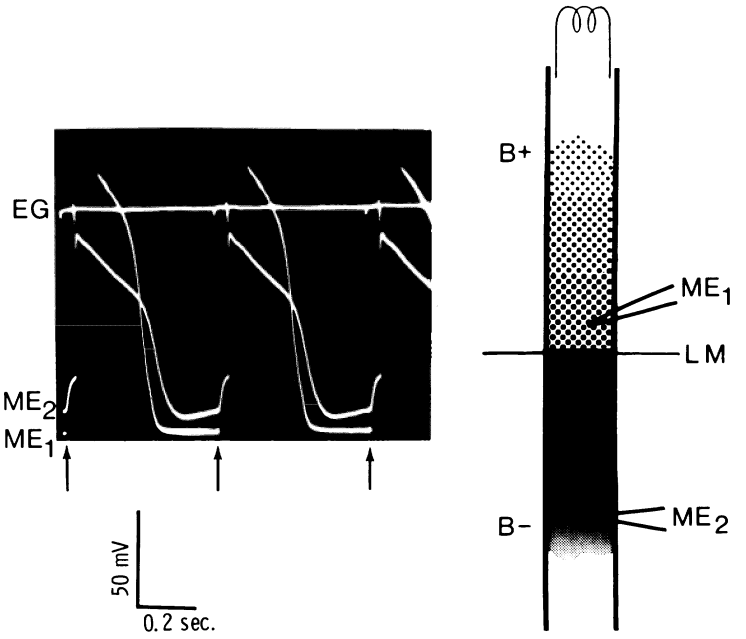

Figure 3. Current depolarization induced first degree block. ME_1 and ME_2 represent proximal and distal transmembrane recordings, respectively. Diagrams at the right show the approximate locations of microelectrodes and the distal block boundary. EG represents the distal electrogram, and the arrows denote the stimulus artifact. Calibrations are as shown.

from the hyperpolarized region of the fiber on the opposite side of the membrane. This was done to insure that some form of hyperpolarization block did not occur. The typical two-component transmembrane potential is seen in the ME_2 recording. The initial component was associated with a transmitted electrotonic potential occurring at the functional proximal block boundary, while the second superimposed component was associated with the active response in the distal electrogram. These data corroborated previous studies of the isolated right bundle branch [25], and it was concluded that these two-component potential recordings were *purely* electrotonic. Therefore, they represented the passive reflection of an active response from those regions proximal and distal to the zone of block.

We next evaluated the waveforms generated under conditions of second degree block. The following two figures represent samples of advanced second degree block and demonstrate the role of these potentials in transmission through blocked zones.

Typical transmembrane potentials taken from the midportion of the depolarized zone under conditions of a 4:1 proximal-to-distal response are seen in

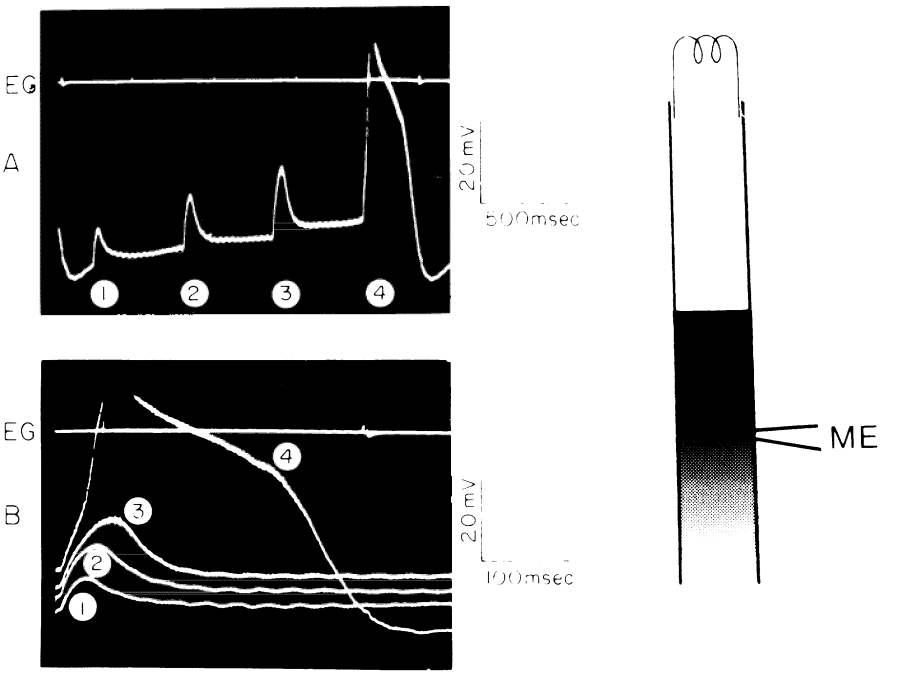

Figure 4. Transmembrane recording and distal electrogram associated with a 4:1 proximal to distal response. Panel A is at a slow sweep speed. One, 2, 3, and 4 represent 4 successive responses to repetitive proximal stimulation. Panel B is a superimposed tracing of responses seen in Panel A at a more rapid time base (5×). Labeling is the same as in Figure 3. Calibrations are as shown. (Adapted from Figure 3, ref. [27].)

Figure 4. Panel A shows a representative cycle, with responses one, two and three being simple, transmitted electrotonic potentials, originating from the functionally proximal block boundary. These potential deviations were of insufficient strength to excite the more active tissue distal to the block. Response 4 is a two-component potential typically derived by excitation of the distally active tissues. Similar to the previous three responses, there is an initial electrotonic potential; however, this response had sufficient strength to influence distally active cells to depolarize, and thereby produce regenerative activity as recorded by the distal electrogram. This distally active response is reflected electrotonically back through the blocked zone forming a two-component electrotonic potential recorded from the blocked region. Figure 5 is a special case of the same experiment and represents an example of an electrotonic potential which has been distorted by additional, local activity. Observe that in response 3 a purely electrotonic potential would follow a voltage and time course as predicted by the dotted line; however, an additional membrane voltage displacement occurs. This type of response, which was only occasionally observed, was never associated with a distally active

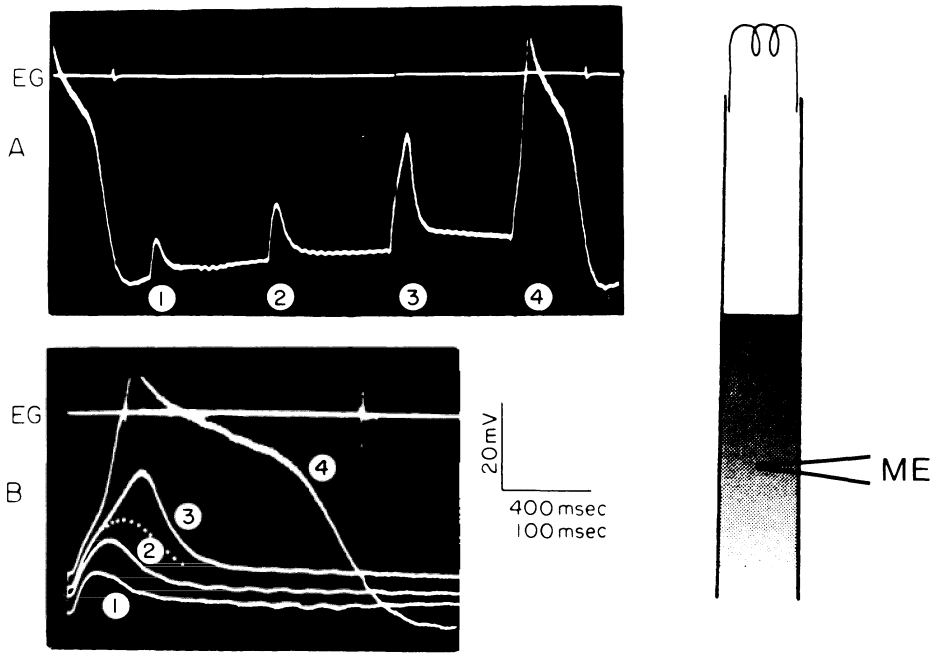

Figure 5. Transmembrane potentials and distal electrogram displaying a 4:1 proximal to distal response. Abbreviation is the same as in Figure 3. Panel A shows 4 successive responses to proximal stimulation. Panel B is a time expanded superimposition of 4 responses in Panel A. The dotted line in response number 3 represents the predicted simple electrotonic waveform. Calibrations are as shown (adapted from Figure 6, ref. [27].)

response, and always remained strictly local in effect. Whether this represented a 'slow channel' type of response or some form of longitudinal dissociation of electrotonic potentials is unclear. The partial engagement of an active process, which fails to progress to an all-or-none response, remains within the confines of that which might be considered as passive. Such has never been observed to be associated with active propagation. For the sake of description, we have termed this phenomenon 'partial local response' [27]. We have objected to the use of the term 'action potential' in reference to those waveforms associated with block, because the 'action' on the part of the fiber is poorly defined [27].

From these and other experiments we have developed a theoretical mechanism for block [26]. This involves the resting membrane potential at the time of initial deflection, as well as the strength of the resultant electotonic potential to explain the manner by which electrical transmission occurs through an 'inactivated zone'. Slow diastolic depolarization, which always existed near the distal block boundary, was intimately involved in the development of all forms of second degree block. It invariably acted to lower the membrane potential of the distal active cells toward threshold potential. This reduction in resting potential, associated with slow diastolic depolarization, produced an increase in membrane impedance at the distal block boundary [27]. This in turn increased the impedance of the inactivated zone and acted to increase the magnitude and upstroke of the transmitted electrotonic potential [27]. At critical lengths of block these two mechanisms added to restore the semblance of transmission. Variation of this same mechanism can be used to explain nearly all forms of second degree block [26, 27].

Although these data seemed conclusive, and the observed potential recordings strongly supported the electrotonic transmission theory, some crucial questions remained. The most important problem was to describe the manner by which the blocking current affected these Purkinje fibers. Could the depolarizing current have distorted 'normal' electrical behavior? It was well known that depolarizing currents could prolong action potential duration and enhance phase 4, slow diastolic depolarization [13, 27]. These alterations might reflect an increased membrane impedance, with the voltage dependence of the process playing an intimate role [28]. Furthermore, the depolarizing current used in these procedures might have produced a cumulative, long-term damage to the tissue. Irreversible block has been produced by prolonged application of a clamping current [27]. Therefore, it was decided that a more conventional model must be tested to discredit criticisms of the current block model. Since chemically induced block had been so widely used in other preparations, it was decided to reproduce a segmentally blocked preparation using chemical modes.

For these studies a special 3-chamber perfusion bath was designed in which

a bundle of myocardial fibers could be positioned and partitioned so that each segment would be perfused by separate solutions [29]. The use of this chamber allowed for the following series of investigations to be performed. We analyzed the electrical activity recorded from a tissue segment in which block was induced by exposure to a sodium deficient milieu, and characterized the means of electrical transmission through this segment.

Canine Purkinje bundles, usually the false tendon associated with the posterior division of the left bundle branch, with attached myocardial bases at each end were placed in the specially designed chamber so that the bundle could be partitioned and perfused from three separate sources. Figure 6 shows a diagram of the preparation situated in the tripartite chamber. The Purkinje bundle is represented as a strand of fibers anchored at each end by a rectanguloid section of myocardium. Following placement of the preparation into the chamber, a proximal stimulating electrode and a distal recording

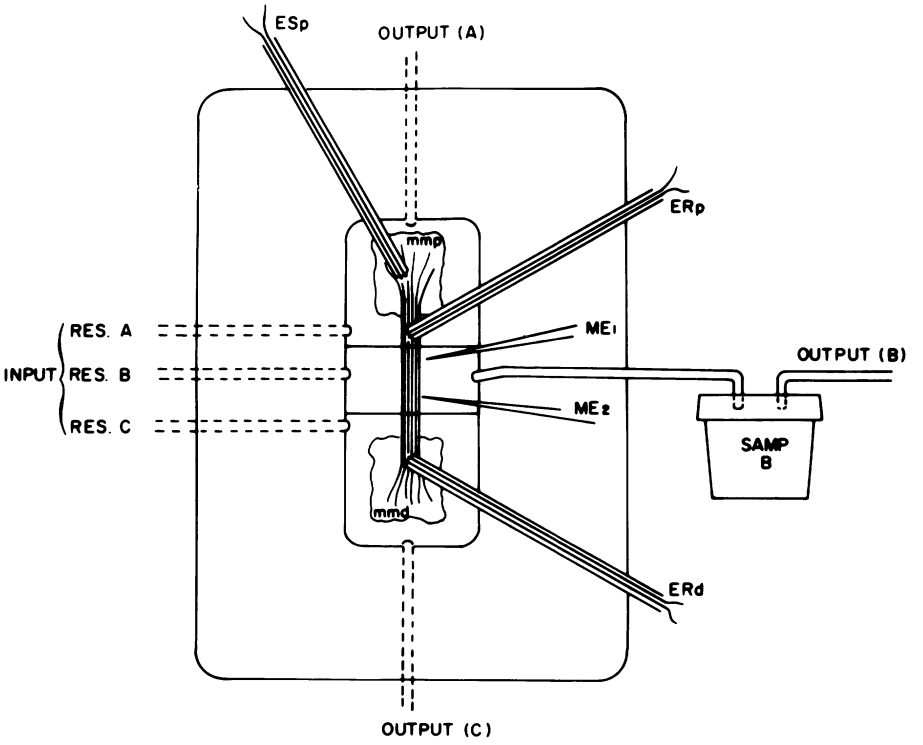

Figure 6. Schematic representation of the three chamber tissue bath with Purkinje bundle in place. Included are proximal and distal muscle bundles (mmp and mmd), two microelectrodes (ME_1 and ME_2), two bipolar extracellular electrodes (ER_p and ER_d), and a bipolar stimulating electrode (ES_p). Inputs to each subchamber are depicted as Input Reservoirs A, B and C, and outputs from each are identified as Outputs A, B and C. A sampling reservoir (Samp B) is located in the outflow line from the middle chamber for effluent analysis.

electrode were positioned on the tissue to monitor the activation time of the fiber during partitioning. If the procedure altered the activation time, the preparation was discarded. Two thin, lucite partitions with milled slots on the bottom edges were coated along the edges that would be touching the preparation with a polyhydrogenated vegetable fat (Crisco) and then lowered into complementary grooves within the sides of the chamber (see Figure 6). The slotted edges were brought into juxtaposition with the chamber floor, and the Purkinje bundle guided into the slots under observation with a dissecting microscope. Crisco acted as a sealant along all edges separating the three chambered system. Each preparation was equipped with a proximal stimulating electrode, ES_p, two extracellular bipolar recording electrodes, one proximal, ER_p, and one distal, ER_d, and two microelectrodes, ME_1 and ME_2, for sampling of transmembrane electrical activity at various sites along the preparation. Each chamber was perfused by its own reservoir, shown as Input Res. A, B and C. The effluent for each bath exited at Outputs A, B, and C. During control readings, all compartments were perfused with oxygenated, normal Tyrode solution ($K^+ = 4.0$ mEq/liter), maintained at 37°C. Following control sampling of electrical activity, the solution in reservoir B, perfusing the middle chamber, was changed to a THAM (trishydroxymethylaminomethane) substituted, zero Na^+ solution to produce block by the elimination of the Na^+ dependent spike. The effluent from the middle chamber was sampled during zero Na^+ perfusion and analyzed to confirm that no leakage from adjacent chambers had occurred. If sodium was detected, the experiment was invalidated. During these studies, preparations were stimulated from 0.5 to 2.0 pulses/sec, while recordings were sampled and amplified by standard methods and stored on magnetic tape. Data were photographed on 35 mm strip film, as well as on Polaroid film. Figure 7 is a photograph of the chamber with the preparation in place. In the center chamber (B), a 3 mm section of Purkinje bundle is exposed to a zero Na^+ environment. Chamber B has been previously perfused with food dye to identify that segment of tissue exposed to the blocking solution.

An example of 1:1 delayed transmission observed during the absence of Na^+ in the middle chamber is shown in Figure 8. EG_p and EG_d represent the proximal and distal electrogram recordings, while ME_1 and ME_2 represent proximally and distally recorded transmembrane potentials. The diagram in the lower right hand corner of the figure is a schematic representation of the Purkinje bundle with the blocked zone indicated by the crosshatched area. ES_p denotes the stimulus source at the proximal end of the preparation, and ME_1 and ME_2 the approximate locations from which transmembrane events were sampled. The numbers at the end of each tracing indicate the resting potentials for those particular recordings. Panel A demonstrates 4 successive responses. Note the two-component character of each transmembrane record-

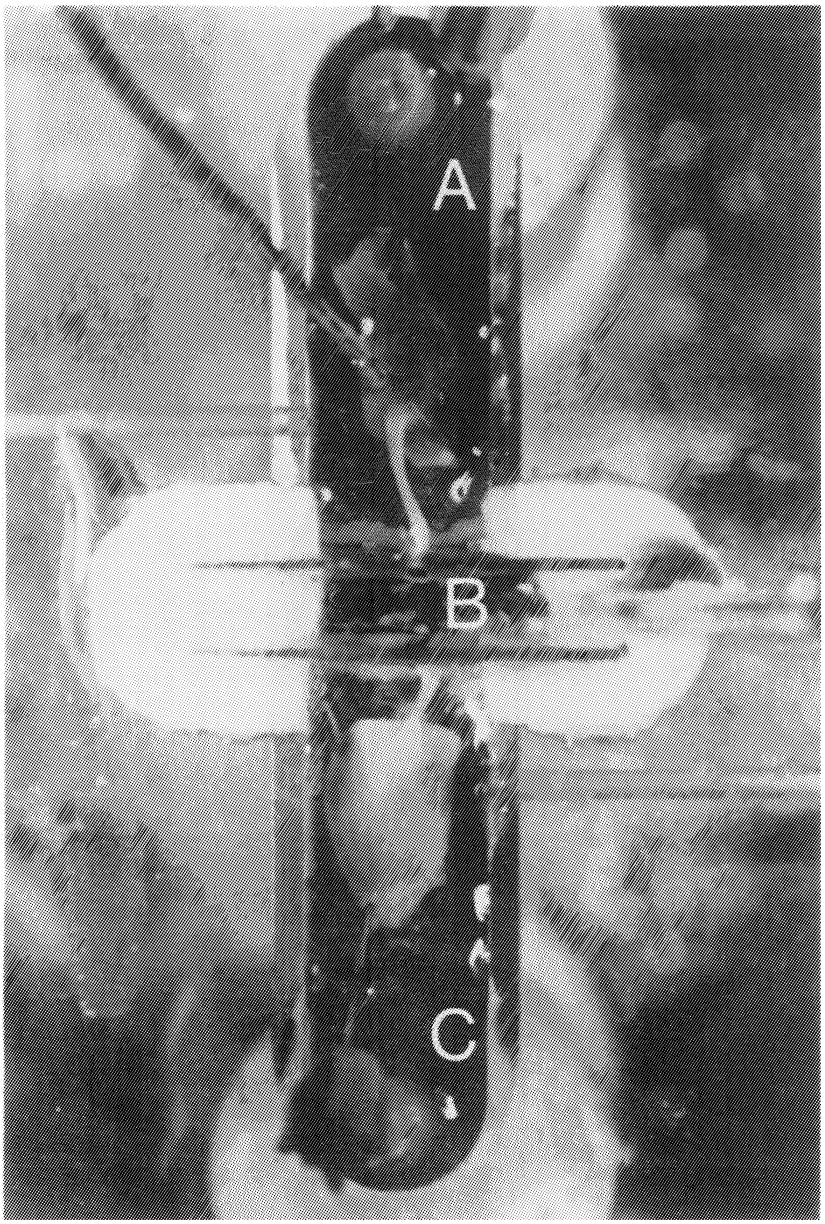

Figure 7. Photograph of the preparation in the tripartite chamber. A, B, and C identify the three separate compartments. The Purkinje bundle is seen coursing through each partition. The darkened color of the Purkinje segment seen in chamber B is secondary to this chamber having been superfused with food dye at the end of the experiment to mark that segment exposed to a Na^+ free solution. Chamber B is 3 mm in length.

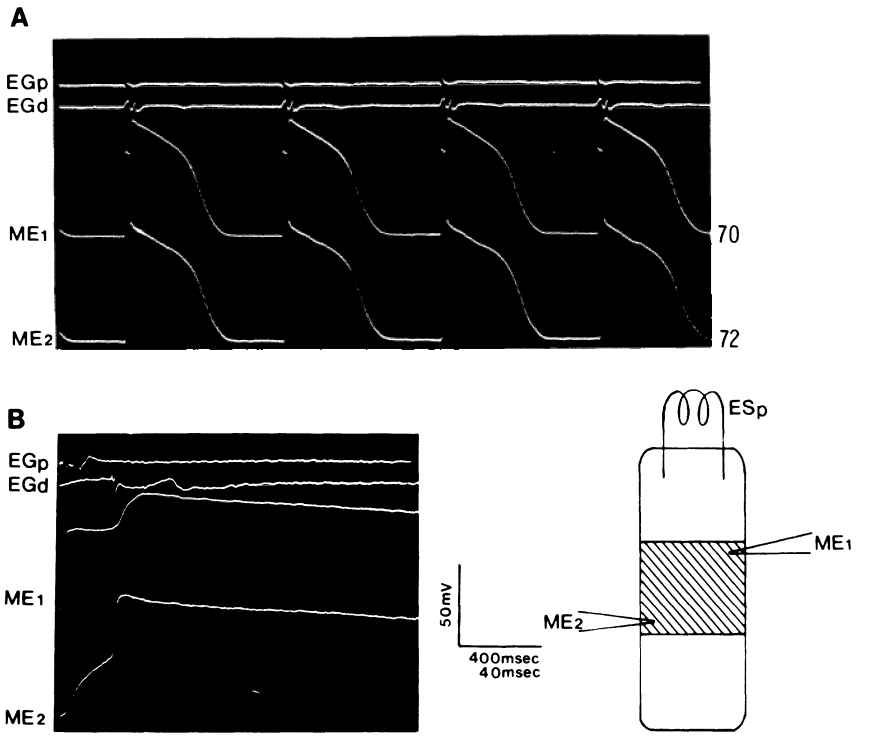

Figure 8. Transmembrane potential recorded from the Na^+ free region under conditions of first degree block. EG_p and EG_d represent proximal and distal electrograms, respectively. ME_1 and ME_2 stand for transmembrane recordings. The diagram at the right is a schematized fiber with the blocked region shown as a crosshatched area. The approximate locations of the microelectrode impalements are as shown. ES_p, the proximal stimulating electrode, is identified for anatomical orientation. Panel A represents 4 sequential responses to proximal stimulation. Panel B is a time expansion ($\times$ 10) of the first response shown in Panel A. The numbers at the end of the traces represent their resting potentials. Calibrations are as shown.

ing; this is more clearly demonstrated in Panel B, showing phase 0 at an expanded time base. Comparing the initial voltage deflections recorded from ME_1 and ME_2, there is the obvious voltage and upstroke attenuation that would be expected from the spatial decay of an electrotonically transmitted potential. The second component of the transmembrane response displays decay in the opposite direction, since this deflection originated from an action potential in the distal zone of active response, and was transmitted electrotonically back through the blocked zone.

From these preparations and from 10 additional experiments, transmembrane electrical activity was mapped under conditions of first, second, and third degree block. Fourteen sites were sampled for each study. Six successively equidistant points, 0.5 mm apart, were sampled through the blocked

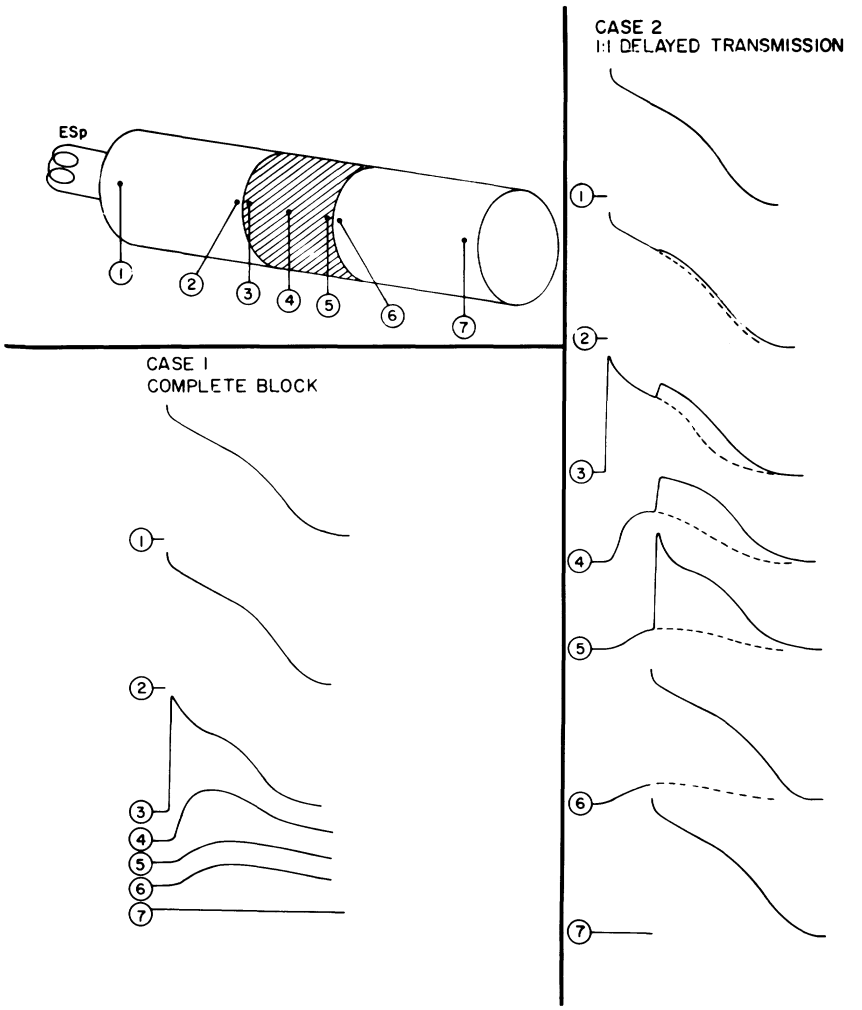

Figure 9. Transmembrane recordings throughout a segmentally blocked Purkinje bundle under conditions of 1° and 3° block. The diagram at the top represents the Purkinje bundle with proximal stimulating electrode (ES_p), and sites of transmembrane potential sampling. Only 7 of the 14 total sites actually sampled are shown. Case 1 represents complete block, while case 2 shows 1:1 transmission with delay. The dashed lines in case 2 represent the voltage/time course of the transmembrane potential that would have prevailed if complete block were present. Data have been retraced from original results. See text for discussion.

region, and 4 points, 1–2 mm apart, were recorded from both proximal and distal normal regions. Effects of proximal and corresponding distal activation upon the potentials recorded from these regions were analyzed to correlate their activity.

Figure 9 typifies a study of this kind and demonstrates the effects that

electrotonic interactions have on waveforms recorded from within and outside of the blocked region. Case 1 demonstrates an example of complete block. The action potential recorded at site number 1 represents the normal transmembrane response to proximal stimulation at 90 pulses/min. From site number 2 an action potential from the vicinity of the proximal block boundary develops within the proximal active zone. With the exception of a slight abbreviation of repolarization, no other changes were noted. Sites 3, 4, and 5 were sampled from within the blocked zone and display electrotonic potentials of progressively decreasing amplitude. An electrotonic potential is also seen in the distal active tissue at site number 6; however, it was of insufficient strength to produce a distal active response. No activity is seen at site number 7. Had this initial transmitted potential reached threshold at the distal block boundary, case 2 would have developed. In this latter situation (first degree block), a characteristic pattern of potentials is recorded near regions of the blocked zone, while only delay is evident between potentials recorded from sites 1 and 7. Site 2 records an active potential with an additional hump on its repolarization phase, which occurs at the exact time of distal activation. This represents the electrotonic influence of distal activation on the configuration of the proximal action potential. Sites 3, 4, and 5 record transmembrane potentials which are similar to case 1 with the initial electrotonic potential; however, a second superimposed electrotonic wave occurs which is coincident with distal activation. This potential demonstrates distance attenuation of upstroke and magnitude through the zone of block in a retrograde fashion. The action potential recorded at site number 6 emerges as an initial transmitted electrotonic potential which managed to attain threshold. This phenomenon of a passive event affecting an active response has been previously reported [30]. It is curious how closely these electrotonic potentials resemble those previously reported as spike and dome type potentials.

Second degree block also resulted in some preparations. Figure 10 shows an example of a 2:1 followed by a 3:2 proximal-to-distal response. If the passively transmitted potential were to fail to reach threshold in the distally active tissue, the recorded event would consist solely of the simple electrotonic waveform. However, when threshold was reached, the resulting active response was superimposed as a second component upon the passively transmitted electrotonic potential. Panel B, a tracing superimposing the three responses, 1, 2, and 3, of the Wenckebach cycle seen in Panel A, graphically illustrates the electrotonic interaction between two active tissues separated by a functionally blocked segment. Only when the initial electrotonic potential (recorded at ME_2) from each proximal activation was capable of eliciting a distal active response, was a second, delayed component present at ME_1. This electrotonic potential was reflected in a retrograde manner through the blocked zone to affect the active tissue proximally. Observe the resultant

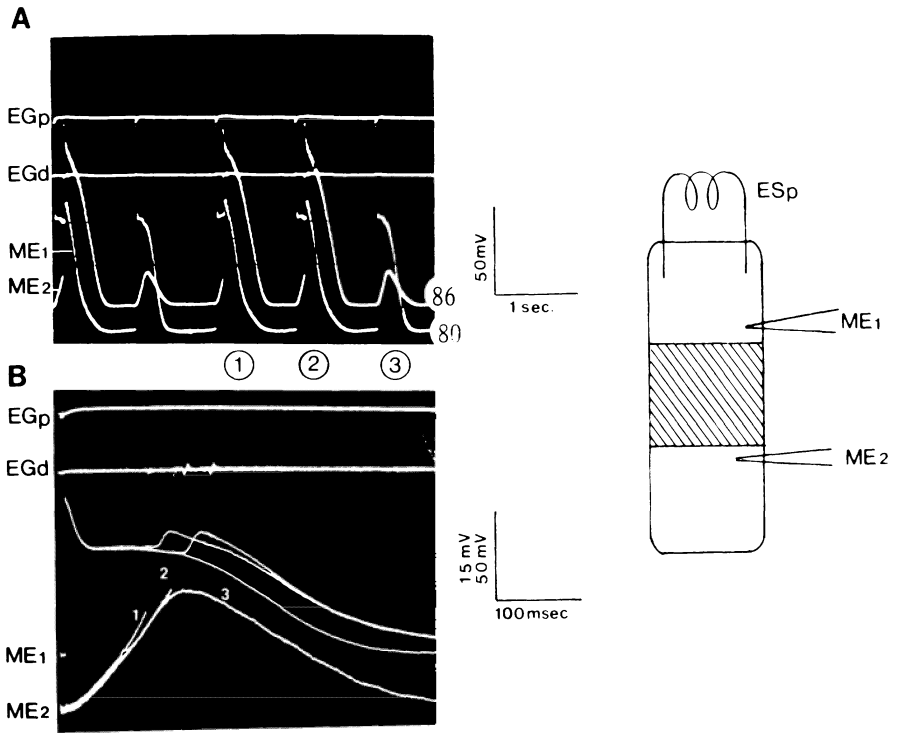

Figure 10. Transmembrane recordings of proximal and distal action potentials during second degree block. Labeling is the same as in Figure 8. Microelectrode locations are shown at the right. Panel A demonstrates a 2:1, Followed by a 3:2, proximal to distal activation. Panel B is a time expanded superimposition of responses labeled 1, 2, and 3 in Panel A. The gain in the ME_2 recording has been increased to demonstrate the progressive decrease in the take-off potential. Calibration is as shown.

electrotonic hump on the recovery phase of the proximal response. The effects of these passively reflected waves upon both depolarization and repolarization phases of normal tissue are evident here. However, the impact of these alterations upon the arrhythmogenicity of depressed myocardial tissues has yet to be described. High grade block, specifically a 5:1 proximal-to-distal response, is illustrated in Figure 11. Panel A demonstrates proximally active responses at ME_1, with only a transmitted electrotonic potential recorded at ME_2. The ongoing slow, diastolic depolarization is of great significance here. Following a distally active response, the tail of which is seen at the left edge of the panel, the resting membrane potential recorded in ME_2 reaches a maximum negative value and begins to undergo slow depolarization. The next regular proximal activation [1] produces an electrotonic potential which fails to reach threshold at the distal boundary; thus an active distal response

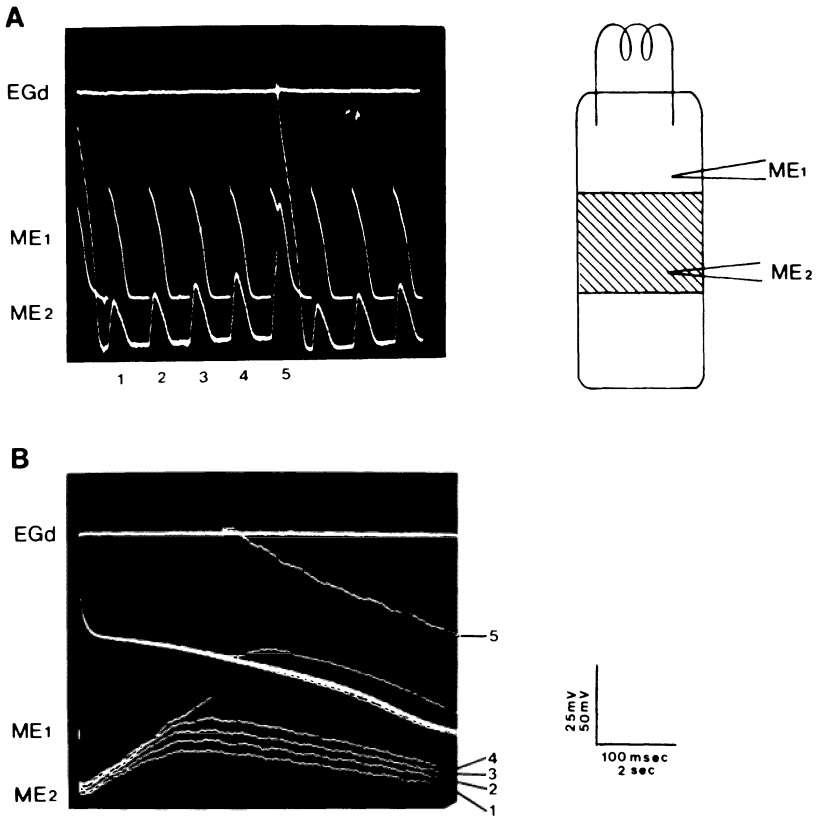

Figure 11. Transmembrane potentials recorded during conditions of high grade block (see Figure 8 for the explanation of labels). Panel A demonstrates a slow sweep speed tracing of a 5:1 proximal to distal response. Only the distal electrogram is shown in this study. ME_1 serves to confirm activation of the proximal active zone. Panel B is a time expanded superimposition of the 5 labeled responses in Panel A. ME_2 is displayed at $2\times$ gain. Calibrations are as shown.

is not attained. Likewise, the next three stimuli [2, 3, and 4] fail to reach threshold. However, the fifth electrotonic response attains threshold distally and causes an actively propagated impulse to proceed forward from that point. This is made evident by a second, delayed, electrotonic component as well as a spike in the distal electrogram. Panel B is a superimposition of the 5 labeled responses seen in Panel A. For each successive stimulation, the magnitude of the resting potential from which the electrotonic potential arises is less negative until a distally active response occurs. The resting potential is then reset to a more negative value, as shown in Panel A. As the initially transmitted electrotonic potential arises from a progressively less negative activation voltage, its magnitude and upstroke increase. This is due to voltage dependent changes in membrane impedance, as previously described by Weidmann [28].

It is important to note the subtle change in take-off potential. Recordings from more proximal regions of the block if made with lower oscilloscopic gain would minimize these small changes.

We found this model to provide an accurate, stable and reversible system for the study of block mechanisms. The results provided evidence supporting our previously proposed concept that transmission through a zone of blocked tissue is electrotonic. Slow, diastolic depolarization also appeared to be necessary to permit impulse transmission through a critically blocked zone of conduction tissue.

We were yet faced with the contrasting interpretations of such data by other investigative groups working with chemically induced blocked segments [14, 22, 23, 32] as well as current depolarized preparations [13, 14]. They invoked a slow Ca^{++} dependent current as responsible for potentials recorded from these depressed tissues. In order to address this problem the following studies were performed in the same preparation. Again the middle chamber was perfused with a zero Na^{+}, THAM substituted Tyrode solution. Techniques for sampling data and chamber effluents were as previously described.

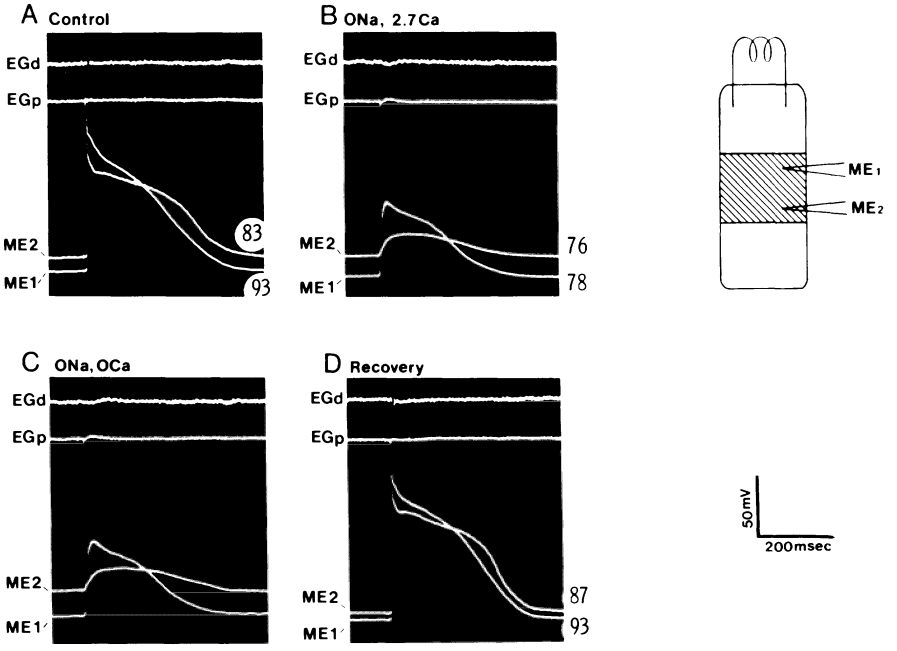

Figure 12. The effects of Ca^{++} depletion on transmembrane potentials recorded from the blocked zone. Panel A is the control. See Figure 8 for labeling details. Panel B displays transmembrane potentials recorded from a zero Na^{+}, 2.7 mEq/liter Ca^{++} environment. Panel C shows potentials recorded from the same two cells following 30 min of superfusion with a zero Na^{+}, zero Ca^{++} solution. Recovery is depicted in Panel D. Calibrations are as shown.

However, alterations in the Ca^{++} concentration of the zero Na^{+} perfusate were made.

Figure 12 demonstrates the effects of calcium depletion on the transmembrane potential recorded from a blocked segment. Panel A is a control photograph with normal Tyrode solution perfused to all three chambers. The data in Panel B were sampled following 30 min of middle chamber perfusion with a sodium free solution containing a normal calcium concentration. Concomitant with complete block, as evidenced by the absence of activity in the distal electrogram, there is a decrease in the resting membrane potential. The recording from ME_1 shows an electrotonic potential which originated at the proximal boundary of the block, and the recording of ME_2 shows this same electrotonic potential with diminution of amplitude and upstroke, secondary to passive decay as a function of distance through the block. Panel C represents activity recorded from these cells following 30 min of perfusion with a Na^{+} and Ca^{++} free solution. There is no observable change in the amplitude or upstroke of these potentials, and only a slight change in the configuration is evident. Panel D shows the recovery of both cells subsequent to middle chamber reperfusion with normal Tyrode solution. Spike activity in the distal electrogram reveals normal propagation.

In addition to evaluating the effects of calcium depletion upon the cardiac impulse, calcium excess was also investigated. Figure 13 represents the effects of altering the calcium concentration perfusing a blocked segment. Panel A is a control tracing with normal transmission throughout the preparation. Panel B exhibits the development of electrotonically transmitted potentials secondary to middle chamber perfusion with a zero sodium, zero calcium solution. Panel C shows the effects 30 minutes following an increase in the calcium concentration of the Na^{+} deficient solution to 2.7 mEq/liter, and Panel D the effects of further increasing the calcium to 16.2 mEq/liter. There are no significant changes in the transmitted electrotonic potentials. Except for an initial reduction of the resting potential, these passive impulses remained minimally changed throughout the course of these solution alterations. Subsequent to the recording taken in Panel D, Verapamil (4 mg/liter), a calcium channel blocker, was added to the 16.2 mEq/liter Ca^{++} superperfusate. Following 30 min of such superperfusion minimal changes were recorded in the waveforms (Panel E). Panel F demonstrates the recovery of both cells following reperfusion with normal Tyrode solution.

During the course of these investigations with blocked zones it was repeatedly noted that if the distally active zone lacked the presence of slow diastolic depolarization there resulted first or third degree block; the presence of second degree block was rare. This was interpreted as additional evidence for the role of take-off potential and slow diastolic depolarization within the distal active zone in the preservation of transmission through a critically

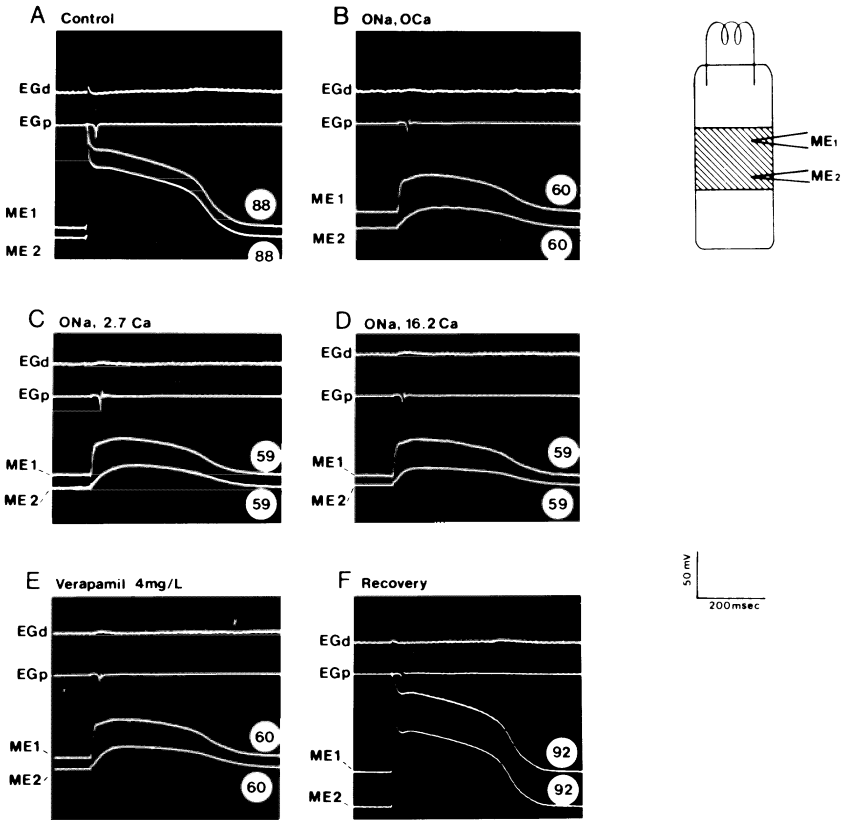

Figure 13. The effects of Ca^{++} depletion, Ca^{++} excess and verapamil on transmembrane potentials recorded from the blocked zone. Abbreviations are the same as those in Figure 8. Panel A represents the control. Potentials recorded from the segment of the fiber exposed for 30 min to a zero Na^{+}, zero Ca^{++} milieu are shown in Panel B. Panel C represents data following a 30 min superfusion with a zero Na^{+}, 2.7 mEq/liter Ca^{++} solution. In Panel D the tissue has been exposed to a zero Na^{+}, 16.1 mEq/liter Ca^{++} solution for 30 min. Following the recordings in Panel D verapamil 4 mg/liter was added to the perfusate, and Panel E represents a recording 30 min following this exposure. Panel F represents recovery of both cells. Calibrations are as shown.

blocked segment [33]. To further validate this hypothesis two additional series of experiments were performed.

Figure 14 demonstrates the results of superfusing the active segment distal to the blocked zone with a solution containing either acetylstrophanthidin or an increased potassium concentration. Panel A is the control photograph during which normal Tyrode solution was superfused to all three chambers. One-to-one transmission was recorded throughout the bundle. Panel B displays 2:1 and 3:1 passive electrotonic transmission resulting from the production of a blocked segment by perfusing the middle chamber (B) with a

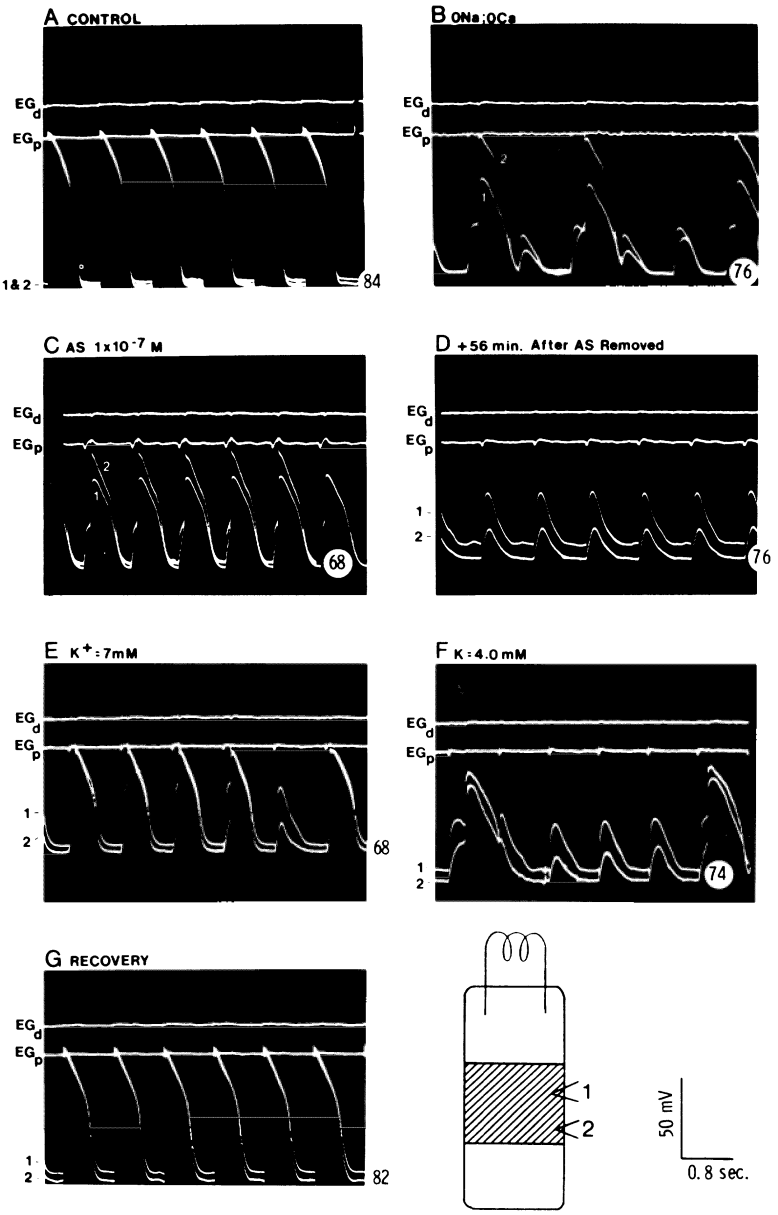

Figure 14. Improvement of transmission through a blocked region by distal chamber perfusion with acetylstrophanthidin (AS) or increased K^+. Labeling is the same as in Figure 8. The last transmembrane potential recorded from ME_2 has been deleted for ease of interpretation. Tracings have been manually separated for clarification. Calibrations are as shown. See text for discussion.

Na^+ and Ca^{++} deficient solution. Immediately following this recording, acetylstrophanthidin in a concentration of 1×10^{-7} M was added to the normal Tyrode solution superfusing the segment of Purkinje fiber distal to the block (chamber C). After 15 min of superperfusion, the transmission ratio was increased (Panel C) and acetylstrophanthidin was discontinued. Panel D was recorded 56 min after washout, and high grade block was now reproduced. At this time the K^+ concentration of the solution perfusing the distal chamber was increased with a resultant enhancement of transmission to a 6:5 ratio (Panel E). In Panel F, following the adjustment of the distal perfusates by returning K^+ concentration to normal, a high grade block was reproduced. Finally, in Panel G we have complete recovery with normal Tyrode solution having been superfused into all three chambers.

Figure 15 similarly demonstrates the effects of altering the resting membrane potential in the distally active tissue upon transmission through the blocked zone. Since in this preparation we recorded from the distal active zone it more dramatically demonstrates the development of phase 4 depolarization. Here Panel A represents the control, and B represents complete block produced by the perfusion of the middle chamber with a Na^+ and Ca^{++} deficient solution. Panel C shows conversion to a 1:1 proximal-to-distal conduction, which is associated with a decreased maximum diastolic potential and an enhanced phase 4 depolarization, as induced by perfusing the distal chamber with acetylstrophanthidin. The spontaneous rate of the distal tissue is slightly slower than the driving rate in Panel C, which had to be increased for overdrive capture. Panel D represents the resumption of block following washout of acetylstrophanthidin and Panel E the recovery following reperfusion of the middle chamber with normal Tyrode solution.

Along with previous studies these results supported the tenet that 'transmission' through a blocked zone of tissue is electrotonic. Acetylstrophanthidin, a substance which enhances phase 4 depolarization as well as decreasing resting potential, was successful in improving transmission through a blocked zone in 9 out of 11 experiments. By increasing the K^+ concentration in the distally active zone, improvement of transmission through the blocked region occurred in 8 out of 12 experiments. The reduction of the resting membrane potential is probably the most important factor responsible for improvement of transmission through the critically blocked zone when this reduction is induced by increasing $[K^+]_0$. The role of impedance and its interaction with the transmitted electrotonic potential is more complex in this case because increasing $[K^+]_0$ in the distal active zone reduces terminal impedance which in turn reduces the characteristic impedance of the critically blocked region. This would act to worsen transmission with an associated increase in the spatial decay of the transmitted electrotonic potential. However, this effect is reduced by voltage dependent alterations in membrane impedance which

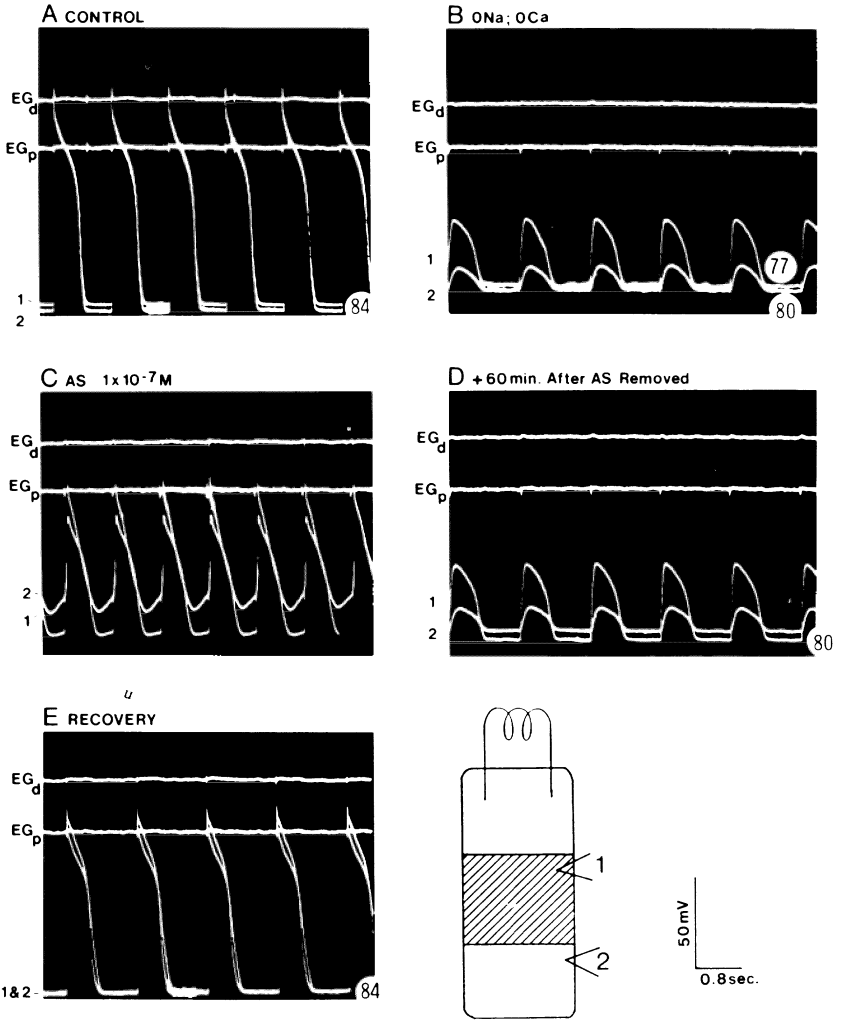

Figure 15. Improvement of transmission through a blocked zone associated with acetylstrophanthidin-induced phase 4 depolarization. See Figure 8 for an explanation of labels. The last potential recorded from ME_1 has been deleted in Panel C for ease of clarification. Maximum and minimum diastolic potentials for transmembrane responses recorded in panel C are 79/77 for ME_1 and 68/62 for ME_2. Calibrations are as shown (see text for discussion).

work to increase membrane impedance as the resting membrane potential is reduced. The net impedance change will be the result of these two interacting mechanisms. Thus, with impedance being attenuated by the effects of increased $[K^+]_0$, the preservation of transmission is most likely related to the reduction of resting membrane potential toward threshold potential in the distal active zone.

Electrotonus as a mode of transmission through cardiac tissue has been extensively evaluated in two separate models of block, with the data highly complementary. We have observed and reconfirmed the following findings in these two models:

(1) Transmission through a blocked zone behaves electrotonically.
(2) The configuration and magnitude of the potentials existing within the blocked zone are dependent upon location, cable properties of the tissue, and the characteristics of the electrical activity of the syncytial tissue.
(3) Calcium has no significant role in the development of these potentials.
(4) Maneuvers which reduce the resting membrane potential in the active tissue of the distal block boundary assist in preserving transmission through a blocked region. This reduction in membrane potential may act to (a) narrow the potential difference between the resting membrane potential and threshold potential, and (b) increase the membrane impedance, thereby improving the cable properties of the tissue, and likewise the transmitted electrotonic potential.

It is easy to conceptualize that, if a zone of myocardium containing conduction tissue were to become inactivated, a zone of block would be produced and transmission would assume a passive mode. Under these conditions, active tissue distal to the block would be initially quiescent, but with time that tissue would develop slow diastolic depolarization. Depending upon the proximity of this slow depolarization to the distal block boundary, the distally active zone would tend to assist in the transmission of electrotonic potentials emanating from regions proximal to the block. If these potentials attained sufficient voltage at the distal block boundary to excite it, a proximal and distal activation would be coupled.

While it is radical to propose that electrotonus is responsible for all forms of transmission delay in critically depolarized tissue, its participation in block zone activity is well within the realm of possibility. Intercellular transmission is generally assumed to be electrotonic [1, 2]. Intracellular conduction also behaves electrotonically under conditions where the cells' active Na^+ current is obviated. For example, if an active cell were to receive an input current and be driven to threshold by that current the cell would become a self regenerative focus of activity. This transmitted depolarization would create a renewed step current for the next electrically coupled cell. In contrast, if this cell were inactivated, the resultant step current supplied to the next electrically coupled cell would depend upon the passive properties of the inactivated cell and the functional interconnections of all neighboring cells. Since the fiber has cable-like properties [31] with resistance and capacitance elements inherent in its design, there is a time and distance dependent decay in the magnitude and upstroke of the transmitted potential. Depending upon factors such as the

input voltage and frequency, the leakiness of the cable, as well as input and terminal impedance, the magnitude and upstroke of the transmitted potential will vary. If the decay of the potential were large, it might be incapable of depolarizing the subsequent cell in the network, and complete block would ensue. Had it sufficient potential to drive the subsequent cell to threshold, the delay incurred by the impulse would have been wholly determined by the upstroke and magnitude of the transmitted electrotonic potential as viewed by the subsequent cell. This same reasoning might be used to describe the electrical activity of a chain (2 or more) of inactivated cells, which would cause greater decay of the potential, associated increased transmission delay, and a greater propensity for complete block. These studies have convincingly demonstrated that both Na^+ and Ca^{++}, two ions implicated in the slow potential, are unnecessary for the development of the so-called slow potential [34]. Therefore, it would be difficult to call these waveforms anything other than electrotonic.

On the other hand, voltage clamp preparations with cardiac tissue have shown convincing evidence that a delayed, slow, inward Ca^{++} current exists. Its direct relationship to the 'slow potential,' however, has been clouded by the experimental requirements for its development: (1) agents which alter membrane impedance, and/or the ionic currents, I_{Ca}, I_K, I_{Na} (e.g., epinephrine [15, 35], tetra ethyl ammonium substituted Na^+ free solution [12, 14], aminophylline [36], cAMP [36], and caffeine [37]), and (2) large stimulus currents necessary to elicit these slow responses in uniformly depressed preparations. Stimulus intensity frequently has to be increased in order to maintain some form of response [35, 36]. Using physiological levels of stimulation, we have never been able to reproduce a 'slow active response' [38]. Few investigators working with depressed cardiac tissue would deny that great intensities of electrical stimulation are required to elicit these responses. Such currents do not exist in the intact heart, where the best stimulus current available can only be that of an adjacent action potential. In these regards the models described herein represent those most suitable to analyze physiologic conduction and pathophysiologic block for two major reasons. First, the active cells adjacent to a proximal boundary act as the stimulus for the blocked region. This results in the development of an action potential which serves as a 'physiologic' stimulus, much like that which would be present in the intact myocardium. Second, because of the characteristically strong interaction among adjacent cardiac cells, a model which provides conditions for abnormal tissue to be surrounded by, or in juxtaposition to, normal tissue most validly recreates those circumstances of the true pathological condition. It is unreasonable to analyze the response of a single cell without considering all of the activity occurring around it.

Another problem arises with conventional analyses of slow potentials when

one considers the relatively low magnitude of the slow inward Ca^{++} current (1–3 μA). When compared to the rapid, inward Na^{+} current (30–40 μA)[39], there exists an order of magnitude difference. The slow current also demonstrates a delayed time development for the positive current. Both of these characteristics act to decrease the stimulus efficacy possible by this type of response. While such slow potentials may be generated in vitro, their existence in vivo is less likely. Nonetheless, these currents are recordable and their role in abnormal impulse generation and propagation must be further elevated.

Whether it is an electrotonic response or a 'slow, active impulse' that is the primary mechanism for transmission under conditions of conduction delay or block has yet to be determined. Our data would implicate the former. At the very least, we would propose that a more careful examination be made of the participation of passive membrane properties in the development of functional cell-to-cell conduction abnormalities. The role of active membrane properties in the precipitation of arrhythmias seems rather limited. Alteration of passive membrane properties might prove the more reasonable alternative, with many of the arrhythmogenic effects attributed to changes in active mechanisms possibly due to changes in the cells' passive characteristics.

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16. VOLTAGE MODULATION OF AUTOMATICITY IN CARDIAC PURKINJE FIBERS

VICTOR ELHARRAR and DOUGLAS P. ZIPES

The sino atrial pacemaker dominates subsidiary pacemakers through the process of overdrive suppression. Subsidiary pacemakers that demonstrate spontaneous diastolic depolarization are located in specialized atrial and ventricular fibers. Diastolic depolarization is normally absent in ordinary atrial and ventricular fibers, although under certain experimental conditions, considered by Surawicz [1], these fibers may develop diastolic depolarization and spontaneous automaticity. The purpose of this chapter is to review the mechanisms responsible for diastolic depolarization and spontaneous automaticity in cardiac Purkinje fibers, with particular emphasis on the effects that pharmacologic agents exert on automaticity that occur at high and low levels of transmembrane potential.

The mechanisms underlying diastolic depolarization in Purkinje fibers with a maximum diastolic potential of approximately -90 mV have been extensively studied [2]. Briefly, a sarcolemmal channel specific to K^+ ions (pacemaker channel) and carrying an outward current (IK_2) appears to be responsible for the diastolic depolarization. The steady state activation curve of this channel indicates that it is completely inactivated at potentials negative to -90 mV and completely activated at potentials positive to -60 mV. The dynamic characteristics of the pacemaker channel are voltage dependent such that its rate of opening (or closing) is faster at less negative potentials. Because of this dynamic property, the pacemaker channel is quickly and fully activated early during the plateau of the action potential, and remains so when the cell has repolarized to its maximum diastolic potential (approximately -90 mV). At this membrane potential, the pacemaker channel inactivates at a slow rate with a time constant of approximately 1.5 sec [3], and carries progressively less outward current as it inactivates. In the presence of a background inward current, the gradual decay of the outward current results in an increasing net inward current and diastolic depolarization. As diastolic depolarization proceeds, the degree of opening of the pacemaker channel

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decreases but remains greater than the steady state value at that potential. Concurrent with the voltage change, the steady state degree of activation of the pacemaker channel increases. When the degree of opening of the pacemaker channel equals the steady state, no further change occurs and the membrane potential stabilizes. If, during this process, the transmembrane potential reaches the voltage threshold for all-or-none depolarization, then a rapid upstroke ensues.

Since the rate of deactivation of the pacemaker channel is faster at lower membrane potentials, it follows that the slope of the diastolic depolarization and the rate of discharge should be greater when the maximum diastolic potential is reduced. Furthermore, spontaneous activity arising from inactivation of the IK_2 current should be restricted to potentials negative to -60 mV. Another feature of this mechanism is that the slope of the diastolic depolarization depends also on the magnitude of the background inward current which is, at least in part, carried by Na^+ ions. Vassalle [4] has shown that a decrease in the external Na^+ concentration leads to a decrease of the rate of discharge of Purkinje fibers, presumably by reducing the background inward current. Conversely, an increase in the external Na^+ concentration accelerates the rate of discharge.

Except for the sinus node, spontaneous activity arising from potentials positive to -60 mV is considered abnormal. Such automaticity cannot be due to inactivation of the IK_2 current. The apparent abnormality may simply be due to the fact that the cell is unable to reach and maintain its normal resting potential, and not necessarily due to the fact that it generates automatic activity when the membrane is maintained at potentials positive to -60 mV. For the purpose of this presentation, we will first consider the features of the depolarized Purkinje fibers and then those of the automatic activity they generate.

1. THE LOW LEVEL OF TRANSMEMBRANE POTENTIAL

Various authors [5–8] have shown that two stable levels of transmembrane potential may exist in Purkinje fibers. Under these circumstances, the fiber generates a rapid upstroke and comes to a halt after a slight repolarization. The transmembrane potential remains at a steady value ranging between -50 and -20 mV due to the failure of repolarization to proceed. At temperatures ranging between 15 and 19 °C, a slight temperature change can act as a means to shift the transmembrane potential from one level to the other [5]. The transition between the high and low level of transmembrane potential is always rather sudden and occurs following a rapid depolarization. For this reason, fibers that are not spontaneously active are not susceptible to arrest at

the low level of potential. Likewise, fibers treated with tetrodotoxin (TTX) or exposed to low $[Na^+]_0$ superfusate will not arrest at the low level of potential, when superfused at low temperatures.

Most of the experimental models resulting in a low level of transmembrane potential are based on alterations of the ionic milieu of otherwise normal fibers. While similarities are present between these models, substantial differences may also exist. It is well established that at $[K^+]_0$ below 10 mM the transmembrane potential of Purkinje fibers deviates from values predicted by the Goldman equation, assuming a constant ratio of membrane permeability to Na^+ and K^+ ions. At $[K^+]_0$ below 2.7 mM, the transmembrane potential may assume either of two levels; one level is relatively close to that predicted by the Goldman relation, whereas the second departs quite markedly in the depolarizing direction and ranges between -40 and -30 mV. The transition between the two levels is triggered by an upstroke followed by a partial repolarization [6–8]. Occasionally, one may observe such behavior in Purkinje fibers bathed in a normal Tyrode's solution ($[K^+]_0$ between 2.7 and 5.4 mM), usually soon after the dissection procedure. A sudden rapid depolarization may shift the transmembrane potential from the high level to the low level. An equally sudden repolarization may bring the membrane potential back to the high level.

Once at the low level, the effects of manipulations of the ionic milieu are of interest because they reveal the possible mechanisms underlying the depolarization. Lee and Fozzard [8] have performed experiments to determine the relative permeabilities of Purkinje fibers brought to the low level of potential by reduction of $[K^+]_0$ to 1 mM. A tenfold reduction of $[Na^+]_0$ from 153 to 15.9 mM hyperpolarized the membrane from -34 to -63 mV. A similar change in $[Na^+]_0$ in the presence of 5 mM of $[K^+]_0$ hyperpolarized the membrane from -81 to -87 mM. This indicates that the relative membrane permeability to Na^+ ions is much greater at the low level of membrane potential. In contrast, it was found that the low level of membrane potential was insensitive to changes in $[Ca^+]_0$ (from .18 to 9 mM) and changes in $[Cl^-]_0$ (from 146 to 73 mM) indicating that the relative membrane permeability to these ions is quite small.

The effects of chloride substitution were studied in sheep Purkinje fibers superfused with K^+-free solutions [6]. Under these conditions, the resting membrane potential was stable at -40 mV. When chloride ions were replaced by acetylglycinate (a less permeable anion) the membrane potential tended to depolarize further. Substitution by nitrate, a more permeable anion, caused hyperpolarization by more than 20 mV. It was concluded that the chloride ions become important electric charge carriers when the membrane is depolarized, in contrast to the findings of Lee and Fozzard [8].

Gadsby and Cranefield [7] determined the critical range of $[K^+]_0$ over which

the membrane may be at the low level. Within that range (1–4 mM), the membrane potential was relatively insensitive to changes in $[K^+]_0$, indicating a decrease in the membrane permeability to K^+. Consistent with this finding was the decreased membrane slope conductance observed at the low level of membrane potential [5, 9].

There is reason to suspect that Ca^{++} ions may carry part of the inward current at the low level of membrane potential. Imcomplete inactivation of the slow Ca^{++} channel at potentials positive to -60 mV has been suggested by various authors [10–12]. On this basis, it was expected that reduction or substitution of Ca^{++} ions would result in hyperpolarization and that an increase in $[Ca^{++}]_0$ would result in further depolarization. The experimental results have indicated that the low membrane potential is insensitive to variations of $[Ca^{++}]_0$ [8, 9]. However, in some experiments [9] an increase in $[Ca^{++}]_0$ precipitated the transition from the low to the high level of transmembrane potential. It was postulated that the increase in $[Ca^{++}]_0$ led to an increase in intracellular Ca^{++} concentration, which in turn increased the membrane K^+ permeability and the outward repolarizing current [13, 14].

In summary, at the low level of membrane potential the membrane slope conductance is decreased. The membrane permeability to Na^+ ions is increased and that to K^+ ions is decreased. The membrane permeability to Ca^{++} ions is probably increased, whereas that to Cl^- ions is still unsettled. Both the inward and outward components of the net current are small. Therefore, small changes in the membrane permeabilities may result in disproportionate changes of the transmembrane potential.

Of interest are those pharmacologic agents that can induce a maintained shift of the transmembrane potential from the low to the high level. In this respect, there is a paucity of information regarding the effects of pharmacologic agents on the low level of membrane potential. The effects of acetylcholine have been studied by Gadsby et al. [15]. They showed that acetylcholine, at a concentration of 10^{-6} M had a significantly greater hyperpolarizing effect at the lower than at the higher level of membrane potential. Concentrations of 10^{-5} M often produced a maintained shift from the lower to the higher potential.

Arnsdorf [16, 17] studied the effects of lidocaine, procainamide and tolamolol on a series of short Purkinje fibers bathed in physiologic Tyrode's solution. These fibers were selected because they demonstrated two stable levels of transmembrane potential. Procainamide (5 μg/ml) and tolamolol (0.1 μg/ml) failed to restore the transmembrane potential to the high level. In contrast lidocaine (5 μg/ml) was able to restore the membrane potential to the high level. The low level of transmembrane potential could not be sustained after exposure to lidocaine.

The existence of two levels of transmembrane potential is related to the

steady state current–voltage relationship. This relationship is N shaped with a region of negative slope conductance between approximately −60 and −40 mV [18]. The number and position of intercepts of this relationship with the zero current axis can vary from 1 to 3, as shown in Figure 1A–E. Voltages at which the steady state current–voltage relationship cross the zero current axis represent stable transmembrane potentials since no net current flows through the membrane at these voltages. In cases A and E, only one stable transmembrane potential is possible, either at the low (A) or at the high level (E). Two stable levels of transmembrane potential are possible in cases B and D, whereas in C three stable levels can potentially exist. Reduction of $[K^+]_0$ in Purkinje fibers results in a downward shift of the curve (from E to A) in addition to a shift of the curve toward the left (not shown in Figure 1)[19]. Within a certain range of $[K^+]_0$, the steady state current–voltage relationship is of the type depicted in B–D, at which more than one stable level of transmembrane potential is possible.

An upward shift of the steady state current–voltage relationship (by increasing the outward current component or decreasing the inward current component) is a possible mechanism to restore the high level of transmembrane potential (from A to E). In cases B–D, it is also possible to induce a shift between the stable levels of transmembrane potential without a vertical shift

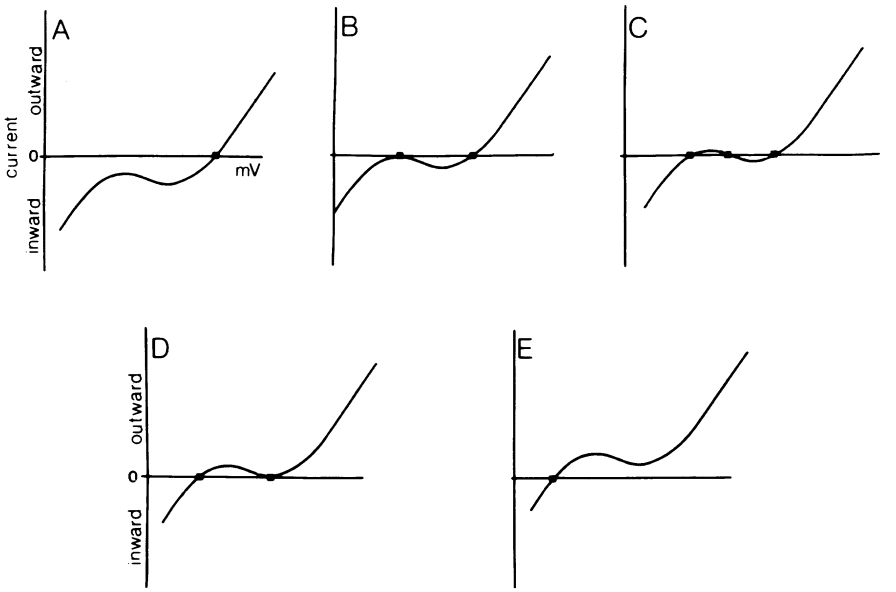

Figure 1. Postulated changes in the steady state current-voltage relationship to account for the existence of one or more stable transmembrane potentials. A progressive upward shift of the curve produced the transition from panels A to E.

of the steady state current–voltage relationship. Transient disturbances of the membrane currents by injection of depolarizing or hyperpolarizing current pulses are commonly used to induce such a shift in the transmembrane potential. Another possibility includes loss of inward going rectification responsible for the N shape of the steady state current–voltage relationship.

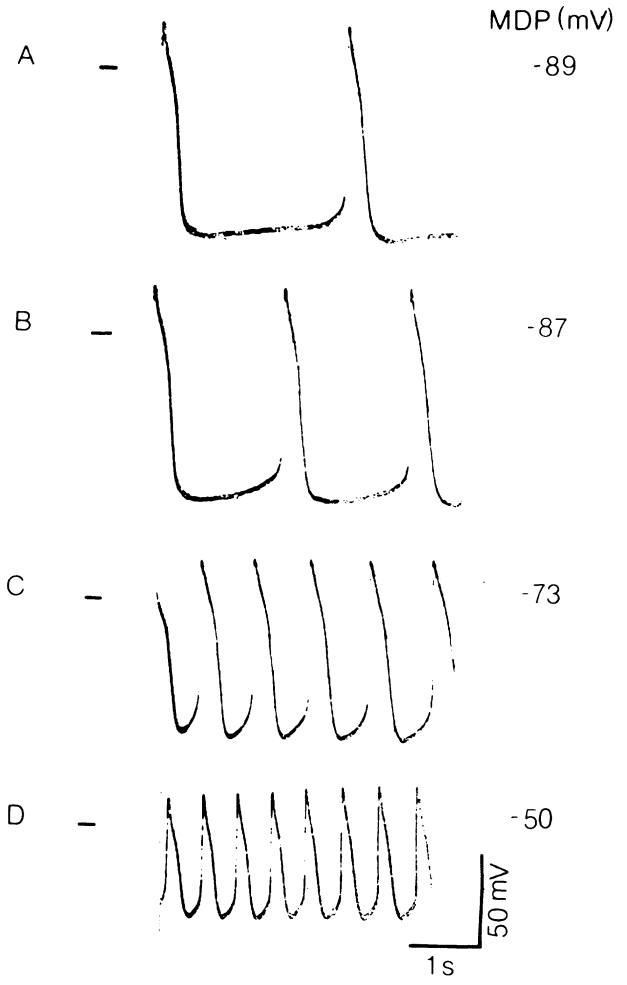

Figure 2. Intracellular recordings from a canine cardiac Purkinje fiber discharging automatically. The maximum diastolic potential (MDP) was −87 mV in the absence of current injection (B). A hyperpolarizing current was applied in A and depolarizing currents in C and D to modify the maximum diastolic potential.

2. AUTOMATICITY

2.1. Modulation by current injection

Figure 2A–D illustrates alterations in spontaneous rate of discharge produced by current injection in a canine cardiac Purkinje fiber. In the absence of current injection (B), the fiber had a maximum diastolic potential of −87 mV and discharged spontaneously at cycle length of 2100 msec. A hyperpolarizing current pulse (A) reduced the rate of discharge while depolarizing current pulses (C–D) increased the slope of the diastolic depolarization and the rate of discharge. The intensity of the applied current determined the magnitude of change in the spontaneous activity.

In this experiment, current was injected using the single sucrose gap technique. Despite certain limitations, this method constitutes a relatively simple means for graded alteration of the transmembrane potential (Figure 3). The

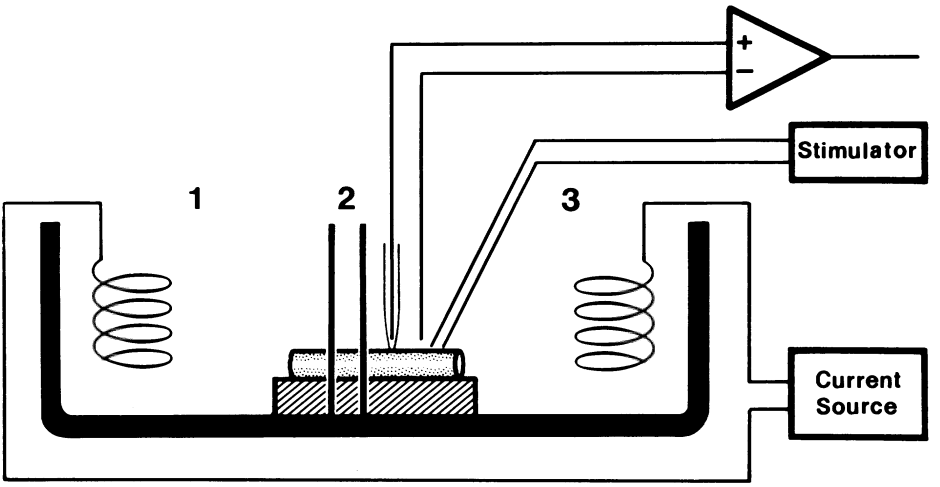

Figure 3. The single sucrose gap arrangement. The three chambered bath consists of a middle chamber (1 mm long) separated from the side chambers (1 and 3) by thin rubber membranes. A cylindrically shaped Purkinje fiber (5–7 mm long) is passed through a small hole in the rubber membranes. Chambers 1 and 3 are superfused with normal Tyrode's solution whereas chamber 2 is superfused with sucrose (300 mM) dissolved in distilled water. The later solution is electrically non-conductive. The electrical activity is recorded from the middle of the test chamber (chamber 3) using standard microelectrodes. The fiber can be regularly stimulated through external bipolar stimulating electrodes. The current is injected from a constant current source between chambers 1 and 3. In chamber 2, the current is forced across the cellular membrane because the electrical conductivity of the extracellular milieu (sucrose) is much lower than that of the membranes. Hyperpolarization or depolarization of the fiber is controlled by the direction of the current. The degree of polarization is controlled by the current intensity. At the surface of the fiber, within chamber 2, there is a layer of conductive fluid obtained by diffusion of ions from the intracellular milieu to the sucrose solution. Therefore, all the current injected will not flow intracellulary; a certain fraction will still flow within the extracellular compartment. This leak limits the value of the current–voltage relationship obtained using the sucrose gap method.

principal limitation of this technique relates to the lack of homogeneity of the transmembrane potential along the fiber during current injection. The degree of inhomogeneity can be reduced in Purkinje fibers whose lengths are short relative to their length constant. In the present experiments, Purkinje fibers less than 7 mm long (3–4 times the length constant) were used, and approximately 2 mm were exposed in the test compartment. A second limitation of the method exists. The fraction of current which flows intracellularly cannot be measured accurately, thus limiting the value of the current–voltage relationship obtained using this method. For this reason, we will consider the maximum diastolic potential, rather than the current intensity, as the independent variable.

Figure 4 relates the cycle length of discharge to the maximum diastolic potential. This relation is continuous and non-linear. It extends from −90 to −20 mV in this particular fiber, and its slope is markedly decreased at

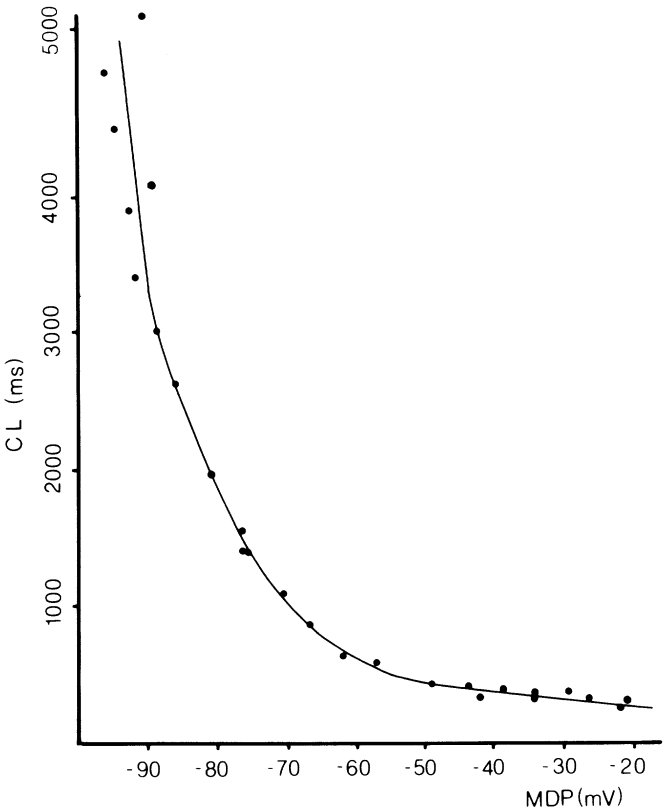

Figure 4. Relation between the cycle length of spontaneous discharge (ordinate) and the maximum diastolic potential (abcissa) in a representative canine cardiac Purkinje fiber. The maximum diastolic potential is controlled by current injection through a sucrose gap.

potentials positive to approximately -65 mV. At $[K^+]_0$ of 2.7 mM, automaticity was present and persisted to a maximum diastolic potential of -18 ± 2.1 mV (mean $\pm$ SEM, $n = 18$). In the potential range of -85 to -70 mV, the slope of the relation was -148 ± 6.7 msec/mV. The slope was reduced to -3.7 ± 1.2 msec/mV (mean $\pm$ SEM, $n = 18$) in the potential range of -50 to -20 mV. At maximum diastolic potentials positive to approximately -20 mV, automaticity was either absent or non-sustained.

The relationship shown in Figure 4 does not reveal, or suggest the need to incriminate, two mechanisms responsible for automaticity. The existence of two mechanisms can be demonstrated, however, by the differential effect of pharmacologic agents.

2.2. Effects of pharmacologic agents

Figure 5A–D illustrates in a representative experiment the effects of lidocaine (3 mg/liter) and of verapamil (3×10^{-6} M) on a canine cardiac Purkinje fiber. Under control conditions (A), the maximum diastolic potential was -86 mV and the fiber discharged spontaneously at a cycle length of 2900 msec. When current injection reduced the maximum diastolic potential to -50 to -55 mV, the discharge cycle length decreased to 1200 msec. After 15 minutes of superfusion with lidocaine (B), the spontaneous discharge cycle length arising from the high level of transmembrane potential lengthened to

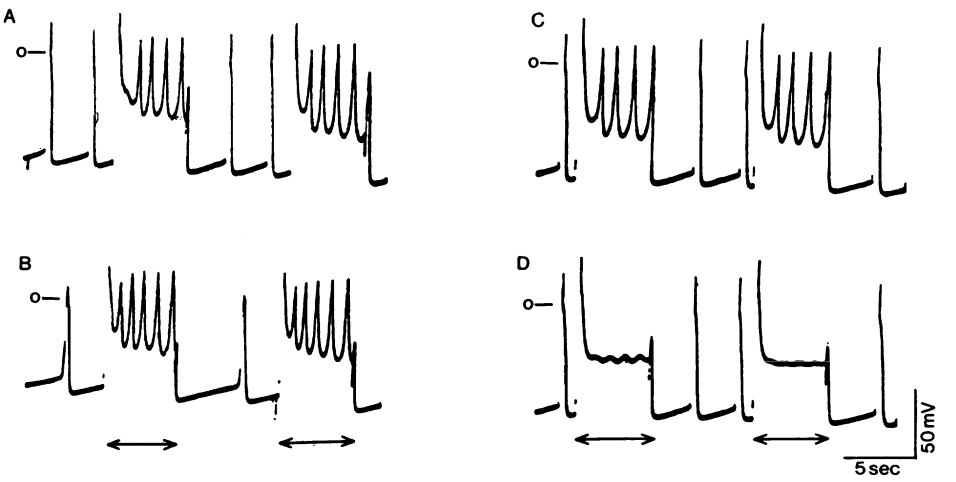

Figure 5. Differential effects of lidocaine (3 mg/liter) and of verapamil (3×10^{-6} M) on automaticity at the high and low levels of transmembrane potential. (A): Control. (B): Under superfusion with lidocaine. (C): Return to control after 30 minutes of wash out. (D): Under superfusion with verapamil. Current is injected between the arrowheads to depolarize the fiber between -50 and -55 mV.

5200 msec, whereas that arising from the low level of transmembrane potential was not altered. Panel C was obtained 30 minutes after washout of lidocaine and represents return to the control conditions. Superfusion with verapamil did not alter the cycle length of discharge at the high level of transmembrane potential but suppressed automaticity at the low level (panel D). Similar observations were obtained in 8 fibers using lidocaine, and in 6 fibers using verapamil. Effects analogous to those of verapamil were observed in 6 fibers when Mn^{++} ions (2×10^{-3} M) were added to the Tyrode's solution.

Figure 6 illustrates the effects of lidocaine and verapamil on the relationship between the cycle length of spontaneous discharge and the maximum diastolic potential in another fiber. Lidocaine did not affect the cycle length of discharge at potentials positive to -60 mV but increased it at potentials negative to -70 mV (Figure 6, left). Conversely, verapamil (right) suppressed the automaticity at potentials positive to -60 mV, and did not alter automaticity arising at potentials negative to -70 mV.

These observations suggest the existence of two different mechanisms that are responsible for spontaneous automaticity occurring in different ranges of transmembrane potential. The first mechanism, probably based on deactiva-

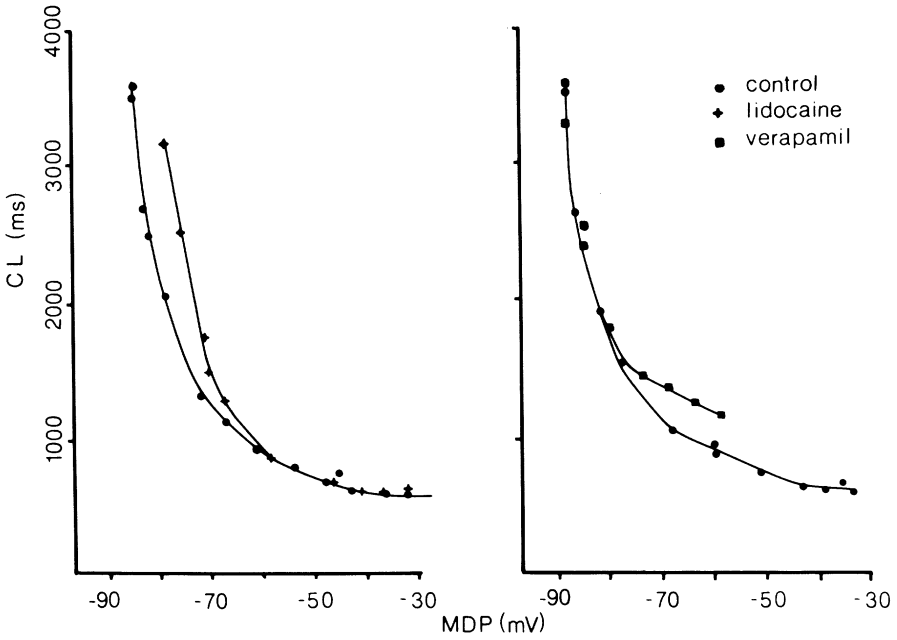

Figure 6. Effects of lidocaine (3 mg/liter) and of verapamil (3×10^{-6} M) on the relationship between the cycle length of spontaneous discharge (ordinate) and the maximum diastolic potential (abcissa).

tion of the IK_2 current, functions at potentials negative to -60 to -70 mV and is sensitive to lidocaine. The second mechanism operates at potentials positive to -60 to -70 mV and is suppressed by agents that block the slow channel. The transition between these two mechanisms is gradual and occurs between -70 and -60 mV. An alternative explanation is that slow channel blockers affected the ability of the cell to generate an action potential rather than the mechanism of diastolic depolarization at the low level of transmembrane potential. The features of the action potential arising from the low level of transmembrane potential in these experiments are similar to those found in 'slow response' potentials which have been repeatedly shown to be suppressed by verapamil and Mn^{++} ions [20–22]. However, prior to suppression of automaticity at the low level of transmembrane potential, we observed in

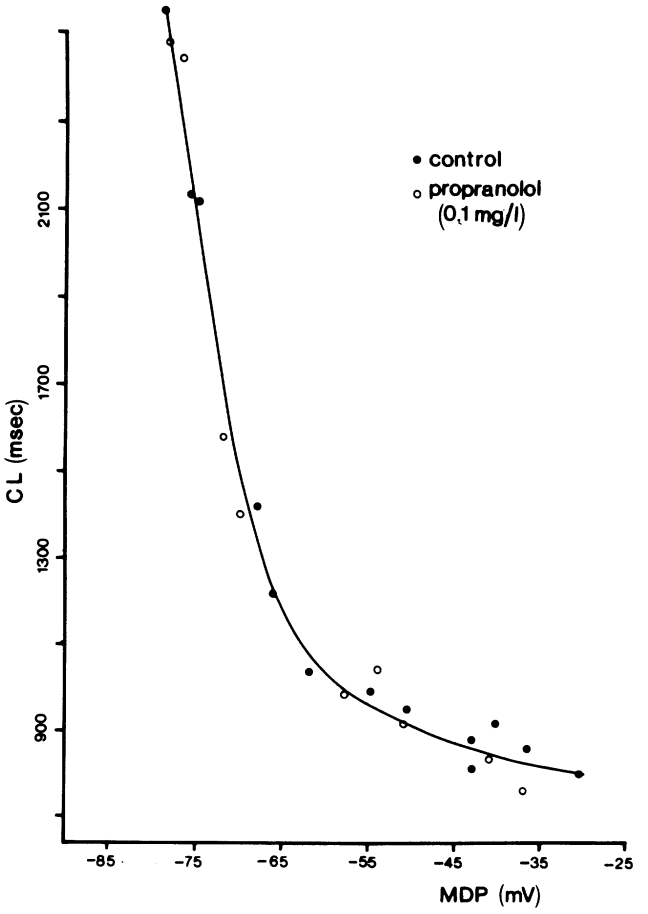

Figure 7. Lack of effects of propranolol on the relationship between the spontaneous discharge cycle length (ordinate) and the maximum diastolic potential (abcissa).

all experiments a gradual prolongation of the cycle length, indicating that the mechanisms responsible for diastolic depolarization were also altered by blockers of the slow channel.

We studied the effects of propranolol (0.1 mg/liter) in three Purkinje fibers. At this concentration, propranolol has no direct negative inotropic effect on Purkinje fibers and has no observable effects on the characteristics of the transmembrane action potential. This concentration, however, suppresses the positive inotropic and the electrophysiologic effects produced by isoproterenol at a concentration of 10^{-7} M (unpublished observations). In all three fibers studied, propranolol had no effect on the relation between the maximum diastolic potential and the cycle length of spontaneous discharge. A representative experiment is shown in Figure 7.

2.3. Effects of ionic manipulations

Potassium: At both ranges of transmembrane potential, an increase in the external K^+ concentration lengthened the spontaneous discharge cycle length. Figure 8 illustrates the effects of increasing $[K^+]_0$ from 4 to 8 and 12 mM on the relationship between the discharge cycle length and the maximum diastolic potential. In the absence of current injection, the resting transmembrane potential was -82 mV at $[K^+]_0$ of 4 mM, -70 mV at $[K^+]_0$ of 8 mM and -63 mV at $[K^+]_0$ of 12 mM. The fiber discharged spontaneously at a cycle length of 2080 msec when superfused with $[K^+]_0$ of 4 mM, and was quiescent at higher $[K^+]_0$. Increasing $[K^+]_0$ shifted upward and to the right the relationship obtained during current injection. The threshold potential at which automaticity could be induced when current was injected was increased to -43 mV at $[K^+]_i$ of 8 mM and to -31 mV at $[K^+]_0$ of 12 mM. These threshold values were substantially positive to the resting potential of the fiber, at the respective $[K^+]_0$. Similar findings were obtained in a total of 5 Purkinje fibers.

These observations may help understand events produced by myocardial ischemia. It has been postulated that cellular depolarization occurs during myocardial ischemia due to release of intracellular potassium. Hill and Gettes [23] have reported extracellular K^+ concentrations exceeding 8–10 mM within the ischemic zone following occlusion of a coronary artery in the pig. The depolarization, coupled with an increase in the release of catecholamines from adrenergic nerve terminals may lead to 'slow channel' dependent action potentials and possibly to enhanced spontaneous automaticity. Under conditions believed to prevail within the ischemic myocardium, the high K^+ concentration in the extracellular space would, in fact, suppress automaticity.

Similar effects of $[K^+]_0$ were also reported in ordinary atrial and ventricular

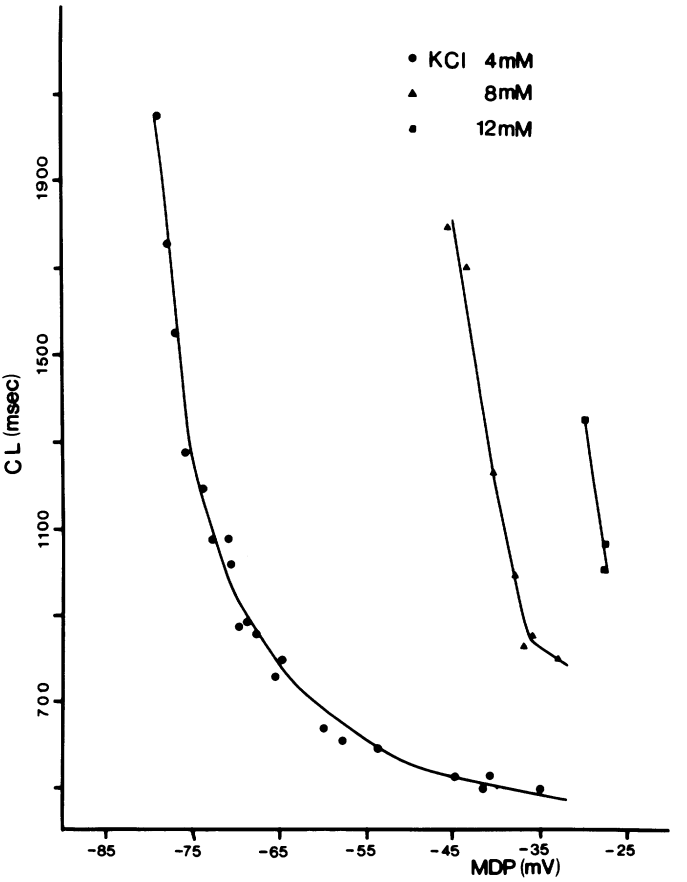

Figure 8 Effects of extracellular potassium concentration on the relation between the spontaneous discharge cycle length (ordinate) and the maximum diastolic potential (abcissa).

fibers (for review see [1]). This also might explain the absence of automatic properties in K^+-depolarized, isoproterenol-treated Purkinje fibers.

Calcium: Figure 9 illustrates the effects of changes in $[Ca^{++}]_0$ from 2.7 to 0.67 mM (left panel) and from 2.7 to 10.8 mM (right panel). A cross over of the curves was observed when $[Ca^{++}]_0$ was decreased due to opposite effects on the discharge cycle length at high and low transmembrane potentials. The discharge cycle length was decreased at potentials negative to -70 mV and increased at potentials positive to this value. However, when $[Ca^{++}]_0$ was increased to 10.8 mM, the discharge cycle length was increased at all transmembrane potentials. Thus at the low level of transmembrane potential, change of $[Ca^{++}]_0$ below and above 2.7 mM led to an increase in the discharge cycle length (Figure 10).

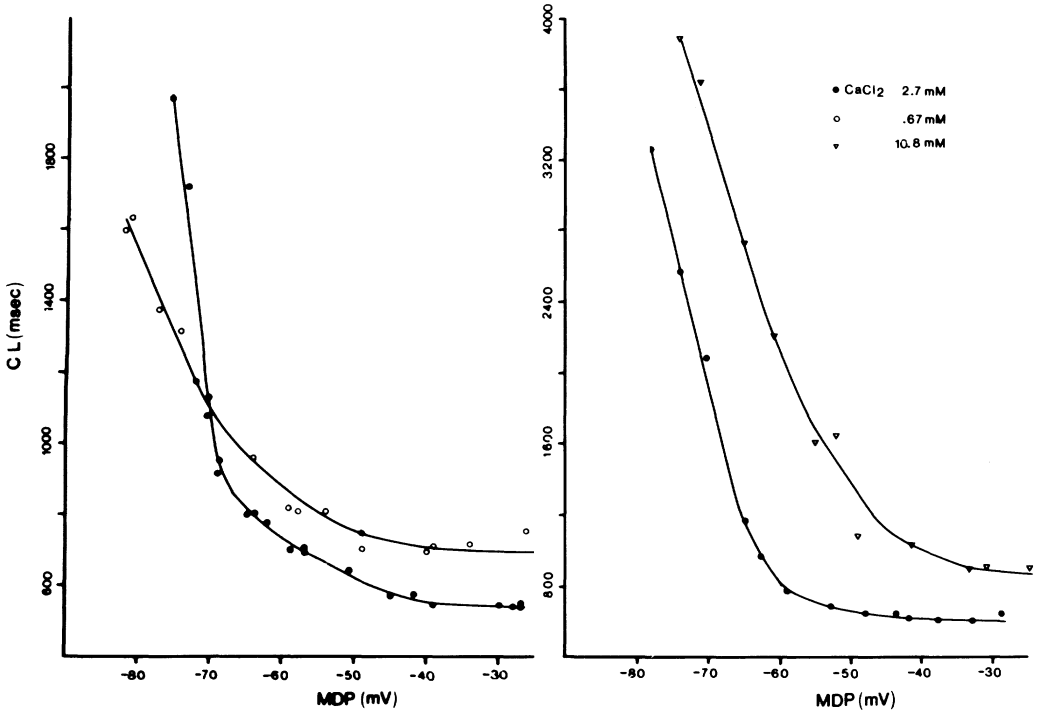

Figure 9. Effect of extracellular calcium concentration on the relation between the spontaneous discharge cycle length (ordinate) and the maximum diastolic potential (abcissa).

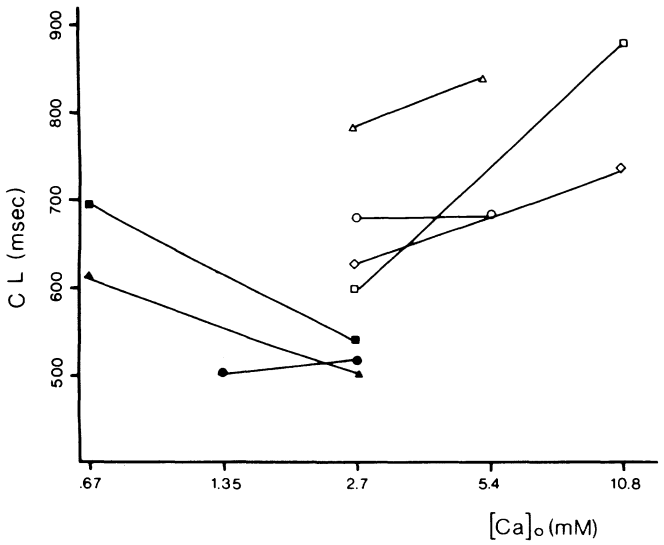

Figure 10. Relation between the spontaneous discharge cycle length at the low level of transmembrane potential (ordinate) and the external calcium concentration (abcissa).

Using a single sucrose sap method, Imanishi [24] studied the effects of Ca^{++} ions on Purkinje fibers of puppies. He observed that an increase in $[Ca^{++}]_0$ from 1.8 to 7.2 mM enhanced the diastolic slow depolarization at the low level of transmembrane potential, and that higher concentrations had a variable effect. These observations were made in Na^+-free solutions. Biphasic effects of Ca^{++} ions on the rate of discharge were also reported in the sinus node by various authors [25, 26]. In the sinus node, the optimal concentration of Ca^{++} ions at which the frequency of firing is maximum ranges between 1.8 and 3.6 mM [26], close to the value we found.

In many respects, the features and properties of spontaneous automaticity at the low level of transmembrane potential in cardiac Purkinje fibers are similar to those reported in ordinary myocardial fibers and in sino atrial fibers. The similarity appears to hold also for the mechanisms underlying the diastolic depolarization at the low level of transmembrane potential.

2.4. Mechanisms

Hauswirth et al. [27] studied the oscillatory activity of short Purkinje fibers that failed to recover their normal high resting potential. The voltage clamp technique was used to determine the membrane currents responsible for diastolic depolarization at this low level of transmembrane potential. It appeared that the oscillatory activity was generated by changes in the same outward current, IX_1, that is responsible for the phase 3 repolarization in more normal fibers. When the inward component of the membrane current is high, the net ionic current becomes zero, and then inward at a less negative transmembrane potential. The IX_1 current deactivates slowly at these low levels of transmembrane potentials, and constitutes the decaying outward current component during diastole.

This mechanism is valid as long as the transmembrane potential does not reach values negative to the reversal potential for IX_1. The reversal potential for IX_1 was found to be approximately -70 mV [28], close to the potential at which the transition between the two mechanisms of automaticity occurs. In addition, this mechanism requires a higher than normal inward current component. In our experiments, this current is provided by the current pulse. This mechanism of diastolic depolarization appears to apply also to ordinary myocardial fibers and to the sinus node [29, 30]. It is analogous to the initial mechanism postulated by Noble [31] to account for the long duration and the diastolic depolarization in cardiac Purkinje fibers.

3. CONCLUSIONS

Spontaneous activity arising from the low level of transmembrane potential does not appear to be due to abnormal mechanisms. It is a property of otherwise normal cells when the inward current component is increased. It is not known if, or under which conditions, such cellular behavior occurs in vivo. In vivo, virtually all injuries of the myocardium result in some degree of cellular depolarization. Depending on the degree and location of the injury, cardiac arrhythmias due to disorders of impulse propagation and/or formation may result. The observations on spontaneous automaticity in canine Purkinje fibers that we report in this chapter are potentially important clinically, since they suggest two mechanisms that can be responsible for the genesis of cardiac arrhythmias. If the second mechanism of automaticity occurs in man, it can produce ventricular arrhythmias that may not respond to cardioactive drugs that affect the IK_2 current; in this situation, slow channel blockers may be effective. The clinical situations where such automaticity plays a role have yet to be defined.

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17. DEPOLARIZATION-INDUCED AUTOMATICITY IN ATRIAL AND VENTRICULAR MYOCARDIAL FIBERS

BORYS SURAWICZ

1. INTRODUCTION

It is well known that automatic activity of cardiac pacemaker fibers can be suppressed by various interventions that increase K^+ conductance (g_K), e.g., administration of acetylcholine, raising extracellular potassium $(K^+)_0$ concentration, or overdrive suppression. Such interventions effectively transform the automatic pacemaker fibers into quiescent fibers devoid of phase 4 diastolic depolarization. Perhaps less well known is a reverse process where normal nonpacemaker atrial or ventricular myocardial fibers acquire phase 4 depolarization and become transformed into spontaneously firing pacemaker fibers. Such induction of automaticity is usually associated with depolarization and decrease in g_K. Sperelakis concluded from his studies of embryonic heart cells that 'all nonpacemaker myocardial cells in culture are capable of being converted into pacemaker cells by decrease in g_K and all pacemaker cells can be converted into nonpacemaker cells by increasing g_K' [1]. Cranefield reviewed the procedures which various investigators have used to change the myocardial nonpacemaker fibers into pacemaker fibers [2]. The list of these procedures includes: (1) decrease in $(K^+)_0$ alone, or together with decrease in $(Ca^{++})_0$, (2) application of barium salts, (3) application of aconitine, (4) stretch, and (5) passage of electrical currents. The purpose of this chapter is to review the above experimental methods, to discuss the underlying mechanisms, to describe some of the pertinent drug effects, and to comment on the possible clinical implications of automatic activity in depolarized myocardium.

2. EXPERIMENTAL METHODS

2.1. Low $(K^+)_0$

In 1961, Antoni induced automatic activity in guinea pig atrial strips by lowering $(K^+)_0$, with addition of acetylcholine, and then driving the preparation at a rapid rate [3]. The author reported that automaticity appeared after driving was stopped but he did not comment on the possible mechanism of

D.P. Zipes, J.C. Bailey, V. Elharrar (eds.), The Slow Inward Current and Cardiac Arrhythmias, 375-396.

this phenomenon [3]. In 1962, Gettes et al. [4] published records of ventricular action potentials from isolated rabbit hearts perfused with low $(K^+)_0$ solution. These records show a smooth continuum from the diastolic depolarization toward the upstroke of the subsequent ventricular action potential (Figure 1).

In 1965, Müller [5] elicited spontaneous automatic activity in calf and sheep ventricular trabeculae superfused with $(K^+)_0$ and $(Ca^{++})_0$-free solution [5]. He explained this phenomenon by a decrease in g_K due to low $(K^+)_0$ and an increase in g_{Na} due to calcium deficiency [5]. Also, Carmeliet used low $(K^+)_0$ perfusion to transform nonpacemaker fibers of guinea pig left atrial myocardium into pacemaker fibers [6]. He first depolarized these fibers to -40 mV or less by application of K^+-free solution for 4 h and then reintroduced Tyrode's solution containing 2.7 mM $(K^+)_0$. When the membrane potential (MP) increased to about -60 mV, pacemaker activity appeared, and persisted for hours if the K^+ concentration was kept sufficiently low [6].

In discussing possible mechanisms, all investigators postulate that low $(K^+)_0$ decreases g_K and causes depolarization which permits sustained automatic activity in the presence of a sufficient background inward current. Judging from the reported levels of depolarization, the inward current is

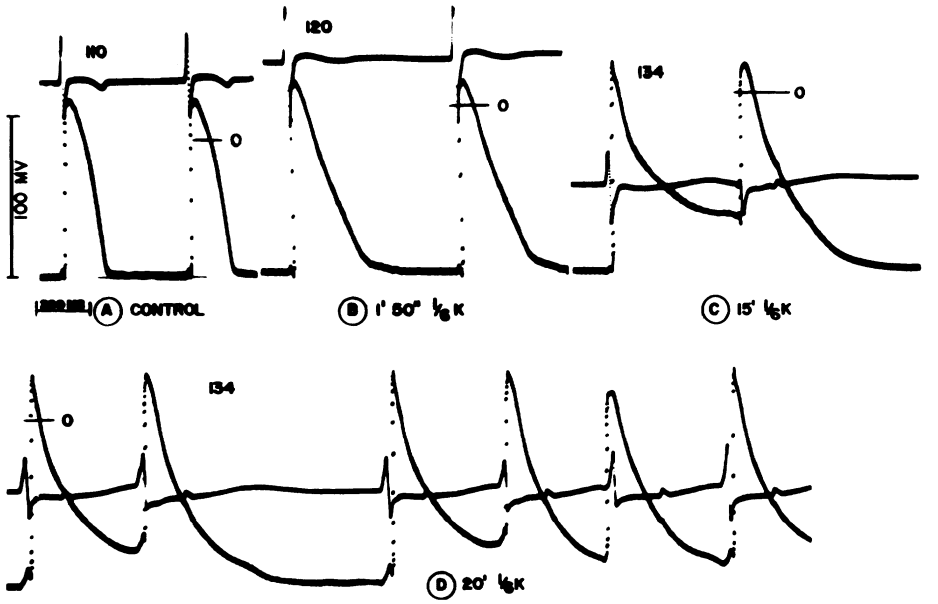

Figure 1. Ventricular action potentials and ECG before (A) and during (B–D) perfusion with low K^+ solution in an isolated rabbit heart. Note prolongation of action potential and slow, smooth transition from repolarization into depolarization of the next action potential in the second and fourth complex in D (from ref. [4], by permission).

probably supplied by the incompletely inactivated rapid inward sodium system.

2.2. *Application of barium salts*

Antoni and Oberdisse studied barium-induced automaticity in atrial and ventricular fibers of rabbits and guinea pigs [7]. They found that the atria were less sensitive to barium than the ventricles [7]. In the ventricles automaticity occurred after application of 2–4 mM barium while in the atria it appeared later and required a higher barium concentration, i.e., 8 mM (Figure 2). In the experiment shown in Figure 2 automaticity occurred in myocardium depolarized to a potential less negative than −60 mV. This level of maximum diastolic potential and the slow upstroke of the action potential in this figure suggest that the depolarization is probably slow-channel dependent. In these

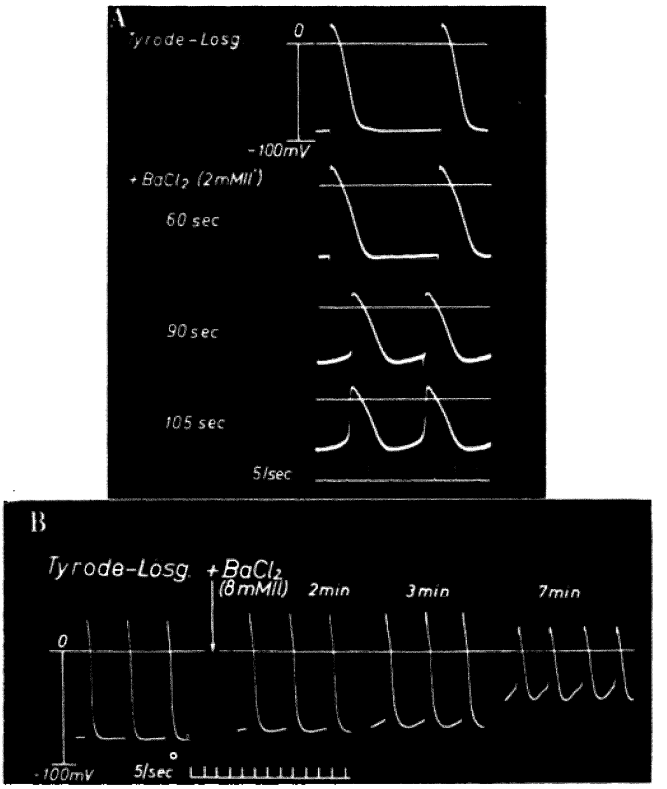

Figure 2. Transformation of a nonautomatic ventricular fiber from rabbit papillary muscle (A) and rabbit atrial trabeculum (B) into an automatic pacemaker fiber after application of 2 mM barium in A and 8 mM barium in B. In each panel single cell impalement. See text (from ref. [7], by permission).

experiments, automatic activity was suppressed by acetylcholine, cooling, increasing $(K^+)_0$ to 23 mM, or increasing $(Ca^{++})_0$ to 18 mM. The activity was enhanced by adrenaline and warming. The authors attributed automaticity to a combination of a decrease in g_K, and an increase in g_{Na}. The latter was attributed to the removal of Ca^{++} by Ba^{++}. Reid and Hecht induced automaticity in dog ventricular myocardium by application of 0.75–5 mM barium which depolarized the fibers to about -60 mV [8]. They showed that the membrane slope resistance increased during diastolic depolarization, and attributed this effect to a decrease in g_K [8]. Similar effect of 5–10 mM barium on membrane input resistance was found in depolarized embryonic chick cells [9].

Toda studied automaticity in the rabbit left atrial fibers depolarized by barium (1–4 mM) to the level of about -50 mV, and reported that automatic activity was suppressed by increasing $(K^+)_0$, $(Ca^{++})_0$, or $(Mg^{++})_0$ concentrations. The author commented on the possible role of calcium current in the genesis of barium-induced automatic depolarizations [10]. Mascher studied the effects of barium (0.5–4.0 mM) in cat papillary muscle which developed pacemaker activity following depolarization to a potential less negative than -60 mV [11]. The author attributed automaticity to the slow depolarizing mechanism unmasked by partial or complete inactivation of rapid inward sodium current [11]. This interpretation is consistent with the results of other reported studies although the exact mechanism by which barium causes depolarization is not entirely clear.

2.3. Aconitine

Topical application of aconitine to myocardium is known to produce rapid ectopic depolarizations. Heistracher and Pillat have shown that 10^{-7} gm/liter aconitine in tissue bath caused slow diastolic depolarization and pacemaker activity in cat papillary muscle [12]. The authors did not comment on the possible mechanism but reported that the automatic activity was not suppressed by quinidine [12].

2.4. Stretch

Kaufmann and Theophile [13] found that stretch transformed normal atrial and ventricular monkey fibers into pacemaker fibers (Figure 3). The critical stretching force was about 3.8 gm, and the extension of the stretched preparation averaged $141 \pm 15\%$ [13]. Less stretch was needed to induce automaticity in thin than in thick muscle bundles. The process was reversible. The automaticity was associated with depolarization, but Figure 3 shows that the maximum diastolic potential was more negative than the assumed threshold

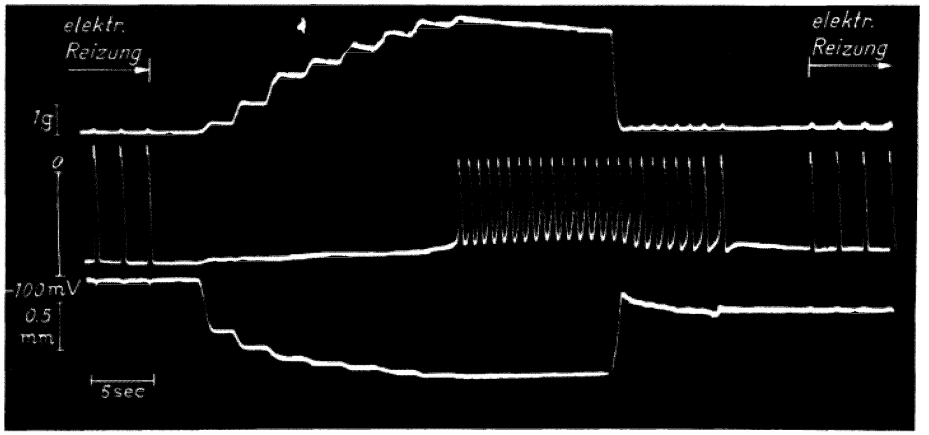

Figure 3. Transformation of a nonautomatic ventricular fiber from monkey papillary muscle into automatic pacemaker by stretch. From top to bottom: stretching force in grams (g), action potential, and change in length. See text (from ref. [13], by permission).

for the inward current passing through the slow channel. The stretch-induced automaticity may be due to a mechanism different from those discussed previously because stretch did not increase but decreased the membrane input resistance, presumably due to increase in g_{Na} [13].

2.5. *Application of electrical currents* [14–26]

Brown and S. Noble observed pacemaker activity in naturally quiescent fibers from atrial trabeculae following application of depolarizing current pulses of several seconds duration across the sucrose gap [14]. The automatic frequency was maximum at about -50 mV, and the inward membrane current involved in this process was tetradotoxin-insensitive [14].

Antoni and coworkers published the earliest reports of automatic activity in the mammalian ventricular myocardium [15–18]. In the early experiments they applied hyperpolarizing 10–30 mA current pulses of 1–30 sec duration in monkey papillary muscles mounted in a sucrose gap [15]. In this preparation, automatic activity occurred when hyperpolarization was succeeded by spontaneous depolarization (Figure 4). Subsequently, they produced similar effects in rabbit atria and monkey papillary muscles by application of depolarizing dc [16], or alternating 50 cycle current [17] (Figure 5). Although the authors did not investigate the ionic mechanism of automatic activity in these studies, they could simulate the phenomenon in a computer model using only the Na^+ and K^+ but not the Ca^{++} or Cl^- currents [18].

Reuter and Scholz described automatic depolarizations in sodium-free solution in depolarized ventricular trabeculae from sheep and calves [19]. The

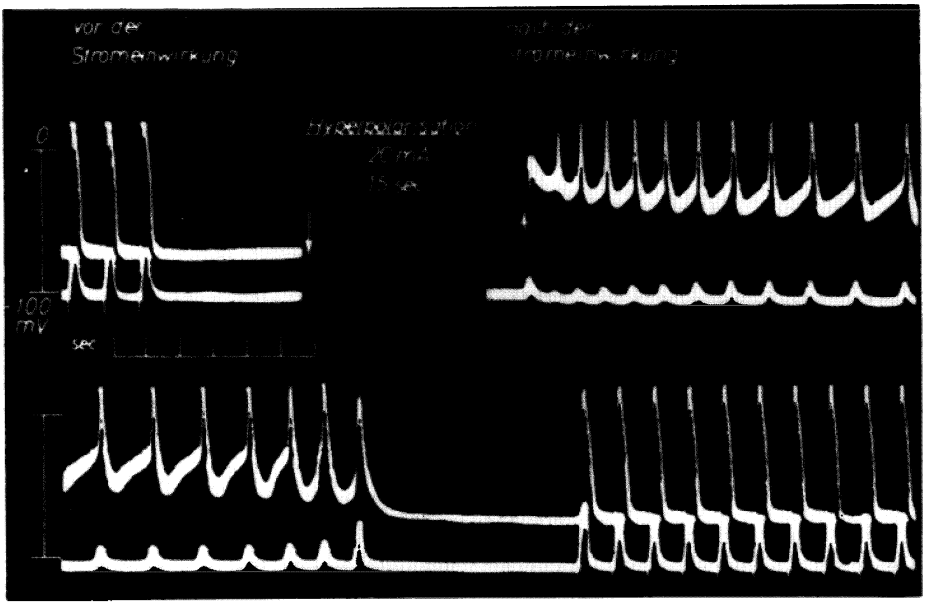

Figure 4. Transient transformation of a nonautomatic ventricular fiber from monkey paillary muscles into automatic pacemaker fiber during depolarization which occurs after hyperpolarization by a 20 mA current pulse of 15 sec duration (from ref. [15], by permission).

phenomenon was calcium-dependent since both the applitude and the $(dV/dt)_{max}$ of automatic depolarizations increased with increasing $(Ca^{++})_0$ concentration.

3. ELECTROPHYSIOLOGIC PROPERTIES

3.1. Threshold potential and maximum diastolic potential (MDP)

We have seen already that the MDP of automatic depolarizations varied considerably in different types of experiments. Although some of these variations could be attributed to differences in the experimental protocols, or to differences in fiber types, the results differed even in similarly designed experiments. Thus, in guinea pig papillary muscles studied by Katzung at $(Ca^{++})_0 = 1.8$ mM [20, 21], the MDP ranged from -75.4 to -2.9 mV, and the threshold potential for automatic depolarizations from -67.3 to -10.3 mV. In the same preparation and the same ionic milieu, Imanishi and Surawicz found that rapid automatic depolarizations appeared at an average MDP of -35.2 ± 7.5 mV, and ceased at MDP of $+4.0 \pm 9.2$ mV [22]. In their study the threshold potential for automatic depolarizations was close to the

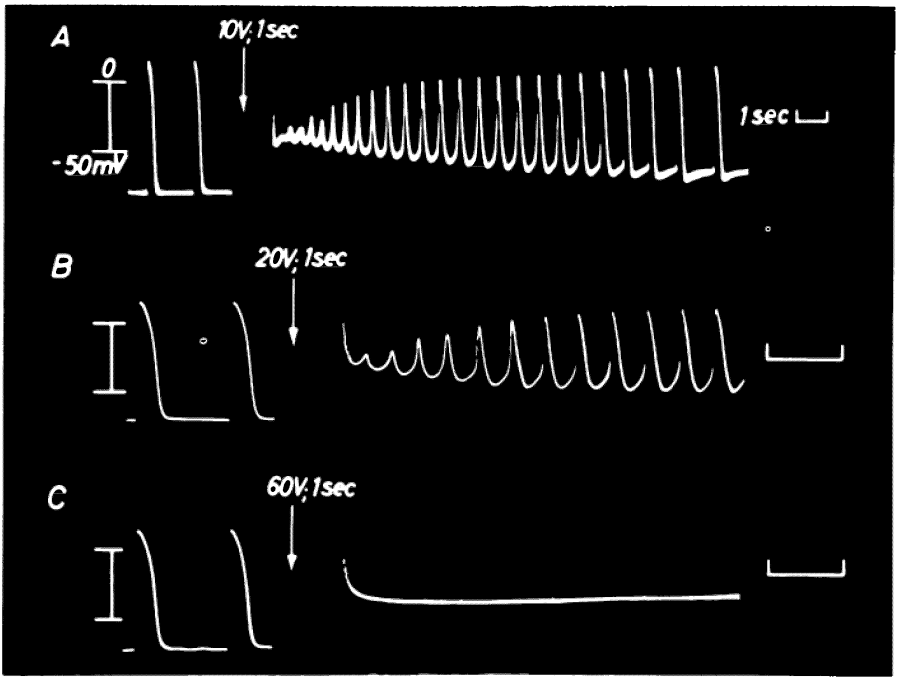

Figure 5. Induction (A, B) and suppression (C) of automaticity by alternating current of variable strength in Purkinje fiber (A) and papillary muscle from a rhesus monkey (B, C). In B, a 20 V pulse induces automaticity in depolarized ventricular myocardium, and in C a 60 V pulse causes standstill (from ref. [17], by permission).

threshold potential for the slow inward current in guinea pig myocardium [22] (Figure 6). These values differed from those reported by Katzung [21]. In the latter study the threshold potential was more negative than the threshold potential for the slow inward current and also more negative than the potential at which the rapid inward sodium current would undergo complete inactivation. Even though Katzung used a sucrose gap [21] and Imanishi and Surawicz a vaseline gap [22], the differences between their results are probably not due to these differences in technique. More likely, the myocardial fibers are inherently capable of developing automatic depolarizations at several different levels of MDP. Other studies support this conclusion. Arita and coworkers reported that in canine papillary muscles mounted in a sucrose gap spontaneous activity occurred at MDP within a range between -65 and -10 mV [23, 24]. In cat papillary muscles, Sakson et al. [25] described two zones of MDP at which automatic activity appeared, one after depolarization by less than -30 mV, and another after depolarization by more than -30 mV [25]. They attributed these results to two different ionic mechanisms [25].

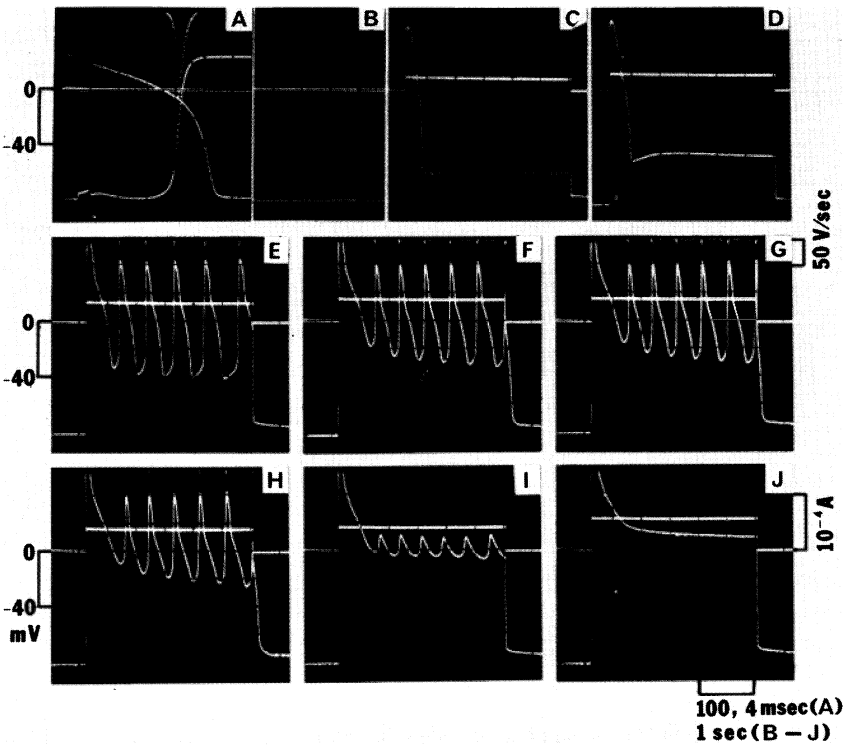

Figure 6. Effect of depolarizing currents of 4 sec duration (from B to J) on the membrane potential of guinea pig papillary muscle (single cell impalement). A: control. B–J: increasing current strength. In each panel the membrane potential is at the bottom, the current trace is in the middle, and the $(dV/dt)_{max}$ at the top. Note automatic depolarizations in E, F, G, H, I but not in B, C, D and J. See text (unpublished experiments of S. Imanishi and B. Surawicz).

3.2. Rate, $(dV/dt)_{max}$ and overshoot

Table 1 summarizes the effect of MDP on the cycle length, maximum dV/dt and the overshoot of rhythmic automatic depolarizations in the study of Imanishi and Surawicz [22]. The oscillations of amplitude lower than -10 mV induced by depolarization to MDP less negative than -10 mV were not included in the analysis of their results. The average cycle length within the ranges of MP from -40 to -10 mV and -10 to $+20$ mV were similar. The average maximum values for $(dV/dt)_{max}$ within these two MDP ranges also were not significantly different from each other. Within the MDP range from -50 to -40 mV the average cycle length, $(dV/dt)_{max}$ and overshoot were significantly different from the corresponding average values at other levels of

*Table 1**. Cycle length, $(dV/dt)_{max}$, and overshoot of rhythmic automatic depolarizations at different levels of membrane potentials..

Membrane potential range (mV)	*Cycle length (msec)*	$(dV/dt)_{max}$ *(V/sec)*	*Overshoot (mV)*
−50 −40	460.0±44.3 (11)**	28.1±9.1 (10)**	45.2±4.7 (11)**
−40 −10	399.9±62.2 (114)	18.0±7.1 (103)	51.7±10.0 (106)
−10 +20	402.0±47.6 (38)	17.1±7.5 (31)	

Results are expressed as average ±SD. The number of preparations is in parentheses.
* Reprinted from ref. 22, by permission.
** Significantly different ($P<0.05$) from values obtained at other membrane potential ranges. See text.

depolarization. This suggests that an incompletely inactivated rapid sodium current could have contributed to the genesis of automatic depolarizations within this potential range [22].

3.3. Slope resistance

Imanishi and Surawicz [22] found that during the slow diastolic depolarization the membrane resistance gradually increased, reaching maximum within 69 ± 19 msec after the point of MDP, and that it decreased again prior to the onset of the rapid upstroke which occurred within 112 ± 22 msec after the point of MDP (Figure 7). Other investigators reported only an increase in membrane slope resistance during diastolic depolarization [21, 23]. The interpretation of such an increase is difficult when the measurements are made within the range of MP associated with anomalous (inward-going) rectification. However, Imanishi and Surawicz measured membrane resistance at membrane potentials less negative than −20 mV, i.e. within a potential range in which the membrane displays not an inward-going but a time-dependent delayed (outward-going) rectification [22]. Their results suggest, therefore that the initial increase in slope resistance was the cause rather than the result of diastolic depolarization [22].

4. MECHANISMS

4.1. Role of calcium

Reuter and Scholz were first to establish the role of calcium in automatic depolarizations in ventricular trabeculae from sheep and calves [19]. They studied the relation between the amplitude of automatic depolarizations and the calcium concentration in sodium-free solution [19]. A 30 mV increase in

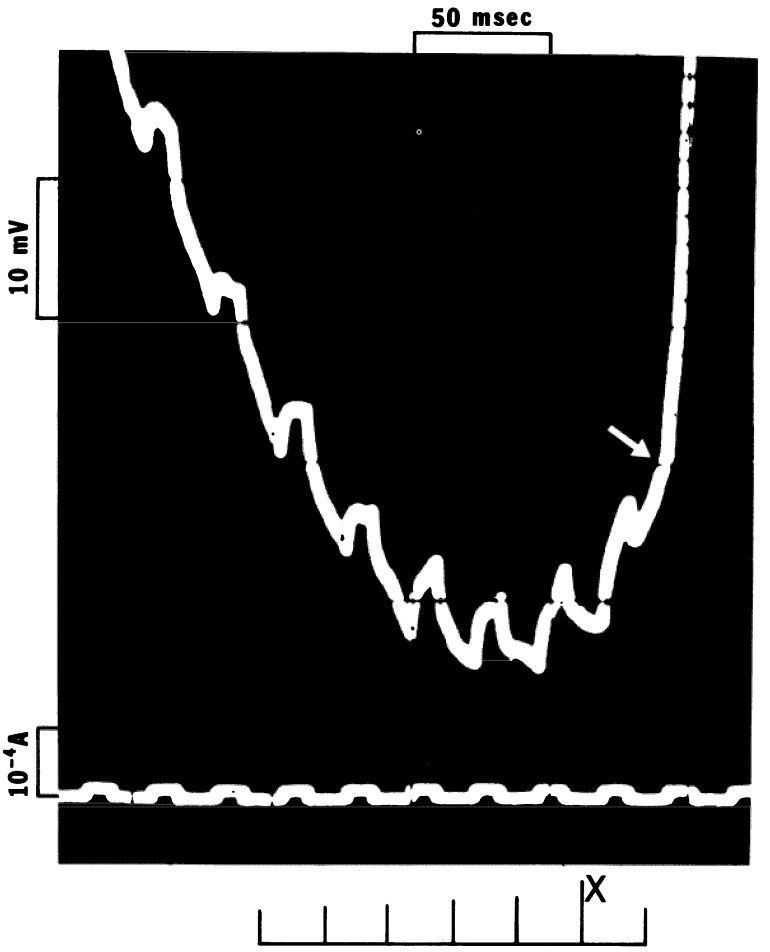

Figure 7. Measurement of slope resistance during slow diastolic depolarization in a depolarized ventricular muscle fiber. Magnified portion of terminal repolarization and the ensuing rhythmic automatic depolarizations from a single response (depolarization to −30 mV) with the superimposed square hyperpolarizing pulses of 20 msec duration. Lower trace shows the strength of these pulses. The vertical bars beneath the photograph show the amplitudes of 7 hyperpolarizing pulses superimposed on the responses; each bar is drawn at the time of the corresponding pulses inscription; X indicates the response with the largest amplitude, i.e., the highest membrane resistance. The arrow points to the onset of the rapid response (from ref. [22], by permission).

amplitude would be expected for a 10-fold increase in $(Ca^{++})_0$ if the depolarizations were due to purely calcium-permeable membrane. However, they found only an increase of 18 mV, a deviation attributed either to a change in calcium driving force, or to contributions of other ions. Imanishi and Surawicz found that in Tyrode's solution the calculated change in amplitude of

the overshoot for a 10-fold change in $(Ca^{++})_0$ was 22.7 mV, i.e., a 7.3 mV deviation from the MP calculated from the Nernst equation [22].

The above studies show that calcium current is not the sole contributor to the automatic depolarizations. In keeping with this assumption, Katzung found that automatic depolarizations persisted even when $(Ca^{++})_0$ was reduced to 10% of normal concentration [21]. However, low calcium

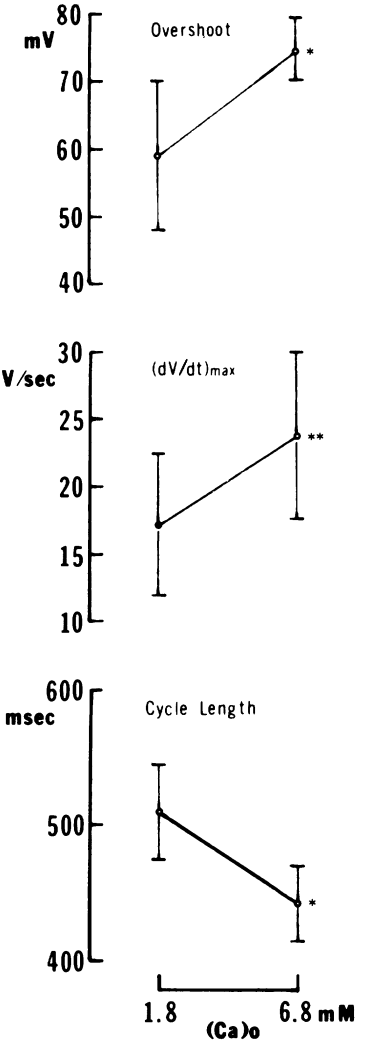

Figure 8. Overshoot, maximum dV/dt, and the cycle length of rhythmic automatic depolarizations (RAD) at two different Ca^{++} concentrations in 5 preparations. Circles indicate mean values and vertical bars standard deviations. *$P < 0.001$, **$P < 0.01$. See text (from ref. [22], by permission).

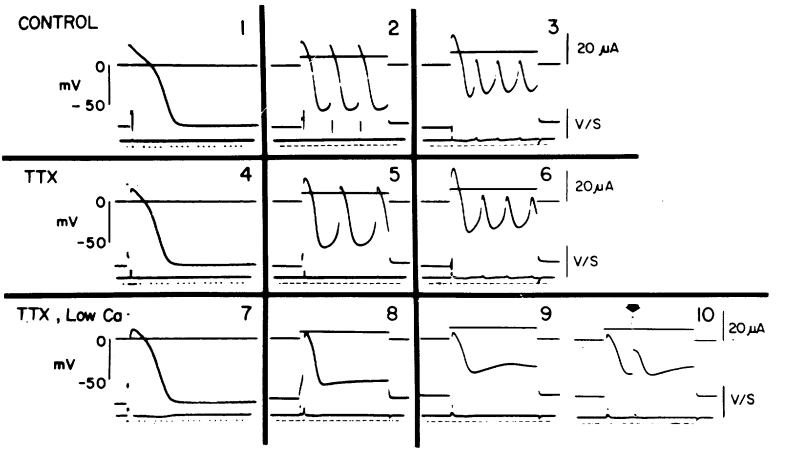

Figure 9. Application of depolarizing currents in guinea pig papillary muscle. Effects of tetrodotoxin (TTX) and calcium reduction; 1–3, control Tyrode's solution; 4–6, 10^{-6} g/ml TTX; 7–10, 10^{-6} g/ml TTX in low calcium (0.18 mM) solution. Note absence of suppression of automatic depolarizations by TTX alone and suppression by combination of TTX and low Ca. See text (from ref. [21], by permission).

decreased the slope of phase 4, constricted the range of MP at which automatic depolarizations occurred, and slowed phase 3 repolarization. The range of threshold potentials for automatic depolarization was -67.3 to -10.3 mV when $(Ca^{++})_0$ was 1.8 mM, and -60.3 to -32 mV when $(Ca^{++})_0$ was decreased to 0.18 mM. High calcium concentration (5.4 mM) increased the slope of phase 4, the overshoot, and the $(dV/dt)_{max}$, accelerated phase 3 of repolarization and broadened the range of MP at which automatic depolarizations occurred [21]. In canine preparations studied by Arita et al. increase in $(Ca^{++})_0$ increased $(dV/dt)_{max}$ and rate of automatic depolarizations but had no effect on the overshoot [23]. Imanishi and Surawicz found that an increase in $(Ca^{++})_0$ increased the average overshoot from 59.6 ± 11.5 to 72.2 ± 5 mV, increased the average $(dV/dt)_{max}$ from 17.3 ± 5.6 to 23.7 ± 6.8 V/sec and decreased the average cycle length from 510.0 ± 36.0 442.3 ± 32.1 msec (Figure 8). A decrease in $(Ca^{++})_0$ to 0.6 mM decreased the rate, the overshoot, and the $(dV/dt)_{max}$, while a decrease in $(Ca^{++})_0$ to 0.4 mM abolished the rhythmic automatic depolarization. This indicates that the automatic depolarizations in fibers depolarized to less negative potentials in the study of Imanishi and Surawicz [22] were more sensitive to changes in $(Ca^{++})_0$ than the automatic depolarizations in fibers depolarized to more negative potentials in the study of Katzung [21].

4.2. *Role of sodium*

Katzung found that a 50% decrease in sodium concentration decreased overshoot and $(dV/dt)_{max}$ of the automatic depolarizations in the guinea pig papillary muscles. The effect of tetradotoxin resembled the effect of low sodium, and a combination of tetradotoxin and low calcium suppressed automatic activity (Figure 9)[21]. Similar effect was produced by a combination of tetradotoxin (2×10^{-5} g/ml) and manganese[21]. Low sodium also constricted the MDP range of -70.3 to -24.8 mV to a range of -62.5 to -34.8 mV[22], and the threshold potential range of -64.3 to -9.5 mV to a range of -46.5 to -16.5 mV[22]. In the study of Arita et al., lowering sodium to one-quarter of normal concentration decreased the rate and the overshoot of automatic depolarizations, while tetradotoxin had no such effect[23].

4.3. *Role of potassium*

Imanishi and Surawicz found that low $(K^+)_0$ increased the rate of automatic depolarizations due to more rapid repolarization and steeper slope of phase 4 of automtic depolarizations[22]. In their study, the increase of $(K^+)_0$ to 15.4 mM either depressed the rate of automatic depolarizations, or abolished it entirely, while the 40.0 mM $(K^+)_0$ concentration always abolished the automatic activity[23]. The suppression was due to increased $(K^+)_0$ and not to K^+ induced depolarization, because it persisted when the membrane potential was held at the level of the control resting potential by means of conditioning hyperpolarizing pulse (Figure 10). Figure 10 also shows that the effects of high $(K^+)_0$ were reversible after return to control solution.

Similar results were reported by Arita et al.[24] and Sakson et al.[25]. In these studies automaticity was suppressed by 2-fold[24] or 3-fold[25] increase in $(K^+)_0$ respectively. Katzung and Morgenstern found that increased $(K^+)_0$ reduced the slope of phase 4 and suppressed automaticity in depolarized guinea pig myocardium[26].

4.4. *Is rhythmic activity in depolarized myocardium automatic?*

In many published records of rhythmic depolarizations, the transition from phase 4 to phase 0 is smooth and gradual[21, 22], and the responses are of uniform shape and duration[21, 22]. This strongly suggests that the activity is due to true automaticity rather than to circus movement or reentry resulting from inhomogeneous depolarization. Transmembrane potentials recorded simultaneously with two pairs of microelectrodes have shown a homogeneous and synchronous activity at the opposite ends of preparation (Figure 3 in

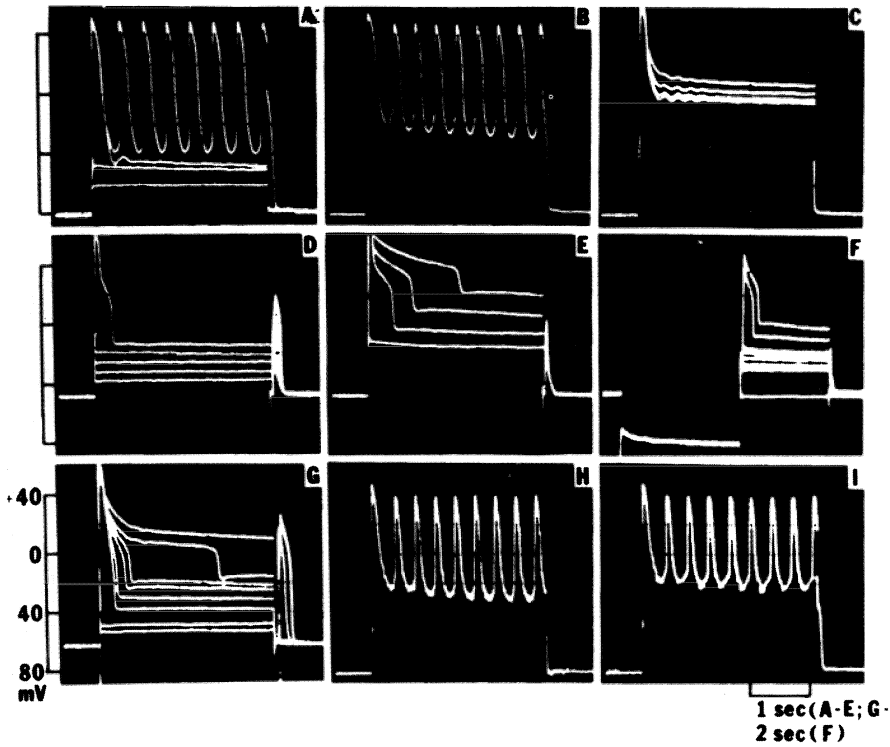

Figure 10. Effect of high $(K^+)_0$ on rhythmic automatic depolarizations (RAD) in depolarized ventricular muscle fiber (single cell impalement). A, B, and C: control ($(K^+)_0 = 5.4$ mM): rhythmic automatic depolarizations (RAD) occur after depolarization to -40 mV (A) and -26 mV (B) and oscillatory responses after depolarization to -2 mV (C). D, E, and F: 30 min after superfusion with 40.4 mM $(K^+)_0$. Note absence of RAD and oscillation. G: 20 min after superfusion with 15.4 mM $(K^+)_0$. H and I: 30 min after return to control. See text (from ref. [22], by permission).

ref. [22] and Figure 1 in ref. [26]). These observations support the assumption that the rhythmic activity in the depolarized myocardium is caused by automatic depolarizations.

4.5. Role of slow inward current

Brown and Noble studied pacemaker activity in depolarized fibers from atrial trabeculae [14] and concluded that the process was dependent on the current which became activated at a potential about 20 mV positive to the threshold of rapid inward sodium current [14].

In the study of Imanishi and Surawicz, the most negative membrane potential at which rhythmic, automatic depolarizations appeared was very close to the threshold potential for the slow inward current in guinea pig

myocardium (reference in ref. [22]), and, in normal Tyrode's solution, the overshoot amplitude was close to the calculated reversal potential of calcium current (references in ref. [22]). Similarly, the maximum dV/dt was within the range of depolarization velocities dependent on the slow inward current [22].

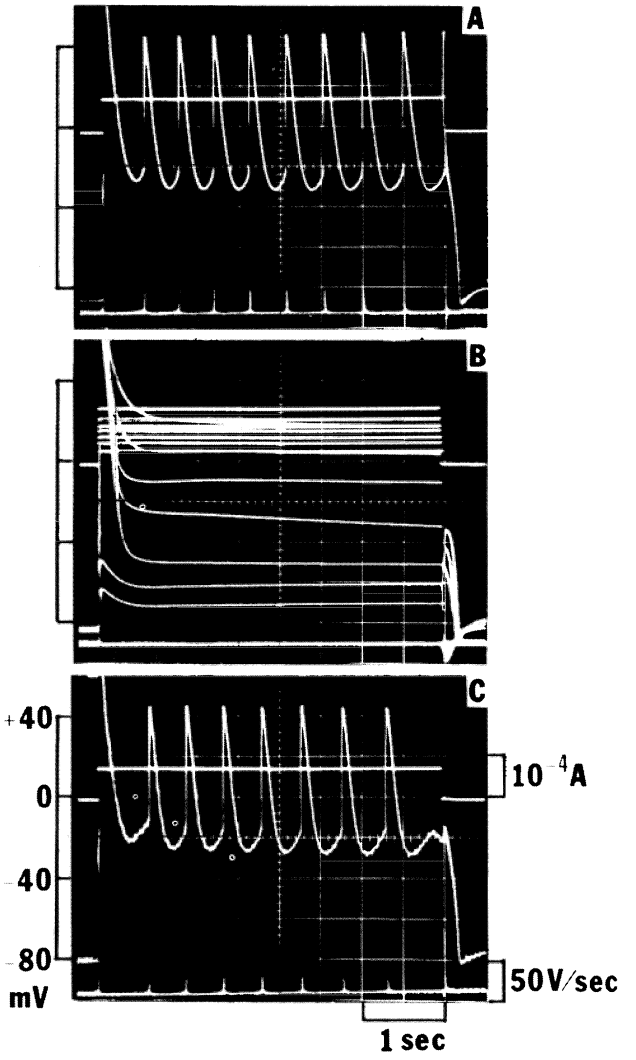

Figure 11. Effect of Mn^{++} on rhythmic automatic depolarizations (RAD) in a depolarized ventricular muscle fiber. At the top is shown the strength of the applied depolarizing current pulses; in the middle, the transmembrane potentials; and at the bottom, maximum dV/dt. A: control: RAD occur after depolarization to −32 mV. B: 20 min after application of 6 mM $MnCl_2$; depolarizations to membrane potentials ranging from −70 to +18 mV do not elicit RAD. C: 30 min after return to control solution without Mn^{++}, RAD occur again after depolarization to −32 mV (from ref. [22], by permission).

Other evidences supporting the role of the slow iward current are: (1) sensitivity to extracellular calcium concentration [21–23]; (2) suppression by manganese [20, 22, 23] (Figure 11); and (3) enhancing effects of adrenaline [23, 20] and isoproterenol [22]. Imanishi and Surawicz have shown that isoproterenol (5×10^{-7} to 10^{-6} g/ml) increased the rate, the overshoot, and the $(dV/dt)_{max}$ in active preparation and induced activity in quiescent depolarized preparations [22] (Figure 6).

4.6. Role of outward potassium current

It is reasonable to attribute the repolarization of rhythmic automatic action potentials to the time-dependent outward K^+ current which has been recorded in the ventricular myocardium in various species (reference in [23]). Katzung and Morgenstern used a voltage clamp to study the characteristics of this current activated by the action potential in cat papillary muscle [26]. Their study showed that the reversal potential of this current was close to the normal resting potential. Hence, at the level of resting potential, the current was either negligible or directed inward. In the depolarized myocardium, the current deactivated slowly. Therefore, one could postulate that, in the presence of reactivated sodium or calcium inward current, phase 4 depolarization could reach threshold and produce a spike [26]. Katzung and Morgenstern attributed the suppression of rhythmic automatic activity by high $(K^+)_0$ to an increase in g_K and to a decline of the time-dependent current caused by shift in the reversal potential of this current [26]. They postulated that automaticity would be induced by any procedures which reduced diastolic potential without simultaneously shifting the reversal potential of the time-dependent K^+ outward current [26].

4.7. Summary of the proposed mechanisms

To explain fully the occurrence of automatic depolarizations in the depolarized myocardium, one must understand the mechanism of the following underlying events: (1) background depolarization, (2) slow diastolic depolarization, (3) spike, and (4) repolarization following the spike. The background depolarization induced by chemical substances, electrolytes, or drugs is usually caused by low g_K. The depolarization induced by application of electric currents brings the membrane into a region of decreased resistance. The slow diastolic depolarization can occur within two regions of membrane potential [25]. In a region of more negative membrane potential, depolarization is caused by the incompletely inactivated sodium current. In another region of less negative membrane potential where the rapid inward sodium current is inactivated, the depolarization is apparently due to the activation of the slow

(calcium and sodium) current. In both ranges, the slow diastolic depolarization is apparently aided by the slow deactivation of the potassium current. The spike must be attributed either to the rapid or to the slow inward current depending on the level of the maximum diastolic potential. The repolarization is probably due to the activation of the time-dependent outward K^+ current.

5. THE EFFECTS OF ANTIARRHYTHMIC DRUGS

Verapamil is probably the most effective of all drugs tested for their ability to suppress rhythmic automatic depolarizations in depolarized ventricular myocardium. In the tissue bath, automaticity was abolished by verapamil concentrations of 2–4 μg/ml in dog [24], and 5 μg/ml in guinea pig papillary muscles [27, 28]. The effect of verapamil was most pronounced at less negative MDP [27]. At more negative MDP, verapamil concentrations of 1–5 μg/ml did not suppress automatic activity but decreased the rate and the overshoot [27]. Grant and Katzung reported that epinephrine reversed the depressing effects of verapamil [28]. In the study of Imanishi et al., verapamil concentrations of

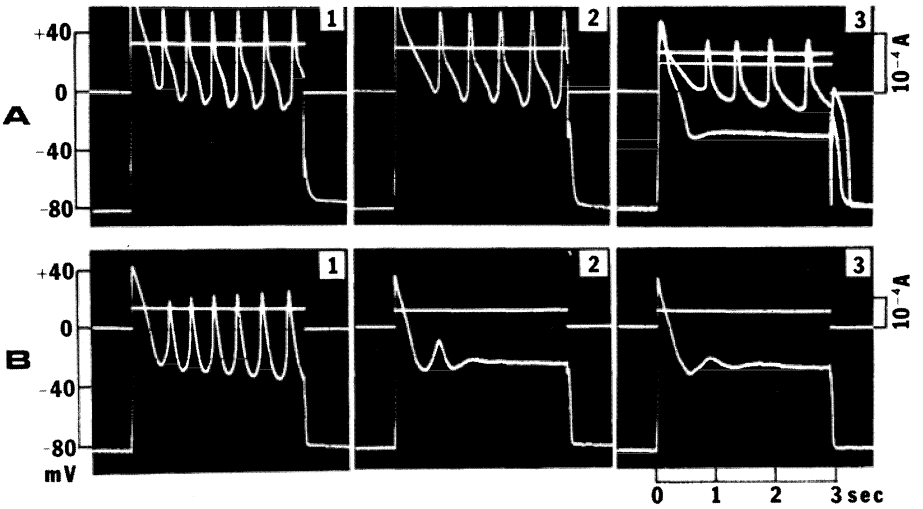

Figure 12. Effects of two different verapamil concentrations (2 mg/liter in A and 4 mg/liter in B) on RAD in depolarized ventricular muscle fibers in two different experiments. In each panel, the applied current trace is at the top and the membrane potential is at the bottom. A1: RAD without verapamil during depolarization to −12 mV; A2 and A3: Depolarizations to the same level after 20 and 30 min superfusion with 2 mg/liter of verapamil. A3 includes an additional current trace of lower strength which causes a single action potential from a more negative membrane potential. B1: RAD without verapamil during depolarization to −32 mV; B2 and B3: depolarizations to the same level of membrane potential after 20 and 30 min of superfusion with 4 mg/liter of verapamil. See text (from ref. [29], by permission).

2 μg/ml suppressed, and those of 3–4 μg/ml abolished rhythmic automatic depolarizations in guinea pig myocardium (Figure 12)[29]. The effect of verapamil in vivo was studied in papillary muscles of guinea pigs pretreated with nonlethal doses of the drug and sacrificed at different time intervals after the administration of the drug[29]. The depressing effects of verapamil on the rate, overshoot, and $(dV/dt)_{max}$ parallelled the plasma and the myocardial concentrations of verapamil[29]. The effects of verapamil in preparations from animals pretreated with nonlethal doses of verapamil were similar to the effects of superfusion with in vitro verapamil concentration of 2 μg/ml which decreased the rate of automatic depolarizations but did not suppress them entirely[29]. Imanishi et al. found differences between the effects of verapamil on the overshoot and the rate of rhythmic automatic depolarizations. The increase in overshoot parallelled the dissipation of verapamil effect in the ventricular muscle fibers of pretreated animals, but the change in the rate of automatic depolarizations showed no such parallelism (Figure 13). This is in keeping with the hypothesis that the overshoot of rhythmic automatic depolarizations may depend predominantly on the slow inward current, while the

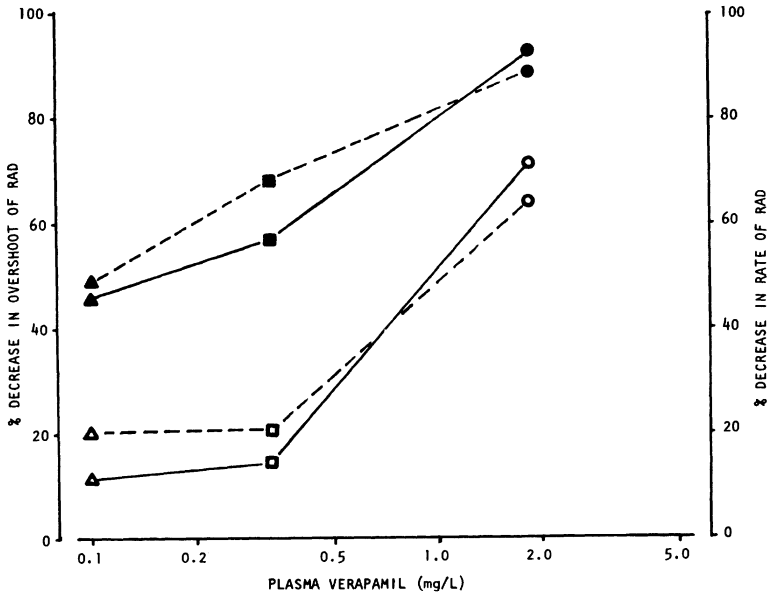

Figure 13. Relation between mean in vivo plasma verapamil concentrations at the time of sacrifice (abscissa) and the decrease in the overshoot of automatic depolarizations and the rate of automatic depolarizations (ordinates) in preparation from animals sacrificed 30 min (circles), 120 min (squares), and 180 minutes (triangles) after verapamil (30 mg/kg) injection. Open symbols indicate the rate and closed symbols the overshoot of automatic depolarizations. Solid lines connect data from fibers depolarized to levels of −50 to −40 mV, and dashed lines data from fibers depolarized to levels of −39 to −10 mV. The 100% values are taken from Table 1. See text (from ref. [29], by permission).

slope of phase 4 may be due to a more complex interaction of depolarizing and repolarizing currents [22, 26].

The only drug other than verapamil which suppressed the automatic depolarizations was propranolol. Sakson et al. reported that propranolol (2–4 μg/ml) suppressed the automaticity arising from myocardium depolarized by more than 30 mV but not the automaticity arising from myocardium depolarized by less than 30 mV [25].

The antiarrhythmic drugs which do not belong to the category of slow channel-blocking agents caused small and variable effects on the automatic depolarizations. These effects could be due to increase in g_K or to the depression of the rapid inward sodium channel in preparations where the latter plays a role in the automatic activity. Grant and Katzung found that quinidine (2–4 μg/ml) reduced the slope of phase 4 and the overshoot, but these effects were less pronounced than those of verapamil [28]. Automatic activity was also slowed by 1×10^{-5} M droperidol, a concentration which is allegedly antiarrhythmic in vivo [30]. These droperidol effects were reversible by adrenaline. Katzung reported that lidocaine (5 μg/ml) reduced the rate of automatic depolarizations [27], while Arita et al. found no effect of 4–16 μg/ml lidocaine concentrations [24]. Imanishi et al. studied the effects of three lidocaine concentrations (4, 8 and 16 μg/ml), none of which had significant effects on the $(dV/dt)_{max}$ and the overshoot. However, the 16 μg/ml concentration decreased the rate of automatic depolarizations (Table 2) [29], presumably due to the increase in g_K by lidocaine (references in ref. [29]).

*Table 2**. Effect of lidocaine on spontaneous rhythmic depolarizations in depolarized guinea pig ventricular myocardium.

	Cycle length Av. ±S.D. (msec)	$(dV/dt)_{mlx}$ Av. ±S.D. (V/sec)	*Overshoot* Av. ±S.D. (mV)
Control	417.1±31.5 (7)	12.7±0.5 (3)	58.3±4.8 (7)
Lidocaine 4 µg/ml	420.0±31.2	12.7±1.6	58.4±2.0
Control	412.3±40.0 (13)	14.6±3.3 (5)	55.5±2.4 (11)
Lidocaine 8 µg/ml	433.8±40.3	13.2±2.6	55.9±2.1
Control	365.9±54.1 (17)	10.1±3.2 (7)	40.4±10.4 (10)
Lidocaine 16 µg/ml	422.4±93.4**	10.1±3.0	41.5±10.5

* Reprinted from ref. 29, by permission.
** $P < 0.05$.
Numbers in parentheses = no. of preparations.

6. CLINICAL IMPLICATIONS

Cranefield [2] suggested at least two ways in which spontaneous depolarization may occur in nonpacemaker fibers in situ, namely by the injury current that

flows between an infarct and the adjacent tissue or by current spreading electrotonically across the region of the block. Katzung et al. created experimental conditions conducive to the development of spontaneous automaticity in vitro in cat papillary muscle mounted in a sucrose gap using 145 mM K Tyrode's solution to depolarize the gap compartment to 0 mV, while the test compartment contained 2 mK K^+ which increased the membrane resistance [31]. In this preparation the depolarization of the myocardium in the test compartment by K^+ diffusing through the gap sometimes induced spontaneous activity [31]. When K^+ concentration in the test compartment was 3.5 mM, spontaneous activity could be induced following application of small subthreshold depolarizing current pulses or after addition of epinephrine [31]. No spontaneous activity occurred at $(K^+)_0$ exceeding 3.5 mM, even upon addition of epinephrine [32]. These expermients suggested that spontaneous activity required a combination of a strong depolarizing 'source' and a high resistance 'sink.'

The occurrence of automatic activity in the depolarized ischemic myocardium has not yet been conclusively demonstrated. The suggestive evidence stems from experiments showing that arrhythmias in the depolarized ischemic myocardium may arise from the epicardium which is presumably devoid of Purkinje fibers [32]. Solberg et al. observed pacemaker-like activity in depolarized ventricular myocardial fibers from the infarcted dog heart and from the ischemic human ventricle [33]. Hordof et al. described spontaneous rhythmic activity in depolarized human atrial fibers [34]. This activity was resistant to procainamide but was suppressed by verapamil [34]. However, the evidence for automaticity in this and similar studies is not conclusive because the studied preparations may contain some unrecognized specialized, potentially automatic fibers, and because the possibility of reentry cannot be ruled out unless the preparation is small and homogeneously polarized.

7. SUMMARY

The procedures used by various investigators to change the ventricular or atrial myocardial fibers into automatic pacemakers have been reviewed. Common to all procedures is depolarization induced by application of electric currents, or interventions which lower K^+ conductance. Role of depolarizing and repolarizing membrane currents in the induction and suppression of automaticity has been discussed in relation to the specific effects of ions and drugs on the depolarization-induced automatic activity. Finally, clinical implications of the automaticity in atrial and ventricular myocardium have been outlined.

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IV. ROLE OF THE SLOW INWARD CURRENT IN THE GENESIS OF CARDIAC ARRHYTHMIAS

18. ROLE OF THE SLOW CURRENT IN THE GENERATION OF ARRHYTHMIAS IN ISCHEMIC MYOCARDIUM

RALPH LAZZARA and BENJAMIN J. SCHERLAG

In the past decade an upsurge of investigation into the mechanisms of arrhythmia development during myocardial ischemia has amassed a body of data implicating both reentry and abnormal automaticity [1–4]. In the experimental animal most commonly studied, the dog, occlusion of a major coronary artery initiates a sequence of electrophysiologic abnormalities in both myocardial and Purkinje cells in the ischemic region. Certain of the abnormalities of cells in specific locations have been singled out as especially instrumental in causing arrhythmias. The most marked conduction delays and irregularities of activation have been detected in the *least* ischemic regions – the superficial subepicardial layers of ordinary myocardial cells. These layers form essential links in reentry circuits that generate the malignant arrhythmias in the first half hour after coronary occlusion [2, 5, 6]. Conduction in these layers improves after the early outburst of arrhythmias but the potential for the formation of reentry circuits in this locale remains for one to two weeks, especially when the heart is stressed at high rates [7–9]. In dogs with coronary occlusion subendocardial layers of ordinary myocardial cells have not been shown to be important sources of arrhythmias despite the fact that they are so severely ischemic that they do not survive the first day. It is not clear why the most ischemic layers proceed to quiescence and death without showing severe delays and irregularities of conduction. On the other hand, there are indications from catheter recordings in humans that marked fragmentation and delay of activation in the subendocardial layers may cause reentry in chronic ischemic heart disease, especially in the presence of ventricular aneurysms [10]. Intramural layers have not been probed extensively, but abnormalities of conduction and refractoriness have been described in dogs [11].

The specialized conducting cells in the ischemic region are affected after several hours of ischemia in a heterogeneous fashion. They are depolarized and upstroke velocity and conduction speed are depressed. Repolarization is prolonged, and diastolic depolarization and automaticity are enhanced [12–14]. These abnormalities are most pronounced in the first day after occlusion and persist for several days [15, 16]. After the abnormalities recede, many of the cells survive in a normal electrophysiological state on the endocardial surface

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of the scar. During the period of abnormality the afflicted Purkinje cells probably generate automatic and reentrant arrhythmias. There are clues that Purkinje cells in infarcted regions of human hearts are affected similarly. Accelerated ventricular rhythm is common in the first several days of myocardial infarction in humans [17]. Its behavior suggests enhanced automatic firing. Surviving Purkinje networks are discernible in chronic infarcts in humans by microscopic examination [18].

This period of intensified interest and investigation in the electrophysiology of ischemic myocardium has coincided with the discovery that the slow inward current of cardiac cells can under certain conditions generate propagated and automatic action potentials, called 'slow responses' [19–23]. In this discussion the slow current will also be referred to as the inward calcium current, although other ions, for example sodium, may provide substantial components of the current under certain circumstances [24]. By and large a common circumstance for the generation of action potentials by the slow current has been the excitation of cells at levels of membrane potential more positive than -55 mV, levels where the normal rapid sodium current is inactivated [25]. The generation of action potentials by such depolarized cells generally requires the coexistence of factors that intensify slow current, such as high concentration of extracellular calcium, or beta adrenergic agonists. One experimental model for reentry that has received considerable attention consists of canine Purkinje fibers depolarized by high extracellular concentration of potassium to inactivate the rapid sodium channels and exposed to high concentrations of beta adrenergic agonists to enhance inward calcium current [2]. The electrophysiological behavior of this model displays the phenomena that occur when excitation and propagation depend on the slow current [26, 27]. The intensity of the slow current is weak and its kinetics are slow; action potentials generated by it conduct very slowly, block readily, and form reentry circuits easily even over minute distances.

Under some experimental circumstances the slow current may be an important generator of diastolic depolarization and upstrokes of action potentials of depolarized cells firing automatically [28]. There is evidence that the slow current may be an important but not yet clearly defined factor in afterdepolarizations and triggerable automaticity [29].

Ischemic myocardial cells have been observed to have some of the same abnormal electrophysiological characteristics as cells operating under experimental conditions that produce slow responses. Specifically ischemic cells have been observed to generate propagated and automatic action potentials at levels of membrane potential where the rapid current would be expected to be inactive [4, 14, 30]. Also, there is evidence that an environment known to promote slow responses (an environment of high concentrations of beta adrenergic mediators and extracellular potassium) may occur during ischemia,

especially during the early period of severe ischemia [31–33]. These and other observations have collaborated in the formulation of the hypothesis that slow responses generated by ischemic cardiac cells produce reentrant and automatic arrhythmias [4, 28, 34].

Lacking a convenient means of directly monitoring slow current in ischemic myocardium, investigators have tested the hypothesis by observing the effects of blockers of the slow or fast current [35], and the effects of stimulators of slow current, on the electrical activity of ischemic tissue. Such experiments suffer from the fact that the agents that have been used are not specific in their actions on slow or fast currents [36]. Therefore, the results are amenable to various interpretations based on the different actions of the agents.

The effect of the slow channel blocker, verapamil, on the epicardial conduction delay during the early phase of ischemia after coronary occlusion in dogs was studied by Elharrar and coworkers [37]. To their surprise verapamil improved conduction in the ischemic tissues, as opposed to impairing it, as expected if slow responses were operative. Their findings are illustrated in Figures 1 and 2 reproduced from their manuscript. They speculated that other effects of verapamil, hemodynamic or metabolic actions, might reduce the degree of ischemic damage and offset the direct action of the agent on the

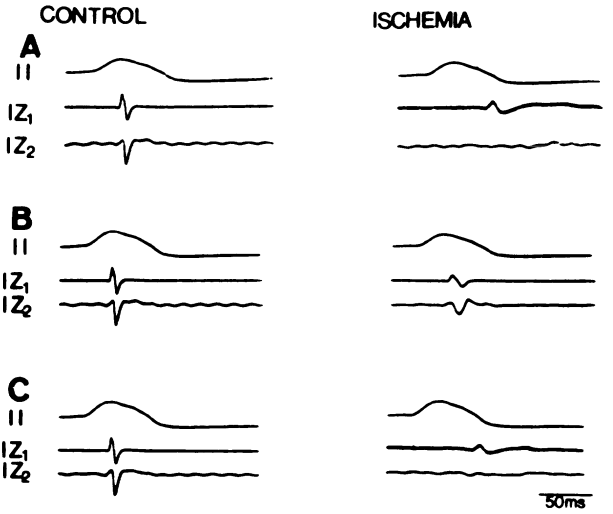

Figure 1. Effects of verapamil on ischemia-induced conduction delay. $1Z_1$ and $1Z_2$ are two epicardial bipolar electrograms obtained from the ischemic zone during pacing at the base of the right ventricle at a cycle length of 500 msec. The left and right panels were obtained before and 5 min after coronary occlusion, before (A), 11 min after (B) and 47 min after (C) administration of verapamil (0.2 mg/kg). Note that 11 min after administration of verapamil, the extent of conduction delay at $1Z_1$ and $1Z_2$ was less than either before or 47 min after verapamil administration. (Reproduced by permission from Elharrar et al: Amer J Cardiol 39:544, 1977.)

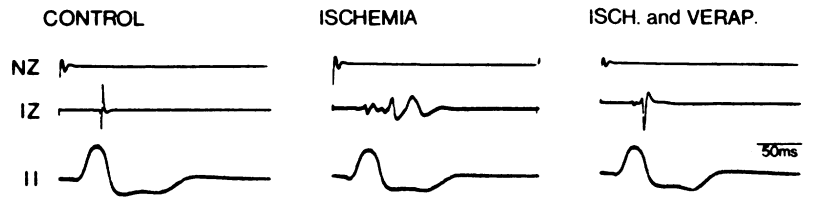

Figure 2. Effect of verapamil on the fractionation of the epicardial electrogram recorded from the ischemic zone (1Z) after 5 min of occlusion of the left anterior descending coronary artery during pacing at a cycle length of 500 msec. Left panel, before coronary occlusion; center and right panels were obtained 5 min after coronary occlusion, before (center) and 11 min after (right) administration of verapamil. Note that verapamil (right panel) prevented the fractionation recorded from the ischemic zone (middle panel). (Reproduced by permission from Elharrar et al: Amer J Cardiol 39:544, 1977.)

slow current. In another study Fondacaro and co-workers also found that verapamil alleviated conduction delay in acutely ischemic subepicardial myocardium [38]. In addition, they found that the firing rate of ventricular escape pacemakers in dogs with heart block was depressed by verapamil during acute ischemia. However, there was no indication that these pacemakers were located in the ischemic region or that they were abnormally affected by the ischemia. Other studies have indicated that the automaticity of Purkinje fibers in the ischemic zone is not enhanced until hours of ischemia have transpired [2, 16]. Therefore, these effects of verapamil on ventricular automaticity may represents the response of normal automaticity to this agent rather than the response of abnormal automaticity of ischemic tissues.

In a study reported in abstract, verapamil caused greater delay of conduction in the ischemic myocardium of the dog heart [39]. A full report of this study has not been published. The reasons for the different findings are not known.

The effects of verapamil on electrical activity of ischemic myocardium during later periods of ischemia after coronary occlusion in the dog also have been studied. El-Sherif and Lazzara reported that delay and fractionation of epicardial electrograms were mitigated by verapamil [40]. In their experimental model, delay and fractionation of epicardial electrograms were monitored with a 'composite electrode,' which essentially sums the electrical potentials from many bipolar recording sites [6]. The resultant recordings reflect the duration of the sequence of activation over the epicardium of the ischemic zone but information about topographic localization of the activation process is lost. The advantage of this method is that the duration of the fractionated electrogram on the composite recording is highly correlated with the occurrence of ventricular ectopic complexes. Therefore in this model, fractionated electrical activity extending throughout systole is a necessary condition and a reliable marker of reentry. Delay and fractionation of activation are exquisitely dependent on heart rate, because of great prolongation and heterogeneity of refrac-

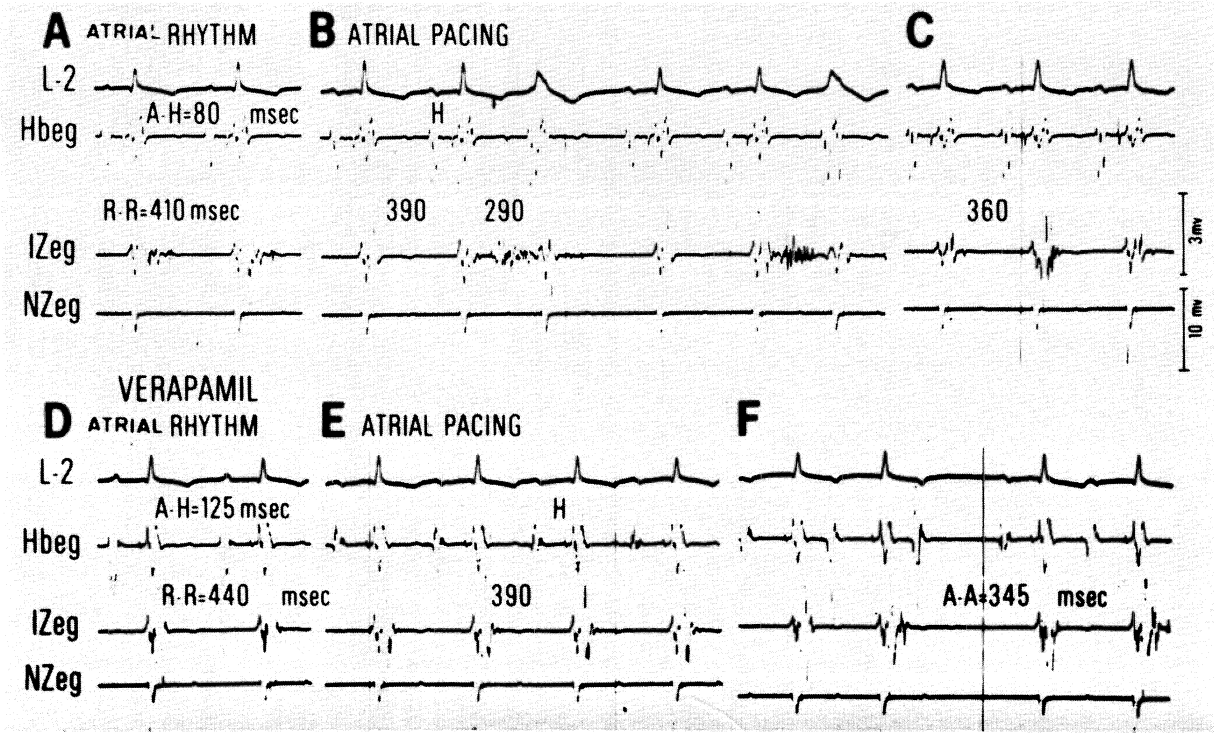

Figure 3. Amelioration by verapamil of delay and fractionation of activation in ischemic myocardium. Explanation and interpretation of the recordings is included in the text. The traces at the top (A–C) were recorded before, and those at the bottom (D–F) 2 min after the administration of a bolus of verapamil (0.5 mg/kg). Hbeg = His bundle electrograms; IZeg = composite electrogram from the infarcted zone; NZeg = composite electrogram from the normal zone; H = His bundle potential. Time lines are set at 1 sec intervals. (Reproduced by permission of the American Heart Association, Inc. from El-Sherif N et al: Circulation 60:605, 1979.)

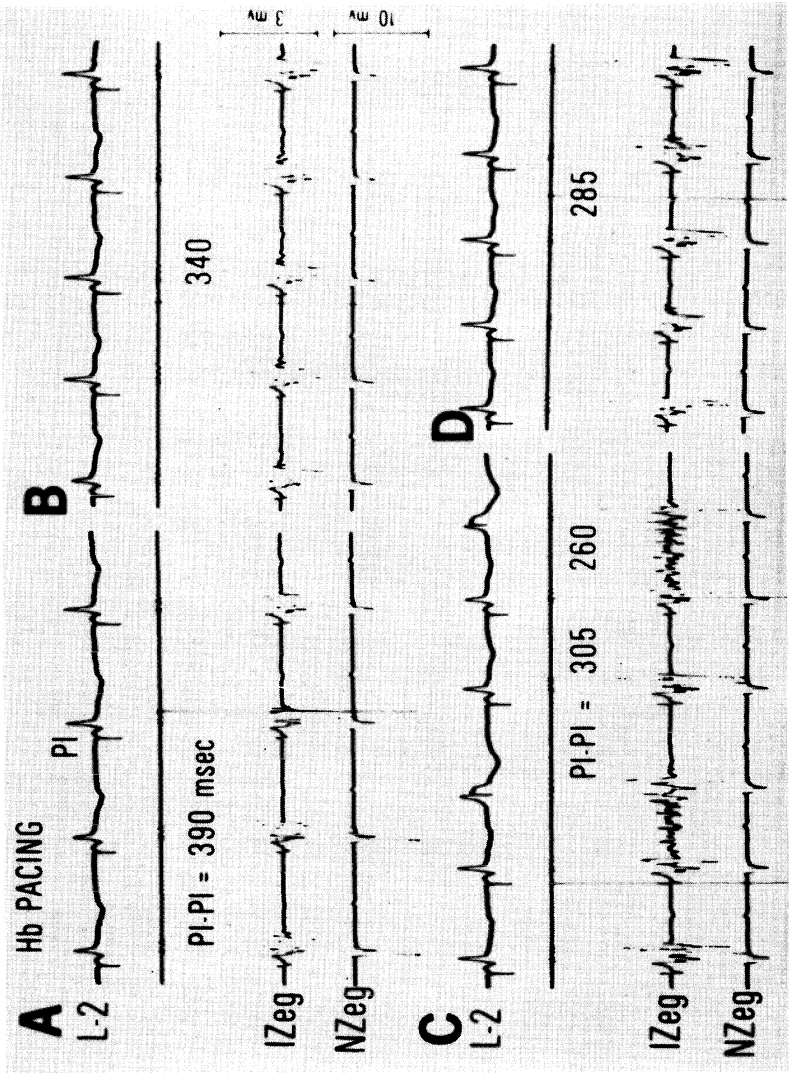

Figure 4. Recordings during pacing of the His bundle 3 min after verapamil (0.5 mg/kg). In the same experiment shown in Figure 3. Pacing of the His bundle at cycle lengths as short as 305 msec (C) caused a Wenckebach pattern of sequential conduction delay in the ischemic zone and reentrant ventricular complexes every 3rd beat. See Figure 3 for an explanation of the abbreviations. (Reproduced by permission of the American Heart Association, Inc., from El-Sherif et al: Circulation 60:605, 1979.)

toriness in the ischemic zone. Consequently, the severity of conduction delay and fractionation must be referenced to the heart rate. The effect of verapamil was to ameliorate the impairment of conduction at any given heart rate. This effect is shown in Figures 3 and 4 from the publication by El-Sherif and Lazzara.

The top set of recordings in Figure 3 (A–C) were made before the administration of verapamil at a driving cycle length of 410 msec. The fractionated electrogram (IZeg) did not extend beyond the T wave and there was no arrhythmia. At a shorter cycle length of 410 msec (3B) there were the rudiments of Wenckebach type of sequence of delayed conduction in the ischemic zone (IZ). These sequences were aborted in the second beat of the sequence when the fractionation extended considerably beyond the T wave and led to an ectopic complex. As a rsult of this repetitive pattern, the surface electrocardiogram showed ectopic complexes every third beat. Further shortening of the cycle length (3C) to 360 msec caused the most delayed portions of the activation pathways to block completely on alternate beats. Block of conduction in the most delayed regions shortened the total activation time of those beats. The blocked portions of the pathways had an effective cycle length (720 msec) twice that of the basic driving cycle length, so there was more time for recovery and better conduction (lesser fractionation and delay) on the other beats. As a consequence, neither of the alternate beats showed sufficiently delayed activation of the ischemic zone to effectuate reentry. After verapamil (Figure 3D–E) there was abbreviation of the duration of the fractionation at any given cycle length. At a cycle length of 390 msec (3E) there was minimal fractionation and delay and no sign of progressive delay of the Wenckebach type. In this experiment attempts to shorten the ventricular cycle length further failed because of AV nodal block (Wenckebach cycles). To circumvent this problem, the His bundle was paced. The results are shown in Figure 4. As the cycle length was shortened to 340 msec (4B) conduction delay in the ischemic zone increased slightly but there was no progressive beat by beat increase until the cycle length was reduced to 305 msec (4C). At this cycle length ectopic complexes occurred every third beat, a pattern similar to that occurring before verapamil at a longer cycle length (390 msec). Further shortening of the cycle length to 285 msec resulted in 2 to 1 block of portions of the activation sequence in the ischemic zone. These recordings demonstrate that verapamil facilitated recovery from activation of ischemic myocardium – in other words, shortened refractoriness. Conclusions about the effect of verapamil on basic responsiveness and conduction cannot be made from these observations. It would be necessary to compare electrograms recorded at slow rates when the cycle lengths exceed the duration of postrepolarization refractoriness.

The data from all the experiments are summarized in graphical form in

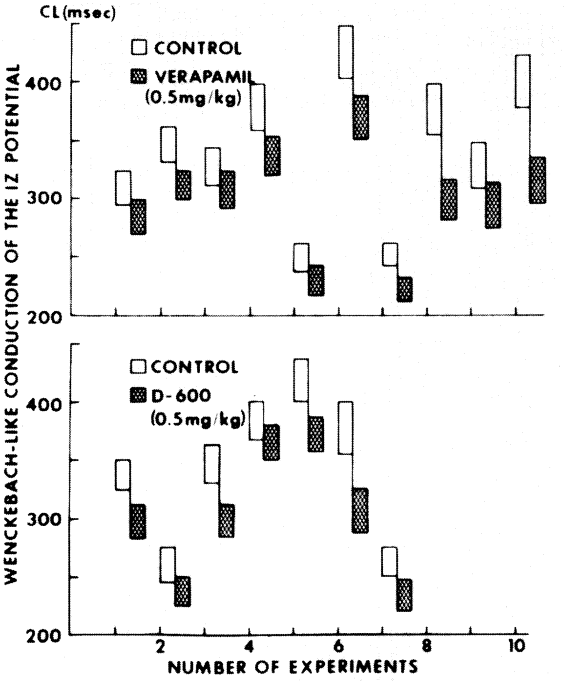

Figure 5. The effects of verapamil and D600 on conduction in the ischemic zone as expressed by the relationship between cycle length and Wenckebach pattern of delay of activation in the ischemic zone. Both compounds improved conduction at any given cycle length with the result that Wenckebach pattern of delay and reentry occurred at shorter cycle lengths. (Reproduced by permission of the American Heart Association. Inc. from El-Sherif et al: Circulation 60:605, 1979.)

Figure 5, which shows the range of heart rates associated with Wenckebach-like conduction delays and reentrant ventricular activation before and after administration of verapamil and D600, a methoxy derivative of verapamil. In these experiments propranolol had insignificant influence on conduction in ischemic myocardium. Since slow current is potentiated by adrenergic agonists [41], this observation also undermines the hypothesis that slow responses are present in this preparation.

Intracellular recordings from ischemic myocardial cells and Purkinje fibers have shown action potentials with very slow upstrokes like the upstrokes of slow responses. The subepicardial layers of ischemic myocardium when isolated in vitro during the period from one day to several weeks after coronary occlusion remain abnormal for hours during superfusion despite the relatively 'normal' environment provided by the superfusate. The persistence of the abnormalities in vitro permits detailed studies of the cellular electrophysiologic properties of these cells. Like the tissues in vivo these isolated preparations also show delayed and irregular conduction greatly sensitive to driving

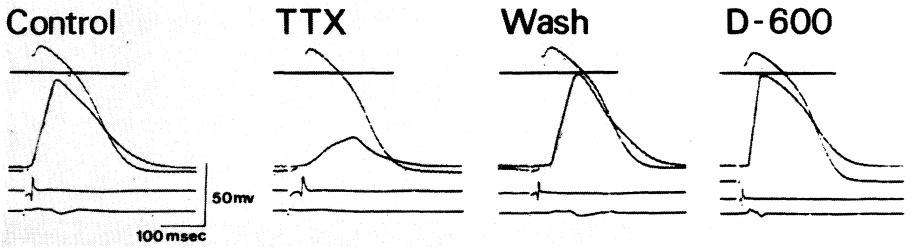

Figure 6. The effects of D600 and tetrodotoxin (TTX) on *apparent* slow responses generated by an abnormal subepicardial myocardial cell in the ischemic zone of tissues isolated three days after coronary occlusion. Action potential of a cell in the adjacent normal region and bipolar electrograms from normal (upper electrogram) and ischemic (lower electrogram) zones are also shown. Tetrodotoxin depressed the action potential of the normal cell, but D600 improved it slightly. The concentration of TTX was 5×10^{-7} g/ml and that of D600 was 10^{-6} g/ml.

rate. When depressed cells with action potentials resembling slow responses were exposed to D600, the upstrokes did not deteriorate further; they usually improved slightly. An illustration of this finding is provided in Figure 6. On the other hand tetrodotoxin, a blocker of rapid current, produced marked suppression of these slow upstrokes (Figure 6). The improvement in the action potentials usually continued even after D600 was washed out of the chamber. This continued improvement is shown in Figure 7. These observations indicated that apparent 'slow responses' were generated by depressed rapid channels in these ischemic cells. The rapid channels (identified by tetrodotoxin sensitivity) were not only depressed in these cells but there appeared to be an alteration in the voltage-dependence of the inactivation gates, since action potentials could be generated at membrane potentials more positive than -55 mV, levels at which the normal rapid channels should be inactive.

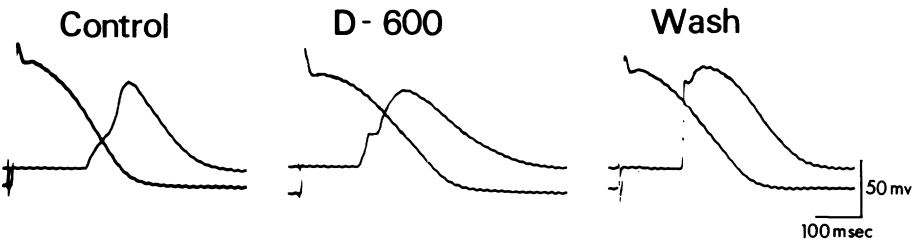

Figure 7. Progressive improvement by D600 in action potentials generated by a cell in the ischemic zone, continuing after washout of D600. Recordings made from epicardial preparation isolated 5 days after coronary occlusion. Action potentials generated by a cell bordering the ischemic zone are also shown. The recordings on the right were made 30 min after D600 was washed out of the chamber. The upstroke of the abnormal cell improved appreciably during the period of washout. The effects of D600 are not washed out rapidly in vitro as illustrated by the action potentials of the bordering cell recorded after 30 min of washout. Repolarization still shows the effects of D600 although there has been regression of the effect toward control in comparison with the middle recordings.

The failure of D600 to depress even the most abnormal action potentials in these ischemic epicardial preparations was consistent. Nevertheless there remains the possibility that true slow responses were present but missed by the microelectrode exploration. We constructed detailed maps of the sequence of activation before and after exposure to D600. These maps did not show regions of slow conduction that were further slowed or blocked by D600. Maps from one representative experiment are shown in Figure 8. Comparison of the map after D600 with the one before demonstrates that conduction in the ischemic region was not retarded by D600 in any portion of the ischemic zone; it was slightly facilitated. On the other hand tetrodotoxin consistently slowed conduction both in the normal and ischemic regions of these preparations. An example of this effect is shown in Figure 9.

Blocking the inward calcium current restored not only the upstrokes but also the abnormal refractoriness of the ischemic cells. After D600 ischemic cells could respond with better action potentials at more rapid rates. Improvement in response of ischemic cells to rapid pacing is illustrated in Figure 10. The marked degradation of response of the cells to rapid facing shown in Figure 10 is characteristic of these preparations. After D600 the response was better even at higher rates. It is apparent that a slow and irregular upstroke is not in itself a reliable indicator of a true slow response. A similar conclusion

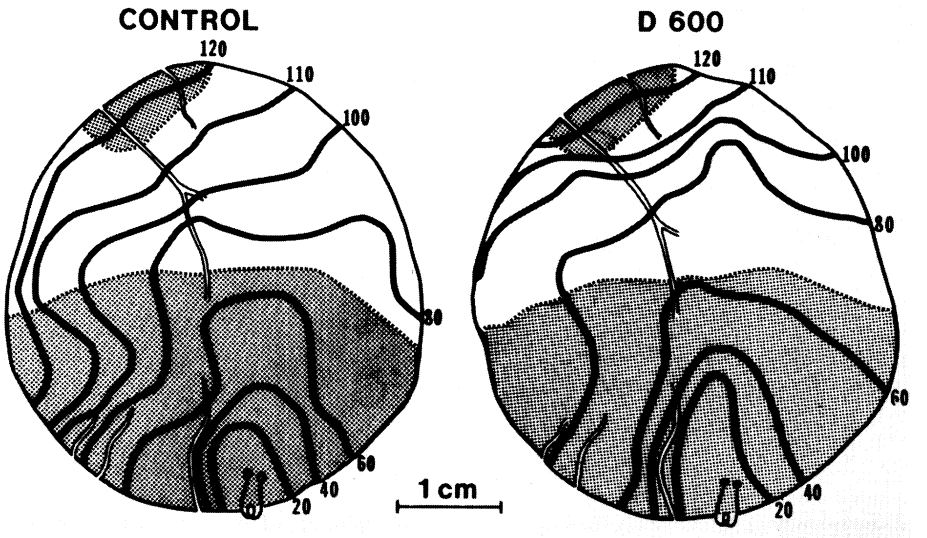

Figure 8. The effect of D600 on the sequence of activation of an epicardial preparation containing ischemic (stippled) and normal zones isolated 3 days after occlusion of a coronary artery. The tissue was driven at a cycle length of 2000 msec from a site in the ischemic zone. The total duration of activation of the preparation (approximately 125 msec) was not changed by D600. The duration of activation of the ischemic zone was decreased.

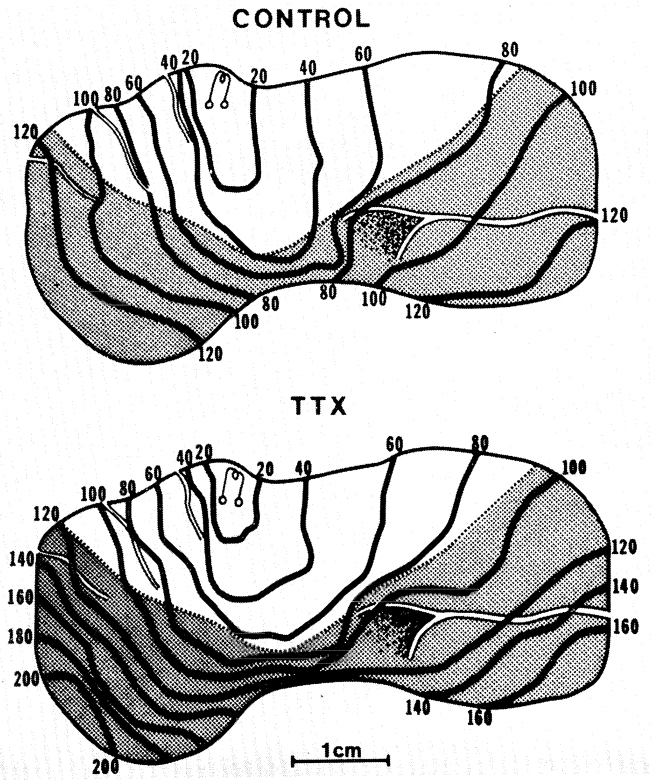

Figure 9. Impairment of conduction by tetrodotoxin (TTX) in normal and ischemic zones of an epicardial preparation isolated 4 days after coronary occlusion. Tetrodotoxin slowed conduction in both normal and infarcted regions but more in the ischemic tissues. The tissue was driven at a cycle length of 2 000 msec.

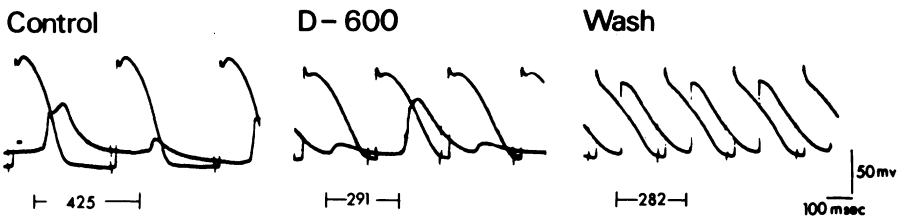

Figure 10. Improvement by D600 of the response of an ischemic myocardial cell to rapid pacing. The epicardial preparation was excised 3 days after coronary occlusion. The pacing cycle length that caused 2:1 block of the abnormal cell was less after D600. The improvement in response continued even after 40 min of washout of D600 (Wash), and one to one response occurrence at an even shorter cycle length.

was offered by Chen and Gettes who found that greatly depressed upstrokes generated by potassium-depolarized cells were sensitive to tetrodotoxin [42].

The improvement of the depressed action potentials of ischemic cells exposed to blockers of slow current is not readily explained. Elharrar and co-workers offered the explanation that alleviation of ischemic damage [43] by 'hemodynamic and metabolic changes' might overshadow the direct electrophysiological effects on the slow channel. In the isolated preparations hemodynamic factors are inoperable but the metabolic effects of reducing calcium entry and cytosolic calcium concentration might have a beneficial effect on the energy balance in the cell. This point will be discussed below.

These findings do not eliminate the possibility that slow responses exist in these preparations in minute regions and form microcircuits for reentry. If this were so, maps of the sequence of activation of the preparation during reentrant excitation should present a series of circumferential waves spreading centrifugally from a focus. In general, this pattern has not been observed on the occasions when the activation sequence was traced during spontaneous rhythms in these preparations. Circuits of activation of appreciable dimen-

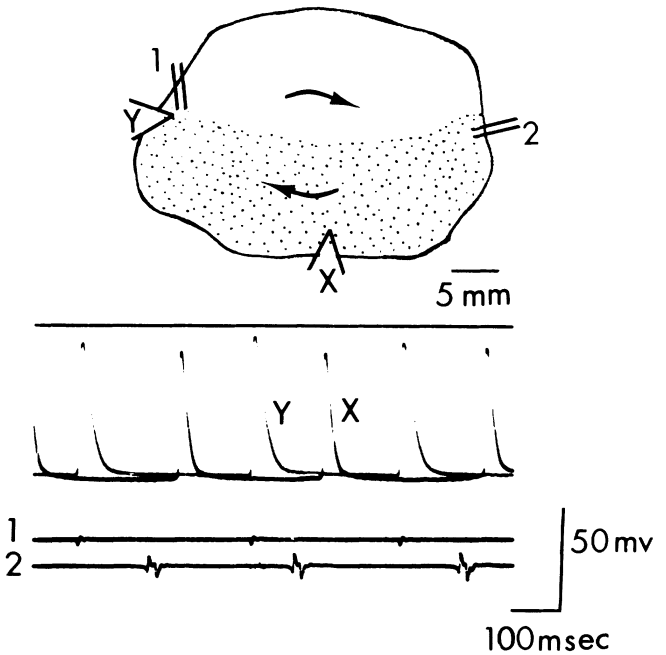

Figure 11. Self-sustaining rhythm induced by rapid pacing of an epicardial preparation isolated 5 days after ligation of the left anterior descending coronary artery. The stippled area at the bottom corresponds to the ischemic zone. The impulse was propagated along a large circuit in the general direction of the arrows. Recordings from other sites besides the ones illustrated verified the general orientation and clockwise rotation of the circuit but the sequence of activation was not traced in detail.

sions could be discerned during these rhythms. In other words, *macro*reentry appears to be the rule. An example of a reentry circuit involving the entire preparation is shown in Figure 11.

The various observations described above suggest the conclusions that depressed action potentials, slow and fractionated activation, and reentry occur in the ischemic subepicardial layers of myocardium in the dog heart in the acute and subacute stages of coronary occlusion; and that the slow calcium current is not the critical factor, rather the 'rapid' sodium current which has been depressed and retarded by ischemia.

The observations and conclusions presented so far apply to circumscribed experimental conditions, namely reentrant ventricular arrhythmias caused by abnormal conduction in ischemic subepicardial layers of myocardium in the dog heart with an occluded coronary artery. The role of the slow current in the abnormal automaticity of Purkinje fibers in the ischemic regions has not been investigated. The slow current could be a factor in diastolic depolarization or the upstrokes of the action potentials of these fibers.

Greatly depressed action potentials sensitive to verapamil have been observed in subendocardial fibers of infarcts taken from human hearts with ventricular aneurysms [44]. These apparent slow responses were generated automatically as well as conducted and stimulated. Figure 12 is reproduced from the report by Spear and co-workers. It shows depressed action potentials identified as slow responses. The investigators concluded that abnormal automaticity was shown by ordinary myocardial fibers as well as Purkinje fibers within the aneurysm. This conclusion was based on gross and microscopic examination of the regions where the recordings were made. The slow responses and abnormal automaticity did not correlate with arrhythmias. None of the patients whose tissues showed these phenomena had consequential arrhythmias. The automatic firing rates in vitro were slow (approximately 45/min in the figure shown) and the slow responses could not be stimulated at rates much above 75/min in the illustration published. The investigators were hesitant to infer that the slow responses they observed in vitro had any role in the generation of arrhythmias during life.

If slow responses play a vital role in the generation of arrhythmias in humans with ischemic heart disease, agents that block slow current would be expected to suppress ischemic arrhythmias. This inference is based on the supposition that the agents would cause block within reentrant circuits or suppression of abnormal automaticity. On the other hand, depression without complete suppression of slow responses might induce or worsen arrhythmias by further slowing conduction and promoting reentry in ischemic myocardium. Most difficult to reconcile with the hypothesis that slow responses cause arrhythmias is the actual clinical observation that blockers of slow current do not affect the ventricular arrhythmias of ischemic heart disease. In

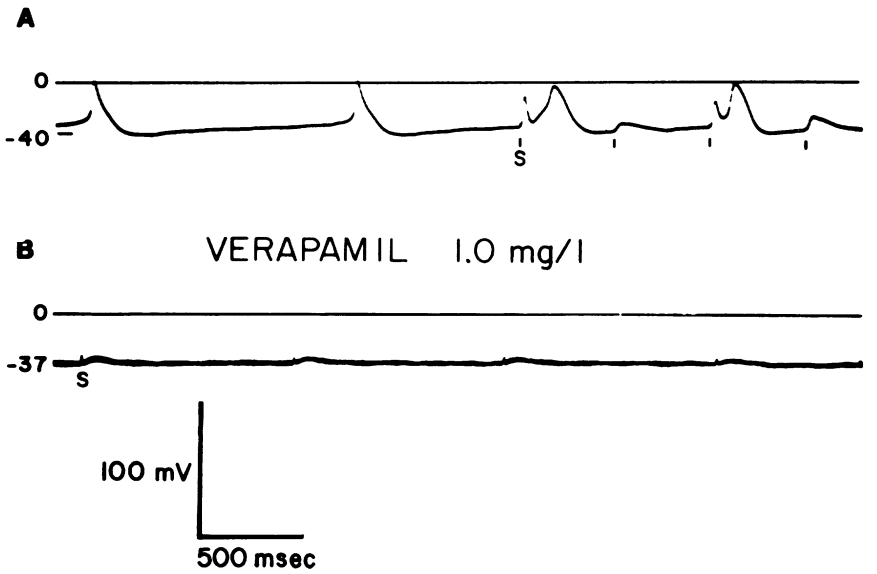

Figure 12. Effect of verapamil on spontaneous and paced action potentials recorded from a subendocardial cell in a human aneurysm. The recording was made from a region containing tissue that histologically appeared to be ventricular myocardium. Verapamil abolished stimulated and spontaneous responses. At a paced cycle length of 450 msec, this cell responded every other beat. (Reproduced by permission of the American Heart Association, Inc. from Spear JF et al: Circulation 59:247, 1979.)

general clinical investigators have reported that verapamil had little if any effect in early trials on ventricular arrhythmias in patients with ischemic heart disease [45–47], although dissident observations have recently appeared [48].

The failure of experimental and clinical observations so far to substantiate an essential role for the slow current in the arrhythmias of ischemia, prompts consideration of the relationships between slow current and metabolism. There are numerous observations of depression of slow current and slow responses by hypoxia, metabolic inhibition, ischemia and excess hydrogen ion [49–54]. There are indications of a direct relationship between the magnitude of the slow current and the intracellular concentrations of cyclic adenosine 3′,5′-monophosphate (cAMP) and ATP [55]. It has been postulated that cAMP mediates the phosphorylation by a protein kinase of specific sites on the membrane to open slow channels [55–57]. ATP provides both cAMP and high energy phosphate. Some have offered the hypothesis that ATP may facilitate inward calcium current by chelating calcium near the inner surface of the membrane, thereby augmenting the effective gradient of concentration [50]. There is rapid and pronounced fall in the concentration of ATP in myocardial cells during ischemia [58]. With continued ischemia there must

occur eventually a fall in cAMP, but some investigators have reported a transient initial rise (xithin 1–3 min) of this compound caused by local catecholamine release [59]. The depletion of these compounds could account for the depression of slow current during hypoxia, metabolic inhibition and ischemia, except perhaps during the very early stages of ischemia when cAMP may be high, and ATP not yet fallen to very low levels. Depression of inward calcium current may protect the cell during ischemia by mitigating the rise of cytosolic free calcium which occurs because of lack of energy for the uphill transport of calcium into the sarcoplasmic reticulum and extracellular space [55, 60]. Rise in cytosolic free calcium promotes the consumption of ATP by activating various reactions that use ATP, including the contractile mechanism, hastening the drop in ATP to lethal levels. The fall of ATP during ischemia brakes itself by reducing calcium inflow. This interplay between cytosolic calcium and ATP during ischemia may be the basis for the improvement of electrophysiologic abnormalities and of other signs of ischemic damage by blockers of slow current.

If these consideratins are valid the failure to demonstrate involvement of slow responses in ischemic arrhythmias is understandable. Perhaps their detection in human aneurysms reflects adequate energy supply of subendocardial cells from chamber blood (or from the superfusate) coexisting with partial depolarization of the fibers due to stretch. In any case the observation of slow responses in human aneurysms cautions against a hasty dismissal of the importance of slow responses in ischemic arrhythmias and advocates continuing investigation of the question. The numerous complex metabolic changes that result directly and indirectly from ischemia are not completely described and understood. At this stage of incomplete understanding, abnormally functioning rapid channels appear to be more important in the generation of arrhythmias than slow responses, but all conditions have not been explored.

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19. DIGITALIS-INDUCED DELAYED AFTERDEPOLARIZATIONS *

MICHAEL R. ROSEN and PETER DANILO, JR.

Until recently, descriptions of mechanisms for cardiac arrhythmias usually referred to two mechanisms; conduction block and/or enhanced automaticity [1]. With respect to digitalis, conduction block was assumed to result from a combination of the direct effects of digitalis on cardiac fibers and, particularly at the atrioventricular node the indirect effects of digitalis (both parasympathomimetic and sympatholytic). The enhanced automaticity was assumed to result from increased phase 4 depolarization.

In the early 1970's several groups of investigators described oscillations in membrane potential induced by digitalis [2–6] that, although depicted in earlier experimental records [7, 8], had not explicitly been studied. These reports have necessitated a reconsideration of the mechanisms whereby digitalis induces arrhythmias. The oscillations described probably do not result from the slow inward current that induces the slow response action potential and is the subject of this book. Rather, they appear to be due to a different spectrum of ionic events as shall be described subsequently.

Before discussing these newer observations, it would be worthwhile to review earlier information concerning the actions of digitalis on cardiac fibers. The effects of digitalis on normal cardiac fibers have been studied in a variety of tissues including sinus node, atrium, AV node and ventricle. Perhaps the most intensive study has been of fibers in the Purkinje system. Here, low concentrations of digitalis have little effect [9]. Their major action appears to be a change in the repolarization phase of the action potential, initially prolonging and subsequently decreasing it. This action has been attributed to an initial decrease followed by a subsequent increase in potassium permeability [10]. Similar effects of digitalis have been shown to occur in ventricular myocardium.

The effects of digitalis on atrial specialized fibers and on the sinus and AV nodes are somewhat different; these are mediated through the parasympathetic nervous system as well as directly. Digitalis has been shown to depress impulse initiation in sinus node cells without changing transmembrane poten-

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tial significantly [11]. Moreover, sinus slowing induced by digitalis is blocked by atropine [12]. This action is attributed to a parasympathomimetic effect of digitalis mediated by the vagus nerve [13]. Digitalis also induces conduction block in the region of the AV node [11]. This action although attributed in part to cholinergic mechanisms [9] is also accompanied by a decrease in the resting potential, action potential amplitude and upstroke velocity [11]. The latter effects are thought to be direct actions of digitalis. The sympatholytic effect of digitalis would tend to enhance its parasympathomimetic actions in the atrium and AV node.

Digitalis has additional effects on diseased fibers. In a study of ouabain action on diseased human atrial fibers Hordof et al. [14] superfused fibers that were markedly depolarized with ouabain, 2×10^{-7} M. Although this concentration ultimately had toxic effects it initially hyperpolarized the fibers, decreased phase 4 depolarization and depressed spontaneous impulse initiation. The hyperpolarization also was associated with normalization of conduction throughout the atrial myocardium. These actions all were blocked by atropine.

When cardiac fibers are superfused with toxic concentrations of digitalis several changes occur. Most prominent, perhaps, is a marked decrease in the resting membrane potential [15]. This effect is concentration-dependent and if sufficient concentrations are maintained for sufficient periods of time the fiber ultimately will attain a low level of resting potential and become inexcitable. The change in resting potential has been attributed to the poisoning of Na, K-ATPase which results in intracellular accumulation of sodium and loss of potassium from the cell [16, 17]. As resting potential decreases in the presence of digitalis so do action potential duration, amplitude and upstroke velocity. In some preparations there is also an increase in the slope of phase 4 depolarization and the onset of automaticity [8]. The latter occurs most frequently at low extracellular potassium concentrations. It has been our experience and apparently that of other investigators that the effect of digitalis on the slope of phase 4 is best seen in situations where stimulus cycle length is long [18].

Of major interest in recent years have been the oscillatory changes in membrane potential induced by digitalis. These events were described nearly simultaneously by investigators at several institutions and were initially called enhanced diastolic depolarization [2], transient depolarizations [5, 6] or low amplitude potentials [3, 4]. Subsequently two parallel terminologies have been developed: the descriptors used now are oscillatory afterpotentials [19] or delayed afterdepolarizations [20]. The latter term will be used here.

The most extensive study of digitalis-induced delayed afterdepolarizations has been in canine Purkinje fibers. However, studies of canine [21] and human [14] atrium and canine ventricular myocardium [22] have been reported

as well. It appears that the observations made for canine Purkinje fibers hold for the other tissues and so the Purkinje fiber will be used as the major example here.

Delayed afterdepolarizations commence when Purkinje fibers have been depolarized to approximately -70 to -85 mV. They are seen *only* following an action potential (i.e., they are dependent on the preceding action potential for their initiation) and may occur singly or as trains of two or more (Figure 1). Although Figure 1 presents what most investigators would accept as being 'typical' delayed afterdepolarizations, it is important to recognize that the oscillatory behavior which occurs as a result of the digitalis effect is not always as clear-cut. For example, in Figure 2 are presented records of oscillatory activity in a Purkinje fiber following cessation of drive at three different cycle lengths. In the upper panel, at CL = 320 msec, the first event following cessation of the drive appears to be a 'slow response' action potential, having an amplitude as great as that of the action potential plateau. This is followed by two delayed afterdepolarizations. In the second panel, at CL = 320 msec, the initial oscillation is not as great as that seen in the first panel. Is this oscillation a delayed afterdepolarization or is it a variant of the slow response action potential? From the records presented it is difficult, if not impossible, to tell. Similarly, it is difficult to state to what extent such an event is due to the transient inward current which is presumed responsible for delayed afterdepolarizations (see below) as opposed to the slow inward current to which the slow response is attributed. At cycle lengths of 250 and 200 msec, cessation of the drive is followed by action potentials, and these in turn are

Figure 1. Delayed afterdepolarizations. In this and all subsequent figures, delayed afterdepolarizations were induced by approximately 30 min of superfusion with ouabain, 2×10^{-7} M. The first three action potentials in this sequence are driven. After the third action potential the drive stimulus is discontinued and three delayed afterdepolarizations of progressively decreasing magnitude are seen. The membrane then hyperpolarizes and there is quiescence. Vertical calibration = 20 mV; horizontal 1 sec. Maximum diastolic potential = -82 mV. The amplitude of the first delayed afterdepolarization in the sequence is measured from the maximum diastolic potential to the peak of the afterdepolarization (solid arrows); the amplitude of the second, from the membrane potential at which it is initiated to its peak voltage (broken arrows). Coupling of delayed afterdepolarizations is measured from the peak of the preceding action potential to the time of peak amplitude.

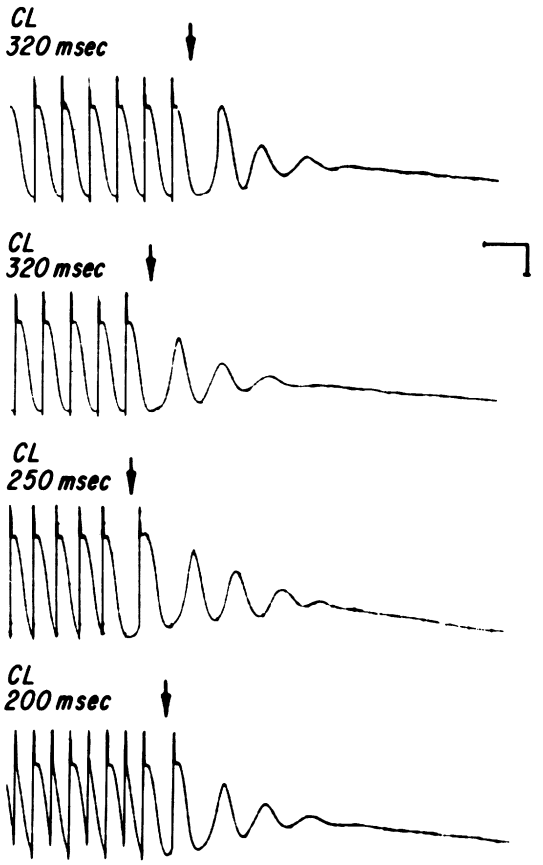


Figure 2. Purkinje fiber driven at cycle lengths of 320, 250 and 200 msec. The arrows indicate discontinuation of the drive stimulus. Vertical calibration = 25 mV; horizontal = 500 msec. Maximum diastolic potential in upper panel is −79 mV. For discussion, see text.

followed by delayed afterdepolarizations of decreasing magnitude. Again, the amplitudes of the initial afterdepolarizations are sufficiently great to question whether they are in fact 'classic' afterdepolarizations or a variant of slow responses.

The relationship between delayed afterdepolarization amplitude and cycle length to the drive cycle length for a preparation is reviewed in Figure 3A. Note that, as stimulus cycle length decreases, the amplitude of the first delayed afterdepolarization in the sequence increases, reaching a peak at a cycle length of 600 msec. It then diminishes in magnitude. The amplitude of the second delayed afterdepolarization continues to increase as cycle length decreases until it attains threshold potential and initiates propagated action potentials (indicated by asterisks).

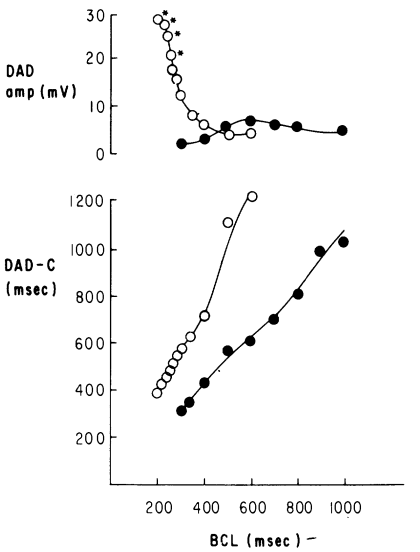


Figure 3A. The relationship between delayed afterdepolarization amplitude and coupling interval to drive cycle length for a ouabain-superfused Purkinje fiber bundle. The ordinates are delayed afterdepolarization amplitude in mV (upper) and delayed afterdepolarization coupling interval in msec (lower). The abscissa is the drive cycle length for the preparation. The filled circles represent the initial delayed afterdepolarization in a sequence; the unfilled circles represent the second delayed afterdepolarization in a sequence; the asterisks indicate those afterdepolarizations which attain threshold potential and initiate action potentials.

The coupling of delayed afterdepolarizations to the preceding action potential is also conditioned by the drive cycle. It is approximately equal to the drive cycle length for the first delayed afterdepolarization in the sequence, and approximately twice the drive cycle length for the second (in the case of three delayed afterdepolarizations, the interval for the third would be approximately 3 times the drive cycle length). Delayed afterdepolarizations tend more to attain threshold potential and initiate spontaneous rhythms at short rather than at long cycle lengths. Ferrier et al. [5] have stated that the greatest frequency of arrhythmias induced by delayed afterdepolarizations is at cycle lengths of about 600 msec. Our own observations concur with these with respect to the occurrence of *sustained* arrhythmias induced by delayed afterdepolarizations. However, almost all preparations we have studied generate single action potentials or brief bursts of action potentials at cycle lengths of 200–300 msec.

It may appear from Figure 3A that the initial delayed afterdepolarization which diminishes in magnitude at CL $\leq$500 msec ultimately 'disappears.' In fact, what happens is that the initial afterdepolarization, as it occurs earlier, simply 'moves into' phase 3 and arises simultaneously with the repolarization

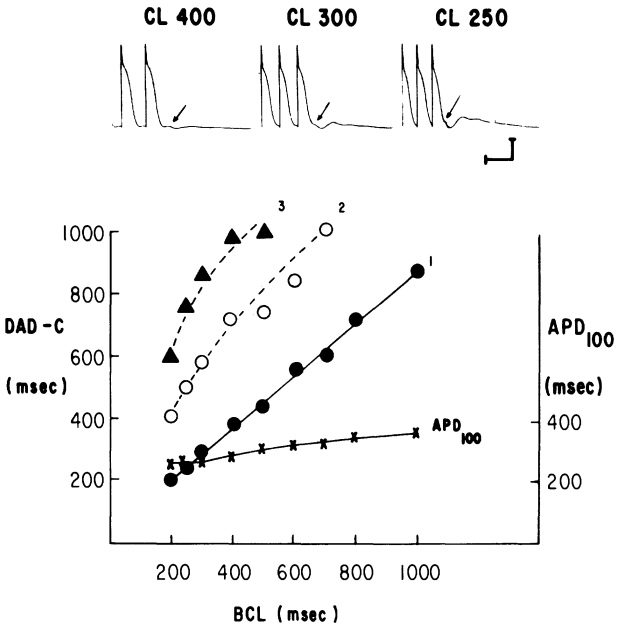


Figure 3B. The upper panels show the relationship between the first, second and third delayed afterdepolarizations in a sequence and the repolarization phase of the action potential. The ordinate on the left is the delayed afterdepolarization coupling interval in msec. The ordinate on the right is the action potential duration measured to full repolarization in msec. The abscissa is basic cycle length in msec. The filled and unfilled circles and filled triangles are respectively the first, second and third afterdepolarizations in a sequence. The crosses represent action potential duration measured to full repolarization. Note that the lines connecting the data points for the first afterdepolarization in a sequence and the action potential duration measured to full repolarization intersect indicating that at very short cycle lengths the first afterdepolarization is occurring before full repolarization in the fiber. This sequence of events is shown in the inserts at cycle lengths 400, 300 and 250 msec indicated by the arrow. Vertical calibration for the inserts is 25 mV, Horizontal calibration is 400 msec. MDP = −83 mV.

phase of the inciting action potential. As shown in Figure 3B, the first afterdepolarization in a sequence occurs after full repolarization at a cycle length of 400 msec, and before full repolarization at shorter drive cycle lengths. As the first afterdepolarization becomes concurrent with phase 3 it decreases in magnitude, and the second delayed afterdepolarization increases in magnitude.

Just as sustained drive at varying cycle lengths affects the magnitude and coupling of delayed afterdepolarizations, so does the occurrence of single premature depolarizations. However, the relationship here is more complex than that for the sustained drive (Figure 4). For example, whereas driving a preparation at a cycle length of 200 msec results in a delayed afterdepolarization having a cycle length of 440 msec, the delivery of a single premature beat

of 200 msec cycle length to a preparation which is being driven at 600 msec, results in a delayed afterdepolarization whose cycle length is 480 msec (i.e., between the drive and premature cycle lengths). In other words, the coupling interval of the delayed afterdepolarization is shorter than that seen at drive cycle length = 600 msec. This observation suggests that the occurrence of a single premature depolarization will result in a change in delayed afterdepolarization characteristics from those of the basic drive. In effect, a 'window' is established, bounded on the one hand by the basic drive cycle, and on the other by the coupling interval that would be established by the premature cycle *if it were the basic drive cycle.* The coupling interval of the delayed afterdepolarization falls within this range. This means that if one were attempting to predict the time of occurrence of a delayed afterdepolarization-induced beat in the presence of single premature depolarizations, one would be able to select a *range* of cycle lengths within which it would occur, but defining the interval more precisely does not appear possible with our present knowledge.

When premature beats occur in sequence, then the delayed afterdepolarization coupling interval and amplitude become less similar to those that occur during the control cycle length and more like that of the premature beat. Following approximately 5–10 'premature' beats in sequence, the coupling interval and amplitude of the delayed afterdepolarizations are identical to those seen when preparations are driven at drive cycle lengths equal to the premature coupling interval.

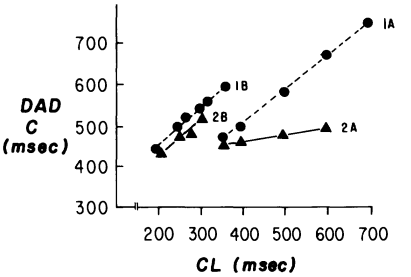


Figure 4. Relationship between the coupling interval of ouabain-induced delayed afterdepolarizations (DAD–C in msec on the ordinate) and the drive cycle length for the preparation(on the abscissa). Curves 1A and 1B refer respectively to the first and second delayed afterdepolarizations in a sequence occurring at the drive cycle lengths indicated. Curves 2A and 2B show the result of interposing a single premature depolarization of cycle length 200 msec in the drive cycle. Note that for very short cycle lengths (i.e., less than 350 msec) the curve for a single premature and that for the second delayed afterdepolarization in the sequence are nearly superimposable. At longer drive cycle lengths, however, the coupling interval of the afterdepolarization following a single premature beat at cycle length 200 msec is intermediate between that which would occur were 200 msec the basic cycle length for the preparation and the drive cycle length of the preparation itself (modified after Rosen et al: Can accelerated junctional escape rhythms be explained by delayed afterdepolarizations, Amer J Cardiol 45:1272-1284, 1980).

Once delayed afterdepolarizations attain threshold potential, they initiate action potentials which can occur singly or as trains of impulses. If a sustained arrhythmia occurs it often increases in rate over several cycles. However, the rate it ultimately attains may be stable or variable. A major factor determining the variability in rate appears to be the events ocurring in electrical diastole from one cycle to the next. Whether these events reflect changes in the delayed afterdepolarization or in some other ionic current flowing during phase 4 is not certain. As shown in Figure 5, a change in the rate of depolarization during phase 4 (possibly due to a change in amplitude and/or slope of a delayed afterdepolarization) may not only cause variations in spontaneous rate, but may also modify the type of action potential that occurs. In Figure 5 the impulses initiated at a less negative membrane potential (associated with changes in phase 4) have lost much of the rapid phases of their upstrokes and might, as a result, be expected to propagate more slowly than the other action potentials. The expected result would be not only a change in the rate of impulse initiation, but in its conduction velocity as well.

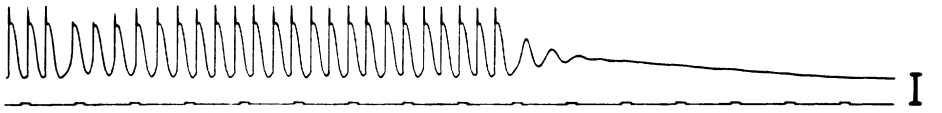


Figure 5. The effects of changes in the voltage–time course of phase 4 on the subsequent action potentials. Following the third beat in the sequence there is a change in the amplitude and slope of phase 4 (which may or may not be due to changes in a delayed afterdepolarization, see text). The result is an action potential which arises later, at a lower level of membrane potential and with a lower amplitude and overshoot than occurred previously. Following several cycles the membrane hyperpolarizes again resulting in a changed slope during phase 4 and an increase in action potential amplitude. Subsequently an afterdepolarization fails to reach threshold and electrical activity ceases. Vertical calibration is 40 mV; horizontal calibration is 1 sec. MDP = −80 mV.

Cessation of delayed afterdepolarization-induced tachyarrhythmias may occur either suddenly, with failure of a delayed afterdepolarization to reach threshold, or gradually with a decrease in spontaneous rate and, finally, failure of delayed afterdepolarizations to reach threshold (Figure 6). Attempts to terminate the tachyarrhythmias by overdrive or by single premature beats may have several results. With respect to interpolation of a single premature depolarization, we have found that it is difficult and often impossible to bring about cessation of an afterdepolarization-induced rhythm. The usual occurrence is a transient resetting of the rhythm (Figure 7, top). Attempts to end the rhythm with overdrive may meet with two possible results: there usually is 'capture' of the rhythm by the overdrive pacemaker. On cessation of the

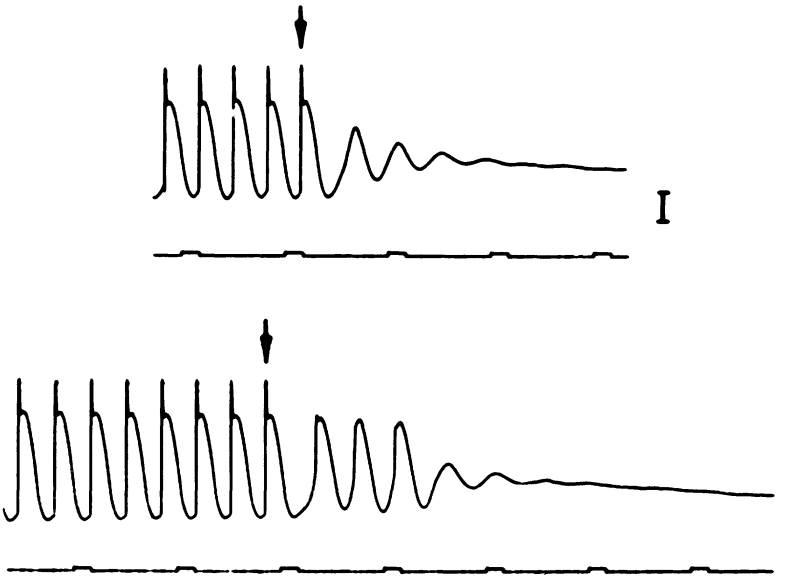


Figure 6. Two mechanisms for cessation of delayed afterdepolarization-induced rhythms. In the upper panel, following 5 afterdepolarization-induced beats occurring at roughly the same cycle length, the 6th delayed afterdepolarization fails to reach threshold. Subsequently afterdepolarizations of diminishing magnitude occur and there is no further electrical activity. In the lower panel afterdepolarization-induced action potentials of approximately equivalent cycle lengths occur until after the 8th beat, when a longer interval for the afterdepolarization is followed by an action potential arising at a lower level of membrane potential and having a small overshoot. The cycle length for this and the subsequent 2 action potentials which appear to have the characteristics of the slow response are quite variable. Subsequently an afterdepolarization fails to attain threshold and electrical quiescence follows. Vertical calibration 25 mV; horizontal axis, 1 sec. MDP = −78 mV.

drive stimulus, the preparation may have been 'entrained' by the overdrive, resulting in maintenance of the overdrive rate *or* gradual slowing of rate to that of the initial tachyarrhythmia. If 5–10 beat bursts at very fast overdrive rates are applied (CL \200 msec) the arrhythmia usually can be halted. In this instance, cessation of the overdrive pacemaker usually is followed by the spontaneous occurrence of 1–3 beats, at varying cycle lengths and then by quiescence (Figure 7, bottom).

Even when delayed afterdepolarizations do *not* attain threshold, they modify conduction. As stated above, delayed afterdepolarizations can vary in magnitude from one cycle to the next. As their amplitude changes so does the level of membrane potential at which action potentials are initiated. This in turn modifies the amplitude and upstroke velocity of successive action potentials and their velocity of propagation. Under appropriate circumstances conduction may be blocked completely. A number of variations on the changes in conduction that are induced by delayed afterdepolarizations are

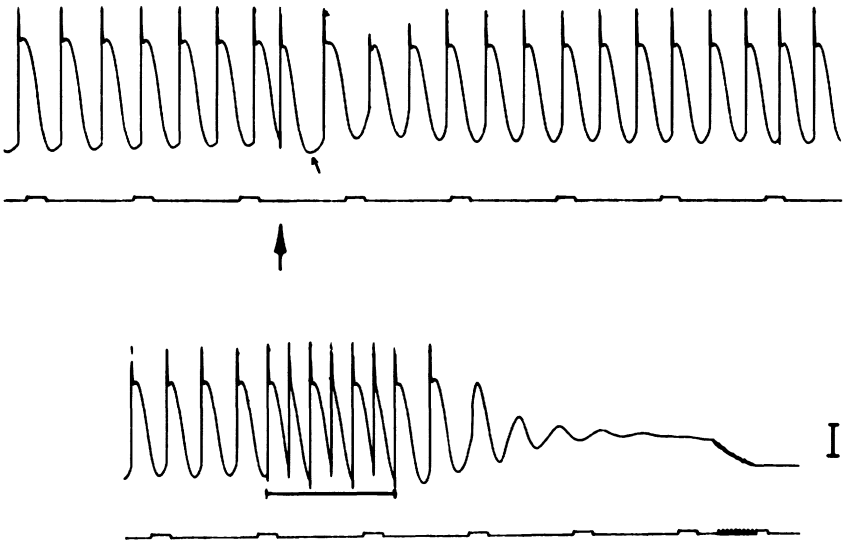
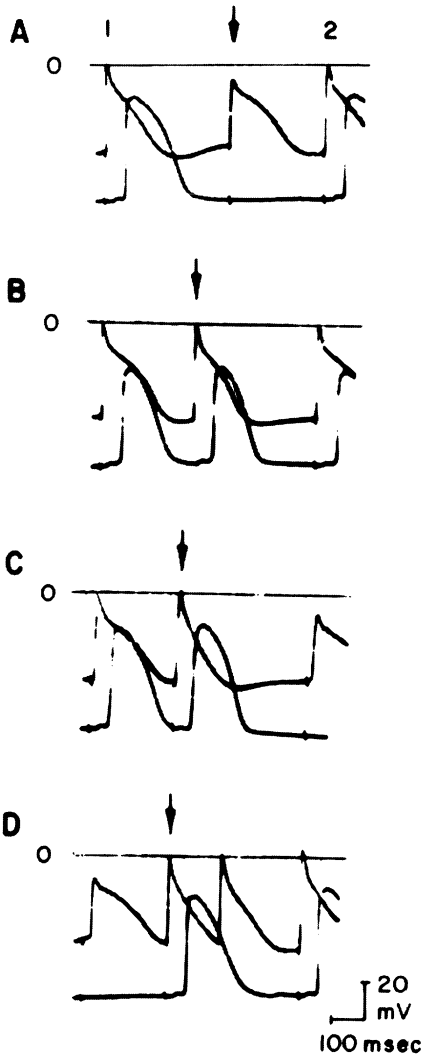


Figure 7. The effects of single premature depolarizations and short bursts of premature depolarizations on afterdepolarization-induced rhythms. In the upper panel a delayed afterdepolarization-induced stable rhythm is occurring. At the large arrow a single premature depolarization is induced. This is followed by an interval that is less than twice the coupling interval for the preceding afterdepolarization-induced beats but more than the ectopic interval. The subsequent few cycles are irregular but the afterdepolarization-induced rhythm than becomes regular again. In the lower panel an afterdepolarization-induced rhythm is interrupted by a burst of 7 driven action potentials occurring at a short cycle length (indicated by horizontal line). Cessation of the drive stimulus is followed by a propagated action potential having characterestics similar to those prior to the drive. The next action potential has characteristics of the slow response and subsequent afterdepolarizations fail to reach threshold and gradually decrease in magnitude. Vertical calibration 25 mV; horizontal calibration, 1 sec. MDP = −85 mV (small arrow, upper panel).

Figure 8. The effect of delayed afterdepolarizations on propagation of action potentials. The upper trace in panels A–D is recorded from a Purkinje fiber bundle, the lower trace is from a myocardial fiber distal to the Purkinje fiber bundle. The numbers 1 and 2 indicate the drive cycle length; the arrow indicates a premature depolarization induced in the Purkinje fiber at various intervals following the basic drive, and therefore at different levels of membrane potential as dictated by the amplitude of the delayed afterdepolarizations. In panel A the initial action potential propagates to the myocardium. The prematurely induced beat occurs at a lower level of membrane potential, has a low amplitude and fails to propagate. The second driven action potential, occurring at a high level of membrane potential propagates to the myocardium. The premature beat, then, results in concealed conduction in the Purkinje system. In panel B the premature depolarization is occurring earlier than in A, and therefore before the afterdepolarization has had a chance to attain peak magnitude. As a result, it occurs at a higher level of membrane potential and has a higher amplitude than previously, and propagates to the myocardium as do both the basic drive beats. In panel C the premature beat is occurring still earlier in the cycle. The first basic drive beat propagates to the myocardium as does the premature depolarization. The subsequent basic drive occurs at a low level of membrane potential as the



result of the afterdepolarization generated by the premature response, and it fails to propagate to the myocardium. Both B and C might be considered as different examples of bigeminy. In C there is, as well, concealed conduction of the second basic drive beat in the Purkinje system. In panel D the initial drive beat is identical to that in panel C. The premature beat occurs still earlier in the cycle and propagates to the myocardium. Subsequently there is propagation of the impulse from the myocardium back to the Purkinje system resulting in another depolarization seen on the Purkinje fiber record. As a result of this action potential the subsequent basic drive, which ordinarily might have been expected to occur at a low level of membrane potential and be blocked, occurs at a high level of membrane potential and is propagated to the Purkinje system. Here then we have an example of probable concealed reentry within the Purkinje system and subsequent facilitation of conduction of the next basic drive beat (reprinted, by permission of the American Heart Journal, Inc. from Rosen MR et al: Electrophysiology and pharmacology of cardiac arrhythmias. Amer Heart J 89:397, 1975).

reviewed in Figure 8. The types of patterns that have been described include bigeminy, concealed conduction and Wenckebach periodicity (not shown).

The ionic events responsible for delayed afterdepolarizations initially were studied by Ferrier and Moe using $MnCl_2$ [23] and Rosen et al. [24] using verapamil. Both studies suggested that an inward Ca^{++} current was important to the occurrence of delayed afterdepolarizations. More recently, the putative Ca^{++} blocker, AHR-2666, has been shown to depress delayed afterdepolarizations as well [25].

Lederer and Tsien [26] and Aronson and Gelles [27] have used a voltage clamp technique to study the ionic mechanisms responsible for delayed afterdepolarizations. Both groups agreed that a transient inward current is responsible for delayed afterdepolarizations and that this current is either superimposed on or 'supercedes' the normal i_{K2} pacemaker. Review of our own data shows that, in most fibers which initially depolarize during phase 4, development of delayed afterdepolarizations in the presence of digitalis is associated with a decrease in the slope of phase 4 (Figure 9). In other instances, however, ouabain both induces delayed afterdepolarizations and *increases* the

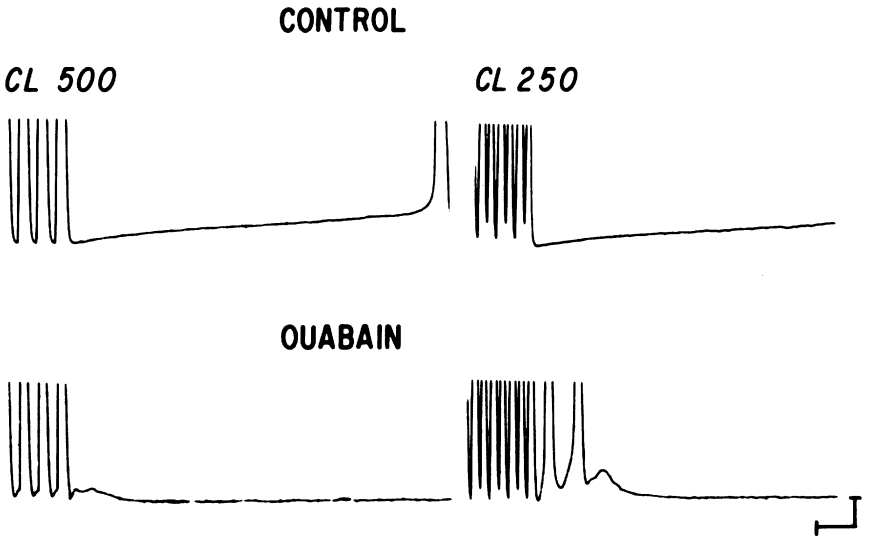


Figure 9. The upper panels show records from a Purkinje fiber bundle driven, on the left, at cycle length 500 msec, and on the right, at cycle length 250 msec. In both of these panels cessation of the drive is followed by depolarization during phase 4. Maximum diastolic potential here is −96 mV. Following superfusion with ouabain (the lower two panels), maximum diastolic potential is decreased to −84 mV. In the left lower panel, at a cycle length of 500 msec, 2 delayed afterdepolarizations occur on cessation of the drive and are followed by quiescence. There no longer is phase 4 depolarization. In the right lower panel, after driving at a cycle length of 250 msec, cessation of the drive is followed by 2 afterdepolarizations which initiate action potentials. Subsequently there is a single afterdepolarization and then repolarization of the membrane. Again there is no phase 4 depolarization. Vertical calibration is 15 mV; horizontal calibration is 1 sec.

slope of phase 4 (Figure 10). Those experiments suggest that, whereas digitalis usually induces delayed afterdepolarizations, it may either diminish or enhance the slope of phase 4 depolarization. Whether the effect on phase 4 is the result of digitalis induced changes in i_{K2} or some other mechanism is uncertain. That the increased automaticity is *not* the result of the i_{K2} associated mechanism to which normal Purkinje fiber pacemaker activity is attributed [28], is suggested by the fact that the resultant automaticity is enhanced rather than suppressed by overdrive. (Because overdrive suppression would be expected with a pacemaker generated by i_{K2}, it is reasonable to assume that it is an abnormal automatic mechanism that is being activated by digitalis in this instance.) Tse and Han [18] found that phase 4 depolarization induced by digitalis is depressed by $MnCl_2$ and by verapamil. This is consistent with our

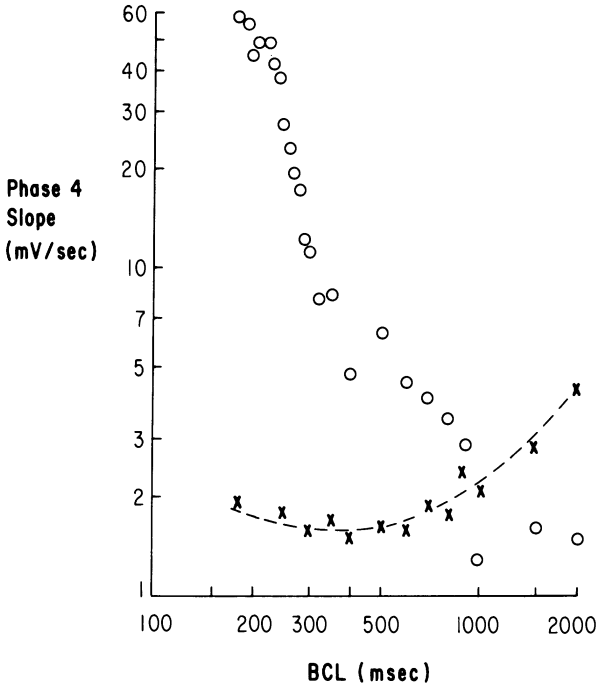


Figure 10. Enhancement of phase 4 depolarization as a result of superfusion of a Purkinje fiber with ouabain. The vertical axis is the slope of phase 4 depolarization in mV/sec; the horizontal axis is the drive cycle length in msec. The crosses represent the slope of phase 4 depolarization during control. The line is fit to the data points by eye. Note that at cycle length 2000 msec the slope of phase 4 depolarization is 4.6 mV/sec but at cycle lengths of 500 or less msec the slope of phase 4 has decreased. Hence, there has been overdrive suppression of the pacemaker. The unfilled circles represent the slope of phase 4 depolarization following ouabain superfusion. Note that there is overdrive enhancement of the slope of phase 4 depolarization, with an increased slope of phase 4 at short cycle lengths. At cycle lengths shorter than 800 msec phase 4 depolarization resulted in an attainment of threshold potential and a spontaneous rhythm.

own study of verapamil [24]. Tse and Han suggested that the mechanism for phase 4 depolarization that occurs as a result of ouabain effect is abnormal and may be due to an inward current carried by Ca^{++}.

Tsien and Carpenter [29] have attempted to define the sequence of ionic events involved in the transient inward current responsible for delayed afterdepolarizations. They suggest that, as digitalis inhibits pump-related Na^+ transport, resulting in intracellular Na^+ accumulation, there is a reduced gradient for Na^+ entry and, in consequence, a decrease in Ca^{++} extrusion via Na^+–Ca^{++} exchange. They suggest that this event, and possibly release of Ca^{++} from mitochondria, results in an increase in intracellular Ca^{++} from the sarcoplasmic reticulum resulting both in the afterdepolarization and an accompanying aftercontraction. This underlying oscillation of Ca^{++} is assumed to increase cation conduction, resulting in Na^+ entry which induces the afterdepolarization, itself. Tsien and Carpenter [29] were able to decrease the magnitude of the transient inward current that accompanies the afterdepolarization by lowering the Na^+ content of their superfusate, and assumed therefore that Na^+ was an important contributor to the afterdepolarization. Although they and Kass et al. [30] reported that the transient inward current was TTX-insensitive, preliminary results by Vassalle and Scida [31] and by us [32] and a preliminary reference to additional work by Kass et al (in ref. [33]) have shown that TTX does diminish the magnitude of the delayed afterdepolarizations. Whether it exerts this action directly on the transient inward current or by depressing sodium entry during phase 0 of the action potential, thereby decreasing intracellular Na^+ accumulation (the event that predisposes to intracellular Ca^{++} accumulation in Tsien and Carpenter's hypothesis), is uncertain.

Other agents, too, modify the occurrence of delayed afterdepolarizations. Acetylcholine depresses digitalis-induced delayed afterdepolarizations occurring in atrial tissue [21]. The mechanism here is uncertain but possibilities include [1] the fact that ACh, itself, can block Ca^{++} current [34] and (2) the fact that ACh hyperpolarizes cardiac fibers. Because the occurrence and magnitude of delayed afterdepolarizations are determined at least in part by the level of membrane potential at which they are initiated the hyperpolarization induced by ACh may be a major factor in decreasing their amplitude.

Another agent which decreases the magnitude of delayed afterdepolarizations is lidocaine [32]. The basis for this action is unclear. Again two possibilities exist. On the one hand, lidocaine depresses the amplitude and upstroke velocity of action potentials that are already depressed as a result of ouabain toxicity. Such an action would tend to depress Na^+ entry and would have an effect similar to that of TTX, described above. Another possibility is based on the observation that under appropriate experimental conditions lidocaine can increase steady-state outward current and decrease background inward cur-

rent [35]. In this way lidocaine also could decrease the magnitude of delayed afterdepolarizations.

There has been a great deal of speculation concerning the types of clinical arrhythmias that might be induced by delayed afterdepolarizations. The initial area of speculation involved the repetitive ventricular response type of arrhythmia induced by digitalis toxicity in dogs, as described by Lown and his associates [36, 37]. Included amongst its characteristics are: (1) an increased likelihood of occurrence with increasing digitalis toxicity, (2) greater ease of arrhythmia induction (measured as a lesser threshold requirement for inducing a single premature beat) with increasing digitalis toxicity and (3) greatest likelihood of inducing the arrhythmia when the premature stimulus that induces it is delivered in the early portion of diastole following termination of the T wave. Although these characteristics of the repetitive ventricular response could be satisfied by delayed afterdepolarizations, there has been no fully convincing attempt to test this. Perhaps the most successful efforts are seen in the studies by Zipes et al. [38] and by Wittenberg et al. [39]. Zipes et al. [38] studied ouabain-treated anesthetized dogs in whom ventricular escape intervals were measured after vagally-induced atrioventricular block, after premature stimulation of the ventricle, and after pacing the heart at various cycle lengths. The characteristics of the ventricular rhythm that followed these interventions led the inventigators to suggest that delayed afterdepolarizations – rather than reentry or overdrive-suppressible automaticity – were the underlying mechanism. For example, as heart rate increased, the escape interval following vagal stimulation shortened and the rate of the subsequent ventricular rhythm increased. With overdrive pacing, as the drive cycle length decreased, so did the escape interval. These investigators indicated that abnormal or enhanced automaticity also could explain such behavior, but pointed out that their observations of the in situ heart were similar, if not identical, to what would be expected with delayed afterdepolarizations.

In an earlier study of dogs with complete heart block induced by formalin injection of the His bundle, Wittenberg et al. found that the rate of the initial idioventricular rhythm decreased during digitalization [39]. However, at 72% of the toxic dose of ouabain, overdrive pacing was followed by acceleration of the idioventricular rhythm, rather than the previously seen overdrive suppression. They interpreted their results as consistent with digitalis-induced enhancement of automaticity. This just as well could be the result of an abnormal automatic pacemaker or delayed afterdepolarizations.

More recently attempts have been made to relate clinical cardiac arrhythmias to delayed afterdepolarizations. In part the attempt stems from increased recognition of the fact that standard 'rules' for clinical diagnosis of reentry and for identification of overdrive suppressible automaticity do not explain the spectrum of clinically occurring tachyarrhythmias. As a result, investiga-

tors have commenced reviewing the characteristics of certain accelerated rhythms to determine whether delayed afterdepolarizations might explain them. At a recent arrhythmia conference (Hilton Head Conference on Cardiac Arrhythmias, sponsored by G.D. Searle) the likelihood that either accelerated junctional escape rhythms or accelerated ventricular tachycardias might be delayed afterdepolarization-induced was discussed. It was agreed that we are now in need of (1) 'rules' that might describe the characteristics of clinically occurring tachyarrhythmias induced by delayed afterdepolarizations and (2) performance of clinical electrophysiologic studies that might employ such rules to differentiate delayed afterdepolarization-induced arrhythmias from those due to other causes.

The differentiation of delayed afterdepolarization-induced arrhythmias and those due to overdrive suppressible automaticity should be not difficult. The former would tend to be captured and enhanced by overdrive (i.e., would tend to persist for at least a brief period after discontinuation of the overdrive pacemaker). The latter would tend to be suppressed by overdrive pacemakers. A more difficult differentiation is between reentrant and delayed afterdepolarization-induced arrhythmias. Just as reentrant arrhythmias will tend to occur at certain cycle lengths and a 'window' for their occurrence can be identified, so delayed afterdepolarization-induced arrhythmias tend to occur at a critical cycle length. A major difference is that the delayed afterdepolarization induced rhythm should, on driving at a faster rate, tend to follow and persist at that faster rate, at least for a short period of time. Unfortunately, driving reentrant rhythms at a faster heart rate can result in propagation through a new reentrant pathway. Hence, for the individual patient, the differentiation of delayed afterdepolarization-induced and reentrant rhythms may be difficult. However, for groups of patients, the consistent finding of entrainment of the arrhythmia and its persistence at the entrainment rate on cessation of overdrive pacing would be more suggestive of delayed afterdepolarizations than reentry as a mechanism.

A final problem to be considered is the type of abnormal automaticity that is seen in depolarized fibers, particularly those in the Purkinje system (such as that induced experimentally by Ba^{++} [40]). The characteristics of this rhythm are such that, like a delayed afterdepolarization-induced rhythm, it is overdrive enhanced and therefore its entrainment might be expected. The clinical counterpart of such a mechanism has not yet been defined although any accelerated rhythm might be explained in this way.

To summarize, although it is likely that certain clinically occurring arrhythmias are induced by delayed afterdepolarizations, we are in need of better means for distinguishing delayed afterdepolarizations from reentry and abnormal automaticity before we can say, for the individual patient, which mechanism has caused his arrhythmia.

The pharmacotherapy of delayed afterdepolarization-induced arrhythmias has been a subject of interest. Because of the observations that delayed afterdepolarizations are calcium-induced oscillations, it initially was assumed that Ca^{++} blockers might be able to suppress them. The observation that verapamil [24] and, more recently, AHR-2666 [25] depress delayed afterdepolarizations in vitro might lead one to suspect that such arrhythmias might be susceptible to the effects of Ca^{++} blockers in situ. Interestingly, although no complete clinical study of verapamil effects on digitalis-induced arrhythmias is presently available, those anecdotal observations we do have suggest that it is not a particularly useful drug here. This observation, coupled with the excellent clinical efficacy of phenytoin [41, 42] and lidocaine [43], might lead one to suspect that rather few clinical arrhythmias induced by digitalis are initiated by delayed afterdepolarizations. However, several years ago it was shown that phenytoin depressed delayed afterdepolarizations in vitro (perhaps due to Ca^{++} blockade) [44] and more recently it has been shown that lidocaine has a similar effect on delayed afterdepolarizations [32] (for mechanism vide supra). Hence the ability to treat clinical digitalis-induced arrhythmias successfully with phenytoin or lidocaine is entirely consistent with delayed afterdepolarizations as the etiology.

In closing, we have reviewed the characteristics of digitalis-induced delayed afterdepolarizations, and the mechanisms presumed responsible for their occurrence. We have shown that there are a number of factors which modify the abilities of delayed afterdepolarizations to reach threshold and induce arrhythmias, and that the characteristics of resultant arrhythmias may be somewhat variable. We still are not able to identify with complete confidence the mechanisms which are responsible for delayed afterdepolarizations, nor are we able to extrapolate comfortably from cellular studies to specific clinical arrhythmias that might result from these oscillations. What is required if we are to learn more about the clinical importance of delayed afterdepolarizations are more stringent rules for clinical electrophysiologic testing and more selective pharmacological tools for investigating and identifying their mechanism.

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20. TRIGGERED ACTIVITY *

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1. INTRODUCTION

A non-driven action potential that arises from an afterdepolarization following a preceding action potential has been referred to as 'triggered' [1–4], not only because the non-driven action potential is triggered by the previous action potential but also because a quiescent fiber can be triggered into sustained rhythmic activity by even a single excitation. For descriptive purposes, it is convenient to divide depolarizing afterpotentials into two kinds. Those that interrupt repolarization of the action potential, occurring before the membrane potential has returned to the level characteristic of diastole, are called early afterdepolarizations; those that occur after repolarization is complete, are called delayed afterdepolarizations [4]. It is also convenient to consider whether the action potential that evokes the afterpotential arises from a high or a low level of membrane potential.

2. EARLY AFTERDEPOLARIZATIONS AND TRIGGERING

2.1. Early afterdepolarizations that result in arrest of repolarization

An action potential arising from a high resting potential (−80 to −90 mV) may fail to repolarize, the membrane potential becoming arrested at about −50 mV (Figure 1A). This is an example of the phenomenon of two stable levels of resting potential [5, 6] but the persisting depolarization may equally be regarded as an early afterdepolarization since the membrane potential may subsequently return to the more negative level of resting potential (Figure 1B). The arrest of repolarization at the low level of resting potential can be arrhythmogenic in either of two ways. On the one hand, a sufficiently large segment of myocardium thus switched to a low level of resting potential might become a locus of sufficiently slow conduction to permit the occur-

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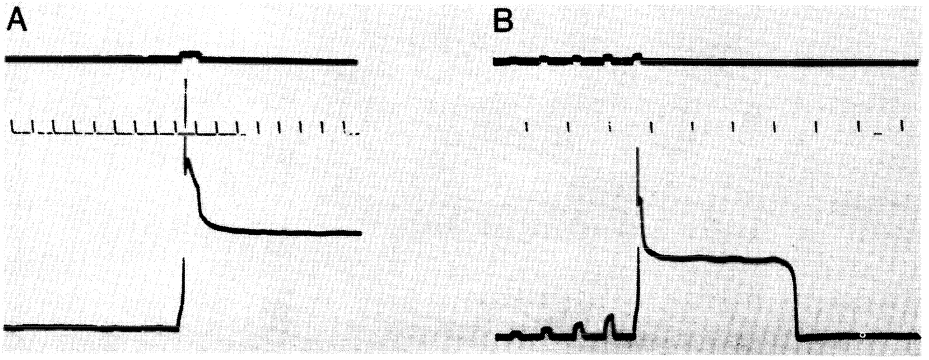

Figure 1. Two levels of resting potential in canine cardiac Purkinje fibers exposed to 4 mM K^+, low-Cl (isethionate) solution. A: the action potential initiated by the 15 nA depolarizing current pulse (shown on the upper trace) failed to repolarize fully, the membrane potential remaining near −50 mV. The potential was steady at that level for at least 2 min before subsequent current pulses were applied. B: prolonged 'action potential' elicited by a 25 nA depolarizing current pulse, in a different preparation exposed to 10^{-6} g/ml tetrodotoxin. The other 4 current pulses were 5, 10, 15 and 20 nA in amplitude. The high level of resting potential was −91 mV in A and −93 mV in B. The time marks occur at 1 sec intervals in A and at 5 sec intervals in B, and in both records the time marks arise from a line marking the zero reference potential.

rence of reentry; on the other hand, fibers switched to the low level of resting potential may become rhythmically active or may be liable to show triggered activity.

2.2. *Early afterdepolarizations that lead to second upstrokes or rhythmic activity*

An action potential arising from a high resting potential may have its repolarization interrupted by a single 'second upstroke' (Figure 2A). That second upstroke, which might propagate as a coupled extrasystole, is triggered in the sense that it is evoked by an early afterdepolarization which follows and is caused by the preceding action potential. Such a second upstroke may in turn be followed by one or more action potentials (Figure 2B). The 'second upstroke' shown in Figure 2 and the rhythmic activity that may follow it, emerge from an early afterdepolarization and have in common the fact that they arise from a low level of membrane potential. Both the 'second upstroke' and the rhythmic activity that may follow it, can be said to be triggered by the initial action potential. However, when rhythmic activity occurs at the low level of resting potential, it is presumably sustained because the original second upstroke and all subsequent action potentials are followed by suprathreshold *delayed* afterdepolarizations [4].

Early afterdepolarizations have been observed in isolated, superfused, heart muscle preparations under many different experimental conditions. These

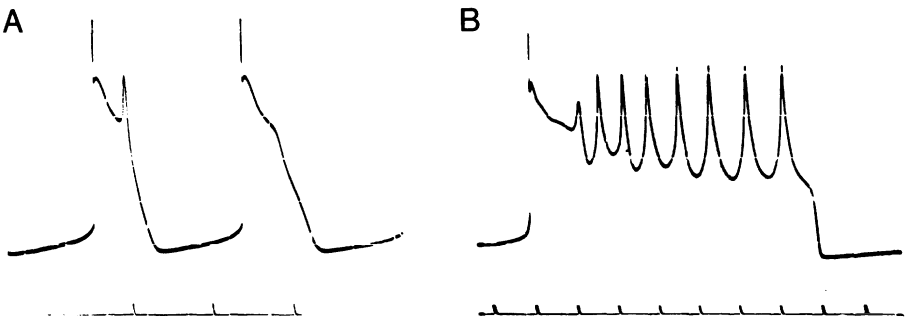

Figure 2. Early afterdepolarizations, second upstrokes and repetitive activity in canine cardiac Purkinje fibers exposed to 4 mM K^+, Cl-containing Tyrode's solution. The two spontaneous action potentials in A show, respectively, an early afterdepolarization, or 'hump', during the repolarization phase (right hand action potential and a second upstroke arising from an early afterdepolarization (left hand action potential). These action potentials were recorded while the fiber was recovering from the effects of a brief exposure to norepinephrine. B shows a spontaneous action potential recorded in another preparation shortly after replacing the bicarbonate/carbon dioxide buffering system with HEPES (N-2-hydroxy-ethylpiperazine-N'-2-ethanesulfonic acid). The second upstroke is followed by a 'burst' of rhythmic activity arising from a low level of membrane potential. The maximum diastolic potential was −84 mV in A and −87 mV in B. The second upstroke in A and the later slow response action potentials in B peaked near 0 mV. The time marks in both A and B occur at 1 sec intervals.

include (1) exposure of ventricular muscle or Purkinje cells to a variety of drugs such as catecholamines [7], aconitine [8], or cardiac glycosides [9] (see Chapter 20 for a discussion of triggered activity caused by digitalis compounds); (2) alterations in the cation content of the superfusate, such as a reduction in K^+ [10] or Ca^{++} [11] concentration; (3) hypoxia [12]; (4) elevated pCO_2 [13]. Most of these studies have been reviewed previously [2]. Since catecholamines, hypoxia and elevated pCO_2 may be present in an ischemic or infarcted region of the ventricle it is possible that early afterdepolarizations may cause some of the arrhythmias which occur soon after onset of myocardial ischemia. This possibility deserves further investigation.

Early afterdepolarizations can occasionally be seen in Purkinje fibers superfused with a normal Tyrode's solution, soon after they have been excised from the heart ([14, 15], Gadsby and Cranefield, unpublished observations). These early afterdepolarizations might be caused by relatively nonspecific inward current flowing via incompletely healed cuts made at the ends of the fibers, or through other regions injured by stretching or crushing during the dissection. It has been suggested that injury produced by stretching Purkinje fibers in situ might cause early afterdepolarizations and extrasystoles [16].

There are several ways in which the slow inward current might play a role in the phenomena seen in association with early afterdepolarizations. First, the presence of a low level of resting potential such as that following an arrest

of repolarization usually requires that g_K fall with depolarization (inward-going rectification) but always requires the presence of a steady-state inward current [6]. At least part of that 'background' inward current is probably carried via non-inactivated, fast Na^+ channels, but part may also be carried via the slow channel [6]. Second, the 'second upstroke' and any subsequent action potentials that arise from the lower levels of membrane potential (low enough to inactivate the 'fast' sodium channels) are presumably slow responses, i.e., the inward current responsible for the upstroke flows via the slow channel. Finally, there is some connection, though probably an indirect one, between the size of the slow inward current and the size of the delayed afterdepolarizations which sustain the rhythmic activity at the low resting potential; this is considered further below.

2.3. Early afterdepolarizations and arrhythmias

As already mentioned, extrasystoles which are caused by early afterdepolarizations should occur with relatively fixed coupling (by electrocardiographic criteria) to the preceding beat. Bigeminal rhythms may occur if only one extra action potential is triggered; tachycardia may occur when triggered activity is sustained. Scherf showed that a single beat can initiate the ventricular extrasystoles or tachycardia caused by aconitine [17]. If such extra-systoles are coupled to sinus impulses, they disappear when the SA node is arrested by vagal stimulation. Extrasystoles arising from early afterdepolarizations caused by factors other than aconitine should also be abolished by vagal stimulation, if vagal stimulation prevents the appearance of the triggering impulse. Many atrial and ventricular arrhythmias encountered in patients with heart disease do appear to be evoked by a preceding beat and thus may be triggered. The requirement of an initiating impulse cannot be used to distinguish activity caused by early afterdepolarizations from that caused by reentry since reentrant activity also requires an initiating impulse [14].

3. DELAYED AFTERDEPOLARIZATIONS AND TRIGGERING

3.1. Delayed afterdepolarizations and triggering in partially depolarized fibers

The first triggerable fibers identified in atria were fibers of the simian mitral valve that have a resting potential of about -60 mV and slow action potential upstrokes [3]; cells with similar properties have since been found in the human mitral valve [18]. The myocardial cells in mitral valves have an ultrastructure which is typical for atrial muscle, suggesting that cells which can be triggered need not have a specialized structure at least at the electronmicros-

copic level [18, 19]. The afterdepolarizations which can lead to triggering in mitral valve fibers have been observed so far only in the presence of catecholamines (Figure 3). In the absence of catecholamines, repolarization is followed by an afterhyperpolarization but the membrane potential thereafter declines monotonically to a steady diastolic value.

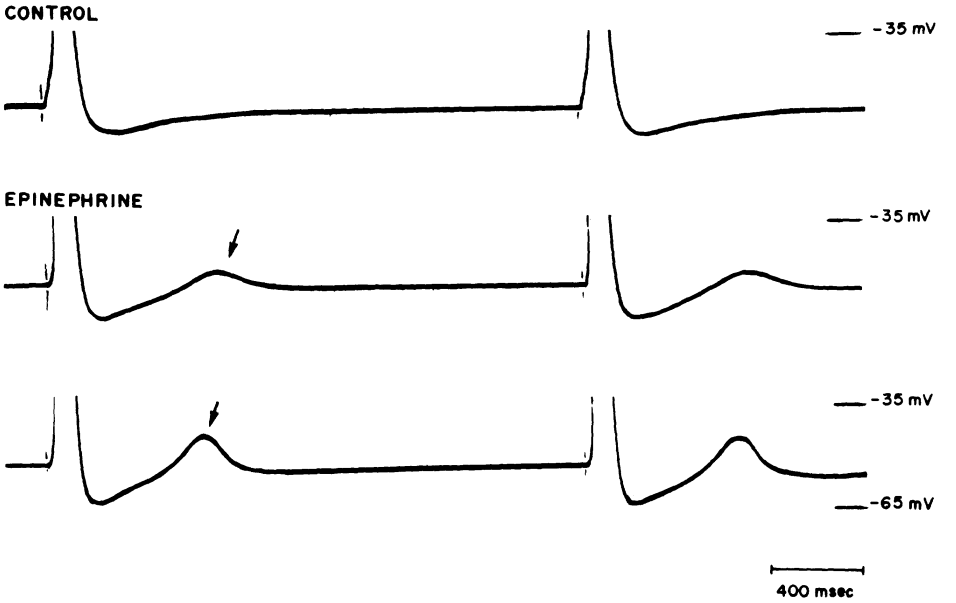

Figure 3. Delayed afterdepolarizations caused by epinephrine in simian mitral valve fibers. The records were taken at a high gain to show the afterpotentials; as a result most of the action potential is off-scale. The top trace shows the transmembrane potential recorded from a fiber in a Tyrode's superfusate without catecholamines. Repolarization is followed by an afterhyperpolarization and the membrane potential then decays to a steady diastolic level. The records shown in the middle trace were obtained about 30 sec after epinephrine was introduced into the tissue bath to give a final concentration of about 1 μg/ml. The afterhyperpolarization is now followed by a small delayed afterdepolarization indicated by the arrow. The records shown in the bottom trace were obtained about 1 min later. The delayed afterdepolarization indicated by the arrow has now reached its maximum size.

Apparently similar electrical behavior, i.e., an afterhyperpolarization followed by a delayed afterdepolarization that leads into another upstroke, has been seen in human atrial fibers showing very low diastolic membrane potentials (less than -60 mV) and slow response action potentials [20]; these preparations were obtained from dilated and diseased atria. Hordof et al. have proposed that the slow response action potentials recorded in diseased atrial cells in some way result from the chronic dilation [21]. Atrial disease has been linked with triggering in a report by Boyden et al. [22] who found fibers showing delayed afterdepolarizations in the presence of catecholamines randomly scattered throughout the dilated left atria of cats with hypertrophic

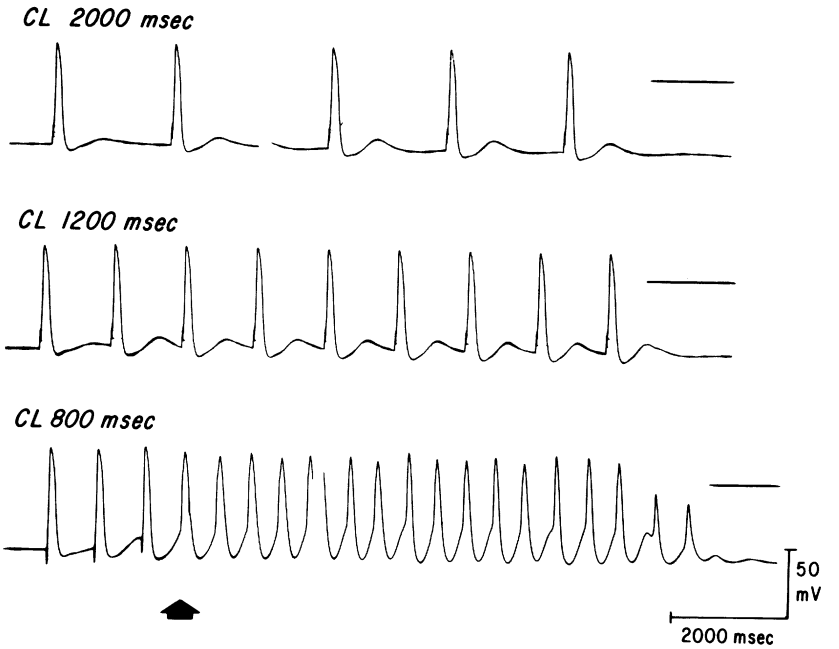

Figure 4. Triggered activity in an atrial fiber in a preparation isolated from the left atrium of a cat with hypertrophic cardiomyopathy. The preparation was superfused with 1.0 μg/ml of norepinephrine in Tyrode's solution. Resting potential of this fiber is -50 mV and the action potential has a slow upstroke. At the right of each panel the 0 reference is indicated by the horizontal line. The top panel shows the action potentials and afterdepolarizations when the preparation was stimulated at a cycle length (CL) of 2000 msec. The first two action potentials at the left were recorded just after stimulation began, the three action potentials at the right were recorded about 10 sec later. In the middle panel the records show action potentials recorded during stimulation at a cycle length of 1200 msec. In the bottom panel, when the preparation was stimulated at a cycle length of 800 msec, triggered activity began at the arrow, after the third stimulated action potential. This panel shows the first 5 triggered impulses. Triggering then continued for several minutes (during the break in the record). The last 12 impulses during triggered activity are shown in the right half of the panel. (Reproduced from ref. 38 with permission of the publishers.)

cardiomyopathy (Figure 4). The resting potentials of these diseased fibers were significantly lower than those of fibers in normal atria, and triggerable fibers were rarely found in normal atria.

How myocardial disease leads to the development of afterdepolarizations and triggering in atrial fibers which cannot normally be triggered is not yet understood, but the reduction in membrane potential may be an important factor. A decrease in membrane K^+ conductance may result from, or may be the initial cause of the fall in resting potential and the reduced conductance would be expected to enhance the depolarizing effect of small inward currents. It is also possible that the transient inward current underlying delayed afterdepolarizations is itself enhanced in the diseased cells, or that other membrane conductances are altered.

3.2. Delayed afterdepolarizations at a high level of resting potential

In fibers of the coronary sinus, in the presence of catecholamines, an action potential that arises from a moderately high resting potential (about −80 mV) demonstrates normal repolarization and is followed by a delayed afterdepolarization that can reach threshold and so evoke non-driven action potentials [23]. However, preliminary observations suggest that the resting potential of coronary sinus cells may, under certain conditions, shift between two relatively stable levels and that delayed afterdepolarizations may arise at either level following excitation (Figure 5). In coronary sinus cells exposed to 4 mM K^+ solution, the higher level of resting potential is usually −80 to −90 mV, whereas the lower level is at −60 to −70 mV. Fibers can be triggered at either level of potential.

Purkinje fibers provide another example of cells which have been shown under certain conditions to have two stable resting potentials [5, 6], and in which delayed afterdepolarizations can be evoked from a wide range of potential levels. Delayed afterdepolarizations arising from the high level of resting potential (−80 to −90 mV) are seen in Purkinje fibers exposed to

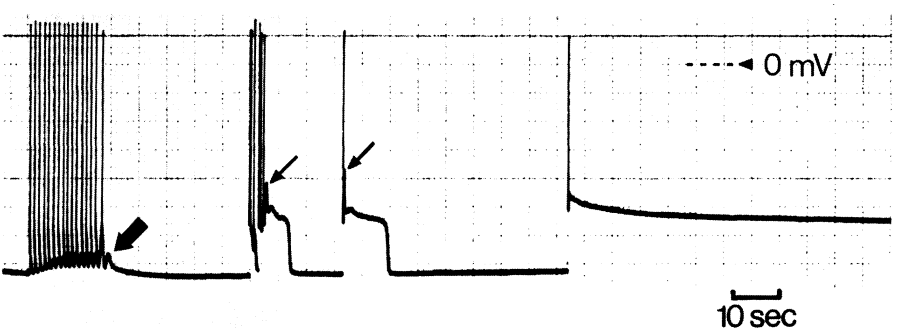

Figure 5. Two levels of resting potential and delayed afterdepolarizations arising at either level, in a fiber of the canine coronary sinus. The preparation was superfused with 4 mM K^+, low-Cl (isethionate) solution and had been transiently exposed to norepinephrine 2 min before this record was obtained. The fiber was stimulated at 1/sec for 15 sec to elicit the action potentials shown at the left. Each action potential was followed by a delayed afterdepolarization which increased in amplitude with each stimulus until, on stopping the stimulation, the fifteenth action potential was followed by a suprathreshold afterdepolarization and the resulting non-driven action potential was followed by a subthreshold afterdepolarization (thick arrow). The second group of action potentials is more complex and results from only 4 stimuli applied at 1/sec. Two of the 4 driven action potentials were followed by second upstrokes. The fourth driven action potential (from a take-off potential near −70 mV) was followed by a subthreshold delayed afterdepolarization (thin arrow); after a slower subthreshold oscillation the membrane returned to about −90 mV. Some 10 sec later, a single stimulus elicited an action potential which failed to repolarize completely and which was followed by a subthreshold afterdepolarization (thin arrow) arising from between −60 and −70 mV. The membrane potential slowly increased towards −70 mV over the next 10 sec and then abruptly repolarized to about −90 mV. The final stimulus, applied 40 sec later, gave rise to an action potential after which the membrane potential drifted to its lower resting level, about −65 mV, where it remained for more than 10 min.

high Ca^{++} concentrations [24] and during the early stages of digitalis toxicity [9]. Delayed afterdepolarizations can also be evoked in Purkinje fibers from much lower potential levels (−50 to −60 mV), e.g., during later stages of digitalis toxicity [1], in high Ca^{++}, Na^{+} free solution [1] or in high K^{+} solution in the presence of catecholamines [25].

There are functionally important differences between delayed afterdepolarizations that arise from high or low levels of potential, particularly with regard to the triggered activity they may initiate. Thus, triggered action potentials arising from low membrane potentials may be slow responses whereas those evoked at more negative potential levels may have rapid fast channel-dependent upstrokes. An additional distinction may be that, as previously noted [4], afterdepolarizations that arise from lower resting or diastolic potentials seem invariably to follow early afterhyperpolarizations, whereas it now seems that afterdepolarizations arising from high levels of resting potential (e.g., in Purkinje fibers [9], in rabbit atrial fibers [26] and in coronary sinus fibers as shown in Figure 5) need not be preceded by early afterhyperpolarizations. Nevertheless, the basic mechanisms underlying these various delayed afterdepolarizations may be the same. Studies of action potentials, delayed afterdepolarizations and triggering, as well as voltage clamp studies, strongly suggest that calcium entry via the slow channel, during depolarizations to plateau levels of potential, is essential to the later appearance of delayed afterdepolarizations. Furthermore, all evidence accumulated to date suggests that the amplitudes both of afterdepolarizations and of the underlying transient inward currents are increased under conditions in which Ca^{++} entry into the cells is enhanced and the intracellular Ca^{++} concentration thereby raised [4], e.g., in the presence of catecholamines [3, 23], cardiac steroids [9], or raised external Ca^{++} concentration [1, 24], or when the rate of stimulation is increased [3, 9, 23]. Blockade of the slow inward Ca^{++} current by verapamil, D600 or Mn^{++} reduces Ca^{++} entry, and thus cellular Ca^{++} content which might explain why those agents diminish or abolish delayed afterdepolarizations [3, 9, 23]. The connection between the slow inward current and afterdepolarizations is thus indirect: afterdepolarizations cannot simply be explained by a phasic increase in slow channel current [27, 28]. For example, as described above, delayed afterdepolarizations may occur at voltages well negative to the threshold for the slow inward current (about −40 mV); moreover, the time course of 'repriming' of the slow inward current is thought to be monotonic, whereas that of the transient inward current underlying afterdepolarizations appears to be oscillatory [29]. The apparently essential role of Ca^{++} entry through the slow channel in the occurrence of delayed afterdepolarizations then seems to be to 'load' cellular Ca^{++} stores.

Although in the remainder of this chapter we mainly consider examples of delayed afterdepolarizations and triggering arising at moderately high levels of

membrane potential, much of the general discussion of the effects of rate, premature stimulation, catecholamines and other agents may well apply to all forms of triggering that depend on delayed (as against early) afterdepolarizations, irrespective of the membrane potential at which such triggering occurs.

3.3. Delayed afterdepolarizations and triggered activity in various cardiac preparations in vitro

We have already discussed triggered activity in mitral valve fibers. As mentioned above, myocardial fibers lining the coronary sinus in the canine heart can also be triggered, but catecholamines usually must be present for afterdepolarizations, and hence triggering, to occur [23]. When these fibers are stimulated at rates of around 100/min in vitro in the absence of catecholamines, action potentials with fast upstrokes may arise from a high level of diastolic potential (−80 mV). Repolarization of these action potentials is followed by a small afterhyperpolarization but no afterdepolarization. During a period of stimulation at a much lower rate (less than about 60/min), or in the absence of stimulation, the resting potential often decreases to a level between −60 and −70 mV and the fibers become inexcitable [23]. During exposure to low concentrations of norepinephrine at this low level of membrane potential, the resting potential increases rapidly, and the fibers become excitable, generating action potentials which are followed by afterhyperpolarizations and afterdepolarizations: the latter may reach threshold and initiate triggered activity (Figure 6).

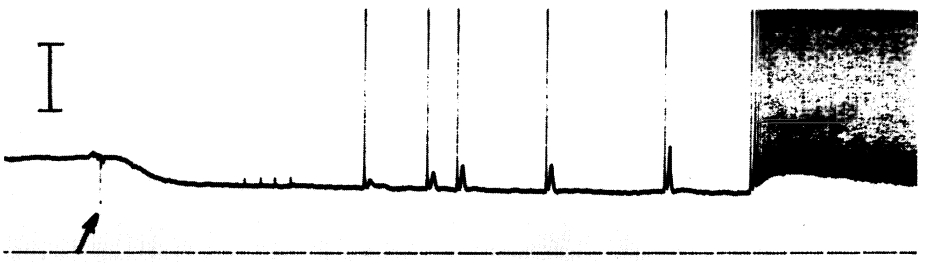

Figure 6. Effects of norepinephrine on membrane potential of a coronary sinus fiber. At the left of the record, membrane potential has fallen to −60 mV after the fiber was not stimulated for 5 min. At this low resting potential the fiber was inexcitable. At the arrow, a small amount of norepinephrine was added directly to the bath. Membrane potential quickly increased and the fiber responded to extracellular stimuli. Each stimulated action potential is followed by a delayed afterdepolarization. The afterdepolarization of the sixth stimulated action potential reached threshold and triggered activity occurs (at the right). The voltage calibration at the left indicates 35 mV. The top of the calibration indicates the 0 reference. The bottom trace shows a time calibration, the discontinuities in the trace occur every 10 sec.

Fibers in the upper pectinate muscles bordering the crista terminalis in the rabbit heart provide another example of normal atrial cells that can be triggered [26]. Saito et al. [26] suggested that the electrical characteristics of these cells and their approximate locations indicate that they may be branches of the sinoatrial ring bundle or they may be transitional fibers between the SA ring bundle and ordinary pectinate muscle. These fibers thus may not be ordinary working atrial myocardial cells but they do show resting potentials characteristic of normal rabbit atrial fibers (73 ± 7 mV) and the action potentials that lead to afterdepolarizations arise from this high resting potential. The afterdepolarization does not appear to follow an afterhyperpolarization but instead begins just prior to complete repolarization of the action potential. The presence of catecholamines does not seem to be required for afterdepolarizations and triggering to occur in these atrial fibers.

Apparently normal fibers in human atrial myocardium can show delayed afterdepolarizations and can be triggered [20]. The fibers are believed to be normal, even though some of the preparations were obtained from diseased hearts, because, while being driven, they showed diastolic potentials of -70 to -75 mV and action potential upstroke velocities of about 100 V/sec, values consistent with those obtained in previous studies of normal human atria [30]. However, the possibility still exists that the observed triggering is associated with some cardiac abnormality since the resting potential decreases to around -60 mV when stimulation is discontinued [20]. These atrial fibers differ from fibers of the coronary sinus in that they become automatic because of spontaneous diastolic depolarization which appears when the fibers become depolarized (Figure 7). The rate of automatic firing is low in such fibers. Delayed afterdepolarizations are superimposed on the phase 4 depolarization that underlies the automatic activity and when a delayed afterdepolarization reaches threshold it triggers sustained activity at a rate greater than that of the automatic rhythm. Spontaneous activity and triggered activity can

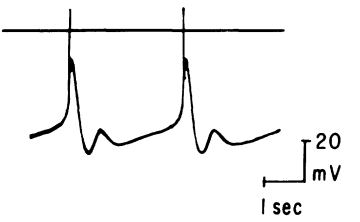

Figure 7. Transmembrane potentials recorded from a human atrial fiber. The tissue was obtained from the right atrium of a patient with rheumatic disease and superfused with a Tyrode's solution containing 1 μg/ml epinephrine. Repolarization is followed by a delayed afterdepolarization. Following the afterdepolarization the membrane potential declines until threshold is reached and a spontaneous action potential occurs. When the afterdepolarization reaches threshold (not shown) a more rapid rhythm is triggered. (previously unpublished record obtained from Dr. Luc Mary-Rabine.)

thus coexist in the same fibers. The action potentials elicited by spontaneous activity give rise to afterdepolarizations which lead to triggering so that, in effect, the cells trigger themselves.

The triggering in human atrial fibers just discussed is not dependent on the addition of catecholamines to the superfusing fluid. However, catecholamines can cause the appearance of delayed afterdepolarizations and triggered activity in human atrial fibers which do not show these properties in their absence [20]. Since catecholamines also induce automatic activity in these fibers, spontaneous diastolic depolarizations and afterdepolarizations may be present in the same fibers so that triggering may occur without extrinsic drive.

Delayed afterdepolarizations and triggered activity caused by toxic concentrations of cardiac glycosides have been widely studied in Purkinje and atrial fibers and in ventricular cells [9]. These studies are discussed in Chapter 20. Delayed afterdepolarizations and the triggered activity they cause also have been studied in canine Purkinje fibers exposed to Na^+-free, Ca^{++}-rich solutions [1], bovine Purkinje fibers exposed to a K^+-rich solution containing catecholamines or caffeine [25], in ventricular muscle fibers exposed to K^+-free, Ca^{++}-rich solutions [31] and ventricular muscle fibers exposed to high levels of catecholamines [32].

In addition, Vassalle has described a phenomenon he calls 'overdrive excitation' which may be related to the triggered activity we are discussing [33]. Ovine Purkinje fibers exposed to low concentrations of norepinephrine do not show automaticity but they become rhythmically active after a period of rapid drive [33]. Similarly, rapid ventricular rhythms can be induced in the in situ dog heart, with AV block, by overdrive of the idioventricular rhythm [34, 35]. It has not been shown that this activity induced by stimulation of the Purkinje fibers or in situ ventricle is dependent on delayed afterdepolarizations although we believe it might be because its behavior is quite similar to that of triggered activity. Thus, increasing the rate of stimulation results in the abrupt onset of a new rhythm, the appearance of the new rhythm is facilitated by catecholamines [35] and by raising the Ca^{++} concentration, and the 'triggered' rhythm can be terminated by prolonged overdrive stimulation [34] (see below).

Vassalle and coworkers have reported striking interactions between the rate and duration of drive, and intrinsic rhythmicity in these preparations [33–35]. A moderate rate or duration of overdrive can lead to suppression of the rhythmic activity that is sustained by spontaneous diastolic depolarization, via overdrive suppression, but a slightly higher rate or longer duration of overdrive may lead to triggering and thus cause 'overdrive acceleration.' As might be expected, a still longer period of overdrive can cause overdrive suppression of the triggered rhythm, presumably because the resulting increase in the rate

of electrogenic sodium extrusion gives rise to a hyperpolarizing (outward) current which is temporarily large enough to suppress both spontaneous diastolic depolarization and the delayed afterdepolarizations that sustain the triggered arrhythmia.

3.4. Initiation of triggered activity by increasing the driving rate

When triggerable fibers show subthreshold afterdepolarizations (either in the presence or absence of catecholamines), triggering may result if the rate at which the fiber is driven is increased [1, 3, 9, 20, 22, 23, 25, 26]. The peak amplitude of the afterdepolarizations increases as the drive rate increases. At a sufficiently high drive rate the afterdepolarizations reach threshold so that triggering occurs (Figure 8). A decrease in the length of even a single drive cycle, i.e., a premature impulse, results in an increase in the amplitude of the delayed afterdepolarization following the premature action potential. The sooner the premature impulse occurs after the previous impulse, the greater is the amplitude of the premature afterdepolarization which may reach threshold and initiate triggered activity [3, 23]. As mentioned earlier, in human atrial fibers which show both automatic activity and subthreshold delayed afterdepolarizations, an increase in the spontaneous rate causes a similar increase in the amplitude of the afterdepolarizations and may lead to triggering in the same way as an increase in the extrinsic drive rate [20].

3.5. Characteristics of membrane potentials during triggered activity

A triggered impulse is initiated when the delayed afterdepolarization reaches the threshold potential level. The resulting non-driven triggered action potential will also be followed by an afterdepolarization which may or may not reach threshold. If it does, then the non-driven action potential may be followed by a train of non-driven action potentials each of which arises from the afterdepolarization caused by the preceding one. This may not be immediately apparent, however, since the rising phase of the afterdepolarization may merge smoothly into the upstroke of the action potential, so that the fiber may show phase 4 depolarization during triggered activity which is practically indistinguishable from that seen during automatic activity (Figure 8). On the other hand, the failure of the afterdepolarization to merge smoothly with the upstroke of the action potential might suggest that the cell under investigation is being excited by propagation from a nearby fiber, just as a latent pacemaker in the SA node is excited by impulses propagating from the dominant pacemaker.

The characteristics of the action potentials of most triggerable atrial fibers, but particularly those with fairly high maximum diastolic potentials and fast

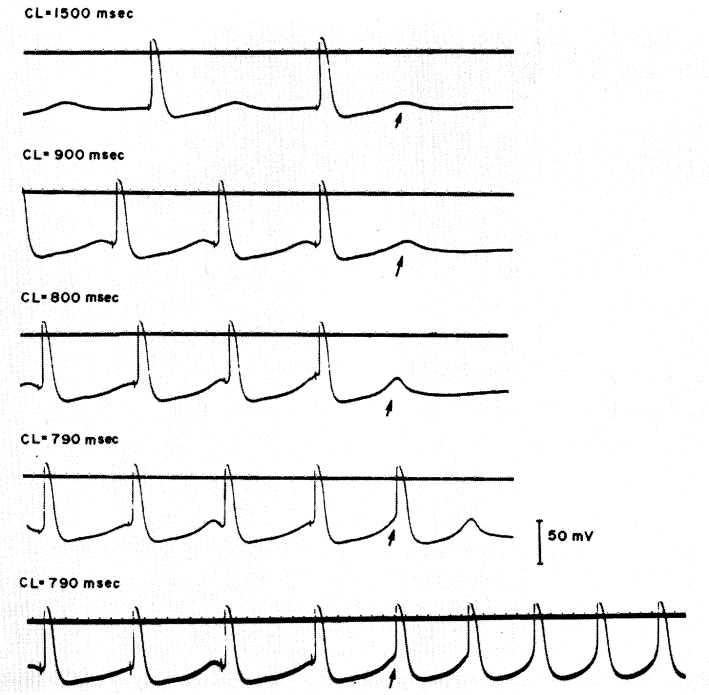

Figure 8. The effect of a decrease in basic cycle length on the amplitude of delayed afterdepolarizations. The transmembrane potentials were recorded from a simian mitral valve fiber superfused with Tyrode's containing 1 μg/ml of norepinephrine. The preparation was stimulated 15 times and the stimulus was then turned off; the last several driven action potentials of the series of 15 are shown in each panel. In the top panel the stimulus cycle length is 1500 msec and the amplitude of the delayed afterdepolarization (arrow) is quite small. The next two panels show records obtained after the stimulus cycle length was decreased to 900 and then 800 msec. There is an increase in the amplitude of the afterdepolarization, both during stimulation and after the last stimulated action potential (arrow). At a stimulus cycle length of 790 msec the delayed afterdepolarization following the last stimulated action potential reached threshold and a triggered action potential occurred (arrow). The afterdepolarization of the triggered action potential was sometimes subthreshold and triggered activity was not sustained but in the bottom panel the afterdepolarization of the first triggered impulse (arrow) reached threshold and so did that of each subsequent impulse causing sustained triggered activity. (Reproduced from ref. 14 with permission of the publishers.)

upstroke velocities, change quite markedly after triggering is initiated. These changes are best exemplified by fibers in the coronary sinus [23]. Maximum diastolic potential decreases during the initial period of triggered activity and, as it does, action potential upstroke velocity and amplitude markedly decrease (Figure 9). The decrease in upstroke velocity and amplitude are presumably caused by inactivation secondary to the depolarization. During triggered activity the action potentials may come to resemble sinus node action potentials,

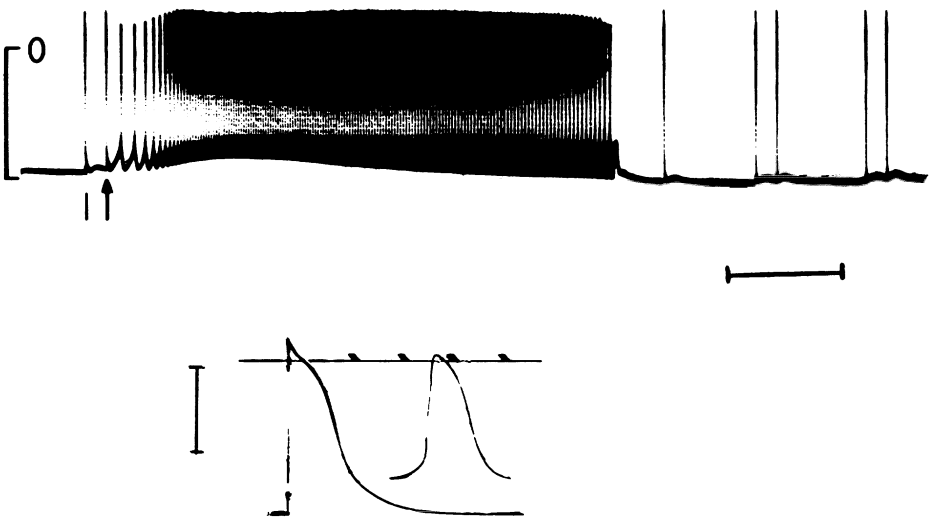

Figure 9. Changes in maximum diastolic potential and action potential of coronary sinus fibers following triggering. In the top panel the fiber was stimulated twice after a period of quiescence. The second stimulated action potential, indicated by the arrow, was followed by an afterdepolarization which reached threshold and triggered activity occurred. The rate of triggered activity accelerated gradually and maximum diastolic potential decreased. After about 20 sec maximum diastolic potential began to increase, rate slowed and triggered activity finally stopped. The last triggered impulse was followed by a delayed afterdepolarization. The fiber was then driven at a slow rate. The voltage calibration to the left equals 90 mV and the top of the calibration bar is the 0 reference potential. The horizontal time calibration = 15 sec. The bottom panel shows the changes in configuration of the action potential recorded from another coronary sinus fiber. The action potential at the left was recorded when the fiber was driven at a regular rate which eventually caused triggering. Action potential configuration resembles that of an atrial plateau fiber. The action potential at the right was recorded from the same fiber after it was triggered and the maximum diastolic potential had decreased to its minimum level. The action potential now resembles that of a nodal fiber. The top trace is the 0 reference potential and also shows time pips at 200 msec intervals. The vertical voltage calibration to the left = 50 mV.

whereas prior to triggering the action potentials resemble those of atrial plateau fibers. This is because the maximum diastolic potential may decrease from about -80 mV to as low as -60 mV.

3.6. Changes in rate during triggered activity

The first triggered impulse arises when the delayed afterdepolarization becomes large enough to bring the membrane potential to the level of the threshold potential. The coupling interval between the first non-driven impulse and the last driven impulse is, therefore, approximately equal to the time between the upstroke of the last driven impulse and the peak of that afterdepolarization; that coupling interval is usually 500–1000 msec. If triggered activity is sustained there is a further decrease in the cycle length

(Figure 9). In coronary sinus fibers [23] and rabbit pectinate muscle fibers [26], this decrease is gradual, occurring during the first 5–15 impulses, after which cycle length becomes relatively constant. In human atrial fibers a constant cycle length seems to be reached sooner [20].

The increase in amplitude of afterdepolarizations with reduction in diastolic interval is probably largely responsible for perpetuation of triggered activity, once the first non-driven action potential has occurred. If the first non-driven action potential arises from the peak of the delayed afterdepolarization, then, as already stated, the coupling interval (cycle length) is the same as the interval between the peak of that afterdepolarization and the upstroke of the previous action potential. This coupling interval is often shorter than the drive cycle length which caused the first non-driven action potential, i.e., the first non-driven action potential is itself 'premature'. Hence, the afterdepolarization following the first non-driven action potential will be even larger than the afterdepolarization from which it arose and a second non-driven action potential will, therefore, occur at a shorter coupling interval. This process is recursive and has been called 'the rhythm of development' [36]. Why afterdepolarizations increase in amplitude when triggerable fibers are driven more rapidly is not known, but may be related to the larger Ca^{++} influx occurring per unit of time at higher stimulation rates, which leads to greater 'loading' of cellular stores of Ca.

3.7. Termination of triggered activity

Triggered activity often terminates spontaneously, even in the presence of a maintained level of catecholamines. The rate usually slows gradually before the termination. This gradual slowing is accompanied by a progressive increase in maximum diastolic potential in fibers of the canine coronary sinus [23], rabbit pectinate muscle [26] and human atria [20] (Figure 9). A delayed afterdepolarization usually follows the last triggered impulse. Maximum diastolic potential at the time triggered activity ceases may be about the same as it was when triggering began, or it may be more negtive. Maximum diastolic potential may continue to increase for some seconds after triggered activity has stopped, but then it returns slowly, over the next few minutes, to the control level.

The spontaneous termination of triggered activity in canine coronary sinus fibers is caused, at least in part, by an increase in the rate of electrogenic sodium extrusion [37]. Sodium pump activity is presumably enhanced by the increase in intracellular Na^+ concentration which results from the increase in Na^+ influx during the period of rapid triggered activity. An increase in outward sodium pump current would be expected both to increase the maximum diastolic potential and to reduce the rate of triggered activity; a suffi-

cient increase in sodium pump current would thus terminate the triggered activity.

3.8. Delayed afterdepolarizations and triggered activity as a cause of arrhythmias

Triggered activity can readily be induced in vitro in both normal and diseased atrial fibers by exposing them to an environment not unlike that expected to exist under certain circumstances in situ. Therefore, since there is little or no reason to believe that isolation and superfusion of cardiac tissue in vitro drastically changes its basic electrophysiological properties, there is every reason to believe that triggered activity should occur in the atria in situ and cause arrhythmias [38].

Vassalle's studies suggest that triggering can also occur in the ventricles in Purkinje fibers with reasonably high levels of membrane potential and can cause ventricular arrhythmias [33–35]. Triggered activity also occurs at the low level of membrane potential in Purkinje fibers as already discussed. The conditions under which Purkinje fibers in situ might have this low level of membrane potential have not yet been identified but some types of cardiac disease might result in a shift of the resting potential of Purkinje fibers from the high to the low level. Some other in vitro conditions which cause triggered activity in Purkinje fibers and ventricular muscle might not exist in situ. Nevertheless, these studies have shown that the fibers can become triggerable when membrane properties are altered. Since we do not know precisely how cardiac disease affects the membrane of cardiac cells, the possibility exists that appropriate alterations are caused by disease to cause triggering. In short, we strongly believe that triggered activity causes at least some atrial extrasystoles and supraventricular tachycardias and may cause some ventricular extrasystoles and tachycardias.

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21. EFFECT OF ANTIARRHYTHMIC DRUGS ON THE SLOW INWARD CURRENT SYSTEM *

LEONARD S. GETTES and TOMOAKI SAITO

1. INTRODUCTION

Thus far, the chapters in this book have been concerned with the characteristics of the slow inward current and its role in the electrical and mechanical characteristics of the individual cardiac cells and of the intact heart. An appreciation of these characteristics is essential to an understanding of the effects of antiarrhythmic drugs on the slow inward current on the intact heart and on clinically encountered arrhythmias.

To recapitulate briefly what has been stated in earlier chapters, the slow inward calcium-sensitive current is present in all cardiac cells and the predominant depolarizing current in cells within the sinoatrial node and the N-region of the atrioventricular node. In all cells, the slow inward current contributes to the overshoot of the action potential upstroke and to the amplitude and duration of the action potential plateau [1, 2]. Agents which alter the slow inward current would be expected to affect the rate of rise of the action potentials in the SA and AV nodal regions and the action potential overshoot and plateau voltage in all cells. Changes in plateau voltage will result in changes in the magnitude of the outward plateau currents. Moreover, agents which alter the slow inward current may also exert an effect on the intracellular calcium concentration which is an important regulator of the outward currents [3, 4]. Thus, the effect of these agents on plateau and total action potential depend on the balance of the changes in the inward and outward currents. The slow current agonists and antagonists may also alter the conductance variables of the rapid inward current, threshold voltage and current, pacemaker currents and passive membrane properties. For these reasons, the effect of these agents on the electrophysiology of the single cell and the intact heart is complicated and unpredictable. Their understanding requires (1) a consideration of the influence of these agents on the conductance variables and kinetics of the slow inward current, (2) their direct and indirect effect on other current systems and passive membrane properties and

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(3) the reflection of these effects on the electrophysiology of the intact heart.

For purposes of this discussion, it is convenient to group the agents into those which enhance or stimulate the slow inward current and those which inhibit or block the slow inward current. Many of these agents may be considered to be antiarrhythmic and/or arrhythmogenic.

Included in the group of agents which stimulate the slow inward current are an increase in extracellular calcium concentration, catecholamines, theophylline, caffeine and possibly the digitalis glycosides. Included in the group which inhibit or suppress the slow inward current are a decrease in extracellular calcium concentration, acetylcholine, other bi- and tri-valent cations, the verapamil group of drugs, and possibly the quinidine-like drugs.

2. AGENTS WHICH ENHANCE THE SLOW INWARD CURRENT (Table 1)

2.1. Calcium ion

An increase in extracellular ionized calcium causes an increase in the magnitude of the slow inward current [1, 2] which results in an increase in the rate of rise of slow channel dependent action potentials, an increase in overshoot and plateau voltage of both rapid channel and slow channel dependent action potentials [5–8], and an increase in the rate of spontaneous diastolic depolarization within the slow channel dependent range (-40 to -9 mV) [6–8]. The effects of an increase in extracellular calcium on action potential duration are dependent on the fiber type and the balance between the action potential lengthening and shortening effects mentioned above. The increase in the magnitude of the slow inward current would, by itself, tend to lengthen plateau duration. However, the inactivation time constant of the slow inward current is shortened by an increase in extracellulr calcium [9], an effect which would shorten the plateau duration. The increase in plateau amplitude results in an increase of the voltage and time dependent outward plateau current

Table 1. Effect of increasing extracellular calcium, catecholamines and digitalis on various ionic currents and action potential plateau duration. In this and Table 2, iS_i = slow inward current, ix_1 = time and voltage dependent outward plateau current, iK_1 = time independent outward current, iK_2 = time and voltage dependent outward pacemaker current, APD = action potential duration, ↑ = increase, ↓ = decrease, ↔ = no change, ? = information not available.

	iS_i	ix_1	iK_1	iK_2	APD
↑Ca^{++}	↑	↔↓	↑	↓	↓
Catecholamines	↑	↑	↔	↓	↓↑
Digitalis	?	↔↓	↔	↓	↓

(ix_1)[10, 11]. In addition, the voltage dependent but time independent outward background current (iK_1) is also increased by an increase in extracellular calcium[11]. The usual result of these multiple effects on the action potential of cells with rapid channel dependent uptrokes is shortening of the action potential plateau and total duration[12].

Calcium shifts the conductance variables for the rapid inward channel[13] and shifts the threshold potential for the rapid inward current to less negative values[13]. In this way, it tends to slow the rate of spontaneously depolarizing but rapid channel dependent fibers[12, 13] even though the slope of spontaneous diastolic depolarization may increase[14]. In addition, by increasing the voltage difference between resting and threshold potentials and causing no change or a slight decrease in the maximum rate of rise of the action potential upstroke, an increase in extracellular calcium tends to slow conduction velocity[15]. An increase in intracelular calcium also causes cell-to-cell uncoupling. This will also slow conduction velocity[16].

In the intact heart, an increase in extracellular calcium shortens the QT interval of the electrocardiogram[17, 18]. High calcium may also increase the duration of the QRS complex by virtue of its ability to slow conduction velocity[17, 18]. When hypercalcemia is severe, variable degrees of AV block may occur[19]. In large part, however, the effects of calcium on the electrophysiologic properties of the heart depend upon potassium concentration. For instance, AV conduction disturbances and ventricular fibrillation induced by increasing extracellular potassium can be abolished or prevented by simultaneously increasing extracellular calcium concentration[19]. High calcium by itself does not appear to increase the incidence of ventricular premature beats in man although an increase in premature beats and the development of ventricular fibrillation has been reported in the experimental animal.

2.2. Catecholamines

As indicated elsewhere in this book a number of studies have illustrated that the slow inward current is enhanced by isoproterenol, norepinephrine and epinephrine (reference in 1 and earlier chapters). This effect is mediated by a catecholamine-induced increase in cyclic AMP[20]. The effect on the slow inward current explains the ability of the catecholamines to stimulate depolarization in fibers depolarized to the level of -40 mV, to increase the rate of spontaneous depolarization within the slow channel range and to potentiate the development of triggered automaticity. In addition, the catecholamines increase the outward plateau currents by increasing the plateau voltage which results from the increase in the slow current[21–23] and by shifting the current–voltage relation of the time- and voltage-dependent outward plateau current (IX_1)[11]. These effects overcome the action potential lengthening

effect which might be anticipated from the increase in the slow inward current and cause action potential duration shortening [24, 25]. The catecholamines also increase the rate of spontaneous depolarization within the range of the rapid channel [26, 27]. As a result of these various changes, the beta stimulating catecholamines increase the rate of sinus [28], AV nodal, and His Purkinje pacemaker activity [26], speed AV conduction [29, 30], and shorten the refractory period in all fiber types [30]. The shortening of action potential duration has recently been shown to be more marked in premature than nonpremature responses [31] and may contribute to the ability of the catecholamines to assist in the induction of arrhythmias by ventricular stimulation techniques [32]. The well-known relationship between ischemia-induced arrhythmias and sympathetic stimulation [33] and the ability of the catecholamines to potentiate the development of triggered automaticity [1] and of digitalis-induced arrhythmias [34], can all be attributed to the effect of the catecholamines on these various electrophysiologic properties.

Other drugs which increase the slow inward current by increasing cyclic AMP include the methylxanthines, caffeine and theophylline [35]. These agents are believed to exert their effect by inhibiting phosphodiesterase activity.

2.3. Digitalis

The digitalis glycosides exert a variety of effects on the single cell which suggest that they may influence the slow inward current. As discussed elsewhere in this book, the most suggestive evidence is the ability of glycosides to cause or increase the calcium-sensitive transient depolarizations [36, 37]. However, while these transient depolarizations may depend on the release of calcium from intracellular stores, they are not thought to depend upon the slow inward current per se [38–41] and a direct effect of the digitalis glycosides on the slow inward current system has not been clearly shown. Digitalis increases the slope of spontaneous diastolic depolarization in the range of -90 to -60 mV [12] and causes a gradual depolarization of the resting potential. Digitalis also shorten the plateau and total action potential duration. These directly mediated effects underlie the shortening of atrial and ventricular refractory periods in the intact heart, the shortening of the QT interval on the electrocardiogram and the ability of digitalis to induce ectopic beats and rhythms.

In addition to the directly mediated effects of the digitalis glycosides, they also enhance vagal tone. It is this effect which accounts for the prolongation of AV nodal conduction time and refractoriness, events which imply an effect on the slow inward current system. Appreciation of these events requires an understanding of the effects of acetylcholine. In this way, digitalis serves as a

convenient bridge between those agents which enhance and those which inhibit slow channel activity.

3. AGENTS WHICH INHIBIT THE SLOW INWARD CURRENT (Table 2)

3.1. Acetylcholine

The ability of acetylcholine to hyperpolarize the membrane, to shorten action potential duration, and to suppress diastolic depolarization has long been appreciated [12]. These effects can be attributed to an increase in potassium permeability [12, 42–44]. Acetylcholine also decreases the rate of rise of the action potential upstroke and decreases the overshoot in cells of the sinoatrial and atrioventricular nodes [28, 45–48]. These effects may be explained by a documented depressant effect on the slow inward current [49, 50] as well as by the increase in the outward current which would cancel some of the inward current. The slowing of the upstroke caused by inhibition of the slow current causes the slowing of the AV conduction which characterizes vagal stimulation. A variety of drugs exert an effect which is mediated by a secondary increase in vagal tone and, thus, may be thought of as indirect slow channel depressants. These agents include the alpha sympathetic catecholamines, the beta sympathetic antagonists, edrophonium and physostigmine as well as digitalis. The alpha sympathetic agonists increase vagal tone by increasing systemic blood pressure. The beta sympathetic antagonists increase vagal tone by preventing the opposing effects of beta sympathetic stimulation. Edrophonium increases vagal tone by inhibiting choline-esterase activity and preventing the metabolism of acetylcholine at nerve endings, and physostigmine acts directly. The effect of each of these agents is to slow the sinus node pacemaker and to slow conduction in the perisinus nodal tissues and in the AV junction. It is these effects which underlie the ability of these agents to

Table 2. Effect of acetylcholine, manganese, verapamil, quinidine and lidocaine on various ionic currents and action potential plateau duration. iS_i = slow inward current, ix_1 = time and voltage dependent outward plateau current, iK_1 = time independent outward current, iK_2 = time and voltage dependent outward pacemaker current, APD = action potential duration, ↑ = increase, ↓ = decrease, ↔ = no change, ? = information not available.

	iS_i	ix_1	iK_1	iK_2	APD
ACh	↓	↑	↑	↑	↓
Mn^{++}	↓	↓	?	↔	↓
Verapamil	↓	↓	↑	↔	↔↓
Quinidine	↔↓(?)	↓	?	↑	↓ and ↑
Lidocaine	↔	↑	?	↑	↓

abolish AV junctional reentrant arrhythmias and to slow AV junctional conduction in atrial fibrillation and flutter.

Acetylcholine also influences the action potentials of Purkinje and ventricular muscle fibers. These effects include a decrease in overshoot and a decrease in the rate of diastolic depolarization and usually a decrease in action potential duration [51–53]. However, an increase in action potential duration has also been reported [54, 55]. These multiple effects may be modulated, at least in part, via the drug's depressant effect on the slow inward current. The ability of acetylcholine to antagonize the effects of isoproterenol in Purkinje fibers [56] also suggests an effect on factors which control the slow inward current.

3.2. Low calcium, calcium chelating agents and other bi- and tri-valent ions

As indicated earlier, the effects of low calcium are complicated because of the importance of calcium on the regulation of the rapid inward currents and the outward plateau currents. In general, the effects of reduction in extracellular calcium ions are the reverse of those of an increase. Low extracellular calcium, induced by calcium deficiency or calcium chelating agents, decreases the magnitude of the slow inward current and the rate of spontaneous diastolic depolarization [8, 12]. In cells made slow-channel dependent by depolarization, this leads to a decrease in maximum upstoke velocity and in the overshoot [6, 8].

In all cells, low calcium decreases action potential overshoot and the voltage of the action potential plateau [12]. Low extracellular calcium prolongs the action potential plateau and total duration, an effect which accounts for lengthening of the QT interval on the electrocardiogram [17]. Within physiologic limits, a decrease in extracellular calcium has no significant effect on the electrocardiogram other than to prolong the QT interval. However, a decrease in extracellular calcium induced by EDTA is capable of suppressing atrial and ventricular premature beats in approximately 50% of patients [57]. Moreover, in the rabbit, lowering extracellular calcium by 80–90% is capable of preventing the ventricular arrhythmias induced by a similar lowering of the extracellular potassium concentration [58]. However, it is unclear if these antiarrhythmic effects are the result of a decrease in slow inward current or the effects of lowered extracellular calcium on the other current systems.

Other bi- and tri-valent ions are capable of competing with calcium for binding sites on the membrane. These include manganese [59–63], magnesium [64–66], barium [64], cobalt [62], nickel [62], strontium [64], and lanthanum [67]. Whether or not these ions become charge carriers themselves depend upon the permeability in the membrane. For instance, lanthanum, nickel and cobalt block the slow channel but do not carry charge across the mem-

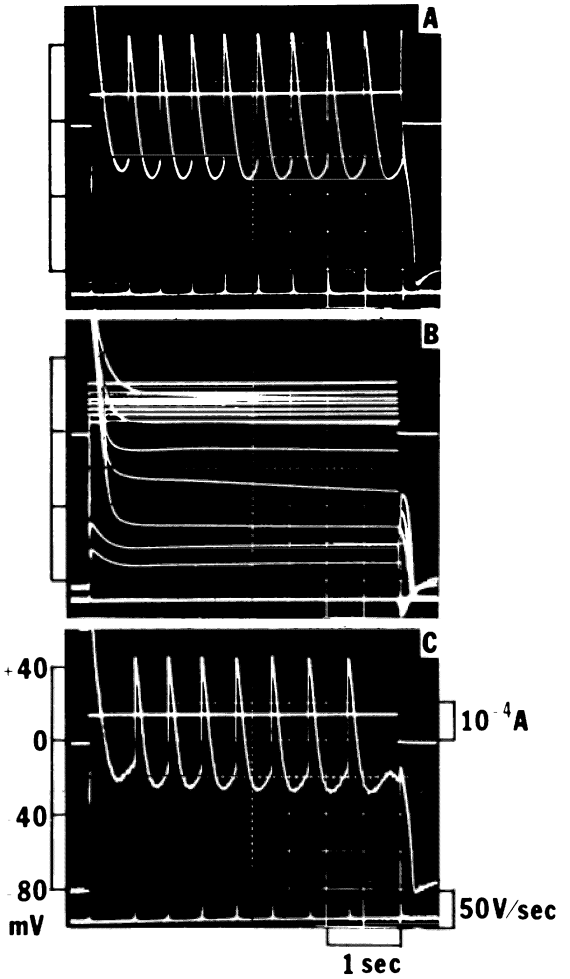

Figure 1. Effect of Mn^{++} on spontaneous depolarization induced in guinea pig papillary muscle by the injection of depolarizing current. In each panel, the current pulse is shown at the top, the transmembrane action potential in the middle and the maximum rate of rise of the upstroke at the bottom. A: control: spontaneous depolarizations occur when the membrane is depolarized to −32 mV. B: twenty minutes after addition of 6 mM $MgCl_2$: no spontaneous depolarizations occur. C: return to control: spontaneous depolarization again occur. From Imanishi and Surawicz: Circulat Res 39:751-759, 1976.

brane [62, 67]. Barium and strontium are capable of carrying charge [64, 68, 69]. Manganese in the presence of calcium and sodium behaves as a blocking agent (Figure 1) but, in the absence of these ions, becomes a charge carrier [64]. The ability of lanthanum and manganese to slow sinus rate and AV conduction in the dog without altering His–Purkinje conduction

provides further evidence for the slow channel dependency of these supraventricular structures [70].

3.3. Verapamil

Of the drugs which inhibit the slow inward current, verapamil is perhaps the most important and in many ways, the most interesting. Initially induced as a coronary vasodilator and antianginal agent [71–74], it was found capable of suppressing AV conduction and of preventing adrenaline and ouabain-induced arrhythmias in the experimental animal [72]. Clinical studies showed that it was almost uniquely effective in converting AV junctional reentrant arrhythmias to sinus rhythm and in slowing the ventricular rate in atrial fibrillation and flutter [75, 76] (Table 3). This early experience has since been duplicated by many investigators.

Table 3. Effect of verapamil, 10 mg iv, on supraventricular and ventricular arrhythmias in 181 patients. From Schamroth, Krikler and Garrett: Brit med j 1:660–662, 1972.

Arrhythmia	*Number cases*	*Response*
Sinus tachycardia	3	Slowing – all
AV junctional tachycardia	3	Conversion to sinus rhythm – all
Paroxysmal supraventricular tachycardia	20	Conversion to sinus rhythm – all
Atrial flutter	15	Conversion to sinus rhythm – Increased AV block – 11
Atrial fibrillation	115	Increased AV block –111
Idioventricular tachycardia	1	Conversion to sinus rhythm
Ventricular tachycardia	1	No effect
Ventricular extra beats	23	No effect – 12 Decreased frequency – 11

The definition of the clinical utility of verapamil was paralleled by an understanding that its mode of action was mediated, at least in large part, by its unique ability to block the calcium sensitive slow inward current [77–80]. Thus, the simultaneous discovery of the slow inward current and of a clinically useful, predominantly selective calcium blocking agent has combined to enhance our understanding of a previously poorly defined area of normal and abnormal cardiac electrophysiology, cardiac contractility and coronary vascular reactivity. The racemic mixture of verapamil and its hydroxy derivative, D600, decrease conductance of the slow inward current over a wide range of voltages [77, 81, 82]. This occurs without shifting the steady state inactivation relationship along the voltage axis [81, 82]. Verapamil prolongs only slightly the kinetics of activation of the slow current and does not alter its inactiva-

tion [81, 82]. However, it does prolong the kinetics of recovery from inactivation [82], an effect which is partially reversed by increasing extracellular calcium and by isoproterenol. As a result of these slow channel blocking properties, the drug depresses the upstroke of SA and AV nodal fibers [83, 84] (Figure 2) and fibers in which the rapid channel is totally inactivated by depolarization with potassium, DC current, tetrodotoxin, or exposure to sodium-free medium [79, 82, 85, 86] (Figures 3 and 4). Verapamil also prevents spontaneous diastolic depolarization in these fibers [85, 86] (Figure 2). Verapamil does not prevent spontaneous depolarization within the range of -90 to -60 mV, i.e., when the spontaneous activity is primarily dependent on the time dependent decay of the outward potassium channel (iK_2). Verapamil, like manganese, prevents the oscillatory afterpotentials induced by digitalis [36, 87]. However, as indicated above, the mechanism for this suppression is

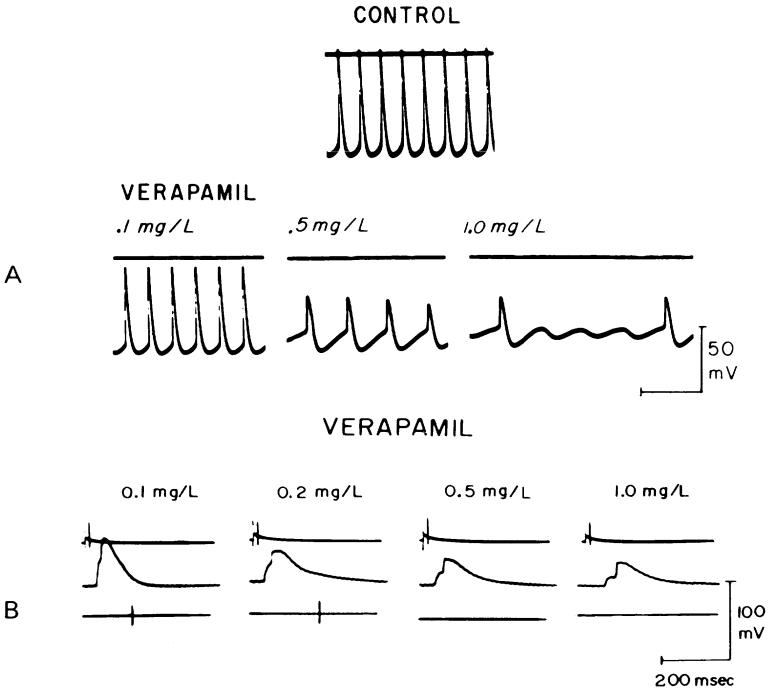

Figure 2. A: action potentials recorded from the rabbit sinus node. The control tracings (top) were recorded from the periphery of the node. The lower three tracings, showing the effects of verapamil, were recorded from the same fiber, but not from the fiber showing the control tracings. Increasing concentrations of verapamil cause progressive decrease in rate, amplitude and maximum diastolic potential of the sinus node action potentials. The sharp angle from the end of phase 4 and of phase 0 indicate that these are not the pacemaker fibers. B: effect of increasing concentrations of verapamil on action potentials from the upper region of the rabbit AV node. The atrial electrogram is above the action potential and the His bundle electrogram is below. From Wit and Cranefield: Circulat Res 35:413-425, 1974.

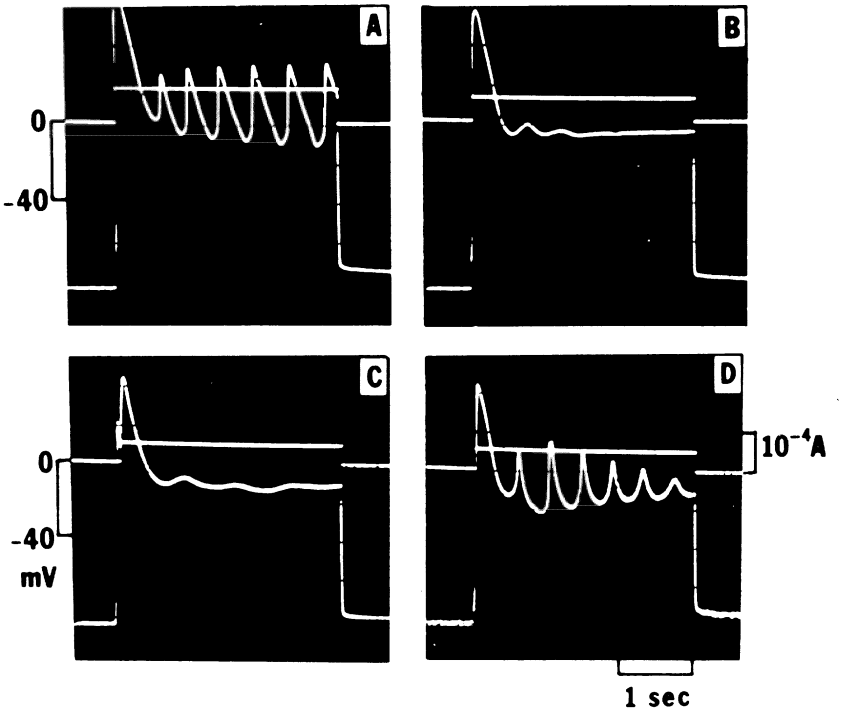

Figure 3. Effect of verapamil 3 mg/liter on spontaneous depolarizations induced in guinea pig papillary muscle by injection of depolarizing current. A: control. B: thirty minutes after addition of verapamil. C: thirty minutes after return to control D after increase of calcium in perfusate from 1.8 mM to 6.9 mM. From Imanishi, McAllister and Surawicz: J Pharmacol exp Ther 207:294-303, 1978.

not clear since the slow inward current is not thought to be primarily responsible for these potentials.

Were the electrophysiologic effects of verapamil purely related to blockade of the slow inward current, the effects of the drug on the single cell and intact heart would be entirely predictable. However, verapamil also increases the time independent outward current [81, 88]. This effect, coupled with the inhibition of the slow inward current, would be expected to shorten action potential duration. Indeed, such shortening of the plateau in action potential duration has been reported in normal Purkinje and ventricular fibers [79, 86, 87]. However, the degree of shortening is rather minimal and, in some studies, verapamil has been without significant effect [84, 89, 90]. In fibers depolarized by increased concentrations of extracellular potassium concentration, but in which the upstroke remains rapid channel dependent, verapamil causes dramatic shortening of the plateau and total action potential

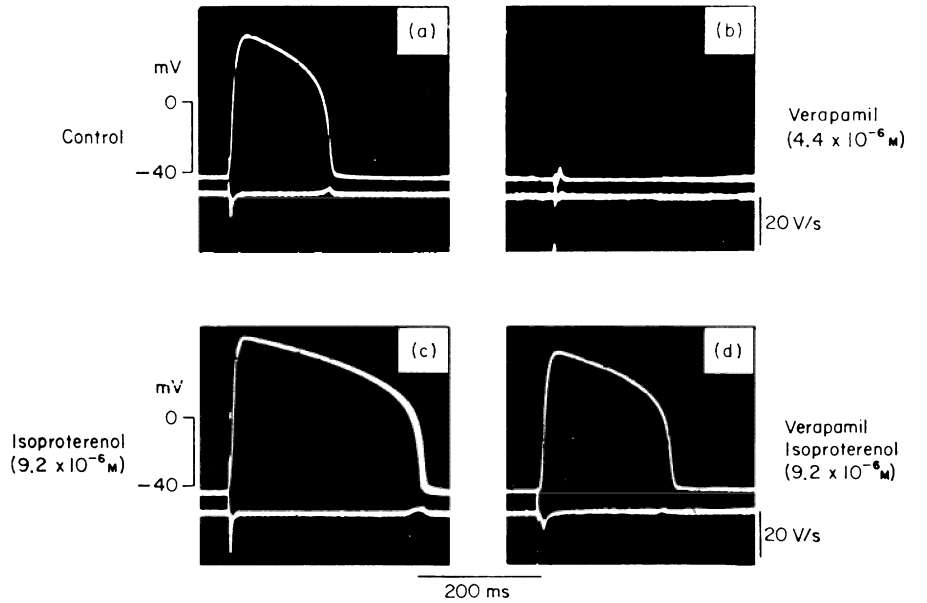

Figure 4. Effect of verapamil and isoproterenol on slow channel dependent action potentials from guinea pig papillary muscle. In each panel, the fiber is depolarized to −40 mV by current. The differentiated upstroke is shown in the lower trace of each panel. a: control. b: after verapamil, the fiber is inexcitable. c: effect of isoproterenol (without verapamil). Note the increase in the maximum rate of rise in the upstroke and increased duration of the action potential. d: effect of isoproterenol after verapamil. Note the partial restoration of the action potential. From Kohlhardt and Mnich: J mol Cell Cardiol10:1037-1052, 1978.

duration without altering either the steady state or recovery characteristics of the upstroke [90] (Figures 5 and 6).

In the intact heart, verapamil slows sinus rate and prolongs AV conduction and refractoriness [70, 91–93] (Figures 7 and 8), but does not change the electrophysiologic characteristics of the remainder of the conduction system [92]. Since the sinus and AV nodal regions are largely dependent upon the slow inward current, these effects of verapamil are predictable. Verapamil, by virtue of its ability to inhibit calcium influx, is also capable of interfering with excitation contraction coupling in myocardium and in vascular smooth muscle [95, 96]. This causes a decrease in peripheral vascular resistance and a decrease in contractility in isolated cardiac muscle fibers [91]. However, in intact experimental animals, the effect of verapamil on myocardial contractility appears dose dependent [93, 97–100]. In lower doses when plasma concentrations are below 150 ng/ml, verapamil either does not change, or increases cardiac contractility [93]. At this plasma level, verapamil's electrophysiologic effects are evidenced by prolongation of AV conduction and abolition of VA conduction [93]. At higher blood levels, verapamil's depressant effect on con-

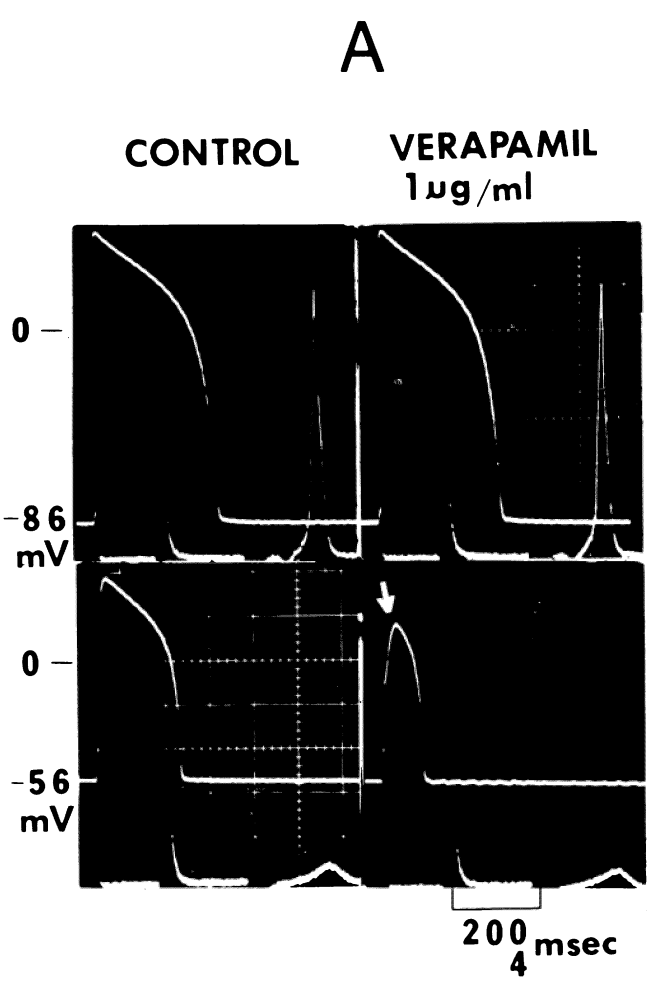

Figure 5. Effect of verapamil on action potentials recorded from the same guinea pig papillary muscle when K^+ in the perfusate is 5.4 mM (top) and 12 mM (bottom). The maximum rate of rise of the action potential upstroke, shown by the magnitude of the differentiated upstroke spike to the right of each action potential, is 300 V/sec at $K^+ = 5.4$ mM (top) and 25 V/sec at $K^+ = 12$ mM (bottom). Note that verapamil has no effect on action potential upstroke at either K^+ value, and no effect on action potential overshoot or duration when $K^+ = 5.4$ mM. When K^+ is 12 MMM and resting potential is −56mV, verapamil markedly decreases action potential overshoot and duration. From Chen and gettes: J Pharmacol exp Ther 209:415-421, 1979.

tractility is more evident (Figure 8). In man, the effects of intravenous doses of up to 10 mg are somewhat controversial and represent the opposing direct negative inotropic effect and the secondary effects of the decrease in afterload. Most studies have shown either an increase [101] or no change [102, 103] in cardiac output, even in patients with heart disease. However, its use in patients with congestive heart failure should clearly be undertaken with

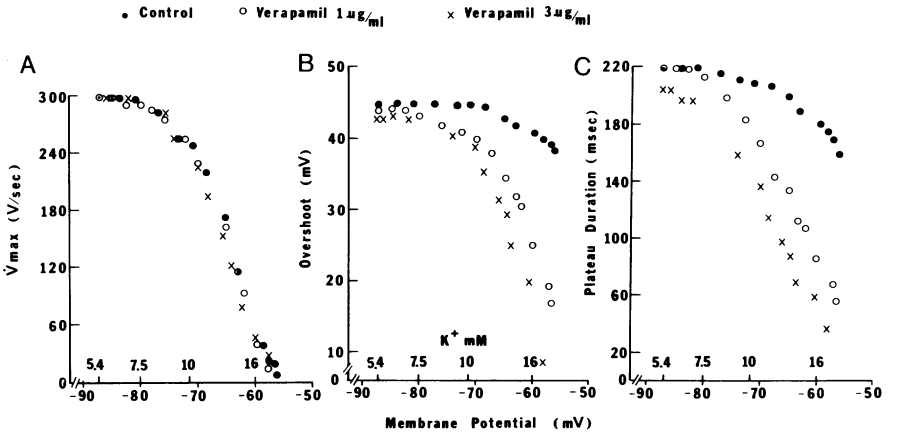

Figure 6. Graphic representation of effects of verapamil, 1 and 3 μg/ml, on maximum rate of rise of the action potential upstroke (V_{max}) (A), overshoot (B), and plateau duration (C), as the resting potential is made progressively less negative by the progressive increase in K^+ concentration in the perfusing solution. Note that in these rapid channel dependent action potentials, verapamil has no effect on V_{max} even when it is decreased to approximately 10 V/sec, but markedly decreases overshoot and plateau duration when K^+ is > 7.5 mM and resting potential is less than −80 mV. From Chen and Gettes: J Pharmacol exp Ther 209:415-421, 1979.

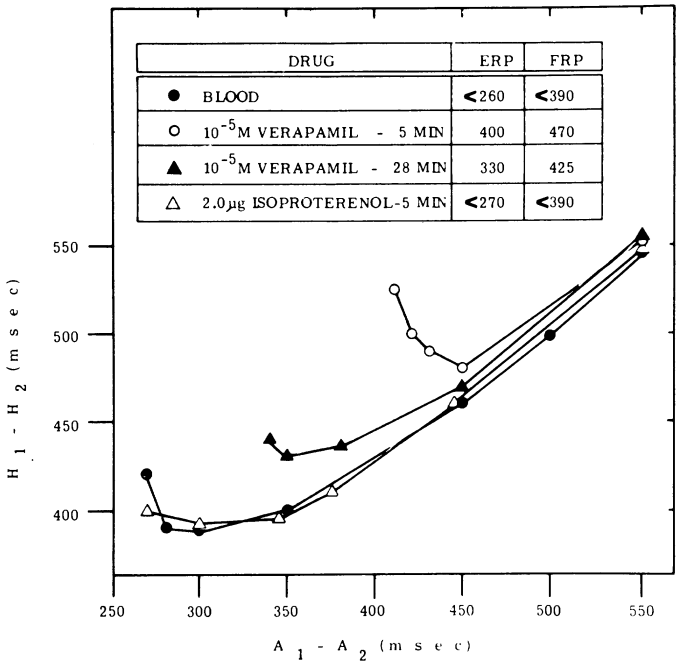

Figure 7. Effects of verapamil and isoproterenol, injected into the AV nodal artery of the dog, on refractory periods of the AV node. The isoproterenol was injected after verapamil. Note the prolongation of AV nodal refractoriness induced by verapamil and the reversal by isoproterenol. From Zipes and Fischer: Circulat Res 34:184-192, 1974.

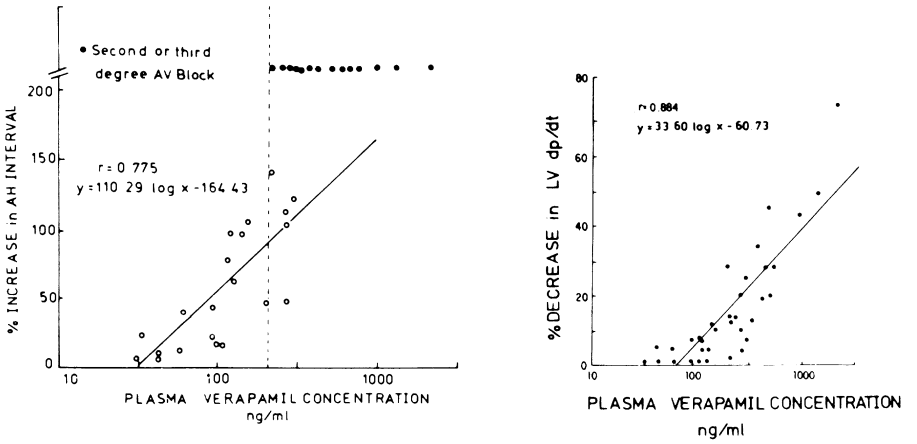

Figure 8. Correlation of plasma verapamil to AH interval (left and left ventricular dp/dt (right) in the dog. Note that significant AH prolongation occurs at plasma levels which do not alter dp/dt. From Mangiarde et al: circulation 57:366-372, 1978.

extreme caution. It is of interest that whereas increasing extracellular calcium restores some of the action potential changes and restores the contractility, reversal of verapamil's effects on AV and sinus node electrophysiology is incomplete, even when calcium is elevated beyond that required to reverse the contractility changes [104].

The electrophysiologic effects detailed above provide the mechanisms for verapamil's well documented ability to prolong the PR interval, to slow the ventricular rate in atrial fibrillation and flutter, and to terminate AV nodal reentrant arrhythmias. The drug is less effective in treating ventricular arrhythmias [76], suggesting that these rhythm disturbances are less dependent on the slow inward current for their expression than are the arrhythmias involving the AV junction. Alternatively, these arrhythmias may be slow channel dependent but may arise from areas of decreased coronary blood flow, preventing the achievement of an effective drug concentration at the site of arrhythmogenesis. It is also important to bear in mind that, in the intact heart, the effects of verapamil are dependent on the level of autonomic tone [105, 106], and the combined direct and indirect effects of the drug on the electrical and mechanical characteristics of the heart and peripheral circulation. Any effect which tends to increase beta sympathetic tone such as the depression of contractility will tend to increase slow channel activity and, thereby, counteract the effect of the drug. Thus, the anticipated decrease in sinus rate and prolongation of AV conduction and refractoriness may be blunted or absent in the presence of an increase in sympathetic tone [107].

In the experimental animal, pretreatment with verapamil prevents ventricular fibrillation induced by coronary occlusion [108] and, in the ischemic myocardium, both speeding and slowing [109, 110] of conduction have been reported following verapamil administration. The reasons for these apparently contradictory results have not been elucidated but may relate to the time of verapamil administration, given before [109] or after [110] coronary artery occlusion. It is possible that the marked shortening of the action potential of K^+ depolarized but rapid channel dependent fibers might account for the speeding of conduction whereas depression of the upstroke of slow channel dependent action potentials might cause conduction slowing. Also to be considered is the ability of verapamil to dilate coronary vessels and, thus, to alter coronary flow [71] and its ability to diminish the extent of necrosis by interfering with calcium entry into the ichemic cells [111].

The next few years will probably witness the re-emergence of verapamil and other slow channel ihibitors as the treatment of choice for patients with anginal pain due to coronary spasm [112–114]. Several drugs in addition to verapamil have been identified as possessing these capabilities and are currently under study. These include nifedipine, which has been reported effective in the treatment of Prinzmetal's angina [115] and Diltiazem, which has been found to have electrophysiologic effects on the single fiber similar to those of verapamil [116], to be a potent coronary vasodilator [117], and to minimize some of the consequences of coronary occlusion on myocardial metabolism [118]. At this time, there is inadequate data on which to compare the efficacy of these agents to that of verapamil in the treatment of cardiac arrhythmias and vasospastic angina or to compare their effects on cardiac function.

3.4. Other antiarrhythmic drugs

Most of the commonly used direct acting antiarrhythmic drugs other than verapamil influence the rapid inward sodium current and the potassium dependent outward currents [119]. These effects are thought to be their primary modes of action. The effect of many of these drugs on the slow inward current has not been carefully studied. Quinidine has been shown to suppress slightly the rate of spontaneous diastolic depolarization occurring within the range of -75 to -20 mV in guinea pig papillary muscles depolarized by current injection [85] (Figure 9). This effect is more marked than that of verapamil in the -70 to -50 mV range, but less marked than verapamil's the -50 to -20 mV range. Moreover, the effects of quinidine on overshoot are less marked than those of verapamil and the ability of calcium and catecholamines to reverse these effects are also less marked. These results suggest that quinidine may possess minimal slow channel blocking capabili-

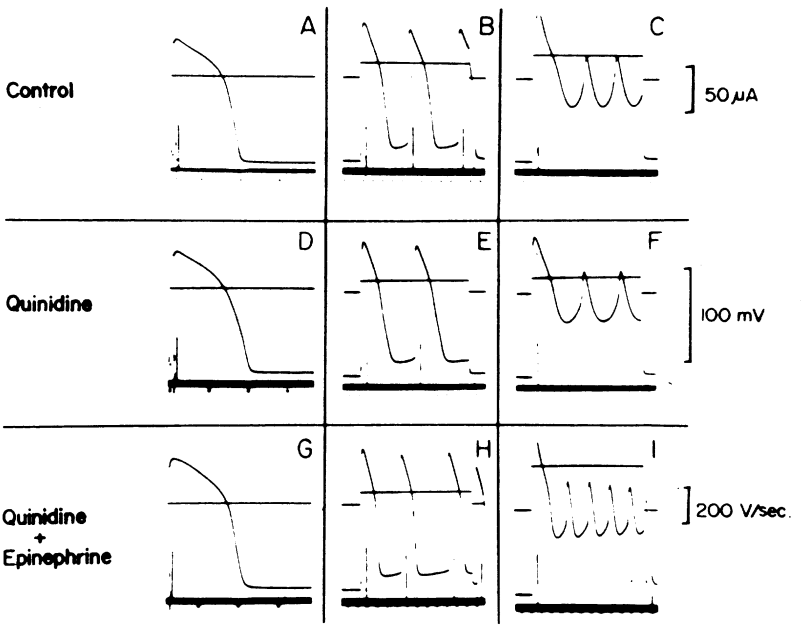

Figure 9. Effect of 7.6 mM quinidine and quinidine plus 10 mM epinephrine on spontaneous activity induced in guinea pig papillary muscle by depolarizing current. Quinidine depresses slightly the rate of the spontaneous activity in both the −50 to −70 mV range (B vs E) and −20 to −40 mV range (C vs F). These effects are reversed by epinephrine (H and I). From Grant and Katzung: J Pharmacol exp Ther 196:407-419, 1976.

ties which contribute to its antiarrhythmic effects although these effects are less marked than those of verapamil. In man, quinidine's effect on sinus node and AV junction is determined not only by its direct effect, but also by its vagolytic capabilities. These effects are antagonistic to the directly mediated effects. Moreover, the direct effects of quinidine on the rapid inward current could also contribute to the drug's ability to slow AV conduction and prolong AV nodal refractoriness by influencing the rapid channel dependent cells which surround the slow channel dependent portion of the AV node.

Lidocaine does not alter either the rate of rise or the overshoot of slow channel dependent action potentials in guinea pig and cat papillary muscles [86, 120] and slows only slightly the rate of spontaneous diastolic depolarization the range of −40 to −10 mV [86] (Figure 10). However, the lack of effect of the drug on the rate of rise or overshoot of these action potentials suggests that this effect on spontaneous activity is due to factors other than blockade of the slow inward current [86]. Lidocaine's lack of effect on the slow inward current system helps to explain the drug's inability to alter sinus rate or AV conduction in man. It is also of interest that lidocaine does not decrease the rate of the escape ventricular pacemaker in patients with AV

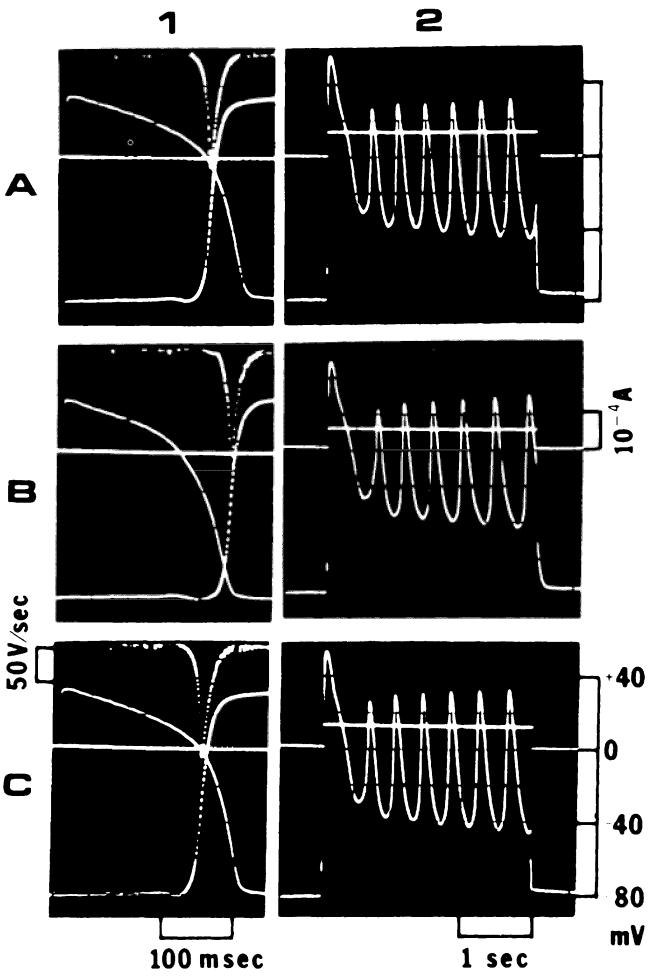

Figure 10. Effect of lidocaine on spontaneous activity induced in guinea pig papillary muscle by depolarizing current. A: control. B: after addition of 8 mg/liter lidocaine to perfusing solution. C: return to control. Note that lidocaine has only a minimal effect on the rate of the spontaneous depolarization and no effect on amplitude. From Imanishi, McAllister and Surawicz: J Pharmacol exp Ther 207:294-303, 1978.

block located below the location of the His bundle potential [121]. This observation, coupled with the knowledge that the rate of these pacemakers is enhanced by isoproterenol, suggests that they, like sinus and AV nodal pacemakers, may be slow channel dependent.

Aprindine suppresses digitalis induced after depolarizations [122] much like verapamil, manganese, and a decrease in extracellular calcium. This effect suggests that aprindine may affect the sodium calcium exchange mechanism

which has been implicated in the genesis of these events (see above). Aprindine also slows sinus rate and AV conduction [123] and is effective in the treatment of AV nodal reentrant tachycardias even in the absence of WPW [124]. While it is tempting to assume that agents which are capable of suppressing these AV nodal arrhythmias, of slowing sinus rate, and of prolonging AV conduction are doing so by virtue of an effect on the slow channel system, it has been shown that aprindine did not suppress the slow channel activity induced in K^+ depolarized fibers by isoproterenol [120, 122]. These results suggest either that the in vivo and in vitro effects of the drug differed or that it is impossible to separate slow channel from rapid channel events on the basis of clinically observed effects. While it is possible that disopyramide and amiodorone may, like quinidine, owe part of their clinical usefulness to slow channel blocking capabilities, it is obvious that specific testing is required before the effects of these drugs on the slow inward current can be ascertained.

In conclusion, it is clear that the slow inward current can be enhanced or inhibited by a variety of ions and currently used arrhythmogenic and antiarrhythmic drugs. Nonetheless, a lack of studies in which an effect of a large number of cardioactive drugs on the slow current has been specifically sought and our inability to determine with certainty which arrhythmias owe their genesis to the slow current system render our knowledge incomplete. The intense interest in the role of the slow current system in the genesis of cardiac arrhythmias and coronary vasospasm predicts that significant advances in our understanding of this system and of the drugs which influence it will be forthcoming in the near future.

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V. CLINICAL IMPLICATIONS

22. THE ROLE OF THE SLOW INWARD CURRENT IN THE GENESIS AND MAINTENANCE OF SUPRAVENTRICULAR TACHYARRHYTHMIAS IN MAN *

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1. INTRODUCTION

Tachyarrhythmias result from disorders of impulse formation, impulse conduction or combinations of both mechanisms. Since cells dependent on the slow inward current possess the properties of impulse formation and conduction [1], it is quite plausible to consider that the slow inward current may serve as an important ionic substrate in fibers responsible for, or at least participating in, the genesis and maintenance of various supraventricular tachyarrhythmias that occur clinically. Such ionic characterization can be made on experimental preparations and ample evidence, reviewed in other chapters of this book, supports the role of the slow inward current in the generation of action potentials from normal and abnormal fibers in the atrium. For example, the data strongly indicate that the normal sinus and atrioventricular (AV) nodes are slow-channel dependent. In addition, other atrial fibers, some made abnormal by a variety of methods, may be slow-channel dependent. However, our present diagnostic tools do not permit unequivocal determination of the electrophysiologic mechanisms responsible for most clinically occurring tachyarrhythmias, i.e., reentry or automaticity, and certainly do not allow convincing differentiation into which arrhythmias may be slow-channel dependent. Nevertheless, extrapolating from experimental animal observations, from in vitro studies on human atrial tissues, and from the response of arrhythmias to the clinically available slow-channel blocker, verapamil [2], in this chapter we will consider the evidence supporting the possible participation of the slow inward current in the genesis of supraventricular tachycardia (SVT). Tachycardias considered are SVTs related to (presumed) reentry in the AV node, sinus node, atrium and over an accessory pathway; (presumed) automatic atrial tachycardia, and nonparoxysmal junctional tachycardia, and atrial flutter and atrial fibrillation. We will assume that

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the slow inward current may play a role in the genesis or maintenance of the SVT if it can be established that the SVT originates in fibers known to be slow-channel dependent; requires such fibers as part of its conduction pathway; exhibits rate responses consistent with automaticity thought to be due to the slow inward current; and/or is affected by verapamil. A brief analysis of these criteria follows.

2. CRITERIA

(1) Origination of the SVT in fibers thought to generate slow-channel dependent potentials. We will apply this criterion to SVTs thought to be caused by automaticity and consider whether they originate in the coronary sinus, mitral or tricuspid valves, or possibly other atrial sites that disease has rendered slow-channel dependent.

(2) Rate responses consistent with automaticity that responds differently from 'normal' automaticity. In some instances, such automaticity may be due to the slow inward current. We will apply this criterion to SVTs thought to be caused by automaticity and consider whether the SVT exhibits an 'anomalous' response to pacing, such as triggering or overdrive acceleration. Triggering refers to initiation of sustained rhythmic activity in which a fiber is triggered to depolarize by an impulse arising elsewhere and each subsequent discharge emerges from the afterdepolarization of the preceding impulse. Such triggering can be observed in transmembrane recordings but, as yet, not from extracellular recordings in vivo. Triggered activity may arise from fibers with high or low resting membrane potentials and its relationship to the slow inward current remains obscure in spite of the ability of verapamil to suppress triggered activity in several preparations. Overdrive acceleration is defined for our purposes as capture of the SVT by pacing or by a spontaneous rhythm (e.g., sinus) at faster rates than the SVT, with persistence or emergence of the SVT at a faster rate after loss of capture. As with triggering, fibers that exhibit overdrive acceleration behave differently than fibers with 'normal' automaticity, but the relationship to the slow inward current yet remains to be defined.

(3) Initiation or maintenance of SVT due to conduction over fibers known to be slow-channel dependent. We will apply this criterion to SVTs thought to be caused and maintained by reentry, and consider whether the reentry occurs within the sinus or AV nodes, or possibly over other atrial sites that disease has rendered slow-channel dependent.

(4) Alteration of the SVT by verapamil. Although termination of electrical activity by verapamil cannot be used as the sine qua non of slow-channel dependence, it is one of the best clinical estimates we have and we will

assume that verapamil is a relatively specific slow-channel blocker, knowing that the racemic mixture, drug concentrations, and tissue types all may affect the response produced by verapamil.

3. AV NODAL SUPRAVENTRICULAR TACHYCARDIA

3.1. Electrophysiological mechanisms

Mines [3] postulated from studies on turtle hearts that reentry within AV connections might be responsible for some arrhythmias, while Iliescu and Sebastiani [4] suggested that paroxysmal SVT might be due to a circus movement. Lewis [5] disagreed with this hypothesis because he felt that conduction velocity in atrial muscle should result in faster rates than those found for typical AV nodal tachycardia. However, Barker et al. [6] pointed out that the slower rates could be explained if part of the reentrant pathway involved the sinus or AV nodes. Subsequent animal studies have supported the conclusion that the AV node can undergo functional longitudinal dissociation and thus provide the anatomic and functional substrate for reentry to occur [7–17].

More recently, electrophysiologic evidence from studies in animals [18, 19], as well as in man [20–24], suggest strongly that AV nodal SVT is due to reentry within the AV node. The pathways used are, as yet, incompletely defined. Studies on isolated rabbit preparations suggest that circus movement occurs in the upper portion of the AV node [16, 18, 19]. These observations naturally do not eliminate reentry at other AV nodal sites, particularly in different species. The necessary role of atrial participation in the reentrant circuit is still debated. Mendez et al. [12] demonstrated the need for the atrium in generating ventricular echoes caused by AV nodal reentrant activity in dogs. Since then, studies in animals [14] and patients [25, 26] have challenged their interpretations and have suggested that ventricular echoes due to AV nodal reentry can occur without activation of the atrium. Figure 1 represents an example of apparent dissociation of the atrium with uninterrupted continuation of the SVT, leading to the conclusion that the atrium is not a necessary part of the reentrant circuit. However, since it is quite clear that rhythms in the atria may become dissimilar [27] and that a portion of the atrium may control, or participate in, the cardiac rhythm without recognition of this fact from scalar ECG or, indeed, from recordings within the right atrium or coronary sinus [28], data refuting the role of the atrium as a necessary link in the reentrant pathway must be obtained from studies in which both atria are carefully mapped for the presence of localized atrial activity, particularly near the upper AV node [18, 19, 29].

Several lines of evidence suggest the presence of more than one AV nodal

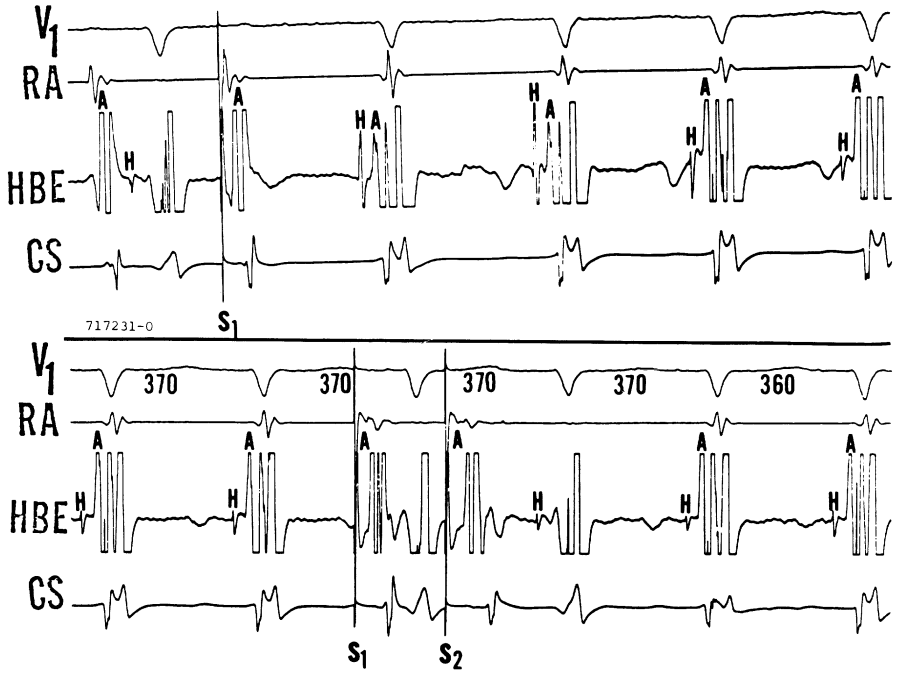


Figure 1. Dissociation of atria from ventricles without interruption of AV nodal reentrant supraventricular tachycardia. During sinus rhythm, a single premature atrial complex (S_1, top panel) conducted with AV nodal delay and initiated an AV nodal reentrant supraventricular tachycardia. Two premature atrial stimuli (S_1 S_2, bottom panel) captured the atrium on both occasions without altering the regular cycle length of the AV nodal reentrant supraventricular tachycardia. Abbreviations V_1, scalar lead; RA, right atrial electrogram; HBE, His bundle electrogram; CS, coronary sinus electrogram.

pathway in many patients with AV nodal reentrant SVT. Finding a 'discontinuous' AV nodal curve [30], during which a marked increase in the interval between consecutive His bundle responses occurs with little or no shortening of premature atrial intervals (Figure 2), has been interpreted to represent conduction over two AV pathways, one having slower conduction and shorter refractoriness than the other. When block occurs anterogradely in the fast pathway and proceeds to the ventricle over the slow pathway, the fast pathway then may be invaded retrogradely to produce atrial echoes or sustained SVT. Less commonly, the fast pathway may be used anterogradely, and the slow pathway retrogradely [31]. Both pathways have electrophysiologic responses in the anterograde direction characteristic of AV nodal fibers. However, in some patients with AV nodal reentrant SVT, the electrophysiologic features of the retrogradely conducting pathway differ greatly from those of the anterogradely conducting pathway, not only in response to atrial or ventricular pacing, but also in response to various drugs [32–37]. Thus, it is

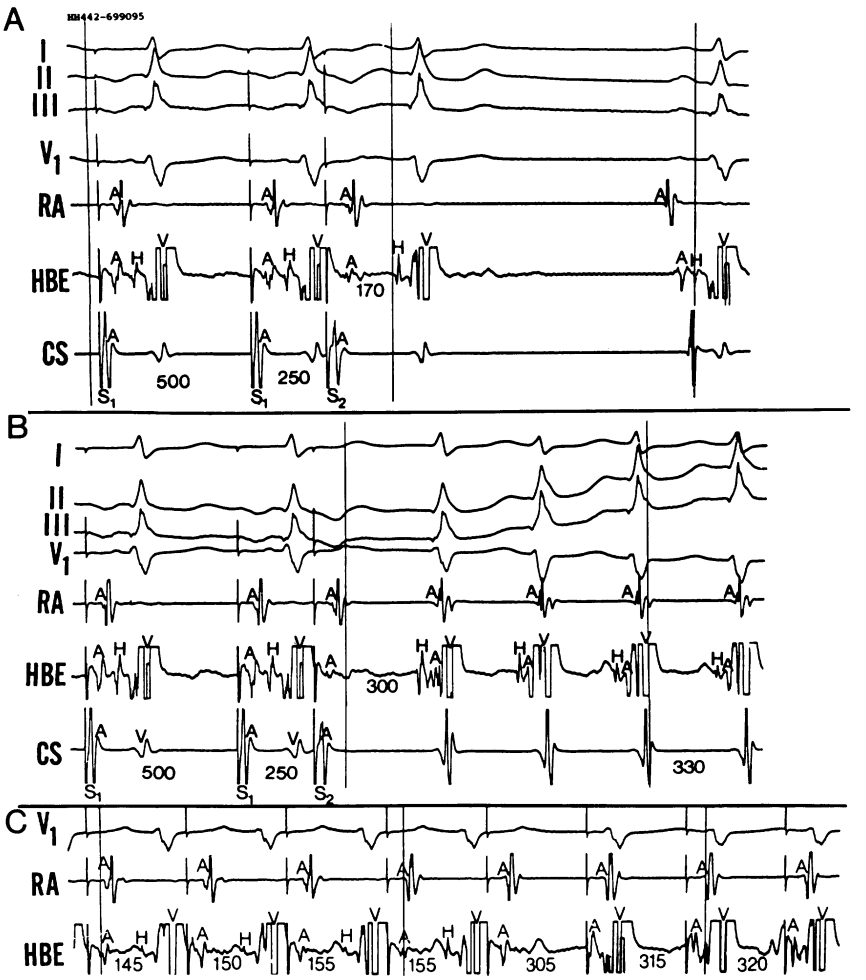


Figure 2. 'Discontinuous' AV nodal response. A premature stimulus (S_2) delivered to the coronary sinus at an interval of 250 msec following a basic cycle length of 500 msec conducted with an AH interval of 170 msec (panel A). At the same premature interval (panel B), the premature atrial complex resulted in sudden prolongation of the AH interval to 300 msec and initiation of an AV nodal reentrant supraventricular tachycardia at a cycle length of 330 msec. In panel C, pacing the coronary sinus at a fixed cycle length of 340 msec resulted in a reasonably stable AH interval of approximately 150 msec for several complexes. Suddenly, the AH interval lengthened to >300 msec (middle), consistent with a discontinuous AV nodal response due to block in the fast pathway. Supraventricular tachycardia was prevented by continued atrial pacing. Reproduced with permission from the American Heart Association, Inc., reference 37.

not clear whether the retrogradely conducting pathway is extranodal, intranodal but insulated from the rest of the AV node, or includes only a small amount of AV nodal tissue. It is also unsettled whether the retrogradely

conducting pathway is distinct from the fast and slow anterogradely conducting pathways, which appear to be composed of AV nodal fibers. The geometry and direction [38] of conduction, effects of summation [39], autonomic tone, as well as the electrophysiologic characteristics of the fibers, all may influence conduction and the response to drugs. Some patients may have three (or more) pathways. It is likely that the anatomic, as well as the electrophysiologic, features of AV nodal pathways may not be the same in all patients with AV nodal reentrant SVTs. Recent evidence suggests that, in some patients with AV nodal reentrant SVT, the retrogradely conducting pathway may exhibit electrophysiologic properties consistent with AV nodal tissue, but is located in an extranodal site since it gives rise to an abnormal sequence of retrograde atrial activation [40].

In summary, the weight of evidence implicates reentry as the mechanism responsible for initiation and maintenance of AV nodal SVT [41] and places the site of the anterogradely conducting pathway(s) in the AV node. The site of the retrogradely conducting pathway, as well as the geometry and electrophysiology of its fibers, is still being defined and may not be the same for all patients with AV nodal reentrant SVT.

3.2. Effects of verapamil

Slow channel blockers, such as manganese [42] and verapamil [43–47], strikingly depress AV nodal propagation and lengthen AV nodal refractoriness, without significantly affecting conduction in the His–Purkinje system, ventricular or atrial muscle. Tetrodotoxin fails to inhibit AV nodal activity, while suppressing activity in the His bundle, atrial [42] and ventricular muscle. In an isolated rabbit AV nodal preparation, verapamil prevented AV nodal reentry and initiation of atrial tachycardia, by causing premature atrial impulses to block rather than to conduct with the delay needed to initiate reentry [45]. By this mechanism, verapamil may terminate AV nodal reentrant SVT in man.

Verapamil, administered intravenously, has been shown repeatedly to terminate effectively episodes of AV nodal reentrant SVT in man [2, 33, 37, 46, 48, 49]. In a recent study, we have confirmed the fact that verapamil terminated AV nodal reentrant SVTs by causing prolongation and eventual block of anterograde AV nodal conduction (Figure 3) [37]. We also found that verapamil only affected conduction and refractoriness in the anterogradely conducting pathway, exerting no significant effect on the pathway used for retrograde conduction during the SVT (Figure 4) [33]. This observation lends further credence to the concept that, at least in some patients with AV nodal reentrant SVT, the retrogradely conducting pathway may not be composed of typical AV nodal fibers [35].

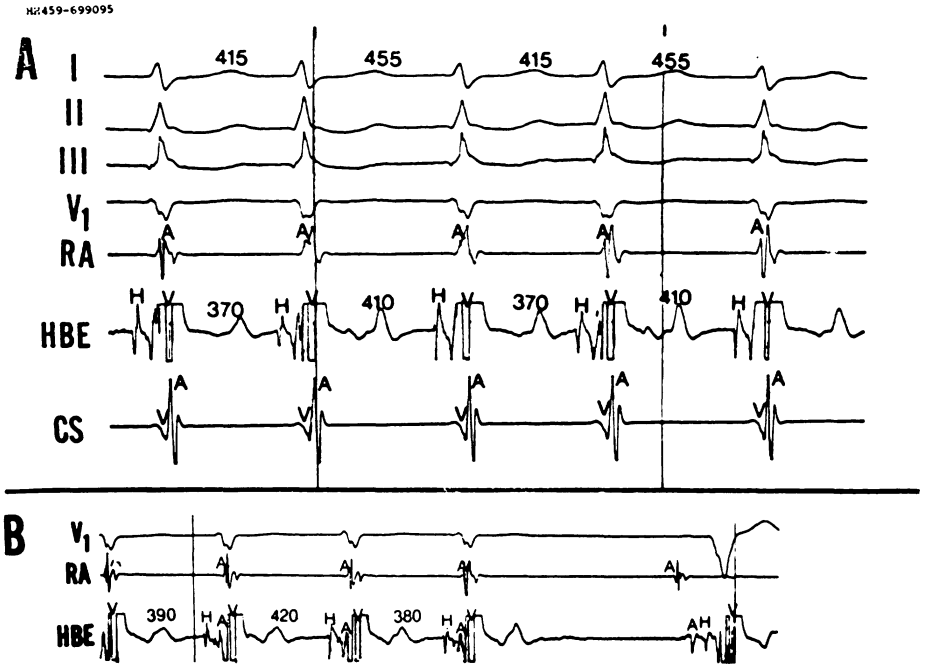


Figure 3. Cycle length alternans and termination of AV nodal reentrant supraventricular tachycardia following verapamil administration. Alternation in AV nodal conduction time (AH intervals) caused alternation in the ventricular cycle length prior to termination of SVT (panel A). SVT terminated during anterograde conduction, with block of the atrial impulse proximal to the His bundle recording site (panel B). Reproduced with permission from the American Heart Association, Inc., reference 37.

Cycle length alternans [50, 51] is not uncommon just prior to termination of SVT, particularly after verapamil administration [52], and several of our patients exhibited such a response. Cycle length alternation during any tachycardia must be due to alternation in the rate of discharge of an automatic focus, to alternation in the pathways used, or to alternation of conduction time in the pathways [50]. Since reentry is presumably responsible for AV nodal SVT, alternation in the rate of discharge of an automatic focus is not tenable, and alternation in conduction time or pathways must be responsible. Alternation in AH intervals caused the cycle length alternans, as seen in a patient with a discontinuous AV nodal curve (Figure 3). Such alternation can be explained by postulating the presence of three pathways: anterograde conduction alternating between a slow and a fast pathway, with constant retrograde conduction over the third, perhaps non-AV nodal pathway. However, unless many patients with AV nodal reentrant SVTs have at least three pathways, the relatively frequent occurrence of cycle length alternans after verapamil administration suggests that the third explanation, that is, alternation in conduction time over only one pathway anterogradely, may be more

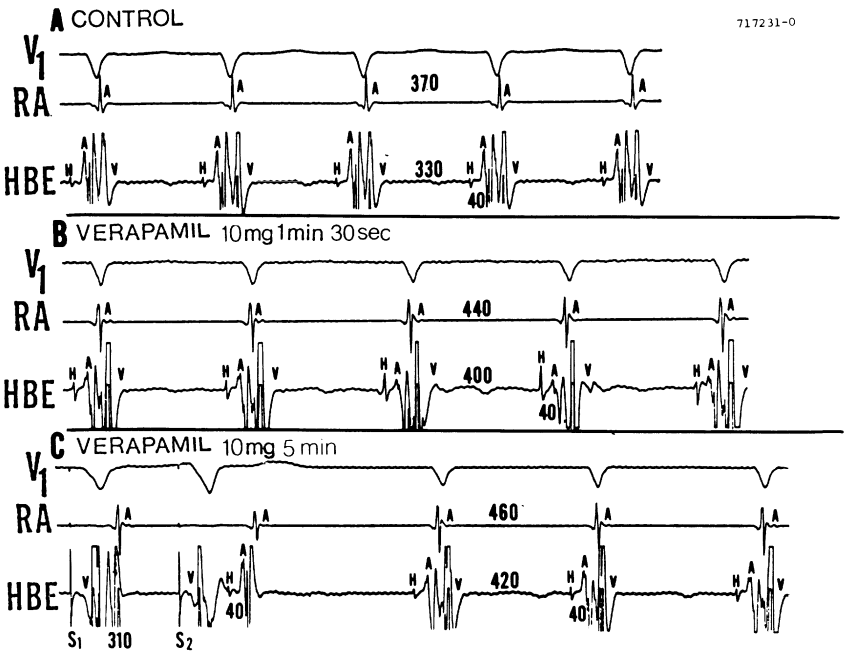


Figure 4. Lack of effect of verapamil on retrograde conduction in the AV nodal pathway during SVT. Verapamil administration during AV nodal reentrant supraventricular tachycardia (panel A) markedly prolonged AV nodal conduction time without changing HV or HA intervals (panel B). Tachycardia was reinitiated with premature right ventricular stimulation (panel C). S_2 produced retrograde His delay allowing easy measurement of HA interval, which was the same as that during SVT. Tachycardia resumed with markedly prolonged AH interval but no change in HA interval. Reproduced with permission from the American Heart Association, Inc., reference 37.

likely. Why this might occur is unclear and several explanations are possible. It is important to note that AV conduction time remains fixed. The cycle with the shorter AH interval might allow the succeeding cycle insufficient time for complete recovery of AV nodal refractoriness, thus prolonging the AH interval in the following cycle. The subsequent long AH interval might provide more time for complete recovery of AV nodal refractoriness and permit a shorter AH interval in the next cycle. Since action potential duration and refractoriness of AV nodal cells may lengthen with slow conduction [16], this explanation may be too simplistic. Other factors, such as electrotonic influences, can be responsible for alternating cycle lengths [53] under certain circumstances.

Conduction in the His–Purkinje system can become dissociated [54–56], and it is possible that reentry in the His–Purkinje system may be responsible for some SVTs. However, if His–Purkinje reentry caused SVT, and the supporting data are not strong, the role of the slow current in such an SVT is unknown. Ordinarily, the fast channel is responsible for depolarization in the

His–Purkinje system. Under various conditions, the fast channel may become suppressed and allow emergence of depolarizations dependent on the slow response [1]. It is often difficult to differentiate in vitro between depressed fast responses and slow responses and little or no information exists regarding the clinical differentiation of such events in the His–Purkinje system. Thus, although SVT owing to reentry in the His–Purkinje system may exist, the evidence is far from compelling and the role of the slow inward current, if any, is unknown.

3.3. Conclusion

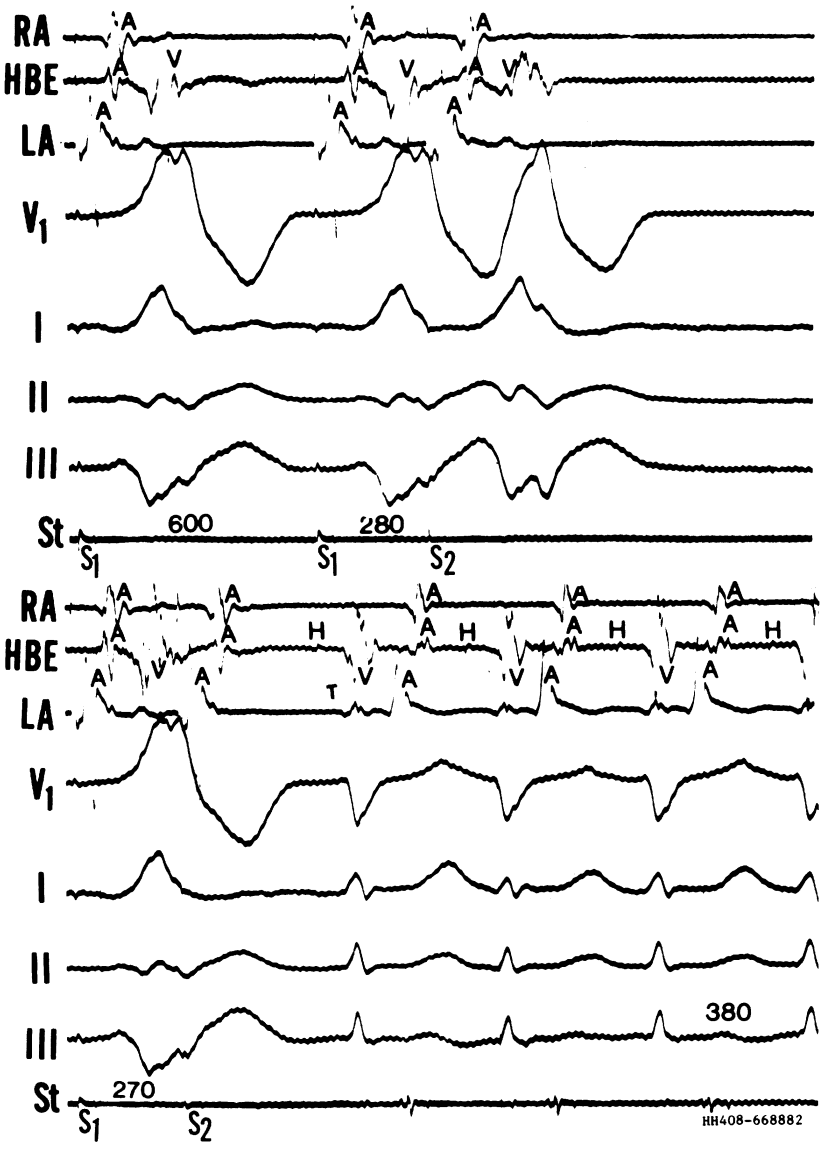
The present evidence indicates that SVT involving the AV node is most probably due to reentry; that at least the anterogradely conducting pathway or pathways are composed of AV nodal tissue; and that verapamil effectively blocks conduction in this pathway to terminate the SVT. Thus, we conclude that criteria 3 and 4 are applicable to AV nodal reentrant SVT, and therefore the slow inward current plays a role in the genesis and maintenance of this SVT.

4. SUPRAVENTRICULAR TACHYCARDIA RELATED TO THE WOLFF–PARKINSON–WHITE (WPW) SYNDROME

4.1. Electrophysiological mechanisms

For none of the clinically occurring supraventricular and ventricular tachycardias are the accumulated anatomic and electrophysiologic data more supportive of a reentrant mechanism than for SVT related to an accessory pathway [57, 58]. Although exceptions occur, the usual activation wave during SVT in a patient with an accessory pathway travels anterogradely over the normal AV node–His–Purkinje system and retrogradely over the accessory pathway, resulting in normal ventricular activation (Figure 5). In some patients, the accessory pathway may be capable only of retrograde conduction [59, 60].

In addition to the response of the SVT to premature stimulation, one of the strongest lines of evidence supporting a reentrant mechanism for SVT associated with an accessory pathway is that interrupting the presumed reentrant loop at widely separated points, i.e., by surgically cutting the normal AV node–His bundle pathway *or* the accessory pathway, eliminates the ability to develop SVT. Cranefield (personal communication) has suggested an alternate hypothesis to explain that observation: the presence of a triggerable focus with unidirectional entrance and exit block. Either a precisely placed lateral

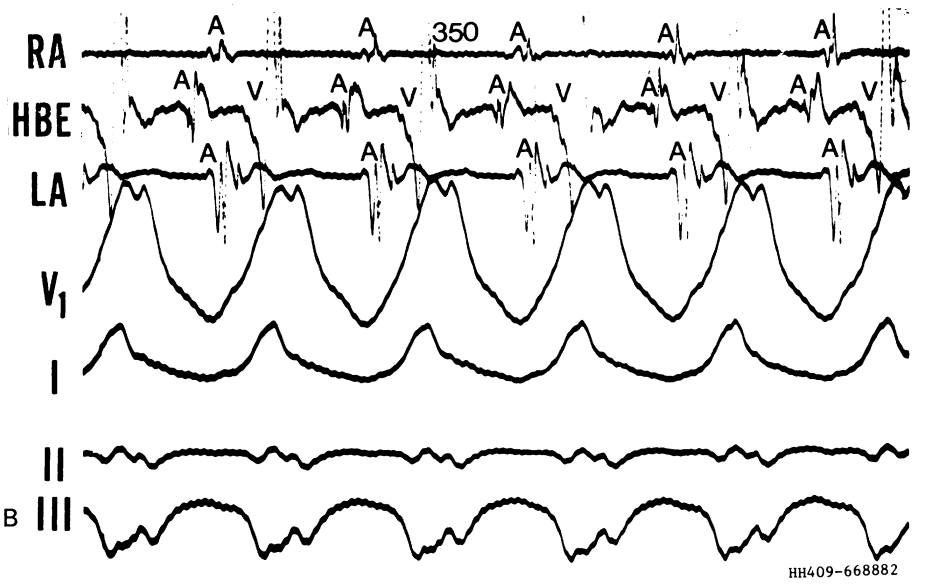


A

Figure 5. A: initiation of supraventricular tachycardia in a patient with Wolff–Parkinson–White syndrome. Premature left atrial stimulation (S_2 top panel) at cycle length of 280 msec following a basic cycle of 600 msec (S_1–S_1) conducted over the accessory pathway to the ventricles. Premature left atrial stimulation at cycle length 270 msec (S_2 bottom panel, basic cycle unchanged) blocked anterogradely in the accessory pathway, conducted to the ventricle over the normal pathway and was followed by initiation of supraventricular tachycardia. Activation during tachycardia occurred anterogradely over the normal AV node His–Purkinje route and retrogradely over the accessory pathway, resulting in left atrial excitation (LA) before low right atrial (HBE) or high right atrial (RA) excitation. B: the same patient as in A. On another occasion, premature atrial

incision, or an incision interrupting the normal pathway, could prevent initiation and maintenance of SVT (Figure 6), without having to postulate reentry. It is of interest to note that Holzman and Scherf[61], in addition to the usually accepted accessory pathway concept which they and Wolferth and Wood[62] postulated, offered an alternate explanation which assumed that atrial contraction stimulated an excitable center within the ventricle, to cause preexcitation. While presently no evidence exists to support this hypothesis, it is interesting and perhaps makes a reentry explanation somewhat less airtight.

Patients with the WPW syndrome also may have SVT due to any of the other mechanisms responsible for SVT in the absence of an accessory pathway, i.e., AV nodal reentry, sinus nodal reentry, and so forth. In addition, variants of the WPW syndrome, involving conduction over atrio-His, nodofascicular or nodo-ventricular pathways, may provide alternate routes for reentry[63]. However, for the stated purpose of this chapter, i.e., whether the slow inward current plays a role, it is clear that SVT in the WPW syndrome most often involves conduction over the AV node anterogradely, and thus



stimulation initiated supraventricular tachycardia with QRS contour identical to that which was produced by conduction over the accessory pathway. In addition, retrograde atrial activation sequence displayed low right atrial activation preceding left or high right atrial activation. Pathway of activation during this tachycardia was a far less common type: anterograde conduction over the accessory pathway and retrograde conduction over the normal AV node His–Purkinje route (one cannot exclude retrograde conduction over a second accessory pathway located in the atrial septum). Retrograde His activation is not apparent, probably lost within inscription of ventricular activation.

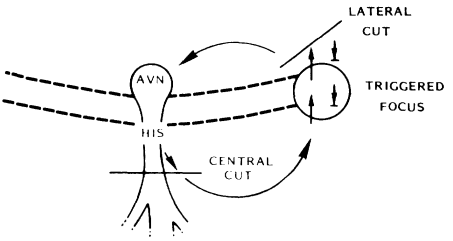


Figure 6. Triggered focus with unidirectional entrance and exit conduction as a hypothetical cause of supraventricular tachycardia in Wolff–Parkinson–White syndrome. The triggered focus can only be entered from the ventricle, and can only exit to the atrium. Therefore, a lateral cut or a central cut can eliminate tachycardia. AVN, atrioventricular node; His, His Bundle.

involves slow-channel dependent fibers in this portion of the reentrant loop. The remaining fibers in the reentrant pathway, atrium, ventricle or specialized fibers, are normally fast-channel dependent.

4.2. Effects of verapamil

Verapamil exerts no significant electrophysiologic effects on the accessory pathway and terminates SVT in patients with WPW syndrome by slowing and blocking conduction over the normal AV node [2, 37, 64]. Thus, SVT in WPW most commonly terminates after verapamil administration during anterograde conduction. If verapamil does not produce block in the AV node during SVT the tachycardia continues at a slower rate, due to prolongation of AV nodal conduction time.

Some data suggest that the AV node in patients with WPW syndrome may conduct more rapidly and exhibit a shorter refractory period than in patients without the WPW syndrome [65]. We have found that verapamil prolonged AV nodal conduction time, functional and effective refractory periods of the AV node to a lesser extent in patients with WPW who had SVT, than in patients who had SVT due to AV nodal reentry [37]. The reason for the reduced electrophysiologic response of the AV node to verapamil in patients with WPW syndrome is unknown.

4.3. Conclusion

The present evidence indicates that SVT associated with an accessory pathway in patients with WPW syndrome is due to reentry, involving anterograde conduction over the AV node and retrograde conduction over the accessory pathway in most patients. Verapamil terminates the SVT by blocking AV nodal conduction. Thus, the AV node forms one link of the reentrant pathway and, in that regard, the SVT is slow-channel dependent, meeting criteria 3 and 4.

5. SUPRAVENTRICULAR TACHYCARDIA DUE TO SINUS NODAL RE-ENTRY

5.1. Electrophysiologic mechanisms

The sinus node shares with the AV node several similar electrophysiologic features, such as reduced resting potential and rate of rise of phase zero, slow propagation and the potential for dissociation of conduction [66]. In many ways, the sinus node is a prototype of a slow-channel dependent automatic fiber, and its activity is suppressed by slow-channel blockers [1, 43–47].

Premature stimulation can produce slow propagation in the sinus node, block in some areas, and the development of repetitive responses, most likely due to reentry. Evidence from a number of studies in man [67–71] and

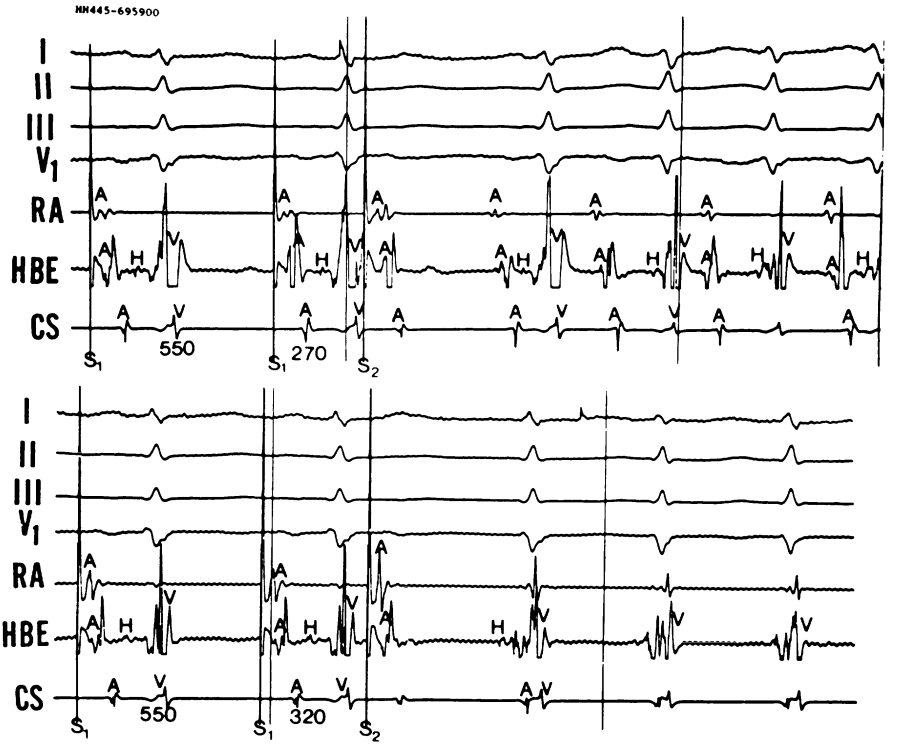


Figure 7. Sinus nodal reentry. Premature right atrial stimulation (S_2, top panel) at a cycle length of 270 msec blocked proximal to the His bundle recording site and was followed by a supraventricular tachycardia in which the atrial activation sequence was high right atrium followed by low right atrium and then left atrium, very similar to that which was produced by high right atrial pacing or during sinus rhythm. Such a supraventricular tachycardia is probably due to sinus nodal reentry. Premature right atrial stimulation at a longer coupling interval (S_1S_2 320 msec bottom panel) was followed by prolonged AV nodal conduction time and initiation of supraventricular tachycardia in which low right atrial activation preceded high and left atrial activation, consistent with AV nodal reentry. Reproduced with permission from the American Heart Association, Inc., reference 37.

animals [11, 66, 72, 73] support the concept that sustained reentry in the sinus node can occur and cause SVT. Recently, Allessie et al. [73] showed that the reentrant circuit in the isolated rabbit preparation was located entirely within the sinus node, and that the atrium was not an essential link.

SVTs thought to be due to sinus nodal reentry generally have a slower rate than SVTs due to AV nodal reentry or reentry over an accessory pathway. P waves appear in front of the QRS complex and exhibit a contour similar to the P wave during sinus rhythm with the expected sequence of activation beginning in the upper right atrium and progressing caudally (Figure 7).

While it is possible that under some circumstances the sinus node responds to premature stimulation with an increase in its discharge rate, as a form of triggered sustained rhythmic activity or overdrive acceleration, and that SVTs thought to be caused by sinus nodal reentry actually are due to triggered activity, experimental evidence does not support this conclusion. The sinus node ordinarily manifests overdrive suppression following a period of rapid pacing [74, 75] a characteristic that may separate it from automaticity due to other slow response models. An increase in sinus nodal discharge rate may occur transiently at times following rapid pacing, possibly due to adrenergic stimulation in response to the pacing-induced rate increase. Premature stimulation of the sinus node may shorten or lengthen the return cycle, depending on the coupling interval of the premature beat, which determines whether the sinus node is reset, is influenced electrotonically [76–79] or if a pacemaker shift occurs. Often, the atrial event does not reflect the true response to premature stimulation of the sinus nodal pacemaker cells. However, triggered sustained rhythmic activity in the sinus node as a cause of persistent SVT does not seem likely, and the weight of evidence suggests that reentry in the sinus node is responsible for the SVT.

5.2. Effects of verapamil

The effects of verapamil in patients with SVT due to sinus nodal reentry have not been investigated systematically. In dogs, a recent study [80] evaluating the effects of verapamil given intravenously suggested a possible differential response of the sinus and AV nodes. Verapamil prolonged AV nodal conduction time and produced AV block at plasma concentrations that did not significantly alter the spontaneous discharge rate of the sinus node. Verapamil's effect on conduction within the sinus and AV nodes was not compared. Similar observations have been made in man [81]. When given following beta-adrenergic blockade, verapamil achieves more significant depression of sinus nodal automaticity. It is possible that a reflex increase sympathetic tone prevents verapamil, when given alone intravenously, from significantly altering sinus nodal automaticity [32].

Curry and Krikler [71] found that verapamil terminated SVT due to sinus nodal reentry promptly in two patients and prevented reinitiation of the tachycardia in five. We [37] have given verapamil to two patients with SVT due to AV nodal reentry. One patient, before verapamil administration, and that patient, plus another patient after verapamil administration, also exhibited an SVT due to sinus nodal reentry (Figure 7). In both patients, the SVT due to sinus nodal reentry persisted several minutes after verapamil had terminated the AV nodal reentrant SVT. It would appear that the sinus nodal reentrant SVT resisted the suppressing effects of verapamil (10 mg intravenously) more than did the AV nodal reentrant SVT. In fact, in the second patient, verapamil may have even caused the sinus nodal reentrant SVT, that could not be produced prior to verapamil administration, by producing partial conduction delay in the sinus node.

5.3. Conclusion

The present evidence indicates that the SVT involving the sinus node is probably due to reentry within the sinus node. The effects of verapamil on this SVT must be regarded as preliminary, but it would appear that verapamil does suppress sinus nodal reentrant SVT, although in some instances the SVT may be more resistant than AV nodal reentrant SVT. Thus, sinus nodal reentrant SVT meets criteria 3 and 4 and is therefore considered to be dependent on the slow inward current.

6. ATRIAL REENTRY

6.1. Electrophysiological mechanisms

Reentry within the atrium, unrelated to the sinus node, has been shown experimentally to occur in the rabbit [83–85] and dog [86] atrium, with or without an anatomical obstacle, and may be a cause of SVT in man. Examples of SVT purported to be due to atrial reentry have been cited [41, 69, 87, 88] but their relative infrequency in the published literature suggests it is not a commonly recognized cause of SVT in man. Atrial reentry differs from sinus nodal reentry by a P wave morphology and atrial sequence of activation unlike that found during sinus rhythm. Distinguishing atrial tachycardia due to automaticity from atrial tachycardia sustained by reentry over very small areas, i.e., micro-reentry, is very difficult and therefore conclusions regarding electrophysiologic mechanisms of this SVT based on presently available information must be accepted cautiously.

6.2. Effects of verapamil

One can speculate that verapamil might terminate the SVT if the latter were due to reentry over atrial fibers possibly altered by disease to be slow-channel dependent. However, to our knowledge, no available information exists regarding the clinical effects of verapamil on SVT due to atrial reentry. In experimentaly-induced reentrant atrial tachycardia in the rabbit, verapamil exerted no significant effect [89].

6.3. Conclusions

The clinical importance of reentry in the atrium, unrelated to the sinus node, as a cause of SVT is presently unknown, as is the role of the slow-channel in its initiation or maintenance.

7. AUTOMATIC ATRIAL TACHYCARDIA

7.1. Electrophysiological mechanisms

Several types of atrial fibers possess the capacity for spontaneous diastolic depolarization [90, 91]. When enhanced, this spontaneous diastolic depolarization could be responsible for SVT in some patients. Of interest has been the demonstration that fibers in the rabbit [92], caniné [93], simian [94] and human [95] atrioventricular valves, and in the canine coronary sinus [96], when superfused with norepinephrine, exhibit the capacity for triggered sustained rhythmic activity [97]. In general, the fibers exhibited delayed afterhyperpolarizations, followed by delayed afterdepolarizations that were capable of reaching threshold and triggering sustained rhythmic activity [98]. Action potentials of the mitral valve possessed less negative resting potentials and slower upstrokes than action potentials in the coronary sinus and more clearly resembled slow-response type action potentials [94]. Verapamil suppressed the triggered activity in these preparations, and it is possible that the slow inward current played a role in their genesis. However, the membrane potential of these fibers, particularly those in the coronary sinus, was not sufficiently depressed to have inactivated the fast response or to have activated the slow inward current [1, 47].

Saito et al. [99] recorded oscillatory potentials and triggered sustained rhythmic activity in fibers of the upper pectinate muscle and in the pectinate muscle along the crista terminalis in the rabbit right atrium. Norepinephrine was not necessary to trigger the activity. A train of stimuli sometimes increased the rate of discharge of the spontaneous activity rather than suppressing it [100]. The effects of slow-channel blockers were not tested. Trig-

gered activity can also be found in diseased human atrial myocardium (see Chapter 20).

In man, SVTs interpreted to be automatic by a variety of criteria have been described [86, 101–104], but remain incompletely characterized. Their differentiation from other forms of SVT, such as sinus nodal reentry (if the P wave of the automatic atrial tachycardia resembles the sinus-initiated P wave) or atrial reentry (particularly if caused by micro-reentry), may be difficult. However, in view of the experimental findings of triggered activity from a variety of atrial fibers, including human mitral valve, it is possible that such triggered activity occurs in man.

We have performed electrophysiologic studies on three patients who exhibited SVT that met the previously established criteria for automaticity [37]. Overdrive suppression following a period of rapid pacing occurred in all patients; capture of the SVT by atrial pacing was not followed by a faster SVT rate when pacing was discontinued. Premature stimulation was not followed by shorter return cycles and the return cycle was inversely, not directly, related to the premature cycle. In all three patients, the SVT could not be initiated or terminated by premature or rapid atrial pacing. The SVT originated in the lower right atrial septum in one patient and in the high right atrium in two patients. Its origin in or near the tricuspid valve or coronary sinus could not be established. Two additional patients were treated with verapamil, but did not undergo electrophysiologic study.

Our therapeutic experience with these 5 patients is as follows: In one patient, the SVT achieved rates of 240/min with 1:1 atrioventricular conduction, and was refractory to conventional or experimental drugs. This patient was finally treated by surgical creation of AV block and insertion of an atrial synchronous pacemaker [105]. In one patient each, the SVT has been suppressed by aprindine and by amiodarone; in two patients the ventricular rate has been controlled by digitalis, which has not affected the SVT.

7.2. Effects of verapamil

In our group of 5 patients, verapamil given intravenously (10 mg to one patient, 5 mg to two patients) resulted in AV nodal block with persistence of the SVT at an unchanged rate (Figure 8). Administered orally (480 mg/day) to the remaining two patients, verapamil also had no effect on the rate of atrial discharge during the SVT.

7.3. Conclusion

The experimental observations of triggered activity in isolated preparations of the mitral valve, coronary sinus, normal and diseased atria, with suppression

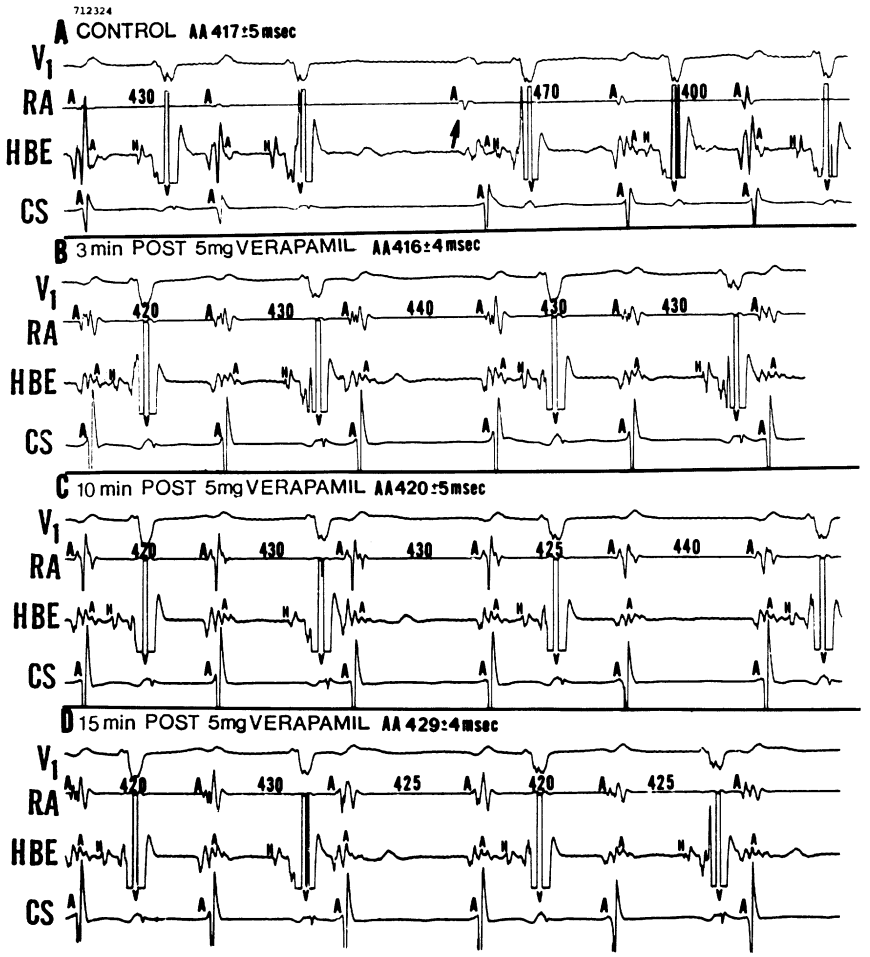


Figure 8. Effects of verapamil in a patient with supraventricular tachycardia due to enhanced automaticity. During tachycardia, atrial activation was first recorded in the HBE lead and then in the high right atrial and coronary sinus leads (panel A). In the midportion of the tracing, the supraventricular tachycardia terminated briefly and a single sinus beat exhibited the normal sequence of atrial activation (arrow), just prior to resumption of the supraventricular tachycardia. Verapamil administration (panels B, C) resulted in type 1, second degree (Wenckebach) AV block but did not slow the discharge rate of the supraventricular tachycardia. Reproduced with permission from the American Heart Association, Inc., reference 37.

by verapamil, suggest but do not prove that the slow inward current may play a role in the genesis of this triggered activity. However, the clinical information, though meager, at present does not support that conclusion, and none of the criteria implicating the slow inward current are applicable to the clinically-occurring SVTs. Further data are necessary to delineate whether automaticity figures as an important cause of clinically occurring SVTs and whether they are slow-channel dependent.

8. NONPAROXYSMAL AV JUNCTIONAL TACHYCARDIA

8.1. Electrophysiologic mechanisms

Nonparoxysmal AV junctional tachycardia (NPJT) is often due to cardiac surgery, acute rheumatic heart disease, digitalis intoxication or myocardial infarction [106–108] (Figure 9). Sometimes no etiology can be obtained and the patient may be otherwise healthy [108]. Conversely, NPJT at very rapid rates has been described in 4 children, and was found to be a very serious, difficult to control SVT [109]. The mechanism responsible for NPJT is generally assumed to be enhanced automaticity of the His bundle, or a contiguous structure that possesses automatic activity. It is possible that NPJT originates in atrial fibers, without recognition of the latter's role in the scalar ECG or invasively, unless a careful search is made [28]. Wenckebach periods may occur, but the presence of exit block has not yet been demonstrated clinically, and the block may be in the AV node, with the origin of the NPJT proximal to the site of His bundle recording [28, 110]. From one interesting report of accelerated junctional escape beats was the observation of shorter junctional escape intervals following premature atrial complexes, raising the possibility of overdrive acceleration in these fibers. Pacing studies and the effects of verapamil were not tested [111].

Several types of junctional rhythms have been produced experimentally in the dog [112]. Recent studies have related the site of presumed His bundle automaticity to the presence of P cells demonstrated histologically [113].

8.2. Effect of verapamil

Clinical information regarding the effects of verapamil on NPJT is negligible. Automaticity in the His bundle is generally thought to be similar to that found in Purkinje fibers and therefore not slow-channel dependent. However, it is certainly possible that some of the interventions responsible for NPJT may create conditions in which the slow inward current is operative.

8.3. Conclusion

NPJT appears to originate in or near the His bundle and seems to be due to enhanced automaticity. Data from one clinical study suggest that it may exhibit overdrive acceleration [111], a finding consistent with some forms of triggered activity, some of which may be slow-channel dependent. The effects of verapamil on NPJT are unknown. We conclude that, although criterion 2 may be satisfied in one study, there are no other data at present indicating that the slow inward current plays a role in NPJT.

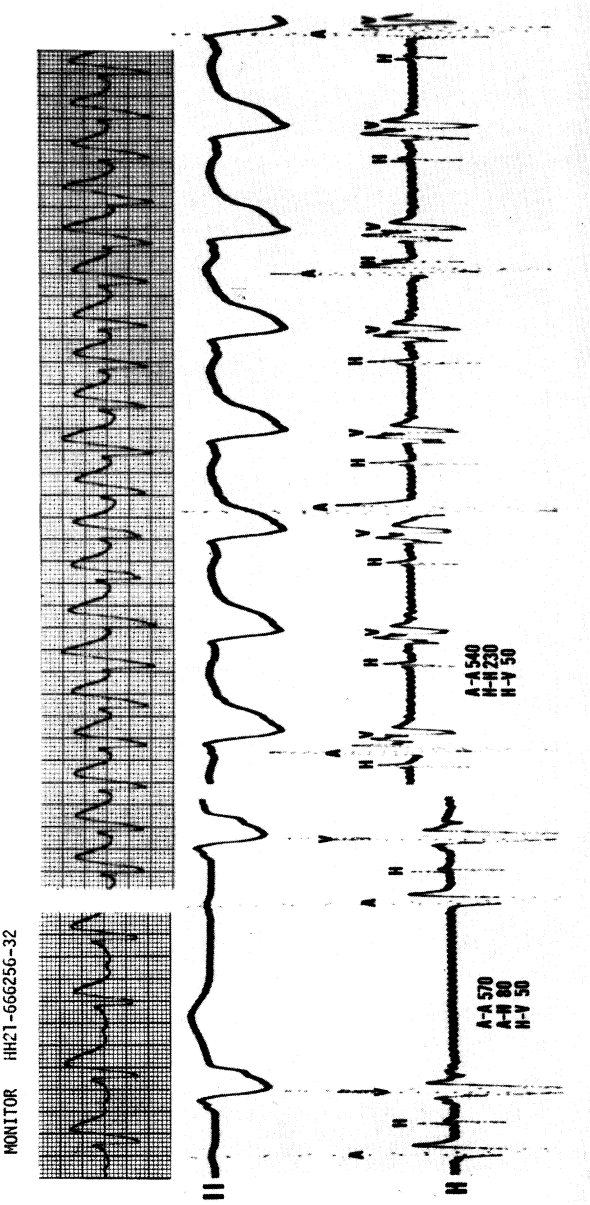


Figure 9. Nonparoxysmal AV junctional tachycardia. His bundle activation at an HV interval identical to that which occurred during sinus rhythm (left panels) resulted in a rapid (150–260/min) supraventricular tachycardia with AV dissociation.

9. ATRIAL FLUTTER AND ATRIAL FIBRILLATION

9.1. Electrophysiological mechanisms

Whether reentry, automaticity or both factors are responsible for atrial fibrillation is still a debated issue and will not be settled here [114]. Much evidence supports the role of reentry as a cause of atrial flutter [114] but abnormal automaticity cannot be excluded (for review, see [86]). Since sustained atrial flutter or atrial fibrillation occur uncommonly in patients without heart disease, it is possible that the atria in these patients manifest abnormal electrophysiologic characteristics, some of which may be consistent with slow-channel dependent action potentials. At present, however, insufficient data prevent any definite conclusions.

9.2. Effect of verapamil

Verapamil reportedly converts to sinus rhythm approximately 30% of episodes of atrial flutter and 16% of episodes of atrial fibrillation [2]. These may be optimistic figures. On our own series of 12 patients with atrial fibrillation, two developed sinus rhythm at a time (2 and 5 h after drug administration) beyond the peak drug effect of IV verapamil, raising questions about causality. The two patients with atrial flutter whom we treated with verapamil developed atrial fibrillation, and one patient transiently had sinus rhythm. The consistent effect of verapamil in patients with atrial flutter or atrial fibrillation was a prompt decrease in the ventricular response, lasting at least 30 min [37].

9.3. Conclusion

In some patients with atrial flutter or atrial fibrillation, the slow response may play a role in the genesis and maintenance of the arrhythmias. However, that conclusion is based on consideration of in vitro observations of transmembrane potentials recorded from diseased human atrial fibers. No clinical information is available to support the importance of the slow inward current in these SVTs and none of the 4 criteria can be satisfied at present.

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23. THE ROLE OF THE SLOW INWARD CURRENT IN THE GENESIS OF VENTRICULAR TACHYARRHYTHMIAS IN MAN

HEIN J.J. WELLENS, JERÓNIMO FARRÉ and FRITS W. BÄR

1. INTRODUCTION

The mechanisms of cardiac arrhythmias are extremely difficult to evaluate in the intact human heart. This can only be accomplished under exceptional circumstances such as in the Wolff–Parkinson–White syndrome. Here an anatomically determined pathway can be identified in which propagation of an impulse leads to the typical circus movement tachycardia of the syndrome. Intracellular potentials cannot be obtained in the intact human heart at the present time, making discussions on the possible role of the slow response in the genesis of clinically occurring ventricular arrhythmias purely speculative. We want to stress therefore that we cannot draw any practical conclusions about consequences of the slow response concept in relation to the treatment of ventricular arrhythmias in man. In discussing the possible role of the slow response as mechanism of clinically occurring ventricular arrhythmias we have to rely heavily upon the notion that this mechanism can be blocked by verapamil [1].

2. VENTRICULAR ARRHYTHMIAS DURING CARDIAC ISCHEMIA

As pointed out elsewhere in this volume, partial membrane depolarization during cardiac ischemia may predispose to activation of the slow inward current. During ischemia the presence of partially depolarized fibers favoring slow conduction and increased levels of cathecholamines promotes the emergence of arrhythmias based upon enhanced automaticity, reentry or both.

Prior to the hypothesis of the slow response Schmid and Hanna [2], Kaumann and Aramendia [3] and Kupersmith et al. [4] reported on the effectiveness of verapamil in the dog heart in reverting ventricular arrhythmias following coronary artery ligation. Elharrar et al. [5] showed that verapamil given intravenously prior to coronary artery occlusion in the dog reduced epicardial conduction delay and the propensity to develop ventricular arrhythmias caused by ischemia. Fondacaro et al. [6] demonstrated that ventricular fibrillation following coronary occlusion could be prevented by giving verapamil.

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As far as ventricular arrhythmias in the human heart are concerned Heng et al. [7] reported on the effects of verapamil in 4 patients with ventricular tachycardia in the setting of acute myocardial infarction. Intravenous verapamil restored sinusrhythm in only one patient. Filias [8] described a reduction in the number of ventricular premature beats following acute myocardial infarction in 28 patients receiving verapamil intravenously either intermittently or by infusion. Fazzini et al. [9] also reported on the effectiveness of verapamil in suppressing ventricular premature beats following acute myocardial infarction. They noted that 0.10 mg verapamil/kg bodyweight given during 2 min resulted in complete abolition of ventricular premature beats in 7 of 8 patients who presented with persistent and frequent ventricular premature beats (more than 10/min) during the first 48 h after myocardial infarction.

The number of reports on the effect of verapamil in ventricular arrhythmias is limited. No controlled studies are available. The observation that verapamil reduces or suppresses ventricular premature beats during acute cardiac ischemia does not necessarily mean that these arrhythmias are based upon a slow response mechanism. By inhibiting transmembrane fluxes of calcium, verapamil also has coronary vasodilation properties and affects excitation–contraction coupling [10]. The negative inotropic properties of verapamil suggest caution in their use in the early phases of myocardial infarction.

3. DIGITALIS INDUCED VENTRICULAR ARRHYTHMIAS

Several authors [11–13] have suggested that the slow response is responsible for cardiac arrhythmias due to digitalis intoxication. Suppression of digitalis induced ventricular arrhythmias in the dog heart by verapamil has been reported by Melville et al. [14] and Rodriguez-Pareira and Viana [15]. The only clinical data on the effect of verapamil on ventricular premature beats which were considered to be the result of digitalis intoxication are from an uncontrolled study by Filias [8]. He reported suppression or marked reduction in premature beats following the intravenous and oral administration of verapamil. Controlled studies on the effect of slow response blocking agents on digitalis-induced ventricular arrhythmias are indicated to establish the clinical value of these agents in the treatment of digitalis intoxication. In these patients the potentiating effects of, for example, verapamil on AV nodal refractoriness and conduction time in digitalis intoxicated patients should be realized.

4. PATIENTS WITH CHRONIC RECURRENT VENTRICULAR TACHYCARDIA

We have evaluated the effects of intravenous verapamil on ventricular tachycardia in patients in whom the ventricular tachycardia can be reproducibly initiated and terminated during programmed electrical stimulation of the heart. In 7 of 8 patients, 10 mg of verapamil given intravenously during the tachycardia died not affect the ventricular rate of the tachycardia (Figure 1). In these patients tachycardia persisted and the same number of premature ventricular beats at identical intervals were required to terminate the arrhythmia as prior to verapamil administration[16]. In one patient, a 23-year-old male with a cardiomyopathy suffering from frequent attacks of ventricular

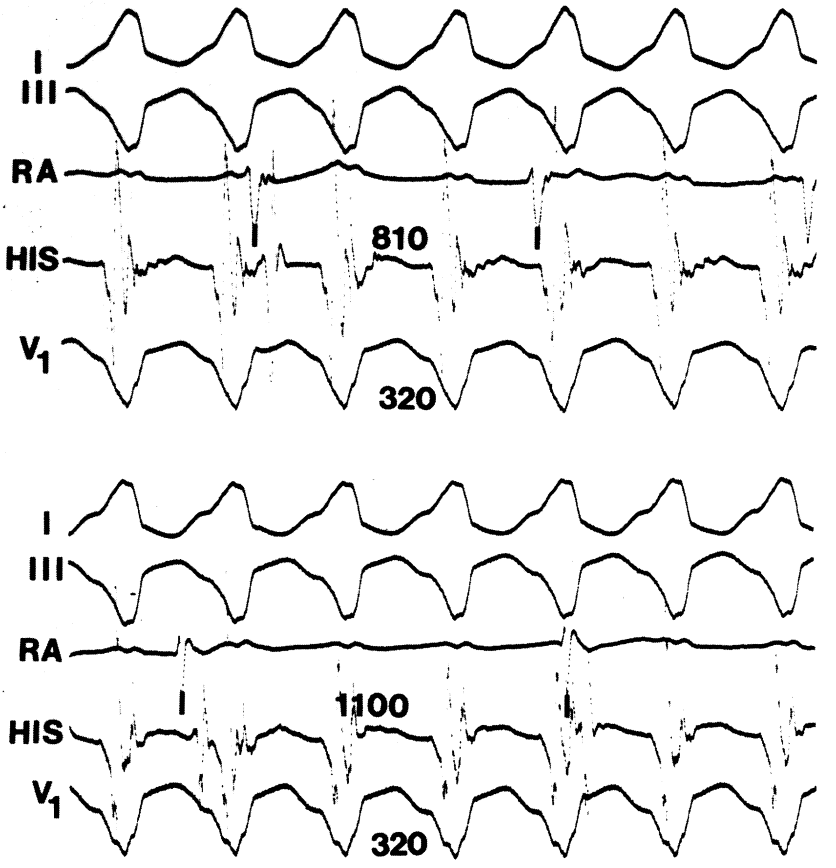

Figure 1. Chronic recurrent ventricular tachycardia induced during programmed electrical stimulation of the heart before (upper panel) and after (lower panel) 10 mg verapamil intravenously. Note that there is no change in ventricular rate. Only slowing in discharge rate of the sinus node is observed resulting in an increase of the P–P interval from 810 to 1100 msec.

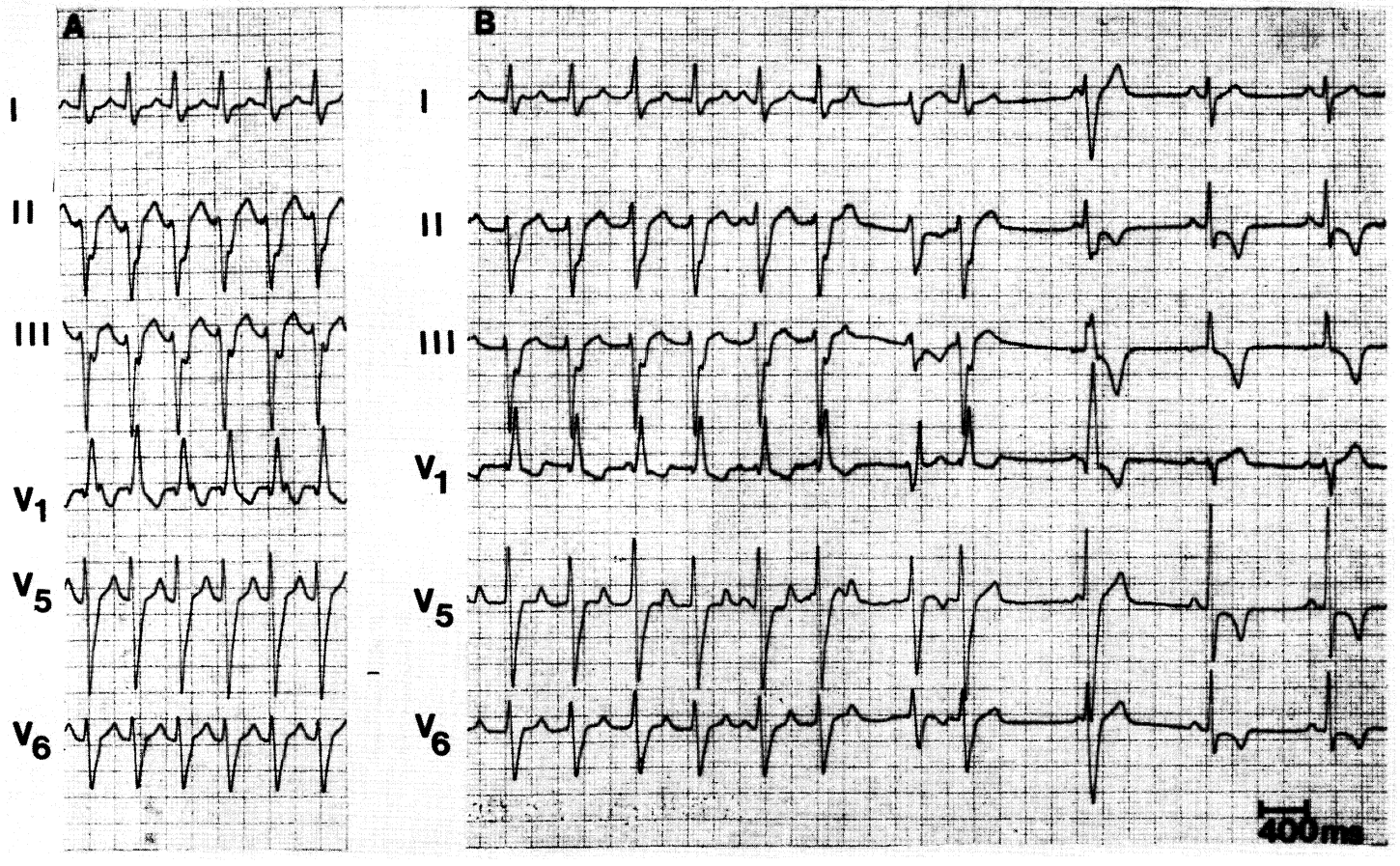

Figure 2. Ventricular tachycardia in a 23-year-old male with cardiomyopathy. In panel A the arrhythmia is shown before drug administration. The ventricular rate is 170/min. Ten mg of verapamil intravenously results in slowing in rate of the tachycardia followed by its termination (panel B).

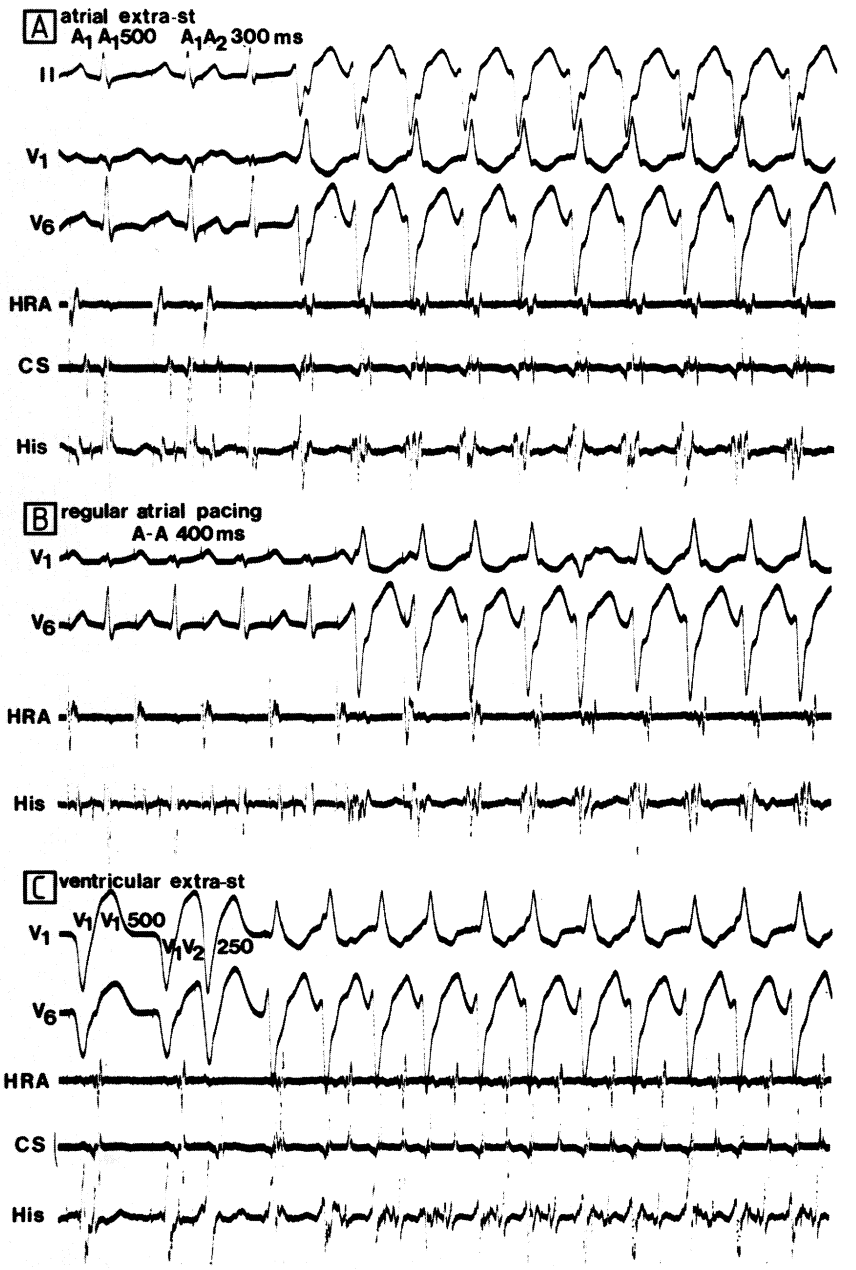

Figure 3. Same patient as Figure 2. The three different mode of initiation of ventricular tachycardia during programmed electrical stimulation of the heart are shown. Panel A: after a single atrial premature beat given after 300 msec during atrial pacing with a basic cycle length of 500 msec. Panel B: during regular pacing of the atrium with a rate of 150/min. Panel C: by a single ventricular premature beat given after 250 msec during ventricular pacing with a basic cycle length of 500 msec. Leads II, V_1 and V_6, a high right atrial (HRA) lead, a coronary sinus (CS) lead and a His bundle lead were simultaneously recorded.

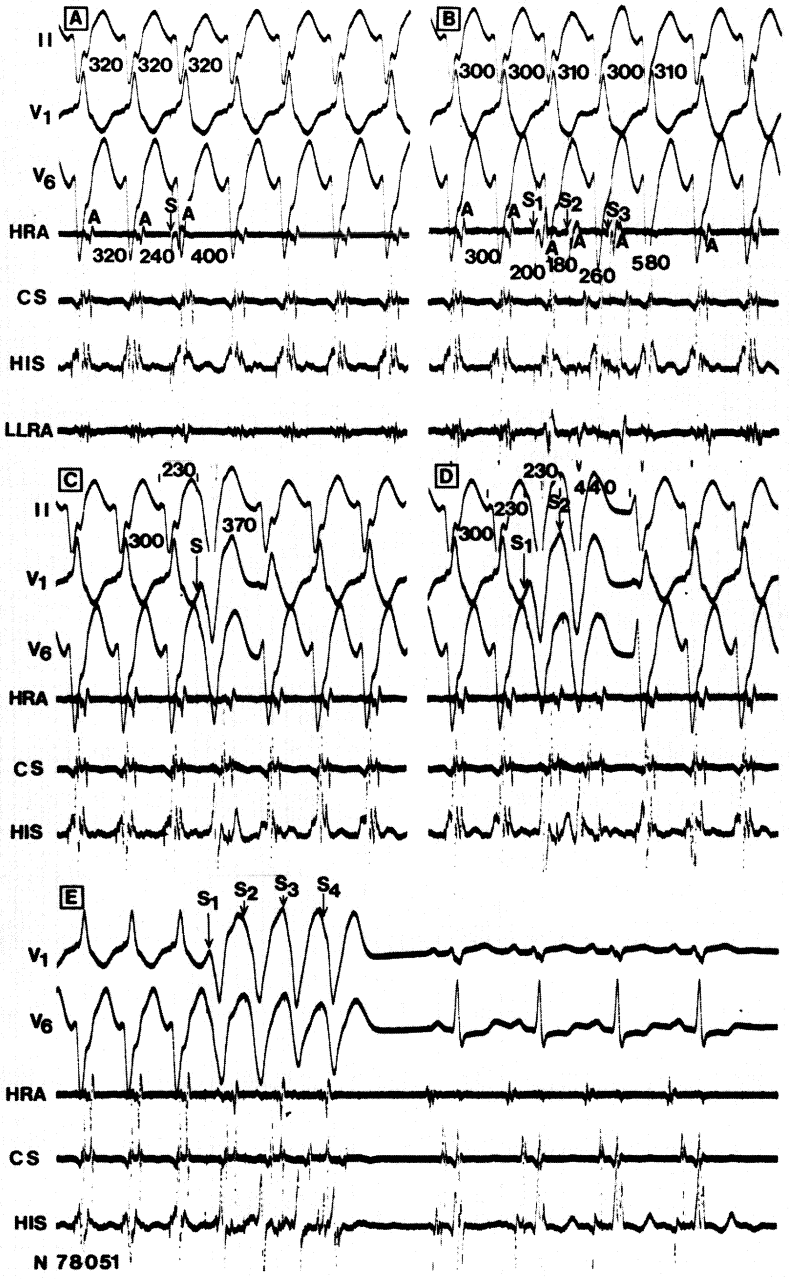

Figure 4. Same patient as Figure 2. Five panels are shown. Panels A and B show the effect of one (panel A) and three (panel B) atrial premature beats during tachycardia.
Note that the ventricular rate is not affected. Panels C, D and E show the effect of one, two and four ventricular premature beats on the arrhythmia. One or two ventricular premature beats do not influence the time relations during tachycardia. Four premature beats are required to terminate the arrhythmia. The same leads are shown as in Figure 3.

tachycardia, the tachycardia terminated during the injection of verapamil. This had already repeatedly been observed clinically during previous admissions to our hospital (Figure 2). As shown in Figure 2 following verapamil slowing in rate of tachycardia was followed by termination of the arrhythmia. Figures 3 and 4 are taken from the programmed stimulation study in this patient. As shown in Figure 3 the ventricular tachycardia could be initiated by a single atrial premature beat during atrial pacing, a single ventricular premature beat during ventricular pacing and by incremental atrial pacing on reaching a critical pacing rate. The tachycardia could only be terminated by 4 ventricular premature beats given in close succession (Figure 4). The latter finding explained why during previous admissions to the hospital the ventricular tachycardia could not be terminated by cardioversion. The mode of initiation by a single ventricular premature beat during ventricular pacing revealed shortening of the interval between the ventricular premature beat and the first QRS of tachycardia on shortening the interval of the tachycardia initiating ventricular premature beat. This is in contrast to our previous observations during initiation of ventricular tachycardia by a single premature beat and seems to argue against reentry as the mechanism of the arrhythmia [17]. It may suggest that our patient represents an example of triggered automaticity at the ventricular level as recently reported by Zipes et al. [18]. From a practical point of view there is need for further intensive evaluation of drug effects in relation to the mechanisms of cardiac arrhythmias as observed during programmed stimulation studies.

5. CONCLUSION

The clinical implications of the slow response concept as mechanism of some of the clinically occurring ventricular arrhythmias are not clear. Further studies on the clinical characteristics of ventricular arrhythmias responding to slow channel blocking agents are required both from a theoretic and a therapeutic point of view.

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