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LETHAL ARRHYTHMIAS RESULTING FROM MYOCARDIAL ISCHEMIA AND INFARCTION

[PROCEEDINGS OF THE SECOND RAPPAPORT SYMPOSIUM]

EDITED BY MICHAEL R. ROSEN
and
YORAM PALTI

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This volume is dedicated to those who made the symposium possible: the investigators, who generously donated their time and shared their intellect, and the Rappaport family, who graciously supported the enterprise.

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PARTICIPANTS IN THE SYMPOSIUM

Ofer Binah, Ph.D.
Departments of Biophysics and Physiology
Rappaport Family Institute
for Research in the Medical Sciences
Technion-Israel Institute of Technology
Faculty of Medicine
Efron Street-Post Office Box 9697
Haifa 31096
Israel

Dr. Günter Breithardt
Professor of Medicine (Cardiology)
Direktor
Medizinische Klinik und Poliklinik
Innere Medizin C
Westfalische Wilhelms
Universitat Munster
Albert-Schweitzer-Strabe 33
D-4400 Münster
West Germany

William Clusin, M.D., Ph.D.
Assistant Professor of Medicine
and Physiology
Falk Cardiovascular Research Center
Stanford University School of Medicine
300 Pasteur Drive
Stanford, California 94305

Ira S. Cohen, M.D., Ph.D.
Professor of Physiology and Biophysics
Health Sciences Center
State University of New York at Stony Brook
Stony Brook, New York 11794

Peter B. Corr, Ph.D.
Associate Professor of Medicine and Pharmacology
Department of Internal Medicine
Cardiovascular Division
Washington University
School of Medicine
660 South Euclid Avenue
St. Louis, Missouri 63110

Leonard S. Gettes, M.D.
Director, Divison of Cardiology
University of North Carolina at Chapel Hill
349 Clinical Sciences Building, 229-H
Chapel Hill, North Carolina 27514

Dr. David J. Hearse
Professor
The Rayne Institute
The Medical School
St. Thomas' Hospital
London SE1 7EH
England

Dr. Anton P.M. Gorgels
Experimentele Cardiologie Academisch
Rijksuniversiteit Limburg
Annadal 1
Maastricht 6201BX
The Netherlands

Brian Hoffman, M.D.
David Hosack Professor and Chairman
Department of Pharmacology
College of Physicians and Surgeons
Columbia University
630 West 168th Street
New York, New York 10032

Jose Jalife, M.D.
Professor
Department of Pharmacology
College of Medicine
State University of New York
Health Science Center
766 Irving Avenue
Syracuse, New York 13210

Dr. Michiel J. Janse
Professor
Klinische en Experimentele Cardiologie
Academisch Ziekenhuis Bij de
Universiteit van Amsterdam
Academisch Medisch Centrum
Meibergdreef 9
1105 AZ Amsterdam
The Netherlands

Dr. Andre G. Kléber
Physiologisches Institut
Universitat Bern
Buehlplatz 5
3012 Bern
Switzerland

W. Jonathan Lederer, M.D., Ph.D.
Associate Professor
Department of Physiology
University of Maryland
School of Medicine
660 West Redwood Street
Baltimore, Maryland 21201

Matthew N. Levy, M.D.
Chief, Investigative Medicine
The Mt. Sinai Medical Center
One Mt. Sinai Drive
Cleveland, Ohio 44106

Robert Myerburg, M.D.
Professor of Medicine and Physiology
Director, Division of Cardiology
University of Miami
School of Medicine
Post Office Box 16960
Miami, Florida 33101

Yoram Palti, M.D., Ph.D.
Director, Rappaport Family Institute
for Research in the Medical Sciences
Technion-Israel Institute of Technology
Efron Street, Post Office Box 9697
Haifa 31096
Israel

Michael R. Rosen, M.D.
Professor of Pharmacology and Pediatrics
Department of Pharmacology
Columbia University
College of Physicians and Surgeons
630 West 168th Street, P&S 7-517
New York, New York 10032

Peter J. Schwartz, M.D.
Associate Professor of Medicine
Instituto di Clinical Medica Generale
e Terapia Medica
Universita di Milano
Centro di Fisiologia Clinica e Ipertensione
Via Fancesco Sforza 35
20122 Milano
Italy

Harold Strauss, M.D.
Department of Medicine
Duke University Medical School
Box 3845
Durham, North Carolina 27710

Richard L. Verrier, Ph.D.
Professor of Pharmacology
Georgetown University
School of Medicine
Medical-Dental Building C-Y02
4900 Reservoir Avenue
Washington, D.C. 20007

Albert L. Waldo, M.D.
Pritchard Professor of Cardiology
and Professor of Medicine
Department of Medicine
Case Western Reserve University
School of Medicine
University Hospitals of Cleveland
2074 Abington Road
Cleveland, Ohio 44106

August M. Watanabe, M.D.
Professor of Medicine and Pharmacology
Indiana University School of Medicine
1100 West Michigan Street
Indianapolis, Indiana 46223

Andrew L. Wit, Ph.D.
Professor
Department of Pharmacology
Columbia University
College of Physicians & Surgeons
630 West 168th Street
New York, New York 10032

Douglas P. Zipes, M.D.
Professor of Medicine
Director, Cardiovascular Research
Indiana University School of Medicine
1100 West Michigan Street
Indianapolis, Indiana 46223

EDITORS' INTRODUCTIONS

For some years I have wondered what it would be like to gather a small group of outstanding investigators and to place them in a room for a few days with nothing to do but talk to one another. Therefore, I was elated when Yoram Palti telephoned me two years ago with just this plan in mind. The format we agreed to was simple—to bring such a group of investigators to the Rappaport Institute of the Technion, to closet them in a conference room, and to set aside a period of time during which each could express his views about what he considered to be a critical aspect of his research. No slides would be permitted—only verbal communication and liberal use of a blackboard. Moreover, interruptions would not only be permitted but also encouraged; after each investigator began to talk, others in the group could interrupt at will. We felt that if the group dynamics worked out appropriately, then no one would be able to complete his planned remarks, but the resultant discussions would lead us to new insights and directions concerning research.

Since the order of the meeting was obviously based on chaos, we belived that some form of structure had to be imposed to maintain an overall focus. The first attempt at structure was the theme of the meeting itself: Lethal Arrhythmias in the Setting of Myocardial Ischemia and Infarction. Certainly, this is an important topic, but having settled on it, we decided to interpret it in the loosest possible sense. That is, rather than consider arrhythmias alone, we included a diversity of factors that might modulate them: neuroendocrine,

metabolic, and ionic. This approach, in turn, required the participation of a diversity of investigator interests and skills. Hence, we invited clinical and basic investigators whose research interests ranged from studies of membrane patches to studies of the patient. This diversity of interests concerned us. How often have we attended meetings where the clinical scientists talk only to each other, the basic scientists do the same, and there is a minimum of interaction? This concern led us to the second aspect of the structure; that is, all participants were requested to remain in the meeting room throughout the duration of the meeting. It is of interest that the request was followed, with a minimum of comings and goings, and clinically and basically oriented individuals found a variety of ground for common discussion. As for the third aspect of the structure, you are holding it in your hands. We believed that to prepare for the meeting, it would be helpful if each individual would write a position paper. We requested an essay, not highly illustrated or referenced, and not necessarily balanced in terms of one's field of research. Ideally, we wanted it to highlight exciting areas of investigation now occurring and to stress directions for future research. These papers were distributed to all participants, one month before the meeting, and provided an initial framework for the participants' consideration.

No tapes or other permanent records were made at the meeting, except for the continual taking of notes by all participants. Based on the discussions and ideas generated, as well as the active participation by all present, my prejudice is to think that the meeting was successful. This book, which contains only a fraction of the ideas stated, nonetheless, serves as an indicator and partial transcript of what transpired.

There are many people for me to thank: first of all my co-editor and co-director of the meeting, Yoram Palti, without whose interest the meeting could never have been conceived and carried out; second, the Rappaport family and the Technion, for their support of the meeting; third, Haia Ganzarski and Rina Banks, whose tireless administrative efforts were required to arrange arrival, departure, and all that occurred in between. Finally, and most important, I would like to thank the participants themselves, for their efforts in preparing for the meeting (including submitting their papers for this volume), for their willingness to share their ideas and fantasies—armed only with blackboard and chalk—before a highly critical cohort of peers, and without whose abilities at communication and criticism there simply would have been no meeting.

Michael R. Rosen
Haifa
March 17, 1988

The research program of the Rappaport Institute for Research in the Medical Sciences gives priority to the cardiovascular system. Since this area is staffed at the Institute by relatively young scientists, we asked Mike Rosen for assistance with its development. Mike provided us not only with developmental guidance, but also served as the driving force and spirit behind the organization of the second Rappaport Symposium, which was dedicated to Lethal Arrhythmias Resulting from Myocardial Ischemia and Infarction.

The symposium topic, which is of prime medical importance, is of special interest to our cardiovascular researchers, most of whom are concerned with cardiac electrophysiology and/or ischemia. I believe the panel of scientists that assembled here represented the ultimate researchers in their fields and were well balanced between clinical and basic sciences. With his charming but firm leadership, Mike managed to conduct the meetings to use the talents, experience, wisdom, and humor of the participants to the utmost. Thus, I feel that we have experienced a stimulating, informative, and at the same time, pleasant meeting.

I am taking this opportunity to thank the participants in the symposium for their scientific contributions and for the friendship they extended to us. I am sure that I speak for all of them when I thank the Institute staff that helped to organize the symposium. I especially want to thank the Meeting Secretary Haia Ganzarski who put forth tireless efforts, used good taste, and exhibited high spirits to ensure that every moment was an enjoyable one.

I hope that the meeting will encourage further interactions and cooperation among all the participants and will benefit each individual's scientific progress and the progress of the medical community as a whole.

Finally, we are grateful to the Rappaport Family for supporting this symposium among their many projects that support medical science in Israel and elsewhere.

Yoram Palti
Haifa
March 17, 1988

I. IONIC DETERMINANTS OF ELECTRICAL ACTIVITY

1. CALCIUM CURRENT AND EXCITATION-CONTRACTION COUPLING IN HEART

W.J. LEDERER, J.R. BERLIN, N.M. COHEN, C.G. NICHOLS, G.L. SMITH, AND M.B. CANNELL

The calcium current (I_{Ca}) plays an important role in regulating calcium flux across the sarcolemma in heart muscle and, therefore, significantly influences the time course and magnitude of the intracellular calcium transient. In addition to *directly* contributing to the rise in intracellular calcium by means of its sarcolemmal calcium flux, I_{Ca} may contribute *indirectly* as the rise in intracellular calcium associated with the calcium flux triggers additional calcium release from the sarcoplasmic reticulum ("calcium-induced calcium release") [1, 2]. Other indirect contributions of I_{Ca} may also be important. The depolarization of the membrane alone may contribute to sarcoplasmic reticulum calcium release ("voltage-dependent calcium release") independently of the initial elevation of cytosolic calcium due to calcium influx [3, 4]. Such a mechanism need not depend on any change in the calcium current itself since other currents contribute to the depolarization of the sarcolemma. The mechanisms that regulate normal excitation-contraction coupling may also influence contractile behavior during ischemia and anoxia, but it is also possible that additional or alternative mechanisms may be involved.

This chapter presents data that support the conclusion that *both* indirect mechanisms appear to play a role in normal excitation-contraction coupling. Our work, therefore, suggests that direct and indirect contributions of I_{Ca} play important roles in controlling force in the normal heart. Additionally, we find that in anoxic rat myocardium, the complete failure of the twitch is primarily due to effects on the action potential mediated by potassium chan-

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nels [5]. However, additional effects of anoxia may be mediated by altered I_{Ca} and modification of excitation-contraction coupling mechanisms.

METHODS

Single Cells

Experiments were carried out using acutely dissociated single adult rat heart cells [4] and neonatal rat heart cells incubated for three days [6, 7]. Cells used for the experiments described were quiescent and exhibited no propagated waves of contraction [8–10] before the experiments were started.

Voltage-clamp Experiments

The experiments were performed with voltage-clamp control of the membrane potential. A rapid-change, low-noise experimental chamber has been used in all experiments [11]. Pipettes with resistances of between 1 and 4 Mohms (adult cells) and between 3 and 5 Mohms (neonatal cells) were used to voltage clamp the single cells. They were filled with intracellular solutions as described later in the figures. Standard voltage-clamp electronics were employed (List LPC/7 and Dagan 8900).

Intracellular Calcium Measurements

Intracellular calcium was measured by lightly loading the intracellular compartment of the cells under investigation with the potassium salt of fura-2. This was done by adding 30 to 75 μm fura-2 to the pipette filling solution. After making a gigaohm seal between the pipette and the cell, the patch of membrane under the pipette was ruptured by the application of additional suction. Approximately ten minutes after breaking into the cell, the fluorescent indicator was sufficiently elevated and stable in concentration to begin making measurements. The cells were illuminated alternatively with 340 nm light and 380 nm light and the fluorescence at 505 nm was measured. After background subtraction was performed, the ratio of fluorescence at 340 nm to that at 380 nm was obtained. The intracellular calcium was estimated from an in vitro calibration curve. For more complete methods see Cannell et al.[3] and Lederer et al. [10].

Length Measurements

Length was measured using a video edge length measuring device obtained from Crescent Electronics (Salt Lake City, Utah). A video image of the cell under investigation was recorded on video tape and played back through the measuring device. Voltage signals proportional to cell length and to cell shortening were produced.

RESULTS AND DISCUSSION

In a normal rat heart, depolarization of the membrane potential leads to the development of a calcium transient and a contraction. Figure 1-1 shows a

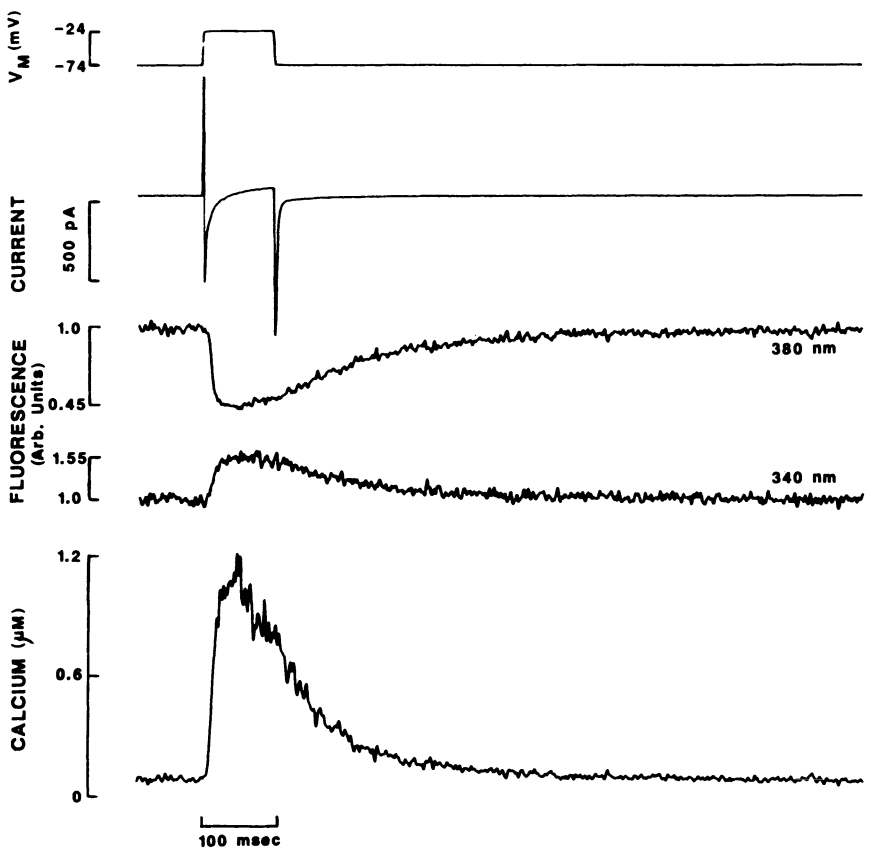


Figure 1-1. Calcium transient in isolated rat heart ventricular myocyte under control conditions. Fluorescence at 505 nm was obtained during illumination with 380 nm and 340 nm light and displayed here in arbitrary units. The record of $[Ca^{2+}]_i$ was obtained by calculating the ratio of fluorescence during 340 nm illumination to that during 380 nm illumination and making use of an in vitro calibration curve. Reprinted from Cannell, Berlin, and Lederer [4], by permission.

normal calcium transient. From the calcium transient alone, it is impossible to determine what fraction is due to the calcium influx across the sarcolemma and how much is due to calcium released from the sarcoplasmic reticulum. We applied ryanodine to remove the transient contribution from the sarcoplasmic reticulum in order to reveal the relative contribution from the calcium current itself as shown in figure 1-2. This figure shows that a large fraction (up to 90 percent) of the calcium transient in adult rat heart cells is due to the calcium released by the sarcoplasmic reticulum.

To investigate the mechanism by which the depolarization leads to the calcium transient in these heart cells, we examined the voltage dependence of the calcium transient and of the calcium current. Figure 1-3 indicates that

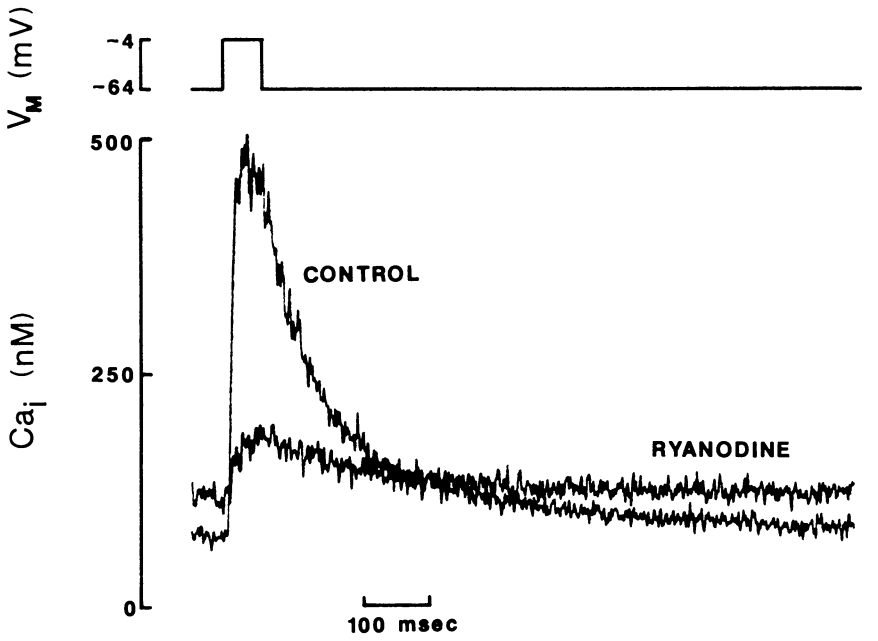
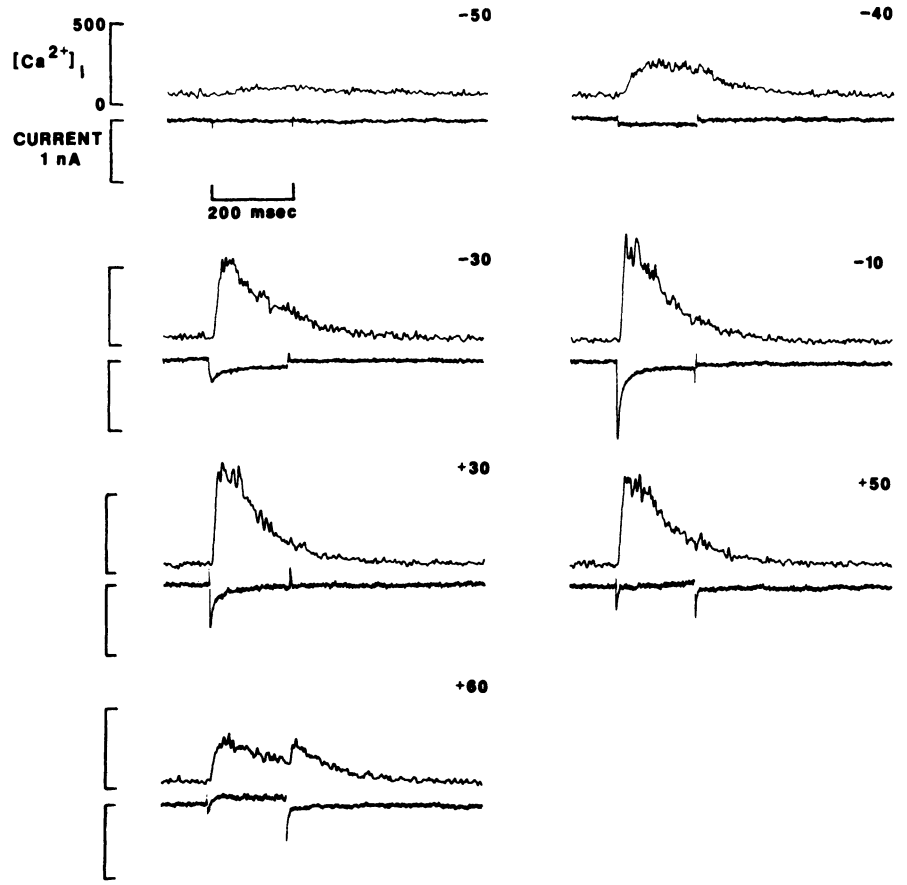


Figure 1-2. The effect of ryanodine on the calcium transient in adult rat heart cells. $[Ca^{2+}]_i$ transients elicited by depolarizations from −64 mV to −4 mV for 50 msec are shown under control conditions and after the addition of 25 μM ryanodine. Ryanodine also caused a rise in $[Ca^{2+}]_i$ (from 60 nM to 100 nM) at the holding potential. Reprinted from Berlin, Cannell, and Lederer, *Biophys. J.* 53:606A, with permission.

there is a small but clear calcium transient in the absence of any measurable calcium current. This observation is reminiscent of recent findings in sheep cardiac Purkinje fibers. In calcium-overloaded cardiac Purkinje fibers, it is also possible to obtain a clear twitch in the absence of any measurable calcium current [12]. In the case of the Purkinje fiber experiments, the calcium current was blocked with the calcium channel antagonist D600 (25 μm). Figure 1-3 and the Purkinje fiber experiments (not shown) indicate that if calcium-induced calcium release (CICR) is the only link between depolarization and release of calcium from the sarcoplasmic reticulum, then the mechanism responsible for CICR is extremely sensitive to calcium fluxes. Further investigation of the links between excitation and contraction involve examining the duration dependence of calcium release. Figure 1-4 shows that brief depolarizations lead to small calcium transients, whereas longer depolarizations lead to larger calcium transients. Long after the calcium current has reached its peak, there is still a dependence of the peak of the measured calcium transient on the duration of the depolarization. This finding is hard to reconcile with a simple and exquisitely sensitive CICR mechanism. These



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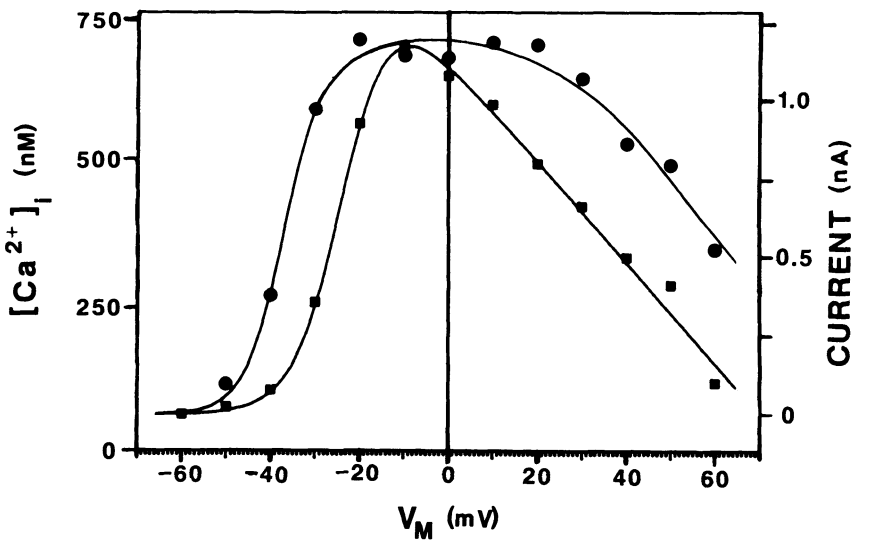

B

Figure 1-3. Voltage-dependence of the calcium transient in adult rat heart cells. A. Original Records. B. Peak $[Ca^{2+}]_i$ (circles) and peak calcium current, I_{Ca} (squares) plotted as a function of depolarization voltage step. The calcium current is measured as the D600 (25 um) sensitive current. Reprinted from Cannell, Berlin, and Lederer [4], by permission.

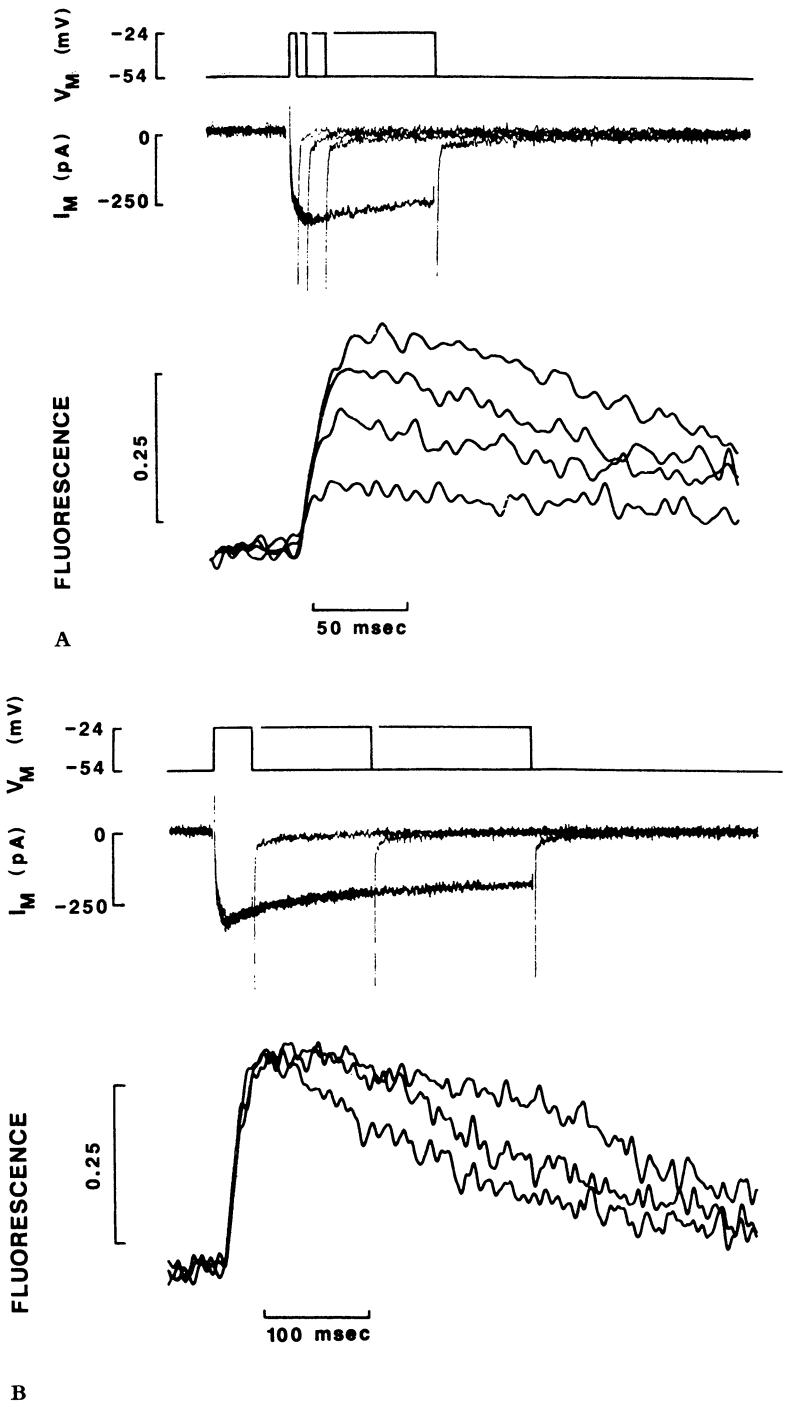

Figure 1-4. The effect of depolarization duration on the time course of the $[Ca^{2+}]_i$ transient in adult rat heart cells. The cell was depolarized from −54 mV to −24mV for 100 msec every 1.5 seconds. During every fourth depolarization the duration was varied. Panel A shows records during 5, 10, 20, and 80 msec depolarizations and panel B shows records during 40, 160, and 320 msec depolarizations. In each panel, the top record shows the voltage protocol, the middle record shows membrane current corrected for capacity and leak currents, and the lower record shows changes in fluorescence measured at 505 nm during illumination with 380 nm light. Reprinted from Cannell, Belin, and Lederer, [4], by permission.

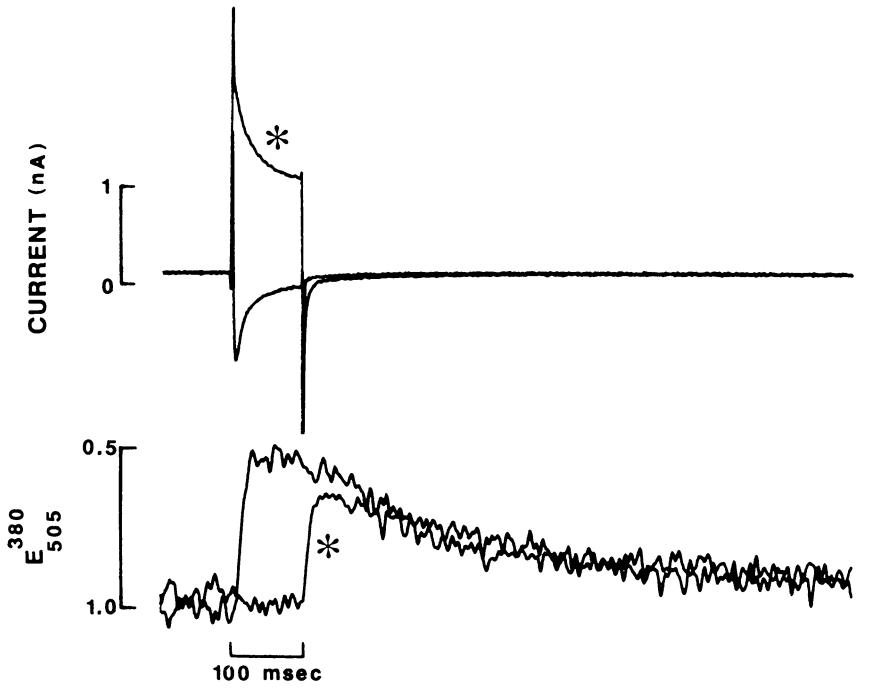

Figure 1-5. Repolarization $[Ca^{2+}]_i$ transient. Membrane current (top) and fluorescence at 505 nm with illumination at 380 nm (bottom) are shown. Records during and following depolarizations for 100 msec to +10 mV and to +100 mV (*) are shown. There is no $[Ca^{2+}]_i$ transient associated with the depolarization to +100 mV while there is a clear $[Ca^{2+}]_i$ on repolarization to the holding potential from +100 mV. Reprinted from Cannell, Berlin, and Lederer [4], by permission.

results are quite compatible with a simple voltage-dependent mechanism controlling calcium release from the sarcoplasmic reticulum; a more complicated CICR mechanism could also be made to accommodate these findings.

Careful inspection of figure 1-3 shows that on repolarization from +60 mV there is a clear "repolarization transient" of calcium. This repolarization transient is shown even more dramatically in figure 1-5. Depolarization to +10 mV produces a normal calcium transient, and repolarization to the holding potential terminates the calcium transient. Depolarization to +100 mV, however, produces no calcium transient. This finding is not readily explained by a simple voltage-dependent mechanism controlling calcium release from the sarcoplasmic reticulum. This result also suggests that a sodium-calcium exchange mechanism as it is usually envisioned [13] is not responsible for activating CICR, since depolarization is thought to favor calcium influx on a sodium-calcium exchange mechanism. The massive "repolarization transient" of calcium seen on repolarization from +100 mV

could be readily explained by an I_{Ca} tail current acting on a simple CICR mechanism. Nevertheless, if one were to consider a more complicated voltage-dependent calcium release process, it could be made to accommodate these findings.

PRELIMINARY CONCLUSIONS

Information about I_{Ca} During Development

The principal goal of this investigation is to obtain primary evidence regarding the characteristics of I_{Ca} during development. Our hope is that this information will contribute to our understanding of excitation-contraction coupling.

Examination of I_{Ca} in neonatal rat hearts when compared to similar examination in adult rat hearts indicates that one of the significant differences lies in the inactivation properties of I_{Ca}. I_{Ca} from the adult can be made to look more like that of the neonate by the application of ryanodine, but the I_{Ca} from rat neonatal heart cells is not altered by ryanodine. We plan to continue to investigate whether the action of ryanodine depends simply on the alteration of calcium released from the sarcoplasmic reticulum in the adult (the neonatal rat heart cell has little *functional* sarcoplasmic reticulum) or whether its action may depend on some other mechanism.

Anoxia

When complete metabolic blockade is brought about by treatment of dissociated adult rat ventricular muscle cells with 10 mm deoxyglucose and 2 mm cyanide, the twitch is abolished. Electrical recordings indicate that the action potential and the twitch are abolished in parallel before rigor contracture develops [14]. However, initial voltage-clamp experiments indicate that it is not the loss of the calcium current that underlies this early contractile failure in rat heart, although reduction of I_{Ca} may play a small role. Additional voltage-clamp experiments examining the mechanism of this early contractile failure are in progress. We seek to determine if we can attribute the early contractile failure to alteration of cytosolic calcium, intracellular calcium stores, intracellular pH, regulatory mechanisms, other membrane currents, or some other process.

SUMMARY

We present evidence supporting three simple roles for the calcium current in excitation-contraction coupling in normal heart muscle: (1) Calcium influx via I_{Ca} directly elevates cytosolic calcium $[Ca^{2+}]i$; (2) calcium influx via I_{Ca} indirectly elevates $[Ca^{2+}]i$ by activating a CICR mechanism; (3) the depolarization of the membrane due to I_{Ca} and other current components indirectly elevates $[Ca^{+}]i$ by activating the release of calcium from the sarcoplasmic reticulum. Our data suggest that in addition to any simple CICR mechanism,

other factors influence excitation-contraction coupling; a voltage-dependent calcium release mechanism is one such possible mechanism. We also suggest, based on preliminary evidence, that an alteration in I_{Ca} is not a necessary element in the early contractile failure observed during anoxia.

REFERENCES

1. Fabiato, A 1985. Simulated calcium current can both cause calcium loading in and trigger calcium release from the sarcoplasmic reticulum of a skinned caniune cardiac Purkinje cell. *J. Gen. Physiol.* 85:191–320.
2. London, B. and Krueger, J.W. 1986. Contraction in voltage-clamped, internally perfused single heart cells. *J. Gen. Physiol.* 88:475–505.
3. Cannell, M.B., Berlin, J.R., and Lederer, W.J. 1987. Calcium in heart muscle function assessed using digital imaging fluorescence microscopy and fura-2 In *Cell Calcium and Control of Membrane Transport*, ed. Mandel, L.J., and Eaton, D.C. eds., New York, Rockefeller University Press.
4. Cannell, M.B., Berlin, J.R. and Lederer, W.J. 1987. Effect of membrane potential changes on the calcium transient in single rat cardiac muscle cells. *Science* 238:1419–1423.
5. Nichols, C.G., Smith, G.L., and Lederer, W.J. 1988. A decline in action potential duration underlies disappearance of the twitch in single rat ventricular myocytes exposed to metabolic blockade. *Biophys* 53:401A
6. Cohen, N.M., and Lederer, W.J. 1987. Calcium current in isolated neonatal rat ventricular myocytes. *J. Physiol.* 391:169–192.
7. Cohen, N.M., and Lederer, W.J. 1987. Developmental changes in the steady state kinetic parameters of the calcium current in isolated rat heart cells. *J. Gen. Physiol.* 88:17a.
8. Capogrossi, M.C., and Lakatta, E.G. 1985. Frequency modulation and synchronization of spontaneous oscillations in cardiac cells. *Am. J. Physiol.* 248:H412–H418.
9. Wier, W.G., Cannell, M.B., Berlin, J.R., et al. 1987. Fura-2 fluorescence imaging reveals cellular and sub-cellular heterogeneity of $[Ca^{2+}]_i$ in single heart cells. *Science* 235:325–328.
10. Lederer, W.J., Cannell, M.B., and Berlin, J.R. 1987. Intracellular calcium measurements in heart using fura-2: Calcium fluctuations linked to arrhythmias and negative inotropy. in *Initiation and Conduction of the Cardiac Pacemaker Response*, Giles, W.R, ed. New York, A.R. Liss.
11. Cannell, M.B. and Lederer, W.J. 1986. An experimental chamber for single-cell voltage-clamp and patch-clamp experiments with temperature and flow control and low electrical noise. *Pflügers Arch.* 406:536–539.
12. Berlin, J.R., Cannell, M.B., and Lederer, W.J. 1987. The regulation of twitch tension in sheep cardiac Purkinje fibres during calcium overload. *Am. J. Physiol.* 253:H1540–H1547.
13. Eisner, D.A. and Lederer, W.J. 1985. Na-Ca exchange: stoichiometry and electrogenicity. *Am. J. Physiol.* 248, C189–C202.
14. Lederer, W.J., Nichols, G.G., and Smith, G.L. 1987. Mechanical and electrical responses of isolated rat cardiac myocytes to metabolic blackade. *J. Physiol.* In 384:34P.

2. ROLE OF CYTOSOLIC CALCIUM IN THE NORMAL AND ISCHEMIC HEART: POTENTIAL NEW INSIGHTS FROM THE SECOND GENERATION INDICATOR, INDO-1

WILLIAM T. CLUSIN, RAJENDRA MOHABIR, AND HON-CHI LEE

A pivotal issue in the understanding of myocardial ischemia is the role played by cytosolic calcium ions. In addition to their well-known role in excitation-contraction coupling, calcium ions regulate several different ionic currents that flow across the cell membrane. Inability of the ischemic myocardium to sequester or extrude calcium could have a variety of effects, the most serious of which would be the subversion of electrical activity in the nonischemic portion of the heart through the genesis of ventricular arrhythmias.

Knowledge about the role of calcium in cardiac ischemia has been elusive. In nerve cells and erythrocytes, the application of metabolic inhibitors causes a prompt increase in $[Ca^{++}]_i$, which leads to a predictable opening of calcium-activated potassium channels [1, 2], but this sequence of events has been more difficult to verify in the ischemic heart. Four factors have contributed to the difficulty of studying $[Ca^{++}]_i$ during ischemia.

First, the metabolic status of cardiac preparations is sometimes difficult to control. Isolated papillary muscles or Purkinje fibers often develop an ischemic central core, in which extracellular potassium can be markedly elevated [3]. Dissociated cardiac myocytes do not present this problem, but may develop metabolic abnormalities as a result of enzymatic treatment. It has been argued that pre-existing metabolic abnormalities may lead to a spurious increase in $[Ca^{++}]_i$ during simulated ischemia [4]. As an alternative, metabolic abnormalities might conceal a $[Ca^{++}]_i$ increase that would have been prominent if the tissue were intially healthy [5].

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Second, in vitro preparations that truly simulate cardiac ischemia have been elusive. Exposure of superfused preparations to hypoxia or metabolic inhibitors does not mimic ischemia because there is little accumulation of potassium, CO_2, or lactate, It is also possible that substances released from vascular endothelium play a role in the ischemic response. Although lack of washout can be simulated in nonperfused tissues, the contribution of the vasculature to the ischemic response can only be observed in perfused tissue.

Third, previous studies of ischemia have tended to use slow stimulation rates, typically 20 to 30/minute. The choice of stimulus rate is often dictated by technical factors, such as the fragility of isolated myocytes, the tendency of microelectrodes to dislodge during vigorous stimulation, and the problem of central ischemia in nonperfused fibers. Nevertheless, it is clear that beat frequency drastically influences the severity of the ischemic response [6].

Finally, the small size and the motility of cardiac cells limits the optimum use of certain calcium indicators. Aequorin, for example, can easily detect the resting $[Ca^{++}]_i$ of giant axons or muscle fibers [7] because the injection of a large amount of the indicator overcomes the limitations imposed by nonlinearity (i.e., minimum sensitivity at low $[Ca^{++}]_i$). In contrast, the amount of indicator that can be injected in cardiac cells is much smaller. This fact makes the diastolic or "resting" signal more difficult to interpret, particularly when the signal is flat or fails to increase during some intervention.

Despite these obstacles, considerable progress has been made in elucidating the behavior of $[Ca^{++}]_i$ during ischemia. Part of this progress is due to the development of new calcium indicators, which can be loaded nondisruptively into perfused tissues or rapidly beating cells. In addition, experiments using aequorin have also advanced, so that responses not previously seen with that indicator are now being reported [5, 8]. This chapter reviews recent advances in the measurement of $[Ca^{++}]_i$ during ischemia and related conditions. Particular emphasis is placed on the newer indicators, such as indo-1, which can be used to record $[Ca^{++}]_i$ transients in intact, rapidly beating mammalian hearts [9].

DOES THE $[Ca^{++}]_i$ INCREASE PRECEDE CONTRACTURE? A CHANGING CONSENSUS

One of the most striking effects of metabolic energy deprivation in cardiac tissue is the development of sustained contracture. Energy deprivation contracture could be due to a rise in $[Ca^{++}]_i$, which would promote interaction of actin and myosin, or to a fall in intracellular ATP, which would produce rigor, due to failure of cross-bridge dissociation. The temporal relation of a $[Ca^{++}]_i$ increase to contracture is particularly important in judging the significance of the increase: Any $[Ca^{++}]_i$ increase that precedes the contracture is reversible by virtue of the remaining cytosolic ATP. A reversible $[Ca^{++}]_i$ increase during ischemia would coincide with the period in which ischemic arrhythmias are most likely to occur. In contrast, a $[Ca^{++}]_i$ increase that did

not occur until after the development of rigor would be an irreversible late event—a manifestation of cell death.

Initial experiments using aequorin failed to detect a $[Ca^{++}]_i$ increase during the initial phase of hypoxia or metabolic inhibition. In quiescent myocytes exposed to metabolic inhibitors, aequorin luminescence undergoes an explosive increase several minutes after the development of rigor, but no increase before rigor [10]. A similar result has been obtained in aequorin-injected papillary muscles exposed to hypoxia or metabolic inhibition [11]. These experiments show that rigor *can* occur in the absence of a $[Ca^{++}]_i$ increase, but they do not rule out a $[Ca^{++}]_i$ increase during ischemia or during other conditions that mimic ischemia.

Newer experiments indicate that a $[Ca^{++}]_i$ increase can precede the development of rigor when appropriate conditions are chosen. In their most recent work, Allen et al. [5] report that exposure of papillary muscles to hypoxia usually *does* increase the amplitude of the $[Ca^{++}]_i$ transients during the first five minutes. The increase in the transients is abolished when the muscle is run down by repeated hypoxic trials. The run-down effect is reversed by addition of 50 mM glucose, so that hypoxia again increases the amplitude of the transients. The implication of this report is that the negative results obtained in earlier studies (i.e., failure of the $[Ca^{++}]_i$ transients to increase) were due to unrecognized metabolic deterioration. Allen et al. [8] have performed a similar study in which ischemia is simulated by combined exposure to hypoxia and restricted extracellular fluid (gas perfusion). In this situation, a progressive and quite dramatic increase in $[Ca^{++}]_i$ transients occurs over 30 minutes, during which time contractile force declines. Simulated ischemia also increases the duration of the $[Ca^{++}]_i$ transients, which would be expected if the effects on $[Ca^{++}]_i$ were due to accumulation of acid metabolites, discussed later.

Results similar to those just described have been obtained by Barry et al. [12] in chick embryonic myocytes loaded with the fluorescent $[Ca^{++}]_i$ indicator, indo-1. In these cells, metabolic inhibitors (cyanide + 2 deoxyglucose) cause cessation of beating, followed by progressive contracture. Onset of the contracture is accompanied by a simultaneous increase in $[Ca^{++}]_i$. By producing the contracture in zero sodium, and then restoring sodium, it is possible to elicit partial relaxation. It is concluded that $[Ca^{++}]_i$ increases promptly during metabolic inhibition and that the $[Ca^{++}]_i$ increase is partly responsible for the contracture.

MEASUREMENT OF $[Ca^{++}]_i$ IN INTACT HEARTS WITH THE NMR PROBE 5,5'-DIFLUORO BAPTA

A clear advance over the use of simulated-ischemia models would be the ability to record $[Ca^{++}]_i$ transients from intact myocardium under conditions of coronary occlusion or zero flow. An important step toward this goal has been the use of NMR spectroscopy in hearts loaded with 5,5'-difluoro BAP-

TA [13, 14], a calcium chelating agent, which is derived from BAPTA, the parent compound of the tetracarboxylate calcium indicators. These compounds can be loaded into myocardial cells nondisruptively as the acetoxymethylester form and can be loaded into intact hearts by arterial perfusion.

5,5'-difluoro BAPTA has several advantages. Signals can be calibrated to give quantitative measurements of $[Ca^{++}]_i$. Spectral shifts for calcium are unique, which avoids contamination of the signal by other metals; and motion artifact is absent. Difficulties with the technique include poor time resolution and buffering of the contraction by the large amount of indicator that is required to obtain adequate signals. Data obtained to date have been time-averaged measurements acquired during 5-minute periods. Determination of the time course of the $[Ca^{++}]_i$ transient is possible by gating of the signal [15], although a longer period of signal averaging is then required.

Recordings of 5,5'-difluora BAPTA spectra show a clear increase in time-average $[Ca^{++}]_i$ after 10 to 15 minutes of ischemia [13, 14]. Typical experiments show a three-fold increase in $[Ca^{++}]_i$, to values exceeding 1. μM. This effect is fully reversible upon reperfusion, although there is a tendency for values to remain elevated during the first few minutes of reperfusion. $[Ca^{++}]_i$ does not appear to increase during the first 5 minutes of ischemia in these experiments, although a transient $[Ca^{++}]_i$ increase would not, as yet, be detectable, owing to the requirement for time averaging.

A further feature of the 5,5'-difluoro BAPTA recordings that must be considered is buffering of intracellular calcium by the indicator. The intracellular 5,5'-difluoro BAPTA concentrations achieved in both rat and ferret heart range from 0.1 to 0.65 mM [13, 14]. These concentrations produce profound suppression of contraction. Marban et al. [14] were able to restore contraction by elevation of external calcium to 8 mM, but the resulting contractions still had a prolonged relaxation phase, as expected when intracellular buffering is present [16]. It is not clear what effect, if any, the buffering of $[Ca^{++}]_i$ on a beat-to-beat basis would have on the more gradual $[Ca^{++}]_i$ increase during ischemia.

CHARACTERISTICS OF INDO-1 TRANSIENTS IN INTACT RABBIT HEARTS

To study the effects of ischemia on $[Ca^{++}]_i$ transients, it is necessary to develop a method of recording these transients in perfused tissue. This has recently been accomplished by Lee, et al. [9] using the fluorescent $[Ca^{++}]_i$ indicator, indo-1. Indo-1 is chemically related to quin-2, fura-2, and to the parent compound, BAPTA. Like these compounds, it can be introduced nondisruptively into isolated cells or arterially perfused tissues as the acetoxymethyl derivative, indo-1 AM. However, indo-1 has the unique feature of exhibiting a marked shift in the fluorescence emission spectrum upon binding calcium [17]. Binding of calcium causes a decrease in fluorescence over a broad peak centered near 490 nm, while causing an increase in fluorescence that is centered at 400 nm. When fluorescence from these two frequency

bands is recorded simultaneously, a ratio can then be constructed, which is particularly sensitive to $[Ca^{++}]_i$. Use of indo-1 in this manner has several advantages over the use of other cell permeant indicators, such as fura-2:

1. The $[Ca^{++}]_i$-dependent fluorescent ratio can be determined instantaneously, for indo-1, without exchange of filters.
2. Instantaneous determination of the ratio allows movement artifact to cancel out, since movement produces a "common mode" signal at the chosen emission wavelengths.
3. The electronic and mechanical equipment necessary to determine the fluorescence ratio is simpler and less expensive than that required for fura-2.
4. There is some evidence that the strategy of loading cells with the membrane permeant acetoxymethylester works better for indo-1 than for fura-2. For example, Steinberg et al. [18] report a tendency of fura-2 to accumulate selectively in mitochondria, producing a granular pattern of flourescence. In contrast, indo-l produces a diffuse pattern of fluorescence that would be expected if the indicator were widely distributed throughout the cytoplasm.

Typical fluorescence transients recorded from an intact rabbit heart containing indo-1 are shown in figure 2-1. In this recording, fluorescence recorded at 400 ± 12.5 nm is shown in the top trace, fluorescence at 550 ± 20 nm is shown in the bottom trace, and the F400/F550 ratio is shown in the middle trace. The 550 nm wavelength, which gives a notably clear fluorescence decrease during each transient, has been used instead of the in vitro peak for calcium-free indo-1 (490nm) because 550 nm does not overlap with the fluorescence of indo 1 AM, which is retained in the tissue to some extent and could contaminate recordings at 490nm [19].

$[Ca^{++}]_i$-dependent fluorescence transients obtained from indo-1-loaded rabbit hearts resemble those obtained from single cells containing indo-1 [20, 21] or fura-2 [22, 23]. These transients have a rapid upstroke followed by a much slower decay, which can last more than 1 second when the interbeat interval is prolonged. The slow decay of indo-1 transients stands in contrast to transients recorded with aequorin, which decay fully within a few hundred milliseconds (see for example Allen et al. [24]). The slow decay of indo-1 transients is not due to buffering of $[Ca^{++}]_i$ by the indicator because there is no reduction in the strength of contraction and no prolongation of the relaxation phase [14, 16]. The slow decay of the transients is also not due to the off-kinetics of indo-1, which have been measured in vitro and found to be much faster [25].

Slow decay of the indo-1 transients is of no practical consequence when sufficiently slow heart rates are chosen. In this case, each transient will decay fully to the baseline, so that the end-diastolic $[Ca^{++}]_i$ is equivalent to the

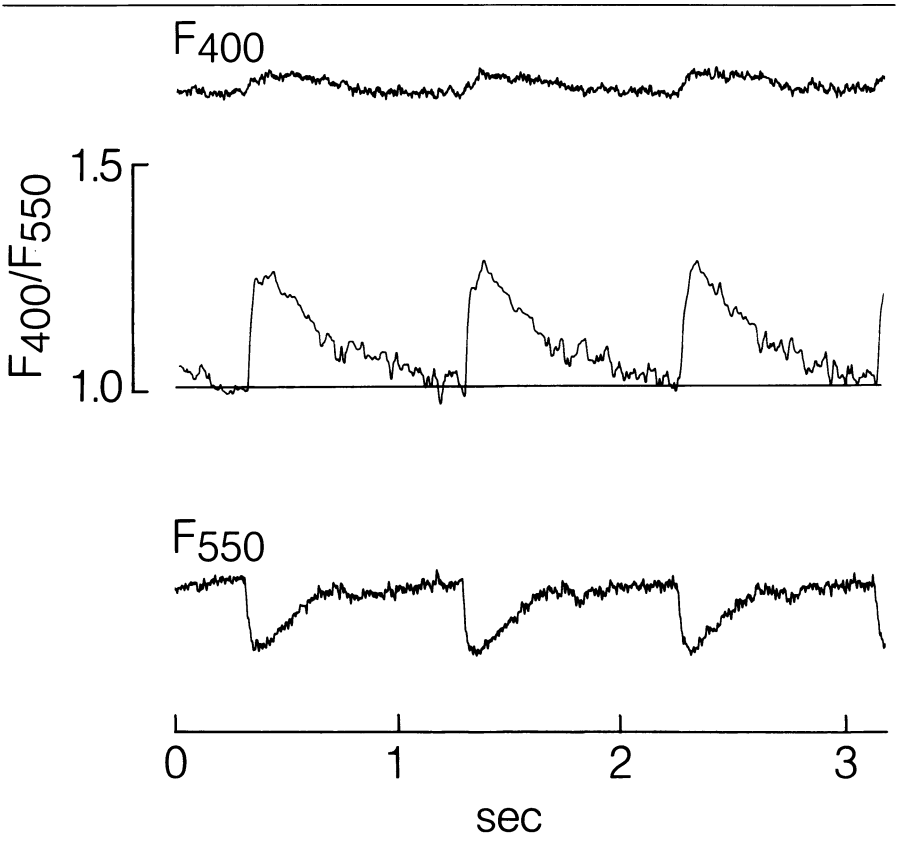

Figure 2-1. $[Ca^{++}]_i$-dependent fluorescence transients in a rabbit heart loaded with indo-1. Each beat produces a phasic fluorescence increase at 400 nm (top trace) and a fluorescence decrease at 550 nm (bottom trace). The middle trace shows the fluorescence ratio (F_{400}/F_{550}), which is calculated by an analog circuit and is known to be a monotonic function of $[Ca^{++}]_i$. The upstroke of each transient is rapid, but decay of the transients continues for hundreds of milliseconds. Reduction of interbeat interval to about 1,200 msec is necessary for the fluorescence ratio to reach steady state. (Same experiment as figure 1 of Lee et al. [9] but displayed at faster sweep speed.)

resting level. However, when physiologic heart rates are chosen, the falling phase of each transient is interrupted by the onset of the next transient, so that the end-diastolic $[Ca^{++}]_i$ is significantly higher than the resting level. Moreover, the absolute level of $[Ca^{++}]_i$ at end-diastole is not constant, but can change quite substantially from beat to beat as a function of the interbeat interval. This is illustrated in figure 2-2, where the onset of rapid right ventricular pacing causes an immediate elevation of end-diastolic $[Ca^{++}]_i$ compared to the level attained during spontaneous beating (horizontal line).

A less obvious consequence of the slow decay of the $[Ca^{++}]_i$ transients is that maneuvers having a positive inotropic effect will also increase end-

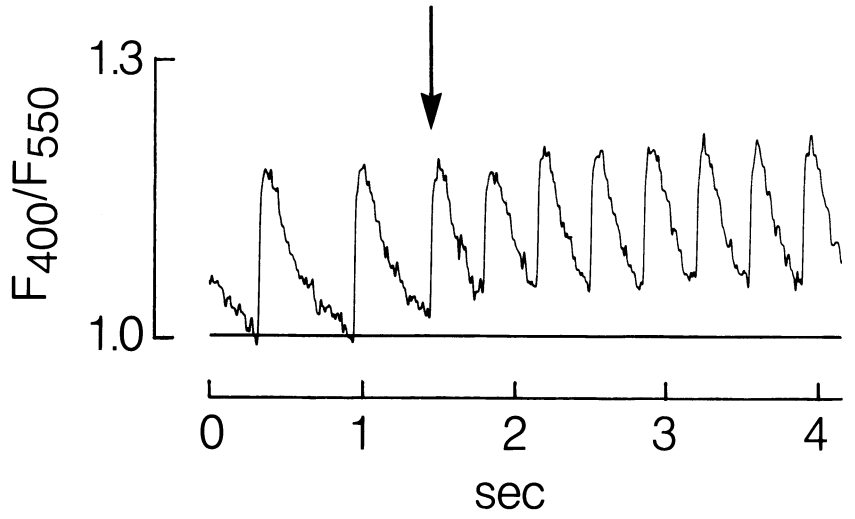

Figure 2-2. $[Ca^{++}]_i$ staircase in an indo-1-loaded rabbit heart. The recording shows the F_{400}/F_{550} ratio during spontaneous beating (left two transients) and during stimulation of the right ventricle by an epicardial plunge electrode (arrow). Onset of stimulation produces an immediate elevation of end-diastolic $[Ca^{++}]_i$ above the usual level. A staircase then ensues in which there is progressive, stepwise elevation of both systolic and diastolic $[Ca^{++}]_i$.

diastolic $[Ca^{++}]_i$. Studies with aequorin have established that a wide variety of positive inotropic agents exert this effect by increasing the peak amplitude of the $[Ca^{++}]_i$ transient [26]. Since aequorin transients decay rapidly (within the electrical refractory period), the associated increase in the duration of the transients is inconsequential and does not lead to elevation of $[Ca^{++}]_i$ at the onset of the next action potential. In contrast, indo-1 transients decay more slowly and are still decaying at the onset of the next beat. As a result, any maneuver that scales up the transient to a higher peak amplitude will increase $[Ca^{++}]_i$ at every other point in time, including the point at which the next beat arises. Exceptions to this rule occur only if there is a compensatory reduction in beat frequency or an acceleration in the time course of calcium removal.

Experimental observations confirm the variability of end-diastolic $[Ca^{++}]_i$ during a wide variety of experimental interventions. In figure 2-2 for example, the onset of rapid pacing is followed by a staircase phenomenon, in which there is progressive elevation of the peak of the $[Ca^{++}]_i$ transient. As previously proposed, there is an associated increase in end-diastolic $[Ca^{++}]_i$. A similar phenomenon has been observed in dissociated chick embryonic cardiac cells [27], where the increase in diastolic $[Ca^{++}]_i$ is associated with a similar increase in persisting contractile deformation. Parallel changes in peak systolic and end-diastolic $[Ca^{++}]_i$ are also observed during exposure of

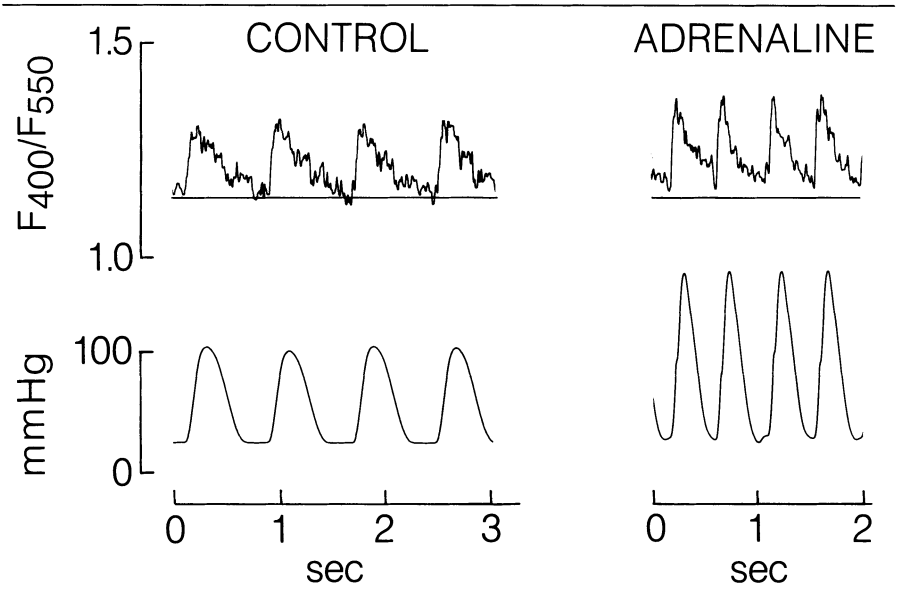

Figure 2-3. Effects of adrenaline (1 μg/ml for 90 sec) on $[Ca^{++}]_i$ transients (top trace) and contraction in an indo-1-loaded heart. Isovolumic left ventricular pressure (bottom trace) is measured by a fluid-filled latex balloon that contains a fiberoptic pressure transducer. Infusion of adrenaline produces a prompt increase in the amplitude of the $[Ca^{++}]_i$ transient and in the contraction. Decay of both signals is accelerated, presumably due to more rapid reuptake of calcium by the sarcoplasmic reticulum. There is slight elevation of end-diastolic $[Ca^{++}]_i$ above the baseline, which is partly due to the increase in beat frequency. Reprinted from Lee, et al. [9].

myocardial cells to elevated extracellular calcium [21] or during treatment with the calcium agonist, Bay K8644 [27]. In contrast, reduction in the amplitude of the $[Ca^{++}]_i$ transient by verapamil (0.3 μM) produces a parallel reduction in end-diastolic $[Ca^{++}]_i$ in rabbit hearts paced at a constant beat frequency [28].

Drugs that have specific actions on the sarcoplasmic reticulum can alter diastolic $[Ca^{++}]_i$ in ways not explained by concurrent changes in the systolic value. Figure 2-3, for example, shows the effects of adrenaline (1 μg/ml/for 90 sec.) on the $[Ca^{++}]_i$ transient (top trace) and contraction (bottom trace). Adrenaline causes a substantial acceleration in beat frequency and an elevation of peak systolic intraventricular pressure, together with elevation of the peak systolic $[Ca^{++}]_i$. The duration of the contraction is abbreviated by adrenaline, and there is obvious acceleration of the rate at which the $[Ca^{++}]_i$ transient decays. End-diastolic $[Ca^{++}]_i$ is slightly elevated in this experiment, but it is not nearly as elevated as it would have been had no acceleration occurred in the decay of the transient. Adrenaline is known to accelerate calcium sequestration through effects of cyclic AMP on the sarcoplasmic reticulum calcium pump [29]. The result in figure 2-3 documents this effect

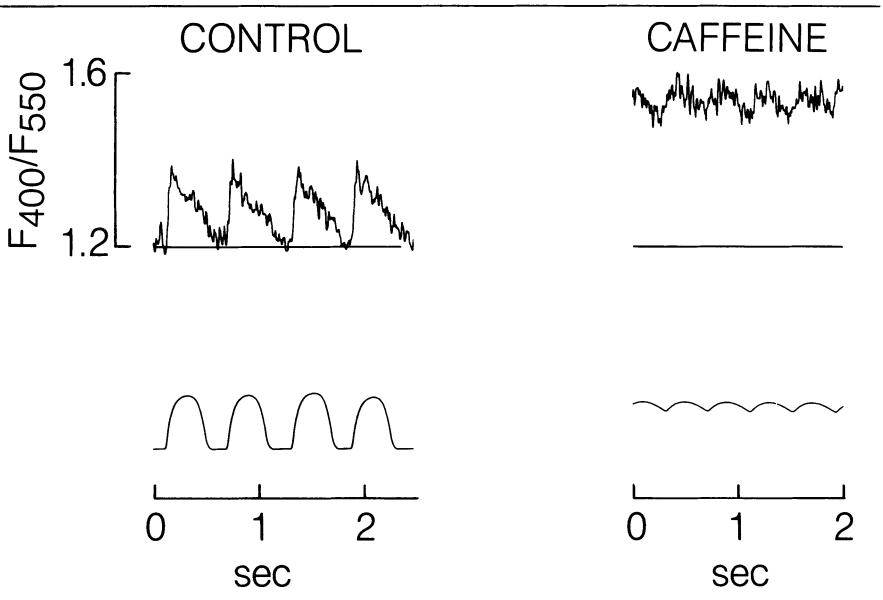

Figure 2-4. Effects of caffeine (20 mM for 30 seconds) on contraction (bottom trace) and on the $[Ca^{++}]_i$-dependent fluorescence ratio (F_{400}/F_{550}) in a rabbit heart loaded with indo-l. Caffeine infusion (right column) causes a marked elevation of diastolic and systolic $[Ca^{++}]_i$ due to impaired calcium uptake by the sarcoplasmic reticulum. This is accompanied by sustained contracture. Modified from Lee et al. [9].

in the intact heart, and confirms that indo-1 fluorescence does, indeed, measure changes in intracellular free calcium within the myofilament space.

A converse effect to that shown in figure 2-3 is the impairment of calcium sequestration that can be observed when cardiac muscle is exposed to high concentrations of caffeine. Rapid exposure to caffeine causes transient elevation of $[Ca^{++}]_i$ in myocardial cells that are voltage clamped at negative potentials. [30, 31]. When action potentials continue during caffeine exposure, one observes either a slower relaxation of twitch tension or else sustained contracture. Which result is observed depends on the species and beat frequency that is tested. Figure 2-4 shows the effects of infusion of 20 mM caffeine into the coronary arteries of a rabbit heart loaded with indo-1. Infusion of caffeine for 30 seconds causes fusion of the systolic twitches into a sustained contracture (bottom trace). A similar pattern is seen in the simultaneous recording of the indo-1 fluorescence ratio (top trace). Here the peak value of the $[Ca^{++}]_i$ transient is increased, and removal of calcium between beats is impaired, so that successive $[Ca^{++}]_i$ transients become fused with one another. The sustained elevation of $[Ca^{++}]_i$ in figure 2-4 depends to some extent on beat frequency. In hearts with slow beat frequencies $[Ca^{++}]_i$ and tension do decline to usual diastolic levels in the presence of caffeine,

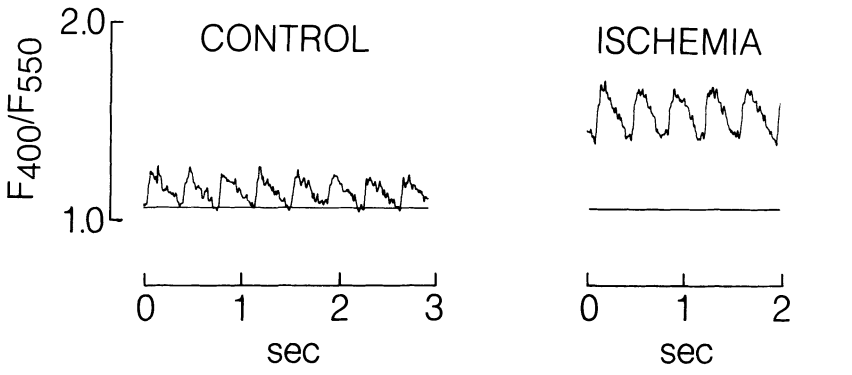

Figure 2-5. Effect of global ischemia on $[Ca^{++}]_i$-dependent fluorescence transients. Ischemia is produced by cessation of coronary perfusion for 90 seconds while the heart is paced at 180 beats/min by an epicardial plunge electrode. Ischemia elevates systolic and diastolic $[Ca^{++}]_i$ (right panel). Ischemia also broadens the peaks of the $[Ca^{++}]_i$ transients and increases their net amplitude. Data from Lee et al. [9].

but the rate of decline is abnormally slow, so that the twitches and $[Ca^{++}]_i$ transients are abnormally broad.

The result in figure 2-4 suggests that the effects of caffeine on contraction are mediated by changes in $[Ca^{++}]_i$. Despite some evidence that caffeine can increase the sensitivity of the myofilaments to calcium [32], this is not apparent from the indo-1 fluorescence changes. In figure 2-4, the strength of the contracture is approximately the same as the normal systolic twitch, whereas the level of $[Ca^{++}]_i$ in the presence of caffeine is somewhat above the systolic value.

EFFECTS OF ISCHEMIA ON $[Ca^{++}]_i$ TRANSIENTS

A primary reason for recording $[Ca^{++}]_i$ transients in the intact heart is to determine the effects of ischemia, which cannot occur in superfused cells [39]. Lee et al. [9] have recently studied the effects of ischemia on fluorescence transients in rabbit hearts containing indo-1. Hearts were paced at 180 beats/min. for these experiments because elevation of beat frequency to this level drastically accelerates the electrophysiologic manifestations of ischemia [6]. Results of a typical experiment are illustrated in figure 2-5, which shows the F_{400}/F_{550} fluorescence ratio before (left panel) and after (right panel) a 90-second ischemic trial. Ischemia produces a prompt increase in the peak amplitude of the $[Ca^{++}]_i$ transients, along with a parallel increase in diastolic $[Ca^{++}]_i$. The overall excursion of the $[Ca^{++}]_i$ transients is increased, and there is a change in the shape of the transients, with broadening of the peak and acceleration of the terminal decay. The effects shown in figure 2-5 are generally maximal during the second minute of ischemia and tend to decay if

the ischemic period is continued beyond 2 minutes. The maximum increase in $[Ca^{++}]_i$, therefore, occurs during the period when systolic tension is *declining*, in agreement with the result obtained by Allen et al. [5] in simulated ischemia.

The effect of ischemia on indo-1 fluorescence transients is reversed by reperfusion, with transients returning to their baseline configuration after 1 minute of reperfusion. A reperfusion-induced increase in $[Ca^{++}]_i$ is neither prominent nor consistently observed, but a brief further increase in the fluorescence ratio sometimes does occur, which peaks after about 10 seconds of reperfusion. The absence of a more prominent reperfusion effect is not surprising, since most studies implicating a role of calcium overload during reperfusion involve much longer periods of ischemia.

The effects of ischemia are reproducible during a series of three to five ischemic trials in the same heart and have also been reproduced in a series of hearts. Figure 2-6 shows the mean values of the F_{400}/F_{550} ratio in five hearts studied by Lee et al. [9]. Ischemia increases the net amplitude of the $[Ca^{++}]_i$ transients, with the largest net amplitude being observed between 45 and 75 seconds of ischemia. As in figure 2-5, ischemia increases both the diastolic and systolic values of the F_{400}/F_{550} ratio, so that the mean end-diastolic value slightly exceeds the peak systolic value prior to ischemia. The overall effects of ischemia and reperfusion are highly significant by the Friedman test ($P < 10^{-6}$ for systolic and end-diastolic values).

EFFECTS OF ISCHEMIA ARE NOT DUE TO AUTOFLUORESCENCE CHANGES OR TO DIRECT EFFECTS OF pH ON INDO-1

Effects of ischemia on the indo-1 fluorescence transients cannot be ascribed to changes in autofluorescence for several reasons. Changes in the F_{400}/F_{550} ratio are not observed during ischemia in hearts that do not contain indo-1. Changes in the autofluorescence at single wavelengths (F_{400} and F_{550}) are often undetectable, and when they are detected, they are always in the same direction (a slight increase in fluorescence) so that cancellation occurs in the ratio. The effects of autofluorescence are further minimized when the heart is loaded with indo-1. Loading with indo-1 increases total fluorescence by 5- to 12-fold. The gain of the recordings, therefore, must be reduced. Although the signal-to-noise ratio is thereby improved, the absolute noise level (which is equal to the square root of total fluorescence) increases by a factor of 2.2 to 3.5. Changes in autofluorescence, which are barely perceptible above the noise level in unloaded hearts, therefore, will become imperceptible in the presence of indo-1. Finally, it is obvious that a steady increase in autofluorescence could not explain the increase in the net amplitude of the fluorescence transients during ischemia. An increase in autofluorescence at 400 and 500 nm should *diminish* the transients observed in the ratio mode, since the beat-to-beat fluctuations in indo-1 fluorescence would constitute a smaller proportion of the total light.

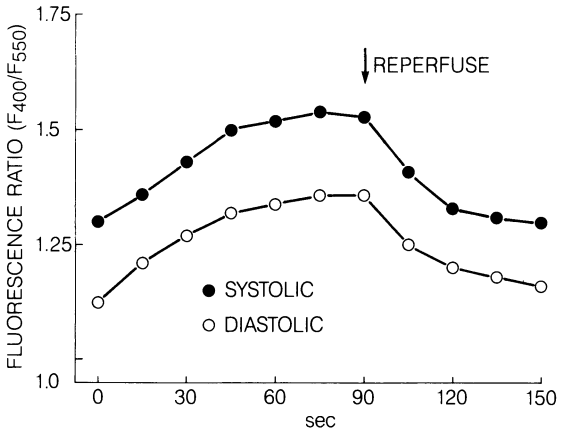

Figure 2-6. Mean values of the F_{400}/F_{550} ratio during ischemia and reperfusion in five rabbit hearts. Filled circles show peak systolic values, while unfilled circles show end-diastolic values. A progressive increase in the fluorescence ratio begins promptly after the onset of ischemia, reaching a steady state value after 90 seconds. Ischemia also increases the net amplitude of the transients. Effects of ischemia are reversed by reperfusion. Modified from Lee et al. [9].

The effects of ischemia on indo-1 fluorescence are also not due to direct effects of cytoplasmic acidification on the properties of the indicator. As pointed out by Grynkiewicz et al. [17] exclusion of a direct pH effect requires that pH-sensitivity be tested separately for the calcium-bound species, the calcium-free species, and for a solution in which the indicator is only partly saturated. This experiment has been performed for indo-1 [28] and revealed no increase in the F_{400}/F_{550} ratio for acidification to pH values as low as 6.0. Cytoplasmic acidification, therefore, is not the explanation for the increase in the F_{400}/F_{550} ratio during brief periods of myocardial ischemia.

WHAT ARE THE CAUSES OF THE ISCHEMIA-INDUCED $[Ca^{++}]_i$ INCREASE?

Cytoplasmic Acidificaton

The early increase in $[Ca^{++}]_i$ depicted in figures 2-5 and 2-6 is presumably not a consequence of ATP depletion because it occurs too rapidly and is not associated with energy deprivation contracture. An alternative explanation is that accumulation of abnormal metabolites somehow impairs the ability of the cell to regulate $[Ca^{++}]_i$.

Among the most prominent effects of ischemia is accumulation of acid metabolites, such as lactate and CO_2. Measurements of tissue pCO_2 in the ischemic canine heart show a detectable increase during the first 10 seconds after coronary occlusion and a doubling of pCO_2 after 2.5 minutes [33]. These results are obtained in open-chest dogs in the absence of rapid pacing.

Although the effects of heart rate on ischemic CO_2 accumulation have not been measured, the corresponding effect of heart rate on extracellular pH is known. Couper et al. [34] have found that three minutes of ischemia in the rabbit ventricular septum reduces extracellular pH by 0.4 units at 75 beats/min but by only 0.1 units at 12 beats/min. Although the effects of much higher heart rates have not been reported, it seems quite possible that ischemic trials at 180/beats/min would produce significant acidification during the first minute of ischemia, in agreement with effects on $[Ca^{++}]_i$ (figures 2-5 and 2-6).

The relationship between intracellular acidification and increased $[Ca^{++}]_i$ is well established in cardiac cells. One way in which this may occur is through competition of protons and calcium ions for common cytoplasmic binding sites. Acidification also appears to facilitate release of calcium from the sarcoplasmic reticulum, so that the total calcium within the myofilament space would increase as well [35].

Intracellular acidosis probably increases $[Ca^{++}]_i$ during both systole and diastole. Bers and Ellis [36] used ion selective electrodes to measure pH_i and $[Ca^{++}]_i$ in quiescent Purkinje fibers and find that removal of transiently applied NH_4Cl (20 mM) acidifies the myoplasm from 7.4 to 7.15, and increases resting $[Ca^{++}]_i$ from roughly 300 to 1,700 nM. $[Ca^{++}]_i$ transients cannot be resolved with ion selective electrodes, but based on studies with other indicators, the resting $[Ca^{++}]_i$ of 1,700 nM that is reported during acidification is somewhat greater than the level that is normally reached during the peak of the $[Ca^{++}]_i$ transient. The result shown in figures 2-5 and 2-6, in which ischemia increases the end diastolic fluorescence ratio to the normal systolic level is, therefore, consistent with the Bers and Ellis result.

Recordings obtained with aequorin confirm that $[Ca^{++}]_i$ increases during acidification of cardiac fibers, [37] but these recordings are surprising because they show an increase in the peak amplitude of the $[Ca^{++}]_i$ transient with no detectable change in the diastolic signal. A similar pattern is seen when aequorin-loaded fibers are exposed to hypoxia [8] or simulated ischemia [5]. The failure of these studies to show an increase in diastolic $[Ca^{++}]_i$ during acidification is not due to any inherent limitation in the use of aequorin. Lea and Ashley [7] have studied the effects of acidification in quiescent barnacle muscle fibers—where it is possible to inject large amounts of aequorin—and find a large increase in resting luminescence, analogous to the increase observed in cardiac Purkinje fibers with ion selective electrodes [36]. Injection of aequorin into multicellular cardiac fibers, however, is more difficult than in giant barnacle fibers, and it is possible that the amount of indicator injected is not sufficient to produce a measurable physiologic resting glow. In the absence of a resting glow, release of calcium from the sarcoplasmic reticulum could produce repetitive $[Ca^{++}]_i$ transients, but associated changes in diastolic $[Ca^{++}]_i$ might escape detection (see Clusin [31]).

Extracellular ATP

A second factor that could cause $[Ca^{++}]_i$ to increase during myocardial ischemia is release of ATP into the extracellular space. Vial et al. [38] have shown that exposure of perfused rat hearts to moderate hypoxia causes abrupt appearance of ATP in the coronary venous effluent. The amount of ATP released rises rapidly during the first 2 to 5 minutes of hypoxia and then declines. Although the amount of ATP in the coronary effluent is small (about 1 nM), the amount present in the vicinity of the myocytes is probably 100-fold higher, since over 99% of extracellular ATP is known to be degraded by ecto-ATP-ases during a single passage through the heart. The site of ATP release is presumably the capillary endothelial cells, but other possible sources include "purinergic nerves" that might be present in the heart or the myocardial cells.

The potential importance of ATP release during ischemia lies in the recent observation that ATP can produce a dramatic increase in myocardial cell $[Ca^{++}]_i$, apparently by acting on purinergic receptors located on the surface membrane. This phenomenon was first discovered by Sharma and Sheu [39] who found that exposure of quin-2-loaded rat ventricular myocytes to 50 uM ATP produced an approximate doubling of $[Ca^{++}]_i$. The initial $[Ca^{++}]_i$ increase occurred very rapidly—in less than 1 minute—and was followed by partial desensitization, with a decline in $[Ca^{++}]_i$ to an intermediate value after 5 minutes. Similar results have been obtained by DeYoung and Scarpa [40] using fura-2. In their study, the effect of ATP was markedly potentiated by norepinephrine. This observation is especially pertinent to ischemia since release of endogenous norpinephrine is an important consequence of ischemia in the intact heart.

The effects of ATP described above are consistent with the rapid time course of the $[Ca^{++}]_i$ increase observed in indo-1-loaded rabbit hearts during ischemia (figures 2-5 and 2-6). The desensitization of the ATP effect is consistent with the gradual decline in $[Ca^{++}]_i$ observed during more sustained periods of ischemia. Mediation of these effects by ATP could also explain why the $[Ca^{++}]_i$ increase observed in other models is so much slower. Whereas cessation of arterial flow should release ATP in a rapid, spatially synchronized manner, this would not occur during exposure of superfused myocytes or muscle fibers to hypoxic saline or metabolic inhibitors. Absence of rapid ATP release would not be a problem in hearts loaded with the NMR probe 5,5'-difluoro BAPTA, but the effect of ATP on $[Ca^{++}]_i$ would not be detected with current technology, since this effect desensitizes in less time than is required for time-averaging of the NMR signal.

The mechanism of the ATP-induced $[Ca^{++}]_i$ increase is uncertain, but could involve either influx of calcium from the external medium or release of calcium from intracellular stores. In arterial smooth muscle cells, ATP is known to open a receptor-operated calcium channel that is thought to

play a role in the postsynaptic response [41]. Calcium channels responsive to ATP have not been identified in cardiac cells, but they could be present. An alternative possibility is that ATP causes mobilization of calcium from intracellular stores. ATP is known to release sequestered calcium in both hepatocytes [42] and Ehrlich acites tumor cells [43], and in both of these cell types, the response appears to be mediated by increased synthesis of inositol 1,4,5-triphosphate. Involvement of sequestered calcium would explain the potentiation of the ATP response by norepinephrine, since norepinephrine enhances calcium sequestration and, therefore, would increase the availability of calcium for release. The postulated release of sequestered calcium by an ATP-IP_3 cascade could contribute to the elevation of diastolic $[Ca^{++}]_i$ in figures 2-5 and 2-6, which is a prominent feature of the response to ischemia.

CONCLUSIONS

Experiments using the second generation $[Ca^{++}]_i$ indicator, indo-1, have provided considerable new insight into the role of calcium in the normal physiology and pathophysiology of the heart. At physiologic heart rates, $[Ca^{++}]_i$ decreases continuously during diastole and the level attained at end diastole is a function of the interbeat interval. Maneuvers that have a positive inotropic effect (adrenaline, acetylcholine, rapid pacing) tend to increase both systolic and diastolic calcium. A drug, such as adrenaline, which enhances calcium uptake by the sarcoplasmic reticulum, accelerates the decay of the $[Ca^{++}]_i$ transient. Conversely, a drug such as caffeine, which inhibits uptake of calcium by the S-R, produces sustained elevation of $[Ca^{++}]_i$. These observations show that modulation of $[Ca^{++}]_i$ can occur during portions of the cardiac cycle when residual calcium was previously believed to be absent.

Ischemia causes a rapid and reproducible increase in both systolic and diastolic calcium. This early increase in intracellular calcium may be mediated by accumulation of acid metabolites, which increase the peak amplitude of the aequorin luminescence transient, as well as the resting $[Ca^{++}]_i$ measured by ion selective microelectrodes. As an alternative, the early increase in $[Ca^{++}]_i$ could be mediated by release of ATP into the extracellular space.

An early increase in $[Ca^{++}]_i$ would not have been anticipated from the effects of ischemia on contraction, since the first few minutes of ischemia are characterized by a rapid reduction in developed pressure. It is now clear that ischemia reduces the sensitivity of the contractile filaments to calcium and that this reduced sensitivity prevents the $[Ca^{++}]_i$ increase from being inferred.

The early $[Ca^{++}]_i$ increase also coincides with the development of electrophysiologic abnormalities, and therefore, may contribute to arrhythmogenesis. Interventions directed at reducing intracellular calcium (diltiazem, reduction of extracellular calcium) have been shown to retard ischemia-induced fibrillation in the dog [44]. This protective effect is correlated with

amelioration of injury potentials, conduction changes, and potassium accumulation in the extracellular space [45, 46]. It is possible that the changes in membrane permeability that mediate these effects are under direct control of $[Ca^{++}]_i$ [1]. Further studies of $[Ca^{++}]_i$ changes during ischemia may provide important information about a common mechanism of sudden death in humans.

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REFERENCES

1. Gardos, G. 1958. The function of calcium in the potassium permeability of human erythrocytes. *Biochim. Biophys. Acta.* 30: 653–654.
2. Clusin, W.T., and Bennett, M.V.L. 1977. Calcium-activated conductance in skate electroreceptors—current clamp experiments. *J. Gen. Physiol.* 69:121–182.
3. Cohen, I., and Kline, R. 1982. K^+ fluctuations in the extracellular spaces of cardiac muscle: evidence from the voltage clamp and extracellular K^+ selective microelectrodes. *Circ. Res.* 50:1–16.
4. Allen, D.G., and Orchard, C.H. 1987. Myocardial contractile function during ischemia and hypoxia. *Circ. Res.* 60:153–168.
5. Allen, D.G., Lee, J.A., and Smith, G.L. The effects of simulated ischaemia on intracellular calcium and tension in isolated ferret ventricular muscle. *J. Physiol.* 401:81P.
6. Blake, K. Smith, N.A., and Clusin, W.T. 1986. Rate dependence of ischaemic myocardial depolarisation: evidence for a novel membrane current. *Cardiovasc. Res.* 20:557–562.
7. Lea, T.J., and Ashley, C.C. 1978. Increase in free Ca^{++} in muscle after exposure to CO_2. *Nature* 275:236–238.
8. Allen, D.G., Lee, J.A., and Smith, G.L. Changes in calcium transients during repeated exposures to anoxia in isolated ferret ventricular muscle. *J. Physiol.* 400:21P.
9. Lee, H., Smith, N., Mohabir, R., and Clusin, W.T. 1987. Cytosolic calcium transients from the bearing mammalian heart. *Proc. Natl. Acad. Sci. USA* 84:7793–7797.
10. Cobbold, P.H., and Bourne, P.K. 1984. Aequorin measurements of free calcium in single heart cells. *Nature* 312:444–446.
11. Allen, D.G., and Orchard, C.H. 1983. The effect of hypoxia and metabolic inhibition on intracellular calcium in mammalian heart muscle. *J. Physiol.* 339:102–122.
12. Barry, W.H., Peeters, G.A., Rasmussen, C.A.F., and Cunningham, M.J: 1987. Role of changes in $[Ca^{++}]_i$ in energy deprivation contracture. *Circ. Res.* 61:726–734.
13. Steenbergen, G., Murphy, E., Levy, L., and London, R.E. 1987. Elevation in cytosolic free calcium concentration early in myocardial ischemia in perfused rat heart. *Circ. Res.* 60:700–707.
14. Marban, E., Kitakaze, M., Kusuoka, H., et al. 1987. Intracellular free calcium concentration measured with ^{19}F NMR spectroscopy in intact ferret hearts. *Proc. Natl. Acad. Sci. USA* 84:6005–6009.
15. Pike, M.M., Kitakaze, M., Chacko, V.P., and Marban, E. 1988. Ca^{2+} transients in intact perfused hearts observed by ^{19}F NMR spectroscopy. *Biophys. J.* 53:649a.

16. Ashley, C.C., Potter, J.D., Strang, P., et al. 1985. Kinetic investigations in single muscle fibres using luminescent fluorescent Ca^{++} probes. *Cell Calcium* 6:159–181.
17. Grynkiewicz, G., Poenie, M., and Tsien, R.Y. 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260:3440–3450.
18. Steinberg, S.F., Bilezikian, J.P., and Al-Awqati, Q. 1987. Fura-2 fluorescence is localized to mitochondria in endothelial cells. *Am. J. Physiol.* 253:C744–747.
19. Luckhoff, A. 1986. Measuring cytosolic free calcium concentration in endothelial cells with indo-1: the pitfall of using the ratio of two fluorescence intensities recorded at different wavelengths. *Cell Calcium* 7:233–248.
20. Peeters, G.A., Hlady, V., Bridge, J.H.B., and Barry, W.H. 1987. Simultaneous measurement of calcium transients and motion in cultured heart cells. *Am. J. Physiol.* 253:H1400–1408.
21. Lee, H., and Clusin, W.T. 1987. Cytosolic calcium staircase in cultured myocardial cells. *Circ. Res.* 61:934–939.
22. Cannell, M.B., Berlin, J.R., and Lederer, W.J. 1987. Effect of membrane potential changes on the calcium transient in single rat cardiac muscle cells. *Science* 238:1419–1423.
23. Barcenas-Ruiz, L., and Wier, W.G. 1987. Voltage dependence of intracellular $[Ca^{++}]_i$ transients in guinea-pig ventricular myocytes. *Circ. Res.* 61:148–154.
24. Allen, D.G., Eisner, D.A., Lab, M.J., and Orchard, C.H. 1983. The effects of low sodium solutions on intracellular calcium concentration and tension in ferret ventricular muscle. *J. Physiol.* 345:491–407.
25. Jackson, A.P., Timmerman, M.P., Bagshaw, C.R., and Ashley, C.C. 1987. The kinetics of calcium binding to fura-2 and indo-1. *FEBS Lett.* 215:31–35.
26. Endoh, M., Yanagisawa, T., Taira, N., and Blinks, J.R. 1986. Effects of new inotropic agents on cyclic nucleotide metabolism and calcium transients in canine ventricular muscle. *Circulation 73* III:117–113.
27. Lee, H., and Clusin, W.T. 1987. Factors governing the duration of cytosolic calcium transients and contraction in embryonic myocardial cell aggregates. *Circulation* 76:IV–471.
28. Lee, H., Mohabir, R., Smith, N., Franz, M.R., and Clusin, W.T. 1988. Effect of ischemia on calcium-dependent fluorescence transients in rabbit hearts containing indo-1: Correlation with monophasic action potentials and contraction. *Circulation.* In press.
29. Katz, A.M. 1983. Cyclic adenosine monophosphate effects on the myocardium: a man who blows hot and cold with one breath. *J. Am. Coll. Cardiol.* 2:143–149.
30. Clusin, W.T. 1983. Caffeine induces a transient inward current in cultured cardiac cells. *Nature* 301:248–250.
31. Clusin, W.T. 1985. Do caffeine and metabolic inhibitors increase free calcium in the heart? Interpretation of conflicting intracellular calcium measurements. *J. Mol. Cell. Cardiol.* 17:213–220.
32. Wendt, I.R., and Stephenson, D.GJ. 1983. Effects of caffeine on Ca-activated force production in skinned cardiac and skeletal muscle fibres of the rat. *Pflugers Arch.* 398:210–216.
33. Case, R.B., Felix, A., and Castellana, F.S. 1979. Rate of rise of myocardial pCO_2 during early myocardial ischemia in the dog. *Circ. Res.* 45:324–330.
34. Couper, G.S., Weiss, J., Hiltbrand, B., and Shine, K.I. 1984. Extracellular pH and tension during ischemia in the isolated rabbit ventricle. *Am. J. Physiol.* 247:H916–H927.
35. Orchard, C.H., Houser, S.R., Kort, A.A., et al. 1987. Acidosis facilitates spontaneous sarcoplasmic reticulum Ca^{++} release in rat myocardium. *J. Gen. Physiol.* 90:145–165.
36. Bers, D.M., and Ellis, D. 1982. Intracellular calcium and sodium activity in sheep heart Purkinje fibers. Effect of changes in external sodium and intracellular pH. *Pfluger's Arch.* 393:171–178.
37. Orchard, C.H. 1987. The role of the sarcoplasmic reticulum in the response of ferret and rat heart muscle to acidosis. *J. Physiol.* 384:431–449.
38. Vial, C., Owen, P., Opie, L.H., and Posel, D. 1987. Significance of release of adenosine triphosphate and adenosine induced by hypoxia or adrenaline in perfused rat heart. *J. Mol. Cell. Cardiol.* 19:187–197.
39. Sharma, V.K., and Sheu, S.S. 1986. Micromolar extracellular ATP increases intracellular calcium concentration in isolated rat ventricular myocytes. *Biophys. J.* 49:351a.
40. De Young, M.B., and Scarpa, A. 1987. Extracellular ATP induces Ca^{2+} transients in cardiac myocytes which are potentiated by norepinephrine. *FEBS Lett.* 223:53–58.

41. Benham, C.D., and Tsien, R.W. 1987. A novel receptor-operated Ca^{2+}-permeable channel activated by ATP in smooth muscle. *Nature* 328:275–278.
42. Charest, R., Blackmore, P.F., and Exton, J.H. 1985. Characterization of responses of isolated rat hepatocytes to ATP and ADP. *J. Biol. Chem.* 260:15789–15794.
43. Dubyak, G.R., and De Young, M.B. 1985. Intracellular calcium mobilization activated by extracellular ATP in Ehrlich tumor cells. *J. Biol. Chem.* 260:10653–10661.
44. Clusin, W.T., Bristow, M.R., Baim, D.S., et al. 1982. The effects of diltiazem and reduced serum ionized calcium on ischemic ventricular fibrillation in the dog. *Circ Res.* 50:518–526.
45. Clusin, W.T., Buchbinder, M., Ellis, A.K., et al. 1984. Reduction of ischemic depolarization by the calcium channel blocker diltiazem—correlation with improvement of ventricular conduction and early arrhythmias in the dog. *Circ. Res.* 54:10–20.
46. Blake, K., and Clusin, W.T. 1986. Effect of diltiazem on ischemic myocardial depolarization and extracellular K^+ accumulation. *Eur. J. Pharmacol.* 127:261–265.

3. REPETITIVE ACTIVITY: ORIGIN OF THE Na^+ LOAD AND ITS PHYSIOLOGIC EFFECTS

IRA S. COHEN, FANG CHANG, AND RICHARD P. KLINE

At a constant heart rate, the heart is in a steady state. This means that with each beat the Na^+, Ca^{2+}, and K^+, which cross the membrane during each action potential, must be returned to their original site inside or outside the cell before the next beat. An obvious corollary of this steady state is that the concentrations of each of these ions in each intracellular and extracellular compartment remains unaltered.

When the heart rate increases, the flux of Na^+, Ca^{2+}, and K^+ across the sarcolemma is altered such that an increased entry of Na^+ and Ca^{2+}, and an increased efflux of K^+ occurs [1–4]. Initially, given the magnitude of the concentration changes intracellularly, the rate of active pumping of Na^+ and Ca^{2+} are largely unaltered. This imbalance between influx and efflux leads to a net accumulation of Na^+ and Ca^{2+} intracellularly [1–3] and K^+ extracellularly [4]. These concentration changes increase the Na/K and Na/Ca exchange rates (as well as other transport processes) so that Na^+, K^+, and Ca^{2+} are ultimately returned to a new steady state. This new steady state occurs at an increased intracellular $[Na^+]$ and $[Ca^{2+}]$ and also with increased fluxes in both directions across the membrance for each ion.

It is our purpose to discuss the dynamics of the effects of drive rate changes on the membrane current of the Purkinje stand. The pathways by which Na^+ is loaded into the cell during a period of enhanced heart rate will be consi-

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dered. We will also discuss the effects that an increased $[Na^+]_i$ has on the important electrophysiologic parameters of maximum diastolic potential, action potential duration, and automaticity.

THE DYNAMICS OF Na^+ ENTRY AND REMOVAL: SETTING UP A NEW STEADY STATE WITH ALTERATIONS IN HEART RATE

The rapid upstroke of the Purkinje fiber action potential is generated by the influx of Na^+ through a TTX sensitive channel. A minimum estimate of the Na^+ entry during the upstroke can be obtained by estimating the charge necessary to be applied to the membrane capacitance to alter the membrane voltage by 120 mV (roughly the amplitude of a Purkinje fiber action potential).

$$Q = CV = 1.2 \times 10^{-12} \text{ moles}/\mu F \tag{3.1}$$

The typical Purkinje cell has a 15μ radius and is 180μ in length. Assuming it to be a right circular cylinder, its total capacitance is

$$S = (2\pi rh + 2\pi r^2)\ 1.54 = 2.8 \times 10^{-4}\ \mu F \tag{3.2}$$

where impedance studies have shown 1.54 $\mu F/cm^2$ of apparent surface area observed with light microscopy [5].

Thus, during a single action potential, the Na^+ entry in moles during the upstroke of a 120 mV action potential is at a minimum

$$Na^+ \text{ entry} = 1.2 \times 10^{-12} \frac{\text{moles}}{\mu F} \times 2.8 \times 10^{-4}\ \mu F = 3.4 \times 10^{-6} \text{ moles} \tag{3.3}$$

This causes a concentration change of

$$[\Delta\ Na^+]_i = \frac{3.4 \times 10^{-16} \text{ moles}}{1.3 \times 10^{-10} \text{ liters}} = 2.6 \times 10^{-6}\ M \tag{3.4}$$

where 1.3×10^{-10} liters is the volume of the Purkinje myocyte and is equal to $\pi r^2 h$ for a right circular cylinder.

Thus, the internal $[Na^+]$ is raised by at least 2 to 3 μM with each action potential upstroke. This calculation ignores the osmotically active volume fraction [6], Na^+ entry via the Na^+ window [7] and slowly inactivating Na^+ currents [8, 9] that occur with each action potential plateau. It further ignores that the Na^+ entry during the upstroke must exceed all background outward currents before any depolarization takes place [10]. Thus, more realistically the intracellular Na^+ content changes by at least 5 to 10 μM with each beat, considering only Na^+ entry through the TTX sensitive channel.

How does a myocyte reach a new steady state when the beating frequency is altered? To understand this process it is necessary to consider the major efflux route from the intracellular compartment. The major process remov-

ing Na^+ is the Na/K exchange pump [11]. Recent studies have suggested in cardiac myocytes that it is a saturable function of $[Na^+]_i$ and is probably best represented by a cubic [12, 13].

A reasonable formalism might be

$$\frac{d[Na^+]}{dt} = -V_{max}\left[\frac{[Na^+]}{[Na^+] + K_{Na}}\right]^3 \tag{3.5}$$

K_{Na} is in the range of about 10 mM [14].

Let us assume the resting Na^+ activity, indicated herein as $[Na^+]_i$, is about 6 mM at a 1 Hz beating frequency [2]. Then each second roughly 10 μM/L of Na^+ enter the myocyte and are extruded. Thus,

$$\frac{d[Na^+]}{dt} = \frac{10\ \mu M}{L \cdot sec} = V_{max}\left[\frac{6}{16}\right]^3 \tag{3.6}$$

$$V_{max} = \frac{10\ \mu M}{L \cdot sec}\ \frac{1}{.052} = \frac{192\ \mu M}{L \cdot sec}$$

When the beating rate rises, the Na^+ entry increases, but Na^+ extrusion is instantaneously unaltered. Up to 10 μM/sec of Na^+ might accumulate intracellularly (if Na^+ entry at the new higher rate is twice that at 1 Hz). This accumulation will continue until the elevated Na^+ entry is matched by an equally elevated Na^+removal rate (at which time a new steady state is achieved).

Thus, if the Na^+ entry is doubled:

$$\frac{20\ \mu M}{L \cdot sec} = \frac{192\ \mu M}{L \cdot sec}\left[\frac{[Na^+]}{[Na^+] + 10}\right]^3 \tag{3.7}$$

$$[Na^+] \cong 9\ mM$$

When, the $[Na^+]_i$ increases to 9 mM, the extrusion rate will have doubled and will match the higher influx. It is this continuous response of the Na/K pump to changes in Na^+ influx that allows for Na^+ homeostasis in the face of changing heart rates.

PATHWAYS FOR Na^+ LOADING

The preceding discussion suggested that with an increase in heart rate a net entry of Na^+ occurs. Although the discussion considered TTX-sensitive channels, there are several pathways by which this load could occur.

Cohen et al. [15], Falk [16], Falk and Cohen [17], and Chang and Cohen [18] investigated the role the fast and slow inward current, as well as steady state depolarizations played in the current induced by the Na^+ load, and Glitsch et al. [19] and Boyett et al. [20] extended this investigation to further

include measurements of a_{iNa} and the contribution of the pacemaker current i_f to the Na^+ load. Next we summarize the evidence demonstrating the capability of each of these ionic pathways to contribute as a means of Na^+ loading.

The TTX Sensitive Na^+ Current

The largest single source of Na^+ entry during an action potential is the TTX sensitive Na^+ current. The current has several kinetic components. The largest fraction is rapidly inactivating so that its role is terminated within a millisecond or two of its initiation [21]. This rapid phase is followed by a more slowly inactivating current [8, 9] and also a steady state window current [7].

Gintant et al. [8] estimate that the fast inward current is at least 100-fold and maybe more than 1,000-fold larger than the other two components in canine Purkinje fibers. Thus, why even consider them all? The answer is simple. What is lost in amplitude is made up in duration. Although the rapidly inactivating fraction extends only for the 1 or 2 ms of the action potential upstroke, the other components contribute throughout the several hundred milliseconds of the action potential plateau. The net result is that Na^+ loading from each of these sources can be significant.

Cohen et al. [15] and Falk and Cohen [17] investigated the role played by the TTX sensitive Na^+ current in generating a Na^+/K^+ pump current following a period of enhanced activity. They showed that with trains of action potentials, or with voltage clamp pulses from -75 to -40 mV, a dihydroouabain (DHO) sensitive decaying outward current would exist following the period of rapid activity. If the same set of voltage clamp pulses were applied in the presence of 10^{-5}M TTX, little or no postdrive current was observed. Further, a reduction of the duration of the depolarizing voltage clamp step from 50 to 10 ms of every 100 ms had only a small effect on the magnitude of the postdrive Na/K pump current. This last observation suggests that most of the Na^+ load occurs in the first 10 ms of a 50 ms protocol.

Na^+ window current was shown to contribute to Na^+ loading in two ways. First, Eisner et al. [22] demonstrated that the application of lidocaine, a local anesthetic antiarrhythmic agent, lowered intracellular Na^+ levels. This was presumably through a reduction of TTX-sensitive window current that lidocaine had earlier been reported to block [23, 24]. The second line of evidence involved Na^+ loading by a period of exposure to 0 mM K^+. The absence of K^+ in the Tyrode solution eliminates the Na/K exchange pump activity. The unbalanced background Na^+ influx then elevates $[Na^+]_i$ so that when the Purkinje fiber is returned to normal Tyrode an elevated $[Na^+]_i$ increases Na/K pump activity [25, 26]. The total additional change Q_p extruded by the pump allows an estimate of Iin_B [5]. Verdonck and Bosteels

[27] used this method with and without TTX in rabbit Purkinje fibers to show that the Na^+ window current contributes appreciably to Na^+ loading.

Pump Current Elicited by Repeated Activation of I_{Ca}

Although the major source of the Na^+ load with repetitive activity is Na^+ entry via the TTX-sensitive channel, it is not the only inward current that loads Na^+. Falk and Cohen [17] and Chang and Cohen [18] applied trains of depolarizing voltage clamp pulses from −50 mV. These pulses activated i_{Ca} but did not activate the TTX-sensitive Na^+ current since almost all the Na^+ channels were inactivated. Nevertheless, following the repetitive train of voltage clamp pulses, a decaying outward current was observed. This current was eliminated by DHO suggesting that the postdrive current was Na/K pump current. The postdrive current was also blocked by D600, suggesting that the calcium channel was necessary for the Na^+ load. TTX had no effect.

Chang and Cohen [18] investigated the origin of this Na^+ load through the Ca^{2+} channel in more detail. As suggested by Falk and Cohen [17], reducing Tyrode $[Ca^{2+}]$ from 4 or 2 mM to 0.1 mM virtually eliminated the postdrive current. This was not due to an alteration in the Na/K pump time constant since repetitive activation of TTX sensitive Na^+ current resulted in an unaltered postdrive pump current when the $[Ca^{2+}]$ was similarly reduced. This suggests that Ca^{2+} entry is necessary to generate the postdrive current. One possible mechanism by which calcium entry could raise $[Na^+]_i$ is via Na/Ca exchange. The procedure by which this would occur is illustrated in the following schematic:

$$\begin{array}{l}\text{Ca entry via} \\ \text{D600 sensitive} \\ \text{channel}\end{array} \rightarrow [Ca^+]_i \uparrow \xrightarrow[\text{exchange}]{\text{Na:Ca}} [Na^+]_i \uparrow \rightarrow \begin{array}{c}\text{Na/K} \\ \text{pump current}\end{array} \qquad (3.8)$$

Experiments by Kimura et al. [28] and Poitreau et al. [29] have demonstrated that Sr^{2+} is a poor substitute for Ca^{2+} in the Na:Ca exchange process, whereas Ba^{2+} is totally ineffective. Chang and Cohen [18] showed that Sr^{2+} was a poor substitute for Ca^{2+} in generating postdrive pump current by repetitive Ca^{2+} channel activation, whereas Ba^{2+} was almost totally ineffective. Addition of Ba^{2+} or Sr^{2+} to a normal Ca^{2+} Tyrode had no effect on postdrive current. These results further support the possible involvement of the Na:Ca exchanger in converting a Ca^{2+} load into a Na^+ load.

Chang and Cohen [18] also investigated the effect of potential on the ability to generate a Na/K pump current via the Ca^{2+} channel. A 50 ms step from −40 to 0 mV was followed by 250 ms at positive or negative potentials. Then the fiber was returned to −40 mV to allow I_{Ca} to recover from inactivation. The more polarized the test potentials the smaller the Na^+ load as indicated by the integral of the postdrive current. This result is expected if the Na:Ca exchanger depends on potential in the manner predicted by Mullins [30].

In summary, repetitive activation of the Ca^{2+} current leads to a Na^+ load. Present evidence suggests that the Na^+ load results indirectly from a Ca^{2+} load, possibly through Na:Ca exchange.

Na^+ Loading Through the Pacemaker Channel

The reinterpretation of the ionic selectivity of the pacemaker current by DiFrancesco [31, 32] necessitated a consideration of the role i_f might play in Na^+ entry. Since i_f is partly Na^+ selective repetitive activation might load Na^+. Glitsch et al. [19] demonstrated in sheep Purkinje fibers that activation of pacemaker current increased the intracellular $[Na^+]$ and that block of i_f by 2 mM Cs^+ could reduce $[Na^+]_i$.

Boyett, et al. [20] investigated the role i_f plays in Na^+ loading with repetitive voltage clamp pulses. They applied repetitive depolarizing voltage clamp pulses in the absence or presence of 2 mM Cs^+. Since this low concentration of Cs^+ has a blocking action on i_f with only small actions on other currents they argued that since they observed a reduced Na^+ load in Cs^+ containing solution i_f must be a contributor to the Na^+ load observed with repetitive activity.

Background Na^+ Loading

In normal Tyrode Solution, the Purkinje fiber resting potential is not at E_K but somewhat more positive [33]. This difference between E_M and E_K is not eliminated by TTX, which has little or no effect at potentials negative to −80mV. Thus, a non-TTX sensitive inward background current must exist.

Long depolarizing pulses alter TTX sensitive, D600 sensitive, and other inward background currents; therefore, it is not possible to characterize the non-TTX or D600-sensitive components except by total pharmacologic blockade. This experiment has yet to be performed in Purkinje fibers. Direct measurements of $[Na^+]_i$ with Na^+ sensitive electrodes show that Na^+ declines on depolarization in sheep Purkinje fibers [11, 20, 34]. It is presumed that this is due to the reduction in driving force. Eisner et al. [11] suggested from measurements of steady state $[Na^+]_i$ at various potentials in the absence of pharmacologic blockade that the background Na^+ current could be reasonably well fit by a constant P_{Na} and Goldman rectification.

It is interesting to note that the membrane current becomes less outward in sheep Purkinje fibers during prolonged depolarization to plateau potentials, presumably due at least in part to the time-dependent decline in $[Na]_i$ reducing the resting pump current [11]. In contrast, in canine Purkinje fibers, prolonged depolarization results in an increasing outward current [35]. This is probably due to a slowly increasing $[Na^+]_i$, since Gadsby [36] has demonstrated an increased inward background current at plateau as compared to diastolic potentials by examining the Na/K pump current following 0 mM K^+ exposures at plateau and diastolic potentials.

In summary, an inward background current exists and contributes to the

potential dependence of $[Na^+]_i$. This potential dependence is not yet well characterized, but is at least somewhat species dependent.

PHYSIOLOGIC CONSEQUENCES OF A PERIOD OF ENHANCED ACTIVITY

When the stimulation rate increases, the action potential shortens, the maximum diastolic potential first decreases and then increases, and the cleft potassium first accumulates and then returns to baseline.

The action potential duration decreases for at least three reasons during repetitive activity. First, repetitive activation of the delayed rectifier with a decreased diastolic interval results in maintained levels of outward current through the delayed rectifier [37, 38]. Second, increased outward currents result in cleft K^+ accumulation, which also provides even more outward membrane current because of the crossover in the membrane current-voltage relationships [39]. Third, the increase in $[Na^+]_i$ results in an increase in electrogenic Na/K exchange that provides yet more outward currents to shorten the action potential [40]. The net result of these three components is to provide substantial additional outward current during the train that dramatically shortens the action potential duration.

The maximum diastolic potential during the train is influenced by the same three factors. Residual activation of the delayed rectifier provides enhanced K^+ selectivity (since i_K is largely K^+ selective in Purkinje myocytes and fibers [41, 42]. K^+ accumulation increases background g_{K1} forcing E_m nearer to E_K; however, the decrease in driving force initially dominates and causes some depolarization [4, 43]. With longer trains, the K^+ accumulation declines as Na/K exchange increases due to a rise in $[Na^+]_i$, resulting in a return to control levels. Following the train, the cleft $[K^+]$ is reduced below control levels as the enhanced Na^+/K^+ exchange driven by the elevated $[Na^+]_i$ removes K^+ from the clefts at an elevated rate, while K^+ entry into the cleft returns towards control levels. The net result is a substantial postdrive hyperpolarization that decays only slowly with the time course of the drive induced Na/K pump current [4, 40].

The cleft $[K^+]$ is determined by a balance of diffusion from cleft to bulk, active transport, and K^+ efflux through transmembrane K^+ channels [39, 44]. When the train is initiated, the intracellular $[Na^+]$ changes slowly (minutes), but the maintained activation of the delayed rectifier is rapid (seconds). Thus, an increased K^+ efflux is not balanced by active transport resulting in extracellular K^+ accumulation. However, with sustained stimulation $[Na^+]_i$ rises and K^+ influx and cleft to bulk diffusion ultimately matches K^+ efflux and begins to return the cleft $[K^+]$ toward bulk $[K^+]$ levels. Following the train as previously explained, cleft $[K^+]$ declines below bulk levels since influx via the Na/K pump remains elevated while efflux through membrane K^+ permeability declines [4, 39].

The rate of pacemaker depolarization is determined by the sum total of all the ionic currents flowing across the cardiac membrane. The major effect of

the period of activity is to increase $[Na^+]_i$ and thus the Na/K pump current [4, 15]. This outward current has a time constant of 80 seconds and should suppress the pacemaker throughout its decay. A secondary effect of this increased pump current is that cleft $[K^+]$ is depleted below bulk levels. This $[K^+]_o$ depletion could hyperpolarize or depolarize the membrane (provide more or less outward current) depending on the membrane I-V relationships, the $[K^+]_o$ dependence of the pacemaker current, and the position of the crossover [39]. The sum total of these effects is a suppression of pacemaker activity throughout the pump current decay.

CONCLUSION AND FUTURE DIRECTIONS

When the stimulation rate increases, $[Na^+]_i$ rises. Some of this Na^+ is loaded directly via TTX-sensitive, or pacemaker channels, and some is loaded indirectly following a calcium load via i_{Ca}; presumably by activation of Na^+/Ca^{2+} exchange.

This Na^+ load decreases AP duration and ultimately hyperpolarizes the MDP. Following the drive, a depletion of cleft $[K^+]$ results. The Na^+ load also generates a postdrive Na/K pump current that suppresses automaticity resulting in overdrive suppression of the Purkinje pacemaker [45, 46].

Although a reasonable description of the pathways for Na^+ loading exists, much less is known about the relative contributions of each of these pathways during typical physiologic alterations in heart rate. Also little is known about the alterations in these ionic pathways in response to pathologic conditions. The absence of this basic physiologic and pathologic information remains an important area for further fruitful research.

REFERENCES

1. Cohen, C.J., Fozzard, H.A., and Sheu, S. 1982. Increase in intracellular sodium ion activity during stimulation in mammalian cardiac muscle. *Circ. Res.* 50:651–662.
2. Boyett, M.R., Hart, G., and Levi, A.J. 1987. Factors affecting intracellular sodium during repetitive activity in isolated sheep Purkinje fibres. *J. Physiol.* 384:405–429.
3. Sheu, S., and Fozzard, H.A. 1982. Transmembrane Na^+ and Ca^{2+} electrochemical gradients in cardiac muscle and their relationship to force development. *J. Gen. Physiol.* 80:325–351.
4. Kline, R., Cohen, I., Falk, R., and Kupersmith, J. 1980. Activity dependent extracellular K^+ fluctuations in canine Purkinje fibers. *Nature* 286:68–71.
5. Cohen, I.S., Datyner, N.B., Gintant, G.A., et al. 1987. Properties of an electrogenic Na/K pump in isolated canine Purkinje myocytes. *J. Physiol.* 383:251–267.
6. Hauser, S., and Freeman, A. 1980. Volumetric properties of intracellular compartments in canine cardiac Purkinje cells. *Am. J. Physiol.* 238:H561–H568.
7. Attwell, D., Cohen, I., Eisner, D., et al. 1979. The sodium "window" current in cardiac Purkinje fibres. *Pflugers Arch.* 379:137–142.
8. Gintant, G.A., Datyner, N.B., and Cohen, I.S. 1984. Slow inactivation of a tetrodotoxin sensitive current in canine cardiac Purkinje fibres. *Biophys. J.* 45:509–512.
9. Carmeliet, E. 1985. Slow decay of sodium current and effect of quinidine in rabbit cardiac Purkinje fibers. In *Cardiac Electrophysiology and Arrhythmias*, D.L. Zipes, ed., pp. 207–215. New York, Grune & Stratton.
10. Cohen, I. 1979. Some difficulties in the experimental use of V_{max} as a measure of g_{Na} in cardiac membranes. *Circ. Res.* 45:309–312.
11. Eisner, D.A., Lederer, W.J., and Vaughan-Jones, R.O. 1981. The effects of rubidium ions

and membrane potential on the intracellular sodium activity of voltage-clamped sheep cardiac Purkinje fibres. *J. Physiol.* 317:189–205.
12. Cohen, I.S., Kline, R.P., Pennefather, P., and Mulrine, N.K. 1987. Models of the Na/K pump in cardiac muscle predict the wrong intracellular Na^+ activity. *Proc. Roy. Soc. London* (Biology) 231, 371–382.
13. Baumgarten, C.M., and Desilets, M. in press. Isoproterenol intracellular Na^+ activity, and the Na/K pump in isolated ventricular myocytes. In *Recent Studies of Ion Transport and Impulse Propagation in Cardiac Muscle*, W.R. Giles, ed., New York, Alan R. Liss, Inc.
14. Fozzard, H.A., Sejersted, O.M., and Wasserstrom, J.A. 1986. Sodium activation of the Na, K pump in isolated sheep cardiac Purkinje strands. *J. Physiol.* 381:91P.
15. Cohen, I., Falk, R., and Kline, R. 1981. Membrane currents following activity in canine cardiac Purkinje fibers. *Biophys. J.* 33:281–288.
16. Falk, R. 1983. Membrane Current Following Activity in Canine Cardiac Purkinje Fibers. Ph. D. dissertation. SUNY Stony Brook.
17. Falk, R.T., and Cohen, I.S. 1984. Membrane current following activity in canine cardiac Purkinje fibers. *J. Gen. Physiol.* 83:771–799.
18. Chang, F., and Cohen, I.S. in press. Na/K pump current elicited by repetitive activation of I_{Ca} (abstr.). *Biophys. J.*
19. Glitsch, H.G., Pusch, H., and Verdonck, F. 1986. The contribution of Na and K ions to the pacemaker current in sheep cardiac Purkinje fibres. *Pflugers Arch.* 406:464–471.
20. Boyett, M.R., Hart, G., and Levi, A.J. 1987. Factors affecting intracellular sodium during repetitive activity in isolated sheep Purkinje fibres. *J. Physiol.* 384:405–429.
21. Colatsky, T.J. 1980. Voltage clamp measurements of sodium channel properties in rabbit Purkinje fibres. *J. Physiol.* 305:215–234.
22. Eisner, D.A., Lederer, W.J., and Sheu, S.S. 1983. The role of intracellular sodium activity in the antiarrhythmic action of local anesthetics in sheep Purkinje fibres. *J. Physiol.* 340:239–257.
23. Colatsky, T.J. 1982. Mechanisms of action of lidocaine and quinidine on action potential duration in rabbit cardiac Purkinje fibres. *Circ. Res.* 50:17–27.
24. Carmeliet, E., and Saikawa, T. 1982. Shortening of the action potential and reduction of pacemaker activity by lidocaine, quinidine and procaineamide in sheep cardiac Purkinje fibers. *Circ. Res.* 50:257–272.
25. Eisner, D.A., and Lederer, W.J. 1980. Characterization of the electrogenic sodium pump in cardiac Purkinje fibres. *J. Physiol.* 303:441–474.
26. Gadsby, D.C. 1980. Activation of electrogenic Na^+/K^+ exchange by extracellular K^+ in canine cardiac Purkinje fibres. *Proc. Nat. Acad. Sci.* (USA) 77:4035–4039.
27. Verdonck, F., and Bosteels, S. 1985. The effects of membrane potential and extracellular K^+ on Na^+ pump activity and intracellular Na^+ activity in rabbit cardiac Purkinje fibers. *J. Gen. Physiol.* 86:30a.
28. Kimura, J., Miyanae, S., and Noma, A. 1987. Identification of sodium-calcium exchange current in single ventricular cells of guinea pig. *J. Physiol.* 384:199–222.
29. Poitreau, D., Richard, S., Nargeot, J., and Raymond, G. 1987. Tension activation and relaxation in frog atrial fibres: evidence for direct effects of divalent cations (Ca^{2+}, Sr^{2+}, Ba^{2+}) on contractile proteins and Na–Ca exchange. *Pflugers Arch.* 410:326–334.
30. Mullins, L.J. 1981. *Ion Transport in Heart.* New York, Raven Press.
31. DiFrancesco, D. 1981. A new interpretation of the pacemaker current in calf Purkinje fibres. *J. Physiol.* 314:359–376.
32. DiFrancesco, D. 1981. A study of the ionic nature of the pacemaker current in calf Purkinje fibres. *J. Physiol.* 314:377–393.
33. Dresdner, K.P., Kline, R.P., and Wit, A.L. 1987. Intracellular K^+ activity, intracellular Na^+ activity and maximum diastolic potential of subendocardial Purkinje cells from one-day-old infarcts. *Circ. Res.* 60:122–132.
34. January, C.T. and Fozzard, H.A. 1984. The effects of membrane potential, extracellular potassium and tetrodotoxin on the intracellular sodium ion activity of sheep cardiac muscle. *Circ. Res.* 54:652–665.
35. Cohen, I., Falk, R., and Kline, R. 1983. Voltage clamp studies on the canine Purkinje strand. *Proc. Roy. Soc. London* (Biology) 217:215–236.
36. Gadsby, D.C. 1983. B-adrenoreceptor agonist increase in membrane K^+ conductance in cardiac Purkinje fibres. *Nature* 306:691–693.

37. Hauswirth, O., Noble, D., and Tsien, R.W. 1972. The dependence of plateau currents in cardiac Purkinje fibres on the interval between action potentials. *J. Physiol.* 222:27–49.
38. Cohen, I.S., Datyner, N.B., Gintant, G.A., and Kline, R.P. 1986. Time dependent outward currents in the heart. In *The Heart and Cardiovascular System*, H.A. Fozzard, eds., New York, Raven Press.
39. Cohen, I., and Kline, R. 1982. K^+ fluctuations in the extracellular spaces of cardiac muscle; evidence from the voltage clamp and extracellular K^+ selective microelectrodes. *Circ. Res.* 50:1–16.
40. Gadsby, D.C., and Cranefield, P. 1982. Effects of electrogenic sodium extrusion on the membrane potential of cardiac Purkinje fibers. In *Normal and Abnormal Conduction in the Heart*, P. de Carvalho, B. Hoffman, and M. Lieberman, eds., New York, Futura Publishers.
41. Gintant, G.A., Datyner, N., and Cohen, I. 1985. Gating of delayed outward rectifiers in acutely dissociated canine cardiac Purkinje myocytes: evidence for a single channel mechanism. *Biophys. J.* 48:1059–1064.
42. Bennett, P., McKinney, L., Kass, R., and Begenesich, T. 1985. Delayed rectification in the calf cardiac Purkinje fiber: evidence for multiple state kinetic. *Biophys. J.* 48:553–568.
43. Kline, R.P., and Kupersmith, J. 1982. Effects of potassium accumulation and sodium pump activation on automatic canine Purkinje fibres. *J. Physiol.* 324:507–533.
44. Attwell, D., Eisner, D., and Cohen, I. 1979. Voltage clamp and tracer flux data: effects of a restricted extracellular space. *Q. Rev. Biophys.* 12:213–261.
45. Gaskell, W.H. 1884. On the innervation of the heart with especial reference to the heart of the tortoise. *J. Physiol.* 4:43–127.
46. Vassalle, M. 1970. Electrogenic suppression of automacity in sheep and dog Purkinje fibers. *Circ. Res.* 27:361–377.

4. IONIC CHANGES ASSOCIATED WITH ACUTE ISCHEMIA

LEONARD S. GETTES, TIMOTHY A. JOHNSON,
WILLIAM F. FLEET, AND ICHIRO WATANABE

Harris et al. [1] were the first to appreciate that occlusion of a coronary artery is followed by an increase of potassium in the extracellular space of the area deprived of its circulation. They then performed a series of experiments, which led them to conclude that the increase in extracellular potassium was a major factor, perhaps *the* major factor in the pathogenesis of the acute ventricular arrhythmias, including ventricular fibrillation, which accompany coronary occlusion [1, 2]. Later, it was shown that acute coronary occlusion leads to a fall in extracellular pH [3] and a rise in extracellular pCO_2 [4] within the ischemic zone. Within the last decade, a variety of new techniques, including ion selective electrodes, nuclear magnetic resonance, and voltage and ion sensitive dyes, have permitted the more precise characterization of the intracellular and extracellular ionic changes that occur when the coronary circulation is abruptly or progressively interrupted and a more accurate correlation of these ionic changes to the associated metabolic, electrical, and mechanical changes. As is usually the case, each new observation has served to bring into sharp focus the limits of our understanding and has spawned a series of new questions. In this presentation, we will briefly review the characteristics, causes, and effects of the potassium and pH changes that occur when coronary flow is interrupted and some of the factors that modify these changes. We will then identify some of the questions that have evolved

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as our understanding of the ionic changes has expanded. We will also touch upon the changes in sodium and calcium that occur in association with the changes in potassium and pH.

DESCRIPTION OF IONIC CHANGES

Intracellular

Most of the information pertaining to the changes in intracellular pH has been derived from nuclear magnetic resonance studies of shifts in the phosphate spectral peaks in isolated hearts. These studies have uniformly shown that within 30 minutes of interrupting coronary blood flow, intracellular pH falls from 7.2 to approximately 6.0 [5–7]. Intracellular potassium (K^+) and sodium (Na^+) have been studied primarily by using ion selective microelectrodes in isolated intact hearts made globally ischemic and in single fibers superfused with solutions modified to simulate ischemia. The simulation is accomplished by altering K^+, pH, pO_2, and glucose either alone or in combination. Nakaya et al. [8] studied canine epicardial ventricular fibers and reported that within 30 minutes of exposure to hypoxic (pO_2 <50 mm Hg), acidotic (pH:6.6), and glucose-free solutions, having a normal (4.0 mM) K^+ concentration, intracellular K^+ activity fell by 25 to 30%. However, Kleber et al. [9] have reported that for the intact ischemic heart, intracellular K^+ activity need decreases by less than 5% in order to cause the reported increase in extracellular K^+. Nakaya et al. [8] also reported that in their experiments, intracellular Na^+ did not change. Wilde and Kléber [10] noted a dependence of the change of intracellular Na^+ on the K^+ concentration in the superfusate. They reported that intracellular Na^+ rose by 3 to 4 mM from a control value of 7.5 mM when the K^+ concentration of the modified superfusate was not increased, but by only 2 mM when the K^+ concentration in the modified superfusate was raised from 4.7 mM to 11.5 mM. They attributed the lesser effect observed when K^+ was increased to the decrease in the Na^+ inward current resulting from the K^+-induced depolarization of the membrane. In Langendorff-perfused isolated guinea pig hearts made globally ischemic, intracellular Na^+ did not change significantly following the total interruption of coronary flow [11] These studies suggest that the Na^+/K^+ pump is only partially suppressed by the conditions of the experiments [8, 10, 11]. Most recently, however, nuclear magnetic resonance studies employing a shift reagent to separate intracellular and extracellular Na^+ peaks have revealed an increase in intracellular Na^+ from 5 to 20 mM after 28 minutes of global ischemia in the isolated rat heart. During the same time period, intracellular pH fell from 7.14 to 6.20 [12].

Changes in intracellular calcium have also been determined using nuclear magnetic resonance techniques in Langendorff-perfused rat hearts. These recently reported studies employing the chelating agent 5-F BAPTA [13] have shown that after 15 minutes of global ischemia, cytosolic free calcium increased from a control of approximately 250 nM in the arrested heart and

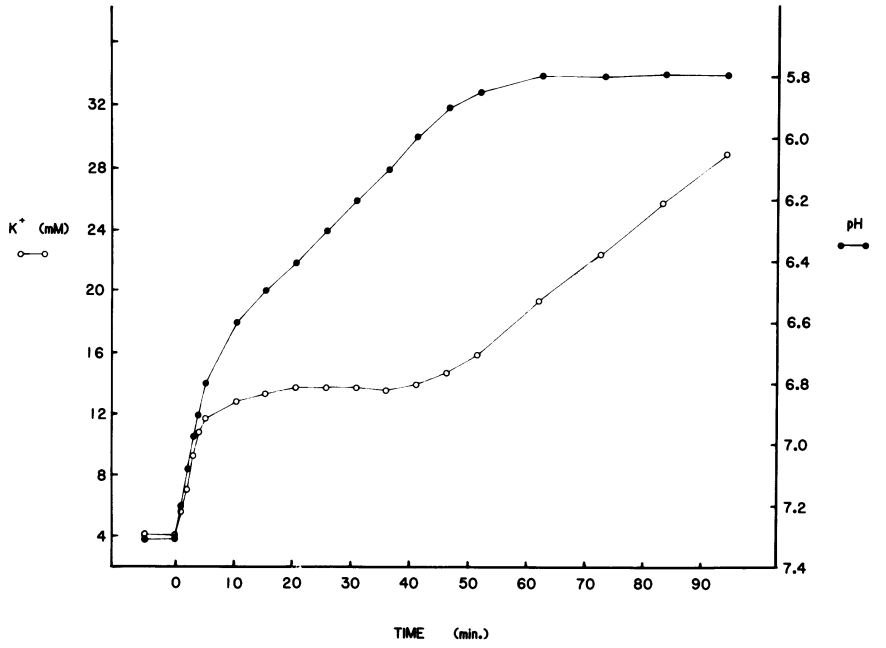

Figure 4-1. Time course of changes in midmyocardial extracellular K^+ (open symbols—left vertical axis) and pH (closed symbols—right vertical axis) recorded from the center of the ischemic zone following ligation of the left anterior descending coronary artery (time = 0 min) in the open-chested, anesthetized pig. Note that the third, or slowly rising phase of the K^+ change occurs when pH has fallen to approximately 6.0 and that pH does not fall below 5.8. Reprinted from Gettes [26], by permission.

600 nM in the beating heart to 2.7 μM during 11 to 15 minutes of total ischemia. The authors determined that the increase in cytosolic free calcium preceded the loss of plasma membrane integrity and was fully reversible upon reperfusion.

Extracellular Ionic Changes

In the extracellular space, the predominant ionic changes following coronary occlusion are an increase in K^+ [11, 14–18] and a fall in pH [16, 18]. Potassium rises in a triphasic fashion (figure 4-1). An initial rapidly rising phase lasting 10 to 15 minutes is followed by a plateau phase, which lasts approximately 20 to 30 minutes. The K^+ level may actually decrease during this plateau phase. A third phase then follows during which potassium rises slowly but continuously. This third, or slowly rising phase, denotes irreversibility and loss of membrane integrity.

The fall of extracellular pH follows a somewhat different time course (figure 4-1). A rapidly falling phase corresponds to the rapidly rising phase of the K^+ change. This is followed by a more slowly falling phase that corres-

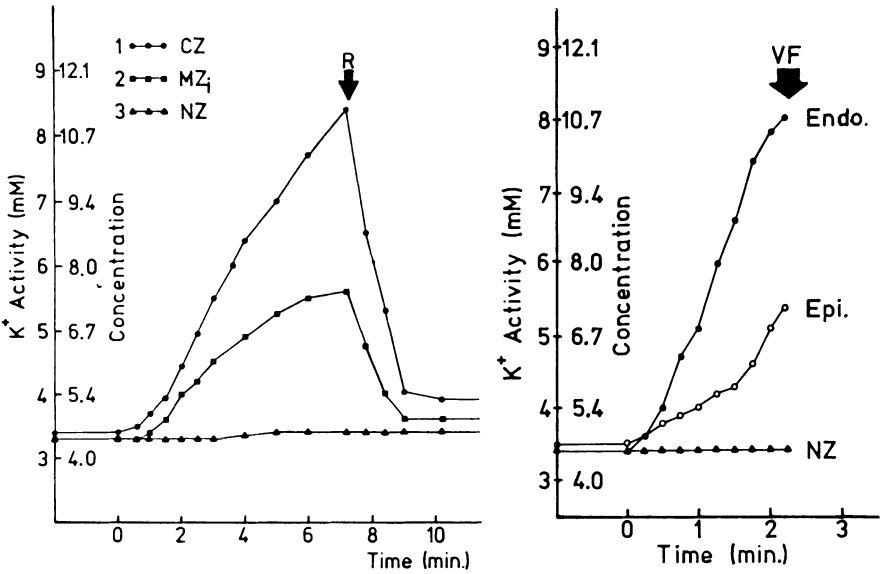

Figure 4-2. The panel on the left shows the midmyocardial K^+ change in the center of the ischemic zone (CZ-top curve), 5 mm inside the lateral margin of the ischemic zone (MZ_i-middle curve) and in the non- ischemic zone (NZ-bottom curve) following ligation of the left anterior descending coronary artery in the open chested, anesthetized pig at time = 0 min. R = the release of the ligation. The right panel shows the K^+ changes recorded in the subendocardium and subepicardium of the center of the ischemic zone in a different but similar experiment. At the arrow marked VF, ventricular fibrillation occurred. Reprinted from Hill and Gettes [14], by permission.

ponds to the plateau phase of the K^+ change. Extracellular pH then plateaus during the third or slowly rising phase of the K^+ change. In the regionally ischemic heart, the magnitude of the changes in extracellular K^+ and pH depend upon the site being sampled. This site specificity reflects the fact that the changes are less profound at the borders of the ischemic zone than in the center [14], due, at least in part, to diffusion of the ions from the margin into the nonischemic area [19]. In addition, at any point in time there are transmural differences [14] (figure 4-2). The changes in the subendocardium are greater than the changes in the midmyocardium which, in turn, are greater than the changes in the subepicardium. These transmural differences are due, most likely, to the wavelike spread of the ischemic process from the endocardium to the epicardium [20]. Figure 4-1 illustrates that in the midmyocardium within the center of the ischemic zone, extracellular K^+ rises to 10 to 15 mM within the first or rapidly rising phase. During this time, extra cellular and intracellular pH fall to approximately 6.5. The third or slowly rising phase occurs when extracellular and intracellular pH have decreased to approximately 6.0. During this phase, K^+ continues to rise, reaching levels in excess of 30 mM 96 minutes after occlusion, but the pH falls only

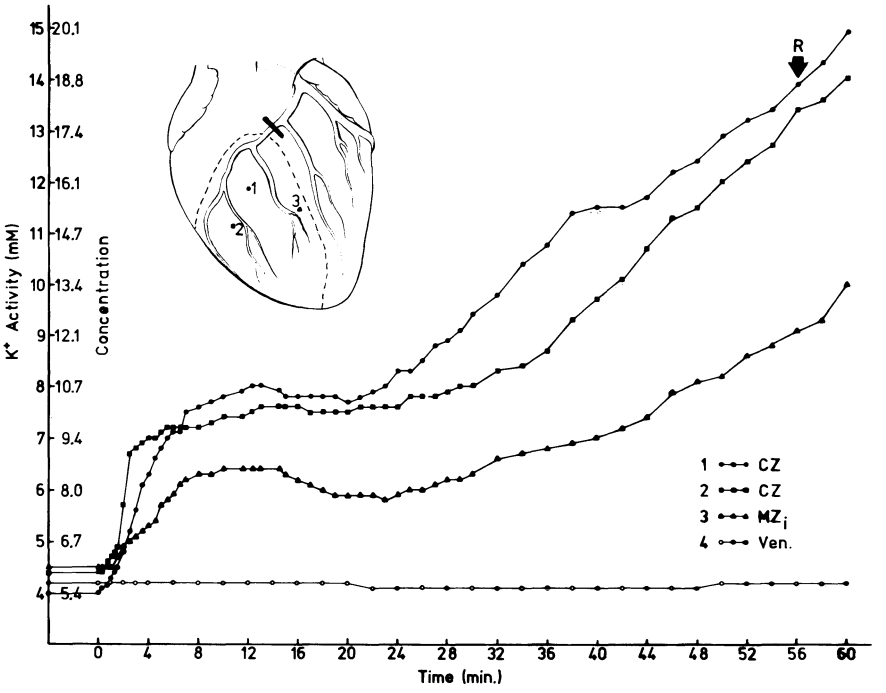

Figure 4-3. Changes in midmyocardial extracellular K^+ recorded from the ischemic zone in hearts of open-chested, anesthetized pigs. The insert shows the location of the left anterior coronary artery ligation (solid bar) the of the ischemic area (broken line), and the location of the K^+ sensitive electrodes. The results from electrodes one and two located in the center of the ischemic zone (CZ) are the upper two curves. The middle curve was recorded from electrode three located at the inside margin of the ischemic zone (MZ_i). The bottom curve was recorded from electrode four (not shown) located in the right ventricle. Note that the third, or slowly rising phase of the K^+ change occurs 24 minutes following ligation in both the center and margin. The arrow marked R denotes the release of the occlusion.

slightly. The various phases of the changes in extracellular K^+ in the center and margin occur at the same time following coronary occlusion, although the changes in the margin are of lesser magnitude (figure 4-3) [14]. The similarity of the time course in the two regions is more consistent with the hypothesis that the margin is composed of the interdigitation of normal and ischemic cells rather than partially ischemic cells.

When coronary flow is sequentially reduced rather than abruptly interrupted, the changes in local myocardial K^+ and pH occur in concert with changes in the TQ and ST potentials of the locally recorded electrograms and are the most sensitive markings of the onset of ischemia.[21] These changes occur first in the subendocardium, then in the midmyocardium, and finally appear at the epicardium reflecting the transmyocardial redistribution of coronary flow that occurs in this setting [22]. These changes frequently

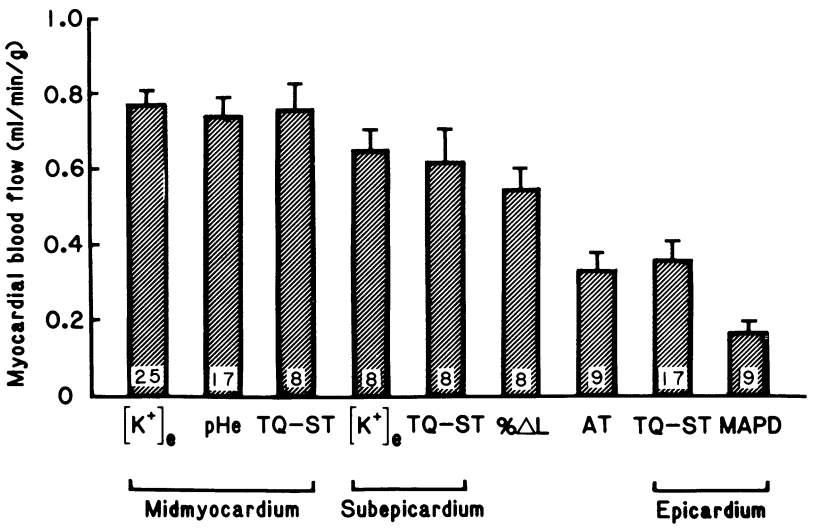

Figure 4-4. Myocardial blood flow per gram of tissue associated with the first change in the various parameters shown along the horizontal axis as determined in the center of the ischemic zone of open-chested, anesthetized pigs subjected to stepwise reduction in the left anterior descending coronary blood flow. $[K^+]_e$ = extracellular K^+, ph_E = extracellular pH, TQ–ST = changes in the locally recorded electrogram, %ΔL = change in segmental myocardial fiber shortening, AT = activation time, MAPD = monophasic action potential duration. Reprinted from Watanabe et al. [21], by permission.

precede changes in contractility. Changes in impulse propagation require an increase in extracellular K^+ to approximately 8 mM and are thus less sensitive than the ionic changes as a marker of ischemia. Changes in the duration of the epicardial monophasic action potential and epicardial TQ and ST segments are the most insensitive of these various markers of ischemia. Figure 4-4 illustrates the myocardial flow per gram of tissue associated with the first change in the ionic, electrical, and mechanical indices of ischemia recorded in the midmyocardium, subepicardium, and epicardium.

The differences in the extracellular ionic changes between the center and margin and between endocardium and epicardium represent macro inhomogeneities within the myocardial extracellular space. In addition, there are significant inhomogeneities in the ionic changes at each location (i.e., center or margin of the ischemic zone, figure 4-5) [14, 18]. These represent micro inhomogeneities and are greater within close proximity of the visible cyanotic margin than in the center of the ischemic zone. Although this might be anticipated on the basis of proximity to the normal zone, our ongoing investigations suggest that other, as yet undetermined, factors contribute to the micro inhomogeneities.

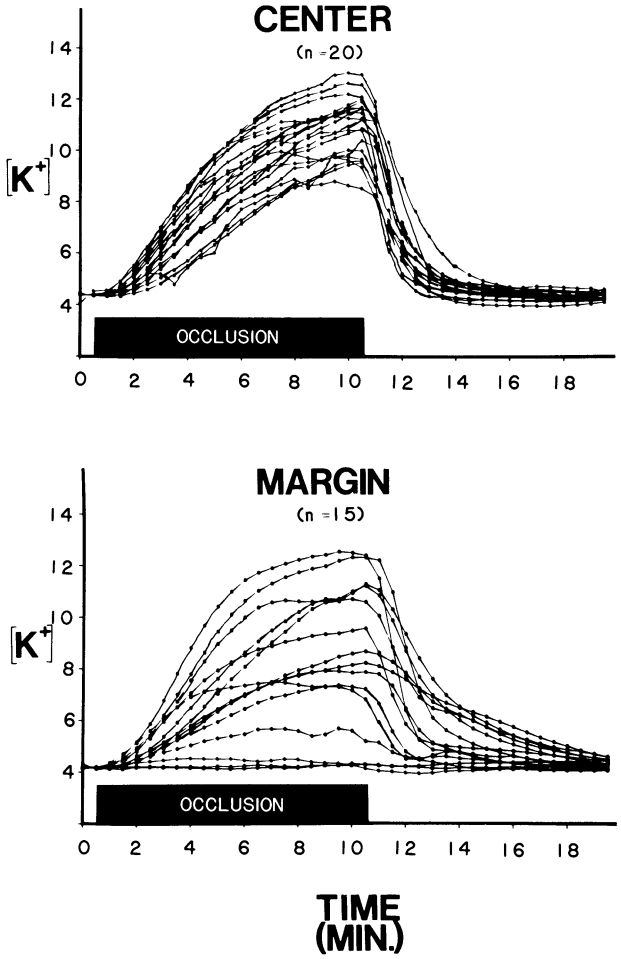

Figure 4-5. Inhomogeneity in midmyocardial K^+ change as recorded simultaneously by 20 K^+ sensitive electrodes in the center of the ischemic zone and 15 electrodes within 5 mm of the visible cyanotic border following occlusion of the left anterior descending coronary in the open-chested, anesthetized pig. We have labeled the differences in each area the "micro"-inhomogeneities.

CAUSE OF THE IONIC SHIFTS

The acute cessation of coronary arterial blood flow results in the immediate deprivation of oxygen and substrate to the myocardial cells. Metabolism becomes anaerobic, glycolysis occurs, and there is a rapid decrease in the high energy phosphate stores. [23]. Lactate is produced [24] and intracellular pH falls [5–7]. It is likely that development of intracellular acidosis is at least

partially responsible for a functional defect of the sarcoplasmic reticulum that, when coupled with a possible pH independent depression of myofibrillar calcium-magnesium ATPase activity [25], results in the recently described increase in intracellular calcium [13] and a decrease in contractility. Sodium-potassium ATPase activity may be somewhat depressed [10] and may contribute to a tendency for intracellular Na^+ to rise. As previously indicated, this effect may be offset by the decrease in sodium inward current that follows the reduction in resting membrane potential.

The rise in extracellular K^+ has been attributed to the passive effect of potassium movement from the intracellular space in association with lactate and phosphate ions [9]. The lack of washout from the extracellular space allows the K^+ extruded from the cells to accumulate. The diffusion of lactate across the cell membrane leads to the increase in extracellular pCO_2 [4] and the fall in extracellular pH [3, 16, 18].

ELECTROPHYSIOLOGIC EFFECTS

The electrophysiologic changes occurring as a result of the ionic changes have been recently reviewed [26] and will only be highlighted in this section. The major effect of the rise in extracellular K^+ is the depolarization of the resting membrane in accordance with the predictions of the Nernst equation [11]. Thus, each doubling of extracellular K^+ depolarizes the resting membrane by approximately 19 mV (figure 4-6). This depolarization is augmented by the associated decreases in pH and PO_2 [27, 28]. As a result of the change in resting membrane potential, the maximum rate of rise of the action potential ($\dot{V}_{max}$) is slowed and its recovery after a preceding depolarization becomes progressively prolonged [29] leading to the development of time-dependent refractoriness. This prolongs the refactory period beyond the end of the action potential, which itself is shortened by the increase in extracellular K^+, the decrease in pO_2 [30, 31], and the increase in catecholamines [32]. The inhomogeneities in K^+ and pH predict that the changes in conduction and refractoriness will be inhomogeneous. This may contribute to the establishment of reentry circuits. The inhomogeneities existing between the center, margin, and normal areas will also result in injury currents flowing between the nonischemic and ischemic areas [33]. In addition, the micro inhomogeneities within the ischemic area may create local injury currents. The injury currents may contribute to the generation of spontaneous beats and rhythms by inducing spontaneous depolarization [34] and by bringing subthreshold depolarizations to threshold. The injury currents also cause shifts in the T-Q potential of the local and body surface electrogram [33, 35]. However, as illustrated in (figure 4-7), the relationship between changes in K^+ and shifts in the T-Q segment are not consistent. [35] For this reason, the magnitude of the extracellular K^+ change cannot be predicted from the associated T-Q segment shift or vice-versa.

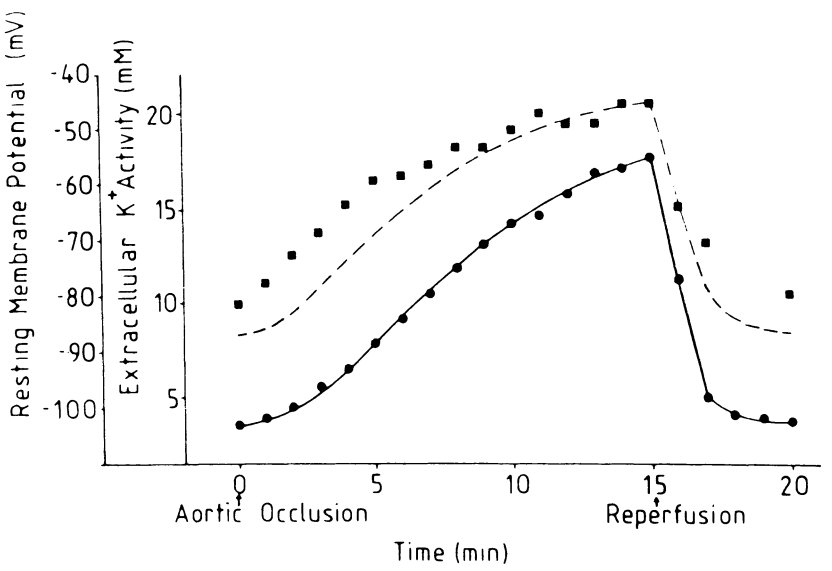

Figure 4-6. Changes in extracellular K^+ activity (unconnected solid squares) and resting membrane potential (connected solid circles) recorded in the perfused isolated guinea pig heart made globally ischemic by aortic occlusion. The broken line is the resting membrane potential calculated from the Nernst equation using the observed values of extracellular K^+ and constant intracellular K^+. Reprinted from Kleber [11], by permission.

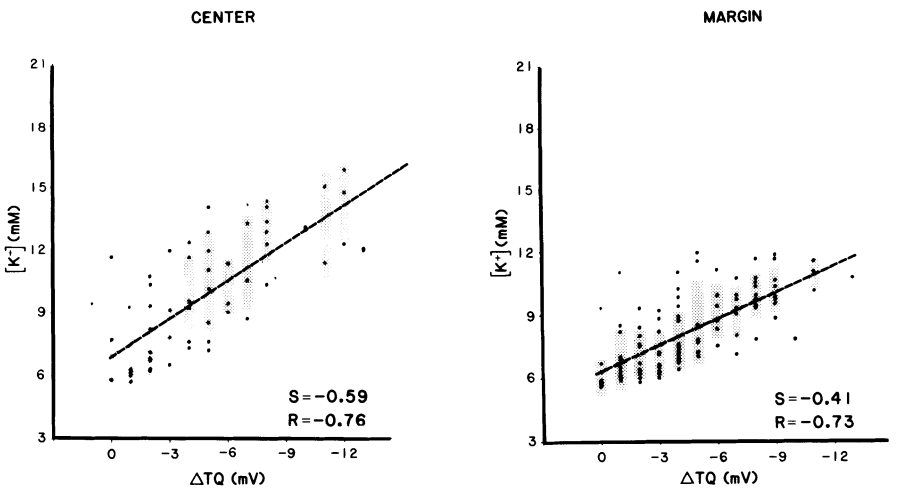

Figure 4-7. K^+-TQ relationships in the center (left panel) and margin (right panel) of the ischemic zone during a ten minute occlusion of the anterior descending coronary artery in the isolated, Langendorff perfused pig heart. Note that for any level of K^+ change in each area, multiple TQ potential shifts are recorded and vice versa. S = the slope of the line defining the overall K-TQ relationship, R = correlation coefficient. Reprinted from Johnson et al. [35], by permission.

The decrease in intracellular pH and the increase in intracellular calcium cause cell-to-cell uncoupling [36, 37]. This factor and the decrease in $\dot{V}_{max}$ of the action potential upstroke are major causes of the slowing and fragmentation of conduction that characterize acute ischemia.

FACTORS THAT INFLUENCE THE IONIC CHANGES

Relatively few studies address the effects of physiologic and pharmacologic interventions on the ionic events reviewed earlier. Studies on the effects of stimulation frequency are limited to changes in extracellular K^+. These studies have shown that in the in situ, regionally ischemic porcine heart, or the isolated, globally ischemic guinea pig heart, increases in rates between 120 and 180 beats/min do not influence the rate or magnitude of the extracellular K^+ accumulation [11, 14]. However, in the isolated perfused rabbit septum, changes in stimulation rate over a wider range do influence the change in extracellular K^+ [17, 38, 39]. At slow rates, the rate and magnitude of the K^+ rise is less than at rapid rates. The administration of acetylstrophanthidin in this preparation augmented the increase the extracellular K^+, presumably by blocking Na^+/K^+ ATPase activity [17], whereas lowering extracellular calcium retarded the rate and magnitude of the extracellular K^+ change in association with a decrease in contractility [17].

It has been recently reported [7] that preventing the rise in extracellular pCO_2 and the fall in extracellular pH retards the rate at which extracellular K^+ rises and with it the changes in resting membrane potential [7].

The calcium channel blocking agent, verapamil, significantly retards the rate and magnitude of the increase in extracellular K^+ and the fall in extracellular pH in the pig [40] (figure 4-8). This effect, which is associated with a lessening of the changes in propagation [40], may be due to the preservation of high energy phosphates and a lessened degree of intracellular acidosis [7]. The beta-adrenergic blocking agent, propranolol, has also been shown to retard the fall in intracellular pH [6] and to slow the rate at which extracellular K^+ increases [15, 41]. However, unlike verapamil, propranolol aggravates the changes in propagation [41].

Ongoing studies in our laboratories suggest that verapamil not only influences the rate and magnitude of the extracellular K^+ and pH changes, but also significantly lessens the inhomogeneities of these changes [40] and slows the rate at which cellular uncoupling occurs. [37]. It is likely that the combination of these effects explains the preservation of impulse propagation [40, 42] and the prevention of ventricular fibrillation [43] that occurs when the calcium channel blocking agents are administered before coronary occlusion in experimental animals.

When administered in the setting of the gradual reduction of coronary flow, verapamil shifts the ischemic threshold to lower levels of coronary flow even when heart rate is maintained constant [44]. Propranolol, however, does not alter the ischemic threshold under these conditions.

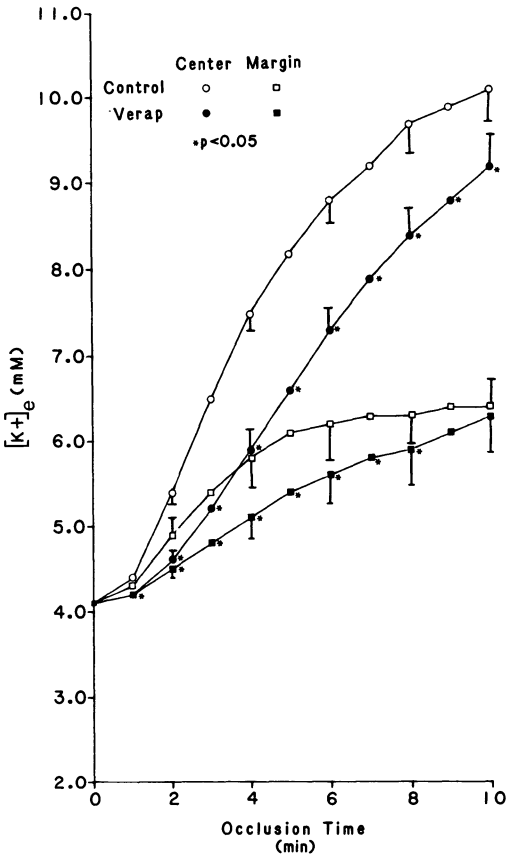

Figure 4-8. Changes in midmyocardial K^+ in the center (circles) and margin (squares) of the ischemic zone following ligation of the left anterior descending coronary artery in the open-chested, anesthetized pig. The white symbols were recorded before, and the black symbols after, the administration of verapamil before the occlusion. Reprinted from Fleet et al. [40], by permission.

These studies, when taken together, indicate that it is possible to modify the ionic changes that characterize acute ischemia and that these effects are reflected by changes in the associated electrical events.

UNRESOLVED QUESTIONS

Many issues relative to the intracellular and extracellular ionic changes that characterize acute ischemia are unsettled. A few of these issues follow:

1. The factors responsible for the rise in intracellular calcium before cell death are still largely speculative. Indeed, the effect of acute ischemia on intracellular Na^+ and K^+ remains controversial and requires further study.

2. The loss in intracellular K^+, although believed to be passive, may also be due to other, as yet unproven, causes.
3. The mechanism responsible for the plateau phase of the extracellular K^+ rise is obscure.
4. The differences in the characteristics of the changes in extracellular K^+ and pH in the margin and center (i.e., the macro inhomogeneities) may be explained by the admixture of different populations of cells, by diffusion of K^+ and pH into areas having more normal values and by the spread of ischemia from endocardium to epicardium. However, there is no obvious reason for the differences in extracellular K^+ and pH observed in regions thought to be equally deprived of arterial flow (i.e., the micro inhomogeneities). Nor is it at all obvious why the relationships between the locally recorded K^+ changes and the T–Q potential shifts should vary to the extent reported.
5. Most of the electrical events that occur during the early phase of acute ischemia can be attributed to changes in active and passive properties of the cell membrane induced by the biochemical and ionic events received above. In addition, recent studies suggest that changes in the electrical characteristics of the extracellular space also contribute to these events [36]. These effects require further definition.
6. A detailed description of the location, magnitude, and effects of the injury currents generated by the macro and micro inhomogeneities in K^+ and pH is lacking. This information may prove important in the understanding of the electrical events that ultimately result in ventricular tachycardia and fibrillation.
7. Our understanding of the effects of physiologic and pharmacologic interventions on the intracellular and extracellular ionic events and on their electrical and mechanical sequellae is incomplete. Such information has important clinical implications.

In conclusion, the studies of the last ten years have greatly expanded our appreciation of the characteristics, causes, and effects of the ionic events that define the ischemic process. However, many important questions remain to be answered before our understanding of these events is complete.

REFERENCES

1. Harris, A.S., Bisteni, A., Russel, R.A. et al. 1954. Excitatory factors in ventricular tachycardia resulting from myocardial ischemia: potassium a major excitant. *Science* 119:200.
2. Harris, A.S. 1966. Potassium and experimental coronary occlusion. *Am. Heart J.* 71:797.
3. Benzing, H., Gerbert, G., and Strohm, M. 1972. Extracellular acidbase changes in the dog myocardium during hypoxia and local ischemia, measured by means of glass microelectrodes. *Cardiology* 56:85–88.
4. Case, R.B., Felix, A., and Castellana, F.S. 1979. Rate of rise of myocardial pCO_2 during early myocardial ischemia in the dog. *Circ. Res.* 45:324–330.
5. Garlick P.B., Radda, G.K., and Seely, P.J. 1979. Studies of acidosis in the ischemic heart by phosphorous nuclear magnetic resonance. *Biophys. J.* 184:547–554.

6. Pieper, G.M., Todd, G.L., Wu, S.T. et al. 1980. Attenuation of myocardial acidosis by propranolol during ischaemic arrest and reperfusion: evidence with 31p nuclear magnetic resonance. *Cardiovasc. Res.* 14:646–653.
7. Lange, R., Ingwall, J., Hale, S.L., et al. 1984. Preservation of high-energy phosphates by verapamil in reperfused myocardium. *Circulation* 70:734–741.
8. Nakaya, H., Kimura, S., and Kanno, M. 1985, Intracellular k+ and na+ activities under hypoxia, acidosis, and no glucose in dog hearts. *Am. J. Physiol.* 249:1078–1085.
9. Kléber, A.G., Riegger, C.B., and Janse, M.J. 1987. Extracellular K+ and h+ shifts in early ischemia: mechanisms and relation to changes in impulse propagation. *J. Mol. Cell Cardio.* 19:Suppl. V 35–44.
10. Wilde, A.A.M., and Kleber, A.G. 1986. The combined effects of hypoxia, high k+, and acidosis on the intracellular sodium activity and resting potential in guinea pig papillary muscle. *Circ. Res.* 58:249–256.
11. Kleber, A.G. 1983. Resting membrane potential, extracellular potassium activity and intracellular sodium activity during acute global ischemia in isolated perfused guinea pig hearts. *Circ. Res.* 52:442–450.
12. Tani, M., and Neely, J.R. 1987. Roles of h+ and na+/h+ exchange in ca++ uptake and recovery of function in ischemic rat heart. *Circulation* 76:Suppl. IV, iv–56.
13. Steenbergen, C., Murphy, E.A., Levy, L., and London, R.E. 1987. Elevation in cytosolic free calcium concentration early in myocardial ischemia in perfused rat heart. *Circ. Res.* 60:700–706.
14. Hill, J.L., and Gettes, L.S. 1980. Effect of acute coronary artery occlusion on local myocardial extracellular k+ activity in swine. *Circulation* 61:768–778.
15. Wiegand, V, Guggi, M, Meesmann, W. et al. 1979. Extracellular potassium activity change in the canine myocardium after acute coronary occlusion and the influence of beta-blockade. *Cardiovasc. Res.* 13:297–302.
16. Hirche, J.H., Franz, C.H.R., and Schramm, M. 1980. Myocardial extracellular K+ and H+ increase and noradrenaline release as possible cause of early arrhythmias following acute coronary artery occlusion in pigs. *J. Mol. Cell Cardiol.* 12:579–593.
17. Weiss, J. and Shine, K.I. 1982. Extracellular k+ accumulation during myocardial ischemia in isolated rabbit heart. *Am. J. Physiol.* 242:H619–H628.
18. Fleet, W.F., Johnson, T.A., Graebner, C.A., and Gettes, L.S. 1985 Effect of serial brief ischemic episodes on extracellular k+, pH, and activation in the pig. *Circulation* 72:922–932.
19. Coronel, R., Fiolet, J.W.T., Wilms-Schopman, F.J.G., et al. 1988. Distribution of extracellular potassium and its relation to electrophysiologic changes during acute myocardial ischemia in the isolated perfused porcine heart. *Circulation* 77:1125–1138.
20. Reimer, K.A., and Jennings, R.B. 1979. The "wavefront phenomenon" of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. *Lab. Invest.* 40:633–644.
21. Watanabe, I., Johnson, TA, Buchanan, J, et al. 1987. Effect of graded coronary flow reduction on ionic, electrical, and mechanical indexes of ischemic in the pig. *Circulation* 76:1127–1134.
22. Fedor, J.M., McIntosh, D.M., Rembert, J.C., and Greenfield, J.C.Jr. 1978. Coronary and transmural myocardial blood flow responses in awake domestic pigs. *Am. J. Physiol.* 235:H435–H444.
23. Reimer, K.A., Hill, M.L., and Jennings, R.B. 1981. Prolonged depletion of atp and of the adenine nucleotide pool due to delayed resynthesis of adenine nucleotide pool due to delayed resynthesis of adenine nucleotides following reversible myocardial ischemic injury in dogs. *J. Mol. Cell Cardiol.* 13:229–239.
24. Case, R.B., Nasser, M.G., and Crampton, R.L. 1969. Biochemical aspects of early myocardial ischemia. *Am. J. Cardiol.* 24:776–781.
25. Hess, M.L., Krause, S., and Kontos, H.A. 1983. Mediation of sarcoplasmic reticulum disruption in the ischemic myocardium: proposed mechanism by the interaction of hydrogen ions and oxygen free radicals. *Adv. Exp. Med. Biol.* 161:377–389.
26. Gettes, L.S. 1986. Effect of ischemia on cardiac electrophysiology. In *The Heart and Cardiovascular System,* H.A. Fozzard, E. Haber, R.B. Jennings, et al. eds., New York, Raven Press, 1317–1342.
27. Kagiyama, Y., Hill, J.L., and Gettes, L.S. 1982. Interaction of acidosis and increased

extracellular potassium on action potential characteristics and conduction in guinea pig ventricular muscle. *Circ. Res.* 51:614–623.
28. Kodama, I., Wilde, A., Janse, M.J., et al. 1984. Combined effects of hypoxia, hyperkalemia and acidosis on membrane action potential and excitability of guinea-pig ventricular muscle. *J. Mol. Cell Cardiol.* 16:247–259.
29. Gettes, L.S., and Reuter, H. 1974. Slow recovery from inactivation of inward currents in mammalian myocardial fibres. *J. Physiol.* 240:703–724.
30. Trautwein, W., Gottstein, U., and Dudel, J. 1954. Der aktionsstrom der myokardfaser im sauerstoffmangel. *Pflugers Arch.* 260:40–60.
31. Carmeliet, E.E. 1984. Myocardial ischemia: reversible and irreversible changes. *Circulation* 70:149–151.
32. Gettes, L.S., and Saito, T. 1980. Effect of antiarrhythmic drugs on the slow inward current system. In *The Slow Inward Current and Cardiac Arrhythmias,* D.P. Zipes, J.C. Bailey, and V. Elharrar eds. The Hague, Martinus Nijhoff Publishers, 455–477.
33. Kléber, A.G., Janse, M.J., van Capelle, F.J.L., and Durrer, D. 1973. Mechanism and time course of s-t and t-q segment changes during acute ischemia in the pig heart determined by extracellular and intracellular recording. *Circ. Res.* 42:603–613.
34. Trautwein, W., and Kassenbaum, D.G. 1961. On the mechanism of spontaneous impulse generation in the pacemaker of the heart. *J. Gen. Physiol.* 45:317–330.
35. Johnson, T.A., Coronel, R., Graebner, C.A., et al. 1987. Relationship between extracellular potassium accumulation and local tq-segment potential during acute myocardial ischemia in the porcine. *J. Mol. Cell Cardio.* 19:949–952.
36. Kléber, A.G., Riegger, C.B., and Janse, M.J. 1987. Electrical uncoupling and increase of extracellular resistance after induction of ischemia in isolated, arterially perfused rabbit papillary muscle. *Circ. Res.* 61:271–279.
37. Fujino, T., Buchanan, J.W. Jr., and Gettes, L.S. 1985. Effects of verapamil on the changes in conduction time, $\dot{V}$max and longitudinal resistance induced by simulated ischemia (abstr.). *Circulation* 72:III-, 1985.
38. Weiss, J., and Shine, K.I. 1982. [K+] accumulation and electrophysiological alterations during early myocardial ischemia. *Am. J. Physiol.* 243:H318–H327.
39. Weiss, J., and Shine, K.L. 1986. Effects of heart rate on extracellular [K+] accumulation during myocardial ischemia. *Am. J. Physiol.* 250:H982–H991.
40. Fleet, W.F., Johnson, T.A., Graebner, B.A., et al. 1986. Effects of verapamil on ischemia-induced changes in extracellular K+, pH, and local activation in the pig. *Circulation* 73:837–846.
41. Gettes, L.S., Symanski, J.D., Fleet, W.F., et al. 1986. The intracellular and extracellular changes associated with ischemia-effects of catecholamines in arrhythmogenesis. *Eur. Heart J.* (Suppl. A):77–84.
42. Elharrar, V., Guam, W.E., and Zipes D.P. 1977. Effect of drugs on conduction delay and incidence of ventricular arrhythmias induced by acute coronary occlusion in dogs. *Am. J. Cardiol.* 39:544–549.
43. Kaumann, A.J., and Aramendia, P. 1968. Prevention of ventricular fibrillation induced by coronary ligation. *J. Pharmacol. Exp. Ther.* 164:326–332.
44. Watanabe, I., Johnson, T.A., Engle, C.L., et al. submitted for publication, 1988. Effects of verapamil and propranolol on changes in extracellular K+, pH, and local activation during graded coronary flow in the pig.

5. MECHANISMS OF REPERFUSION ARRHYTHMIAS

HAROLD C. STRAUSS, RAYMOND YEE, JOSEPH A. HILL, JR.
AND THOMAS L. WENGER

Sudden release of a coronary artery occlusion is known to be a potent arrhythmogenic stimulus, often leading to ventricular fibrillation [1–4]. Although this phenomenon has been the subject of intense laboratory investigation, assessment of its clinical relevance has been difficult. The majority of patients with acute myocardial infarction undergoing coronary thrombolysis and/or angioplasty, or even those patients with Prinzmetal's angina have not experienced significant reperfusion arrhythmias. This is not surprising as experimental data derived from dog studies suggest that the prevalence of reperfusion arrhythmias is related to a range of values for different variables that, if not satisfied, would minimize the expression of such arrhythmias. Practical considerations may reduce the risk for certain factors, such as duration of occlusion, myocardium at risk, and magnitude of reflow during reperfusion, resulting in a nominal risk for most patients [1–5]. Nonetheless, as patient access to thrombolytic therapy is accelerated, the duration of occlusion may be shortened, and expression of these arrhythmias may actually increase. More important, interaction among variables may occur, thereby altering the relationship between prevalence of arrhythmia and the value of a particular variable, so that expression of this arrhythmia may increase. Further, elements of this model may have direct bearing on lethal arrhythmias occurring in patients during other phases of the ischemic heart disease syndrome. This chapter will address recent advances in the field of reperfusion

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arrhythmias, including our own studies on Purkinje fibers and single ion channels, and suggest future directions for research.

The canine reperfusion-ventricular fibrillation model has been characterized in detail and has been used to assess the effectiveness of many antiarrhythmic drugs in the prophylaxis of sudden cardiac death [6–10]. Examination of factors such as duration of occlusion before release and amount of myocardium at risk point toward the amount of ischemic, but not yet infarcted, myocardium as an important determinant of outcome. Specifically, reperfusion-induced ventricular fibrillation is most likely to occur when the duration of previous ligation is 20 to 30 minutes [11]. The prevalence of ventricular fibrillation, which correlates significantly with the amount of myocardium at risk, is uniformly low and high with respectively small and large amounts of myocardium at risk; and a direct correlation for midrange values of myocardium at risk is observed [5]. Further, although other variables, such as heart rate and reperfusion blood flow, also influence outcome [4, 12], elucidation of their role in this model has not established the underlying electrophysiologic mechanism(s) or the site of origin of these malignant arrhythmias in the dog. Other studies have demonstrated that a myriad of metabolic changes occur in this setting. For example, studies performed in a feline model of ischemia and reperfusion have demonstrated a major electrophysiologic role of α-adrenergic stimulation during both coronary occlusion and reperfusion [7, 13, 14]. These findings have been linked to a change in density of α-adrenergic receptors in ischemic myocardium and to potent antiarrhythmic and antifibrillatory effects of α_1-adrenergic blockade during both ischemia and reperfusion [13–15].

In vivo electrophysiologic studies have provided important information concerning electrophysiologic consequences of ischemia and reperfusion. However, our understanding of the electrophysiologic perturbations resulting from these pathologic states is limited by the techniques applicable to in vivo study. For example, measurement of electrograms using extracellular recording techniques, including monophasic action potentials, does not provide the same detailed cellular information as that provided by intracellular transmembrane action potential recordings. Measurements of total ionic content do not accurately reflect changes in the thermodynamically active fraction of intracellular ions, since redistribution of ions between subcellular compartments and buffers may occur. Use of magnetic resonance imaging to measure intracellular cations, such as hydrogen, sodium, and calcium, is limited due to the need to average the weak signal from many pulses. To overcome some of these problems, investigators have developed a variety of in vitro models of ischemia and reperfusion in globally perfused hearts, superfused preparations of papillary muscle, or Purkinje fiber [16–19].

Ferrier et al. [17, 18] developed an in vitro model of ischemia and reperfusion in a preparation that includes both ventricular muscle and Purkinje tissues. Tissues are exposed to conditions simulating ischemia (hypoxia, acidosis, elevated lactate, and zero metabolic substrate for 40 minutes) and

then superfusion with normal Tyrode's solution reinstituted. Exposure to ischemic solutions resulted in depressed excitability and progressive conduction block between muscle and Purkinje tissue. Return to normal Tyrode's solution resulted in a sequence of responses in the Purkinje fibers: prompt hyperpolarization, progressive depolarization to unresponsiveness, and final repolarization to control values. The depolarization phase was accompanied by oscillatory afterpotentials that initiated extrasystoles. The appearance of oscillatory afterpotentials during the reperfusion phase was accompanied by aftercontractions.

To determine whether reperfusion-induced arrhythmias might also arise from ventricular myocardium, Hayashi et al. [19] recently exposed isolated guinea pig papillary muscles to hypoxia (30 to 60 minutes) followed by reoxygenation (30 minutes). Arrhythmic activity following reinstitution of oxygenated superfusion was characterized by aftercontractions and delayed afterdepolarizations that persisted for as long as 15 minutes. The peak of delayed afterdepolarization preceded the peak of aftercontraction by 40 to 100 ms. Arrhythmic activity required sufficiently severe hypoxic conditioning, as well as electrical stimulation during the reoxygenation phase, and was correlated with the degree of rise in resting tension that occurred during the hypoxic phase.

Although the arrhythmic manifestations were not identical in these two in vitro models, the similarities are remarkable despite the differences in composition of superfusate, tissue, and animal species. Further, the temporal sequence of changes in these models provides a reasonable first approximation of the changes underlying the malignant, reperfusion-induced arrhythmias in the in vivo dog heart model. Validation of these models will require further experimentation.

We have studied the Ferrier model [20], because the electrophysiologic changes observed appear to be more consistent with ischemia/reperfusion arrhythmias than any other model. Further, because Ferrier's experiments pointed toward the Purkinje fiber as the source of the reperfusion-induced arrhythmias, ventricular myocardium was not included in our preparations. Although initially one might attribute the cyclic changes in membrane potential seen in the Ferrier model to disturbances in K-channel permeability and/or Na-K pump activity, it is likely that disturbances of other ionic permeation pathways, as well as other membrane transporters, also are important. As a necessary first step in identifying the potential scope of the abnormalities, we set out to characterize the changes in intracellular and extracellular cation (Na^+, H^+, K^+) activity in this ischemia/reperfusion model and to correlate them with the electrophysiologic and mechanical changes observed.

Another approach to this problem has been adopted in our laboratory. The introduction of single channel recording techniques [21] has provided the investigator of cardiac arrhythmias with new methods of approach to electrophysiological problems. Answers to questions obtained from measure-

Table 5-1. Composition of solutions used to superfuse Purkinje fibers

	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	$H_2PO_4^-$	Glucose	Lactate	O_2	N_2	CO_2	pH*
Solution I (control)	149.8	4	2.5	0.5	138	20	0.8	5		95%		5%	7.38–7.42
Solution II (combined hypoxia, lactic acidosis)	149.8	4	2.5	0.5	132	6	0.8		20		95%	5%	6.79–6.82

* Data expressed as range of values.

ments of macroscopic currents are sometimes limited due to the complications resulting from study of multicellular syncytial cardiac preparations with overlap of different ionic currents. Thus, single channel studies can be particularly illuminating by resolving questions about ion channel permeation properties that can only be inferred from macroscopic current studies. For these reasons, we set out to study ion channels from ventricular myocardium and will describe our data from a calcium-activated cation channel. We argue herein that this channel may have direct bearing on the electrophysiologic changes seem in the ischemia/reperfusion model.

METHODS

Purkinje fibers were dissected from both left and right ventricles and studied using both conventional and ion selective microelectrode techniques. Tension was monitored in most experiments. The composition of the solutions used in these experiments is described in Table 5-1. After equilibration in solution I, the preparations were exposed to ischemia (solution II), followed by reperfusion (solution I) for up to 60 minutes. Only those fibers exhibiting a depolarization of maximum diastolic potential to less than −70 mV and loss of excitability upon reperfusion were included in subsequent analysis, and, as a result, data from 25 percent of the fibers were eliminated. Analysis of the variables measured did not allow us to establish why the electrophysiologic changes were not uniformly seen.

The experimental model used in these studies was designed to simulate some of the known effects of myocardial ischemia. It is clear, however, that the conditions imposed in these experiments differ from ischemia in several important respects. Superfusion of isolated tissue with modified electrolyte solutions containing reduced pO_2 results in washout of many metabolites that would accumulate in a closed system, such as amphiphiles, free radicals, purine nucleotides, and extracellular K^+. To partially address this problem, Ferrier et al. [17] examined the effects of elevating potassium concentration in the superfusate during ischemic conditions and determined that the responses of the tissues to this modified protocol were similar to those in which potassium was not elevated. Second, the Purkinje fibers were not exposed to circulating catecholamines and free fatty acids. Third, in myo-

cardial ischemia, Purkinje fibers are exposed to an unknown ionic environment as they continue to be bathed in intracavitary blood, despite the interruption of their perfusion following occlusion. Resolution of this question is important if the mechanisms of reperfusion-induced arrhythmias are to be established. Fourth, the imposition of extracellular acidosis generates a steep, inwardly directed proton gradient, which is directionally opposite to the outwardly directed proton gradient that occurs during ischemia and perhaps is much more severe than could be experienced *in vivo*. Nevertheless, the similarities between the electrophysiologic derangements reported in *in vitro* study and *in vivo* ischemia/reperfusion experiments suggest that important insights into ischemic dysfunction and reperfusion abnormalities can be obtained by studying this model.

To implement the single channel studies, the planar lipid bilayer technique was used [22]. Lipid bilayers were prepared by painting a 1:1 (w/w) phospholipid mixture of phosphatidylethanolamine and phosphatidylserine dissolved in decane across a 300 μm diameter hole separating two chambers (*cis, trans*). Ventricular membrane vesicles were incorporated into the artificial membrane by adding them to the *cis* chamber under conditions promoting fusion. The *cis* chamber was connected to a voltage source, and the *trans* chamber was held at virtual ground by a current-to-voltage converter circuit. Bath calcium activity was calculated in Ca-EGTA solutions using an effective stability constant of $4.31 \times 10^6\ M^{-1}$ (0.1 M ionic strength, pH = 7.1, 25°C).

RESULTS

Transmembrane Potential Changes

Under control conditions, the mean maximum diastolic potential (E_{MDP}) in 19 fibers was -92.6 ± 2.4 mV (figure 5-1). The temporal course of the changes in maximum diastolic potential in response to exposure to the combined hypoxia-lactic acidosis and glucose depletion (solution II) followed by return to control solution (solution I) is illustrated in figure 5-1B. Superfusion with solution II led to an initial small but significant reduction in E_{MDP} and, thereafter, E_{MDP} changed little, so that at the end of 40 minutes a value of -86.0 ± 4.0 mV was recorded. Fibers were then re-exposed to solution I and E_{MDP} rapidly hyperpolarized to -90.0 ± 4.7 mV at 2 minutes. The time to the peak of hyperpolarization (mean = 7.6 ± 2.3 mV) ranged from 1 to 3 minutes. The period of hyperpolarization was then followed by rapid depolarization, which began within 5 minutes of return to solution I. At 10 minutes, E_{MDP} had depolarized to -47.0 ± 7.5mV ($P<.001$), and the tissue became inexcitable; thereafter, gradual recovery occurred. Early in the course of reexposure to solution I, small amplitude oscillatory potentials were seen during diastole; their amplitude progressively decreased during the 60 minute recovery period. At no time did these potentials lead to triggered activity, in contrast to the findings of Ferrier et al. [17] and Hayashi et al. [19]. This

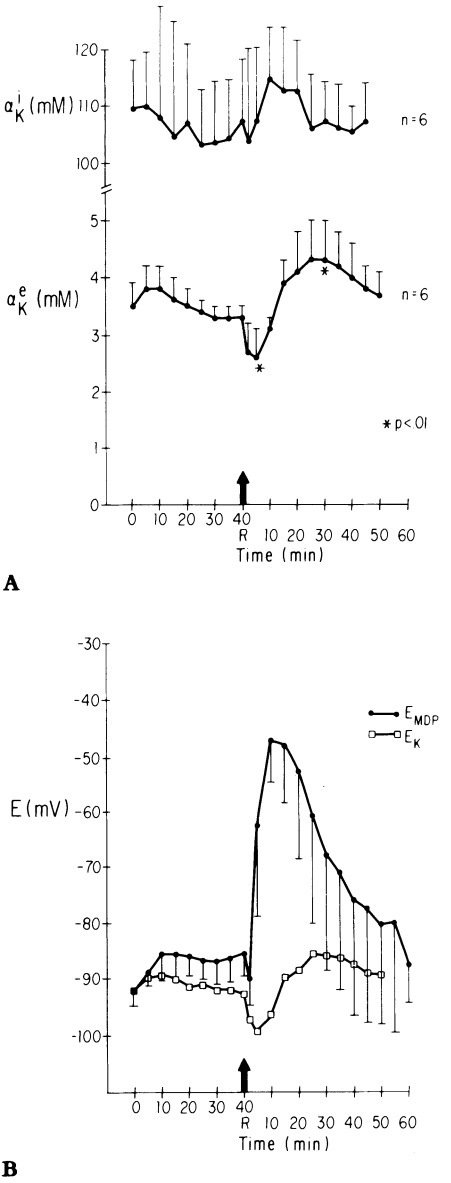

Figure 5-1. Time course of intracellular (α^i_K) and extracellular (α^e_K) potassium activity and maximum diastolic potential (E_{MDP}) obtained from separate sets of experiments during and following 40 minute exposure to a combination of hypoxia, glucose depletion, and lactic acidosis (solution II). Panel A shows activity measurements, and panel B compares maximum diastolic potential (E_{MDP}) with the potassium equilibrium potential (E_K) from the values shown in panel A. Data points at five-minute intervals represent mean ± standard deviation. Arrow in this and subsequent figures indicates return to control Tyrode's solution (solution I). Asterisk in this and subsequent figures denotes values significantly different from control values. From Yee et al. [20] reproduced by permission of the Rockefeller University Press.

difference may reflect the disparities in stimulation protocol employed in the two studies.

Intracellular and Extracellular K^+ Activity, E_K

Intracellular K^+ activity was 109.5 ± 8.7 mM (n = 6) under control conditions, and neither exposure to solution II nor return to control conditions was associated with any significant change in α^i_K (figure 5-1A). No significant change in extracellular K^+ activity during exposure to solution II was seen (figure 1A). Immediately following return to control Tyrode's solution, a small but significant transient fall in α^e_K was observed (0.7 ± 0.5 mM, 5 min, $P < 0.03$).

Because it was not possible to measure α^i_K and α^e_K simultaneously in the same preparations, E_K was calculated using the mean values at each time interval (figure 5-1B). The transient increase in E_K seen following return to solution I reflects the change in α^e_K. However, the depolarization phase was clearly not attributable to a change in α^e_K, as $E_{MDP} - E_K$ increased to 48.9 mV at 10 minutes.

Intracellular Na Activity

The temporal course of the α^i_{Na} values during sequential exposure to solutions I and II is illustrated in figure 5-2. The mean control α^i_{Na} in 10 preparations was 8.7 ± 1.3 mM (figure 5-2). Superfusion with solution II caused a slow and modest rise in α^i_{Na} to a maximum value of 10.9 ± 1.9 mM ($P < .01$) at the end of this period. On return to control conditions, α^i_{Na} rapidly declined, reaching a minimum value of 5.1 ± 2.2 mM ($P < .01$) at 10 minutes. To ascertain the role of the Na-K pump in the changes in α^i_{Na} recorded, acetylstrophanthidin ($4 - 5 \times 10^{-7}$M) was added to solution II during the final 10 minutes of exposure in some experiments, causing a rise in α^i_{Na} to 19.9 ± 3.8 mM (data not shown). Following five minutes of re-exposure to solution I (without acetylstrophanthidin), α^i_{Na} was unchanged and gradually fell to control values at 25 minutes.

Intracellular pH

A striking change in pH_i occurred in these experiments (figure 5-3). The pH_i in eight preparations superfused with Tyrode's solution averaged 7.06 ± 0.05. Exposure to solution II caused a rapid and steady decline in pH_i, so that at the end of 40 minutes pH_i averaged 6.49 ± 0.04 ($P < .001$). Return to solution I resulted in a rapid recovery and ultimately an alkaline overshoot in pH_i.

Resting and Twitch Tension

Because of the experimental system used, absolute values of resting tension (RT) are difficult to quantify accurately. We therefore analyzed the relative change in resting tension corrected for cross-sectional area (RT_c). Control

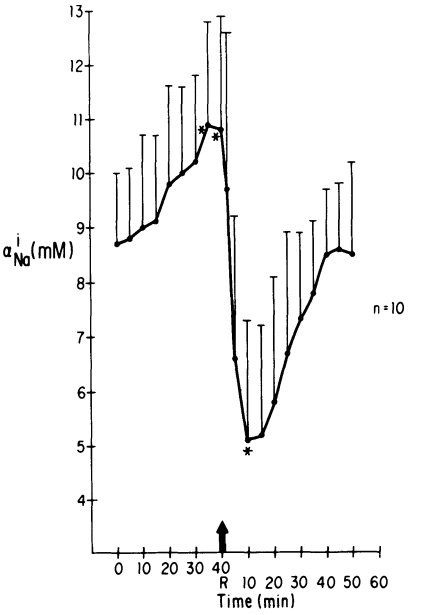

Figure 5-2. Time course of intracellular sodium activity (α^{i}Na) during the 40 minute exposure to Solution II and following return to control Tyrode solution in ten preparations. From Yee et al. [20] reproduced by permission of the Rockefeller University Press.

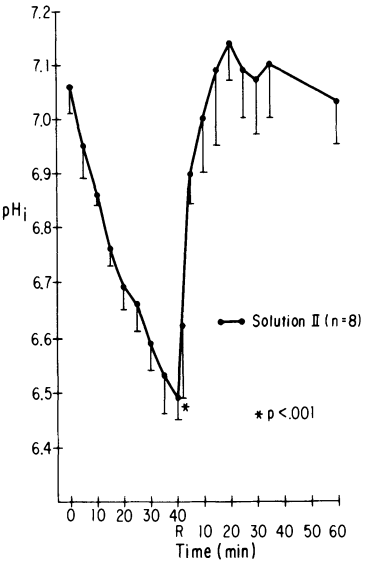

Figure 5-3. Time course of intracellular pH (pH_i) during 40 minutes exposure to combination of ischemia, hypoxia, glucose depletion, and lactic acidosis (solution II, n = 8) and following return to control Tyrode's solution. From Yee et al. [20] reproduced by permission of the Rockefeller University Press.

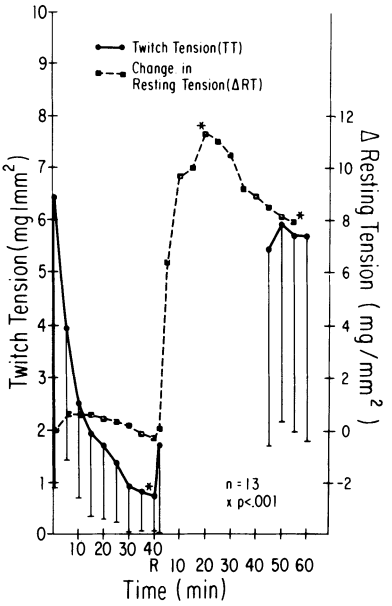

Figure 5-4. Time course of twitch tension and change in resting tension corrected for cross-sectional fiber area during the 40 minute exposure to solution II and following return to solution I (n = 13). From Yee et al. [20] reproduced by permission of the Rockefeller University Press.

values before exposure to combined hypoxia-lactic acidosis were designated as zero. Upon initial exposure to solution II, no significant change in RT_c occurred (figure 5-4). Upon return to control Tyrode's solution, there was a marked rise in RT_c to a peak of 11.3 ± 9.9 mg/mm^2 ($n = 13$, $P < .001$). Thereafter, RT_c slowly declined but remained elevated even after 50 minutes. Early in the recovery period, oscillations in resting tension were observed throughout diastole. These aftercontractions progressively decreased in amplitude throughout the reperfusion period.

In marked contrast to resting tension, twitch tension corrected for cross-sectional fiber area (TT_c) fell from a mean control value of 6.4 ± 5.5 to 0.77 ± 0.71 mg/mm^2 ($P < .001$) during the 40 minutes of superfusion with solution I. TT_c declined by 50 percent within ten minutes of combined hypoxia-lactic acidosis. Simultaneous measurement of pH_i and TT_c enabled us to establish a steep relationship between TT_c and pH_i; a 50% decline in TT_c occurred at a time when pH_i fell 0.20 units.

Properties of a Calcium Activated Nonselective Cation Channel

The representative continuous record of single channel current illustrated in figure 5-5 shows that gating in this channel results in bursts of activity separated by relatively long periods of quiescence. The open state (displayed

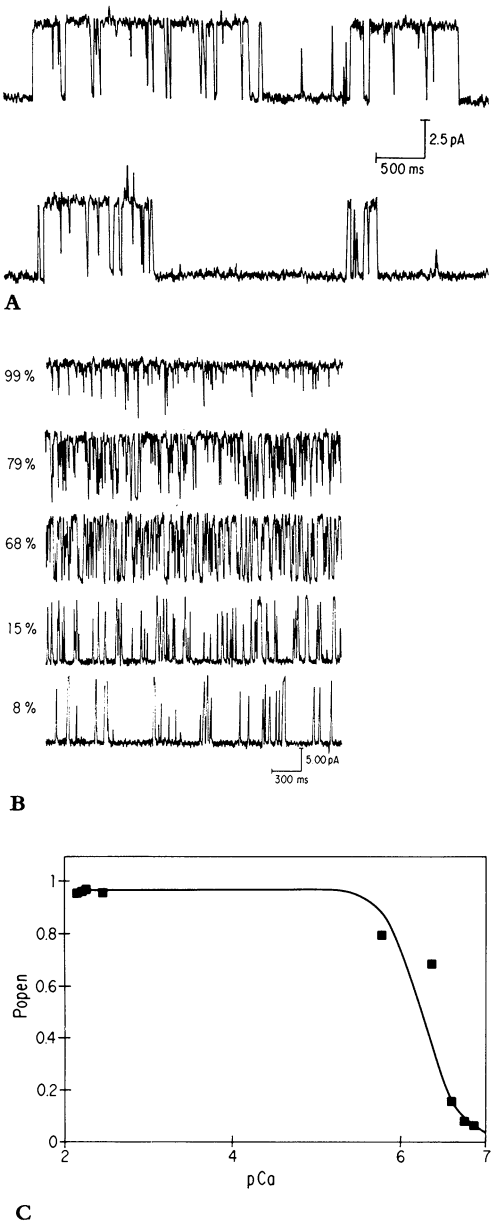

Figure 5-5. Analog recordings of single channel current and derived open probability. Panel A shows a continuous record obtained at a holding voltage = +10 mV, *cis* [KCl] = 73 mM, *trans* [KCl] = 50 mM, [Ca^{++}] < 100 nM. Channel opening is represented by an upward deflection. Panel B shows a series of recordings obtained under symmetrical [KOAc] = 100 mM and *cis* [Ca^{++}] is 10 mM, 1.6 μM, 0.4 μM, 0.25 μM and 0.20 μM. Trans [Ca^{++}] < 100 nM. All recordings were made at −40 mV holding potential and channel openings are represented as upward deflections. Open probability is as listed to the left of each record. Panel C shows open probability plotted as a function of −log ([Ca^{++}]). From Hill et al. [22] reproduced by permission of the American Heart Association.

up) transiently closes repeatedly. Single channel open probability (cumulative open time divided by the sum of cumulative open and closed times) is modulated by *cis* calcium activity (figrue 5-5B). Representative recordings selected from data points depicted in panel C are shown in panel B. Open probability ranges from 8 to 99% as Ca^{2+} in the *cis* chamber increased from 0.2 μM to 10 mM and decreased following addition of ethylene glycol-bis (β-amino-ethylether) N, N, N′, N′-tetracidic acid (EGTA). Open probability varied between approximately unity and zero over the pCa range of 6 to 7 (figure 5-5C). This observation fits well with the experimentally observed values of intracellular calcium in ventricular myocytes under physiologic conditions (approximately 100 nM diastolic Ca^{2+}) and in states of calcium overload (1 μM and upward) [23–26].

Channel gating was also strongly voltage dependent [22]. Open probability varied from approximately zero to one over the voltage range −60 to 0 mV under these conditions. Single channel current was ohmic over the voltage range −80 to +20 mV. In the presence of different KCl activity gradients across the bilayer, the interpolated reversal potential agreed within 3 mV with the Nernst potential for a K^+ electrode (figure 5-6A). Thus, we conclude that the channel selects strongly for cations over anions. We have also measured the reversal potential (+2 mV) under biionic conditions for Na^+ and K^+ (100 mM) (figure 6B). Under these conditions, conductance was 120 picosiemens (pS). Using the Goldman-Hodgkin-Katz equation for biionic conditions to calculate relative ionic permeabilities, P_{Na}/P_K was found to approximate unity. This suggests that the channel conducts current that reverses near 0 mV under physiologic conditions. We interpret the conductance (120 pS) to represent a first approximation (neglecting temperature effects) of the channel's conductance in a physiologic environment. Although at present we are unable to quantify relative calcium permeability, we have estimated it to be small relative to potassium based on those experiments in which calcium concentration was varied in the *cis* chamber.

DISCUSSION

The measurement of intracellular and extracellular cation activities along with electrical and mechanical properties have provided insights into the electrophysiologic changes that occur in the Ferrier model of ischemia and reperfusion. The changes in E_{MDP}, pH_i, tension, α^i_{Na}, and α^e_K support the view that multiple ionic mechanisms underlie the electrophysiologic changes during the different phases of this mode.

Changes in Membrane Potential, α^i_K and α^e_K

Maximum diastolic potential (E_{MDP}) initially depolarized, reaching a plateau ten minutes after the onset of superfusion with solution II. This initial depolarization, which occurred before a marked change in pH_i, is believed to result from an effect of pH_o on outward K^+ current and/or inhibition

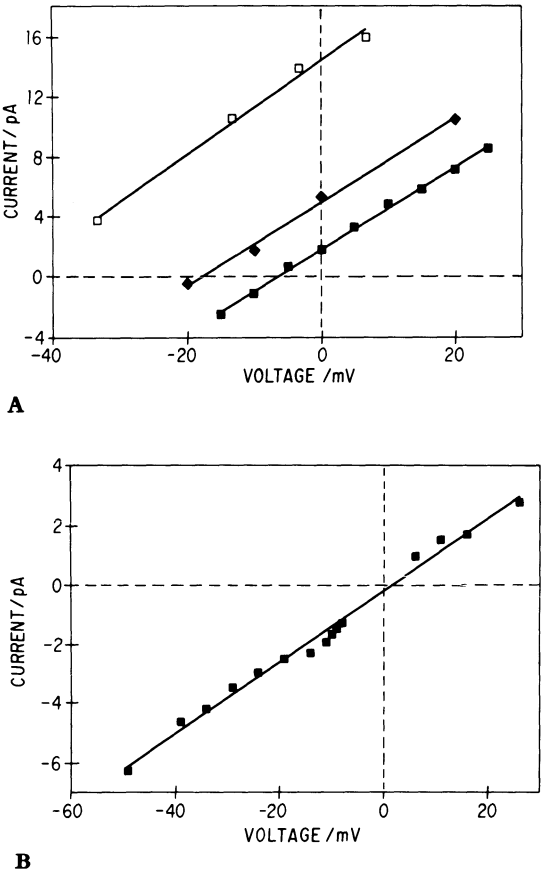

Figure 5-6. Steady state current voltage (I-V) relations. Panel A shows I-V curves recorded under the following conditions (*cis* [KCl]// *trans* [KCl]): 73//50 (filled squares), 96//50 (filled diamonds), 319//50 (empty squares). In each instance $[Ca^{++}] < 100$ nM. In Panel B biionic potential measurement was performed under the following conditions- *cis* [NaOAc]// *trans* [KOAc]:100//100 mM. From Hill et al. [22] reproduced by permission of the American Heart Association.

of the Na-K pump [27]. Our results suggest a small accumulation of K^+ in the extracellular space early during exposure to combined hypoxia-glucose depletion-lactic acidosis solution.

The changes in E_{MDP} after return to control perfusate are best considered in three phases. The initial hyperpolarization can be attributed to a decrease in α^e_K. The subsequent marked decrease in E_{MDP} cannot be attributed to a redistribution of K^+ across the sarcolemma, but may be due, instead, to an increase in inward current. Two possible mechanisms to be considered are an increase in permeability to another cation via a channel that mediates the

transient inward current or an increase in electrogenic Na-Ca exchange [22, 28, 29]. These mechanisms will be discussed in greater detail later. At the end of reperfusion, both E_{MDP} and E_{MDP}-E_K had returned toward control values. Resolution of the underlying mechanism is essential to the identification of an appropriate therapeutic stratagem.

Changes in α^i_{Na}

We have shown a 25 percent (2.2 mM) rise in α^i_{Na} during 40 minutes of exposure to hypoxia, lactic acidosis, and glucose depletion. Similar findings were reported in guinea pig papillary muscles subjected to hypoxia, respiratory acidosis, and glucose-free solutions, albeit with a faster rise in α^i_{Na} noted [30]. The rise in α^i_{Na} seen in our study could be the result of reduced active efflux through partial inhibition of Na-K ATPase activity or an increase in passive influx mediated by Na-Ca exchange, Na-H exchange, or a nonselective cation channel.

It is known that extracellular and intracellular acidosis and hypoxia can inhibit the Na pump. These effects may result from reduced free energy of ATP hydrolysis [31–33]. However, the failure of α^e_K to rise in conjunction with the rise in α^i_{Na} and the effect of acetylstrophanthidin in causing a further marked increase in α^i_{Na} immediately before the end of combined hypoxia-lactic acidosis, indicates that Na-K ATPase inhibition, if present at all, is far from complete. Our efforts to measure ATP and phosphorus metabolite concentrations in Purkinje fibers using magnetic resonance imaging were unsuccessful. Thus, we were unable to confirm if free energy changes in ATP hydrolysis occurred in our experiments.

The Na-Ca exchanger is unlikely to contribute to the rise in α^i_{Na}, as it is inhibited by a fall in pH_i [34]. Thus, in the absence of a significant increase in $[Ca^{2+}]_i$, it is difficult to attribute an increase in α^i_{Na} to the Na-Ca exchanger. As a reduction in pH_o has been shown to cause a fall in α^i_{Na} [34], the Na-H exchanger is unlikely to contribute to Na influx early during exposure to solution II. However, as pH_i continues to fall (figure 5-3), Na influx via the Na-H exchanger would be expected to increase and contribute to the rise in α^i_{Na}. Of considerable interest is the very rapid decline in α^i_{Na}, commencing almost immediately on reintroduction of oxygen and normal pH. This fall in α^i_{Na} begins simultaneously with the hyperpolarization of E_{MDP}, fall in α^e_K, and rapid increase in pH_i pointing toward stimulation of Na-K pump activity. Enhanced Na-K pump activity is likely due to the reestablishment of aerobic metabolism, reduction in inorganic phosphate (Pi) and rapid pH_i recovery in the presence of an elevated α^i_{Na}. We attempted to test this hypothesis by adding acetylstrophanthidin to the ischemic solution 10 minutes before reperfusion with control solution. Identification of the appropriate concentration to inhibit the Na pump, yet not irreversibly damage the fiber, proved difficult. Nonetheless, the concentration selected demonstrated

that the fall in α^{i}_{Na} during reperfusion was delayed. This observation in conjunction with the observed decline in α^{e}_{K} indicates that the early fall in α^{i}_{Na} during recovery was due, at least in part, to enhanced Na-K pump activity.

Although stimulation of the Na-K pump may contribute to the initial rapid fall in α^{i}_{Na}, it does not adequately explain the continued decline in α^{i}_{Na} below control values or the nadir of α^{i}_{Na} occurring 5 minutes after the corresponding nadir for α^{e}_{K}. Other likely contributing factors may include an increase in Na-Ca exchange and/or a decrease in Na influx. As the membrane begins its depolarization two to five minutes after recovery from the combination of hypoxia lactic acidosis and glucose depletion, the rise in pH_i would be expected to enhance the activity of the Na-Ca exchanger [35]. On the other hand, using our measurements of V_m and α^{i}_{Na} to calculate the electrochemical driving force (assuming a stoichiometry of 3Na:1Ca and equilibrium conditions) on the exchanger during the first ten minutes of recovery, a five-fold fall in α^{i}_{Ca}, and thus Na influx, is predicted. However, the absence of continuous measurements of α^{i}_{Ca} and of the relative fluxes of Na and Ca through each of the transport pathways precludes an accurate estimation of the contribution of the Na-Ca exchanger to the fall in α^{i}_{Na}.

Intracellular pH

Control values of pH_i were found to be similar to those reported by other investigators [36–38]: Hypoxia alone evokes a gradual but progressive and modest decline in pH_i [20], which likely results from lactate accumulation secondary to anaerobic glycolysis. Lactic acidosis alone (pH_o = 6.79 − 6.82) causes a steeper decline in pH_i to 6.88 over the first ten minutes; thereafter, pH_i stabilizes as equilibration with the perfusate has occurred. However, combined hypoxia and lactic acidosis results in a progressive and steady decline in pH_i to values lower than those resulting from a simple additive effect of each individual component of the model [20]. Although the inclusion of lactate ions in solution II was intended to inhibit anerobic metabolism, the Purkinje fiber is a glycogen-rich preparation with a high glycolytic rate, and it is likely that glycolysis was not fully inhibited in our experiments. Thus, the observed decline in pH_i may simply be the result of continued lactate production via anaerobic metabolism coupled with reduced lactate efflux, although breakdown of intracellular metabolites such as ATP may also contribute to the pH_i decline [39].

Tension and α^{i}_{Ca}

Under physiologic conditions, the amount of sarcoplasmic free Ca^{2+} is reflected by resting and twitch tension, although the relationship is complex, and other factors, such as pH_i, ATP, and Pi, modulate myofilament sensitivity to free Ca^{2+} [33, 40–42]. In this model, resting tension showed minimal

change during combined hypoxia, acidosis, and glucose depletion, despite the fall in pH_i. This fall in pH_i is known to decrease myofilament sensitivity to Ca^{2+}; therefore, it is likely that α^i_{Ca} rose during this period [41, 43], reflecting either Ca^{2+} displacement from internal buffering sites by H^+ [44] or inhibition of Ca pumps possibly due to a change in free energy of ATP hydrolysis. Twitch tension, which reflects the cyclic release and reuptake of Ca^{2+} by the sarcoplasmic reticulum, falls dramatically during hypoxia and/or lactic acidosis in this model. Simultaneous measurements of twitch tension and pH_i during superfusion with solution II and an acidic solution [20] indicate that the accumulation of $[H^+]_i$ is not solely responsible for the observed negative inotropic effect during exposure to solution II, and accumulation of inorganic phosphate is likely during the ischemic phase of this model.

Upon recovery from hypoxia-lactic acidosis, there is a dramatic rise in resting tension. The extent to which this increase in tension reflects a marked rise in α^i_{Ca}, as opposed to a reduction in the inhibitory effect of Pi and H^+_i on the Ca^{2+} sensitivity of the myofilaments, will need to be ascertained by measurement of free calcium concentration [36, 45]. Although it is likely that both mechanisms are involved, our observations support an increase in α^i_{Ca} as evidenced by the appearance of oscillatory afterpotentials and aftercontractions during the onset of the recovery period. The increase in α^i_{Ca} has been shown under experimental conditions to result in a transient inward current, which some have attributed to inward cation movement through a calcium-activated nonselective cation channel. In fact, two such channels have been identified and tentatively linked to the transient inward current [22, 46]. Activation of such a channel early during the reperfusion phase could explain the dramatic depolarization and marked deviation of E_{MDP} from E_K.

Evidence for a Transient Inward Current Channel and Its Role in the Depolarization Seen During Reperfusion

Although voltage-clamp experiments may provide one approach to analyzing the ionic mechanism underlying the marked depolarization during reperfusion, evidence for a channel mediating the transient inward current may have to come from another line of experiments. For example, one approach would be to identify a highly specific blocking agent that could be used in these experiments; however, even if such an agent could be identified, its ability to block the depolarization phase would depend on whether the inward current is mediated primarily by a nonselective cation channel or by the Na/Ca exchanger. Such an approach could identify the basis of the depolarization during the reperfusion phase and could also provide a new therapeutic approach to the treatment of reperfusion-induced arrhythmias, as well as arrhythmias resulting from calcium overload. To this end, we will review the pertinent data from our own experiments and those of other laboratories.

Our recordings from this channel represent the first description of single calcium-activated channels from working adult myocardium. Colquhoun et al. [46] have described a calcium-activated channel from cultured neonatal rat heart that exhibited some of these same characteristics except that the single channel conductance was markedly lower (viz. 30 to 40 pS) in the presence of physiologic salt solutions. In addition, they observed voltage-independent gating and calcium sensitivity in the 1 to 10 μM range. We speculate that such discrepancies may be due to species and tissue development differences. One report has been published of single channel measurements of a calcium-activated K-selective channel in bovine cardiac Purkinje fibers [47], but its selectivity characteristics differ from those deduced from our recordings. Calcium-activated channels have also been described from cardiac sarcoplasmic reticulum [48, 49], but their properties differ from the one described herein [22].

We were able infrequently to reconstitute this channel into bilayers; this suggests that the channel may be present in the preparation in relatively small numbers. Using single channel and macroscopic current measurements, Hill et al. [22] estimated its density in the sarcolemma to be 1/200 μm^2, which may explain why thus far the cardiac channel has not been observed with the patch clamp technique. Additionally, this channel may be localized to clefts in the sarcolemmal membrane as in skeletal muscle, thereby restricting access to the patch pipette. However, in order to elucidate further its physiologic role, it will be necessary to demonstrate its function directly using either patch clamping or high affinity ligand binding techniques.

Cannell and Lederer [50] have presented evidence that the transient inward current in sheep Purkinje fibers is dependent on channel current (although Na-Ca exchange-mediated current or current from other electrogenic mechanisms may also be involved). The putative transient inward current channel must exhibit calcium-dependent gating over a physiologic range of intracellular calcium, conduct inward current at diastolic membrane potentials, and select for cations over anions. Our measurements of calcium sensitivity, P_{Na}:P_K approaching unity, and chloride impermeability are consistent with these predictions. We have not tried to measure Ca^{2+} current under the same conditions used by these investigators (replacing *trans* monovalent cations with Ca^{2+}); however, absence of substantial calcium permeability in the presence of monovalent cations is seen. For these reasons, we propose that this channel may contribute to transient inward current in canine ventricular muscle and may be involved in development of triggered arrhythmias in calcium-overloaded tissue and in depolarization during the reperfusion phase. Validation of this hypothesis awaits future experimentation.

SUMMARY

Although the discussion thus far has focused on the potential mechanisms underlying the changes in V_m, α^i_{Na}, α^e_K, pH_i, and tension, our data do not

allow us to conclude which ionic translocation represents the primary derangement in this model. Indeed, it is likely that a series of interrelated changes occur throughout both phases of this model and will need to be resolved by future studies. One might speculate that the elevation in α^{i}_{Na} and α^{i}_{Ca} at the end of the ischemic phase are prime determinants of the changes that follow the onset of reperfusion. The rapid rise in pH_i and washout of metabolites enable the Na-Ca exchanger and Na-K pump to respond to the elevated α^{i}_{Na} and to augment their activity to reduce α^{i}_{Na} toward control values. It is difficult to determine the role that the exchanger plays in the reperfusion phase, since the electrochemical gradient and the fraction of total Na and Ca fluxes carried by it across the sarcolemma are unknown. The dramatic increase in resting tension argues for an increase in myofilament sensitivity to calcium as pH_i rises in addition to an increase in cytosolic calcium probably resulting from calcium release from the sarcoplasmic reticulum. The increase in intracellular free calcium concentration could, in turn, activate a nonselective cation channel, leading to an inward current and membrane depolarization. Data provided by Hayashi et al. [19] indicating that reperfusion arrhythmias are dependent on excitation during reperfusion support this view. The magnitude of the α^{i}_{Na} increase during ischemia and the extent to which this increase is dissipated by the Na-K pump may very well help determine the degree of calcium loading during reperfusion and may explain the increased sensitivity of Purkinje fibers to acetylstrophanthidin. Given the changes in pH and pCa during both the ischemic and reperfusion phases, resolution of ionic current changes in a syncytial preparation will be challenging.

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REFERENCES

1. Tennant, R., and Wiggers, C.J. 1935. The effect of coronary occlusion on myocardial contraction. *Am. J. Physiol.* 112:351–361.
2. Balke, C.W., Kaplinsky, E., Michelson, E.L., et al. 1981. Reperfusion ventricular tachyarrhythmias: correlation with antecedent coronary artery occlusion tachyarrhythmias and duration of myocardial ischemia. *Am. Heart J.* 101:449–456.
3. Battle, W.E., Naimi, S., Avitall, B., et al. 1974. Distinctive time course of ventricular vulnerability to fibrillation during and after release of coronary ligation. *Am. J. Cardiol.* 34:42–47.
4. Manning, A.S., and Hearse, D.J. 1984. Reperfusion-induced arrhythmias: mechanisms and prevention. *J. Mol. Cell. Cardiol.* 16:497–518.
5. Austin, M., Wenger, T.L., Harrell, F.E. Jr., et al. 1982. Effect of myocardium at risk on outcome after coronary artery occlusion and release. *Am. J. Physiol.* 243:H340–H345.
6. Naito, M., Michelson, E.L., Kmetzo, J.J., et al. 1981. Failure of antiarrhythic drugs to prevent experimental reperfusion ventricular fibrillation. *Circulation* 63:70–79.
7. Sheridan, D.J., Penkoske, P.A., Sobel, B.E., and Corr, P.B. 1980. Alpha adrenergic contributions to dysrhythmias during myocardial ischemia and reperfusion in cats. *J. Clin. Invest.* 65:161–171.

8. Stephenson, S.E. Jr., Cole, R.K., Parrish, T.F., et al. 1960. Ventricular fibrillation during and after coronary artery occlusion: incidence and protection afforded by various drugs. *Am. J. Cardiol.* 5:77–87.
9. Stewart, J.R., Burmeister, W.E., Burmeister, J., and Lucchesi, B.R. 1980. Electrophysiologic and antiarrhythmic effects of phentolamine in experimental coronary artery occlusion and reperfusion in the dog. *J. Cardiovasc. Pharmacol.* 2:77–91.
10. Stockman, M.B., Verrier, R.L., and Lown, B. 1979. Effect of nitroglycerin on vulnerability to ventricular fibrillation during myocardial ischemia and reperfusion. *Am. J. Cardiol.* 43:233–238.
11. Jennings, R.B., Sommers, H.M., Smyth, G.A., et al. 1960. Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. *Arch. Pathol.* 70:68–78.
12. Lederman, S.N., Wenger, T.L., Harrell F.E. Jr., and Strauss, H.C. 1987. Effects of different paced heart rates on canine coronary occlusion and reperfusion arrhythmias. *Am. Heart J.* 113:1365–1369.
13. Corr, P.B., Shayman J.A., Kramer, J.B., and Kipnis, R.J. 1981. Increased α-adrenergic receptors in ischemic cat myocardium: a potential mediator of electrophysiological derangements. *J. Clin. Invest.* 67:1232–1236.
14. Corr, P.B., Yamada, K.A., and Witkowski, F.X. 1986. Mechanisms controlling cardiac autonomic function and their relation to arrhythmogenesis. In *The Heart and Cardiovascular System*, H.A. Fozzard et al., eds., pp. 1343–1403 New York, Raven Press.
15. Wilber, D.J., Lynch, J.J., Montgomery, D.G., and Lucchesi, B.R. 1987. α-adrenergic influences in canine ischemic sudden death: effects of α_1-adrenoceptor blockade with prazosin. *J. Cardiovasc. Pharmacol.* 10:96–106.
16. Kleber, A.G. 1983. Resting membrane potential, extracellular potassium activity, and intracellular sodium activity during acute global ischemia in isolated perfused guinea pig hearts. *Circ. Res.* 52:442–450.
17. Ferrier, G.R., Moffat, M.P., and Lukas, A. 1985. Possible mechanisms of ventricular arrhythmias elicited by ischemia followed by reperfusion: studies on isolated canine ventricular tissues. *Circ. Res.* 56:184–194.
18. Ferrier, G.R., Moffat, M.P., Lukas, A., and Mohabir, R. 1985. A model of ischemia and reperfusion: effect of potassium concentration of electrical and contractile responses of canine Purkinje tissue. In *Cardiac Electrophysiology and Arrhythmias*, pp. 325–330, New York, Grune and Stratton.
19. Hayashi, H., Ponnambalam C, and McDonald, T.F. 1987. Arrhythmic activity in reoxygenated guinea pig papillary muscles and ventricular cells. *Circ. Res.* 61:124–133.
20. Yee, R., Brown, K.K., Bolster, D.E., and Strauss, H.C. 1988. The relationship between ionic perturbations and electrophysiologic changes in a canine Purkinje fiber model of ischemia and reperfusion. *J. Clin. Invest.* 82:225–233.
21. Hamill, O.P., Marty, A., Neher, E., et al. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Arch.* 391:85–100.
22. Hill, J.A., Coronado, R., and Strauss, H.C. 1988. Reconstitution and characterization of a calcium-activated channel from heart. *Circ. Res.* 62:411–415.
23. Wier, W.G., Cannell, M.B., Berlin, J.R., et al. 1987. Cellular and subcellular heterogeneity of $[Ca^{2+}]_i$ in single heart cells revealed by fura-2. *Science* 235:325–328.
24. Cannell, M.B., Wier, W.G., Berlin, J.R., et al. 1986. Free intracellular calcium in normal and calcium-overloaded rat heart cells: digital imaging fluorescent microscopy using fura-2. *Biophys. J.* 49:466a.
25. Yue, D.T., Marban, E., and Wier, W.G. 1986. Relationship between force and intracellular $[Ca^{2+}]$ in tetanized mammalian heart muscle. *J. Gen. Physiol.* 87:223–242.
26. Valdeolmillos, M., and Eisner, D.A. 1985. The effects of ryanodine on calcium-overloaded sheep cardiac Purkinje fibers. *Circ. Res.* 56:452–456.
27. Lauer, M.R., Rusy, B.F., and Davis, L.D. 1984. H^+-induced membrane depolarization in canine cardiac Purkinje fibers. *Am. J. Physiol.* 247:H312–H321.
28. Kass, R.S., Lederer, W.J., Tisien, R.W., and Weingart, R. 1978. Role of calcium ions in transient inward currents and aftercontractions induced by strophanthidin in cardiac Purkinje fibers. *J. Physiol. (Lond.)* 281:187–208.

29. Grinwald, P.M. 1982. Calcium uptake during post-ischemic reperfusion in the isolated rat heart: Influence of extracellular sodium. *J. Mol. Cell. Cardiol.* 14:359–365.
30. Wilde, A.A.M., and Kleber, A.G. 1986. The combined effects of hypoxia, high K^+, and acidosis on the intracellular sodium activity and resting potential in guinea pig papillary muscle. *Circ. Res.* 58:249–256.
31. Balasubramanian, V., McNamara, D.B., Singh, J.N., and Dhalla, N.S. 1973. Biochemical basis of heart function. X. Reduction in the Na^+-K^+-stimulated ATPase activity in failing rat heart due to hypoxia. *Can. J. Physiol. Pharmacol.* 51:502–510.
32. Russell, J.M., Boron, W.F., and Brodwick, M.S. 1983. Intracellular pH and Na fluxes in barnacle muscle with evidence for reversal of the ionic mechanism of intracellular pH regulation. *J. Gen. Physiol.* 82:47–78.
33. Allen, D.G., and Orchard, C.H. 1987. Myocardial contractile function during ischemia and hypoxia. *Circ. Res.* 60:153–168.
34. Deitmer, J.W., and Ellis, D.W. 1980. Interactions between the regulation of the intracellular pH and sodium activity of sheep cardiac Purkinje fibres. *J. Physiol. (Lond.)* 304:471–488.
35. Philipson, K.D., Bersohn, M.M., and Nishimoto, A.Y. 1982. Effects of pH on Na^+-Ca^{2+} exchange in canine cardiac sarcolemmal vesicles. *Circ. Res.* 50:287–293.
36. Roos, A., and Boron, W.F. 1981. Intracellular pH. *Physiol. Rev.* 61:296–434.
37. de Hemptinne, A. 1980. Intracellular pH and surface pH in skeletal and cardiac muscle measured with a double-barrelled pH microelectrode. *Pflugers Arch.* 386:121–126.
38. Ellis, D., and Thomas, R.C. 1976. Direct measurement of the intracellular pH of mammalian cardiac muscle. *J. Physiol. (Lond.)* 262:755–771.
39. Hoerter, J.A., Miceli, M.V., Renlund, D.G., et al. 1986. A phosphorus-31 nuclear magnetic resonance study of the metabolic, contractile, and ionic consequences of induced calcium alterations in the isovolumic rat heart. *Circ. Res.* 58:539–551.
40. Couper, G.S., Weiss, J., Hiltbrand, B., and Shine, K.I. 1984. Extracellular pH and tension during ischemia in the isolated rabbit ventricle. *Am. J. Physiol.* 247:H916–H927.
41. Fabiato, A., and Fabiato, F. 1978. Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *J. Physiol. (Lond.)* 276:233–255.
42. Kentish, J.C. 1986. The effects of inorganic phosphate and creatine phosphate on force production in skinned muscles from rat ventricle. *J. Physiol. (Lond.)* 370:585–604.
43. Blanchard, E.M., Pan, B.S., and Solaro, R.J. 1984. The effect of acidic pH on the ATPase activity and troponin Ca^{2+} binding of rabbit skeletal myofilaments. *J. Biol. Chem.* 259:3181–3186.
44. Langer, G.A. 1985. The effect of pH on cellular and membrane calcium binding and contraction of myocardium. *Circ. Res.* 57:374–382.
45. Kusoka, H., Porterfield, J.K., Weisman, H.F., et al. 1987. Pathophysiology and pathogenesis of stunned myocardium. Depressed Ca^{2+} activation of contraction as a consequence of reperfusion-induced cellular calcium overload in ferret hearts. *J. Clin. Invest.* 79:950–961.
46. Colquhoun D., Neher, E., Reuter, H., and Stevens, C.F., 1981. Inward current channels activated by intracellular Ca in cultured cardiac cells. *Nature* 294:752–754.
47. Callewaert, G., Vereecke, J., and Carmeliet, E. 1986. Existence of a calcium-dependent potassium channel in the membrane of cow cardiac Purkinje cells. *Pflugers Arch.* 406:424–426.
48. Smith, J.S., Coronado, R., and Meissner, G. 1985. Sarcoplasmic reticulum contains adenine nucleotide-activated calcium channels. *Nature* 316:446–449.
49. Rousseau, E., Smith, J.S., Henderson, J.S., and Meissner, G. 1986. Single channel and $^{45}Ca^{2+}$ flux measurements of the cardiac sarcoplasmic reticulum calcium channel. *Biophys. J.* 50:1009–1014.
50. Cannell, M.B., and Lederer, W.J. 1986. The arrhythmic I_{ti} current in the absence of electrogenic sodium-calcium exchange in sheep cardiac Purkinje fibres. *J. Physiol (Lond.)* 374:201–219.

II. METABOLIC FACTORS IN ISCHEMIA

6. ISCHEMIA AND NA^+/K^+PUMP FUNCTION

ANDRÉ G. KLÉBER AND WAYNE E. CASCIO

Passive sodium influx into rhythmically active heart cells occurs via a variety of parallel transmembrane pathways, such as membrane channels responsible for the upstroke of the action potential and ion exchange systems involved in the regulation of intracellular calcium and H^+. During steady state, the cellular sodium load is counterbalanced by the energy-dependent sarcolemmal Na^+/K^+ pump, which extrudes sodium and imports potassium ions against an electrochemical gradient (figure 6-1).

Myocardial ischemia (arrest of coronary perfusion) or anoxia (O_2 withdrawal from perfusate) will ultimately impair energy-dependent pumping and consequently will result in cellular Na^+ and Ca^{++} overload, [1], rigor [2], and irreversible cellular damage [1]. Several experimental studies, which used a variety of techniques, have shown that development of irreversible damage and impairment of cellular function is not a continuous process. Instead, in the early phase of myocardial ischemia (first 10 to 15 minutes) ionic homeostasis appears to be at least partially maintained. This initial phase is of particular importance because it produces the majority of malignant ventricular arrhythmias leading to sudden cardiac death [3]. An understanding of the pathophysiologic mechanisms underlying this initial, reversible period requires the answer to two major questions: (1) What are the

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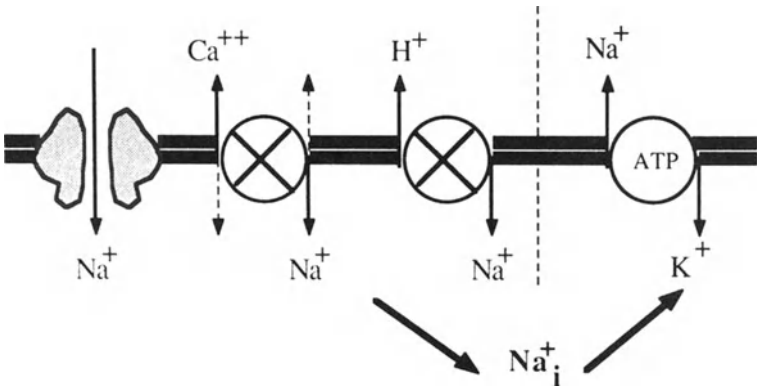

Figure 6-1. Schematic representation of membrane elements that contribute to the ionic homeostasis of sodium in an electrically and mechanically active myocardial cell. The passive inward Na^+ movements include the fast inward Na^+ current, and the Na^+/Ca^{++} and Na^+/H^+ ion exchange systems (left of vertical line). The ion exchange systems use the inwardly directed Na^+ gradient to extrude Ca^{++} and H^+ from the cell. The intracellular Na^+ load is counterbalanced by sodium extrusion accomplished by the energy-dependent (Na^+/K^+) pump (right of vertical line).

metabolic and ionic mediators of the disturbances in impulse conduction that develop extremely rapidly after occlusion of a coronary artery, and (2) is it mechanistically and thermodynamically possible and reasonable to accept experimental data that suggest at least partially maintained intracellular Na^+ and Ca^{++} levels during this initial, highly arrhythmogenic phase?

This chapter specifically addresses the second question. Despite a large quantity of recent experimental data and new techniques, our knowledge of the factors and their interrelationship, which contribute to the maintenance of intracellular sodium (and calcium) levels is incomplete. Moreover, the available experimental data are controversial. Several factors contribute to the inability to provide a unified interpretation of all experimental data: (1) conflicting results obtained in different species, (2) methodological limitations, and (3) different experimental models. The experimental model of myocardial ischemia presents particular difficulties. Arrest of coronary flow produces a closed system that consists of an extra-cellular and an intracellular space uncoupled from the surrounding environment. The lack of extracellular washout is an essential component and explains the considerable difference between the electrical and ionic disturbances observed during anoxia (lack of O_2 alone) and ischemia [3]. Thus, commonly used interventions to test cellular function, such as the application of activators or inhibitors, or changes of the ionic composition of the extracellular fluid, open the system and lead to problems of applicability to the ischemic condition. Therefore, special emphasis will be given in the following paragraphs to possible experimental limitations and species differences.

PUMP FUNCTION AND IONIC REGULATION

Intracellular Sodium

Two experimental studies [4, 5], which used different techniques, have determined the time course of intracellular Na^+ in early myocardial ischemia. Their results appear to conflict. Measurements of intracellular Na^+ activity from subepicardial layers of blood perfused and ischemic guinea pig hearts revealed no significant increase of intracellular Na^+ in the first 15 minutes of ischemia (32.5°C, 1 Hz). These experiments were criticized because the experimental technique did not use an oxygen-free environment adjacent to the epicardial site of the electrode impalement. However, calculation of the oxygen partial pressure as a function of the radius of the ventricle suggested virtual absence of oxygen at a presumed depth of 150 to 200 μm of subepicardial electrode impalements (see Kléber [5]). Moreover experiments in which a N_2 environment surrounded the heart confirmed the lack of sodium increase in this experimental condition (Kléber, unpublished).

An analysis of the different factors that influence the level of intracellular Na^+ in early ischemia (changes in influx versus changes in efflux) was not possible because of methodological limitations (i.e., lack of access to the extracellular space and instability of electrode impalements upon sudden rate changes). Therefore, intracellular Na^+ activity was measured in small superfused guinea pig trabecula in which the main components of early ischemia (extracellular K^+ accumulation, acidosis, anoxia, absence of glucose) were simulated by changing the composition of the superfusate [6]. In this series of experiments, special care was taken to keep the partial oxygen pressure below 5 mm Hg. The result of a typical experiment from this series, which illustrates the effect of the various components on membrane potential and on intracellular Na^+ activity, measured by an ion-selective electrode, is shown in figure 6-2. At time 1, the superfusate (normal Tyrode's solution) was changed to a solution containing an elevated potassium concentration (11.5 mM). This produced the expected changes of electrical activity and the decrease of intracellular Na^+ activity [7, 8]. After 14 minutes (time 2) the superfusate was changed to a solution simulating an ischemic environment, (i.e., containing an elevated $[K^+]$, low pH, and no O_2 or glucose). This produced an immediate increase of intracellular Na^+ within nine minutes. However, the level reached after 9 to 11 minutes of simulated ischemia was not significantly different from the control level. These results suggested that the intracellular Na^+ level in the early phase of myocardial ischemia is determined by two processes with opposing effects: (1) a decrease of active Na^+ efflux (partial pump inhibition) and (2) a decrease of Na^+ influx. The decrease of the Na^+ influx (cellular Na^+ load) was caused by two mechanisms, a decrease of the electrochemical gradient for Na^+ brought about by depolarization and the development of inexcitability, which is equivalent to the transition from beating to resting tissue [9]. In this series of experiments,

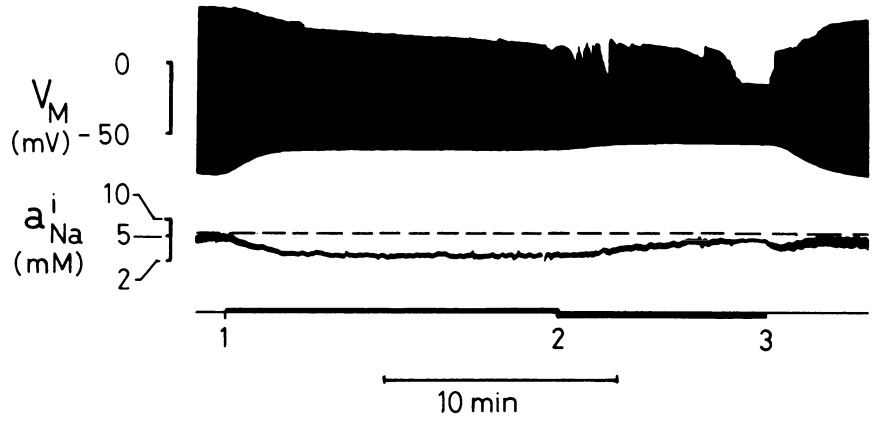

Figure 6-2. A single representative experiment illustrating the effect of a normoxic, or simulated ischemic (i.e., anoxic, glucose-free, acidic) solution on the relationship of the transmembrane potential (V_m) and the intracellular sodium activity (a^i_{Na}) in the presence of an elevated extracellular K^+ concentration. At time 1, superfusion was changed from normoxic Tyrode's solution at 4.7 mM K^+ to normoxic Tyrode's solution at 11.5 mM K^+. The consequent membrane depolarization resulted in a reduction of a^i_{Na} due to reduced passive influx. At time 2 after 14 minutes, the superfusate was changed to the simulated ischemic solution for nine minutes. Partial inhibition of the Na^+/K^+ pump results in a reduction of active Na^+ efflux, and leads to a slight rise in a^i_{Na}. Time 3 indicates return to normal Tyrode's solution. Reprinted from Wilde and Kléber [6] with the permission of the American Heart Association.

anoxia, absence of glucose, and acidosis at a normal extracellular K^+ concentration caused a mean increase in intracellular Na^+ activity by 3.5 mM within 9 to 11 minutes. This increase was attributed to the partial inhibition of active Na^+ pumping because it was reversed by addition of glucose.

Several other groups measured intracellular Na^+ in hypoxic (but not ischemic) myocardium with the same method (ion-selective electrode) afterwards and obtained differing results. Thus, Ellis and Noireaud [10] measured a similar, small increase of intracellular Na^+, whereas, Nakaya et al. [11] observed no change after 30 minutes of hypoxic perfusion. Guarnieri [12] reported a nearly twofold increase of intracellular Na^+ after 20 to 25 minutes of hypoxia. However, a close inspection of his results reveals that the increase was not linear. Rather, sodium remained low initially and increased only after 10 to 15 minutes. Variable degrees of hypoxia may partially explain these differences. The important mutually shared observation is that the increase of intracellular Na^+ activity never exceeded 3 mM in the first 10 minutes of hypoxia. This change is considerably smaller than the changes observed, for example, after administration of cardiac steroids [13] and, as we showed, is cancelled by arresting the heart and increasing extracellular K^+.

Nuclear magnetic resonance (NMR) has also been applied to the measurement of intracellular Na^+ in buffer-perfused and ischemic rat hearts [4], A shift reagent was used to distinguish between the intracellular and the extracellular Na^+ pool. These results, which sampled and averaged spectra within one minute time frames, showed a very fast increase of intracellular Na^+ (by 240 percent after 12 minutes) starting almost immediately after perfusional arrest. This result seems to be in marked contrast to the findings obtained with ion-selective electrodes, and a methodological explanation of the difference would certainly devaluate one or both methods. However, there is strong evidence that the difference is due to the species studied (rat versus guinea pig or rabbit) rather than to the technique. The species dependence will be discussed in the last paragraph of this section (calcium and cellular coupling).

Pump Function and Cellular Potassium

Since the experiments of Harris et al.[14] more than 40 years ago, cellular potassium loss, and consequently, extracellular K^+ accumulation, was considered to be a major determinant of the electrical changes and of the genesis of malignant ventricular arrhythmias following coronary occlusion. The introduction of the ion-selective electrodes allowed the quantification of the change in extracellular K^+ during myocardial ischemia [15, 16] and the correlation with the electrical changes, such as the change in resting membrane potential [5]. Despite considerable experimental efforts, the quantitative contribution of the different (proven or postulated) components leading to net K^+ efflux and extracellular accumulation of K^+ remains unclear (see article by Gettes et al. in this volume). Moreover, experimental treatment of important questions, such as the frequency-dependence of extracellular K^+ accumulation, have yielded controversial results.

Intuitively, one is tempted to attribute cellular potassium loss (net K^+ efflux) to a decrease in active (energy-dependent) unidirectional K^+ influx. However, the early studies, which measured unidirectional potassium fluxes with radioactive tracers, revealed an increase in passive K^+ efflux in the presence of maintained active pumping [17, 18]. These results were used to postulate a dissociation between K^+ loss and the decreased energetic state in hypoxia [18]. The studies of Kline and Morad [19] and Kunze [20] have shown in normoxia that changes in heart rate are associated with transient accumulation or depletion of potassium in the narrow extracellular space of compact ventricular tissue [21]. The transients are caused by a dissociation between instantaneous changes of passive unidirectional K^+ efflux and a delayed adaption of the Na^+/K^+ pump rate and, therefore, can be used as a qualitative test of the activity of the Na^+/K^+ pump. This method was used to assess active Na^+/K^+ pumping in ischemic hearts by Weiss and Shine [22] and by Kléber [5]. A result of the change in extracellular K^+ upon a sudden

change in heart rate is shown in figure 6-3A (absence of cardiac steroid) and figure 6-3B (presence of strophanthidin, 10^{-6} M). During normal perfusion (left panel of figure 6-3A), the increase of extracellular K^+ upon a sudden increase in heart rate is compensated by a delayed increase of Na^+/K^+ pumping. The increased pump rate is also indicated by the transient depletion of extracellular K^+ upon the decrease in rate. Similar changes of extracellular K^+ can be demonstrated during ischemia (figure 6-3A, right panel). Thus, transient K^+ depletion is observed after a sudden decrease in rate during the initial phase of extracellular K^+ accumulation, indicating (qualitatively) that the Na^+/K^+ pump can adapt its rate to an increased ionic load in the early phase of ischemia. This finding is further confirmed by the inhibitory effect of strophanthidin as shown on figure 6-3B.

On the basis of the finding of maintained intracellular Na^+ activity and simultaneous net cellular K^+ loss [5], we have suggested that the net outward movement of K^+ requires compensatory charge movement other than inward transfer of Na^+ (for possible hypotheses of anion movements and an eventual relationship to intracellular acidosis, see Kléber [23] and Kléber et al. [24]. It should be emphasized that the discrepancy between the extracellular rise in K^+ and a steady intracellular Na^+ level does not rule out the possibility that, in the case of ischemia, active K^+ influx decreases as well. The hypothetical relationship among changes in K^+ influx, intracellular Na^+, and pump rate is illustrated in figure 6-4. It is based on the model presented by Cohen et al. for Purkinje fibers (see Cohen et al. [25] or Cohen et al. in this volume) and assumes saturation of the pump by extracellular K^+ at levels above 4.5 mM [26]. At a constant K_m for Na^+ [27], an increase of 3 mM in hypoxia [6] would correspond to approximately 40% inhibition (shift from upper to lower curve on figure 6-4) in this particular model. Consequently, maintained intracellular Na^+ in ischemia at 6.5 mM implicates a decreased pump rate of Na^+ and K^+. Although the exact amount of pump inhibition by hypoxia is difficult to predict (about 20% in the first ten minutes according to Bersohn et al. [28]), the schematic relationship presented on figure 6-4 suggests an essential difference between K^+ influx in ischemia and hypoxia. In early hypoxia, K^+ influx will be maintained [17, 18] by an increase of intracellular Na^+, whereas the additional depolarization in myocardial ischemia will decrease intracellular Na^+ and Na^+/K^+ pumping.

Intracellular Calcium and Cellular Uncoupling

In normal heart cells, the Na^+/K^+ pump is indirectly involved in the regulation of intracellular calcium via the Na^+/Ca^{++} exchange mechanism. Therefore, a change in the activity of the Na^+/K^+ pump might influence intracellular Ca^{++} via Na^+/Ca^{++}exchange. However, the discussion of the link between intracellular Na^+ and Ca^{++} changes is complicated because the Na^+/Ca^{++} exchanger may be affected by O_2 withdrawal as well, and be-

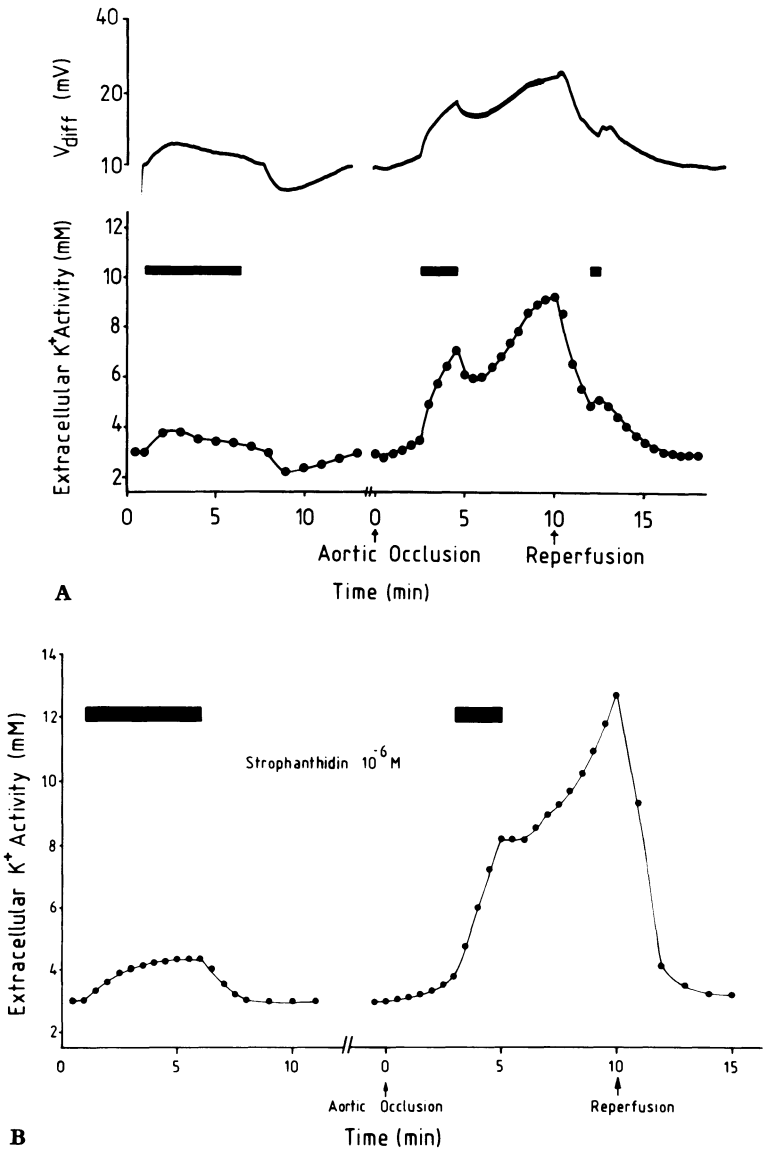

Figure 6-3. (A) Effects of an abrupt change in heart rate on a°_K before occlusion, during occlusion, and during reperfusion in a single experiment. Upper trace shows the potential sensitive to extracellular potassium activity, a°_K (V_{diff}). Lower trace shows a°_K calculated from V_{diff}. The spontaneous heart rate (60 beats/min) was increased to 170 beats/min during the times indicated by the horizontal bars. In the perfused preparation, the time course of reversion of a°_K to the steady state level during and after stimulation (left side of figure) suggested delayed activation and inactivation of the Na^+/K^+pump. Rapid stimulation during ischemia (right side of figure) caused a more pronounced increase in extracellular K^+ accumulation. The decline of a°_K after stimulation implies that Na^+/K^+ pump activity resulted in a net uptake of potassium from the extracellular space. (B) Effect of a change in heart rate in the presence of 10^{-6} M strophanthidin. Rapid stimulation (170 beats/min) is indicated by the bars. During strophanthidin perfusion, block of the Na^+/K^+ pump is suggested by the absence of a decrease in a°_K during, and of an undershoot in a°_K following, rapid stimulation (170 beats/min). During ischemia, rapid stimulation resulted in a steady increase in extracellular K^+ accumulation. After stimulation, a°_K did not decline. Reprinted from Kléber [5] with permission of the American Heart Association.

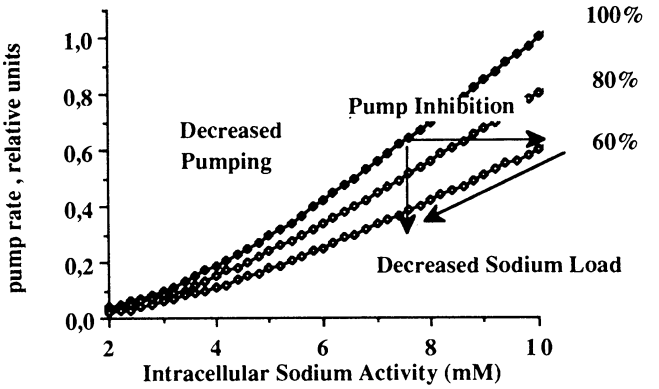

Figure 6-4. Hypothetical relationship between intracellular sodium (a^i_{Na}) activity and Na^+/K^+pump rate in Purkinje fibers based on the model proposed by Cohen et al. [25]. In ischemia, a^i_{Na} is affected by two mechanisms with opposing effects: (1) partial inhibition of the Na^+/K^+ pump leads to an increase of intracellular Na^+ (horizontal arrow), (2) decrease of passive influx is associated with a decrease of intracellular Na^+ (oblique arrow). Maintained intracellular Na^+ activity results of (1) and (2); (vertical arrow).

cause no information is available about changes of the energy-dependent Ca^{++}/H^+ pump, another proposed component involved in the regulation of Na^+ and Ca^{++} movements. Partial inhibition of the Na^+/Ca^{++} exchange by hypoxia is suggested from the data of Guarnieri [12] and Bersohn et al. [28]. Guarneri reported an unchanged intracellular Ca^{++} activity (measured with ion-selective electrodes) during the delayed increase of intracellular Na^+ in hypoxia. Bersohn et al. showed a decrease of the Na_i^+-dependent Ca^{++} uptake in ischemic isolated sarcolemmal vesicles. However, as discussed by Bersohn, interpretation of the importance of a partial inhibition of the Na^+/Ca^{++} exchange is difficult and ultimately depends on whether the exchange is more important for Ca^{++} influx or Ca^{++} efflux. Recent experiments measuring intracellular Ca^{++} by means of aequorin light emission [29] suggest that the Na^+/Ca^{++} exchange is at least partially active during metabolic inhibition (simulated hypoxia and absence of metabolic substrate).

The conflicting experimental data and the complicated relationship between intracellular Na^+ and Ca^{++} underline the importance of direct, reliable measurements of intracellular Ca^{++} in myocardial hypoxia and ischemia. Unfortunately, the available measurements of resting intracellular free Ca^{++} or of Ca^{++} transients during the cardiac cycle seem to be controversial as well. The data on the change of intracellular free Ca^{++} during metabolic blockade clearly showed a delayed increase of resting calcium [29] and a decrease of calcium transients in hypoxia [30]. According to the article by Clusin et al. (this volume), more recent data indicate a transient increase of free calcium during the early reversible stage of hypoxia. Apparently, the

failure of detection of this early increase in the numerous previous studies would have been caused by the use of metabolically "deteriorated" preparations. Such controversial results underline the need for a reproducible definition of a "healthy" or "deteriorated" state of the myocardium during normoxic superfusion and/or perfusion.

Two studies have reported changes in intracellular free Ca^{++} in myocardial ischemia using NMR spectroscopy [31, 32]. These studies have been criticized, because the indicator (5,5'-difluoro BAPTA) is a potent intracellular chelator of calcium. To compensate for this effect, the investigators increased extracellular calcium severalfold, to a level that produced a contractile response similar to that of normal, untreated hearts. Induction of ischemia showed a consistent response in both studies, characterized by an early stable phase during which average intracellular free Ca^{++} remained normal and by a steep subsequent increase. In the case of the rat heart, the increase occurred earlier (after four to five minutes [31]) than in the ferret heart (10 to 15 minutes [32]). This difference is particularly interesting because it suggests a species difference, which also might explain the difference in the early changes of intracellular Na^+ between rat [4] and guinea pig hearts [5]. It is known that normal rat hearts have a significantly higher metabolic rate than hearts from larger species, such as guinea pigs or rabbits [33]. During ischemia, this might cause an earlier exhaustion of the energy sources provided by anaerobic glycolysis in the rat heart.

The data obtained with NMR in ferret hearts are in close agreement with measurements of cellular coupling during early ischemia in the arterially-perfused rabbit papillary muscle [34]. Furthermore, they are closely correlated with the secondary increase of extracellular K^+. In figure 6-5 the time course of the change in longitudinal whole tissue resistance (r_t) is compared to the change in extracellular K^+ concentration in a rabbit papillary muscle after arrest of arterial perfusion. As has been shown [34], the immediate increase of r_t (r_t is equivalent to the extracellular and intracellular longitudinal resistance in parallel) is due to an increase in the extracellular resistance, and cellular uncoupling is indicated by the fast, second increase of r_t after 18 minutes. In all such types of experiments it can be shown that cellular uncoupling, which probably is due to an increase of intracellular free Ca^{++}, starts after 12 to 15 minutes and always coincides with the second phase of extracellular K^+ accumulation. In contrast, the rapid, initial phase of extracellular K^+ accumulation is not associated with a change of coupling resistance.

In this volume, Clusin et al. propose an early, transient increase of intracellular free Ca^{++} in myocardial ischemia as a possible factor for the genesis of malignant ventricular arrhythmias. Such an early change may potentially affect the excitability of the cell or (as needs to be shown) elicit triggered activity. However, our measurements make it unlikely that it will affect the conduction of the impulse through a decrease of cellular coupling.

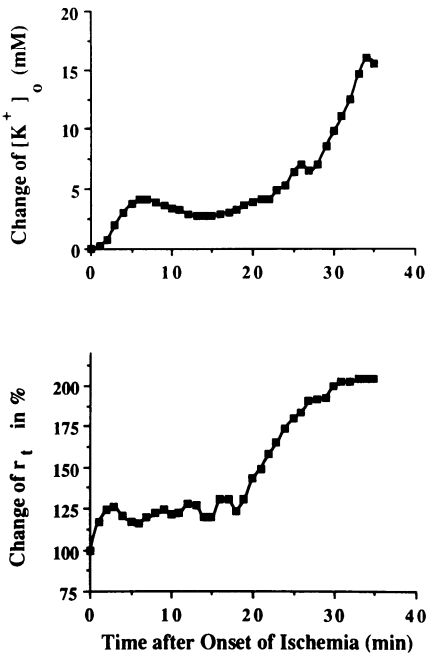

Figure 6-5. Representative single experiment showing the relationship of the change of extracellular K^+ concentration, ($[K^+]_o$), to the whole tissue longitudinal resistance (r_t) in acutely ischemic rabbit ventricular muscle. The initial rise in r_t is due to an increase in the extracellular longitudinal resistance. However, the rapid increase of r_t, which begins at 18 minutes, is due to a rapid increase in the longitudinal intracellular resistance (i.e., cellular uncoupling, see 34), and corresponds closely to the secondary rise in extracellular K^+. Note that r_t reaches a plateau when cellular uncoupling is complete after 30 minutes.

THERMODYNAMIC ASPECTS OF PUMP FUNCTION

Arguments based on equilibrium thermodynamics were used to criticize the experimental observation that sodium homeostasis is maintained in early ischemia. Thus, it was assumed that the decrease of the cytosolic phosphate potential (or free energy change for hydrolysis of ATP, $dG/d\xi_{ATP}$) would implicate an increase of intracellular Na^+ activity [35]. At present it is not possible to provide a profound analysis of energy transfer between the driving process (cytosolic phosphate potential) and the driven process (Na^+/K^+ pumping). Such analysis would necessitate the dynamic measurement of the phosphate potential, the phosphate flux, the electrochemical potential for Na^+, and pump rate.

A simpler analysis involves the measurement of the change in the cytosolic phosphate potential [35, 36] alone. This energy level will set the upper limit for the possible sarcolemmal electrochemical gradients. Figure 6-6 (filled symbols) shows the change in cytosolic phosphate potential during myocar-

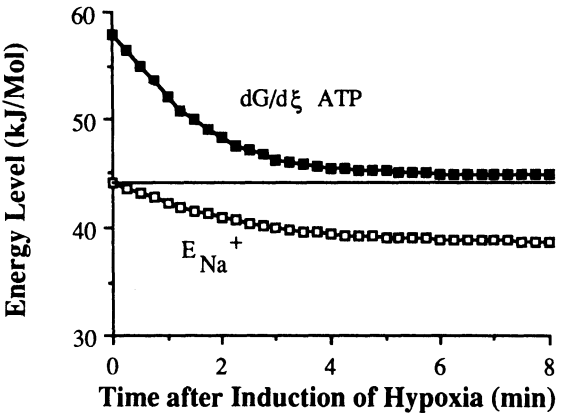

Figure 6-6. Hypothetical relationship of the free energy change for ATP-hydolysis, $dG/d\xi_{ATP}$ to the transmembrane electrochemical gradient of sodium (E_{Na}^+) during hypoxia. The cytosolic phosphate potential, $dg/d\xi_{ATP}$ (filled symbols) is illustrated and based on the study of Kammermeier et al. [36] in rat heart. The calculated electrochemical gradient of sodium (E_{Na}^+) during hypoxia is based on the transmembrane potential and the intracellular Na^+ activity observed by Wilde and Kléber [6] during hypoxia in guinea pig papillary muscle.

dial hypoxia in rat hearts taken from Kammermeier et al. [36]. Astonishingly, there is not a continuous decrease of $dG/d\xi_{ATP}$ after oxygen withdrawal. Rather, $dG/d\xi_{ATP}$ reaches a new steady state after approximately six minutes at a level of approximately 45 KJ/Mol. The calculation of the absolute values of $dG/d\xi_{ATP}$ are based on measurements of cellular creatine and creatinephosphate levels and depend on several assumptions (pH-dependence of creatine kinase equilibrium constant, intracellular compartmentation of inorganic phosphate [35, 36]) and, therefore, may be subjected to some bias. The time course of $dG/d\xi_{ATP}$ corresponds closely to the change of cellular overall ATP concentration observed in myocardial hypoxia [2]. The transient steady level of $dG/d\xi_{ATP}$ raises two questions: (1) What are the thermodynamic limits for the transmembrane ionic gradients? (2) What processes decrease the energy consumption in early ischemia and allow a steady state at a decreased rate of ATP synthesis?

An attempt to answer the first question can be made by calculating the minimal energy required to build up the transmembrane electrochemical gradient for sodium (E_{Na}^+). Calculation of this level is based on a stoichiometry of 1 Mol ATP for 3 Mol Na^+ and involves a chemical term ($=RT \ln[Na^+]_o/[Na^+]_i$), an electrical term ($=F V_m$, V_m= membrane potential) and assumes equilibrium between the membrane potential and the transmembrane potassium distribution (e.g., Fiolet et al. [35]). For the curve of E_{Na}^+ shown in figure 6-6, the data for the change in intracellular Na^+ and membrane potential were taken from Wilde and Kléber [6]. It is important to note that in hypoxia the decrease of E_{Na}^+ is only partly a consequence of the

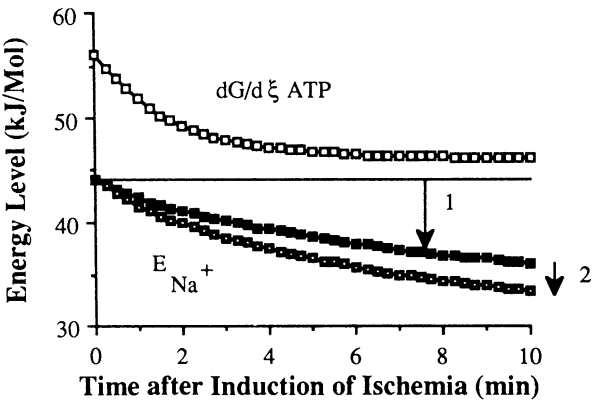

Figure 6-7. Hypothetical relationship of the energy change for hydolysis of ATP, $dG/d\xi_{ATP}$ to the transmembrane electrochemical gradient of sodium (E_{Na}^+) in acute myocardial ischemia. The cytosolic phosphate potential, $dG/d\xi_{ATP}$ (open squares) was taken from Fiolet et al. [35] and is shown to plateau near 45KJ/Mol until a secondary rapid decrease takes place after ten minutes of ischemia. Arrow 1 points to E_{Na}^+ based on the data of Kléber [5], (accumulation of extracellular K^+, no change in intracellular sodium). Arrow 2 points to E_{Na}^+ calculated from the hypothesis that the increase of extracellular K^+ is compensated by an increase in intracellular Na^+.

increase of intracellular Na^+. For example, the level after eight minutes of hypoxia was calculated from an increase of the intracellular Na^+ activity from 6.8 to 9.8 mM and from a positive shift of the resting membrane potential from −80 mV to −70 mV [6]. For this case, the contribution of the depolarization to the decrease of E_{Na}^+ was 52 percent; only 48 percent was due to the increase in intracellular Na^+.

In myocardial ischemia, depolarization of the resting membrane is the main determinant of the decrease in E_{Na}^+. This is shown in figure 6-7 in which the data for the cytosolic ATP potential ($dG/d\xi_{ATP}$, open squares) was taken from Fiolet et al. [35] (rat heart). Similar to hypoxia, $dG/d\xi_{ATP}$ decreases immediately after the onset of ischemia to a plateau level of approximately 45 KJ/Mol until a secondary rapid decrease takes place after ten minutes (not shown). Arrow 1 points to the energy level E_{Na}^+, which was calculated from the measurements of a_{Na}^i and V_m by Kléber [5]. In essence, these measurements showed a depolarization from −80 mV to −52 mV after ten minutes, but no increase in intracellular Na^+ (former paragraph). The lowest curve in figure 6-7 represents E_{Na}^+ calculated from the assumption that the loss of intracellular K^+ is compensated by a gain of intracellular Na^+ (which is contradictory to our experimental findings). Arrow 2 symbolizes the additional decrease of E_{Na}^+ brought about by the increase of a_{Na}^i. Comparison of the curves in figure 6-7 leads to two conclusions: (1) Assessment of time course of $dG/d\xi_{ATP}$ during ischemia does not allow one to

make any predictions about the mechanism of cellular K^+ loss or about the change of $a_{Na}{}^i$. Both solutions for $E_{Na}{}^+$ shown in figure 6-7 are thermodynamically possible. (2) Even if the net cellular K^+ loss in ischemia were to be coupled to a gain in intracellular Na^+, the depolarization (associated with the K^+ loss) would always determine the main component of the decrease in $E_{Na}{}^+$ (75 percent in figure 6-7), because the membrane potential is relatively sensitive to small changes in extracellular K^+ and because any increase of intracellular Na^+ would be associated with a corresponding three-fold increase in extracellular K^+ (extracellular: intracellular volume ratio = 1:3; 21).

REFERENCES

1. Jennings, R.B., Hawkins, H.K., Lowe J.E., et al. 1978. Relation between high energy phosphate and lethal injury in myocardial injury in the dog. *Am. J. Pathol.* 92:187–214.
2. Hearse, D.J., Garlick, P.B., and Humphrey, S.M. 1977. Ischemic contracture of the myocardium: mechanisms and prevention. *Am. J. Cardiol.* 39:986–993.
3. Janse, M.J., and Kléber, A.G. 1981. Electrophysiological changes and ventricular arrhythmias in the early phase of regional myocardial ischemia. *Circ. Res.* 49:1069–1081.
4. Balschi, J.A., Frazer, J.C., Fetters, J.K., et al. 1985. Shift reagent and Na-23 NMR discriminates between extra- and intracellular sodium pools in ischemic heart. *Circulation* 72:(Suppl. 3) 355.
5. Kléber, A.G. 1983. Resting membrane potential, extracellular potassium activity and intracellular sodium activity during acute global ischemia in the isolated guinea pig heart. *Circ. Res.* 52:442–450.
6. Wilde, A.A.M., and Kléber, A.G. 1986. The combined effects of hypoxia, high K+, and acidosis on the intracellular sodium activity and resting potential in guinea pig papillary muscle. *Circ. Res.* 58:249–256.
7. Eisner, D.A., Lederer, W.J., and Vaughan-Jones, R.D. 1981. The effects of rubidium and membrane potential on the intracellular sodium activity of sheep Purkinje fibers. *J. Physiol. (Lond.)* 317:189–205.
8. January, C.T., and Fozzard, H.A. 1984. The effects of membrane potential, extracellular potassium, and tetrodotoxin on the intracellular sodium ion activity of sheep cardiac muscle. *Circ. Res.* 54:652–665.
9. Cohen, C.J., Fozzard, H.A., and Sheu, S.S. 1982. Increase in intracellular sodium ion activity during stimulation in mammalian cardiac muscle. *Circ. Res.* 50:651–662.
10. Ellis, D. and Noireaud, J. 1987. Intracellular pH in sheep Purkinje fibers and ferret papillary muscles during hypoxia and recovery. *J. Physiol. (Lond.)* 383:125–141.
11. Nakaya, H., Kimura, S., and Kanno, M. 1985. Extracellular potassium activity and intracellular sodium activity under hypoxia, acidosis, and no glucose in dog hearts. *Am. J. Physiol.* 249:H1078–H1085.
12. Guarnieri, T. 1987. Intracellular sodium-calcium dissociation in early contractile failure in hypoxic ferret papillary muscles. *J. Physiol. (Lond.)* 388:449–465.
13. Ellis, D. 1977. The effect of external cations and ouabain on the intracellular sodium activity of sheep Purkinje fibres. *J. Physiol. (Lond.)* 273:211–240.
14. Harris, A.S., Bisteni, A., Russell, R.A., et al. 1954. Excitatory factors in ventricular tachycardia resulting from myocardial ischemia. Potassium a major excitant. *Science* 119:200–203.
15. Hirche, H.J., Bös, F.L., Bissig, R. et al. 1980. Myocardial extracellular K^+ and H^+ increase and noradrenaline release as possible causes of early arrhythmias following acute coronary artery occlusion in pigs. *J. Mol. Cell Cardiol.* 12:579–593.
16. Hill, J.L. and Gettes, L.S. 1980. Effect of acute coronary artery occlusion on local myocardial K^+ activity in swine. *Circulation* 61:768–778.
17. Rau, E.E., Shine, K.I., and Langer, G.A. 1977. Potassium exchange and mechanical performance in anoxic mammalian myocardium. *Am. J. Physiol.* 232:H85–H94.

18. Rau, E.E. and Langer, G.A. 1978. Dissociation of energetic state and potassium loss from anoxic myocardium. *Am. J. Physiol.* 235:H537–H543.
19. Kline, R.P. and Morad, M. 1978. Potassium efflux in heart muscle during activity: extracellular accumulation and its implications. *J. Physiol. (Lond.)* 280:537–558.
20. Kunze, D.L. 1977. Rate-dependent changes in extracellular potassium in the rabbit atrium. *Circ. Res.* 41:122–127.
21. Polimeni P.I. 1974. Extracellular space and ionic distribution in rat ventricle. *Am. J. Physiol.* 227:676–683.
22. Weiss, J. and Shine, K.I. 1982. Extracellular K+ accumulation during myocardial ischemia in isolated rabbit heart. *Am. J. Physiol.* 242:H619–H628.
23. Kléber, A.G. 1984. Extracellular potassium accumulation in acute myocardial ischemia. *J. Mol. Cell Cardiol.* 16:389–394.
24. Kléber, A.G., Riegger, C.B., Janse, M.J. 1987. Extracellular K^+ and H^+ shifts in early ischemia: mechanisms and relation to changes in impulse propagation. *J. Mol. Cell Cardiol.* 19 (Suppl V):35–44.
25. Cohen, I.S., Kline, R.P., Pennefather, P., and Mulrine, N.K. 1987. Models of the Na-K pump in cardiac muscle predict the wrong intracellular Na+ activity. *Proc. R. Soc. Lond.* B231:371–382.
26. Gadsby, D.C. 1980. Activation of electrogenic Na^+/K^+ exchange by extracellular K^+ in canine cardiac Purkinje fibers. *Proc. Natl. Acad. Sci. USA* 77:4035–4039.
27. Garay, R.P. and Garrahan, P.J. 1975. The interaction of adenosinetriphosphate and inorganic phosphate with the sodium pump in red cells. *J. Physiol. (Lond.)* 249:51–67.
28. Bersohn, M.M., Philipson, K.D., and Fukushima. J.Y. 1982. Sodium-calcium exchange and sarcolemmal enzymes in ischemic rabbit hearts. *Am. J. Physiol.* 284:C288–C295.
29. Smith, G.L. and Allen, D.H. in press. The effetcs of metabolic blockade on intracellular calcium concentration in isolated ferret ventricular muscle. *Circ. Res.*
30. Allen, D.G. and Orchard, C.H. 1983. Intracellular calcium concentration during hypoxia and metabolic inhibition in mammalian ventricular muscle. *J. Physiol. (Lond.)* 339:107–122.
31. Steenbergen, C., Murphy, E., Levy, L., and London, R.E. 1987. Elevation in cytostolic free calcium concentration early in myocardial ischemia in perfused rat heart. *Circ. Res.* 60:700–707.
32. Kitakaze, M., Pike, M.M., Chacko, V.P., and Marban, E. 1987. Direct measurement of cytosolic free calcium during ischemia and reperfusion in ferret hearts. *Circulation* 76:(Suppl. IV) 381.
33. Loiselle, D.S. 1985. The rate of resting heat production of rat papillary muscle. *Pflügers Arch.* 405:155–162.
34. Kléber, A.G., Riegger, C.B., and Janse, M.J. 1987. Electrical uncoupling and increase of extracellular resistance after induction of ischemia in isolated, arterially perfused rabbit papillary muscle. *Circ. Res.* 61:271–279.
35. Fiolet, J.W.T., Baartscheer, A., Schumacher, C.A., et al. 1984. The change of free energy of ATP hydrolysis during global ischemia and anoxia in the rat heart. Its possible role in the regulation of transsarcolemmal sodium and potassium gradients. *J. Mol. Cell Cardiol.* 16:1023–1036.
36. Kammermeier, H., Schmidt, P., and Jüngling, E. 1982. Free energy change of ATP-hydrolysis: a causal factor of early hypoxic failure of the myocardium? *J. Mol. Cell Cardiol.* 14:267–277.

7. AMPHIPATHIC LIPID METABOLITES AND ARRHYTHMOGENESIS: A PERSPECTIVE

PETER B. CORR AND DAVID J. DOBMEYER

Sudden death associated with ischemic heart disease is primarily a result of an abnormality in cardiac rhythm leading to ventricular tachycardia and fibrillation [1]. A multitude of studies has carefully characterized the electrophysiologic abnormalities underlying these lethal arrhythmias in the ischemic heart. However, until the last decade little effort has been directed at characterizing the discrete, yet critical, alterations in the plasma membrane that elicit the alterations in electrophysiologic function. Although several antiarrhythmic agents are available and have been proposed for the prevention of sudden cardiac death in patients with ischemic heart disease, results of studies to date have been disappointing, probably because currently available agents are, for the most part, nonspecific membrane depressants. More specific and therefore more effective agents are likely to be available only through a thorough understanding of the specific biochemical events underlying the electrophysiologic alterations in the ischemic heart. For reasons outlined in this chapter, we have concentrated our efforts on two amphipathic metabolites, long-chain acylcarnitines and lysophosphatides. Recent data would suggest that manipulation of the activity of the enzymes controlling the synthesis and/or catabolism of these moieties can influence markedly the electrophysiologic alterations and resulting lethal arrhythmias in the ischemic heart. In this perspective, we will highlight recent findings in this area and suggest

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areas and pose questions requiring furture investigation. In particular, the discussion will pertain primarily to how changes in fatty acid and phospholipid metabolism in the ischemic heart disrupt the normal functions of the sarcolemma leading to electrophysiologic instability, arrhythmias, and sudden death. For a more in depth discussion of this area, the reader is referred to additional sources [2, 3].

All cells are encased in a phospholipid bilayer known as the cell membrane. This membrane performs a number of vital functions, such as separating a cell from its surrounding and serving as an anchor for intracellular and extracellular proteins. In myocytes, the cell membrane also serves the critical function of acting as a semipermeable barrier for the maintenance of electrochemical gradients necessary for the electrical excitability of these cells. Normal cellular electrophysiologic function, therefore, is dependent on the biochemical and biophysical properties of the phospholipid bilayer.

MYOCARDIAL FATTY ACID METABOLISM

Myocardial cells are among the most metabolically active cells. Not only must myocytes perform cellular maintenance functions, they must also expend large amounts of energy for sarcomere contraction and for the maintenance of electrochemical gradients across the sarcolemma. Since fatty acids provide more adenosine triphosphate (ATP) on a per mole basis than any other energy source, it is not surprising that myocytes preferentially metabolize fatty acids to meet their energy requirements.

Myocardial metabolism of fatty acids begins when free fatty acids are taken up by the myocyte [2]. Once inside the cell, the free fatty acid is bound to a fatty acid binding protein and transported to the mitochondria or, alternatively, incorporated into triglycerides or phospholipids. At the mitochondria, the fatty acid is then transesterified to coenzyme A (CoA) and in this form is able to cross the outer mitochondrial membrane. CoA esters of fatty acids (acyl CoA), however, are unable to cross the inner mitochondrial membrane for subsequent β-oxidation within the mitochondria (figure 7-1). To accomplish this transfer of acyl CoA, carnitine acyl transferase I, an enzyme on the outer portion of the inner mitochondrial membrane, transesterifies the fatty acid to carnitine, producing acylcarnitine. This long chain acylcarnitine is then transposed to the inside or matrix of the mitochondria by carnitine-acylcarnitine translocase. Here, the acyl group is transferred back to CoA by carnitine acyltransferase II and the fatty acyl CoA then undergoes β-oxidation with sequential removal of two carbon acetyl CoA groups. The acetyl CoA then enters the citric acid cycle to yield nicotinamide adenine dinucleotide, reduced (NADH) that enters the electron transport chain for oxidation to yield ATP. The overall process of fatty acid metabolism is very efficient in terms of energy production. For every molecule of palmitic acid (which contrains 16 carbon atoms), 131 molecules of ATP are produced.

The electron transport chain is oxygen dependent in that the terminal

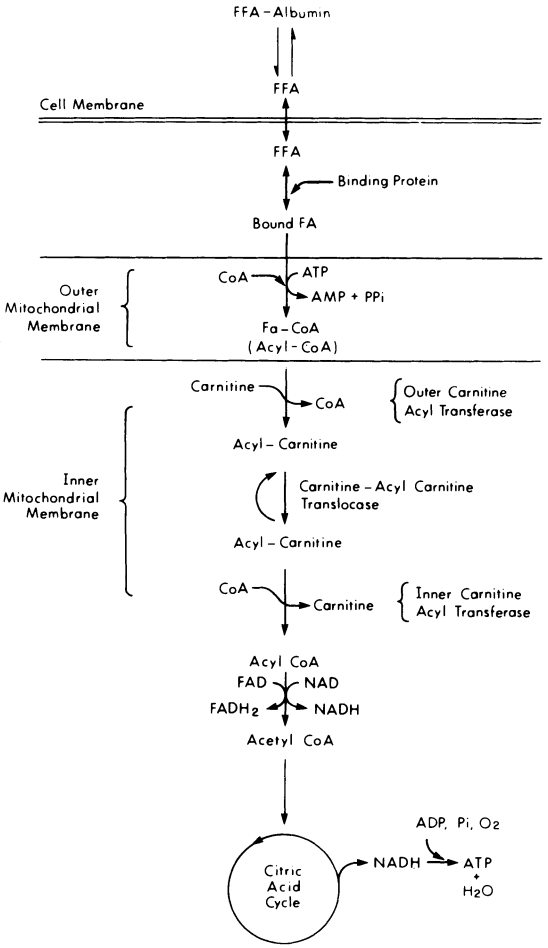

Figure 7-1. Diagrammatic representation of the esterification of free fatty acids and subsequent transport into, and β-oxidation within, mitochondria. Free fatty acids traverse the sarcolemmal membrane and subsequently activate CoA lipases on the outside of the outer mitochondrial membrane. Long-chain thioesters are transported across the inner mitochondrial membrane by sequential transesterification, resulting in the delivery of fatty acyl CoA to the mitochondrial matrix compartment. Fatty acyl CoA in the matrix compartment is oxidized by sequential enzyme reactions, resulting in reducing equivalents, which ultimately result in the production of ATP by oxidative phosphorylation. Reprinted from Corr et al. [2] by permission from the American Heart Association.

enzyme, cytochrome oxidase, must have oxygen present to catalyze the final reaction in the chain. During myocardial ischemia or, alternatively, profound hypoxia, a rapid cessation of function of this terminal enzyme occurs. This results in a rapid increase in the concentration of NADH and flavin adenine dinucleotide, reduced ($FADH_2$) and thus, a shift in the ratio of reduced-to-oxidized coenzymes. A substantial amount of data indicates that the change

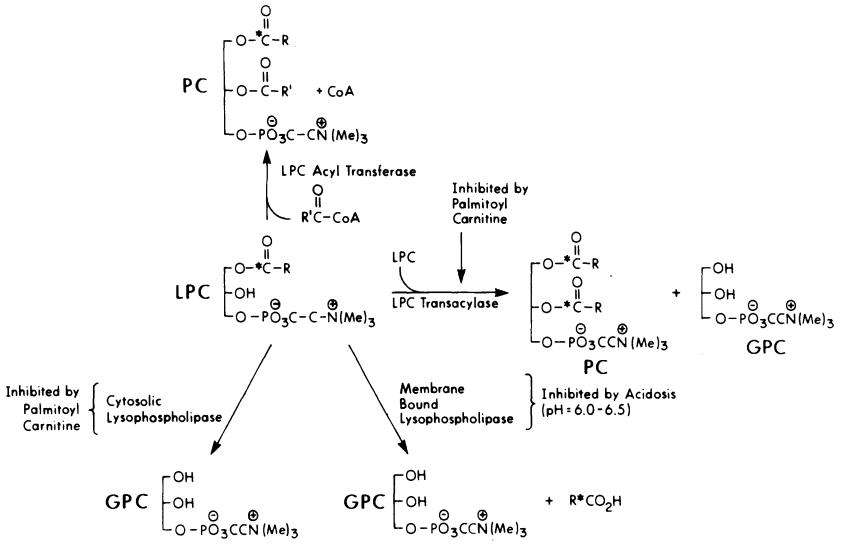

Figure 7-2. Enzymatic pathways for the catabolism of lysophosphatidyl choline (LPC). Clockwise from top: LPC acyl transferase reesterifies the LPC to phosphatidylcholine (PC). LPC transacylase, which is inhibited by palmitoyl carnitine, transfers the fatty acid side chain from one molecule of LPC to another producing PC and glycerophosphoryl (GPC) choline. Lysophospholipase cleaves the remaining fatty acid from LPC leaving GPC and free fatty acid (R^*CO_2H). The membrane bound form of lysophospholipase is inhibited by acidosis, and the cytosolic form is inhibited by palmitoyl carnitine.

in this ratio and increase in reduce NADH and $FADH_2$ effectively inhibits the β oxidation of fatty acids. The formation of long chain acylcarnitines and acyl CoA, however, does not diminish. As a result, levels of these two metabolites increase markedly during the initial minutes of oxygen deprivation. The acyl CoA tends to remain in the mitochondrial matrix, whereas long chain acylcarnitines distribute throughout the entire myocyte [4, 5] and, as will be discussed later, accumulate in the sarcolemma.

MYOCARDIAL PHOSPHOLIPID METABOLISM

Phospholipids are found in all cell membranes and in all organelles encased in a lipid bilayer. Phospholipids are classified according to the chemical moiety on the polar head group. Many chemical species exist, but in myocardial cells, phosphatidyl choline predominates followed by decreasing amounts of phosphatidyl ethanolamine, serine, and inositol.

All diacyl phospholipids (e.g., phosphatidyl choline) contain two fatty acid side chains linked by ester linkages to the sn-1 and sn-2 carbons of glycerol (figure 7-2). In the case of phosphatidyl choline, the sn-3 carbon atom of glycerol is linked to the phosphocholine residue by a phosphoester. Most

commonly, the fattty acid on the sn-1 carbon of glycerol is saturated, and the sn-2 carbon fatty acid is unsaturated.

The combined hydrophilic (polar head group) and hydrophobic (fatty acid side chain) structure of phospholipids makes them suitable for the formation of lipid bilayers in aqueous solutions. In these bilayers, phospholipids are arranged such that their fatty acyl side chains (hydrophobic portion) point in toward one another, whereas the polar head groups (hydrophilic portion) are oriented toward the extracellular or intracellular aqueous environment. This combination of hydrophilic and hydrophobic domains also lends itself to the integration of proteins within and across the phospholipid bilayer. These proteins include receptors, ion channels, and ion pumps, all of which are necessary for the normal electrophysiologic activity of myocytes.

Phospholipid catabolism is complex and, like fatty acid metabolism, can be profoundly affected by ischemia. Breakdown of phospholipids is affected by a diverse group of enzymes known as phospholipases. One of these phospholipases, phospholipase A_2 (PLA_2), catalyzes the cleavage of the sn-2 fatty acid from diacyl phospholipids. As in the case of phosphatidylcholine, the products of this reaction are a molecule of free fatty acid and a molecule of lysophosphatidylcholine (LPC). LPC is capable of being degraded by a number of enzymes (see Corr et al. [2]). One of these catabolic enzymes is lysophospholipase, which cleaves the remaining sn-1 fatty acid, leaving a molecule of free fatty acid and a molecule of glycerophosphorylcholine (GPC). In addition to lysophosphlipase there exist, lysophospholipase-transacylases, which transfer the fatty acid from one molecule of LPC to another leaving a molecule of GPC and a molecule of phosphatidylcholine.

Under normal conditions, LPC is present only in small quantitites within cells because of the overwhelming capacity of the catabolic compared to the synthetic enzymes. During ischemia, however, several events occur that can permit, under the right conditions, the accumulation of LPC. First, local catecholamine release occurs. This release has the capacity to stimulate PLA_2 activity through a β-adrenergic receptor linked mechanism [6]. Second, acidosis to a pH level of 6.5, similar to levels seen during ischemia in vivo, reduces the activity of membrane-bound lysophospholipase by about 88 percent [7]. Third, accumulation of long chain acylcarnitines has been shown to inhibit markedly cytosolic lysophospholipase transacylase [8], as well as cytosolic lysophospholipase [9]. Therefore, during ischemia, a complex series of events combine and intermingle to cause the accumulation of both long chain acylcarnitines and lysophosphatides.

Long chain acylcarnitines and lysophosphatides bear a remarkable structural similarity to one another (figure 7-3). Both are termed amphipathic, which designates that these compounds possess both a hydrophilic region, the carnitine or phosphocholine groups, and a hydrophobic region, the fatty acid side chain. Hence, these compounds will tend to accumulate in phospholipid bilayers, including the sarcolemma. Since these amphipathic metabolites

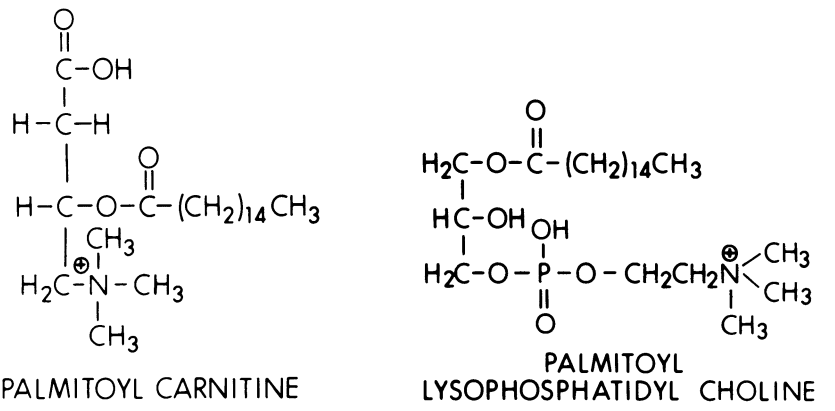

Figure 7-3. A comparison of the structures of long-chain acylcarnitine and lysophosphatidyl choline. Reproduced from Corr et al. [10] with permission of the American Heart Assoication.

contain only one fatty acid, they possess very different physical characteristics compared to diacyl phospholipids. Diacyl phospholipids have a sterically bulky unsaturated fatty acid in the sn-2 postion, which adds "stiffness" to the molecule. The absence of this fatty acid, as in the case of LPC, permits more degrees of freedom of movement of these metabolites in the phospholipid bilayer. Similar effects are seen when long chain acylcarnitines accumulate in the lipid bilayer. This, in effect, increases the fluidity of the membrane, thereby altering its physical characteristics. This change is then translated to integral membrane proteins affecting their normal function, and in the case of myocytes, affects the electrophysiologic properties of the cell.

BASIC ELECTROPHYSIOLOGIC EFFECTS OF LONG CHAIN ACYLCARNITINES AND LYSOPHOSPHATIDES

Long chain acylcarnitines, when superfused over isolated tissue in vitro, elicit significant and reversible electrophysiologic alterations including a marked reduction in $\dot{V}_{max}$ of phase 0, resting membrane potential, and action potential duration [10]. In addition, these changes are enhanced by a decrease in pH to 6.5, a level consistent with that seen during myocardial ischemia in vivo [10]. These alterations are very similar to that seen in the ischemic heart in vivo. However, exogenously applied long chain acylcarnitines do not mimic those found in vivo since the metabolite is never found extracellularly until irreversible cell damage has occurred. To obviate this difficulty and to evaluate the effects of endogenously produced long chain acylcarnitines, myocytes were grown in culture with ^{3}H-carnitine. This labeled carnitine was taken up by the myocytes and was distributed equally throughout all carnitine pools including the free, short and long chain acylcarnitine fractions [11]. This permited analysis and movement of carnitine metabolism in the cells using

electron microscopic (EM) autoradiography. By using the differential acid solubilites of the carnitine species during tissue preparation for EM autoradiography, over 99 percent of the free and short chain acylcarnitines (acid soluble) was removed, leaving the long chain acylcarnitine (acid insoluble) fraction essentially intact. Autoradiograms then demonstrated the subcellular location of the remaining labeled long chain acylcarnitine, the species of interest [11].

Under normoxic conditions, long chain acylcarnitines composed only 2 to 3 percent of total cellular carnitine and this amount was located primarily in the mitochondrial membrane. After ten minutes of severe hypoxia ($PO_2 \leq$ 10 mm Hg), there was a fourfold to fivefold increase in the total cellular long-chain acylcarnitine fraction, with a more than 70-fold increase in the sarcolemmal [11]. The electrophysiologic derangements accompanying hypoxia, namely a decrease in $\dot{V}_{max}$ of phase 0, a fall in the resting membrane potential, and a decrease in action potential duration, paralleled the sarcolemmal accumulation of long chain acylcarnitines. When the cells were pretreated with sodium-2-[5-(4-chlorophenyl)-pentyl]-oxirane-2-carboxylate (POCA), an irreversible inhibitor of carnitine acyltransferase I, not only was the accumulation of long chain acylcarnitines prevented, but the electrophysiologic changes previously induced by hypoxia were markedly attenuated [11].

During myocardial ischemia, several important differences exist between the accumulation of LPC and the accumulation of long chain acylcarnitines. LPC increases substantially in both the venous [12] and lymphatic drainage [13] from ischemic tissue. This increase occurs within minutes of the onset of ischemia and is believed to represent an accumulation of this metabolite in the extracellular space. Hence, exogenous delivery of this metabolite to myocytes may more closely mimic the pathophysiologic processes occurring in vivo. Indeed, the intracellular delivery of LPC in concentrations up to 500 μM has no adverse electrophysiologic consequences in isolated tissue [13].

Delivery of exogenous LPC to myocardial tissue has demonstrated a number of interesting findings. LPC, when superfused over isolated tissue, also results in decreases in $\dot{V}_{max}$ of phase 0, as well as a marked decrease in the resting membrane potential and action potential duration [10, 14, 15]. These changes are greatly enhanced in the presence of acidosis without increased cellular accumulation [10, 12, 16]. The reduction of resting membrane potential elicited by LPC in ventricular muscle appears to be due to a decrease in outward potassium conductance [17]. Since potassium conductance appears to be enhanced during ischemia, contributing in part to the increase in extracllular potassium, the question remains whether the effect of LPC in ischemic cells would elicit the same response. One explanation for the increase in extracellular potassium during ischemia involves stimulation of the ATP-dependent K^+ channel due to loss of cellular ATP at the membrane surface [18]. However, since the level of ATP required to inhibit this channel

is quite low, an alternate explanation could involve an interaction of LPC and/or long chain acylcarnitines with this ATP-dependent K^+ channel, particularly as the ATP level falls. In addition to these electrophysiologic effects, LPC has also been shown to induce delayed afterdepolarizations, which are magnified by catecholamines and increased extracellular Ca^{2+}, and occur even in the presence of acidosis and an increase in extracellular K^+, known to modify or prevent the development of delayed afterdepolarizations in other systems [19]. This finding may be critical in the understanding of the mechanism of lethal arrhythmias in the ischemic heart since nonreentrant mechanisms, potentially mediated by delayed afterdepolarizations leading to triggered rhythms, have been demonstrated to contribute to arrhythmias associated with both myocardial ischemia [20] and reperfusion [21].

Radiolabeled tracer studies, combined with EM autoradiography, have shown that the electrophysiologic effects of LPC are present when as little as 1 mole % or 1 nmol/mg protein is incorporated into the sarcolemma, values easily achieved during ischemia in vivo [16, 22]. Similar to long chain acyl-carnitines, LPC incorporates preferentially into cellular membrane structures, particularly the sarcolemma. Reversal of the electrophysiologic derangements induced by exogenous LPC occur with washout and catabolism of the amphiphile [16].

POTENTIAL MEDIATORS OF THE ELECTROPHYSIOLOGIC EFFECTS OF BOTH AMPHIPHILES

Amphipathic metabolites are known to have a deleterious effect on normal cellular ion channel function [2]. For example, LPC has been shown to markedly increase intracellular Ca^{2+} levels [23] and to increase inotropy, but appears to actually attenuate the voltage-dependent inward current carried by Ca^{2+} [17]. In isolated rat myocytes perfused with Krebs solution containing LPC for only five minutes, total intracellular Ca^{2+} increased as determined by measurements of $^{45}Ca^{2+}$, as well as atomic absorption spectroscopy [23]. Prolonged perfusion of the myocytes for 15 minutes with the LPC resulted in massive intracellular accumulation of Ca^{2+} and irreversible cell damage [23]. One possibility is that the increase in intracellular Ca^{2+} is occurring through nonspecific membrane alterations unassociated with changes in normal calcium channel function. Another possibility is that since LPC can inhibit Na^+-K^+ ATPase [24], the resulting accumulation of intracellular Na^+ may stimulate Na^+-Ca^{2+} exchange, which can lead to an increase in intracellular Ca^{2+} [25]. Therefore, increases in intracellular Ca^{2+} in response to accumulation of LPC in the sarcolemma may not only contribute to malignant ventricular arrhythmias, but may also potentiate ischemic injury by enhancing Ca^{2+} influx.

Long chain acylcarnitines have been shown to activate Ca^{2+} channels in cardiac tissue [26] leading to an actual increase in the voltage-dependent Ca^{2+} inward current [27]. Similar activation of Ca^{2+} channels occurs in neural

tissue in response to membrane insertion of long chain acylcarnitines [28]. Recently, we have shown that insertion of exogenous or endogenous long chain acylcarnitines into the sarcolemma actually increases the number of α_1-adrenergic receptors [29]. In isolated adult canine myocytess, we have shown that ten minutes of hypoxia resulted in a threefold increase in total cellular long chain acylcarnitines. This increase was reversible and was not associated with irreversible cellular damage as assessed by morphologic criteria and intracellular enzyme release. Determination of the number of α_1-adrenergic receptors on the surface of these myocytes revealed that the receptor number also increased twofold to threefold in response to hypoxia [29]. Furthermore, inhibition of carnitine acyltransferase I with POCA not only prevented the accumulation of long chain acylcarnitines, but also prevented the increase in α_1-adrenergic receptors in response to hypoxia [29]. Even more recent data indicate that these receptors are coupled to an increase in inositol trisphosphate (IP_3), based on mass measurements. The increase in IP_3 occurs in response to catecholamine stimulation of this receptor and may also stimulate release of intracellular Ca^{2+} directly. Alternatively, concomitant production of diacylglycerol, which stimulates protein kinase C, may enhance the transsarcolemmal movement of Ca^{2+} secondary to phosphorylation of one or more membrane proteins. The significance of these findings is underscored by the fact that α_1-adrenergic responsivity actually increases in ischemic myocardium in vivo [30], associated with an increase in α_1-adrenergic receptors [31]. Likewise, several studies have shown that α_1-adrenergic blockade is antiarrhythmic in the ischemic heart (see Corr et al. [32]). However, a true cause-effect relationship between acylcarnitine accumulation in the sarcolemma and the exposure of the α_1-adrenergic receptor in vivo during ischemia has not been demonstrated. By the same token, whether stimulation of the exposed α_1-adrenergic receptor contributes to the recently recognized increase in intracellular Ca^{2+} during ischemia [33] has not been evaluated.

ARRHYTHMOGENIC EFFECTS OF LPC AND LONG CHAIN ACYLCARNITINES DURING ISCHEMIA IN VIVO

Studies in vivo have demonstrated the importance of both of these metabolites in the genesis of malignant ventricular arrhythmias during myocardial ischemia. That LPC accumulates rapidly in ischemic myocardium was demonstrated in cats with regional myocardial ischemia produced by proximal occlusion of the left anterior descending coronary artery [34]. At the end of three minutes of ischemia or the initiation of ventricular fibrillation, high speed fast-frozen transmural biopsies were obtained from both the anterior (ischemic) and posterior (nonischemic) zones of the left ventricle. In sham ligated control animals, both ischemic and nonischemic zones exhibited a basal level of LPC of 2.2 nM/mg protein. In those animals that developed ventricular fibrillation with an ischemic interval of $\leq$2.2 minutes, the ische-

mic zone content of LPC had increased to 3.5 nM/mg protein. Those animals that did not demonstrate arrhythmias demonstrated no increase in LPC in the ischemic zone, whereas those that had arrhythmias without ventricular fibrillation, including multifocal premature ventricular complexes, demonstrated an intermediate increase in the level of LPC in the ischemic zone to 2.7 nM/mg protein [34]. This increase in LPC content of 0.5 nM/mg protein correlates closely with the amount of exogenous LPC required to induce electrophysiologic alterations in vitro based on measurements of radiolabeled LPC with EM autoradiography [16, 22].

To evaluate the interrelationship between LPC and long chain acylcarnitines in vivo, we used an identical animal preparation with high speed biopsies obtained from both the ischemic and nonischemic zones of the left ventricle. Similar to the study noted here, five minutes of ischemia induced an even further increase in LPC content in the ischemic zone. Additionally, long chain acylcarnitines also demonstrated a four-fold increase in the ischemic compared to the nonischemic zone [35]. When animals were pretreated with POCA, an inhibitor of carnitine acyltransferase I, a number of critical findings were noted. First, POCA completely inhibited the ischemia induced increase in not only long chain acylcarnitines, but also the increase in LPC [35, 36]. Second, animals treated with POCA failed to demonstrate malignant ventricular arrhythmias in response to ischemia, in contrast to control untreated animals. Third, blood flow maps using radiolabeled microspheres confirmed that the antiarrhythmic and biochemical effects of POCA were not associated with an increase in blood flow to the ischemic region or with significant improvement in the hemodynamic response to ischemia during normal sinus rhythm. Therefore, the salutory effects of POCA were not due to a diminution of ischemia, but were likely secondary to the prevention of accumulation of amphipathic metabolites by inhibition of carnitine acyltransferase I. The most likely explanation for the effect of POCA to prevent the accumulation of LPC is that in the absence of long chain acylcarnitines, the normal catabolism of LPC is facilitated.

This summary of recent in vivo findings provides further evidence to suggest that both amphipathic metabolites, LPC and long chain acylcarnitines, contribute substantially to the deleterious electrophysiologic effects induced by ischemia. The mechanism likely involves accumulation of the amphiphile in the sarcolemma with subsequent disruption of membrane protein function. LPC and long chain acylcarnitines have both been shown to accumulate in ischemic myocardium in amounts that are capable of eliciting electrophysiologic alterations in vitro. In addition, both metabolites accumulate over a time course, within minutes after the onset of ischemia, consistent with the onset of malignant ventricular arrhythmias in vivo. Most important, the inhibition of accumulation of these metabolites attenuates the electrophysiologic effects of ischemia in vitro and modifies arrhythmias associated with ischemia in vivo.

FUTURE DIRECTIONS

Although a substantial amount of experimental evidence supports the causal role of these amphipathic metabolites in the genesis of arrhythmias during ischemia, critical gaps remain in our understanding of several aspects of this hypothesis. As discussed earlier, both LPC and long chain acylcarnitines are very similar structurally, and both produce similar, yet not identical, electrophysiologic effects in vitro. The marked differences in the two agents on the voltage-dependent, inward current carried by Ca^{2+} likely influence their role in arrhythmogenesis. Indeed, the exact mechanism of action of these compounds relative to their effects on membrane function has yet to be delineated. One potential approach would be to use whole cell or patch clamping procedures in which individual ionic channels could be evaluated relative to the effect of exogenously applied or endogenously generated metabolites. Special emphasis should be focused on channels that would have the ability to increase intracellular Ca^{2+} or extracellular K^{+}.

The accumulation of intracellular Ca^{2+} in myocytes during ischemia may also be mediated by mechanisms other than sarcolemmal ion channels. As previously disscussed, myocyte sarcolemmal accumulation of long chain acylcarnitines in response to severe hypoxia results in a three-fold increase in α_1-adrenergic receptors. Recently, these receptors have been shown to be coupled to the modulation of intracellular processes via the hydrolysis of phosphoinositides. Both the hydrolysis products, inositol trisphosphate (IP_3) and diacylglycerol, are capable of causing increases in intracellular Ca^{2+}, the former by stimulating release from the sarcoplasmic reticulum and the latter by stimulating protein kinase C. However, the influence of these mechanisms on arrhythmogenesis in vivo has not been delineated.

Although the site of endogenous production of long chain acylcarnitines has been localized to the myocyte, the site of production of LPC has not been elucidated. LPC is found in high concentrations in lymph and venous blood draining ischemic regions in vivo. These observations suggest that LPC accumulates in the extracellular space in close proximity to the sarcolemma. Although LPC may be produced in myocytes and released into the interstitial space, LPC release from nonmyocytic sources remains a distinct possibility. Possible extramyocytic sources of LPC include endothelial cells, smooth muscle cells, and formed blood cell elements. In an alternative fashion, biochemical modulators acting between cells could have a profound affect on the accumulation of metabolites in the myocardium. Eicosanoids may be important in this respect since leukotriene D_4 (LTD_4) has been shown to increase PLA_2 activity specific for PC in bovine endothelial cells [37] and may contribute to the release of LPC, particularly under conditions such as ischemia, during which catabolism is inhibited. Whether this or similar mechanisms are operative in the ischemic heart remains a possibility.

The accumulation of LPC and long chain acylcarnitines is likely inter-

dependent to some degree. At least one catabolic enzyme of LPC is inhibited by increasing concentrations of long chain acylcarnitines. Additionally, PLA_2, the synthetic enzyme of LPC, is a Ca^{2+}-dependent enzyme. It is possible that the effect of long chain acylcarnitine is to expose the α_1-adrenergic receptor and thereby to modulate the intracellular Ca^{2+} concentration. This may actually enhance the production of LPC secondary to alterations in the activity of PLA_2. To some extent, this mechanism has been shown to exist in pituitary cells where α_1-adrenergic stimulation markedly increases the activity of PLA_2 [38].

The most important future endeavors will need to center on the identification of a clinically useful inhibitor of carnitine acyltransferase I. As was previously noted, POCA, the irreversible inhibitor of the enzyme, prevented the increase in both long chain acylcarnitines and LPC in ischemic myocardium and produced a profound beneficial effect on arrhythmias induced by ischemia. It is possible that similar pharmacologic manipulation in humans would have a major impact on sudden death associated with ischemic heart disease. Potential inhibitors of carnitine acyltransferase I should be reversible and ideally would only be activated in ischemic cells. Modification of the drug to make it sensitive to an acidic environment or become active during changes in the redox potential are, of course, two possibilities.

Although inhibitors of carnitine acyltransferase I would appear to be a promising new approach, other new pharmacologic maneuvers may also be beneficial. It is theoretically possible to inhibit PLA_2 activity, thus limiting the ischemia induced rise in LPC. However, this pharmacologic maneuver may have no effect on the myocardial accumulation of long chain acylcarnitines.

In summary, although we have described recent findings with two amphipathic metabolites that appear to contribute to the electrophysiologic abnormalities and associated malignant arrhythmias in the ischemic heart, there is little doubt that other biochemical alterations contribute as well. Only by detailed characterization of the underlying alterations in membrane function in response to ischemia will improved pharmacologic approaches to their prevention be forthcoming.

REFERENCES

1. Armstrong, A., Duncan, B., Oliver, M.F., et al. 1972. Natural history of acute coronary heart attacks. A community study. *Br. Heart J.* 34:67–80.
2. Corr, P.B., Gross, R.W., and Sobel, B.E. 1984. Amphiphathic metabolites and membrane dysfunction in ischemic myocardium. *Circ. Res.* 55:135–154.
3. Corr, P.B., Saffitz, J.E., and Sobel, B.E. 1987. What is the contribution of altered lipid metabolism to arrhythmogenesis in the ischemic heart. In Life-threatening Arrhythmias During Ischemia and Infarction, D.J. Hearse, A.S. Manning, and M.J. Janse eds., pp. 91–114, New York, Raven Press.
4. Idell-Wenger, J.A., Grotyohann, L.W., and Neely, J.R. 1978. Coenzyme A and carnitine distribution in normal and ischemic hearts *J. Biol. Chem.* 253:4310–4318.
5. Liedtke, A.J., Nellis, S., and Neely, J.R. 1978. Effects of excess free fatty acids on

mechanical and metabolic function in normal and ischemic myocardium in swine. *Circ. Res.* 43:652–661.
6. Franson, R.C., Pang, D.C., and Weglicki, W.B. 1979. Modulation of lipolytic activity in isolated canine cardiac sarcolemma by isoproterenol and propranolol. *Biochem. Biophys. Res. Commun.* 90:956–962.
7. Gross, R.W. and Sobel, B.E. 1982. Lysophosphatidylcholine metabolism in the rabbit heart. Characterization of metabolic pathways and partial purification of myocardial lysophospholipase-transacylase. *J. Biol. Chem.* 257:6702–6708.
8. Gross, R.W., Drisdel, R.C., and Sobel, B.E. 1983. Rabbit myocardial lysophospholipase-transacylase: purification, characterization, and inhibition by endogenous cardiac amphiphiles. *J. Biol. Chem.* 258:15165–15172.
9. Gross, R.W. 1983. Purification of rabbit myocardial cytosolic acyl CoA hydrolase, identity with lysophospholipase and modulation of enzymatic activity by endogenous cardiac amphiphiles. *Biochemistry* 22:5641–5646.
10. Corr, P.B., Snyder, D.W., Cain, M.E., et al. 1981. Electrophysiological effects of amphiphiles on canine Purkinje fibers: implications for dysrhythmia secondary to ischemia. *Circ. Res.* 49:354–363.
11. Knabb, M.T., Saffitz, J.E., Corr, P.B., and Sobel, B.E. 1986. The dependence of electrophysiological derangements on accumulation of endogenous long-chain acylcarnitine in hypoxic neonatal rat myocytes. *Circ. Res.* 58:230–240.
12. Snyder, D.W., Crafford, W.A., Glashow, J.L., et al. 1981. Lysophosphoglycerides in ischemic myocardium effluents and potentiation of their arrhythmogenic effects. *Am. J. Physiol.* 241 (*Heart Circ. Physiol.* 10): H700–H707.
13. Akita, H., Creer, M.H., Yamada, K.A., et al. 1986. The electrophysiologic effects of intracellular lysophosphoglycerides and their accumulation in cardiac lymph with myocardial ischemia in dogs. *J. Clin. Invest.* 78:271–280.
14. Corr, P.B., Cain, M.E., Witkowski, F.X., et al. 1979. Potential arrhythmogenic electrophysiological derangements in canine Purkinje fibers induced by lysophosphoglycerides. *Circ. Res.* 44:822–832.
15. Arnsdorf, M.F. and Sawicki, G.J. 1981. The effects of lysophosphatidyl choline, a toxic metabolite of ischemia, on the components of cardiac excitability in sheep Purkinje fibers. *Circ. Res.* 49:16–30.
16. Gross, R.W., Corr, P.B., Lee, B.I., et al. 1982. Incorporation of radiolabeled lysophosphatidyl choline into canine Purkinje fibers and ventricular muscle: Electrophysiological, biochemical and autoradiographic correlations. *Circ. Res.* 51:27–36.
17. Clarkson, C.W., and Ten Eick, R.E. 1983. On the mechanism of lysophosphatidyl choline-induced depolarization of cat ventricular myocardium. *Circ. Res.* 52:543–546.
18. Weiss, J.N. and Lamp, S.T. 1987. Glycolysis preferentially inhibits ATP-sensitive K^+ channels in isolated guinea pig cardiac myocytes. *Science* 238:67–69.
19. Pogwizd, S.M., Onufer, J.R., Kramer, J.B., et al. 1986. Induction of delayed afterdepolarizations and triggered activity in canine Purkinje fibers by lysophosphoglycerides. *Circ. Res.* 59:416–426.
20. Pogwizd, S.M. and Corr, P.B. 1987. Reentrant and nonreentrant mechanisms contribute to arrhythmogenesis during early myocardial ischemia: Results using three-dimensional mapping. *Circ. Res.* 61:352–371.
21. Pogwizd, S.M. and Corr, P.B., 1987. Electrophysiologic mechanisms underlying arrhythmias due to reperfusion of ischemic myocardium. *Circulation* 76:404–426.
22. Saffitz, J.E., Corr, P.B., Lee, B.I., et al. 1984. Pathophysiologic concentrations of lysophosphoglycerides quantified by electron microscopic autoradiography. *Lab. Invest.* 50:278–286.
23. Sedlis, S.P., Corr, P.B., Sobel, B.E., and Ahumada, G.G. 1983. Lysophosphatidyl choline potentiates Ca^{++} accumulation in rat cardiac myocytes. *Am. J. Physiol.* 244 (*Heart Circ. Physiol.* 13):H32–H38.
24. Karli, J.N., Karikas, G.A., Hatzipavlou, P.K., et al. 1979. The inhibition of Na^+ and K^+ stimulated ATPase activity of rabbit and dog sarcolemma by lysophosphatidyl choline. *Life Sci.* 24:1868–1876.
25. Barcenas-Ruiz, L., Beuckelmann, D.J., and Wier, W.G. 1987. Sodium-calcium exchange in heart: Membrane currents and changes in $[Ca^{2+}]_i$. *Science* 238:1720–1722.

26. Spedding, M. and Mir, A.K. 1987. Direct activation of Ca^{++} channels by palmitoyl carnitine, a putative endogenous ligand. *Br. J. Pharmacol.* 92:457–468.
27. Inoue, D. and Pappano, A.J. 1983. L-Palmitylcarnitine and calcium ions act similarly on excitatory ionic currents in avian ventricular muscle. *Circ. Res.* 52:625–634.
28. Spedding, M. 1985. Activators and inactivators of Ca^{++} channels: New perspectives. *J. Pharmacol (Paris)* 16:319–343.
29. Heathers, G.P., Yamada, K.A., Kanter, E.M., and Corr, P.B. 1987. Long-chain acylcarnitines mediate the hypoxia induced increase in alpha$_1$-adrenergic receptors on adult canine myocytes. *Circ. Res.* 61:735–746.
30. Sheridan, D.J., Penkoske, P.A., Sobel, B.E., and Corr, P.B. 1980. Alpha-adrenergic contributions to dysrhythmias during myocardial ischemia and reperfusion in cats. *J. Clin. Invest.* 65:161–171.
31. Corr, P.B., Shayman, J.A., Kramer, J.B., and Kipnis, R.J. 1981. Increased α-adrenergic receptors in ischemic cat myocardium: A potential mediator of electrophysiological derangements. *J. Clin. Invest.* 67:1232–1236.
32. Corr, P.B., Yamada, K.A., and Witkowski, F.X. 1986 Mechanisms controlling cardiac autonomic function and their relation to arrhythmogenesis. In *The Heart and Cardiovascular System. Scientific Foundations,* H.A. Fozzard, E. Haber, R.B. Jennings, et al., eds., pp. 1343–1403, New York, Raven Press.
33. Lee, H.C., Smith, N., Mohabir, R., and Clusin, W.T. 1987. Cytosolic calcium transients from the beating mammalian heart. *Proc. Natl. Acad. Sci. USA* 84:7793–7797.
34. Corr, P.B., Yamada, K.A., Creer, M., et al. 1987. Lysophosphoglycerides and ventricular fibrillation early after onset of ischemia. *J. Mol. Cell. Cardiol.* 19 (Suppl. V):43–53.
35. Creer, M.H., Sobel, B.E., Saffitz, J.E., et al. 1987. Prevention of accumulation of acylcarnitine, lysophosphatides, and ventricular fibrillation by inhibition of carnitine acyltransferase I in ischemic hearts. *Circulation* 76 (Suppl. IV):IV–111.
36. Creer, M.H., Knabb, M.T., Pogwizd, S.M., et al. 1986. Antiarrhythmic effect of inhibition of accumulation of long chain acylcarnitines with ischemia. *Circulation* 74 (Suppl. II):II–67.
37. Clark, M.A., Littlejohn, D., Conway, T.M., et al. 1986. Leukotriene D_4 treatment of bovine aortic endothelial cells and murine smooth muscle cells in culture results in an increase in phospholipase A_2 activity. *J. Biol. Chem.* 261:10713–10718.
38. Ho, A.K. and Klein, D.C. 1987. Activation of α_1-adrenoceptors, protein kinase C or treatment with intracellular free Ca^{++} elevating agents increases pineal phospholipase A_2 activity. *J. Biol. Chem.* 262:11764–11770.

8. FREE RADICALS AND MYOCARDIAL INJURY DURING ISCHEMIA AND REPERFUSION: A SHORT-LIVED PHENOMENON?

DAVID J. HEARSE

> Any expanding field attracts the charlatans, such as those who make money out of proposing that consuming radical scavengers will make you live for ever or that taking tablets containing superoxide dismutase will enhance your health and sex life. In evaluating these and other less-obviously silly claims, it is useful to understand the basic chemistry of radical reactions.
> HALLIWELL AND GUTTERIDGE [1]

In their excellent book *Free Radicals in Biology and Medicine*, Halliwell and Gutteridge [5] give a clear exposition of the basic chemistry of free radicals and convince the reader that these intermediates can play a major role in the pathophysiology of tissue injury in many organs and many disease processes. In the field of toxicology, agents such as paraquat, carbon tetrachloride, adriamycin, and alloxan, together with ionizing radiation and a whole range of chemical carcinogens (including those in cigarette smoke), have been shown to exert their deleterious effects through the production of free radicals. Free radicals have been shown to play a major role in the natural history of aging, inflammatory diseases such as rheumatoid arthritis, and various autoimmune conditions.

THE NATURE OF FREE RADICALS

Halliwell and Gutteridge [1] define a free radical as "any species capable of independent existence that contains one or more unpaired electrons." Free radicals of many elements, such as carbon and nitrogen, can exist, but the focus of the present chapter is on *oxygen-derived free radicals.*

Most of the molecular oxygen consumed in aerobic biologic systems is tetravalently reduced to water. However, in certain instances univalent re-

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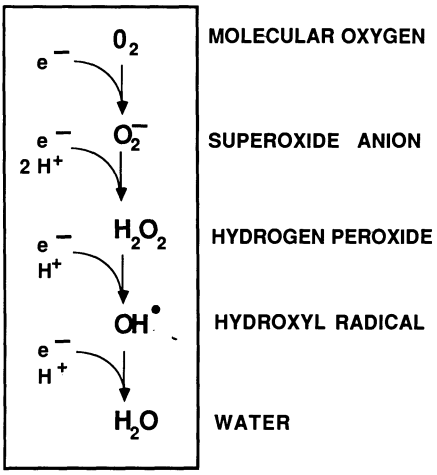

Figure 8-1. The stepwise univalent reduction of molecular oxygen to water.

duction, via single electron transfer, can occur (figure 8-1). In this way molecular oxygen is first reduced to the superoxide free radical (O_2^-); this can be further reduced to hydrogen peroxide (H_2O_2), which itself can be reduced to the hydroxyl radical ($OH^\cdot$). By virtue of their unpaired electrons, these intermediates can be highly reactive, particularly the hydroxyl radical, which reacts with very high rate constants (10^6 to 10^{11} M^{-1} s^{-1}) with almost all biologic compounds [1]. This high reactivity means that many radicals have extremely short half-lives and as such can be exceedingly difficult to detect by anything other than indirect means. Radicals, such as $OH^\cdot$, can interact with, and thus damage, nucleic acids, enzymes, proteins, polysaccharides, and lipids. One of the best known effects of free radicals relates to their effects on membranes where they are capable of initiating chain reactions of lipid peroxidation and damaging the protein complexes of various ion pumps. Either of these effects would be expected to lead to dramatic changes in membrane fluidity, permeability, and function.

Since free radicals are produced continuously by all systems under both normal and pathologic conditions (see below) it is relevant to ask how the cell survives the onslaught of these damaging intermediates. The answer is through the evolutionary development of powerful antioxidant systems with enzymes, such as superoxide dismutase, catalase, and peroxidase, together with organic antioxidants, such as vitamins E and C, all of which are strategically located throughout the cell to scavenge or remove the radicals as soon as they are formed.

Free Radicals and Tissue Injury

Realization that free radicals, although short-lived, may play a crucial role during ischemia and reperfusion owes much to the work of Granger, Parks and colleagues in Alabama [2]. In their many studies of intestinal injury, they drew attention to the possibility that oxygen-derived free radicals may be responsible for the tissue injury associated with ischemia and reperfusion. Their studies focused particularly on the enzyme xanthine oxidase as an important source of superoxide, work that was complemented by the studies of McCord and Fridovitch [3] who did much to enhance our understanding of the importance of xanthine oxidase and the toxic effects of oxygen-derived free radicals. As testified by many reviews [1–8], a massive literature accumulated throughout the 1970s and 1980s to implicate oxygen radicals in ischemia-reperfusion injury in many organs including the gut, brain, lung, liver, pancreas, and skin. From these studies emerged the concept that "anti-free radical interventions" may be used to exert beneficial therapeutic effects.

FREE RADICALS AND THE HEART

Surprisingly, despite their great interest in ischemia and reperfusion, cardiologists were slow to consider free radicals as a potentially important factor in the pathogenesis of myocardial injury. For example, it was not until the mid 1980s that abstracts dealing with free radical-related studies began to appear regularly among the 2,000 or so papers accepted for presentation at the annual meeting of the American Heart Association. In 1985 there were 3 such papers and in 1986 there were 12. Not until the 1987 meeting did free radicals earn the distinction of an index term in the abstracts selected for publication in *Circulation*, and at that meeting in excess of 30 studies were reported. Extrapolating this exponential growth curve, one might expect to see approximately 70 papers in 1988 and an overwhelming number in 1989; fortunately, the growth of any new area of investigation is self-limiting.

AREAS OF CARDIOVASCULAR INVESTIGATION AND EMERGING SKEPTICISM

Studies on free radicals and myocardial injury fall into three distinct groups [9]: myocardial infarct size limitation, cardioplegia, and arrhythmias. In each instance a variety of interventions, which, among their other properties, were known to be capable of limiting free radical-producing reactions or enhancing endogenous antioxidant capacity, were shown to be protective in the ischemic-reperfused myocardium.

In relation to regional ischemia and evolving myocardial infarction, studies in a variety of species including dogs and pigs showed that allopurinol pretreatment (i.e., presumed inhibition of superoxide-producing xanthine oxidase activity), neutrophil depletion or the administration of superoxide dismutase and/or catalase during ischemia, or just before reperfusion, limited

the size of the resultant infarct. In relation to global ischemia and reperfusion in the context of cardiac surgery, studies, again in several species, indicated that allopurinol pretreatment or the addition of allopurinol, antioxidant enzymes (superoxide dismutase, catalase, peroxidase), or organic antioxidants (ascorbate, methionine) to cardioplegic and reperfusion solutions enhanced postischemic recovery of function. Finally it was demonstrated that all of these so-called antifree radical interventions were able to reduce the vulnerability of the rat heart to ventricular fibrillation induced by reperfusion after a short period of regional ischemia in vivo or in vitro. However, in all of these studies, the association between the observed protection and the manipulation of free radicals was indirect and as such could have been circumstantial. In each instance alternative mechanisms of action could have been proposed, a point that was not missed by those who were skeptical about a role for free radicals in myocardial injury. This limitation undoubtedly raises a major challenge for future studies.

Although representing a relatively new and growing area of cardiovascular research, investigators raised the criticism that too much was being attributed to free radical-mediated mechanisms and that many of the observed effects could readily be explained through other mechanisms, particularly calcium imbalance. Some investigators have even gone so far as to question the very relevance of free radicals, suggesting that the lifetime of free radical research, like that of the radicals themselves, may be very short indeed. The 1987 meetings of the American Heart Association and of the International Society for Heart Research featured debates in which eminent investigators questioned the role of free radicals in myocardial injury. For those committed to continuing research into free radicals and the heart, the barrier of skepticism and prejudice appears unusually heavy.

THE PROBLEMS

Why have free radicals been so ignored by the cardiologists and why have the relatively few studies that have been reported been surrounded by so much criticism and skepticism? In considering these questions it is hoped that future possible directions may be defined for research into free radicals and cardiovascular injury.

Associations which are Indirect and Possibly Circumstantial: Alternative Mechanisms of Action?

As already stressed the association between free radicals and myocardial injury is usually indirect, often being based upon the ability of a so-called antifree radical intervention to reduce tissue injury in some carefully controlled model. Although superoxide dismutase, as an example, has been shown in many studies to limit the evolution of myocardial infarction, improve the efficacy of cardioplegic solutions, and reduce the vulnerability of the heart to reperfusion-induced ventricular fibrillation, in none has it been shown to

reduce the content of free radicals in the tissue at the time of induction of injury. It is without doubt important that investigators address the weakness of interpretation in future studies. Ideally this could be achieved by greater exploitation of the technique of electron spin resonance (ESR) for the direct detection, identification, and quantification of radicals. However, as is stressed in the next section, this is a complex and expensive technique that is unavailable to many investigators, and it would seem that alternative approaches should be considered. For instance, one avenue to consider would be an attempt to eliminate alternative mechanisms by which antifree radical interventions might work. For example, could the protective effects of allopurinol be explained by its ability to conserve purines (and hence reduce myocardial stunning)? Could it act by altering coronary flow or its distribution? Is it necessarily working via xanthine oxidase or could it be a direct free radical scavenger as some would suggest? Clearly, in the future a more critical approach must be applied with serious consideration of alternate mechanisms of action.

The Half-life of Free Radicals and the Problem of Their Detection and Measurement

Cardiologists have long been aware of the rapidity of biological processes in the heart: the complex transmembrane ionic cycling that occurs with every beat of the heart and, for those involved in metabolic studies, the short half-lives of adenosine triphosphate and creatine phosphate, which require that freeze-stop procedures be used when sampling tissue for biochemical analysis. Unfortunately, when dealing with free radicals, the problem of half-life increases by several orders of magnitude; the hydroxyl radical, for example, exists only for a fraction of a microsecond and can travel only a few molecular diameters before interacting with another molecule. Conventional chemical methodologies cannot be used to extract, identify, and quantify free radicals; and such indirect methods for detection (such as interaction with cytochrome C) as exist are nonspecific and can be prone to artifact and misinterpretation. It is only by the technique of ESR that radicals can be definitively identified and measured. In this connection, several recent studies [10–12], using differing ESR methodologies, have confirmed that reperfusion, after a brief period of ischemia (e.g., 15 minutes), does indeed result in a sudden burst of radical production.

It must be conceded, however, that ESR is complex, expensive, prone to artifact, and often requires the use of spin-trapping techniques. Inappropriate preparation of tissue, which involves, for example, lyophilization or vigorous pulverization, can create mechanoradicals not originally present in the tissue. ESR spectra often detect secondary radicals (such as the carbon- or peroxy-radicals produced during lipid peroxidation), and these may be many steps removed from the initiating event with a superoxide or hydroxyl radical. Even experts disagree over the merits of the various methodologies and their interpretation. In looking to the future, high priority should be

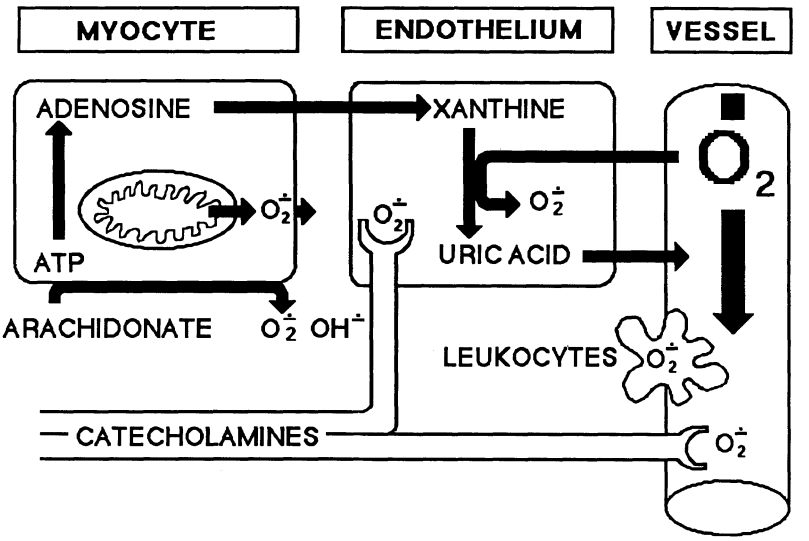

Figure 8-2. Some sources of free radicals in the mammalian myocardium.

given to research aimed at improving and unifying the ESR methods used by cardiologists to detect and characterize the radicals in the heart. In addition, emphasis should perhaps be placed on the exploitation of alternate but specific methodologies for demonstrating the presence and activity of free radicals. One such possibility, (although controversial) advocated by Halliwell and colleagues [1], is the use of small quantities of aromatic compounds that interact with the hydroxyl radical (which cannot be detected directly by ESR) to produce aromatic hydroxylation products. These "chemical fingerprints," which are diagnostic of hydroxyl radical activity, can then be detected by sensitive but readily accessible chromatographic procedures.

Sites of Production and Targets for Radical Interactions

One factor that contributes very significantly to the current controversy and confusion over the importance of free radicals in cardiovascular injury concerns the sources of radical production. Many different cellular processes at a variety of subcellular sites are capable of producing radicals, not only under pathologic conditions, but also in the course of their normal metabolic activity (figure 8-2). Thus, during normal mitochondrial respiration 1 to 2 percent of the consumed oxygen generates superoxide, hydrogen peroxide, and the hydroxyl radical via the univalent leak [5]. Although small in percentage terms, the massive consumption of oxygen by mitochondria (particularly in the heart) represents a large cumulative radical load. The arachidonic acid pathway and the oxidation of catecholamines result in free radical generation as does the activity of a wide variety of oxidase enzymes, such as

reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase. Leukocytes also generate large quantities of free radicals, especially during their respiratory burst when attacking some foreign body.

The question that most often arises is: if these intermediates are so short lived and can travel only minute distances, how can radicals generated in the vascular space, say by leukocytes, or in the endothelium by xanthine oxidase, cause damage to the myocyte? At the present time only a theoretical answer can be given, namely that not all radicals are as spatially confined as the hydroxyl radical; superoxide, for example, can pass from cell to cell (possibly by the anion channel [13]) and hydrogen peroxide (formed from superoxide) can traverse cell membranes and may be transported from its site of formation to other sites where it may produce hydroxyl radicals. It is clear, however, that in future work it will be necessary to demonstrate such phenomena and show that radicals produced at one site can migrate and cause damage at another and that this can be prevented by appropriate interventions.

Xanthine Oxidase in the Human Heart

Another argument that is often leveled against the free radical hypothesis of cardiovascular injury is that xanthine oxidase is not present in the human heart and, therefore, the phenomenon is of little relevance. If this is so then it is of course also true that xanthine oxidase, as a major source for radical-induced injury, is of little clinical relevance. However, I would argue that this does not necessarily lessen its interest, nor does it in any way invalidate the free radical hypothesis, since many other sources of radicals exist. Nonetheless, the xanthine oxidase controversy continues to add unnecessary confusion to the field and as such merits early resolution.

Several studies claim to have detected xanthine oxidase activity (albeit at low levels) in the human heart and several others (some of which used postmortem tissue) claim that no activity could be detected. Part of the controversy may relate to the fact that xanthine oxidase is located [14] in the endothelial cells and as such a high level of activity in the endothelium may well be associated with a low or undetectable level in a whole heart homogenate. In addition, it is possible that in some of the hearts (e.g., hearts that had been destined for transplantation and had been subjected to cardioplegic infusion—a procedure that may damage endothelial cells) the endothelium may have been damaged and the enzyme lost. Since considerable energy is currently being expended in studying xanthine oxidase as a possible contributor to myocardial injury, the status of this enzyme in the human heart should be resolved as soon as possible.

Ischemia or Reperfusion?

Most work to date has shown that free radical-mediated injury occurs during early reperfusion. Indeed, Hess and Manson [15] and McCord [16] have

proposed that ischemia, primes the myocardium for free radical production during the early moments of reperfusion. This proposition is based on the accumulation of purine substrates (xanthine and hypoxanthine) for xanthine oxidase as a consequence of the ischemia-induced degradation of adenine nucleotides, the ischemia-induced conversion of xanthine dehydrogenase to xanthine oxidase, the ischemia-induced loss of endogenous antioxidant activity (e.g., superoxide dismutase and vitamin E), activation of catecholamine and arachidonic acid metabolism, and the stimulation of leukocyte infiltration into the affected tissue. Thus, although ischemia is undoubtedly an important precursor for free radical production in reperfusion, the question increasingly being asked is can radical production occur during the ischemic period itself and if so does it contribute significantly to the pathogenesis of ischemic injury.

On theoretical grounds it would seem probable that disturbances during ischemia of metabolic pathways not dependent on oxygen would favor the increased production of potentially damaging carbon-and nitrogen-centered radicals. In addition, if there is some residual flow to the ischemic tissue then the Michaelis constant (K_m) for a number of radical producing reactions (e.g., xanthine oxidase activity) would be such that substantial production of oxygen radicals could occur even with a very low tissue pO_2. However, the definitive experiments, evaluating the extent and importance of radical production during ischemia, remain to be done.

Cause or Effect?

Although there is considerable evidence that free radicals are produced during the early moments of reperfusion and although there is no doubt that these radicals are potentially capable of inducing tissue injury, it remains to be resolved whether these intermediates actively contribute to the development of reperfusion-induced injury or whether they are secondary to it, being formed as a consequence of severe cellular injury mediated by other triggers. Thus, it could be argued that calcium overload (arising as a consequence of impaired function of the membrane pumps, energy shortage, and mitochondrial reactivation) might induce cellular swelling, activate phospholipases, and cause the membrane to deteriorate. Further, these and other changes (such as mitochondrial calcium uptake in preference to adenosine diphosphate [ADP] phosphorylation), in the face of the readmission of oxygen, might promote the production of radicals secondary to the initial reperfusion-induced injury. In order to place free radicals into correct perspective, future studies must resolve this issue of cause or effect.

Advocates of the involvement of free radicals in reperfusion-induced injury would cite several studies in which various antifree radical interventions, given just before the onset of reperfusion, were shown to limit the extent of injury, part of which was attributable to the act of reperfusion. Furthermore, Hess et al. [17] and other groups have shown that free radical generating systems can

induce changes in the function of membrane pumps (such as a loss of calcium adenosine triphosphate [ATPase] activity) that are similar to those associated with ischemia and reperfusion and that antioxidant enzymes can ameliorate these changes. More recently, Reeves et al. [18] have suggested that reduced oxygen species, such as superoxide, hydrogen peroxide, and the hydroxyl radical, may be able to promote thiol-disulphide interchange in the carrier protein. If this were to occur during early reperfusion, free radicals might be the initiating cause of calcium uptake where the cell may be sodium loaded and depolarized. Since the status of the sodium-calcium exchange mechanism appears to be of great importance in determining the outcome of ischemia and reperfusion, it is vital that future studies exploit electrophysiologic techniques to investigate the possibility that free radicals exert their destructive effects, not via processes such as lipid peroxidation, but by modifying the function of the membrane ionic movement.

CONCLUDING COMMENTS

Consideration of a number of pathologic processes in a variety of organs including the gut, kidney, brain, and lung provides convincing evidence that free radicals play an important role, particularly if the condition involves ischemia and reperfusion. Intuitively one would expect the same to apply to other tissues, especially highly oxidative organs such as the heart. While evidence continues to accumulate that free radicals may play a role in the evolution of myocardial infarction, in the consequences of reperfusion after global ischemic arrest, and in the electrophysiologic perturbations of the membrane, which cause serious ventricular arrhythmias, considerable skepticism is still in evidence. Much of the problem of determining the true role of free-radical-mediated events in cardiovascular disease relates to the difficulty of detecting and measuring these short-lived but highly reactive intermediates. Techniques such as ESR and spin-trapping are foreign to most cardiologists and are inaccessible to most cardiovascular investigators. This has meant that, by necessity, most studies of free radicals in the heart have been indirect and as such any association between observed events and free radicals may be circumstantial. It is undoubtedly important that investigators strive in the future to demonstrate directly that radicals are actually produced at the time of injury, that they are responsible for additional injury, and that this injury and tissue radical content can be coincidently reduced through the use of appropriate interventions. From such studies would emerge the need to determine cause and effect, in particular whether radicals generated during early reperfusion, damage or activate ion exchange processes (e.g., sodium-calcium exchange), which in turn lead to reperfusion-induced injury or whether reperfusion damages the ionic processes leading to calcium overload and tissue injury, which may then promote the production of free radicals secondary to the initial insult.

Although future work will undoubtedly concentrate on events occurring

during the early moments of reperfusion, the possibility that radicals are involved in ischemic injury should not be ignored. It would also be unfortunate if emphasis were to be placed exclusively on oxygen-centered radicals to the exclusion of others, such as those centered on carbon and nitrogen. As more information becomes available, it should be possible to determine the nature of the most important sources of radical production, their subcellular location, and the identity of the radicals that are produced. Thus armed, it should then be possible to develop, on a rational basis, effective antifree radical interventions for the control of tissue injury.

Into the Clinical Arena

If free radicals do prove to play a significant role in myocardial injury, we must determine the clinical circumstances under which their manipulation would be feasible. Readily accessible and relatively easily quantifiable problems are found in cardiac surgery, in particular cardiac transplantation. Here, clinical trials could be designed to assess the effect of various antioxidants on postischemic functional recovery, the incidence and severity of reperfusion-induced arrhythmias, and the maximal periods of cardiac preservation and storage. In the area of thrombolysis, it seems highly unlikely that the mere achievement of reflow will be sufficient; adjunct therapy will also be required to slow ischemic injury before reperfusion (so as to buy more time for effective reflow [19] and also to prevent reperfusion injury at the time of reflow. Antifree radical interventions might be expected to contribute here, and their effects should be measurable. One great advantage that appears to characterize most antioxidant interventions studied to date is the absence of any major direct hemodynamic effect; not only does this render them relatively safe to use but also aids in resolving their likely mechanism of action.

ACKNOWLEDGMENTS

The advice of Dr. M.J. Davies is gratefully acknowledged as is the assistance of Mrs. C. Erlebach.

REFERENCES

1. Halliwell, B. and Gutteridge, J.M.C. 1985. *Free Radicals in Biology and Medicine.* Oxford, Clarendon Press.
2. Granger, D.N., Hallworth, M.E., and Parks, D.A., 1986. Ischemia-reperfusion injury: role of oxygen-derived free radicals. *Acta. Physiol. Scand. Suppl.* 548:47–63.
3. McCord, J.M., and Fridovitch, I. 1968. The reduction of cytochrome C by milk xanthine oxidase. *J. Biol. Chem.* 243:5753–5760.
4. Bulkley, G.B. 1983. The role of oxygen free radicals in human disease processes. *Surgery* 94:407–411.
5. Boveris, A. and Chance, B. 1973. The mitochondrial generation of hydrogen peroxide. *Biochem. J.* 134:707–716.
6. McCord, J. 1985. Derived free radicals in post-ischemic tissue injury. *N. Engl. J. Med.* 312:159–163.

7. Pryor, W.A. 1984. Free radicals in auto-oxidation and in ageing. in *Free Radicals in Molecular Biology, Ageing and Disease,* D. Armstrong, R.S. Sohal, R.G. Cutler, and T.F. Slater, eds., pp. 13–41, New York, Raven Press.
8. Slater, T.F. 1984. Free radical mechanisms in tissue injury. *Biochem. J.* 222:1–15.
9. Hearse, D.J. 1987. Myocardial ischaemia and reperfusion: a possible role for free radicals? in *Free Radicals, Oxidant Stress and Drug Action,* C. Rice-Evans, ed. pp. 13–42, London, Richelieu Press.
10. Garlick, P.B., Davies, M.J., Hearse, D.J., and Slater, T.F., 1987. Direct detection of free radicals in the reperfused rat heart using electron spin resonance spectroscopy. *Circ. Res.* 61:757–760.
11. Kramer, J.H., Arroyo, C.M., Dickens, B.F., and Weglicki, W.B. 1987. Spin-trapping evidence that graded myocardial ischemia alters post-ischemic superoxide production. *Free Rad. Biol. Med.* 3:153–159.
12. Zweier, J.L., Flaherty, J.T., and Weisfeldt, M.L. 1987. Direct measurement of free radical generation following reperfusion of ischemic myocardium. *Proc. Natl. Acad. Sci. USA* 84: 1404–1407.
13. Kontos, H.A., Wei, E.P., Ellis, E.F., et al. 1985. Appearance of superoxide anion radical in cerebral extracellular space during increased prostaglandin synthesis in cats. *Circ. Res.* 57:142–151.
14. Jarash, E.D., Grund, C., Bruder, G., et al. 1981. Localization of xanthine oxidase in mammary gland epithelium and capillary endothelium. *Cell* 25:67–82.
15. Hess, M.L. and Manson, N.H. 1984. Molecular oxygen: friend and foe. *J. Mol. Cell Cardiol.* 16:969–985.
16. McCord, J.M. 1984. Are free radicals a major culprit? in *Therapeutic Approaches to Myocardial Infarct Size Limitation,* D.J. Hearse and D.M. Yellon, eds., pp. 209–218, New York, Raven Press.
17. Hess, M.L., Okabe, E., and Kontos, H.A. 1981. Proton and free oxygen radical interaction with calcium transport system of cardiac sarcoplasmic reticulum. *J. Mol. Cell Cardiol.* 13:767–772.
18. Reeves, J.P., Bailey, C.A., and Hale, C.C. 1986. Redox modification of sodium-calcium exchange activity in cardiac sarcolemmal vesicles. *J. Biol. Chem.* 261:4948–4955.
19. Hearse, D.J. 1988. The protection of the ischemic myocardium: surgical success versus clinical failure. Prog. Cardiovasc. Dis. 6:381–402.

9. THYROID HORMONES AND CARDIAC FUNCTION

OFER BINAH, IRIT RUBINSTEIN, BELLA FELZEN,
YECHIEL SWEED, AND SELA MAGER

Thyroid hormones have profound effects on growth, development, and metabolism of virtually all tissues of higher organisms. Both thyroxine (T_4) and triiodothyronine (T_3) have been shown to induce major effects on cell replication and differentiation, calorigenesis and O_2 consumption, stimulation of enzyme activity, beta-adrenergic responsiveness, and production and secretion of various hormones. As our main interest is in the heart, we will restrict ourselves to issues related to cardiac effects of thyroid hormones. The major changes in cardiac function produced by hyperthyroidism in humans and in experimental animals include marked increases in cardiac output, resting heart rate, and left ventricular ejection fraction, whereas hypothyroid patients have reduced cardiac output, resting heart rate, stroke volume and contractility [1]. These alterations have been attributed both to changes in the peripheral circulation and to direct inotropic and chronotropic effects on the heart. More specifically, the hyperthyroid myocardium is characterized by a decrease in the isometric time to peak tension, an increased shortening velocity and relaxation time, an increased sarcoplasmic reticulum (SR) Ca^{2+} pumping rate, an elevated actin and Ca^{2+}-myosin ATPase activity, and a high percentage of myosin isoenzyme V_1, the fast form of the enzyme. Conversely, the hypothyroid myocardium is characterized by a decreased shortening velocity, decreased myosin ATPase activity, and a reduced percentage of V_1, combined with an elevated percentage of the slower myosin isoenzyme V_3. Whereas the effects of thyroid hormones on cellular mechan-

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isms associated with Ca^{2+} metabolism and the contractile machinery have been thoroughly investigated, electrical properties affected by thyroid hormones have received little attention. Based on our own studies and those by other groups [2–5], it appears that certain electrophysiologic characteristics are modulated by thyroid hormones, and by virtue of this modulation the hormones affect cardiac function. As compared with a major neuroendocrine system that regulates the heart, i.e., the beta-adrenergic system, thyroid hormones seem to induce their effects in a different fashion. This notion is based among other observations on the fact that whereas thyroid hormone levels undergo slow changes in the blood, catecholamine levels change rapidly in response to various stimuli, such as stress, exercise, and alterations in blood pressure. Whereas beta-adrenergic stimulation is frequently associated with relatively short-term alterations in various aspects of cardiac function (heart rate, contractility, etc.), thyroid hormones operate mainly to determine (either directly or indirectly) the basal levels of these variables on which the beta-adrenergic system and other modulators will impose their effects. This idea is exemplified by the effects of thyroid hormones on beta-adrenergic responsiveness. It has been shown that thyroid hormones increase myocardial responsiveness to beta-adrenergic stimulation, by increasing the number of the receptors and by modifying their properties. Hence, at a constant catecholamine level, the magnitude of the response to beta adrenergic stimulation is predetermined by the actions of thyroid hormone, which in fact define the dynamic range of the response. In the next sections, we will discuss our experimental findings and present thoughts and speculations regarding two aspects of thyroid hormone action: interspecies variations in cardiac electrophysiologic and pharmacologic characteristics and effects of thyroid hormones on transmembrane potentials and membrane currents in guinea pig ventricular muscle.

INTERSPECIES VARIATIONS IN CARDIAC ELECTROPHYSIOLOGIC AND PHARMACOLOGIC CHARACTERISTICS; POSSIBLE RELATIONS TO THE THYROID STATE

An important feature we have been suggesting for thyroid hormones is the responsibility for interspecies variations in some ventricular electrophysiologic and pharmacologic characteristics. The initiative to carry out comparative analyses of ventricular action potential characteristics among mammalian species was triggered by the marked differences between the rat action potential duration (APD) and repolarization, and those of other commonly studied mammalian species, such as dog and guinea pig. Whereas in the latter species, APD is approximately 200 to 400 ms and a prominent plateau phase is present, the rat APD is about 50 ms and the plateau is nearly absent. These observations have been traditionally explained by the term "interspecies variability," which does not provide a mechanistic or a physiologic basis for the observed variations. As previous studies have suggested that hyperthy-

roidism and hypothyroidism can modify repolarization and duration in rabbit atrial muscle [4], we hypothesized that some variations in transmembrane potentials among mammalian species result in part from interspecies differences in the individual thyroid state. It should be clearly stated that while the term "thyroid state" is frequently used to describe the level of thyroid hormone activity in individual species, it is not as meaningful a term when it is used across species. However, just as in individual mammals O_2 consumption is a sensitive characteristic of the thyroid state, it is likely that variations in thyroid hormone activity (along with other factors), are responsible for interspecies variations in O_2 consumption. Hence, although O_2 consumption and the thyroid state are probably interrelated in a complex fashion, we think that as a first approximation, it can still be used as an indicator of the thyroid state among different species.

We initially tested our hypothesis in six mammalian species encompassing a wide range of O_2 consumptions, from 0.38 in the dog to 4.05 ml O_2 gr^{-1} hr^{-1} in the shrew (figure 9-1). Note in figure 9-1A that although the superimposed action potentials were recorded under identical experimental conditions, they differ markedly in their overall configuration, duration, and rate of repolarization. An example of the comparative analysis of action potential characteristics among different mammalian species is shown in figure 9-1B, which demonstrates that APD_{50} is inversely correlated with O_2 consumption ($r = -0.91$). Interestingly enough, we found that not only APD but also resting potential and action potential amplitude were inversely correlated with O_2 consumption ($r = -0.92$ and -0.78, respectively). It is possible that the primary relationships are between O_2 consumption and the resting potential, which through its effect on sodium channel availability results in the relationships between O_2 consumption and action potential amplitude.

Although O_2 consumption may not be the most accurate representation of the thyroid state, it is still instructive to discuss the physiologic significance of the relationships between O_2 consumption and APD. It is obvious that in species such as the shrew, with heart rates of over 850 beats/min, APD (in situ) is expected to be much shorter than in the human heart, which beats at a much slower rate. However, heart rate-dependent changes in APD should only be relevant to the in situ heart and can not explain why in vitro, at a cycle length of 2,000 ms (figure 9-1A), APD and repolarization differ so markedly among species. Therefore it was interesting to find that in the shrew, APD increased as cycle length was shortened, an observation in marked contrast with the typical behavior exemplified by the canine and the guinea pig ventricular muscle (figure 9-2). We believe that these interspecies variations (figures 9-1 and 9-2) probably result from differences in intrinsic electrophysiologic characteristics among species, which in part are determined by the thyroid state.

An interesting outcome of the extremely short APD in species with very high O_2 consumption is the occurrence of tetanus in the shrew ventricular

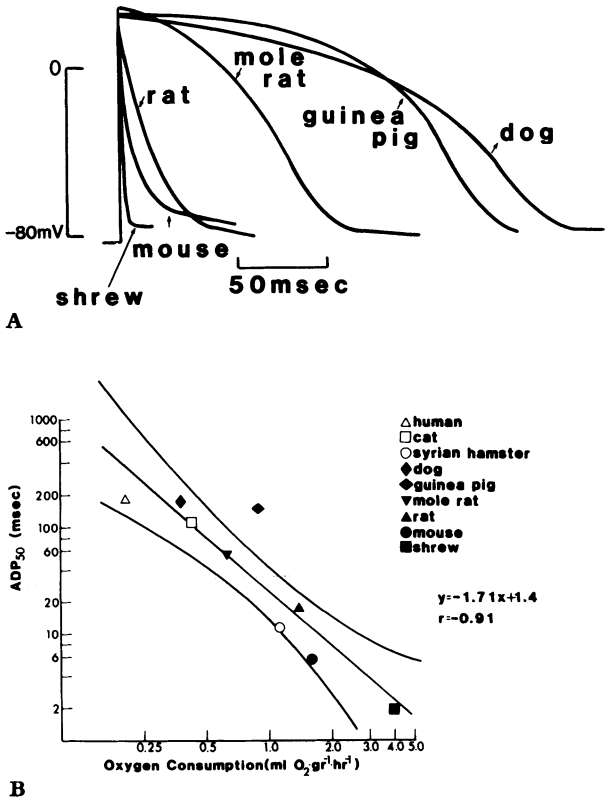

Figure 9-1. A. Typical action potentials from six mammalian species. The traces were reconstructed from the original action potentials. BCL = 2 sec in all experiments. B. Relationships (log-log) between action potential duration at 50% repolarization (APD_{50}) and O_2 consumption. Abscissa log O_2 consumption; ordinate, log APD_{50}. Cycle length = 1000 ms. The straight line and the equation were calculated by means of linear regression analysis. The curved lines mark 95% confidence limits for the line. Inset shows symbols for our data (black symbols) and for data obtained from the literature (white symbols). Human, n = 1; cat, n = 1; golden (Syrian) hamster, n = 1; dog, n = 7; guinea pig, n = 9; mole-rat, n = 8; rat, n = 7; mouse, n = 10; shrew, n = 12.

muscle. As in commonly studied ventricular preparations the electrical refractory period outlasts most of the twitch; neither summation nor tetanus can be induced. However, in the shrew, in which APD_{50} is only 1.9 ms (at a cycle length of 1,000 ms), and the action potential terminates before mechanical activity begins, tetanus can be readily induced (figure 9-3). The special features of the excitation-contraction coupling in the shrew myocardium renders this species an interesting animal model regarding the force-frequency relationships and the contribution of $[Ca^{2+}]_o$ and $[Ca^{2+}]_i$ to the contraction. Moreover, at heart rates of nearly 900 beats/minute, one wonders whether this tiny mammal can increase its heart rate and cardiac out-

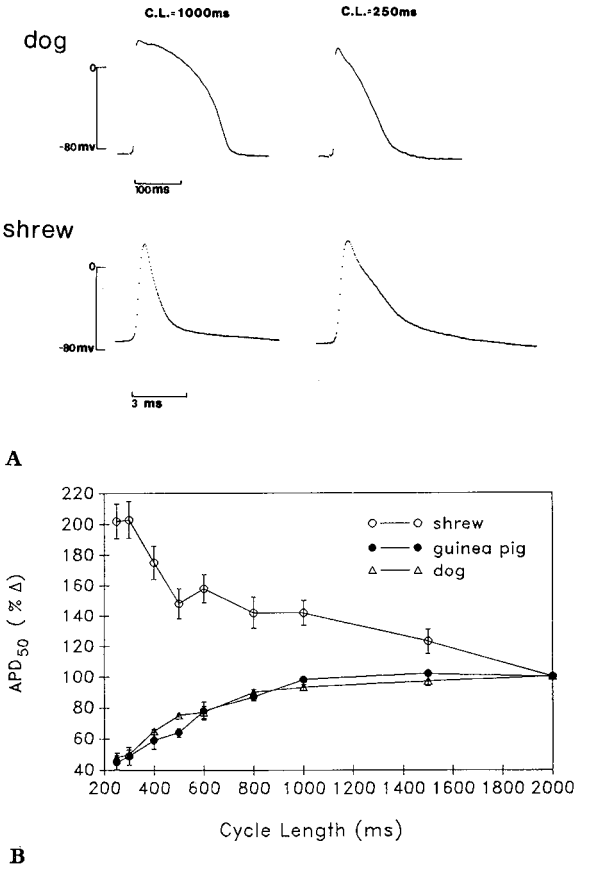

Figure 9-2. Effect of changes in the rate of stimulation on ventricular action potentials of different mammalian species. (A) Representative action potentials recorded from the dog and the shrew ventricular muscle at cycle lengths (C.L.) of 1000 and 250 ms. (B) Relationships between APD_{50} and cycle length. Abscissa, cycle length (ms); ordinate percent change in APD_{50} compared with APD_{50} at cycle length of 2000 ms. Dog, n = 7; guinea pig, n = 9; shrew, n = 12. Results expressed as $\bar{X} \pm SE$.

put, in response to various stimuli, as much as can larger animals. Thus, it would be a challenging experiment to monitor the shrew's heart rate and cardiovascular performance "while the little guy rides his exercise bike," or alternatively, is subjected to sympathetic stimulation or catecholamine infusion.

If the thyroid state does in fact vary among species, then it might also be responsible, at least in part, for interspecies variations in the responsiveness to cardiac glycosides. Whereas in species such as the dog, rabbit, and guinea

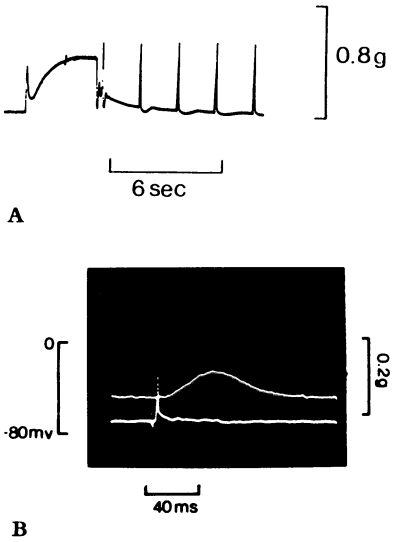

Figure 9-3. Mechanical activity in the shrew ventricular muscle. (A) The basic stimulus (cycle length = 2000 ms), resulting in a twitch was followed by a train of stimuli (cycle length = 15 ms), during which fused tetanus developed. (B) Simultaneous recordings of the twitch (upper trace) and the action potential (lower trace) in a ventricular muscle preparation. Cycle length = 2000 ms.

pig, cardiac glycosides induce a marked positive inotropic effect, the response of the rat myocardium [6, 7] and that of the mouse (Binah et al., unpublished observations) is significantly attenuated. Since $Na^+ - K^+$, ATPase, the receptor for cardiac glycosides, is modulated by thyroid hormones [8, 9], we hypothesized that interspecies variations in the responsiveness to the glycosides might result from the differences in the thyroid state among species. In a recent study [10], we tested this hypothesis by investigating the properties of ouabain-induced delayed afterdepolarizations (DAD) in four species encompassing a wide range of O_2 consumptions (guinea pig, rat, mouse, and shrew) and in guinea pigs in which the thyroid state was modified in opposite directions by T_4 administration and propylthiouracil treatment. DAD were readily induced in guinea pig ventricular muscle after 30 to 45 minutes superfusion with 10^{-6} M ouabain and 5.4 mM Ca^{2+}, whereas in rats, DAD with much smaller amplitude and dV/dt were induced only when the Ca^{2+} concentration was raised to 8.1 mM (figure 9-4). DAD could not be induced in mice and shrews. The thyroid state hypothesis was supported by studies in guinea pigs in which the thyroid state was modified (figure 9-5); as compared with the euthyroid state, DAD amplitude and dV/dt were larger in hypothyroidism and much smaller in hyperthyroidism. To gain a better understanding of the mechanisms whereby thyroid hormones modulate the responsiveness to cardiac glycosides, in future studies we plan to investigate

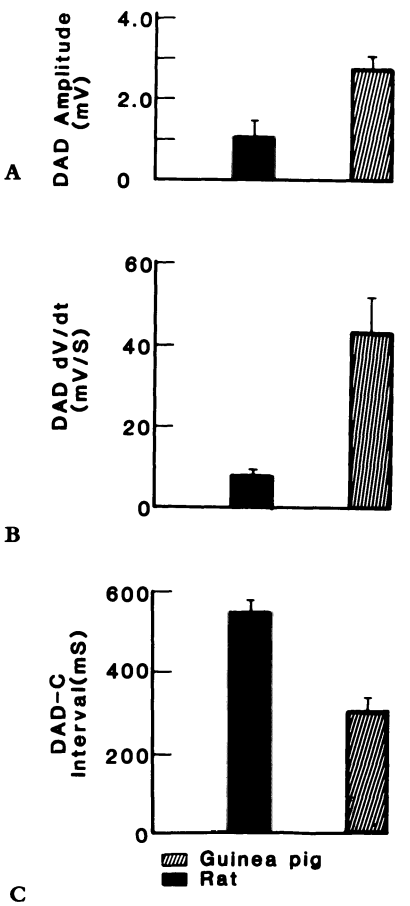

Figure 9-4. Delayed afterdepolarization (DAD) characteristics in guinea pig and rat ventricular muscle. Data for guinea pigs (n = 10) were obtained after superfusing the preparations for 40 to 60 minutes with Tyrode's solution containing 10^{-6} M ouabain and 5.4 mM Ca^{2+}, and in rats (n = 7), after superfusion for two hours with Tyrode's solution containing 10^{-6} M ouabain and 5.4 mM Ca^{2+} (with no DAD induced), followed by 30 minutes of superfusion with 10^{-6} M ouabain and 8.1 mM Ca^{2+}. (A) DAD Amplitude. (B) The slope of the ascending limb of the DAD (DAD dV/dt). (C) DAD coupling interval (DAD-C). Results are expressed as mean ± SEM. Cycle length = 500 ms.

the effects of thyroid hormones on ouabain-induced changes in intracellular ion activities and on the properties of the transient inward current.

ELECTROPHYSIOLOGICAL EFFECTS OF THYROID HORMONES AND THEIR FUNCTIONAL SIGNIFICANCE

The thought that thyroid hormones are important modulators of cardiac electrical properties has led us to investigate various aspects of thyroid hormone action in guinea pig ventricular muscle. The most prominent effect

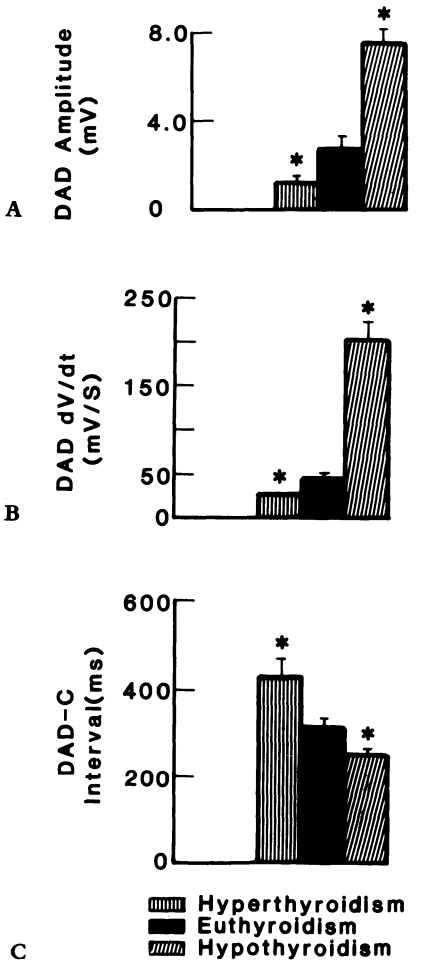

Figure 9-5. Delayed afterdepolarization (DAD) characteristics in guinea pigs with altered thyroid states. Measurements were obtained as indicated in figure 9-4. A. DAD Amplitude. B. The slope of the DAD ascending limb (DAD dV/dt). C. DAD coupling interval (DAD-C). Euthyroid, hypothyroid, and hyperthyroid guinea pigs, n = 10. Results are expressed as mean ± SEM. *, $P < .05$ compared with euthyroid guinea pigs. Cycle length = 500 ms. Hyperthyroidism was induced by 8 to 11 daily injections of T_4, 200 μg/kg. Hypothyroidism was induced by maintaining guinea pigs on regular Purina chow and drinking water containing 0.05% propylthiouracil for 30 to 45 days.

was on APD, demonstrable in papillary muscle preparations and in isolated ventricular myocytes (figure 9-6); APD was decreased in hyperthyroidism and increased in hypothyroidism, whereas the slope of the plateau was increased in hyperthyroidism and decreased in hypothyroidism. Before considering other aspects of thyroid hormone action, we shall consider whether

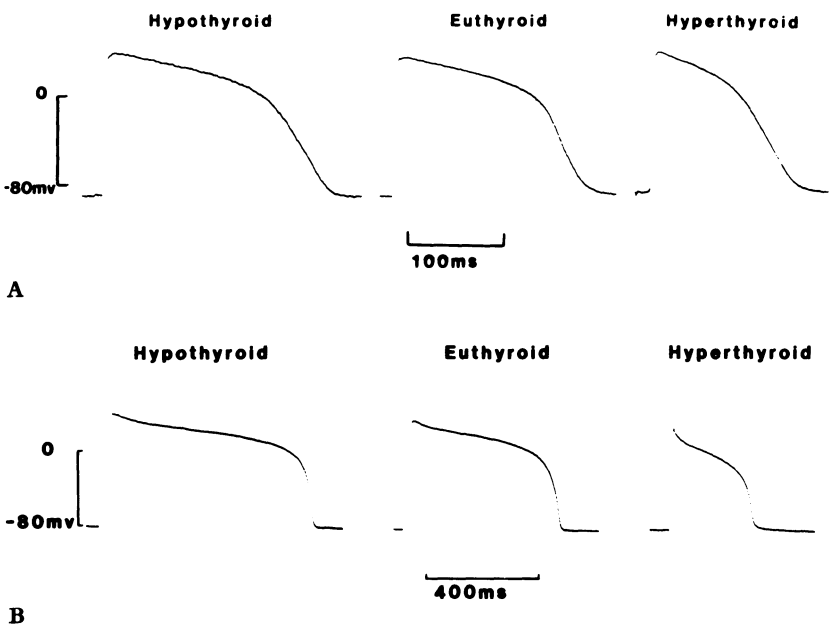

Figure 9-6. Effects of hyperthyroidism (8 to 11 daily injections of T_4 150 μg/kg), and hypothyroidism on the guinea pig action potential recorded from papillary muscle (A), and from isolated ventricular myocytes (B). A. Cycle length = 1000 ms, temperature = 30°C. B. Cycle length = 2000 ms, temperature = 24.5°C. Hypothyroidism was induced as in figure 9-5.

the effect on APD is consonant with the overall action of thyroid hormone on cardiac function. Hyperthyroidism is generally associated with an increase in the basal metabolic rate, which in part can be achieved by modifying intrinsic myocardial properties and by increasing the sensitivity to beta-adrenergic stimulation. This in turn will result in increased heart rate, accompanied by a shortened diastolic interval, which might impair ventricular filling. However, a shorter APD in hyperthyroidism will be associated with a longer diastolic interval and increased ventricular filling, thus compensating for the increased heart rate. The effect of thyroid hormones on $Na^+ - K^+$, ATPase is also compatible with cardiac manifestations of hyperthyroidism; in the presence of higher heart rates and thereby increased Na^+ and K^+ fluxes, a stronger pumping activity would certainly be of advantage to the myocardium.

Following the basic finding that chronic hyperthyroidism (8 to 11 daily injections of T_3 or T_4, 100 to 150 μg/kg) is associated with APD shortening, we became interested in three major questions:

1. Is there linkage between the effects of thyroid hormones on APD and the chronic manifestations of hyperthyroidism?

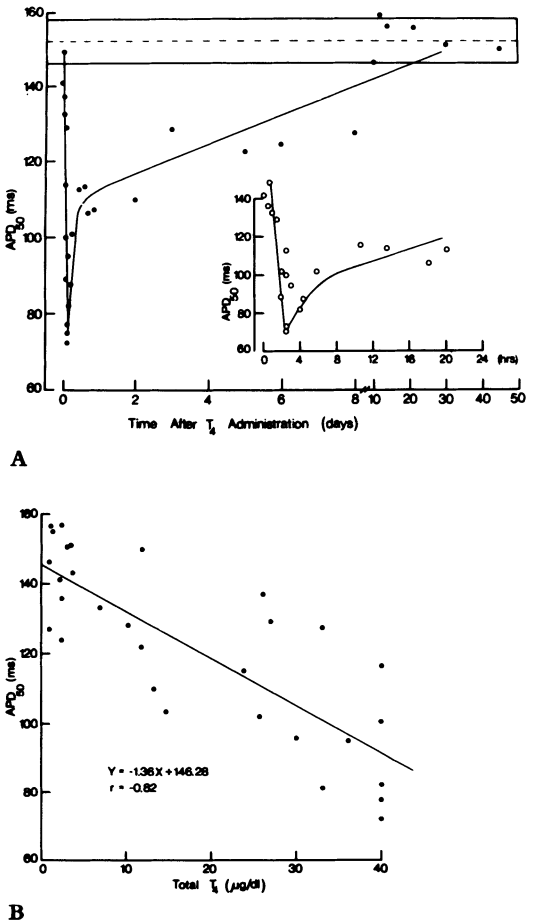

Figure 9-7. Electrophysiologic effects of thyroxine (T_4) in guinea pig ventricular muscle. A The time course of the effect of a single dose of T_4, 100 μg/kg on action potential duration at 50% repolarization (APD_{50}). The broken line and the width of the enclosed area represent the mean ± SEM of APD_{50} in euthyroid guinea pigs (n = 11). The continuous line was fit by eye. The inset depicts an expansion of the first 24 hours after T_4 administration. B Dose response relationships between total T_4 levels and action potential duration at 50% repolarization (APD_{50}). In the regression analysis, total T_4 levels of < 1.0 μg/dl were taken as 1.0 μg/dl, and values that were > 40.0 μg/kg were taken as 40 μg/kg.

2. What is the time course for thyroid hormone action on APD?
3. Are the changes in APD related to thyroid hormone levels?

We have been trying to answer these questions by injecting guinea pigs with a single dose of T_4, 100 μg/kg and monitoring the changes in action potential characteristics and thyroid hormone levels at various intervals after hormone administration (figure 9-7). Regarding the time course of thyroid

hormone action, it was rather surprising to find that 30 to 45 minutes after T_4 administration, APD was already shortened, and within 2 to 3 hours, maximal shortening was attained. These findings suggest that the effect on APD (at least the rapid phase) did not result from alterations in protein synthesis, and furthermore, that it was unrelated to the chronic effects of hyperthyroidism. As to the third question, figure 9-7B demonstrates that the changes in APD_{50} and total T_4 levels are correlated; however, these findings do not necessarily imply that thyroid hormones have a direct effect on specific ionic channels, and detailed experiments are still required before such a conclusion can be drawn. Although these studies provided us with important information, the protocol, which involved intraperitoneal hormone administration and in vitro measurements, is inappropriate for determining the cellular mechanisms of thyroid hormone action. To control the length of exposure to the hormone and its concentration, we are now in the process of developing a cell culture system for adult guinea pig ventricular myocytes. We chose to culture adult guinea pig myocytes rather than the commonly used newborn rats for the following reasons: (1) we have collected a large database on the electrophysiologic effects of thyroid hormones in guinea pig papillary muscle and ventricular myocytes; (2) the properties of various membrane currents are known better in adult guinea pigs than in newborn rats; and (3) we expect that the electrophysiologic characteristics of adult guinea pig myocytes in culture would be more stable than those of newborn myocytes, which will probably undergo developmental changes.

THE EFFECT OF THYROID HORMONES ON MEMBRANE CURRENTS IN GUINEA PIG VENTRICULAR MYOCYTES

The findings discussed thus far have dealt with the effects of thyroid hormones on APD; however, as duration and repolarization are determined by several ionic and pump currents, not much concerning mechanism can be concluded from action potential measurements. To study the currents specifically affected by thyroid hormones, we have begun to use the whole cell recording technique in isolated guinea pig ventricular myocytes [11]. Studies on the effects of thyroid hormones on membrane currents are under way, and some of our preliminary results are presented. The major finding was that thyroid hormones have a marked effect on the slow inward current, i_{si}; it is increased by hyperthyroidism and decreased by hypothyroidism. For example, in euthyroid, hyperthyroid, and hypothyroid guinea pig ventricular myocytes, peak i_{si} ($X \pm SEM$) was 1.19 ± 0.06 nA, $1.89 \pm 0.18^*$ n_a, and $0.80 \pm 0.05^*$ nA, respectively (*, $P < 0.001$). These findings are of major importance since they suggest that aside from affecting cardiac performance by modifying myosin ATPase isoenzymes, thyroid hormones also affect cardiac function by modulating the properties of the voltage–dependent Ca^{2+} channels. Thyroid hormones also affected the steady state outward current, i_k; this was augmented by hyperthyroidism and slightly attenuated by hypothyroidism.

In summary, we have presented experimental evidence suggesting that interspecies variations in cardiac electrophysiologic and pharmacologic characteristics are related to differences in the individual thyroid state among species. We have also discussed the possible physiologic significance of the electrophysiologic effects of thyroid hormones. Finally, effects of thyroid hormone on membrane currents in guinea pig ventricular myocytes were presented.

REFERENCES

1. Morkin, E., Flink, I.L., and Goldman, S. 1983. Biochemical and physiologic effects of thyroid hormones on cardiac performance. *Prog. Cardiovasc. Dis.* 25:435–464.
2. Binah, O., Arieli, R., Beck, R., et al. 1987 Ventricular electrophysiological properties: is interspecies variability related to the thyroid state? *Am. J. Physiol.* 252:H1265–H1274.
3. Binah, O., Rubinstein, I., and Gilat, E. 1987. Effects of thyroid hormone on the action potential and membrane currents of guinea pig ventricular myocytes. *Pflugers Arch.* 409:214–216.
4. Freedberg, A.F., Papp, J.G., and Vaughan Williams, E.M. 1970. The effect of altered thyroid state on atrial intracellular potentials. *J. Physiol. (Lond.)* 207:357–369.
5. Sharp, N.A., Neel, D.S., and Parsons, R.L. 1985. Influence of thyroid hormone level on the electrical and mechanical performance of rabbit papillary muscle. *J. Mol. Cell Cardiol.* 17:119–132.
6. Repke, K., Est, M., and Portius, H.J. 1965. Uber die spercies unter-schiede in der digitalis-empfindlichkeit. *Biochem. Phramacol.* 14:1785–1802.
7. Ku, D.D., Akera, T., Tobin, T., and Brody, T.M. 1976. Comperative species studies on the effect of monovalent cations and ouabain on cardiac Na^+, K^+-adenosine triphosphatase and contractile force. *J. Pharmacol. Exp. Ther.* 197:458–469.
8. Asano, Y., Liberman, U.A., and Edelman, I.S. 1976. Relationships between Na^+-dependent respiration and Na^++K^+-adenosine triphosphatase activity in rat skeletal muscle. *J. Clin. Invest.* 57:368–379.
9. Curfman, G.D., Crowley, T.J., and Smith, T.W. 1977. Thyroid-induced alterations in myocardial sodium and potassium-activated adenosine triphosphatase monovalent cation active transport and cardiac glycoside binding. *J. Clin. Invest.* 59:586–590.
10. Felzen, B. and Binah, O. Interspecies variability in the myocardial responsiveness to cardiac glycosides: possible relationships to the thyroid state, submitted for publication.
11. Hamill, O.P., Marty, A., Neher, E., et al. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Arch.* 391:85–100.

III. NEURAL AND HORMONAL FACTORS

10. RECEPTOR MECHANISMS IN ISCHEMIA AND INFARCTION

AUGUST M. WATANABE

The autonomic nervous system, consisting of the sympathetic and parasympathetic limbs, is the most important system extrinsic to the heart for regulating cardiac function. Much has been learned in the last few years about the molecular mechanisms by which the autonomic nervous system regulates the heart. The components of the autonomic nervous system that regulate the heart include sympathetic and vagal nerve terminals, beta-adrenergic and muscarinic receptors, a transmembrane signal transduction system, protein kinases that phosphorylate various proteins within the myocardial cell, and the proteins themselves whose functions are altered when they are phosphorylated [1–6]. For discussion of regulation and interaction at the level of sympathetic and vagal nerves, see the chapter by Levy in this volume.

The signal transduction system at the plasma membrane of the cells is comprised of three major components: receptors, coupling proteins, and enzymes whose activities are altered when the appropriate receptors are activated. The receptors of interest when considering the autonomic nervous system are the adrenergic and muscarinic receptors. In the heart, beta-adrenergic receptors are stimulated by circulating catecholamines, particularly epinephrine and norepinephrine released from sympathetic nerve terminals. Beta-adrenergic receptors are coupled to adenylate cyclase, via a guanine nucleotide binding and regulatory protein (G-protein), to the catalytic subunit of adenylate cyclase. When beta-adrenergic receptors are occupied

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by catecholamines, adenylate cyclase activity is increased and intracellular levels of cyclic adenosine monophosphate (AMP) increase. The increased cyclic AMP levels within the cells lead to activation of cyclic AMP-dependent protein kinase. Cyclic AMP-dependent protein kinase, in turn, phosphorylates many proteins within the myocardial cells to alter function. Among these proteins are channels, proteins that regulate the activity of certain enzymes, and proteins that are important in modulating the metabolic activities of the cell. Three specific phosphorylated proteins whose phosphorylation leads to altered function of cardiac cells are the calcium channel, phospholamban, and troponin-I. Phosphorylation of phospholamban leads to increased activity of calcium ATPase in sarcoplasmic reticulum. This results in an increased rate of sequestration of calcium into the sarcoplasmic reticulum and an increased rate of relaxation of cardiac muscle. Because of the increased sequestration of calcium by sarcoplasmic reticulum, with subsequent systoles, more calcium is released from sarcoplasmic reticulum, and this may also account, at least partially, for the positive inotropic effect of agents that elevate cyclic AMP levels. Cyclic AMP-dependent protein kinase also leads to phosphorylation of calcium channels located in the sarcolemma of cardiac cells. When such calcium channels are phosphorylated, they are, in effect, recruited to be activated when the membrane depolarizes. Thus, calcium flows through the channels during systole, and this contributes to the positive inotropic effects of agents that elevate cyclic AMP levels. Cyclin AMP-dependent protein kinase can also phosphorylate the inhibitory subunit of troponin. When troponin I is phosphorylated, troponin C becomes less sensitive to the effects of calcium. This leads to reduced disinhibition of actin and myosin interaction and may also partially account for the increased rate of relaxation produced by agents that elevate cyclic AMP levels. However, in experimental preparations, phosphorylation of troponin-I could not be consistently associated with increased rates of relaxation of cardiac muscle, and thus the physiologic role of this potential mechanism remains uncertain.

The heart is regulated by the autonomic nervous system, not only in a stimulatory manner, but also in an inhibitory manner. The inhibitory regulation occurs via the parasympathetic limb of the autonomic nervous system. The parasympathetic nervous system regulates the heart by means of the neurotransmitter acetylcholine, which is released from vagal nerve endings that innervate the heart. When muscarinic receptors are stimulated by acetylcholine, heart rate decreases and myocardial contractility, particularly of atrial myocardium, is reduced. In the mammalian heart, parasympathetic innervation of the ventricles is substantially less than that of the atria, except for the areas surrounding the specialized conducting tissues. Accordingly, the muscarinic-mediated negative inotropic effect on ventricular myocardium is much less than that seen with muscarinic regulation of atrial myocardium.

Muscarinic receptors are also coupled to intracellular effectors by G-

proteins. In contrast to the situation with beta-adrenergic receptors, muscarinic receptors couple to several different sarcolemmal or intracellular effectors. These include adenylate cyclase, potassium channels (at least in atrial myocardium), phosphoinositol, adenylate cyclase, and guanylate cyclase. In the case of all of these intracellular effectors, G-proteins are involved in coupling of the muscarinic receptor to the effector. A possible exception is coupling of the muscarinic receptor to guanylate cyclase.

One family of G-proteins has recently been characterized at a biochemical, molecular, and genetic level. These G-proteins, which mediate hormone action, can be classed according to whether they are substrates for the bacterial toxins of vibrio cholera or bordetella pertussis. These bacterial toxins are enzymes that transfer ADP-ribose from nicotinamide adenine dinucleotide (NAD) to the alpha-subunit of the G-proteins. One group of G-proteins is a substrate for cholera toxin, a second group of G-proteins is substrate for pertussis toxin, a third group of G-proteins is a substrate for both toxins, and a fourth group of putative G-proteins is a substrate for neither. For the latter group, a specific toxin has not yet been discovered which will cause ADP-ribosylation of the protein.

The G-protein that couples beta-adrenergic receptors to adenylate cyclase has been designated G_s, and the alpha-subunit of this protein is a substrate for cholera toxin. The G-protein that couples muscarinic receptors to adenylate cyclase has been designated G_i, and its alpha-subunit is a substrate for pertussis toxin.

Stimulation of muscarinic receptors leads to inhibitory regulation of cardiac function by several complex mechanisms. In fact, the muscarinic inhibitory regulation of cardiac function appears to be more complex than the beta-adrenergic stimulatory regulation of cardiac function. One important mechanism is muscarinic inhibition of adenylate cyclase activity. Occupation of muscarinic receptors by agonists leads, via G_i, to inhibition of adenylate cyclase activity and thus reduced intracellular levels of cyclic AMP. Accordingly, cyclic AMP-dependent protein kinase is activated to a lesser degree; thus, the various proteins that are phosphorylated in cardiac muscle are phosphorylated to a lesser extent. This mechanism is probably important in mediating the muscarinic inhibitory regulation of cardiac function. In atrial myocardium, muscarinic regulation of potassium channels is probably also important in inhibitory regulation of cardiac function. When muscarinic receptors are stimulated, the potassium channels that produce the inward rectifying current are activated, and thus atrial myocardium tends to hyperpolarize and the action potential of atrial myocardial cells is shortened. This results in a decreased rate of impulse initiation by pacemaker cells in specialized pacemaker tissues, as well as in atrial myocardium, and also decreased contractility of atrial myocardium. Muscarinic receptors do not appear to be coupled to potassium channels in ventricular myocardium. It is possible that muscarinic regulation of phosphoinositol turnover also plays a role in

mediating the cellular effects of the parasympathetic nervous system. However, the physiologic consequences of muscarinic modulation of phosphoinositol turnover in heart muscle have not yet been elucidated. Finally, substantial evidence suggests that muscarinic receptor stimulation can modulate the extent of phosphorylation of proteins within the cardiac cells. This modulation appears to be more than can be explained by muscarinic inhibition of adenylate cyclase activity with the resultant regulation of intracellular cyclic AMP levels. Therefore, it has been postulated that activation of muscarinic receptors can lead to regulation of additional enzymes that are important is determining protein phosphorylation; the two enzymes considered are cyclic AMP-dependent protein kinase and phosphoprotein phosphatase. No available evidence suggests that muscarinic agonists can directly regulate cyclic AMP-dependent protein kinase activity. However, indirect and direct evidence supports the conclusion that muscarinic receptors can regulate certain protein phosphatases. The mechanism by which this muscarinic regulation of phosphatase activity occurs is currently under investigation.

The physiologic implication of the dual sympathetic and parasympathetic regulation of cardiac function is that the function of the heart at any given time is the result of the dynamic balance between these two limbs. In individuals who are in good physical condition, vagal tone and muscarinic regulation of cardiac function are augmented. The slow heart rate and normal to low normal blood pressure of such individuals is due not only to augmented vagal tone per se, but also to the vagal inhibition of the cardiac response to sympathetic stimulation by the mechanisms previously discussed. Thus, the increased parasympathetic activity functions physiologically as beta-adrenergic receptor antagonism.

It is reasonable to speculate that the muscarinic inhibitory regulation of cardiac function is important in pathophysiologic states, such as ischemic heart disease. Thus, if the heart during myocardial ischemia is under inhibitory control of the vagal system, myocardial oxygen demands would be less and, as a consequence, the magnitude of ischemia might be minimized. Thus, infarct size might be limited in patients with coronary artery disease who also have high vagal tone. Also, in such patients, high vagal tone might tend to protect the individual from developing cardiac arrhythmias during myocardial ischemia.

With the increasing understanding of the important role of muscarinic inhibitory regulation of cardiac function in protecting the heart from injury in certain diseases, such as ischemic heart disease, and with the increasing knowledge of the biochemical and molecular mechanisms by which the muscarinic system regulates myocardial cell function, one can begin to envision the design of new drugs that would pharmacologically augment this muscarinic inhibitory control. Such drugs might be important as antiischemic and antiarrhythmic agents.

In summary, in the past two decades much has been learned about the

importance of dual regulation of cardiac function by the two limbs of the autonomic nervous system. Also, much has been learned about the biochemical and molecular mechanisms by which the sympathetic and parasympathetic nervous systems modify the biochemical and physiologic properties of myocardial cells. Based on this knowledge, the important physiologic implications of this dual control are being realized, and opportunities for designing new drugs that might modify the functions of the inhibitory regulation are apparent. The pursuit of such opportunities should lead to interesting and exciting research in the years ahead.

REFERENCES

1. Watanabe, A.M., Bailey, J.C., Lathrop, D.A., and Besch, H.R., Jr. 1978. Acetylcholine antagonism of the cellular electrophysiologic effects of isoproterenol, in *Nervous Mechanisms in Cardiac Arrhythmias,* pp. 349–358, P.J. Schwartz, ed., New York, Raven Press.
2. Watanabe, A.M., Jones, L.R., Manalan, A.S., and Besch, H.R., Jr. 1982. Cardiac autonomic receptors: recent concepts from radiolabelled ligand binding studies. *Circ. Res.* 50:161–174.
3. Watanabe, A.M. 1983 Pharmacological modification of cardiac function: cholinergic agonists and antagonists. in *Cardiac Therapy,* B.F. Hoffman and M.R. Rosen, eds., pp. 95–144, Boston, Martinus Nijhoff.
4. Watanabe, A.M. 1984. Cellular mechanisms of muscarinic regulation of cardiac function. in *Nervous Control of Cardiovascular Function,* W. Randall, ed., pp. 130–164, New York, Oxford University Press.
5. Watanabe, A.M. and Lindemann, J.P. 1984. Mechanisms of adrenergic and cholinergic regulation of myocardial contractility. in *Physiology and Pathophysiology of the Heart,* N. Sperelakis, ed., pp. 377–404, Boston, Martinus Nijhoff.
6. Fleming, J.W. and Watanabe, A.M. 1986. Biochemical mechanisms of parasympathetic regulation of cardiac function. in *The Heart and Cardiovascular System,* H.A. Fozzard et al. eds., pp. 1679–1688, New York, Raven Press.

11. SYMPATHETIC-PARASYMPATHETIC INTERACTIONS IN THE NORMAL HEART

MATTHEW N. LEVY

Tonic vagal activity inhibits and tonic sympathetic activity facilitates most aspects of cardiac behavior. These antagonistic effects do not summate algebraically, but complex interactions prevail. Some of these interactions take place within the central nervous system, whereas others occur in the periphery. This chapter will be restricted to the peripheral interactions that take place within the heart. I will attempt to define the current state of knowledge about the autonomic interactions that prevail normally. I will try to emphasize those areas currently being studied most intensively and to identify those problems that merit investigation in the next few years.

EFFECTS ON THE SINUS NODE

Rosenblueth and Simeone [1] reported in 1934 that a given level of vagal stimulation diminished heart rate more in the presence of tonic sympathetic stimulation than in its absence. Other groups of investigators [2–4] have since confirmed their findings. The exaggerated inhibitory effect of vagal stimulation that develops as the level of tonic sympathetic activity increases has been termed accentuated antagonism [5].

Figure 11-1 illustrates the nonlinear summation that we observed when we stimulated simultaneously the sympathetic and vagal nerves to the heart in anesthetized dogs [3]. We stimulated these nerves at various combinations of frequencies, and the combinations were applied in a random sequence. The

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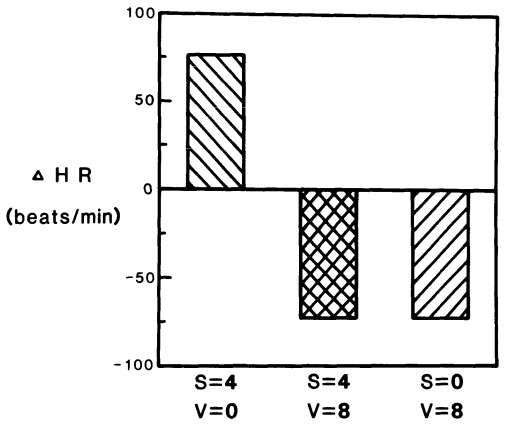

Figure 11-1. The changes in heart rate (ΔHR) elicited by stimulating the cardiac nerves in an anesthetized dog. The numbers below each bar denote the frequencies (in Hz) at which the right stellate ganglion (S) and the left vagus nerve (V) were stimulated simultaneously and supramaximally for 30 seconds. Modified from Levy and Zieske [3].

results of three such frequency combinations are illustrated in the figure. The heart rate increased by 78 beats/min when we stimulated the right stellate ganglion at 4 Hz (S = 4) in the absence of vagal stimulation (V = 0). Conversely, when we stimulated the left vagus nerve at 8 Hz (V = 8) in the absence of sympathetic stimulation (S = 0), the heart rate decreased by 70 beats/min. Had simple algebraic summation prevailed, the response to combined sympathetic stimulation at 4 Hz and vagal stimulation at 8 Hz would have been barely detectable. In fact, the observed response to combined stimulation (S = 4, V = 8) scarcely differed from the response to vagal stimulation alone; that is, the vagal effect overwhelmed the sympathetic effect.

The chronotropic responses of this animal to the various permutations of sympathetic and vagal stimulation frequencies are shown in figure 11-2. As the sympathetic frequency was progressively increased from 0 to 4 Hz in the absence of vagal stimulation (V = 0), the heart accelerated substantially [3]. However, when the vagus nerves were stimulated tonically at a frequency of 8 Hz (V = 8), the same changes in the frequency of sympathetic stimulation were much less effective. Tonic vagal stimulation at a frequency of 4 Hz (V = 4) had an intermediate effect. The extent to which the various curves in figure 11-2 deviate from being parallel reflects the extent of the sympathetic-parasympathetic interaction. The pronounced divergence of these curves denotes the strong autonomic interaction that prevails in the neural control of heart rate.

The following questions have been raised by experts in this area:

1. What characteristics of the autonomic innervation or of the effector cells

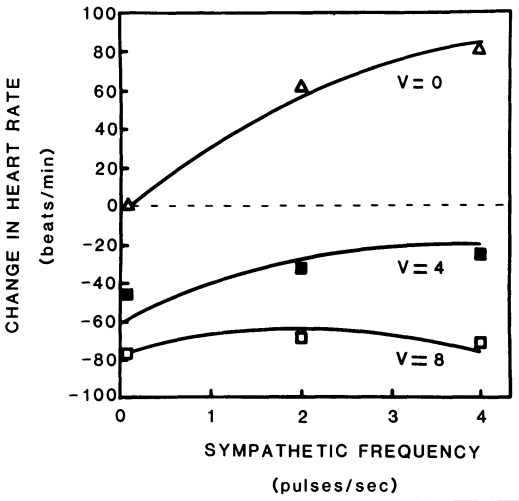

Figure 11-2. The changes in heart rate in an anesthetized dog as the frequency of right stellate ganglion stimulation was varied from 0 to 4 Hz. The responses to stellate stimulation were obtained during concomitant left vagal stimulation at frequencies of 0 Hz (V = 0), 4 Hz (V = 4), or 8 Hz (V = 8). Data are from the same animal from which figure 11-1 was derived. The symbols represent the actual data; the curves were derived by regression analysis. Modified from Levy and Zieske [3].

are responsible for the pronounced sympathetic-vagal interaction? How does the density of nerve fibers vary from region to region? How does the proximity of sympathetic to vagal fibers vary from region to region?

2. Why is nonlinear summation more pronounced in the neural regulation of sinus node activity than it is in the regulation of any other functional region of the heart?
3. What are the relative roles of prejunctional and postjunctional mechanisms in the neural regulation of sinus node automaticity? This question can probably be answered by a modification of the technique of Takahashi and Zipes [6]. These investigators have already used this technique to show that an integral part of the interaction in the sinus node region depends on a prejunctional mechanism. The extent of the postjunctional component remains to be established.
4. What are the intracellular processes that underlie the postjunctional adrenergic-cholinergic interaction in sinus node automatic cells? Are they identical to the processes that prevail in myocardial cells? Is the coupling between receptors and adenylate cyclase in sinus node automatic cells similar to that in other cardiac cells?

EFFECTS ON ATRIOVENTRICULAR AUTOMATICITY

Prominent interactions are also evident in the control of heart rate in animals in which an atrioventricular (AV) junctional rhythm has been induced ex-

perimentally [7]. Figure 11-3 shows the mean changes in heart rate elicited by various combinations of sympathetic and vagal stimulation frequencies in a group of seven anesthetized dogs. An increase in the frequency of sympathetic stimulation from 0 to 1.4 Hz (left upper edge of the response surface) evoked a 55 beats/min increase in heart rate when the vagus nerves were not being stimulated. However, when the vagus nerves were being stimulated tonically at 8.4 Hz (lower right edge of the response surface), the same change in sympathetic stimulation (0 to 1.4 Hz) increased the heart rate by only 32 beats/min. Thus, tonic vagal stimulation attenuated the chronotropic response of the AV nodal pacemakers to a given change in the frequency of sympathetic stimulation. However, the attenuation did not appear to be as pronounced as it was in the sinus node (figure 11-2), although the relative strengths of the interactions in the two nodal regions have not yet been determined systematically.

The following questions remain to be answered:

1. Are the autonomic interactions on automaticity stronger in the sinus node than in the AV junction? If so, what is the basis for the disparity?
2. What is the relative importance of prejunctional and postjunctional mechanisms in each of these nodal regions?

EFFECTS ON ATRIOVENTRICULAR CONDUCTION

In contrast to the prominent interactions that were uncovered in our studies of sinoatrial (SA) nodal [3] and AV junctional [7] automaticity, other experiments from our laboratory [3, 8] failed to disclose a significant autonomic interaction with respect to AV conduction. Changes in the sympathetic stimulation frequency over the range from 0 to 4 Hz (figure 11-4) in our experiments significantly diminished the AV conduction time [3]. Conversely, changes in the frequency of vagal stimulation over the range from 0 to 8 Hz significantly increased the AV conduction time. However, the response to a given change in vagal stimulation frequency was not influenced materially by the prevailing level of sympathetic activity; that is, the interaction was negligible. This is reflected by the fact that the opposite edges of the response surface in figure 11-4 are virtually parallel to each other.

Significant interactions in the autonomic control of AV conduction, however, have been demonstrated by the more recent studies of Takahashi and Zipes [6] and of Urthaler et al. [9]. Perhaps their techniques for detecting interactions were more sensitive than ours. Nevertheless, such autonomic interactions are certainly much stronger in the regulation of cardiac automaticity than in the regulation of AV conduction. Furthermore, the experiments of Urthaler et al. [9] indicate that the nature of the interaction differs for the two cardiac functions. With regard to heart rate control, Urthaler and his collaborators found that the vagal effects predominate over the sympathetic effects, just as in our study [3]. However, with regard to the control of

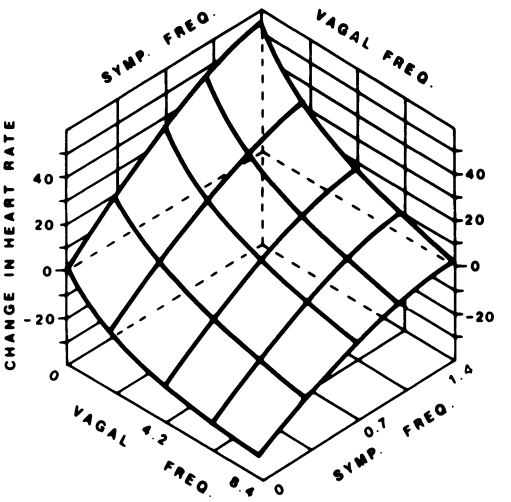

Figure 11-3. The mean changes in heart rate (beats/min) in a group of seven dogs in which an AV junctional rhythm had been induced. The heart rate changes were elicited by various combinations of stimulation frequencies applied concurrently to the stellate ganglia and vagus nerves. Reprinted Wallick et al. [7].

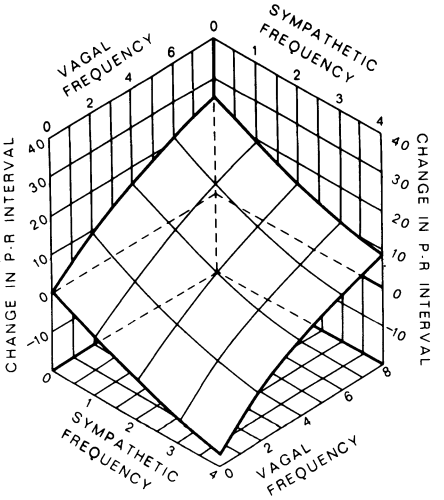

Figure 11-4. The mean changes in AV conduction time (P-R interval) as a function of the frequencies of right stellate ganglion and left vagal stimulation, in a group of 6 anesthetized dogs. Reprinted Levy and Zieske [3].

AV conduction, they found that the sympathetic effects predominate over the vagal influences [9].

The following questions remain to be answered:

1. Why are the autonomic interactions that influence cardiac automaticity so much stronger than those that affect AV conduction?
2. What characteristics of the AV junctional innervation account for our observations of a prominent interaction with regard to AV junctional automaticity, but for our failure to detect any interaction with regard to AV conduction?
3. What characteristics of the autonomic innervation of the heart account for a vagal preponderance in some cardiac structures, but a sympathetic preponderance in other cardiac structures?

EFFECTS ON MYOCARDIAL CONTRACTILITY

The first clear demonstration of an adrenergic-cholinergic interaction in the control of ventricular contractility was provided by the studies of Hollenberg et al. [10] in 1965. When they infused acetylcholine (ACh) into a coronary artery in anesthetized dogs, they elicited only a slight reduction in left ventricular contractile force. However, an identical dose of ACh evoked a much greater reduction in ventricular contractile force when it was infused during tonic sympathetic nerve stimulation or during a norepinephrine (NE) infusion.

My collaborators and I [11] observed similar interactions when we substituted trains of vagal stimuli for the ACh infusions that had been given by Hollenberg et al. [10]. The results we obtained [11] in an isovolumically contracting left ventricle preparation are shown in figure 11-5. When we progressively increased the volume of fluid in a balloon in the left ventricle (panels A, B, and C), vagal stimulation decreased peak left ventricular pressure by 21 percent, regardless of the magnitude of the peak ventricular pressure. We then restored the fluid volume in the left ventricular balloon (panel D) to the volume that prevailed in panel A. We stimulated the left stellate ganglion at a frequency of 2 Hz between the times denoted by event marks 1 and 3. During the tonic stellate stimulation, we stimulated the vagus nerve concomitantly (event mark D) at the same frequency that had been used to evoke the responses shown in panels A to C. Vagal stimulation in the presence of tonic sympathetic stimulation diminished peak left ventricular pressure by 35 percent (panel D). Hence, the negative inotropic effect of vagal stimulation was augmented substantially when the level of background sympathetic activity was enhanced. These effects of vagal stimulation parallel the results obtained by Hollenberg et al. [10] with ACh infusions. Qualitatively, similar autonomic interactions have also been demonstrated in the atrial myocardium [12].

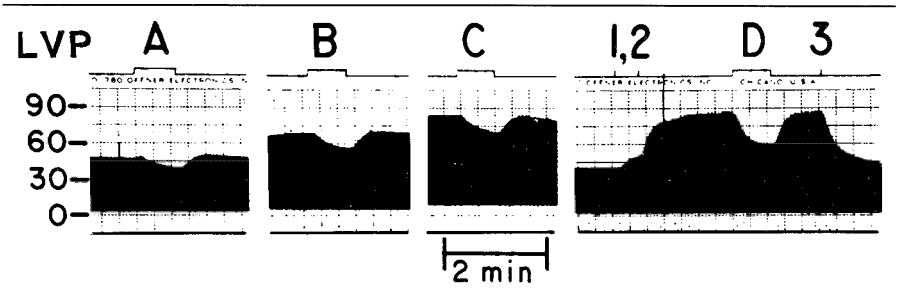

Figure 11-5. The effects of supramaximal vagal stimulation (20 Hz) on left ventricular pressure (LVP, in mm Hg) in a canine isovolumic left ventricle preparation. During event marks A, B, and C, the right vagus nerve was stimulated in the absence of sympathetic stimulation. Between panels A and B and again between panels B and C, additional fluid was added to a balloon in the left ventricle to raise the ventricular diastolic pressure. Between panels C and D, the balloon volume was restored to the level that prevailed during panel A. At event mark 1 in panel D, we began to stimulate the left stellate ganglion at a frequency of 2 Hz, but at a submaximal voltage. Between event marks 2 and 3 in panel D, the left stellate ganglion was stimulated supramaximally at 2 Hz. During event mark D, the right vagus nerve was stimulated supramaximally at 20 Hz, just as it had been during event marks A to C. Reprinted Levy et al. [11] with permission from the American Heart Association, Inc.

Questions that remain to be answered are

1. What is the relative importance of prejunctional and postjunctional mechanisms in the ventricular myocardium?
2. How are the interactions influenced by the level of coronary blood flow?
3. How do the interactions in the atrial myocardium compare with those in the ventricular myocardium? To what extent does any difference in the interactions depend on the fact that the vagal innervation of the atria is abundant, whereas the vagal innervation of the ventricles is more sparse?

UNDERLYING MECHANISMS

Autonomic interactions can occur at prejunctional and postjunctional levels (figure 11-6) of the autonomic neuroeffector junctions in the heart [5, 13–18]. Prejunctionally, the ACh released from vagal endings inhibit the release of NE from neighboring postganglionic sympathetic nerve endings [19–23]. Alternatively, either NE or neuropeptide Y (NPY), released from sympathetic nerve endings, can inhibit the release of ACh from nearby vagal endings [24, 25]. The postjunctional interactions occur at the level of the cardiac effector cells themselves and involve changes in intracellular concentrations of cyclic AMP [13–18].

Prejunctional Mechanisms

Postganglionic vagal and sympathetic fibers often lie close to each other in the walls of the heart, and they may even be wrapped in a common Schwann

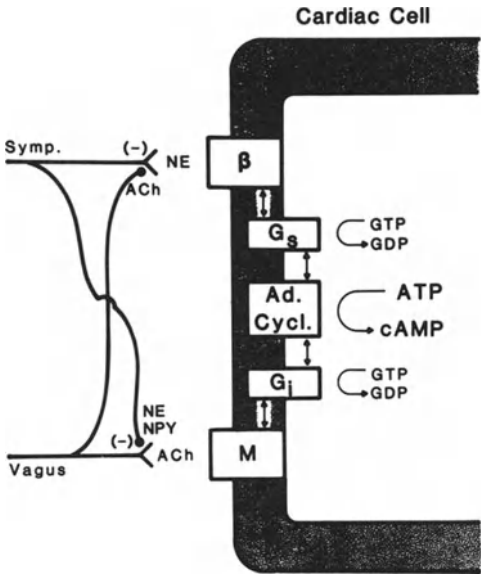

Figure 11-6. Mechanisms responsible for cardiac sympathetic-parasympathetic interactions. Prejunctionally, vagal activity releases acetylcholine (ACh), which inhibits (−) the release of norepinephrine (NE) from sympathetic nerve endings. Also prejunctionally, sympathetic activity releases norepinephrine (NE) and neuropeptide Y (NPY), which both inhibit the release of ACh from vagus nerve endings. Postjunctionally, the NE released from sympathetic nerve endings occupies β-adrenergic receptors (β) on the cardiac cell surface. This process stimulates adenylate cyclase (Ad. Cycl.) to accelerate the synthesis of cyclic AMP (cAMP) from adenosine triphosphate (ATP). The β-adrenergic receptor (β) is coupled to adenylate cyclase through a stimulatory protein, G_s. Acetylcholine (ACh) released from vagal endings occupies muscarinic receptors (M) on the cell surface. These receptors are coupled to adenylate cyclase through an inhibitory protein, G_i. Thus, ACh attenuates the rise in cAMP that would otherwise be induced by the NE reacting with the β-adrenergic receptors. Modified from Levy [18].

sheath [26]. Thus, neurotransmitters and neuromodulators, released from one type of autonomic nerve fiber, may be present in effective concentrations near the terminals of the other type of autonomic nerve fiber.

In isolated atrial preparations, the addition of muscarinic agonists [19, 21] or excitation of vagal nerve fibers [20] diminishes the release of NE from sympathetic fibers. This inhibition of NE release is mediated by muscarinic receptors on the postganglionic sympathetic nerve endings [22]. We decided to determine whether a sympathetic-vagal interaction in the ventricles, similar to that shown in figure 11-5, could be explained at least partially by a prejunctional mechanism. We measured the overflow of NE into the coronary sinus blood of anesthetized dogs under control conditions, during cardiac sympathetic stimulation alone, and during combined sympathetic and vagal stimulation [23]. During sympathetic stimulation alone, the NE overflow was 33.2 ng/min during supramaximal stimulation at 2 Hz (figure 11-7).

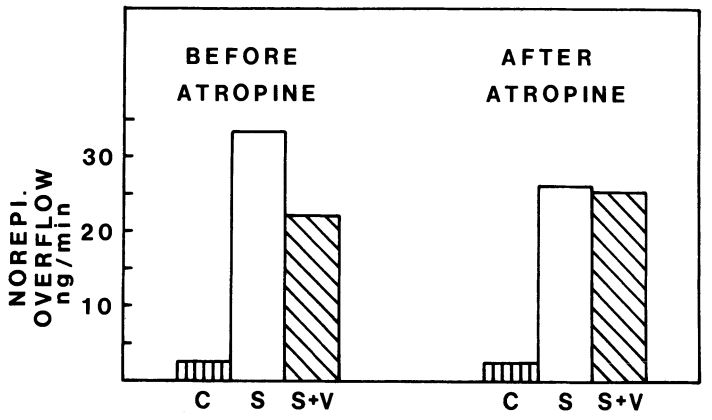

Figure 11-7. The mean overflows of norepinephrine into the coronary sinus blood in a group of seven anesthetized dogs under control conditions (C), during supramaximal sympathetic stimulation (S) at a frequency of 2 Hz, and during combined stimulation (S + V) of the sympathetic (2 Hz) and vagus (15 Hz) nerves. Norepinephrine overflows were determined before and after atropine administration. Modified from Levy and Blattberg [23] with permission from the American Heart Association, Inc.

During concomitant sympathetic and vagal stimulation, the NE overflows were diminished by 33 percent. This inhibition of NE overflow by vagal stimulation was abolished by atropine. These vagally induced changes in NE overflow must have taken place mainly in ventricular tissue because more than 95 percent of the blood that drains into the coronary sinus has perfused ventricular tissue, whereas only 5 percent or less has perfused atrial tissue.

Similarly, neurotransmitters or neuromodulators released from sympathetic nerve endings can inhibit the release of ACh from vagal endings in the heart (figure 11-6). NE and NPY are known to be released from sympathetic nerve endings throughout the body, including those in the heart. These substances are able to inhibit ACh release from vagal terminals in the heart.

Experiments on isolated atria have shown that the catecholamines, NE and epinephrine, inhibit the release of ACh from intramural vagal nerve endings [27, 28]. The inhibition appears to be mediated by α_1-adrenergic receptors on the vagal terminals. ACh release can be inhibited by α_1-adrenergic agonists, such as methoxamine, but not by α_2- or β-adrenergic agonists, such as clonidine or isoproterenol, respectively [28]. Furthermore, the inhibitory influence on ACh release can be antagonized effectively by the α-receptor blocker, prazosin, but α_2- and β-receptor blockers are much less potent as antagonists [28].

The neuropeptide, NPY, is also released along with NE, from sympathetic nerve endings (figure 11-6) in the heart [24, 25]. This neuropeptide can profoundly inhibit the release of ACh from neighboring vagal endings. A brief train of sympathetic stimulation may strongly inhibit the chronotropic

response of the heart to subsequent vagal stimulation (figure 11-8). High frequency stimulation (15 to 20 Hz) of the sympathetic nerves is usually required to evoke significant effects, and the inhibition may persist for about an hour. Intravenous injection of NPY similarly inhibits the chronotropic responses to vagal stimulation (figure 11-8). The magnitude and duration of the inhibition evoked by NPY parallels those induced by sympathetic stimulation.

Postjunctional Mechanisms

Substantial adrenergic-cholinergic interactions occur in the heart in response to infusions of adrenergic and cholinergic agonists, just as they do in response to stimulation of the sympathetic and parasympathetic nerves [5, 10, 13–18]. Hence, the interactions must not be mediated exclusively by the prejunctional inhibition of transmitter release from autonomic nerve fibers (figure 11-6). An important component of the interaction must occur postjunctionally, at the surface of the cardiac effector cell itself [5, 13–18].

The mechanism for the postjunctional antagonism between neurally released NE and ACh is depicted in figure 11-6. The sympathetically released NE combines with β-adrenergic receptors (β) in the cell membrane [14]. Occupation of the β-adrenergic receptors by NE stimulates the membrane-bound enzyme, adenylate cyclase, to catalyze the intracellular synthesis of cyclic AMP (cAMP) from ATP. The facilitation of the adenylate cyclase by the β-adrenergic receptors is mediated by a regulatory protein, G_s. The enzymatic effect of the G_s protein involves the hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP).

The ACh released from cardiac vagal endings antagonizes the facilitatory influence of NE by combining with muscarinic receptors (M) in the effector cell membrane (figure 11-6). The muscarinic receptors inhibit adenylate cyclase, through an inhibitory coupling protein, G_i [15–17]. This regulatory protein also catalyzes the hydrolysis of GTP to GDP, just as does the G_s protein.

The principal postjunctional adrenergic-cholinergic interaction is thus mediated by adenylate cyclase. The facilitatory effects of sympathetic activity are achieved by raising the intracellular levels of cAMP. The ACh released from postganglionic vagal endings antagonizes these effects by inhibiting adenylate cyclase and thereby attenuating the adrenergically induced elevation of the cAMP concentration.

Questions that remain to be answered are

1. What are the relative roles of prejunctional and postjunctional mechanisms in the autonomic interactions that take place in the heart?
2. Do the relative roles vary in the various structures in the heart?
3. Do the relative roles change under various physiologic and pathophysiologic conditions?

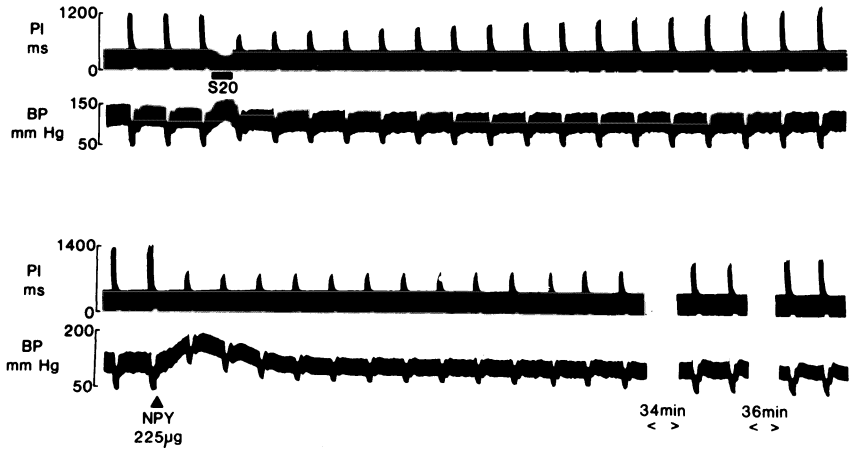

Figure 11-8. The effects of 60 sec train of sympathetic stimulation (horizontal bar) at a frequency of 20 Hz (upper panel) or of an intravenous injection (solid triangle) of 0.225 mg of neuropeptide Y (NPY, lower panel) on the chronotropic responses to repetitive 15 sec trains of vagal stimulation, delivered once every two minutes. The chronotropic responses (upper tracing in each panel) are the changes in pulse interval (PI), in msec, induced by the vagal stimuli. The corresponding changes in arterial blood pressure (BP) are shown in the lower tracing in each panel. Reprinted from Potter [24].

4. Do the relative roles vary with species? What are the relative roles in humans? Do they change with age?
5. The inhibitory effects of NE on ACh release have been demonstrated only in vitro. How effective is this inhibition in vivo?
6. Is the sympathetically mediated inhibition of the chronotropic response to vagal stimulation shown in figure 11-8 truly mediated by NPY? The evidence is highly suggestive, but still indirect, because specific and effective blocking drugs have not yet been developed.
7. A number of other neuropeptides are known to be present in the cardiac autonomic nerves. Many of them are known to have potent effects on specific cardiac activities. What are their actions on the heart under various physiologic and pathophysiologic conditions?
8. Do various physiologic and pathologic states affect the specific postjunctional components of the adenylate cyclase system differentially? For example, what components of this system are affected by endocrine changes (such as hyperthyroidism), by myocardial ischemia, or by chronic volume or pressure overload.

REFERENCES

1. Rosenblueth, A. and Simeone, E.A. 1934. The interrelations of vagal and accelerator effects on the cardiac rate. *Am. J. Physiol.* 110:42.
2. Samaan A. 1935. The antagonistic cardiac nerves and heart rate. *J. Physiol. (London)* 83:332.

3. Levy, M.N. and Zieske, H. 1969. Autonomic control of cardiac pacemaker activity and atrioventricular transmission. *J. Appl. Physiol.* 27:465.
4. Warner, H.R. and Russell, R.O., Jr. 1969. Effect of combined sympathetic and vagal stimulation on heart rate in the dog. *Circ. Res.* 24:567.
5. Levy, M.N. 1971. Sympathetic-parasympathetic interactions in the heart. *Circ. Res.* 29:437.
6. Takahashi, N. and Zipes, D.P. 1983. Vagal modulation of adrenergic effects on canine sinus and atrioventricular nodes. *Am. J. Physiol.* 244:H775.
7. Wallick, D.W., Felder, D., and Levy, M.N. 1978. Autonomic control of pacemaker activity in the atrioventricular junction of the dog. *Am. J. Physiol.* 235:H308.
8. Wallick, D.W., Martin, P.J., Masuda, Y., and Levy, M.N. 1982. Effects of autonomic activity and changes in heart rate on atrioventricular conduction. *Am. J. Physiol.* 243:H523–527.
9. Urthaler, F., Neely, B.H., Hageman, G.R., and Smith, L.R. 1986. Differential sympathetic-parasympathetic interactions in sinus node and AV junction. *Am. J. Physiol.* 250:H43.
10. Hollenberg, M., Carriere, S., and Barger, A.C. 1965. Biphasic action of acetylcholine on ventricular myocardium. *Circ. Res.* 16:527.
11. Levy, M.N., Ng, M., Martin, P., and Zieske, H., Jr. 1966. Sympathetic and parasympathetic interactions upon the left ventricle of the dog. *Circ. Res.* 19:5.
12. Stuesse, S.L., Wallick, D.W., and Levy, M.N. 1979. Autonomic control of right atrial contractile strength in the dog. *Am. J. Physiol.* 236:H860–H865.
13. Levy, M.N. and Martin, P.J. 1979. Neural control of the heart. in *Handbook of Physiology*, section 2, The cardiovascular system, vol. I, The heart, R.M. Berne and N. Sperelakis, eds., p. 581, Bethesda, American Physiology Society.
14. Watanabe, A.M., Lindemann, J.P., Jones, L.R., et al. 1981. Biochemical mechanisms mediating neural control of the heart. in *Disturbances in Neurogenic Control of the Circulation*, F.M. Abboud, H.A. Fozzard, J.P. Gilmore, and D.J. Reis, eds., p. 189, Bethesda, American Physiology Society.
15. Stiles, G.L., Caron, M.G., and Lefkowitz, R.J. 1984. β-Adrenergic receptors: biochemical mechanisms of physiological regulation. *Physiol. Rev.* 64:661.
16. Watanabe, A.M., Lindemann, J.P., and Fleming, J.W. 1984. Mechanisms of muscarinic modulation of protein phosphorylation in intact ventricles. *Fed. Proc.* 43:2618.
17. Jakobs, K.H., Minuth, M., Bauer, S., et al. 1986. Dual regulation of adenylate cyclase. A signal transduction mechanism of membrane receptors. *Basic Res. Cardiol.* 81:1.
18. Levy, M.N. 1988. Sympathetic-vagal interactions in the sinus and atrioventricular nodes. in *Electrophysiology of the Sino-Atrial and Atrioventricular Nodes*, L. Dreifus, ed., New York, Alan R. Liss, Inc., p. 85.
19. Löffelholz, K. and Muscholl, E. 1969. A muscarinic inhibition of the noradrenaline release evoked by postganglionic sympathetic nerve stimulation. *Naunyn-Schmiedebergs Arch. Pharmakol.* 265:1.
20. Löffelholz, K. and Muscholl, E. 1970. Inhibition by parasympathetic nerve stimulation of the release of the adrenergic transmitter. *Naunyn-Schmiedebergs Arch. Pharmakol.* 267:181.
21. Allen, G.S., Rand, M.J., and Story, D.F. 1974. Effect of the muscarinic agonist McN-A-343 on the release by sympathetic nerve stimulation of (3H)-noradrenaline from rabbit isolated ear arteries and guinea-pig atria. *Br. J. Pharmacol.* 51:29.
22. Muscholl, E. 1980. Peripheral muscarinic control of norepinephrine release in the cardiovascular system. *Am. J. Physiol.* 239:H713.
23. Levy, M.N. and Blattberg, B. 1976. Effect of vagal stimulation on the overflow of norepinephrine into the coronary sinus during cardiac sympathetic nerve stimulation in the dog. *Circ. Res.* 38:81.
24. Potter, E.K. 1985. Prolonged nonadrenergic inhibition of cardiac vagal action following sympathetic stimulation: neuromodulation by neuropeptide Y? *Neurosci. Lett.* 54:117.
25. Kilborn, M.J., Potter, E.K., and McCloskey, D.I. 1985. Neuromodulation of the cardiac vagus: comparison of neuropeptide Y and related peptides. *Regulatory Peptides* 12:155.
26. Ehinger, B., Falck, B., and Sporrong, B. 1970. Possible axo-axonal synapses between peripheral adrenergic and cholinergic nerve terminals. *Z. Zellforsch. Mikrosk. Anat.* 107:508.
27. Wetzel, G.T. and Brown, J.H. 1985. Presynaptic modulation of acetylcholine release from cardiac parasympathetic neurons. *Am. J. Physiol.* 248:H33–H39.
28. Wetzel, G.T., Goldstein, D., and Brown, J.H. 1985. Acetylcholine release from rat atria can be regulated through an α_1-adrenergic receptor. *Circ. Res.* 56:763–766.

12. CENTRAL NERVOUS SYSTEM MODULATION OF CARDIAC RHYTHM

RICHARD L. VERRIER

The conceptual framework of this chapter is based on two fundamental assumptions. The first is that behavioral states are associated with distinct patterns of autonomic nervous system activity. The second is that the resulting neurohumoral profile will determine the intermediary mechanisms responsible for arrhythmias in susceptible individuals. Accordingly, the major modalities for arrhythmogenesis could include direct effects of neurotransmitters on the myocardium and its specialized conducting system and impaired perfusion due to inappropriate coronary vasoconstriction and/or enhanced platelet aggregability (figure 12-1).

RATIONALE FOR INVESTIGATING BEHAVIORAL STATES

It is apparent that the experimentalist cannot hope to replicate the entire spectrum of human emotions. Thus, it is necessary to select certain states for exploration based on their relevance to clinical and basic science. On both grounds three behavioral states appear to be particularly worthy of investigation. These are anger, fear, and sleep. With respect to clinical considerations, these states have been associated with the occurrence of angina pectoris and life-threatening arrhythmias [1, 2]. Experimentally, they have the advantage of being amenable to modeling and are associated with distinct, reproducible physiologic responses [3–7]. This chapter will be organized around a discussion of these specific states and will conclude by delineating some directions for future research.

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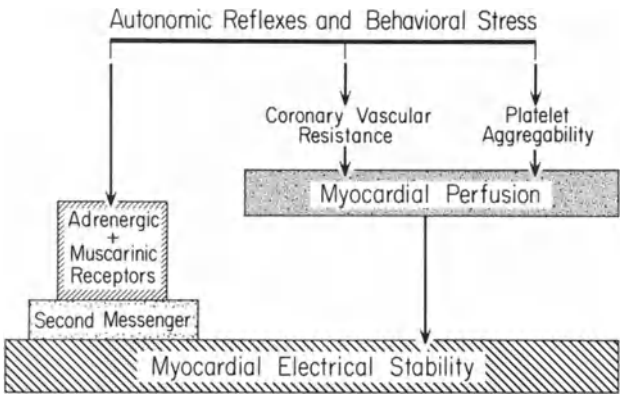

Figure 12-1. Summary of the mechanisms mediating the effects of the autonomic nervous system on ventricular electrical stability.

ANGER

Effects on Myocardial Electrical Stability

A few years ago we undertook to develop an experimental counterpart to anger [4]. This was prompted by clinical reports linking this emotional state with cardiovascular disorders, including angina pectoris and cardiac arrhythmias [1, 2, 8–10]. The experimental paradigm consisted of inducing an anger-like state in dogs by denial of access to food. After an overnight fast, the instrumented dog was brought to the experimental laboratory and allowed to acclimate for 20 to 30 minutes. While secured by a leash, the animal was presented with a dish of food. At this point, the food was moved just out of reach and a second leashed dog was permitted to consume the food. Upon observing this, the first dog almost invariably exhibited an anger-like behavioral state as evidenced by growling and exposing its teeth. At no time were the animals allowed to come into contact. The anger response persisted as long as the animals remained within sight of each other. The behavioral response was associated with consistent increases in heart rate, mean arterial blood pressure, and plasma catecholamine levels and a significant decrease in the repetitive extrasystole threshold (figure 12-2). The latter effect indicates that induction of anger is capable of substantially decreasing the electrical stability of even the normal myocardium. It is reasonable to assume, although it has not yet been established, that in the damaged myocardium major arrhythmias would be precipitated.

Delayed Myocardial Ischemia

Employing the anger model, we made a serendipitous observation regarding the effects of the poststress state on myocardial perfusion [4]. Specifically, we

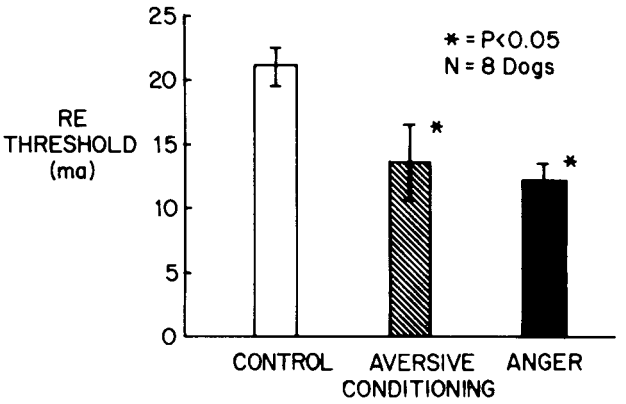

Figure 12-2. Effects of behavioral stress on the repetitive extrasystole (RE) threshold in normal dogs. Both passive aversive conditioning using a mild electric shock and induction of an anger-like state by denial of access to food produced significant reductions in the vulnerable period threshold. Heart rate was maintained constant during cardiac electrical testing by ventricular pacing. Reprinted from Verrier and Lown [24], by permission.

observed that after induction of the anger-like state, there is a progressive increase in coronary vascular resistance that ensues within two to three minutes and persists for 10 to 15 minutes after the anger episode (figure 12-3). The vasoconstrictor state lasts well after heart rate and arterial blood pressure have returned to control levels, indicating primary coronary vasoconstriction. In some animals, the response is so intense as to obstruct flow in the affected vessel completely. The presence of myocardial ischemia is indicated by significant ST segment changes. Although this phenomenon, which we termed "delayed myocardial ischemia," is not fully understood, some important insights have emerged from recent studies. Specifically, it appears that activation of the sympathetic nervous system is a critical factor. This is based on two lines of evidence. The first is that the phenomenon can be induced by direct electrical stimulation of the left stellate ganglion and that the vasoconstrictor response can be averted by alpha-adrenergic blockade with prazosin (figures 12-4 and 12-5) [11]. The second line of evidence is based on the recent observation that bilateral stellectomy prevents delayed ischemia induced by anger [12].

What then is the basis for the delayed nature of the response? A clue was provided by the finding that there is a close temporal association between the onset of ischemia and the return of coronary arterial blood pressure to the control level following anger or sympathetic stimulation [4, 11]. Experimental interventions were carried out to define the role of pressure in delayed ischemia [13]. The first involved preventing the hypertensive response to stellate stimulation by controlled exsanguination. When this proce-

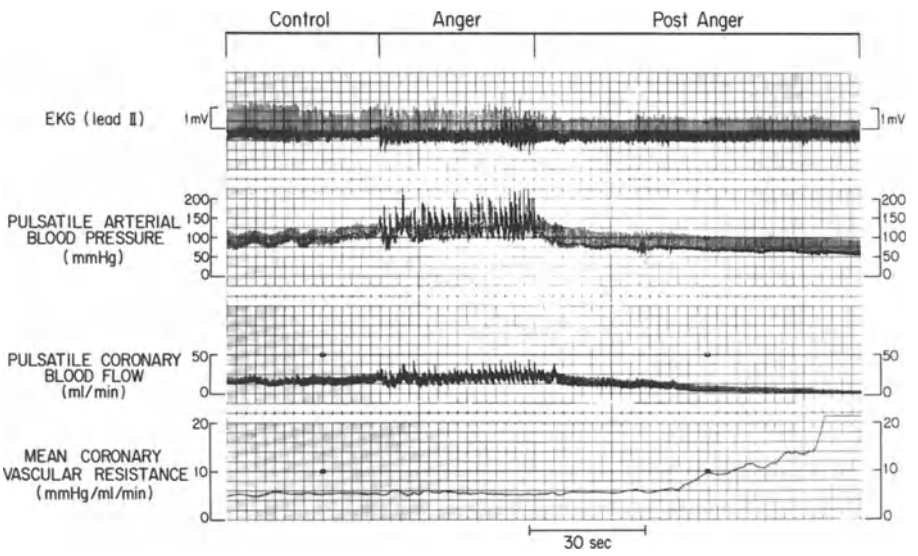

Figure 12-3. Effects of inducing an anger-like state on coronary hemodynamic function in a dog with coronary artery stenosis. During the stress state, coronary arterial blood flow increased substantially and coronary vascular resistance decreased (panels 1 and 2). However, during the poststress recovery phase (panels 3 and 4), a pronounced coronary vasoconstriction was evidenced by a fall in coronary arterial blood flow and an increase in coronary vascular resistance. These changes occurred when heart rate and arterial blood pressure returned to the prestress levels, suggesting primary coronary vasoconstriction. Reprinted from Verrier et al. [4].

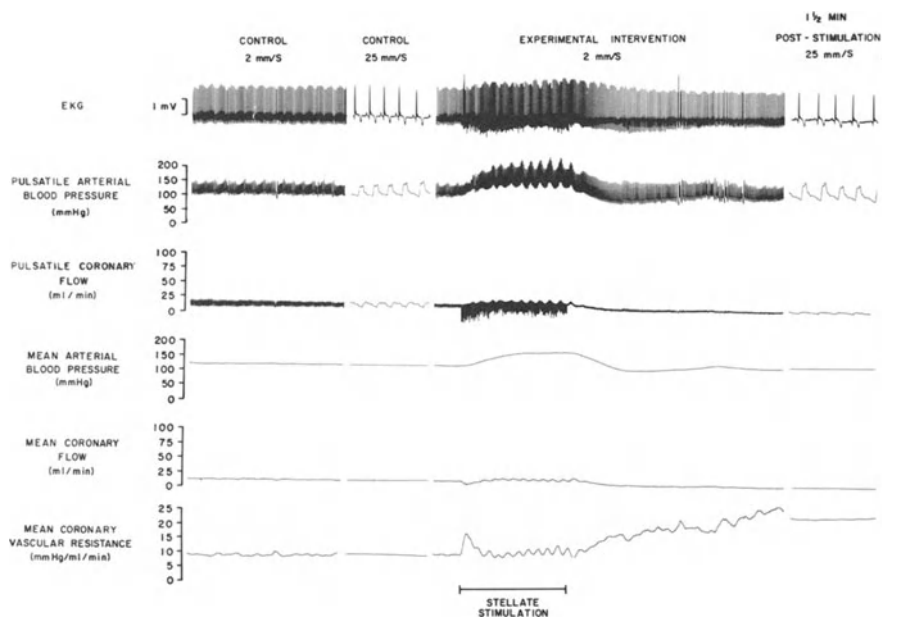

Figure 12-4. Representative tracing that depicts the response to a 30-second period of stellate stimulation. During sympathetic excitation, heart rate and blood pressure increase, but coronary blood flow and vascular resistance remain essentially unaltered. The poststimulation period is characterized by a renormalization of heart rate and blood pressure. At the same time, however, coronary flow decreases appreciably and there is a pronounced elevation in coronary vascular resistance. These changes are accompanied by S-T segment elevation indicative of myocardial ischemia. Reprinted from Hagestad and Verrier [11], by permission.

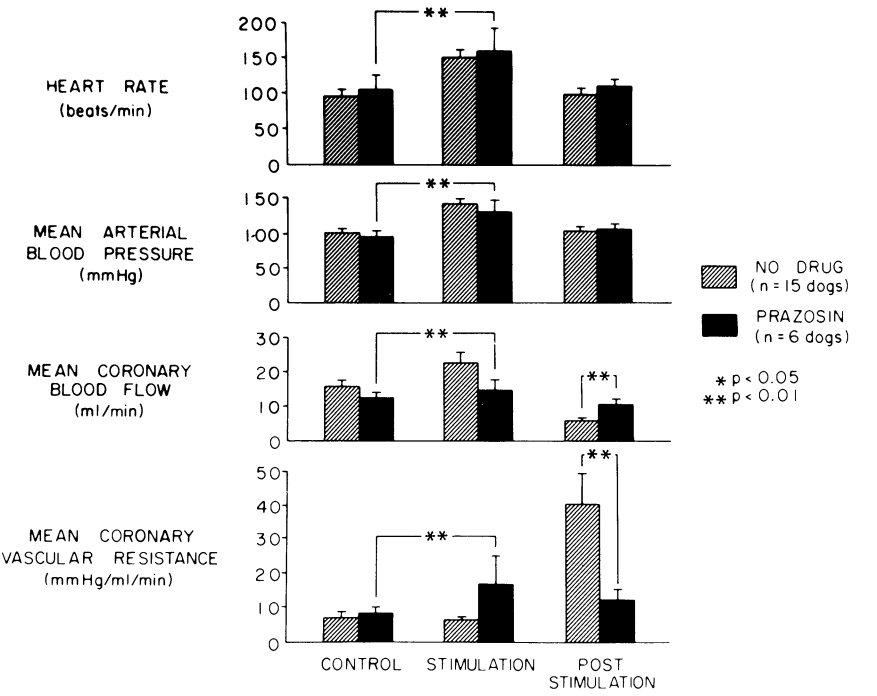

Figure 12-5. Influence of intravenous prazosin (0.3 mg/kg) on heart rate and hemodynamic function before, during, and after left stellate ganglion stimulation. Prazosin completely blunted the alterations in coronary blood flow and vascular resistance during the poststimulation period. These results were obtained from another subset of the control group. Reprinted from Hagestad and Verrier [11], by permission.

dure was carried out, the coronary vasoconstrictor response was not delayed but occurred during stimulation. A series of interventions designed to raise arterial blood pressure was next performed. These entailed stimulating the left stellate ganglion without blood pressure regulation and allowing the delayed coronary vasoconstriction to occur. Thereafter, systemic blood pressure was raised to the stimulation level by occluding the aorta with a snare. Increasing arterial blood pressure in this manner consistently returned coronary arterial flow and intracoronary pressure to the control values. By contrast, elevating systemic pressure by restimulating the stellate ganglion failed to restore flow through the coronary artery.

These findings are in accord with those of Gerova et al. [14]. Specifically, they found using sonomicrometers that stellate stimulation decreased coronary vessel diameter only when the hypertensive response was prevented by regulated exsanguination. They also demonstrated that the vasoconstrictor response to nerve excitation could be abolished by intravenous administra-

tion of phentolamine. Thus it appears that alpha-adrenergic receptor activation was the mechanism responsible for the increase in coronary vascular tone.

Our working hypothesis is that delayed myocardial ischemia results from an interplay between adrenergic and hemodynamic factors and that this interaction is the likely basis for the delayed nature of the ischemic response. The following hypothesis is proposed to account for the available data [4, 11–13]. During activation of the sympathetic nervous system, arterial blood pressure increases in response to either behavioral stress or direct excitation of the stellate ganglia, thereby opposing the vasoconstrictor influence of alpha-adrenergic receptor stimulation on vascular smooth muscle. The net result is that coronary vascular resistance remains unaltered. However, during the postexcitation phase, systemic pressure returns abruptly to the control level, thereby lessening distending pressure within the coronary vessel. We hypothesize that the dissipation of cathecholamines is delayed and thus the active adrenergic vasoconstrictor influence predominates over the passive distending force. The imbalance leads to a decrease in coronary diameter and to an increase in vascular resistance. This formulation is analogous to that proposed by Masuda and Levy [15] to account for a delayed recovery of heart rate and contractility following cessation of sympathetic nerve stimulation. Our findings are summarized in table 12-1.

Clinical Implications of Poststress Ischemia

These findings indicate that several characteristics of the poststress state are conducive to myocardial ischemia and arrhythmias. They include relatively elevated catecholamine levels and reduced coronary distending pressure in the face of lingering neurohumorally mediated vasoconstrictor drive. These factors may be responsible for the delayed onset of ischemia following cessation of exercise or intense emotional arousal. This hazardous coexistence of enhanced neurogenic activity and inadequate coronary distending pressure may also occur under conditions such as heart failure or hemorrhage. Schwartz et al. [16] have postulated that the markedly elevated sympathetic tone and concomitantly low coronary distending pressure may be responsible for the occurrence of coronary insufficiency and myocardial ischemia during hemorrhage [17]. It is clear that experimental modeling of these conditions and those associated with the poststress state could lead to important new insights into the pathophysiology of coronary artery disease.

FEAR

Aversive Conditioning

Fear is another affective state that has also been linked to a number of cardiovascular disorders including angina pectoris and life-threatening arrhythmias [1, 2]. Our early attempts at modeling this state involved a passive

Table 12-1. Mechanism responsible for delayed myocardial ischemia

1. The ischemic state ensues two to three minutes following provocation of stress.
2. Delayed ischemia can be prevented by stellectomy and induced by sympathetic stimulation. The latter effect can be blocked by alpha$_1$-adrenergic blockade.
3. The interaction between coronary distending pressure and adrenergic factors appears to be responsible for the delayed nature of the response.
4. These findings carry important clinical implications as they may help to explain the occurrence of myocardial ischemia in response to hemorrhage and heart failure. Under such conditions, the neurally induced rise in intravascular pressure may not be adequate to offset the coronary vasoconstrictor influence.

aversive shock conditioning paradigm [3, 18]. Essentially, dogs were placed alternately in a sling where they received a mild chest shock and in a nonstressful cage environment where they were left undisturbed. This sequence was repeated for three consecutive days after which time no further shocks were administered. The only behavioral input was the recollection of the previous noxious experience. In the sling environment, the animals exhibited somatic tremor and salivated excessively, and their heart rate and arterial blood pressure were markedly elevated. Most remarkable, however, was the pattern of neurohumoral release. The predominant catecholamine in the plasma was epinephrine rather than norepinephrine (figure 12-6) [19]. In fact, the norepinephrine to epinephrine ratio decreased from a resting level of 1.35 to a stress value of 0.6. This pattern of catecholamine release is in sharp contrast to that observed in response to anger, wherein there is a major increase in plasma norepinephrine with little or no increase in epinephrine [12].

The aversive conditioning paradigm proved to be a valuable biological model. It led to the demonstration that stress was not only capable of lowering the vulnerable period threshold but could precipitate ventricular tachycardias during myocardial infarction [20] and ventricular fibrillation during acute ischemia [21, 22]. Also it was demonstrated that adrenergic factors play a key role as beta-adrenergic blockade and stellectomy prevented stress-induced vulnerability [21, 23]. Another discovery was that spontaneous vagal tone was capable of annulling the adverse effects of enhanced adrenergic activity associated with behavioral stress [24, 25]. This finding provided essential verification of the fact that the mechanism of accentuated antagonism of adrenergic inputs by the vagus nerve [26] operates both in the conscious [24, 25, 27, 28] and anesthetized state [18, 29–31].

Natural Model

A crucial remaining question was whether a fearlike state can be induced without the need for delivering noxious stimuli. Recently obtained data suggest an affirmative answer to this question [32]. The paradigm is as

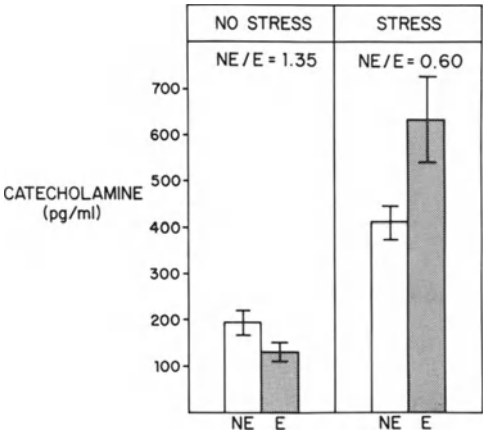

Figure 12-6. Relative plasma levels of norepinephrine (NE) and epinephrine (E) in two different psychologic environments. In the nonstressful cage environment the predominant catecholamine was NE (NE/E ratio = 1.35). In comparison, in the stressful sling setting E became the prevailing catecholamine (NE/E ratio = 0.60). Reprinted from Liang et al. [19], by permission.

follows. First the animals are screened and those that fail to exhibit an anger-like state (anger-negative dogs) are selected. Next the animals are suspended in a Pavlovian sling with their feet three to four inches above the floor. At this point, a leashed anger-positive dog is brought into the room. The dog in the sling immediately exhibits a fearlike state as it is unable to escape. There are major reproducible changes in heart rate, systemic blood pressure, and coronary arterial flow. Most notable, however, is the rise in epinephrine, as well as norepinephrine. Thus, during the same confrontation situation, the dog exhibiting an anger-like state releases predominantly norepinephrine with little change in epinephrine, whereas in the dog expressing fear, both catecholamines are significantly elevated.

Putative Intermediary Mechanisms

In the future we plan to address the following questions: What are the neurochemical and cardiovascular consequences of inducing the fearlike state? Is there habituation with repeated exposure? Can the changes in plasma constituents be related to the observed alterations in coronary hemodynamic and cardiac electrophysiologic function? How do these relationships differ from those discovered using the anger paradigm? Thus, the overall objective of these experiments is to obtain a relatively comprehensive understanding of the impact of a continuum of behavioral states. Since the same animals can be used in the different paradigms, the analytical power of the experimental designs is enhanced.

SLEEP

Despite considerable progress in defining the influence of stress on myocardial perfusion and ventricular electrical stability, comparatively little has been learned about the effects of sleep on these cardiovascular functions. Because sleep is a precisely integrated neurophysiologic state, its study could provide valuable insights into the linkage between the brain and the heart. Several fundamental issues need to be addressed. Specifically, what is the impact of sleep on coronary hemodynamic function and vulnerability to ventricular fibrillation in the normal and ischemic heart? What are the roles of the sympathetic and parasympathetic nervous systems in regulating these variables? To what extent do the two divisions of the autonomic nervous system interact during the various sleep stages? What is the impact of sleep deprivation on myocardial perfusion and ventricular electrical stability?

Effects on Heart Rhythm

Clinical studies indicate that sleep suppresses ventricular arrhythmias [33–41]. Lown et al. [35] found that 45 of 54 subjects undergoing 24-hour ambulatory monitoring exhibited significant reduction in ventricular ectopic activity during sleep. When sleep stages were monitored, these investigators noted reduction of ventricular premature beats during all stages except rapid eye movement (REM) sleep [34]. The most marked lessening of arrhythmias was recorded during slow wave sleep [stages 3 and 4]. The change in ventricular ectopic activity could not be related to changes in heart rate because heart rate remained relatively stable during the various sleep stages. The frequency of ventricular premature beats was similar during the awake and REM periods. Pickering et al. [37, 38] and others [33, 41] reported results comparable to those of Lown et al. [34, 35]. A beneficial effect of sleep on myocardial electrical stability is also suggested by the infrequency of sudden cardiac death during sleep, although it occupies about one third of the diurnal cycle [42, 43]. Ventricular tachycardia and fibrillation, however, have been noted to occur in association with violent or frightening dreams [44, 45]. Nevertheless, it remains highly inferential that when sudden death does occur nocturnally, it is during REM sleep.

Only a few experimental studies have been conducted to define the effects of sleep on susceptibility to cardiac arrhythmias. Skinner et al. [46] have explored specifically the influences of sleep stage on the occurrence of ventricular arrhythmias during left anterior descending coronary artery occlusion in pigs. They found that the period during the early sleep cycle wherein transitional and slow wave sleep alternate was accompanied by an increase in arrhythmias compared to the awake state. This was true both in the acutely infarcted and the recently infarcted pig heart. The maximum increase in ventricular arrhythmias was observed during sustained periods of slow wave sleep. Later when REM sleep predominated, the overall arrhythmia incidence

abruptly diminished. Acute coronary artery occlusion performed after the inception of slow wave sleep reduced the latency in onset of ventricular fibrillation compared with that observed during the awake state. Coronary occlusion during REM sleep was associated with the opposite effect, namely, a delay in the development of ventricular fibrillation.

These investigators reached some unexpected conclusions: (1) slow wave sleep, but not REM sleep, has a deleterious influence on the ischemic heart; (2) REM sleep may be beneficial because it delays the development of ventricular fibrillation during coronary artery occlusion; and (3) the heart rate changes during sleep do not correlate with the effects of slow wave or REM sleep on cardiac rhythm.

The explanation for these changes remains unclear. It is curious that in the pigs with coronary artery occlusion, arrhythmia was reduced not only during REM sleep but also during wakefulness. These investigators cite Baust and Bohnert [47] who found in cats that reduction in sympathetic tone accounts for the slow tonic heart rates during REM sleep, whereas during slow wave sleep, bradycardia was due to increased parasympathetic tone. However, increased sympathetic tone is certainly an attribute of the awake state. Snyder, Hobson, and Goldfrank [48] and others [49, 50] have demonstrated that heart rate and arterial blood pressure are higher during wakefulness than during sleep. There may indeed be hemodynamic concomitants, as well as coronary artery flow changes, linked to neural alterations during sleep stages that influence the electrically unstable ischemic heart.

Recently, we have obtained evidence suggesting that alterations in vagal tone may modulate cardiac electrophysiologic properties during sleep [51]. Specifically, the effects of REM and slow wave sleep on ventricular refractoriness were studied in chronically instrumented cats. Electrodes were implanted to record electrooculograms, electromyograms, and electroencephalograms for sleep stage determination. A right ventricular catheter was employed for cardiac electrical testing using the single stimulus technique. Both REM and slow wave sleep significantly increased the effective refractory period (table 12-2). This effect was independent of alterations in heart rate as this variable was maintained constant by pacing. These alterations were not prevented by bilateral stellectomy. However, when the muscarinic blocking agent atropine methylnitrate was administered, the sleep induced changes were completely abolished. These results suggest that the electrophysiologic changes associated with sleep are mediated through fluctuations in cardiac vagal tone.

Influence of Sleep on Myocardial Perfusion

Clinical studies indicate that phasic changes in coronary blood flow occur throughout the diurnal cycle. In particular, Deanfield et al. [52] have found during 24-hour Holter monitoring in patients with stable angina that ST

Table 12-2. Influence of sleep stage on ventricular refractoriness [51]

	Unpaced heart rate (beats/min)	Effective refractory period (msec) during fixed rate pacing at 180 beats/min
Awake	170 ± 10	114 ± 4
REM	140 ± 7*	124 ± 3*
SWS	140 ± 3*	121 ± 2*

Values are expressed as means ± S.E.M., * P<.05. SWS = slow wave sleep.

segments fluctuate substantially during day and night. Studies to correlate the ST segment variation with the sleep stage would be of considerable interest.

The potential value of such investigations is underscored by a report by King et al. [53] who studied the effects of sleep on the occurrence of Prinzmetal's variant angina. In an individual with angiographically documented coronary artery spasm, they found that the episodes of nocturnal chest pain accompanied by ST segment elevation occurred primarily during the REM stage of sleep. The factors responsible for the episodes of nocturnal angina, however, were not defined.

In animal experiments, Vatner et al. [54] noted that in the nocturnal period, when baboons were apparently asleep, coronary artery blood flow fluctuated by as much as twofold. The periodic oscillations in blood flow were not associated with changes in heart rate or arterial blood pressure and occurred while the animals remained motionless with eyes closed. Since the baboons were not instrumented for electrographic recordings, no data were obtained regarding sleep stage, nor was the mechanism for the coronary blood flow surge defined.

We addressed the issue of sleep-induced coronary blood flow changes in a recent series of experiments carried out in chronically instrumented dogs [55]. The animals were prepared for recording sleep stage and systemic and coronary hemodynamic function. The animals were studied during natural sleep and the cycles were divided into one minute periods of either quiet wakefulness, slow wave sleep, or REM sleep. The results are summarized in table 12-3. The findings indicate that during slow wave sleep there are moderate but significant reductions in heart rate and coronary blood flow and increases in coronary vascular resistance. In REM, the coronary blood flow baseline is moderately elevated compared to slow wave sleep and there are striking, episodic surges in flow. Coronary vascular resistance is reduced correspondingly. Heart rate and mean arterial pressure are also elevated during the flow surges, indicating that an increase in cardiac metabolic activity may be the basis for the coronary vasodilation. Since bilateral stellectomy prevented the surges in coronary blood flow, this response does not

Table 12-3. Differential effects of rapid eye movement (REM) and slow wave sleep (SWS) on coronary hemodynamic function [55].

	Awake	SWS	REM baseline	REM surge
MAP (mm Hg)	107 ± 4	106 ± 4	105 ± 7	111 ± 7
HR (beats/min)	85 ± 11	78 ± 10*	83 ± 10	102 ± 9†
CBF (ml/min)	33 ± 5	30 ± 4*	34 ± 4	52 ± 8†
CVR (mm Hg/ml/min)	3.6 ± 0.7	3.9 ± 0.7*	3.4 ± 0.7	2.5 ± 0.6†

Values are expressed as means ± S.E.M. * $P < .02$ compared to awake and REM baseline, and † $P < .02$ compared to awake SWS and REM baseline. MAP = mean arterial blood pressure; HR = heart rate; CBF = coronary blood flow; CVR = coronary vascular resistance.

appear to be due to nonspecific effects of somatic activity or respiratory fluctuations. Rather, the changes appear to be the direct result of enhanced adrenergic discharge. The influence of sleep on coronary hemodynamic function during coronary stenosis and myocardial infarction is currently under study in our laboratory. Preliminary data suggest that in the presence of coronary stenosis, the phasic increases in sympathetic discharge during REM sleep result in a decrease rather than an increase in coronary arterial blood flow [56].

FUTURE DIRECTIONS

Behavioral states are associated with distinct neurophysiologic responses, which in turn can have a unique impact on coronary hemodynamic and cardiac electrophysiologic function. Whereas it is evident that our understanding of the mechanisms involved is far from complete, important conceptual and methodologic foundations have been established. Consequently, the next few years are likely to witness major advances in both the experimental and clinical sectors. Some promising research approaches are indicated in table 12-4.

Specifically, there is a need to continue diversification of the behavioral models. Some specific states deserving of further study include anxiety, panic, and depression [2, 5], which would provide heuristic models for investigation of psychotropic and cardiovascular drugs.

Significant progress can be made in defining the central nervous system pathways involved in cardiac arrhythmogenesis. The roles of the thalamic gating system and amygdala and hypothalamic control centers in stress-induced vulnerability need to be defined [6, 57, 58]. A number of innovative techniques including reversible cryogenic blockade [57] and localized neuroinjection will be helpful in the pursuit of this objective [6].

The neurohumoral component of brain-heart interactions remains largely unexplored. Pituitary secretion of beta-endorphin, as well as the other hormones, may play a role in long-term fluctuation of cardiac electro-

Table 12-4. Future directions for study of neurocardiac interactions

Research focus	Research approaches
Behavior	Modeling of relevant states such as anxiety, panic and depression
Central nervous system mapping	Application of lesioning and neurochemical techniques to delineate arrhythmogenic centers in the brain. Also, pharmacologic study of the role of neurotransmitters in behaviorally induced arrhythmias.
Cardiac electrophysiologic mechanisms	Use of mapping techniques to define electrophysiologic processes responsible for stress related arrhythmias.
Myocardial perfusion	Use of advanced imaging methods to determine mechanisms involved in stress-induced coronary artery spasm. Also, application of platelet antibodies to characterize the role of platelets in the observed perfusion deficits.

physiologic properties. The recent discoveries of circulating peptides with significant cardiovascular effects (atrial natriuretic factor, gamma-mSH, enkephalins) [59–62] add another promising dimension to the study of neurocardiac interactions.

The application of sophisticated mapping techniques for the electrophysiologic study of tachyarrhythmias during stress could provide insights into mechanisms at the cardiac level [63–66]. The relationship of anatomically inhomogeneous sympathetic and parasympathetic innervation to electrophysiologic function remains to be elucidated. Also, study of the effects of stress in animals with mottled infarcts will provide useful information [67–70]. Indeed, postinfarction arrhythmias represent a substantial clinical management problem that could benefit from further investigation. There is an additional important rationale for exploring the effects of stress in the infarcted myocardium. Namely, as Inoue and Zipes [71] have shown, there may be necrotic damage to the heart's nerve supply that results in supersensitivity to catecholamines. This could be an important factor in stress-induced arrhythmias.

Finally, it is important to explore the role of myocardial perfusion as an intermediary mechanism in the genesis of arrhythmias during behavioral stress. This will require consideration of the effects on platelet aggregability and coronary hemodynamic function. In both disciplines, there have been many new exciting advances, clinically and experimentally, that could accelerate progress in the study of neural modulation of heart rhythm.

REFERENCES

1. Reich, P., DeSilva, R.A., Lown, B., and Murawski, B.J. 1981. Acute psychological disturbances preceding life-threatening ventricular arrhythmias. JAMA 246:233–235.
2. Schwartz, G.E., Weinberger, D.A., and Singer, J.A. 1981. Cardiovascular differentiation

of happiness, sadness, anger, and fear following imagery and exercise. *Psychosom. Med.* 43:343–364.
3. Lown, B., Verrier, R.L., and Corbalan, R. 1973. Psychologic stress and threshold for repetitive ventricular response. *Science* 182:834–836.
4. Verrier, R.L., Hagestad, E.L., and Lown, B. 1987. Delayed myocardial ischemia induced by anger. *Circulation* 75:249–254.
5. Hobson, J.A. 1978. What is a behavioral state? *Aspects of Behavioral Neurobiology*, J.A. Ferrendelli, ed. pp. 1–15, Bethesda Society for Neuroscience.
6. Smith, O.A. and DeVito, J.L. 1984. Central neural integration for the control of autonomic responses associated with emotion. *Annu. Rev. Neurosci.* 7:43–65.
7. Verrier, R.L. 1987. Mechanisms of behaviorally induced arrhythmias. *Circulation Suppl I. Circulation Monograph #6* 76:I48–I56.
8. Heberden, W. 1772. Some account of a disorder of the breast. Read at the College, July 21, 1768. *Medical Transactions*, Published by the Royal College of Physicians and Surgeons [Lond.] 2:59.
9. Kligfield, P. 1980. John Hunter, angina pectoris and medical education. *Am. J. Cardiol.* 45:367–369.
10. Kobayashi, T., Ishikawa, H., and Tawara I. 1970. Psychosomatic aspects of angina pectoris. *Scand. J. Rehabil. Med.* 2:87–91.
11. Hagestad, E.L. and Verrier, R.L. 1988. Delayed myocardial ischemia following the cessation of sympathetic stimulation. *Am. Heart J.* 115.
12. Verrier, R.L., Kirby, D.A., and Papageorgiou, P. 1988. Plasma catecholamines and anger-induced delayed myocardial ischemia [abstr.]. *Circulation*, in press.
13. Papageorgiou, P., Hagestad, E.L., and Verrier, R.L. 1988. Coronary distending pressure and delayed myocardial ischemia. *Am. Heart J.* 116:59–66.
14. Gerova, M., Barta, E., and Gero, J. 1979. Sympathetic control of major coronary artery diameter in the dog. *Circ. Res.* 44:459–467.
15. Masuda, Y. and Levy, M.N. 1985. Heart rate modulates the disposition of neurally released norepinephrine in cardiac tissues. *Circ. Res.* 57:19–27.
16. Schwartz, J.S., Carlyle, P.F., and Cohn, J.N. 1980. Effect of coronary arterial pressure on coronary stenosis resistance. *Circulation* 61:70–76.
17. Master, A.M., Dack, S., Horn, H, et al. 1950. Acute coronary insufficiency due to acute hemorrhage: An analysis of one hundred and three cases. *Circulation* 1:1302–1317.
18. Lown, B. and Verrier, R.L. 1976. Neural activity and ventricular fibrillation. *N. Engl. J. Med.* 294:1165–1170.
19. Liang, B., Verrier, R.L., Melman, J., and Lown, B. 1979. Correlation between circulating catecholamine levels and ventricular vulnerability during psychological stress in conscious dogs. *Proc. Soc. Exp. Biol. Med.* 161:266–269.
20. Corbalan, R., Verrier, R.L., and Lown, B. 1974. Psychological stress and ventricular arrhythmias during myocardial infarction in the conscious dog. *Am. J. Cardiol.* 34:692–696.
21. Verrier, R.L. and Lown, B. 1978. Influence of neural activity on ventricular electrical stability during acute myocardial ischemia and infarction. In *Management of Ventricular Tachycardia: Role of Mexiletine*, E. Sandøe, D.G. Julian, and J.W. Bell, eds., pp. 133–150, Amsterdam, Excerpta Medica (International Congress Series #458).
22. Verrier, R.L. and Lown, B. 1979. Influence of psychologic stress on susceptibility to spontaneous ventricular fibrillation during acute myocardial ischemia and reperfusion [abstr.] *Clin. Res.* 27:570A.
23. Verrier, R.L. and Lown B. 1977. Effects of left stellectomy on enhanced cardiac vulnerability induced by psychologic stress [abstr.] *Circulation* 55/56:III80.
24. Verrier, R.L. and Lown, B. 1984. Behavioral stress and cardiac arrhythmias. *Annu. Rev. Physiol.* 46:155–176.
25. Verrier, R.L. and Lown, B. 1980. Vagal tone and ventricular vulnerability during psychological stress [abstr.] *Circulation* 62:III176.
26. Levy, M.N. 1971. Sympathetic-parasympathetic interactions in the heart. *Circ. Res.* 29: 437–445.
27. DeSilva, R.A., Verrier, R.L., and Lown, B. 1978. The effects of psychological stress and vagal stimulation with morphine on vulnerability to ventricular fibrillation (VF) in the conscious dog. *Am. Heart J.* 95:197–203.

28. De Ferrari, G.M., Vanoli, E., Stramba-Badiale, M., et al. 1987. Vagal stimulation and sudden death in conscious dogs with a healed myocardial infarction [abstr.]. *Circulation* 76: IV107.
29. Kolman, B.S., Verrier, R.L., and Lown, B. 1975. The effect of vagus nerve stimulation upon vulnerability of the canine ventricle: role of sympathetic-parasympathetic interactions. *Circulation* 52:578–585.
30. Zuanetti, G., De Ferrari, G.M., Priori S.G., and Schwartz, P.J. 1987. Protective effect of vagal stimulation on reperfusion arrhythmias in cats. *Circ. Res.* 61:429–435.
31. Rabinowitz, S.H., Verrier, R.L., and Lown, B. 1976. Muscarinic effects of vagosympathetic trunk stimulation on the repetitive extrasystole (RE) threshold. *Circulation* 53:622–627.
32. Verrier, R.L. and Moya-Huff, F.A. 1988. Fear-induced changes in plasma catecholamines and coronary hemodynamic function [abstr.]. Society for Neuroscience Abstracts, in press.
33. Brodsky, M., Wu, D., Denes, P., et al. 1977. Arrhythmias documented by 24-hour continuous electrocardiographic monitoring in 50 male medical students without apparent heart disease. *Am. J. Cardiol.* 39:390–395.
34. DeSilva, R.A. 1982. Central nervous system risk factors for sudden cardiac death. *Ann. N.Y. Acad. Sci.* 382:143–161.
35. Lown, B., Tykocinski, M., Garfein, A., and Brooks, P. 1973. Sleep and ventricular premature beats. *Circulation* 48:691–701.
36. Monti, J.M., Folle, L.E., Peluffo, C., et al. 1975. The incidence of premature contractions in coronary patients during the sleep-awake cycle. *Cardiology* 60:257–264.
37. Pickering, T.G., Goulding, L., and Cobern B.A. 1977. Diurnal variations in ventricular ectopic beats and heart rate. *Cardiovasc. Med.* 2:1013–1022.
38. Pickering, T.G., Johnston, J. and Honour, A.J. 1978. Comparison of the effects of sleep, exercise and autonomic drugs on ventricular extrasystoles, using ambulatory monitoring of electrocardiogram and electroencephalogram. *Am. J. Med.* 65:575–583.
39. Rosenblatt, G., Zwilling, G., and Hartmann, E. 1969. Electrocardiographic changes during sleep in patients with cardiac abnormalities [abstr.]. *Psychophysiology* 6:233.
40. Smith, R., Johnson, L., Rothfeld, D., et al. 1972. Sleep and cardiac arrhythmias. *Arch. Intern. Med.* 130:751–753.
41. Winkle, R.A., Lopes, M.G., Fitzgerald, J.W., et al. 1975. Arrhythmias in patients with mitral valve prolapse. *Circulation* 52:73–81.
42. Friedman, M., Manwaring, J.H., Rosenman, R.H., et al. 1973. Instantaneous and sudden deaths. Clinical and pathological differentiation in coronary artery disease. JAMA 225:1319–1328.
43. Myers, A. and Dewar, H.A. 1975. Circumstances attending 100 sudden deaths from coronary artery disease with coroners' necropsies. *Br. Heart J.* 37:1133–1143.
44. Lown, B., Temte, J.V. Reich, P., et al. 1976. Basis for recurring ventricular fibrillation in the absence of coronary heart disease and its management. *N. Engl. J. Med.* 294:623–629.
45. Skinner, J.E. and Verrier, R.L. 1982. Task force report on sudden cardiac death and arrhythmias. In *Circulation, Neurobiology, and Behavior*, O.A. Smith, R.A. Galosy, and S.M. Weiss, eds., pp. 309–316, New York, Elsevier Science Publishing Co.
46. Skinner, J.E., Mohr, D.N., and Kellaway, P. 1975. Sleep-stage regulation of ventricular arrhythmias in the unanesthetized pig. *Circ. Res.* 37:342–349.
47. Baust, W. and Bohnert, B. 1969. The regulation of heart rate during sleep. *Exp. Brain Res.* 7:169–180.
48. Snyder, F., Hobson, J.A., and Goldfrank, F. 1963. Blood pressure changes during human sleep. *Science* 142:1313–1314.
49. Baccelli, G., Guazzi, M., Mancia, G., and Zanchetti, A. 1969. Neural and non-neural mechanisms influencing circulation during sleep. *Nature* 223:184–185.
50. Coccagna, G., Mantovani, M., Brignani, F., et al. 1971. Arterial pressure changes during spontaneous sleep in man. *Electroencephalogr. Clin. Neurophysiol.* 31:277–281.
51. Francis, G.C., Hagestad, E.L., and Verrier, R.L. 1986. Influence of sleep stage on ventricular refractoriness [abstr.] *Physiologist* 29:163.
52. Deanfield, J.E., Maseri, A., Selwyn, A.P., et al. 1983. Myocardial ischaemia during daily life in patients with stable angina: its relation to symptoms and heart rate changes. *Lancet* 2:753–758.
53. King, M.J., Zir, L.M., Kaltman, A.J., and Fox, A.C. 1973. Variant angina associated with

angiographically demonstrated coronary artery spasm and REM sleep. *Am. J. Med. Sci.* 265:419–422.
54. Vatner, S.F., Franklin, D., Higgins, C.B., et al. 1971. Coronary dynamics in unrestrained conscious baboons. *Am. J. Physiol.* 221:1396–1401.
55. Kirby, D.A. and Verrier, R.L. 1987. Differential effects of rapid eye movement and slow wave sleep on coronary hemodynamic function [abstr.] *Society for Neuroscience Abstracts* 13:740.
56. Verrier, R.L., Kirby, D.A., and Pflugheber, J.D. 1988. Phasic decreases in coronary blood flow (CBF) during rapid eye movement (REM) sleep in the stenosed canine circulation [abstr.] FASEB J. 2:A326.
57. Skinner, J.E. and Reed, J.C. 1981. Blockade of frontocortical-brain stem pathway prevents ventricular fibrillation of ischemic heart. *Am. J. Physiol.* 240:H156–H163.
58. Skinner, J.E. 1985. Regulation of cardiac vulnerability by the cerebral defense system. *J. Am. Coll. Cardiol.* 5:88B–94B.
59. Holtz, J., Sommer, O., and Bassenge, E. 1986. Sympatho-adrenal activity inhibited by atrial natriuretic factor (alpha-ANF) at physiological release rates in conscious dogs [abstr.]. *Circulation* 74:II425.
60. Sander, G.E., Giles, T.D., Kastin, A.J., et al. 1981. Cardiopulmonary pharmacology of enkephalins in the conscious dog. *Peptides* 2:403–407.
61. Carr. D.B., Saini, V., and Verrier, R.L. 1988. Opioids and cardiovascular function: neuromodulation of ventricular ectopy. In *Neurocardiology*, H.E. Kulbertus, and G. Franck, eds., pp. 223–245, Mt. Kisco, NY, Futura.
62. Saini, V., Carr, D.B., Hagestad, E.L., et al. 1988. Antifibrillatory mechanism of the narcotic agonist fentanyl. *Am. Heart J.* 115:598–605.
63. Burgess, M.J. 1982. Ventricular repolarization and electrocardiographic T wave form and arrhythmia vulnerability. In *Excitation and Neural Control of the Heart*, M.N. Levy and M. Vassalle, eds., pp. 181–202, Bethesda, American Physiological Society.
64. Kramer, J.B., Saffitz, J.E., Witkowski, F.X., and Corr, P.B. 1985. Intramural reentry as a mechanism of ventricular tachycardia during evolving canine myocardial infarction. *Circ. Res.* 56:736–754.
65. Janse, M.J., Wilms-Schopman, F., Wilensky, R.J., and Tranum-Jensen, J. 1985. Role of the subendocardium in arrhythmogenesis during acute ischemia. In *Cardiac Electrophysiology and Arrhythmias*, D.P. Zipes and J. Jalife, eds., pp. 353–362, Orlando, FL, Grune & Stratton, Inc.
66. Wit, A.L. and Josephson, M.E. 1985. Fractionated electrograms and continuous electrical activity: fact or artifact. In *Cardiac Electrophysiology and Arrhythmias*, D.P. Zipes and J. Jalife, eds., pp. 343–351, Orlando, FL, Grune & Stratton, Inc.
67. Karagueuzian, H.S., Fenoglio, J.J., Jr., Weiss, M.B., and Wit, A.L. 1979. Protracted ventricular tachycardia induced by premature stimulation of the canine heart after coronary artery occlusion and reperfusion. *Circ. Res.* 44:833–846.
68. Michelson, E.L., Spear, J.F., and Moore, E.N. 1980. Electrophysiologic and anatomic correlates of sustained ventricular tachyarrhythmias in a model of chronic myocardial infarction. *Am. J. Cardiol.* 45:583–590.
69. Michelson, E.L. 1982. Recent advances in antiarrhythmic drug research: studies in chronic canine myocardial infarction-ventricular tachyarrhythmia models. *PACE* 5:90–99.
70. Patterson, E., Holland, K., Eller, B.T., and Lucchesi, B.R. 1982. Ventricular fibrillation resulting from ischemia at a site remote from previous myocardial infarction. A conscious canine model of sudden coronary death. *Am. J. Cardiol.* 50:1414–1423.
71. Inoue, H. and Zipes, D.P. 1987. Results of sympathetic denervation in the canine heart: supersensitivity that may be arrhythmogenic. *Circulation* 75:877–887.

13. INFLUENCE OF ISCHEMIA AND INFARCTION ON CARDIAC VAGAL AND SYMPATHETIC INNERVATION

DOUGLAS P. ZIPES

Although the importance of the autonomic nervous system in modulating the genesis of cardiac arrhythmias has been known for many years and is widely accepted, the precise mechanisms by which such modulation results is incompletely understood. Endpoints, after a neural intervention, such as changes in the spontaneous development or electrical induction of arrhythmias, the fibrillation or repetitive response threshold or overall mortality, for example, provide vital information that a neural manipulation exerted an effect on the arrhythmia substrate, but they do not expose the underlying electrophysiologic mechanism. Knowledge of the latter is critical to formulate ways to intervene discretely and uniquely.

Assuming that normal autonomic input to the normal heart is not arrhythmogenic, the autonomic nervous system may provoke arrhythmias if its input becomes qualitatively or quantitatively abnormal (increased, decreased, or heterogeneous), the response of the myocardial substrate to normal autonomic nervous system stimulation becomes abnormal, or combinations of both. Using this simplistic logic of an exceedingly complex interactive system as a framework in which to consider the problem, one needs to begin by inquiring if and how ischemia/infarction makes cardiac vagal and sympa-

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thetic innervation abnormal, and if and how the ischemic/infarcted myocardium responds inappropriately to autonomic nervous system discharge.

FUNCTIONAL PATHWAYS OF AUTONOMIC NERVOUS SYSTEM INNERVATION OF THE CANINE VENTRICLE

The first objective is to understand the pathways of autonomic innervation to the ventricle so that one can determine whether a particular anatomic lesion, placed at the apex or base of the heart and involving epicardium, endocardium, or both, affects vagal or sympathetic neural transmission of afferent or efferent impulses.

AFFERENT PATHWAYS

Some afferent fibers with endings in the left ventricular wall appear to travel with sympathetic nerves and may be chemically sensitive, mechanosensitive, or both [1]. These sympathetic afferent fibers mediate a pressor response and a pseudoaffective response in animals that can be elicited by epicardial application of bradykinin [2]. Other afferent fibers travel with the vagus nerves and can be activated by epicardial application of nicotine [3]. Little is known of their intraventricular course. However, applied to the left ventricular epicardium, bradykinin increases systemic arterial blood pressure, a response eliminated by stellate ganglia interruption. Epicardial nicotine application decreases systemic arterial blood pressure, which is eliminated by vagotomy. Therefore, the nicotine vasodepressor response requires intact vagal nerves but not intact cardiac sympathetic pathways. The bradykinin vasopressor response depends on intact cardiac sympathetic nerves but not on intact vagi [4].

We found that epicardial application of phenol to the left ventricular free wall interrupted sympathetic afferent transmission and produced regions apical to the treated myocardium that no longer responded to bradykinin. Phenol did not interrupt vagal afferent transmission unless it was painted on the ventricular surface in the AV groove where it abolished sympathetic afferent reflexes as well. Intracoronary bradykinin activated a vasodepressor response followed by a vasopressor response. Phenol in the AV groove totally eradicated the vasodepressor response and phenol on the left ventricular free wall interrupted the vasopressor response. Intracoronary nicotine produced primarily a vasodepressor response unaffected by a phenol circle but eliminated by phenol in the AV groove.

We interpreted these data to indicate that afferent vagal fibers are located intramurally or in the subendocardium and cross the AV groove in the superficial epicardium. In contrast, afferent sympathetic fibers are located in the superficial subepicardium of the left ventricle (figure 13-1).

Further, we found that vagal vasodepressor responses to epicardial nicotine are greater in the canine midposterior left ventricle and are compatible with the higher incidence of bradycardia and/or hypotension during acute infero-

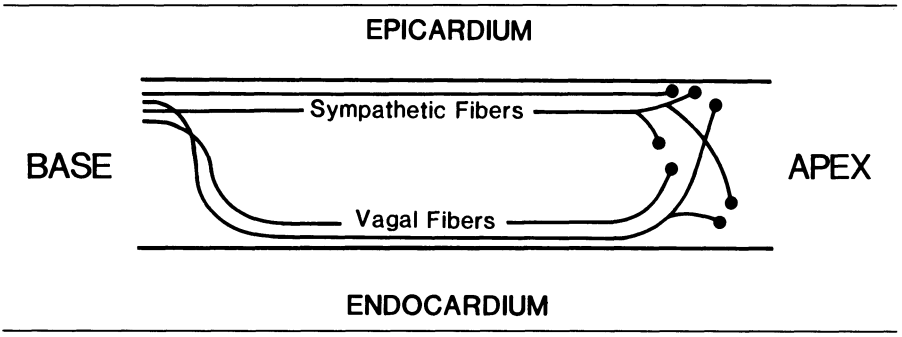

Figure 13-1. Proposed functional pathways of efferent and afferent vagal and sympathetic innervation of the left ventricle, shown in a sagittal view.

posterior myocardial infarction in patients. Sympathetic vasopressor responses to the epicardial bradykinin are not site specific [5].

Future Directions

The previous data provide information on functional pathways of afferent vagal and sympathetic innervation to the canine left ventricle. To our knowledge, there are no histologic-anatomic studies that verify or refute these conclusions. Indeed, it appears to be difficult to identify anatomically which autonomic limb is present when neural structures are found within the myocardium. However, using modern methodology, it is essential that the intramyocardial vagal and sympathetic afferent, as well as efferent (see below), pathways be tracked and the functional observations tested. The greater vasodepressor response to nicotine applied to the midposterior left ventricle confirms related observations by others that have been explained by preferential location of vagal afferent receptors in this area of the ventricle. However, to our knowledge, the actual density of these receptors in different parts of the heart has not been determined.

Effects of Ischemia and Infarction

Myocardial infarction of 90 minutes duration interrupts those vagal and sympathetic afferent responses elicited by nicotine and bradykinin [6]. More recently, we [7] have shown that, within minutes of the onset of transmural myocardial ischemia, the vasopressor response elicited by bradykinin applied within the ischemic zone or apical to it (figure 13-2) becomes attenuated or totally interrupted (figure 13-3). Further attenuation results when the myocardial blood flow in the epicardial test site decreases to about 40 percent or less of the control value. In contrast, nontransmural ischemia does not attenuate the response to bradykinin (figure 13-4). However, nontransmural ischemia produced by occlusion of a diagonal branch does attenuate the vasodepressor response to nicotine applied apically to the nonischemic area

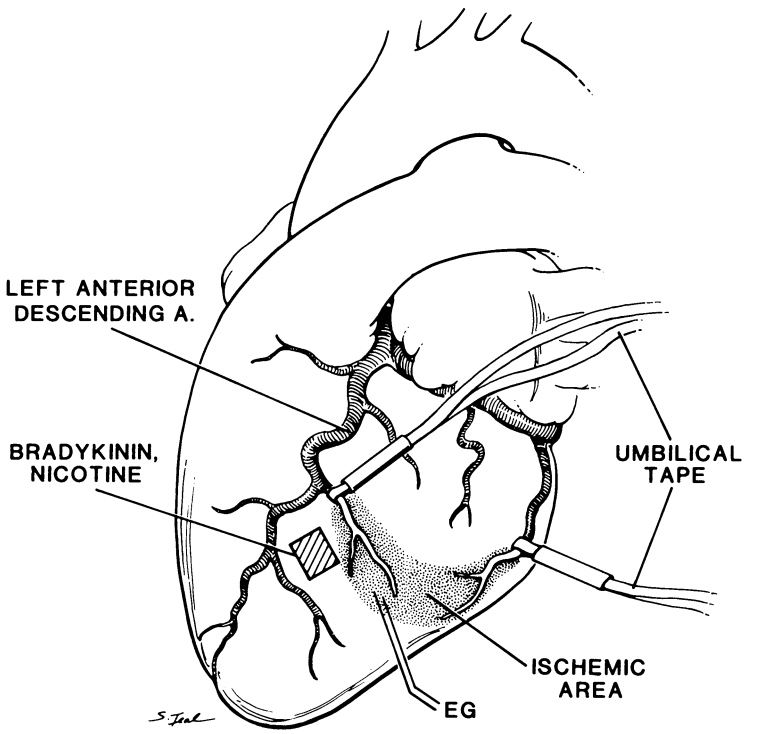

Figure 13-2. Schematic illustrating ischemic area and application of test substances. Reproduced from Inoue et al. [7], by permission.

within minutes (figure 13-5). These observations fit the postulated autonomic pathways shown in figure 13-1.

A 15-minute coronary occlusion followed by reperfusion produces reversible attenuation of both afferent neural responses (figures 13-5 and 13-6). Loss and recovery of these afferent reflex responses is slower than changes in the amplitude of a bipolar electrogram recorded from the ischemic zone, suggesting a different sensitivity to the effects of ischemia on conduction in ventricular muscle compared with neural transmission [7].

Future Directions

Several aspects of these studies appear worthy of future considerations. For reasons unknown, epicardial bradykinin and nicotine consistently fail to elicit afferent reflex responses in about half of the dogs studied [4–7]. The response failure may be due to an insufficient number of epicardial receptors stimulated because intracoronary injections of these drugs elicit expected responses [5]. The reasons why some dogs respond and others do not should be

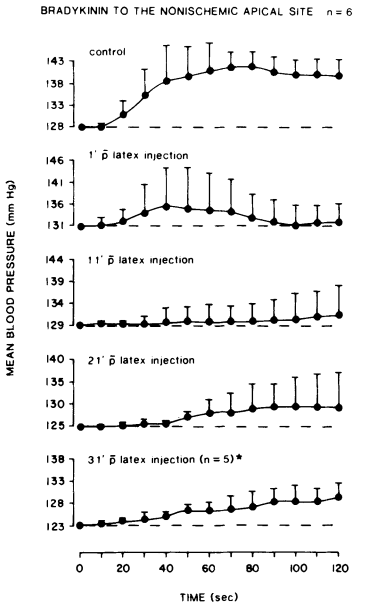

Figure 13-3. Blood pressure response to bradykinin (ordinate) versus time (abscissa) is shown at various intervals after coronary occlusion produced by latex injection. Reproduced from Inoue et al. [7], by permission.

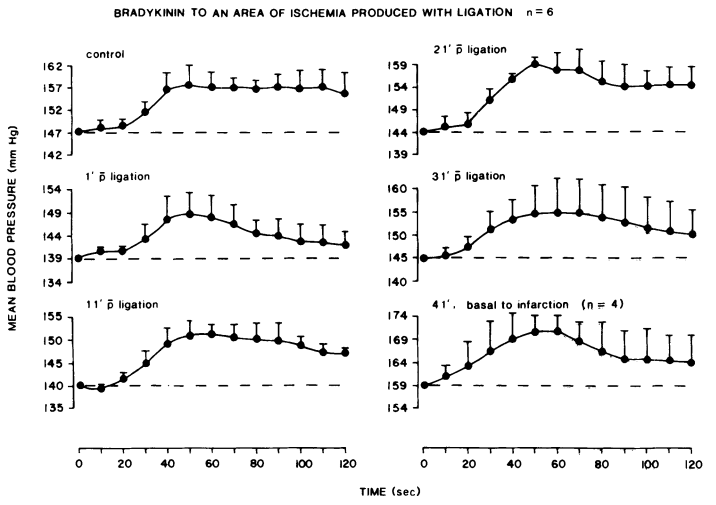

Figure 13-4. Blood pressure response to bradykinin (ordinate) versus time (abscissa) is shown at various intervals after coronary occlusion produced by coronary artery ligation. Reproduced from Inoue et al. [7], by permission.

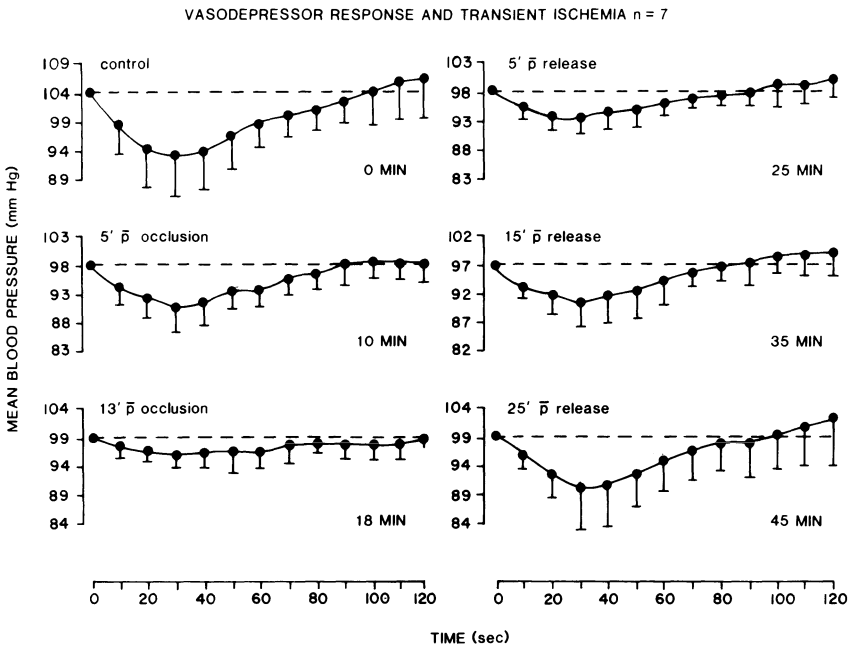

Figure 13-5. Blood pressure response to nicotine (ordinate) versus time (abscissa) is shown at various intervals after coronary occlusion and release. Time at lower right is interval since obtaining control values. Reproduced from Inoue et al. [7], by permission.

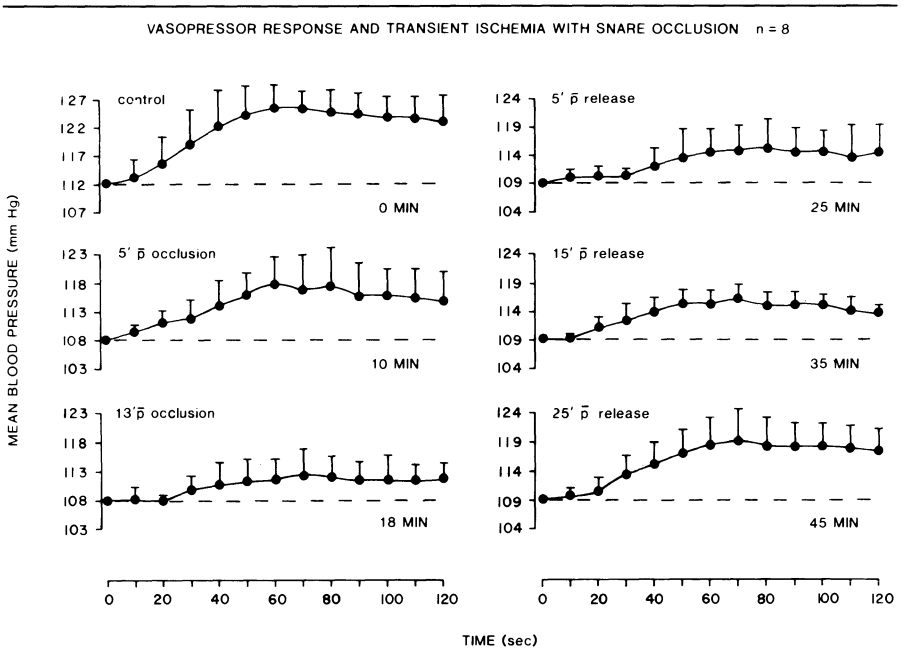

Figure 13-6. Blood pressure response to bradykinin (ordinate) versus time (abscissa) is shown at various intervals after coronary occlusion and release. Time at lower right is interval since obtaining control values. Reproduced from Inoue et al. [7], by permission.

explored in terms of receptor physiology. Perhaps the receptor density in the epicardium varies from dog to dog. The clinical counterpart of this variable response may explain why all patients with posteroinferior ischemia do not develop vasodepressor reactions.

Exploration of interactions between when and why ischemia activates a variety of well-known ischemic-induced afferent reflexes [8–11] and when and why it inhibits other afferent reflexes, as noted here, is important. The resultant physiologic response may be a balance between inhibiting and activating actions. It is possible that activation occurs at ischemic sites at the basal or lateral borders of the ischemic zone, whereas inhibition by the ischemic area itself occurs at more apical locations. Perhaps activation and inhibition have different time courses or different responses to transmural/nontransmural ischemic locations, or to mechanosensitive or chemosensitive stimulation.

These observations may have important clinical relevance. One area of special interest is the clinical presentation of painless ischemia. Since afferent sympathetic fibers appear to mediate cardiac pain sensation [12, 13], it is possible that some patients during angina undergo a form of autodenervation. Ischemia could interrupt afferent neurotransmission and eliminate pain perception. Recovery of neurotransmission would occur with reperfusion, so that another episode of ischemia, perhaps localized differently (e.g., sparing the epicardium and sympathetic afferents) might then produce pain in the same patient.

EFFERENT PATHWAYS

Previous results from our laboratory [14] showed that a circle of phenol of 2 to 3 cm radius applied to the anterior left ventricular epicardium interrupted efferent sympathetic but not vagal innervation within the circle. We concluded that efferent vagal fibers did not travel in the subepicardium but that sympathetic fibers probably did. Subsequently we applied phenol to the left ventricular epicardium along the AV groove and showed that it completely eliminated vagal-induced prolongation of the effective refractory period (ERP) in the subepicardium of the anterior and posterior left ventricle and significantly reduced, but did not completely eliminate, ERP prolongation in the intraventricular septum [15]. Interpretation of these data suggested that the majority of efferent vagal fibers enroute to innervate the anterior and posterior left ventricular epicardium crossed the AV groove within 1/4 to 1/2 mm of the epicardial surface, diving intramurally within 1 to 2 cm of the AV groove. Thus, the efferent pathways were quite similar to the afferent pathways (figure 13-1). Since vagal simulation after phenol application still prolonged the ERP of the intraventricular septum, some vagal efferent fibers must reach the septum and possibly the specialized conducting fibers via a different route.

Recent studies by Bloomquist et al. [16] confirm completely our observations on efferent vagal innervation, extending them to include right ventricular innervation and to suggest that the vagal efferent fibers crossing the AV groove are postganglionic axons having ganglion cells located in the atrium.

It is possible that the vagal fibers are located intramurally in the left ventricle. However, a subendocardial vagal route after crossing the AV groove is supported by our observation that a 2 mm deep encircling apical endocardial incision (2 cm radius) eliminated vagal-induced prolongation of refractoriness not only in the subendocardium within the circle outlined by the cut, but also in the intact epicardium directly above the incision [17]. These data suggest that the subendocardial incision interrupted vagal nerves passing through that subendocardial site enroute to innervate the overlying epicardium on the apical side of the cut.

We have also shown that dissection of the AV groove with or without cryoablation, as is done to interrupt accessory pathways in patients with the Wolff-Parkinson-White syndrome, interrupts vagal innervation [18].

Future Directions

As indicated earlier these functional pathways of efferent innervation need to be confirmed histologically.

Effects of Transmural Myocardial Ischemia and Infarction

Effect Denervation

We have shown that transmural myocardial infarction involving the subepicardial regions of the left ventricle interrupted efferent sympathetic neural transmission over nerves located within the infarction and produced sympathetic denervation at noninfarcted sites apical to the infarction. We have demonstrated functional denervation by using ERP response, by chemical denvervation using myocardial norepinephrine depletion, and by histofluourescent staining of catecholamine-containing nerve fibers and varicosities [19, 20]. Transmural myocardial ischemia/infarction similarly interrupts efferent vagar innervation [20] and depletes choline acetyltransferase [21]. In addition, a subendocardial infarction that spares the epicardium does not interrupt sympathetic transmission [20, 22], although it does interrupt vagal transmission [7, 20, 21].

More recently we have shown that efferent vagal and sympathetic neural responsiveness is attenuated or lost within the ischemic area or in normal, nonischemic myocardium apical to it in 5 to 20 minutes after the onset of ischemia and that denervation occurs heterogeneously and progresses with time (figures 13-7 and 13-8). More sites are totally denervated or exhibit attenuated responses at three hours than at one hour, for example [20].

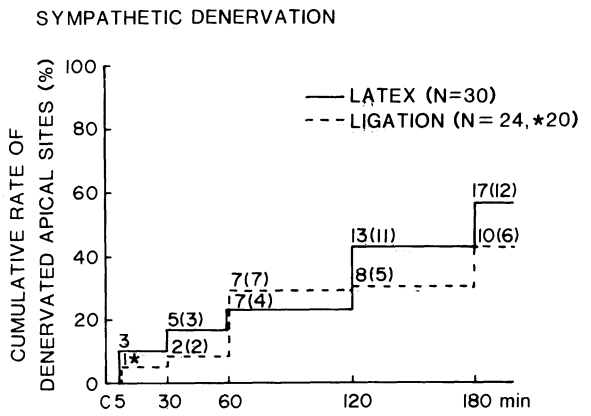

Figure 13-7. Cumulative percentage of sympathetically denervated apical sites divided by the number of total apical test sites (N) is shown on the ordinate as a function of time. A solid line indicates data from dogs with latex injection and a dotted line indicates data from dogs with coronary ligation. Figures without parentheses indicate cumulative number of denervated test sites, that is, the total number of the sites that had shown shortening of effective refractory period ≤2 msec at least once by that moment of determination. Figures in the parentheses indicate number of test sites that showed shortening of effective refractory period ≤2 msec at that moment of determination. Presentation in this fashion is necessary because some sites showed variation in response around the cutoff value. For example, from 120 to 180 minutes after latex injection, 4 new sites (17 minus 13) became denervated, whereas only 12 of the 17 sites actually still exhibited shortening of effective refractory period ≤2 msec at 180 minutes. The remaining five sites that had shown shortening of effective refractory period ≤2 msec at least once by 120 minutes now exhibited refractory period shortening >2 msec at 180 minutes after infarction. The cumulative rate of denervated sites did not differ between dogs receiving latex injection and coronary artery ligation ($P = .41$). C = control, i.e., before coronary occlusion. *Data from one dog that developed ventricular fibrillation were not included at 5 minutes alter ligation. Reproduced from Inoue and Zipes [20].

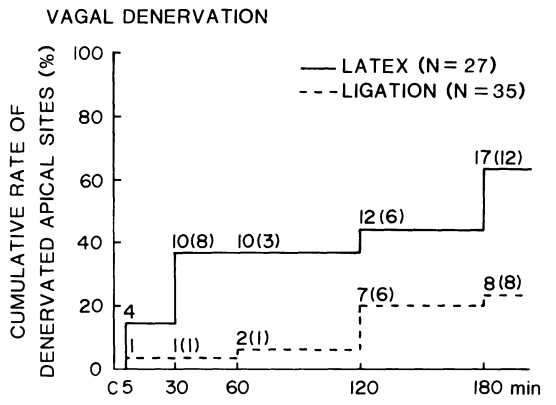

Figure 13-8. Cumulative percentage of vagally denervated apical sites is shown as in figure 13-7 was greater in dogs receiving latex injection than in dogs with ligation of coronary arteries ($P < .002$). Reproduced from Inoue and Zipes [20], by permission.

Future Directions

The activity of tyrosine hydroxylase, a neurochemical marker for sympathetic innervation, decreases in the ischemic left ventricle only after five hours of left anterior descending (LAD) coronary artery ligation. Similarly, choline acetyltransferase activity, a neurochemical marker for vagal innervation, decreases 5 to 25 hours after LAD ligation [23]. The disparity between the time courses for the early functional and later biochemical changes in response to ischemia/infarction needs to be explained. Differences in causal mechanisms may be responsible and need to be explored.

For example, it is not known whether early neural failure is due to direct ischemic injury to the nerves themselves or whether alterations in the ischemic myocardium through which the neural impulses traverse (e.g., hyperkalemia, hypoxia, acidosis, and other substances generated by ischemia and catabolism) are responsible. Given the promptness of the denervation and its reversibility, functional alterations of one kind or another appear most likely. The later reductions in tyrosine hydroxylase and choline acetyltransferase may follow nerve death from ischemic injury.

One additional point is worthy of future study. Although the extent of neural-induced alterations in refractoriness increased at most apical sites (i.e., more myocardium became denervated as the duration of ischemia increased, changes not all that unusual or unexpected with continuing ischemia), some test sites considered initially to be denervated regained neural responsiveness without measurable alterations in the degree of ischemia. Restoration of electrophysiologic responses during ongoing ischemia has been noted [24–26] and ascribed to increased collateral blood flow or diffusion of catabolites, such as potassium, from the ischemic area. The fact that we have observed similar phenomena in vitro [27] and that no changes in blood flow occurred in the present studies raise several interesting possibilities concerning ischemic injury.

Consistent with the observations of Murray et al. [28], we [29] have shown that a series of four five-minute coronary occlusions separated by five minutes of reperfusion reduces the extent of denervation produced by a subsequent three-hour coronary occlusion. The effects of this preconditioning ischemia may be analogous to the reduced mortality found when using the so-called Harris two-stage coronary occlusion and the reversal of autonomic denervation (or electrophysiologic recovery previously noted) during continued ischemia. The mechanism responsible for the effects of preconditioning ischemia is unknown. An increase in collateral blood flow does not occur. The short-term reversible occlusions may diminish the accumulation of noxious catabolites during the subsequent long-term occlusion or may promote the development of a substance or an event that enables the myocardium to withstand the effects of ischemia for a longer time period. Such a phenomenon may be important in patients who have episodes of reversible angina before developing a permanent coronary occlusion.

Denervation Supersensitivity

Tissue deprived of its nerve supply responds in an exaggerated fashion to certain agents, a phenomenon called denervation supersensitivity [30]. Thus, sympathetically denervated tissue may become supersensitive to the effects of infused catecholamines [31]. Supersensitivity in the area of myocardium that was sympathetically denervated by the infarction becomes manifested as an exaggerated shortening of the ERP during both norepinephrine and isoproterenol infusion, with an upward and leftward shift in the dose-response curves in the apical versus basal regions [32]. There is no difference in the density of beta-adrenergic receptors ([^{131}])-cyanopindolol) in the apical versus basal regions. Beta-adrenergic receptor coupling (guanosine triphosphate [GTP] plus isoproterenol-stimulated adenylate cyclase activity) is not different statistically in the apical and basal regions. Muscarinic modulation of beta-receptor coupling (oxotremorine attenuation of GTP plus isoproterenol-stimulated adenylate cyclase activity) also is not significantly different in the apical versus basal sites. These data show that a transmural myocardial infarction produces denervation supersensitivity in areas apical to the infarction, but in this model the supersensitivity is not produced by an increase in the total number of beta-adrenergic receptors. The fact that isoproterenol also produces supersensitive responses suggests a postjunctional mechanism. A future direction is to investigate the mechanism responsible for this form of supersensitivity.

Arrhythmogenesis Related to Denervation Supersensitivity

Because denervation supersensitivity elicits inhomogeneous autonomic and electrophysiologic changes, it may make the heart more vulnerable to the development of ventricular arrhythmias [22]. We found that the prevalence of ventricular arrhythmias elicited by programmed ventricular stimulation was highest in dogs with ligation-induced infarction that received epicardial phenol application to produce denervation supersensitivity. Propranolol attenuated the supersensitive shortening of ERP and decreased the incidence of induction of ventricular fibrillation. From these data we concluded that phenol-induced sympathetic supersensitivity combined with nontransmural myocardial infarction made the canine heart most vulnerable to electrically-induced ventricular fibrillation. This may have been due to the presence of inhomogeneous myocardial infarction, which might produce nonuniform depression of excitability and conduction, plus the differences in refractoriness at the apical denervated and basal innervated sites due to supersensitivity.

Future Directions

The complexity of the preparations used in this study temper conclusions. The data from the phenol-treated dogs suggest that supersensitivity in these animals provided an arrhythmogenic influence. Although supersensitivity

may be arrhythmogenic due to heterogeneous electrophysiologic effects, modest absolute differences in ERP in the apical and the basal segments, the small number of sites studied, and the poor correlation between ERP and induction of ventricular fibrillation prevented definitive statements in this regard. Refractoriness is only one of several electrophysiologic factors, such as conductivity or tissue excitability, that may modulate arrhythmogenesis. The extent of denervation and of infarction must be important but was not determined in the present study. Thus, other explanations may be more appropriate. Also, in this model an effect of denervation supersensitivity on the development of spontaneous ventricular arrhythmias would be an appropriate and desirable endpoint.

The clinical relevance of the observations may be important and testable. For example, we have now shown that sympathetic denervation after myocardial infarction occurs in humans (see next section). Beta-blocker treatment reduces the incidence of sudden cardiac death after myocardial infarction [33, 34], possibly due to antiischemic and antiarrhythmic effects [35, 36]. On the basis of the present data, however, it is tempting to speculate that beta blockers may exert an antiarrhythmic effect in patients after a transmural infarction, due in part to attenuation of the effects of denervation supersensitivity and/or attenuation of the dispersion in ERP or other electrophysiologic variables produced by denervation of sympathetic nerves.

Reinnervation

Reinnervation occurs after postganglionic denervation by myocardial infarction in dogs. Using I^{123} metaiodobenzylguanidine (MIBG), a guanethidine analog taken up by sympathetic nerve terminals, to obtain a scintigraphic image of the sympathetic innervation, image defects occur after epicardial phenol application or myocardial infarction. The site of the defect correlates electrophysiologically with efferent sympathetic denervation and the presence of denervation supersensitivity. After about three months, the MIBG defect disappears, and dogs studied at that time demonstrate sympathetic reinnervation. Interestingly, supersensitivity persists despite reinnervation [37].

Future Directions

Using the MIBG scintigraphic approach combined with thallium perfusion, we have demonstrated sympathetic denervation in patients after myocardial infarction [38]. The significance of this finding, as well as whether reinnervation takes place in humans, awaits further study.

ACKNOWLEDGMENT

The author would like to thank his many colleagues over the years whose work has contributed to the information summarized in this report. Their names can be found in the bibliography.

REFERENCES

1. Coleridge, J.C.G. and Coleridge, H.M. Chemoreflex regulation of the heart. In *The Handbook of Physiology Section 2: The Cardiovascular System*, vol. 1, R.M. Berne, ed., p. 653–676. Bethesda, American Physiology Society.
2. Staszewska-Barczak, J. 1983. Prostanoids and cardiac reflexes of sympathetic and vagal origin. *Am. J. Cardiol.* 52:36A–45A.
3. Sleight, P. 1964. A cardiovascular depressor reflex from the epicardium of the left ventricle in the dog. *J. Physiol. (London)* 173:321–343.
4. Barber, M.J., Mueller, T.M., Davies, B.G., and Zipes, D.P. 1984. Phenol topically applied to left ventricular epicardium interrupts sympathetic but not vagal afferents. *Circ. Res.* 55:532–544.
5. Inoue, H. and Zipes, D.P. 1987. Increased afferent vagal responses produced by epicardial application of nicotine on the canine posterior left ventricle. *Am. Heart. J.* 114:757–764.
6. Barber, M.J., Meller, T.M., Davies, B.G., and Zipes, D.P. 1985. Interruption of sympathetic and vagal mediated afferent responses by transmural myocardial infarction. *Circulation* 72:623–631.
7. Inoue, H. Skale, B., and Zipes, D.P. in press. Effects of myocardial ischemia and infarction on cardiac afferent sympathetic and vagal reflexes in the dog. *Am. J. Physiol.*
8. Malliani, A., Peterson, D.F., Bishop, V.S., and Brown, A.M. 1972. Spinal sympathetic cardiocardiac reflexes. *Circ. Res.* 30:158–166.
9. Ninomiya, I., Matsukawa, K., Honda, T., et al. 1986. Cardiac sympathetic nerve activity and heart rate during coronary occlusion in awake cats. *Am. J. Physiol.* 251:H528–H537.
10. Thames, M.D., Klopfenstein, H.S., Abboud, F.M., et al. 1978. Preferential distribution of inhibitory cardiac receptors with vagal afferents to the inferoposterior wall of the left ventricle activated during coronary occlusion in the dog. *Circ. Res.* 43:512–519.
11. Uchida, Y. and Murao, S. 1974. Excitation of afferent cardiac sympathetic nerve fibers during coronary occlusion. *Am. J. Physiol.* 226:1094–1099.
12. Harken, D.E., Black, H., Dickson, J.F., and Wilson, H.E. 1955. Deepicardialization: a simple, effective surgical treatment for angina pectoris. *Circulation* 955–962; vol. 12.
13. Lindgren, I. and Olivercrona, H. 1947. Surgical treatment of angina pectoris. *J. Neurosurg.* 4:19–39.
14. Martins, J.B. and Zipes, D.P. 1980. Epicardial phenol interrupts refractory period responses to sympathetic but not vagal stimulation in canine left ventricular epicardium and endocardium. *Circ. Res.* 47:33–40.
15. Takahashi, N., Barber, M.J., and Zipes, D.P. 1985. Efferent vagal innervation of the canine left ventricle. *Am. J. Physiol.* 248 (*Heart Cir. Physiol.* 17):H89–H97.
16. Bloomquist, T.M., Priola, D.V., and Romero, A.M. 1987. Source of intrinsic innervation of canine ventricles: a functional study. Am. J. Physiol. 252 (*Heart Circ. Physiol.* 21):H638–H644.
17. Chilson, D.A., Peigh, P., Mahomed, Y., and Zipes, D.P. 1985. Encircling endocardial encision interrupts efferent vagal-induced prolongation of endocardial and epicardial refractoriness in the dog. *J. Am. Coll. Cardiol.* 5:290–296.
18. Inoue, H., Mahomed, Y., and Zipes, D.P. 1988. WPW surgery interrupts efferent vagal innervation to the left ventricle and to the AV node in the canine heart. *Cardiovasc. Res.* 22:163–170.
19. Barber, M.J., Mueller, T.M., Henry D.P., et al. 1983. Transmural myocardial infarction in the dog produces sympathectomy in noninfarcted myocardium. *Circulation* 67:787–796.
20. Inoue, H. and Zipes, D.P. 1988. Time course of denervation of efferent sympathetic and vagal nerves after occlusion of the coronary artery in the canine heart. *Circ. Res.* 62:1110–1120.
21. Kovacs, R.J., Kammerling, J.M., Zipes, D.P., and Bailey, J.C. 1985. Reduction of choline acetyltransferase activity by cardiac parasympathectomy and transmural myocardial infarction (abstr.). *Circulation* 72 (Suppl. III):246.
22. Inoue, H. and Zipes, D.P. 1987. Results of sympathetic denervation in the canine heart: supersensitivity that may be arrhythmogenic. *Circulation* 75:877–887.
23. Schmid, P.G., Greif, B.J., Lund, D.D., and Roskoski, R., Jr. 1982. Tyrosine hydroxylase and choline acetyltransferase activities in ischemic canine heart. *Am. J. Physiol.* 243:H788–795.

24. Scherlag, B.J., El-Sherif, N., Hope, R., and Lazzara, R. 1974. Characterization and localization of ventricular arrhythmias resulting from myocardial ischemia and infarction. *Circ. Res.* 35:372–383.
25. Downar, E., Janse, M.J., and Durrer, D. 1977. The effect of acute coronary artery occlusion on subepicardial transmembrane potentials in the intact porcine heart. *Circulation* 56:217–224.
26. Janse, M.J., Cinca, J., Morena, H., et al. 1979. The "border zone" in myocardial ischemia. An electrophysiologic, chemical and histochemical correlation in the pig heart. *Circ. Res.* 44:576–588.
27. Gilmour, R.F. Jr. and Zipes, D.P. 1980. Different electrophysiological responses of canine endocardium and epicardium to combined hyperkalemia, hypoxia and acidosis. *Circ. Res.* 46:814–825.
28. Murray, C.E., Jennings, R.B., and Reimer, K.A. 1986. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 74:1124–1136.
29. Miyazaki, T. and Zipes, D.P. in press. Protection against a heterogeneous development of autonomic denervation following acute myocardial infarction by ischemic preconditioning. *Circ. Res.*
30. Cannon, W.B. 1939. A law of denervation. *Am. J. Med.* 198:737–750.
31. Vatner, D.E., Lavallee, M., and Amano, J. 1985. Mechanisms of supersensitivity to sympathomimetic amines in the chronically denervated heart of the conscious dog. *Circ. Res.* 57:55–64.
32. Kammerling, J.M., Green, J.F., Watanabe, A.M., et al. 1987. Denervation supersensitivity of refractoriness in noninfarcted areas apical to transmural myocardial infarction. *Circulation* 76:383–393.
33. The Norwegian Multicenter Study Group. 1981. Timolol-induced reduction in mortality and reinfarction in patients surviving acute myocardial infarction. *N. Engl. J. Med.* 304:801–807.
34. Beta Blocker Heart Attack Trial Research Group. 1982. A randomized trial of propranolol in patients with acute myocardial infarction. Part I. Mortality results. *JAMA* 247(12):1707–1714.
35. Frishman, W.H., Furberg, C.D., and Friedewald, W.T. 1984. Beta-adrenergic blockade for survivors of acute myocardial infarction. *N. Engl. J. Med.* 310:830–837.
36. Bigger, J.T., Jr. and Coromilas, J. 1984. How do beta-blockers protect after myocardial infarction. *Ann. Intern. Med.* 101:256–258.
37. Minardo, J.D., Tuli, M.M., Mock, B.H. et al. in press. Scintigraphic and electrophysiologic evidence of canine myocardial sympathetic denervation and reinnervation produced by myocardial infarction or phenol application. *Circulation.*
38. Stanton, M.S., Tuli, M.M., Heger, J.J. et al. in press. Comparative SPECT I-123 metaiodobenzylguanidine (MIBG) and thallium 201 cardiac imaging following myocardial infarction in patients (abstr.) *J. Am. Coll. Cardiol.* 11:81A, 1988.

IV. ARRHYTHMOGENIC MECHANISMS

14. ON THE PROBLEM OF ANISOTROPIC PROPAGATION IN VENTRICULAR MUSCLE

MARIO DELMAR, CARMEN DELGADO, DANTE CHIALVO,
DONALD C. MICHAELS, AND JOSE JALIFE

Mammalian cardiac tissues consist of individual cells connected by low resistance pathways. Such pathways are provided by the gap junctions [1–3], which are comprised of networks of membrane proteins fused together across the intercellular space at points of intimate cell-to-cell contact. Yet, even though gap junctional channels have a relatively low resistance [4–6], they represent a significant myoplasmic discontinuity [7–9], and propagation may be considered stepwise when time and distance are resolved in sufficiently small units.

This idea of a discontinuous nature of electrical propagation has been supported by recent computer simulations using discretized cable models [8–12] to represent the periodic pattern of cell-to-cell communication. Indeed, Heppner and Plonsey [10] have shown that action potential transmission between two coupled cells is critically dependent on the intercellular resistance. Other investigators [11–13] have provided evidence that large increases in the intercellular resistance can decrease propagation velocity even in the presence of an increased $\dot{V}_{max}$. Similar results were obtained by Diaz et al. [8] and more recently by Rudy and Quan [9] in their theoretical model of cylindrical cardiac cells connected end-to-end by intercalated discs, which demonstrated the inability of classical continuous cable theory to describe cardiac impulse propagation in a quantitative manner.

A particularly important problem arises when studying propagation in

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multicellular preparations of mammalian ventricular muscle: Conduction velocity is faster along the longitudinal axis of the cells than it is in the transverse direction [14–16]; that is, propagation is anisotropic. The chief mechanism postulated for explaining such anisotropy is that the effective axial resistivity is lower longitudinally than transversely [13, 14]. Moreover, as demonstrated by Spach et al. [7], this anisotropic property is associated with differences in action potential configuration, depending on whether the excitation wavefront propagates transversely or longitudinally. Greater maximum upstroke velocity ($\dot{V}_{max}$) and amplitude, together with a faster foot potential, are seen during transverse propagation. These results led the authors to suggest that, when active generator properties of the cells are altered, conduction block can occur in the direction parallel to fiber orientation, while perpendicular propagation is maintained; that is, the margin of safety for propagation is greater in the transverse direction. Surface electrode activation mappings in atrial [7] and ventricular tissue [17] support this view.

Recent experimental studies from our laboratory [18] have indicated that under conditions of decreased intercellular coupling induced by heptanol superfusion, conduction in anisotropic ventricular muscle was blocked more readily in the transverse than in the longitudinal direction. Moreover, additional preliminary experiments and computer simulations (Delgado et al., unpublished) have suggested that conditions that diminish active generator currents (e.g., high KCl superfusion in the experiments; decrease in maximum sodium conductance [$\bar{g}_{Na}$] in the simulations) may, in fact, lead to preferential conduction block in the transverse direction.

The evidence thus indicates that anisotropy in cardiac muscle can set the stage for preferential conduction block (a critical factor in the development of reentrant arrhythmias). But, as suggested by our published and unpublished experiments, the directionality of this preferential block will depend on whether active generator properties or intercellular coupling are affected and, perhaps more important, on the conditions for the recovery of excitability of the tissues involved. In this essay, we will present a theoretical analysis of the possible dynamic relationships between active generator properties, intercellular coupling, and conduction velocity in anisotropic ventricular myocardium. We will also present results from our initial studies on the role of recovery of membrane excitability in determining success or failure of propagation in the anisotropic tissue. Finally, we will make some suggestions for potentially useful investigative avenues that, in our opinion, may lead to testable hypotheses about the roles of active and passive membrane properties in anisotropic propagation and about the key factors involved in the development of longitudinal dissociation and reentry in ventricular muscle.

THE AREA OF PROPAGATION

A helpful simplification for understanding the overall problem of anisotropic propagation and preferential conduction block is to consider conduction

velocity and margin of safety (i.e., success or failure of propagation) as dynamic functions of two of the variables previously mentioned: namely, active generator properties (represented by $\dot{V}_{max}$) and axial resistivity (R_a = myoplasmic resistivity + junctional resistivity). The following discussion will be limited to the situation in which action potential propagation occurs uniformly in every direction with respect to fiber orientation. Thus, once propagation has been initiated, conduction velocity is continuous and constant, regardless of its direction.

Consider the three-dimensional relation shown in figure 14-1C, in which we have plotted the log of axial resistivity on the X axis, the log of the squared conduction velocity on the Y axis, and $\dot{V}_{max}$ on a Z axis directed backwards from the plane of the paper. The following initial conditions are established to limit whatever area is to be defined in this space:

1. Axial resistivity may not decrease below a certain minimum value ($R_{a(min)}$), which is that observed under physiologic conditions during longitudinal propagation.
2. The greatest value of conduction velocity cannot exceed that obtained at the minimum value of R_a.
3. When R_a increases, or when the direction of propagation changes from longitudinal to transverse, $\dot{V}_{max}$ increases asymptotically toward a maximum value.
4. The relation between the increase in $\dot{V}_{max}$ and the change in conduction velocity (Θ) is given by:

$$\dot{V}_{max} = A - B \exp K(\Theta) \tag{14.1}$$

   where A, B, and K are proportionality constants that, in ventricular muscle, have the values of 187, 6.5, and 4.7 respectively [7].
5. Increasing R_a leads to a decrease in conduction velocity (figure 14-1A). In the absence of actual experimental data to correlate these two variables, we have obtained some values using the equation for continuous propagation in a unidimensional cable [19]:

$$\Theta^2 = \dot{V}_{max}/R_a \tag{14.2}$$

6. A string of points can be obtained from (1) and (2) that delineate the upper boundary in the three-dimensional relation of figure 14-1C. In theory, this line would be drawn from the point of minimum R_a and maximum Θ^2 backward, downward, and to the right, until it approaches asymptotically the greatest attainable value of $\dot{V}_{max}$. As it does, $\dot{V}_{max}$ changes less and less as R_a extends toward infinity (figure 14-1B), and the function approaches zero Θ^2 asymptotically. For convenience, R_a and Θ^2 are plotted on logarithmic scales in figure 14-1C.

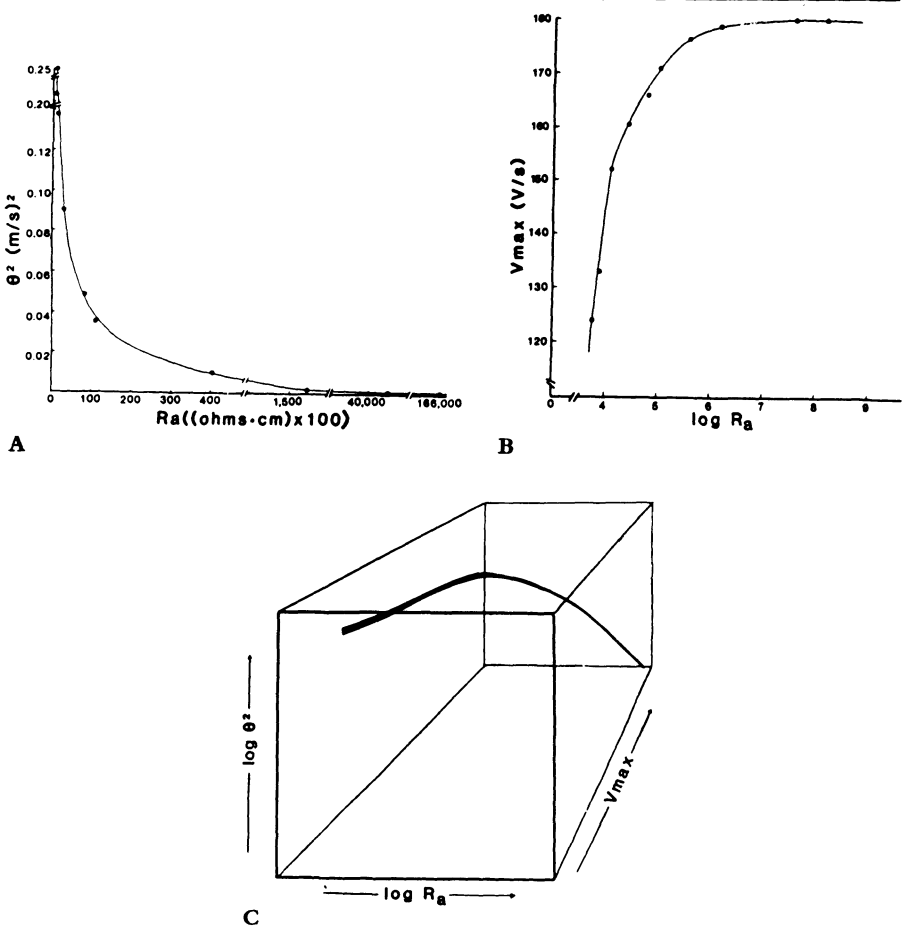

Figure 14-1. Derivation of the three-dimensional relationship between conduction velocity, maximum velocity of action potential upstroke ($\dot{V}_{max}$), and axial resistivity (R_a) for anisotropic ventricular myocardium. Panel A, the squared conduction velocity (Θ^2) is plotted on the ordinate, as a function of R_a. The curve and data points were obtained by numerical solution of the equation for a continuous cable [19]. Panel B, $\dot{V}_{max}$ vs log of R_a, constructed on the basis of data published by Spach et al. [7]. Panel C, three-dimensional relationship between log of Θ^2, log of R_a and $\dot{V}_{max}$. See text for further details.

We will assume that when R_a remains constant there is a linear relation between Θ^2 and $\dot{V}_{max}$ [19]. Although it has not been studied for bidimensional systems, it is generally accepted that such a relationship holds in mammalian ventricular muscle [20]. Hence, each point of the line that defines the upper boundary can be projected linearly (i.e., on the Y vs. Z plane) to its corresponding value of R_a at $\dot{V}_{max} = 0$ and $\Theta^2 = 0$. By so doing, an "area of propagation" is now defined (figure 14-2).

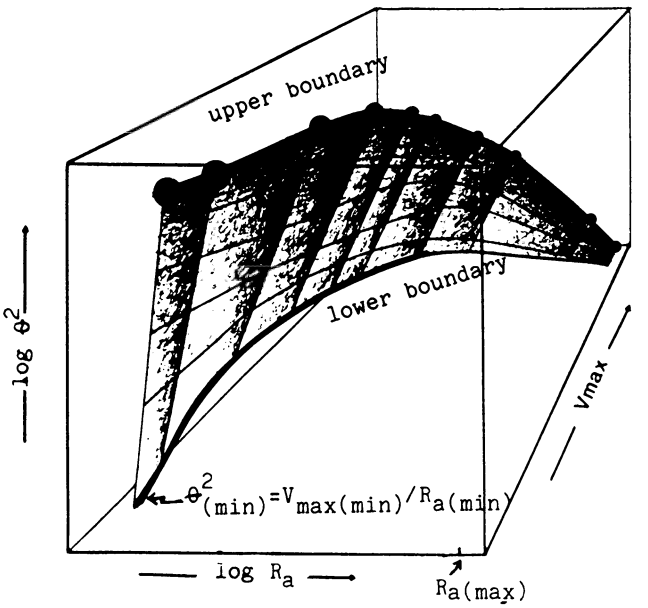

Figure 14-2. The area of propagation for a hypothetical two-dimensional sheet of anisotropic ventricular epicardial muscle. See text for description.

Obviously, Θ^2 cannot extend continuously to zero. In fact, the velocity of propagation can decrease progressively to a limit at which it abruptly stops. Hence, for each value of R_a, there must be a minimum $\dot{V}_{max}$ ($(\dot{V}_{max})_{min}$) compatible with active propagation. Moreover, since block can be induced either by decreasing $\dot{V}_{max}$ or by increasing R_a, or both, a maximum R_a ($R_{a(max)}$) boundary can also be defined.

Finally, the precise function that relates $(\dot{V}_{max})_{min}$ to R_a has not as yet been obtained. Yet, as will become clear below, this is of critical importance for determining the margin of safety for propagation. For present discussion and, in the absence of experimental data, we will use a hypothetical exponential function for $(\dot{V}_{max})_{min}$ vs R_a whose shape is similar to that of the curve defining the upper boundary (figure 14-2). When the corresponding values of minimal Θ^2 ($\Theta^2_{min} = (\dot{V}_{max})_{min}/R_a$) are calculated, a line results that constitutes the lower boundary for the area of propagation.

THE INFLUENCE OF EXCITABILITY

So far, we have limited the discussion to the roles played by $\dot{V}_{max}$ and R_a in determining success or failure of propagation in a unidimenisional or bidimensional array of the excitable cells. However, active propagation depends not only on the ability of the excitable cells to act as current sources, but also on the excitability of neighboring cells. We can define excitability as

the minimum amount of current input needed by a cell membrane to reach threshold and elicit a net flow of regenerative inward membrane current. Of course, in the hypothetical system we are analyzing it would not be possible to determine this value accurately. However, an approximation can be made. In the area of propagation bounded by our three-dimensional space (figure 14-2), the level of R_a is given by the morphologic structure of the system; that is R_a depends on the myoplasmic and gap junctional conductances, as well as on the geometrical arrangements of cell-to-cell contacts. On the other hand, $(\dot{V}_{max})_{min}$ depends indirectly on the minimum amount of current necessary to allow propagation at a given value of R_a. When the excitability is relatively low, the margin of safety for propagation would also be low and, consequently, block would occur at a relatively high value of $\dot{V}_{max}$. Thus, the width of the area of propagation would be small, since the $(\dot{V}_{max})_{min}$ versus R_a boundary would be drawn at relatively high values of $\dot{V}_{max}$. On the other hand, increasing cell excitability would displace the lower boundary downward, and the margin of safety for propagation would increase. If such an increase in excitability is equivalent for all values of R_a, then the displacement of the lower boundary will be parallel to its control. Thus, viewed in this manner, excitability is a dynamic element within the system.

R_a, $\dot{V}_{max}$, AND MARGIN OF SAFETY

We have now obtained an area in the three-dimensional space that defines successful conduction in an excitable medium, as well as some theoretical values of conduction velocity at any given combination of R_a and $\dot{V}_{max}$ compatible with successful propagation. We will now consider three points, A, B, and C, within that system (figure 14-3). We will assume that these points represent individual $R_a/\dot{V}_{max}/\Theta^2$ values obtained from an experiment in a bidimensional piece of epicardial ventricular muscle, when propagation is measured longitudinally (point A), diagonally (point B), and transversely (point C) to the direction of the fibers. We will also assume that, depending on whether $\dot{V}max$ or R_a is altered, these points can move in the space according to the conditions we have established for our three-dimensional relationship.

Let us now ask the following question: which point A, B, or C will be the first to reach the lower or rightmost boundaries, respectively, if either $\dot{V}_{max}$ or R_a is altered? We can answer this question separately for each of the two different perturbations.

Consider first the three-dimensional plot in figure 14-3A. If under optimal initial conditions of propagation the tissue is subject to a manipulation that gradually increases coupling resistance (e.g., superfusion with an alcohol or with a relatively low toxic concentration of ouabain), but does not significantly change active generator properties, each of the points will move

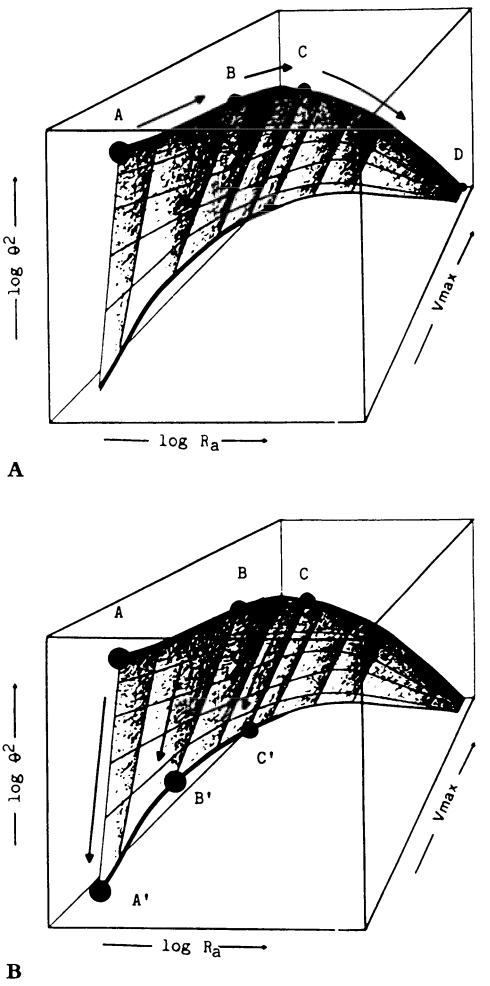

Figure 14-3. Predicted effects of decreasing intercellular coupling and maximum sodium conductance on conduction velocity in anisotropic ventricular muscle, and graphical illustration of the concept of margin of safety for propagation in the transverse and longitudinal directions. Three-dimensional areas of propagation are plotted for conditions that lead to increases in axial resistivity (panel A) or to decreases in $\dot{V}_{max}$ (panel B). In both panels, points A, B, and C indicate initial conditions for longitudinal, diagonal, and transverse propagation, respectively. In panel A, increasing R_a will move all three points along the upper boundary of the surface (arrows). Point D indicates the highest value of R_a that will permit propagation in any given direction. In panel B, gradual decreases in $\dot{V}_{max}$ will also induce slowing of conduction in all directions. However, the changes will now follow three different trajectories, depending on their particular direction, i.e., longitudinal (AA'); diagonal (BB') and transverse (CC').

along the upper boundary of the surface and could eventually exit the area through point D. The position of such a point would be given by the highest value of R_a that is compatible with active propagation; and the pathway followed by A, B, and C would be exactly the same for all three. Yet, starting conditions would be such that C begins to move from a relatively higher value of R_a than A and B. Hence, assuming that electrical uncoupling occurs homogeneously throughout the tissue, and thus all points travel their trajectories at the same rate, C will find the rightmost edge earlier than the other two, and A will be the last to get there. In other words, when intercellular resistivity is increased in a bidimensional anisotropic network of cardiac cells, the margin of safety for successful propagation should be higher for the longitudinal direction (point A) than transversely (point C). This theoretical assumption is compatible with our experimental results [18].

Consider again the same three points, except that now we will follow their trajectories in figure 14-3B; that is, in the case in which $\dot{V}_{max}$ decreases at constant R_a. For example, under the conditions in which the bidimensional piece of muscle is superfused with increasing concentrations of tetrodotoxin (TTX), the pathway followed by each point is not shared by the others. Point A will exit the propagation surface through point A′, B through B′ and C through C′. The question of which one reaches the edge first can be answered by determining the lengths of the individual trajectories (AA′, BB′, and CC′). Again, we will assume that the rate at which each point moves is the same as that of the others. For a given value of R_a (e.g., point A), the length of the trajectory can be calculated in the $\dot{V}_{max}$ versus Θ^2 plane by the equation

$$AA'^2 = (\dot{V}_{max}(A) - \dot{V}_{max}(A'))^2 + (\Theta^2(A) - \Theta^2(A'))^2 \tag{14.3}$$

which is applicable to points B and C as well.

Furthermore, under the conditions of $\dot{V}_{max}$ decrease, the sequence with which conduction block occurs (i.e., longitudinal versus diagonal versus transverse) will depend very strongly on the excitability of the tissue and on the shape of the curve that defines the lower boundary of the area of propagation. For example, if we were to assume that the lower boundary is an exponential function of higher order than that shown in figures 14-2 and 14-3, segment AA′ would be longer than BB′, which would be longer than CC′. Under these conditions, conduction would fail first transversely, then diagonally, and finally, in the longitudinal direction. However, if the lower boundary is defined by a power function with positive constants, then the sequence would be CC′ > BB′ > AA′. The experimental results of Spach et al. [7] and Tsuobi et al. [17] suggest that, under certain conditions, the margin of safety can be greater for transverse than for longitudinal propagation. Yet, our preliminary experiments and computer simulations suggest that the opposite may also be true (see next section) and, to our knowledge,

no attempt has been made to correlate systematically and quantitatively the interaction of the three parameters just discussed.

Hence, according to this analysis, the margin of safety for propagation can be higher or lower in either direction, depending as much on whether active generator properties or cell-to-cell coupling are reduced, as on the excitability of the tissue.

EXPERIMENTAL RESULTS

In this section, we present experiments [18] (Delgado et al., unpublished) in which we studied the respective roles of cell-to-cell coupling and active generator properties in determining the margin of safety for propagation and the onset of preferential blockade.

Electrical Uncoupling and Anisotropic Conduction Block

In a first group of experiments, we exposed thin (<0.5 mm) sheep epicardial muscle preparations to the uncoupler heptanol, while stimulating alternately in the transverse and longitudinal directions [18]. Our results demonstrate that changes in the specific nexal resistance have different effects on anisotropic propagation from those previously demonstrated for sodium conductance decrease [7]; that is, of the two parameters, transverse propagation is more vulnerable to uncoupling, regardless of any directional differences in $\dot{V}_{max}$ observed. Thus, in nine heptanol experiments, conduction block occurred in the transverse direction, while longitudinal propagation was maintained for several minutes [18]. In fact, although the time course of heptanol effects varied from one experiment to another, the time to transverse block was about 28 minutes, whereas longitudinal block was observed at about 44 minutes.

The results demonstrated that when intercellular coupling is impaired, transverse propagation is more vulnerable to block than longitudinal propagation. That difference did not depend on the changes in $\dot{V}_{max}$ observed [18]. However, from these experiments, it was impossible to verify the precise site of block or whether propagation remained uniform with respect to fiber orientation during the development of uncoupling. The stepwise conduction velocity changes [18, figure 3] observed in the course of heptanol superfusion would lead one to speculate that the waveform may have followed an increasingly tortuous path. To study this possibility, we have performed additional experiments with simultaneous intracellular and extracellular recordings. The experimental arrangement was similar to the one described by Delmar et al. [18]; that is, a small, square (6 × 6 × .5 mm) of sheep ventricular epicardium activated alternately by one of two stimulators through bipolar electrodes located at opposite corners on the tissue. An intracellular microelectrode was placed in a third corner, in such a way as to record alternating transverse and longitudinal activities on a beat-to-beat

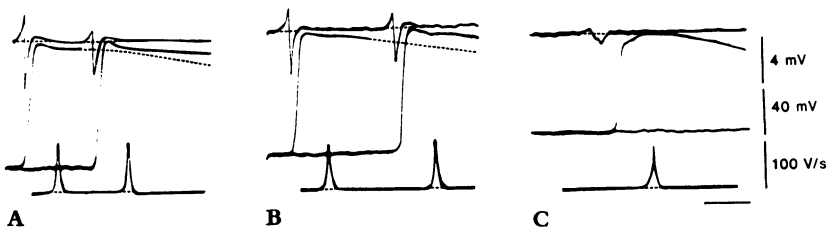

Figure 14-4. Effects of heptanol superfusion (1.5 mM) on conduction velocity, $\dot{V}_{max}$ and extracellular waveforms in a square sheet (dimension 6 × 6 × 0.5 mm) of sheep ventricular epicardial muscle. In all panels, top traces are the extracellular recordings obtained from one corner of the sheet, middle traces are transmembrane potentials recorded through a nearby microelectrode, and bottom traces are the differentiated signals of membrane potential with respect to time (dV/dt). Responses were initiated by repetitive stimuli (BCL 750 msec), applied alternately from one of two bipolar stimulators that were placed at opposite corners from each other (see Delmar et al. [18] for more details). Two superimposed traces are shown in each panel during longitudinal (earliest signals in each panel) and transverse propagation. The broken tracings permit better visualization of the activation patterns. Panel A, control; panel B, recordings obtained ten minutes after heptanol was started; panel C, 1 hour of heptanol superfusion; note the appearance of the longitudinal action potential and failure of activation during stimulation in the transverse direction. Calibrations: vertical, as indicated; horizontal, 20 msec.

basis. The two stimulators were synchronized so that each had a basic cycle length of 1,500 ms, but their firings were out of phase with each other by 750 ms. Hence the effective cycle length was 750 ms. In addition, for these experiments, a thin (50 μm), Teflon-coated, monopolar silver wire electrode was placed on the surface of the tissue, very close to the intracellular microelectrode. Figure 14-4 shows the recordings obtained from one of the experiments. In all panels, two superimposed sweeps are shown. For the sake of clarity, traces were partially redrawn with broken lines. Upper traces are the extracellular recordings, middle traces the action potentials, and bottom traces the electronically differentiated signals. Panel A shows the control. Intracellular and extracellular potentials differed in their respective morphologies depending on whether the excitation wavefront propagated transversely or longitudinally, as described by Spach et al. [7]. Panel B was taken ten minutes after starting superfusion with 1.5 mM heptanol, just before transverse block. Notice that the configuration of the extracellular waveforms did not change, suggesting that the conduction pathways remained constant throughout. Panel C, taken 50 minutes after panel B, and a few beats before longitudinal block, shows a polymorphic extracellular signal. Such a fragmented electrogram probably reflects longitudinal dissociation of the impulse, rather than "zig-zagging" of the wavefront through partially blocked longitudinal and transverse pathways. Nevertheless, additional measurements with multiple intracellular and extracellular recording sites are required to confirm these findings and to determine accurately the site of block.

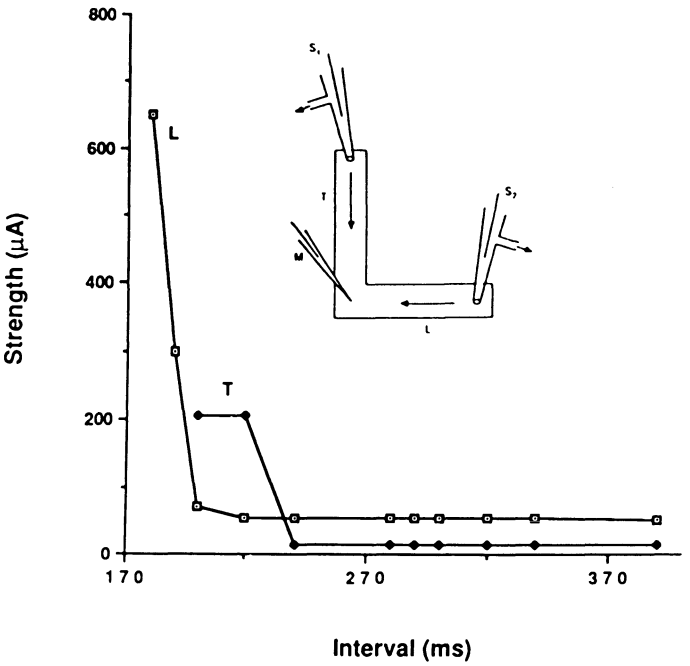

Figure 14-5. Strength-interval curves for longitudinal (L; white symbols) and transverse (T; black symbols) activation in an L-shaped piece of sheep ventricular epicardial muscle (length in each direction was about 8 mm). The inset shows the experimental arrangement. Two suction electrodes (S_1 and S_2) located at opposite ends were used to apply depolarizing current pulses while action potentials were recorded with a microelectrode at the juncture of the L. Care was taken to place the two suction pipettes at about the same distance from the recording electrode. Single test pulses scanning the diastolic interval were applied after every tenth basic stimulus. The curve shows the current strength necessary for obtaining a propagated response at the recording site, as a function of the test interval. Note that current strength is lower for T at long intervals, but there is a crossover of the curves at the shorter intervals.

Generator Properties and Preferential Anisotropic Blockade

We have extended the foregoing observations on the effects of various perturbations on propagation in anisotropic muscle. As previously discussed, because $\dot{V}_{max}$ of the action potential is greater during transverse propagation, it has been suggested that block is less likely to occur transversely than longitudinally [7]. Therefore, we have performed additional preliminary experiments to study the relative role of active membrane properties in determining success or failure of propagation in anisotropic myocardium. After determination of fiber orientation, thin pieces of sheep epicardial ventricular muscle (2 × 2 × 0.5mm) were cut into an "L" shape according to such an orientation (see inset in figure 14-5). Following a 1 hour equilibration period, preparations were exposed to Tyrode solution containing increasing concentrations of KCl, while recordings were obtained simultaneously from T and

L by means of appropriately placed pairs of extracellular unipolar and intracellular microelectrodes. The changes in $\dot{V}_{max}$, T and L effective conduction velocities, and degree of anisotropy (measured as the difference in T and L conduction velocities), as well as time to complete blockade were monitored during and after the transition from 4 to 20 mM KCl. As shown in table 14-1, simultaneous T and L conduction block was observed in one experiment. However, in seven of eight experiments, effective conduction velocity decreased more for T (45 percent) than for L (20 percent) and the time to conduction block was briefer for T (4.9 minutes) than for L (6.7 minutes). Further, contrary to what is predicted by linear cable theory, the relationship between the squared conduction velocity and $\dot{V}_{max}$ was nonlinear and, at the time of T block, $\dot{V}_{max}$ had decreased to 74 and 63 percent for T and L, respectively. These experiments suggest that high KCl increases the degree of anisotropy in ventricular muscle and that, just as in the case of electrical uncoupling, there are certain conditions of impairment of active generator properties (e.g., those resulting from membrane potential reduction) in which the margin of safety for propagation is higher in the longitudinal than in the transverse direction.

In another series of experiments, we have begun to study the influence of excitability in determining success or failure of propagation in anisotropic ventricular muscle. As illustrated in the inset of figure 14-5, an intracellular microelectrode was used to record action potentials from a cell at the junction between longitudinal and transverse fibers in an L-shaped preparation of sheep epicardial muscle, while 2 ms depolarizing current pulses were applied alternately from one or the other suction pipette-electrodes located at either end of the tissue. The figure shows the strength-interval plot where the stimulus strength just necessary for propagation in the L and T directions was determined for single premature stimuli applied after every tenth basic pulse. At the basic cycle length (1,000 ms), a range of stimulus values resulted in T but not L propagation. This was true also for values just above threshold at premature intervals that were 220 ms or longer. However, at briefer intervals, there was a crossover of the T and L curves that resulted in a range of stimulus times at which there was longitudinal but not transverse propagation. These data were independent of changes in the action potential duration. Similar results were obtained in four additional experiments of this type. Our interpretation of these results is as follows: Because of the lower loading conditions associated with transverse propagation, the current strength necessary to initiate propagation at the longer intervals is lower transversely than longitudinally. However, becasuse of the longer latency for activation that is normally present in the transverse direction during the basic beat, the effective refractory period (measured as S_1-S_2 interval; where S_1 is the last of the basic stimuli prior to the premature current pulse, S_2) is longer transversely than longitudinally. Consequently, at the very early intervals, the pulse successfully activates the tissue in the L direction but is unable to

Table 14-1. Effects of increasing concentrations of KC1 (4–20 mM) on conduction velocity and $\dot{V}_{max}$, and preferential blockade in anisotropic ventricular myocardium

	Control								Just before T block			
	Conduction velocity (m/s)		$\dot{V}_{max}$ (v/s)			Time to block (min)			Conduction velocity (m/s)		$\dot{V}_{max}$ (v/s)	
	L	T	L	T		L	T		L	T	L	T
1	0.51	0.14	125	219	1	3.9	3.7	1	0.42	0.024	30	47
2	0.51	0.069	100	93	2	4.9	3.8	2	0.239	0.038	23	26
3	0.63	0.081	135	206	3	5.1	3.7	3	0.63	0.049	40	51
4	0.92	0.143	135	181	4	12.2	7.6	4	0.92	0.089	47	93
5	0.385	0.093	90	108	5	4.0	4.0	5	0.25	0.109	42	56
6	0.66	0.088	65	85	6	8.2	5.8	6	0.35	0.03	23	14
7	0.39	0.060	271	186	7	9.0	7.0	7	0.4	0.031	65	9
8	0.10	0.059	144	161	8	6.0	3.6	8	0.11	0.036	121	23
$\bar{X}$	0.51	0.092	133	155	$\bar{X}$	6.7	4.89	$\bar{X}$	0.41	0.051	49	40
									↓ 20%	↓ 45%	↓ 63%	↓ 74%

initiate a T response. In other words, in spite of the fact that, under normal conditions, current threshold requirements are lower for T than for L, during premature stimulation activation failure is more easily achieved transversely than longitudinally. This interpretation is consistent with our overall hypothesis relating excitability to margin of safety and preferential block (see previous discussion).

PERSPECTIVES

The potential role of preferential blockade in the development of reentrant arrhythmias cannot be overemphazised. Thus, it is of crucial importance to provide accurate predictions as to the effects of various perturbations on active generator properties and conduction velocity in altering the degree of anisotropy in ventricular muscle. Future experiments and computer simulations should be designed to address these specific issues in a systematic manner. Indeed, accurate definition of the role of intercellular communication and of active membrane properties on two-or three-dimensional anisotropic propagation should greatly improve our understanding of the mechanisms of life-threatening cardiac dysrhythmias, which may have important implications in terms of diagnosis and therapy.

Indeed, investigations should be directed toward the study of the relative role of intercellular coupling and of active membrane properties on two-dimensional anisotropic propagation in isolated ventricular cardiac tissues. Such studies should allow one to define experimentally the relationship between conduction velocity, $\dot{V}_{max}$ and intracellular resistivity (see figure 14-2) in a uniform anisotropic medium. To achieve this goal, it might be necessary to measure all three variables directly in the same preparation. It is clear that estimating conduction velocity and $\dot{V}_{max}$ should not present any major difficulty. However, measuring axial resistivity should be more complicated because ventricular muscle behaves like bidimensional, anisotropic networks, and hence, unidimensional cable theory is not applicable to the analysis of its membrane properties.

To our knowledge, the only previous attempt to correlate directly axial resistivity and conduction velocity in ventricular muscle was made by Clerc [14]. In his determinations of passive membrane properties and intercellular coupling, this author used the equations for unidimensional cables [22] and was able to demonstrate that anisotropy was the result of different resistivity in the transverse and longitudinal axes. Although to a large extent Clerc's approximation was good, there are some factors that should be considered. First, those measurements were made at 25° C, and possible effects of temperature on axial resistivity or conduction velocity were not taken into consideration. Second, although determined in the same preparation, longitudinal and transverse resistances were not measured under identical experimental conditions since, for the latter, Clerc had to transfer his prepa-

rations from one chamber to another. Third, in his calculations, the investigator assumed that the fiber radius was the same for the longitudinal and transverse cell axes, although it is well known that ventricular cells are rod-shaped, with the length being as much as ten times greater than the diameter. The impact that this assumption may have had on estimating transverse resistance was not discussed. Fourth, with the method used by Clerc, resistance can only be determined for the two opposite directions (i.e., longitudinal and transverse to fiber orientation). Measurements in the diagonal axis could not be performed.

It is interesting that in that study the time constant of the foot potential was the same during longitudinal and transverse propagation, which is in fact contrary to what has been described more recently [7]. In addition to differences in animal species and tissue type, it is possible that this discrepancy might have resulted from the fact that during determination of the individual parameters in Clerc's experiments, propagation was occurring in one dimension only, in either the longitudinal or transverse direction.

Clerc [14] made an effort to apply current homogeneously so that the system could be defined by unidimensional cable theory. Yet, realistically one should calculate space constant, axial resistivity, and membrane resistance on the basis of analysis that applies specifically to bidimensional system. One possibility would be to use the equations developed by Bukauskas et al. [22] in their analysis of the sinoatrial region and adapt them to ventricular muscle. By measuring these passive properties in the same experiments in which one measures transverse, diagonal, and longitudinal propagation in isolated tissue preparations, one should be able to determine directly and accurately the relative roles of both active and passive properties in anisotropy.

Premature stimulation techniques as well as pharmacologic probes should also be used in future investigations to manipulate specific cell membrane properties and/or intercellular coupling and to elucidate precisely the factors that determine the margin of safety for propagation in the transverse versus longitudinal axes of the ventricular fibers. The specific location of discontinuities in propagation for various conditions of cell excitability and intercellular coupling should be determined as well. Moreover, microelectrode impalements should be made at the site at which fragmentation of extracellular electrograms is detected, with the overall goal of providing a solid mechanistic basis to phenomena such as very slow conduction and functional block in ventricular muscle.

CONCLUSION

We suggest that accurate and detailed information on the electical properties of homogeneous and nonhomogeneous anisotropic myocardium is necessary. Such information will help in the understanding of the mechanistic bases of

the major determinants of reentrant activity in ventricular muscle. In fact, it is our strong contention that only after this information is obtained can a precise definition be formulated of the dynamic events leading to life-threatening ventricular tachycardias and fibrillation; such a definition should aid in the establishment of meaningful strategies for the prevention of sudden cardiac death.

ACKNOWLEDGMENTS

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REFERENCES

1. Sjostrand, F.S. and Andersson, C.E. 1954. Electron microscopy of the intercalated discs of cardiac muscle tissue. *Experientia* 10:369–370.
2. Sjostrand, F.S., Andersson, C.E., and Dewey, M.M. 1958. Ultrastructure of the intercalated disc of frog, mouse and guinea pig cardiac muscle. *J Ultrastruc. Res.* 1:271–287.
3. Gilula, N.B. 1978. Structure of intercellular junctions. In *Intercellular Junctions and Synapses*. J. Feldman, N.B. Gilula and J.D. Pitts, eds., pp. 3–22, London, Champman and Hall.
4. Kameyama, M. 1983. Electical coupling between ventricular paired cells isolated from guinea pig hearts. *J. Physiol.* 336:345–357.
5. Weingart, R. 1986. Electrical properties of the nexal membrane studied in rat ventricular cell pairs. *J. Physiol (Lond.)* 370:267–284.
6. Veenstra, R.D. and DeHaan, R.L. 1986. Measurement of single channel currents from cardiac gap junctions. *Science* 233:972–974.
7. Spach, M.S., Miller, W.T., Geselowitz D.B., et al. 1981. The discontinuous nature of electrical propagation in normal canine cardiac muscle. Evidence for recurrent discontinuities of intracellular resistance that affect membrane currents. *Circ. Res.* 48:39–54.
8. Diaz, P.J., Rudy, Y., and Plonsey, R. 1983 Intercalated discs as a cause for discontinuous propagation in cardiac muscle: a theoretical simulation. *Ann. Biomed. Eng.* 11:177–189.
9. Rudy, Y. and Quan, W. 1987. A model study of the effects of discrete cellular structure on electrical propagation in cardiac tissue. *Circ. Res.* 61:815–823.
10. Heppner, D.B. and Plonsey, R. 1970. Simulation of electrical interaction of cardiac cells. *Biophys. J.* 10:1057–1075.
11. Fozzard, H.A., Levin, D.N., and Walton, M. 1982. Control of conduction velocity in cardiac Purkinje fibers. in *Normal and Abnormal Conduction in the Heart*, A. Paes de Carvalho, B.F., Hoffman, and M. Lieberman, eds., pp. 105–116, New York, Futura Publishing Company.
12. Joyner, R.W. 1982. Effects of discrete electrical coupling on propagation through an electrical syncytium. *Circ. Res.* 50:192–200.
13. Spach, M.S. and Kootsey, J.M. 1983. The nature of electrical propagation in cardiac muscle. *Am. J. Pysiol.* 244:H3–H222.
14. Clerc, L. 1976. Directional differences of impulse spread in trabecular muscle from mammalian heart. *J. Physiol. (Lond.)* 255:335–346.
15. Sano, T., Takayama, N., and Shimamoto, T. 1959. Directional differences of conduction velocity in the cardiac ventricular syncytium studied by microelectrodes. *Circ. Res.* 7:262–267.
16. Woodbury, J.W. and Crill, W.E. 1961. On the problem of impulse conduction in the atrium. in *Nervous Inhibition. Proceedings of the Second Friday Harbor Symposium*. E. Florey, ed., pp. 124–135, New York, Pergamon Press.
17. Tsuobi, N., Kodama, I., Tayama J., and Yamada, K. 1985. Anisotropic conduction properties of canine ventricular muscles. *Jpn. Circ. J.* 49:487–498.

18. Delmar, M., Michaels, D.C., Johnson, T., and Jalife, J. 1987. Effects of increasing intercellular resistance on transverse and longitudinal propagation in sheep epicardial muscle. *Circ. Res.* 60:780–785.
19. Hunter, P.F., McNaughton, P.A., and Noble, D. 1975. Analytical models of propagation in excitable cells. *Progr. Biophys. Mol. Biol.* 30:99–144.
20. Gettes, L.S., Buchanan, J.W., Saito, T. et al. 1985. Studies concerned with slow conduction. in *Cardiac Electrophysiology and Arrhythmias*, D.P. Zipes and J. Jalife, eds., pp 81–87, Orlando, FL, Grune & Stratton.
21. Weidmann, S. 1970. Electrical constants of trabecular muscle from mammalian heart. *J. Physiol. (Lond.)* 210:1941–1954.
22. Bukauskas, F.F., Gutman, A.M., Kisunas, K.J., and Veteikis, R.P. 1982. Electrical cell coupling in rabbit sinoatrial node and atrium: experimental and theoretical evaluation. In *Cardiac Rate and Rhythm*, L.N. Bouman and H.J. Jongsma, eds., pp. 195–216. The Hague, Martinus Nijhoff.

15. ANISOTROPIC REENTRY: A MODEL OF ARRHYTHMIAS THAT MAY NECESSITATE A NEW APPROACH TO ANTIARRHYTHMIC DRUG DEVELOPMENT

ANDREW L. WIT

A number of different arrhythmogenic mechanisms cause the ventricular arrhythmias that result from myocardial ischemia and infarction [1–4]. Reentrant excitation is one of these mechanisms [5, 6]. The role of reentry in the genesis of ischemic arrhythmias has been an area of intensive electrophysiologic investigation during the past 15 years, with a number of new and exciting experimental results being published. Among the most significant of these results is that reentrant circuits have been mapped with high resolution multiple electrode recording systems in the in situ hearts [6–11], an advance that could never have been predicted by those of us who struggled to obtain several simultaneous recordings in reentrant circuits in isolated tissues in the early 1970s [12]. As a result of these mapping studies, reentry as a cause of experimental ischemic arrhythmias has been proven. But how can this help in the clinical therapy of ischemic arrhythmias? To a limited degree the location of reentrant circuits causing ventricular tachycardia by mapping techniques in the clinic has helped by providing a rational basis for surgically cutting the circuit and thereby abolishing arrhythmias [13]. However, this can be accomplished without following precisely the impulse around the circuit. In my opinion the information pertaining to reentry that might have more practical consequences in relation to clinical therapy is yet to come: that is the identity of the cellular electrophysiologic causes for reentry. Why does

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reentry occur? In pursuing these causes for reentry in ischemia, until just recently emphasis has been placed on alterations in the transmembrane action potentials of the ischemic myocardial cells [14]. For example, in acute ischemia, membrane depolarization of ventricular muscle fibers leads to a reduced action potential amplitude and upstroke velocity, inhomogeneous recovery of excitability, slow conduction, and reentry that causes tachycardia and fibrillation [6]. It has also been proposed that similar changes in transmembrane potentials of muscle fibers in subacute and chronic areas of ischemia may lead to reentry by similar mechanisms [15].

Our recent interest in mechanisms of reentry and ventricular arrhythmias in ischemic hearts has been focused in a different direction. The results of our studies in models of subacute and chronic ischemia have suggested to us that abnormalities of the transmembrane potentials are not the primary cause of arrhythmias in the healing or healed infarct, but rather its structure will determine whether arrhythmias occur and the characteristics of the arrhythmias that do occur [16, 17]. If these hypotheses are eventually proven to be correct, they could have implications concerning the development of antiarrhythmic drugs. At the present time much of drug development is aimed at the pathophysiology of the membrane channels that govern the genesis of the action potential. Can drugs also be developed to influence the structural basis for arrhythmogenesis?

STRUCTURE OF A HEALING INFARCT

Our proposal is that as an infarct develops and heals after the period of acute ischemia, abnormalities in the transmembrane potentials that are so prominent during the acute ischemic period diminish and assume less of a role in arrhythmogenesis, whereas structural characteristics assume a more important role. These structural characteristics include: (1) the size of the infarcted area, (2) whether there are surviving myocardial cells in the infarct and the location of these surviving cells, and (3) the geometrical arrangement of the surviving cells.

Survival of Myocardial Cells in Infarcts

It seems logical that there must be surviving myocardial cells in an infarcted region in order for arrhythmias to occur. Otherwise, from where would the abnormal impulses come that cause tachycardia? The implication is that no matter how extensive the infarct, if it is completely transmural and homogeneous, arrhythmias would not arise from the infarcted region. In fact, surviving myocardial cells in both experimentally produced [16–21] and clinically occurring infarcts are a common occurrence [22, 23]. In the canine or feline heart even after complete occlusion of the left anterior descending or circumflex artery, myocardial cells may survive on the epicardial surface of the infarct [16, 17], and myocardial or Purkinje cells may survive on the

endocardial surface [18, 20, 21, 24]. Survival on the epicardial surface may result from collateral flow that persists after the occlusion, whereas endocardial survival may result because of the blood supply from the ventricular cavity. Muscle fibers may also survive intramurally within the infarcted region, particularly if the occlusion is not permanent and blood flow is restored after several hours [25, 26]. Similarly in the course of examining human infarcts from patients with ventricular arrhythmias, we have found surviving myocardial and Purkinje fibers surrounded by the healed infarcts [22, 23].

Transmembrane Potentials of Surviving Cells Improve with Time During Infarct Healing

The characteristics of the transmembrane potentials of the cells surviving in and around infarcts have been studied mostly by excising preparations from hearts and superfusing them in a tissue chamber with a normal physiologic (Tyrode) superfusate. Therefore, there is always some degree of uncertainty whether the transmembrane potentials recorded in vitro are truly representative of the transmembrane potentials in vivo. Assuming that they are, some interesting observations have been made. On the endocardial surface, surviving Purkinje fibers in canine infarcts have abnormal action potentials, which probably cause the automatic arrhythmias that occur 24 to 48 hours after coronary occlusion [18, 20]. Action potential characteristics are restored to normal during the subsequent weeks [21]. Despite the normal transmembrane potentials, abnormal conduction properties may remain because of the structural organization of the surviving myocardial cells [27]. I will return to this point later when I discuss how structure may cause arrhythmias in the absence of cellular electrophysiologic abnormalities. On the epicardial surface where markedly depressed resting potentials and action potentials have been recorded during the acute ischemic phase of arrhythmias [6, 14], the transmembrane potentials of the fibers that survive this acute ischemic phase also improve during the next days [16]. By five days, resting membrane potential recorded in the isolated preparations is only slightly depressed (by approximately 5 to 10 mV) and there is some depression of $\dot{V}_{max}$ of phase 0 [16]. Conduction velocity in these isolated preparations is nearly normal indicating the depression of $\dot{V}_{max}$ is not physiologically very significant. We have also measured conduction velocity in the surviving epicardial muscle fibers in transmural infarcts in situ by constructing high resolution activation maps and have found it to be almost normal [28]. The depression of resting membrane potential and $\dot{V}_{max}$ in vitro, therefore, may be a product of the isolation and superfusion procedures. Nevertheless, arrhythmias arise in the surviving epicardial muscle fibers at the time that conduction velocities are normal and transmembrane potentials might be normal.

We have also recorded transmembrane potentials from the surviving ventricular muscle cells at later times during infarct healing and have found them

to be normal [16, 17]. Normal resting potentials and action potentials, in fact, are associated with marked slowing of conduction in the healed canine infarct that is more than one month old [16, 17]. Calculated conduction velocities are slow enough to cause reentrant excitation in small regions (<.05 m/sec) but the occurrence of reentry was not documented in these experiments. The very slow conduction can be attributed entirely to the structural organization of the surviving myocardium.

Geometrical Arrangement of Surviving Myocardial Fibers in Infarcts Causes Arrhythmias

How can arrhythmias arise in regions of surviving myocardium in infarcts where transmembrane potentials are not very abnormal? We have experimental data that indicate some mechanisms for reentrant excitation in the absence of action potential abnormalities. Reentry is still dependent on the occurrence of slow activation permitting recovery of excitability in the reentrant pathway, but the slow activation is a product of the geometrical arrangement of the surviving myocardial fibers.

In the epicardial region of surviving ventricular muscle fibers (which we call the epicardial border zone [19]), the muscle fibers form a thin sheet of parallel fibers overlying the necrotic infarct during the first several weeks after coronary occlusion (during infarct healing) [16]. When complete transmural necrosis occurs there are no underlying muscular connections to this surviving epicardial muscle; the epicardial border zone is connected to normal muscle only around the margin of the infarct. Impulses conduct over the surface of the infarct in the surviving muscle, and the muscle cannot be activated from intramural regions as occurs before coronary occlusion. The direction of the activating wavefront in relation to the orientation of the muscle fibers has a profound influence on conduction velocity. Conduction velocity parallel to the long axis of the myocardial fibers is two to three times as rapid as that transverse to the long axis in the epicardial border zone [16]. This characteristic is similar to that found in normal myocardium and is known as anisotropy of conduction [29, 30]. The surviving epicardial muscle is said to be anisotropic. This anisotropy can cause reentry even though action potentials are normal. Slow conduction in the transverse direction is probably a consequence of fewer intercellular connections transversely than longitudinally although precise quantitative data are not yet available. In addition, in the canine epicardial border zone at this time, anisotropy is nonuniform [31]; that is, conduction transverse to fiber orientation in some regions is slower than in others. We postulate that this is a result of nonuniform separation of the myocardial fibers either by edema or by the formation of connective tissue septa during infarct healing. The patterns and degree of nonuniformity in anisotropy may be the most important factors that determine whether arrhythmias occur and the kinds of arrhythmias that do occur.

After several weeks, the parallel orientation of surviving fibers in some

regions of healed infarcts may be lost because the fiber arrangement is distorted by the formation of connective tissue [16, 17]. In these regions, intercellular connections may be markedly attenuated, accounting for the very slow conduction despite the normal action potentials [17].

Anisotropic Reentry—A Mechanism for Sustained Ventricular Tachycardia

We have formulated a model for reentrant excitation that is caused by anisotropy. The model was derived from experiments in which we determined the activation patterns in the epicardial border zone of 3-to 5-day-old canine infarcts, caused by permanent left anterior descending artery (LAD) occlusion, during *sustained* ventricular tachycardia induced by programmed stimulation [32]. Reentry occurs because of the nonuniform anisotropy in the surviving parallel muscle fibers. The 196 bipolar electrodes used to record extracellular waveforms were all located on the epicardial border zone of the infarct rather than being distributed throughout the ventricles (figure 15-1). This gave a much higher spatial resolution in the infarct than had been previously used and provided us with information that could not have been obtained with a lower spatial resolution. During sustained ventricular tachycardia, we found activation rotating around narrow long lines of apparent conduction block (figure 15-2). Block was initially suggested because of the marked differences in activation times on either side of the lines. One characteristic of these lines of apparent block was particularly important for the interpretation of our results. The lines were usually oriented parallel to the long axis of the myocardial fibers, which comprised the epicardial border zone. The orientation of a line of apparent block has implications concerning the mechanisms of tachycardia (see below).

We showed that the lines of apparent block during sustained tachycardia were usually not caused by any gross anatomic obstacles, such as a region in which myocardial fibers were replaced by the connective tissue forming in the healed infarct. They appeared to result from functional properties of the surviving muscle fibers in the epicardial border zone. Lines of block were not present in the absence of tachycardia (i.e., during sinus beats or ventricular stimulation).

Although following the sequence of isochrones according to convention suggests that activation occurred around the lines of block, the patterns of the isochrones immediately adjacent to the line are not easily explained if activation spread only around them. Isochrones immediately adjacent to the distal sides of the line of block were often parallel to the lines and extended for a long distance along their length (figure 15-2). This means that activation at the recording sites included within these parallel isochrones occurred nearly simultaneously and could not be accounted for by conduction around the putative line of block. Therefore, it was necessary to consider the possibility that activation occurred across the line of apparent block.

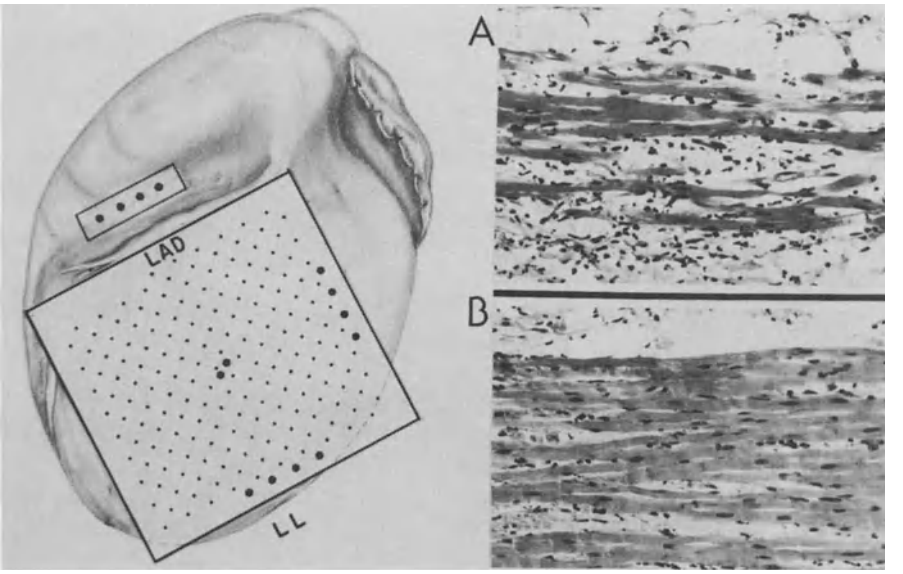

Figure 15-1. The location of the multiple electrode array on the epicardial surface of the canine heart for our mapping experiments of ventricular tachycardia is shown at the left. The margin of the array adjacent to the left anterior descending coronary artery is labeled (LAD), the opposite margin is (LL). The long axis of the surviving epicardial muscle fibers that comprise the epicardial border zone in general courses from the LAD to LL margins. Each small dot represents the location of a bipolar electrode pair, large dots show the location of stimulating electrodes used to demonstrate the anisotropic conducting properties of the epicardial border zone. At the right are micrographs of the parallel surviving muscle fibers in the epicardial border zone. In some regions (a) muscle fibers are widely separated while in others (b) they are packed more closely together. This difference in anatomic organization may determine the nonuniformity of anisotropic conduction properties.

These observations led to our hypothesis that there is slow activation through the line of apparent block, even though there is a large time difference between activation on either side of it. Activation across the line occurs transversely to the long axis of the fibers, which might explain the slow propagation (the influence of anisotropy) and the large time differences on either side of the lines. To prove this hypothesis and to show that anisotropy significantly influenced conduction in regions where lines of block were formed, the epicardial border zone was stimulated during sinus rhythm from the margins and from the center so that activation occurred in different directions. We compared activation times in regions where lines of block were located during tachycardia with activation during stimulation from the different sites and found that stimulated wavefronts traveling transversely to fiber orientation mimicked the activation pattern that occurred in the region of the line during tachycardia [32].

We also carefully examined the electrograms at the lines of apparent block

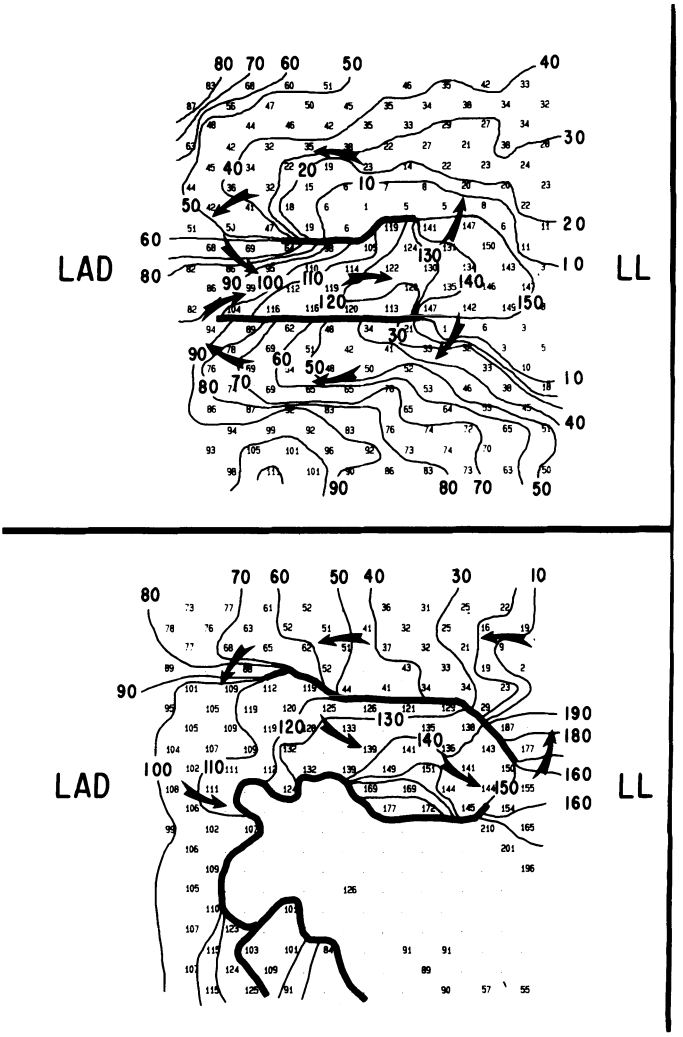

Figure 15-2. Examples of reentrant circuits in the epicardial border zone of two different canine infarcts during sustained ventricular tachycardia. The activation maps are superimposed on the electrode array (see figure 15-1). The LAD and LL margins are labeled. Activation times are plotted at each recording electrode position and are indicated by the small numbers. Isochrones are drawn at 10 msec intervals and indicated by larger numbers. Arrows point out the sequence of excitation beginning at the earliest isochrone (10 msec). In the top panel there are two reentrant circuits in a figure 8 pattern. Activation proceeds initially from the 10 msec isochrone at the LL margin both upward toward the base and downward toward the apex, forming two wavefronts moving in opposite directions. The upper wavefront moves toward the LAD margin, which it reaches after 60 to 80 msec. The lower activation wave also moves toward the LAD margin arriving there after 90 msec. The two activation waves coalesce and activation proceeds back toward the LL margin, completing the reentrant cycle. In the bottom panel only one reentrant circuit is apparent. Activation begins within the 10 msec isochrone at the LL margin and progresses along the base to the LAD margin, which is activated after 100 msec. Activation then occurs back toward the lateral margin.

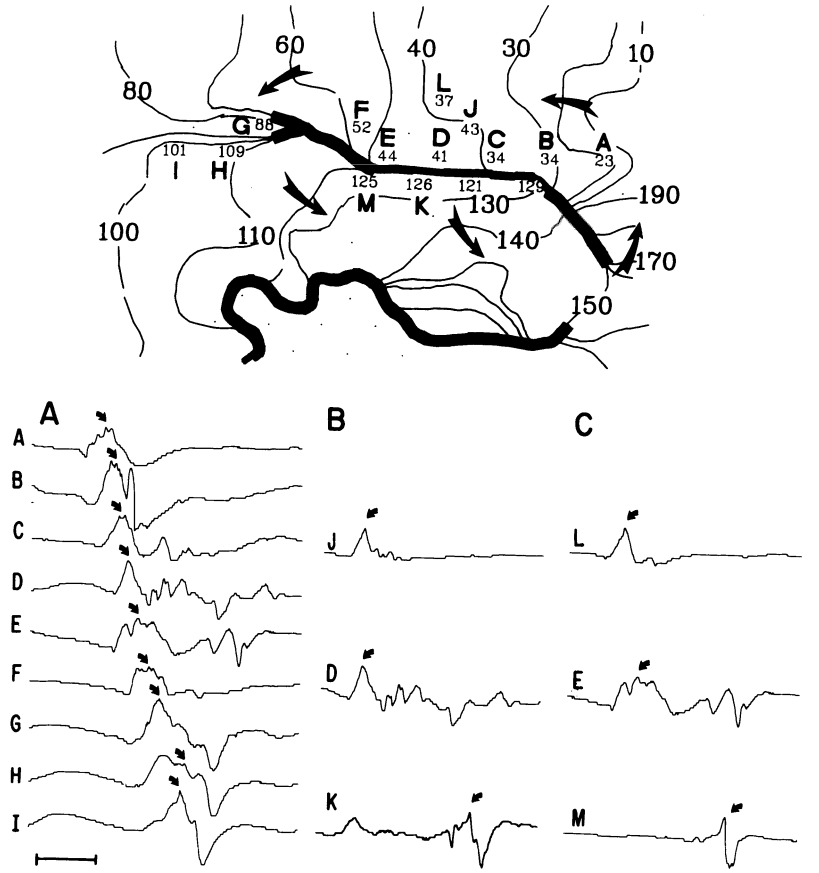

Figure 15-3. Characteristics of electrograms recorded along the line of apparent block in one of the reentrant circuits described in figure 15-2. At the top, the isochrones from the activation map described in the bottom panel of figure 15-2 are shown. The letters indicate recording sites of electrograms shown in panels A, B, and C. Panel A shows electrograms recorded at sites A to I along the line of block. Moving from right to left it is apparent that the electrogram duration and number of components increase as the line is reached (compare electrograms A, B, and C with D and E in panel A). Toward the opposite end of the line (electrogram H and I) duration and fractionation decrease. Panels B and C show electrograms recorded on either side of the line of block. Electrograms J and L were recorded from locations above the line, and K and M below the line. These electrograms have relatively discrete deflections. Between these sites that are on opposite sides of the line, fractionated electrograms of long duration were recorded (D and E) (Reproduced from ref. 32 with permission of the American Heart Assoc.).

in each experiment (figure 15-3). Electrograms along the lines were fractionated and had long durations, whereas at most recording sites that were not at the line of block, electrograms recorded during tachycardia were characterized by relatively discrete deflections. The long duration and fractionated characteristic of electrograms recorded at the lines of apparent block only occurred during tachycardia and represented a dramatic change from the

characteristics of these electrograms during sinus rhythm or ventricular stimulation. On the basis of our in vitro studies on fractionated electrograms, we interpret the long duration fractionated electrograms at the lines of block to mean that activation is occurring during the entire interval of the electrogram [17]. The fractionated components are caused by asynchronous activation of adjacent fiber bundles. Fractionation of electrograms has been shown to occur during conduction in nonuniform anisotropic tissue, when activation occurs in the direction perpendicular to the long axis of the myocardial fibers [30], whereas there is much less or no fractionation during activation parallel to the long axis. Interpreted in conjunction with the observations that adjacent isochrones to the lines of apparent block are parallel to them, it can be concluded that activation proceeds slowly through this region and that the lines do not indicate block of conduction.

These results have led us to formulate a hypothesis for the mechanisms of reentrant excitation in the epicardial border zone. The cornerstone of the hypothesis is that anisotropy is a major cause of reentry. Therefore, we call it *anisotropic* reentry. According to this model (figure 15-4) the conduction properties of the epicardial border zone that enable reentry to occur are a result of the anisotropic structure of this region—parallel bundles of muscle fibers in which there is slow conduction transverse to the long axis of the fibers and fast conduction parallel to the long axis. Impulse propagation during reentry in this anisotropic medium must occur both parallel and transverse to the long axis because of the narrow dimensions of the surviving sheet (10 to 20 cell layers thick). In the regions of the circuits in which activation is occurring rapidly parallel to fiber orientation, isochrones are widely separated and oriented perpendicular to the long axis of the fibers. The region of the circuits in which activation is occurring perpendicularly to the long axis is shown by the isochrones that are bunched closely together indicating slow activation (figure 15-4). These closely bunched parallel isochrones are what were referred to as the lines of apparent block. Therefore, despite the appearance of long lines of apparent block (figure 15-2) (which are not block), reentry is actually occurring around a small central fulcrum (figure 15-4). We have no data relating to the events occurring at this fulcrum; it may be a region of collision and block of centripetal impulses from the circulating wavefront as in the leading circle model of reentry [33], or it may be a small area damaged by the infarction. Because of the anisotropy, activation (conduction velocity) around the circuit is inhomogeneous. During activation parallel to fiber orientation, it is rapid (approximately 0.5 m/sec), but it is very slow transverse to fiber orientation (approximately 0.05 m/sec). The slow activation transverse to fiber orientation is necessary to allow the recovery of excitability of fibers in the circuit that enables reentry to continue.

In some infarcted canine hearts, we have been able to induce sustained ventricular tachycardia and in others, despite the presence of a large infarct,

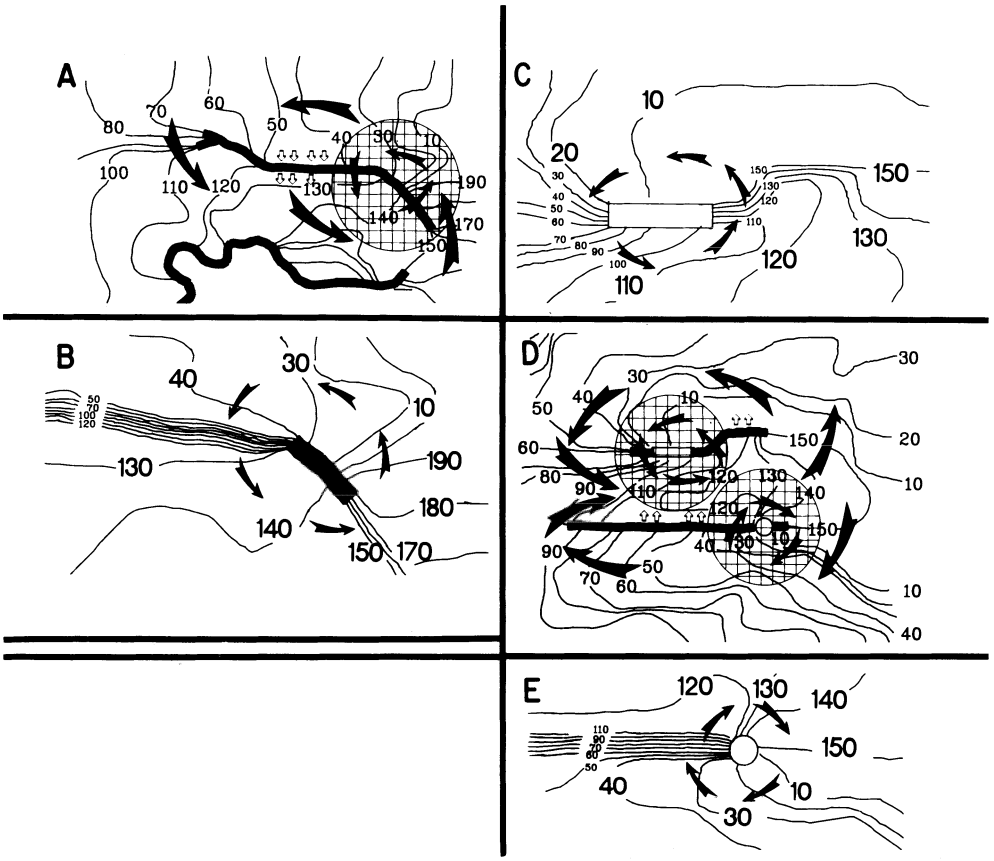

Figure 15-4. Diagrams illustrating the effects of anisotropy on reentrant circuits. The isochrones from the reentrant circuits discussed in figure 15-2 are shown in panels A and D. In panel A the large black arrows point out a sequence of excitation that appears to be progressing around the long line of block, indicated by the horizontal thick black line, as discussed in the description of figure 15-2, bottom panel. From a closer analysis of isochrones adjacent to this line, we have proposed that there is slow activation across it (see text), and, therefore, the center of the circuit is smaller as indicated by the smaller black arrows within the shaded circle. This region is enlarged in panel B where the thick black line of apparent block has been resolved into closely bunched isochrones indicating slow activation in the part of the circuit transverse to fiber orientation. In panel D the large black arrows point out a sequence of excitation that appears to be progressing around two long lines of block, indicated by the horizontal thick black lines as discussed in the description of figure 15-2, top panel. From a closer analysis of isochrones adjacent to the lines of apparent block, we have shown that there is very slow activation across these lines. The smaller arrows in panel D within the shaded circles indicate this proposed activation pattern. As a result, the center of the reentrant circuit may be small. These proposed circuits are shown enlarged in panel C (upper circuit) and E (lower circuit). According to this interpretation, there is rapid activation in the parts of the circuit where activation occurs parallel to the long axis of the fibers and slow activation in the parts of the circuit where activation occurs transversely. The slow activation is indicated by resolving the thick black line of apparent block into the closely bunched isochrones (Reproduced from ref. 32 with permission of the American Heart Assoc.).

tachycardias were not induced. This has forced us to think about the necessary substrate for the occurrence of tachycardia; it is not only the presence of a large infarct. Our proposal is that the appropriate anisotropic properties are crucial. There must be nonuniform anisotropy with a region in which conduction transverse to fiber orientation is slow enough to allow reentry. This region forms the center of the reentrant circuit depicted in figure 15-4. Without the appropriate anisotropic properties, sustained tachycardia could not occur.

Significance of the Anisotropic Reentry Hypothesis

Anisotropic reentry is a result of the geometric interrelationships of the cardiac muscle bundles in the reentrant circuit. These interrelationships cause the slow conduction necessary for reentry to occur—slow conduction that is not dependent on depressed membrane potentials. This mechanism may help us understand the electrophysiologic properties of reentrant circuits and reentrant arrhythmias, as well as provide an impetus for a different approach to the development of antiarrhythmic drugs.

Allessie et al. [33] described the generally expected properties of the two major classifications of reentrant circuits that can result in long lasting arrhythmias: anatomic circuits in which the reentrant impulse travels in a well defined anatomic pathway around an inexcitable (anatomic) barrier and functional reentrant circuits in which the electrophysiologic properties, particularly inhomogeneities in refractory periods, dictate the location of the circuit. The leading circle mechanism for reentry is the best example. (Reflection, which is a third kind of reentry, was not included in this comparison [34].) One property of particular importance for determining both the characteristics of the arrhythmia and its response to antiarrhythmic drugs is the excitable gap. The excitable gap concept, which originated in the studies of Mines [35] at the beginning of this century, is best described in terms of the wavelength of the propagating impulse in the reentrant circuits. The wavelength is a product of the conduction velocity and the effective refractory period [36]. In order for reentry to continue, the wavelength cannot be longer than the path length or the reentrant impulse would block in refractory tissue. If the wavelength is significantly less than the pathlength, a part of the circuit is completely excitable, and this part is the excitable gap. The larger the pathlength with respect to the wavelength, the greater the part of the circuit that remains excitable. In functional reentrant circuits it was predicted and shown that there is little if any completely excitable gap and that "the head of the impulse bites in its refractory tail" [33]. On the other hand, in an anatomic circuit there can be a large and fully excitable gap. The size of the gap in the different kinds of circuits may be important because it determines whether an arrhythmia can be terminated by stimulated impulses and it may determine the stability of a circuit. Leading circle re-

entry without an excitable gap may cause mainly unstable tachyarrhythmias, which either terminate spontaneously or degenerate into fibrillation. Circuits with excitable gaps may more readily cause sustained tachycardias.

Into which classification does anisotropic reentry fit? It is functional in that its occurrence is dependent on the conductile properties of the myocardium. Yet it might also be argued that it is anatomic because it is dependent on the anatomic organization of the fibers and might only occur where there is a particular microscopic anatomy resulting in the necessary amount of nonuniform anisotropy. We also predict on the basis of some computer simulations that an excitable gap is associated with anisotropic reentry [37]. The gap is a result of the sudden change in wavelength that occurs when the impulse turns from the fast conducting part of the circuit (along the long axis of the myocardial fibers) to the slow conducting part of the circuit (transverse to the long axis of fibers). When conduction slows the wavelength should shrink (providing that slowing of conduction is not associated with prolongation of the refractory period), resulting in the appearance of an excitable gap. The gap may result in the stable reentrant circuits that cause sustained ventricular tachycardia. In the absence of a gap, as might occur when the amount of nonuniform anisotropy is insufficient, nonsustained tachycardias might occur. The characteristics and properties of reentry caused by anisotropic tissue structure, therefore may be unique, warranting separate classification of this reentrant mechanism.

That structural features and anisotropy cause reentrant tachycardia obviously has implications concerning the proposed mechanisms of antiarrhythmic drug action. The emphasis in investigations on drug effects has been on membrane channels and currents and has not taken into account influences of axial resistivities caused by structural complexities on conduction and reentry. When this is taken into consideration as a possible cause of slow conduction and block, the influences of drugs on both intercellular coupling and anisotropy become more important, and perhaps the effects on membrane currents become less important. For example, it has been proposed that a primary mechanism of action of (class 1) Na channel blocking drugs on reentry is to diminish the inward current in slowly conducting regions of the circuit to such an extent that conduction blocks and reentry is terminated [38]. However, this effect might only occur if the inward current is initially depressed and this depression is a cause of the slow conduction. If the action potentials in the reentrant circuits are not depressed, as in anisotropic reentry, Na channel-blocking drugs may not decrease Na inward current enough to cause conduction block.

A question that we feel is important to answer is how do presently available drugs affect anisotropic conduction in tissues with normal action potentials having fast upstroke? Are these drugs capable of slowing or blocking conduction? If so, do they act preferentially on conduction in one direction? What is the mechanism(s) of drug effects? A limited amount of data is available.

Spach et al. [39] have shown in isolated preparations of human atria that quinidine and lidocaine produced a greater relative decrease in $\dot{V}_{max}$ and conduction velocity during longitudinal propagation than transverse propagation. Does this mean that effects of such drugs to cause conduction block in reentrant circuits are more likely to occur where activation is occurring in the longitudinal direction in the circuit? On the other hand, if drugs influence gap junctional conductances, conduction in the transverse direction would be expected to be influenced more. Although a number of interventions are used experimentally for this effect, a pharmacologic agent designated for antiarrhythmic use that has this specific action on intercellular junctions has not yet been developed.

In addition to the possibility that anisotropic reentrant circuits might necessitate a new kind of antiarrhythmic drug for the termination of reentry because of the role of intercellular coupling in causing slow conduction, the presence of a large excitable gap as a property of these circuits also should influence antiarrhythmic drug action. For example, if a class III drug is to stop reentry it must increase the wavelength by prolonging action potential duration to promote conduction block. If there is a large excitable gap in anisotropic reentrant circuits, such a prolongation in action potential duration may not be possible.

CONCLUSION

A model of anisotropic reentry in experimental myocardial infarction has been described with properties that are different from previous models of reentry. If anisotropy is cause of clinical arrhythmias, the concept may necessitate a new approach to development of antiarrhythmic drugs.

REFERENCES

1. Gettes, L.S. 1986. Effects of ischemia on cardiac electrophysiology. in *The Heart and Cardiovascular System. Scientific Foundation.* H.A. Fozzard, R.B. Jennings, E. Haber, and A.M. Katz, eds., New York, Raven Press, pp. 1317–1341.
2. Wit, A.L. and Bigger, J.T., Jr. 1975. Possible electrophysiological mechanisms for lethal arrhythmias accompanying myocardial ischemia and infarction. *Circulation* 51 and 52 (Suppl. II):96–115.
3. Wit, A.L. 1982. Electrophysiological mechanisms of ventricular tachycardia caused by myocardial ischemia and infarction in experimental animals. in *Ventricular Tachycardia, Mechanisms and Management,* M.E. Josephson ed., pp. 33–96, Mt. Kisco, NY, Futura.
4. Janse, M.J. and Kleber, A.G. 1981. Electrophysiological changes and ventricular arrhythmias in the early phase of regional myocardial ischemia. *Circ. Res.* 49:1069–1081.
5. Wit, A.L. and Cranefield, P.F. 1978. Reentrant excitation as a cause of cardiac arrhythmias. *Am. J. Physiol.* 235:H1–H17.
6. Janse, M.J. 1986. Reentry rhythm. in *The Heart and Cardiovascular System.* Scientific Foundation. H.A. Fozzard, R.B. Jennings, E.Haber, and A.M. Katz, eds., pp. 1203–1238, New York, Raven Press.
7. El-Sherif, N. 1985. The figure 8 model of reentrant excitation in the canine post infarction. in *Cardiac Electrophysiology and Arrhythmias,* D.P. Zipes and J. Jalife eds., pp. 363–372 New York, Grune and Stratton.
8. Wit, A.L., Allessie, M.A., Bonke, F.I.M. et al. 1982. Electrophysiological mapping to determine the mechanisms of experimental ventricular tachycardia initiated by premature

impulses. Experimental approach and initial results demonstrating reentrant excitation. *A.J. Cardiol.* 49:166–185.
9. Cardinal, R., Savard, P., Carson, D.L., and Page, P. 1984. Mapping of ventricular tachycardia induced by programmed stimulation in canine preparations of myocardial infarction. *Circulation* 70:136–148.
10. Kramer, J.B., Saffitz, J.E., Witkowski, F.X., and Corr, P.B. 1985. Intramural reentry as a mechanism of ventricular tachycardia during evolving myocardial infarction. *Circ. Res.* 56:736–753.
11. Garan, H., Fallon, J.T., Rosenthal, S., and Ruskin, J.N. 1987. Endocardial, intramural and epicardial activation patterns during sustained monomorphic ventricular tachycardia in late canine myocardial infarction. *Circ. Res.* 60:879–896.
12. Wit, A.L., Cranefield, P.F., and Hoffman, B.F. 1972. Slow conduction and reentry in the ventricular conducting system. II. Single and sustained circus movement in networks of canine and bovine Purkinje fibers. *Circ. Res.* 30:11–22.
13. Josephson, M.E. 1978. Recurrent sustained ventricular tachycardia. 2. Endocardial mapping. *Circulation* 57:440–447.
14. Downar, E. Janse, M.J., and Durrer, D. 1977. The effect of acute coronary occlusion on subepicardial transmembrane potentials in the intact porcine heart. *Circulation* 56:217–224.
15. El-Sherif, N. and Lazzara, R. 1979. Reentrant ventricular arrhythmias in the late myocardial infarction period. 7. Effects of verapamil and D-600 and role of the "slow channel".
16. Ursell, P.C., Gardner, P.I., and Albala, A. et al. 1983. Time course of structural and electrophysiologic changes in the epicardial border zone of canine myocardial infarcts during infarct healing. *Cir. Res.* 56:11–23.
17. Gardner, P.I., Ursell, P.C., Fenoglio, J.J., Jr., and Wit, A.L. 1985. Electrophysiologic and anatomic basis for fractionated electrograms recorded from chronically ischemic and infarcted regions of the fheart. *Circulation* 72:596–611.
18. Friedman, P.L., Stewart, J.R., and. Wit, A.L. 1973. Spontaneous and induced cardiac arrhythmias in subendocardial Purkinje fibers surviving extensive myocardial infarction in dogs. *Circ. Res.* 33:612–626.
19. Wit, A.L., Allessie, M.A., Fenoglio, J.J. Jr., et al. 1981. Significance of the endocardial and epicardial border zones in the genesis of myocardial infarction arrhythmias. in Harrison, D., ed., pp. 39–68, *Cardiac Arrhythmias: A Decade of Progress*, Boston, GK Hall.
20. Lazzara, R., El-Sherif, N., and Scherlag, B.J. 1973. Electrophysiological properties of canine Purkinje cells in one day old myocardial infarction. *Circ. Res.* 33:722–734.
21. Friedman, P.L., Fenoglio, J.J., Jr., and Wit, A.L. 1975. Time course for reversal of electrophysiologic and ultrastructural abnormalities in subendocardial Purkinje fibers surviving extensive myocardial infarction in dogs. *Circ. Res.* 36:127–144.
22. Fenoglio, J.J. Jr., Alabala, A., Silva, F.G., et al., 1976. Structural basis of ventricular arrhythmias in myocardial infarction: hypothesis. *Hum. Pathol.* 7:547–552.
23. Fenoglio, J.J. Jr., Pham, T.D., Harken, A.H., et al. 1983. Recurrent sustained ventricular tachycardia: Structure and ultrastructure of subendocardial regions in which tachycardia originates. *Circulation* 68:518–533.
24. Myerburg, R.J., Gelband, H., Nilsson, K., et al. 1977. Long term electrophysiological abnormalities resulting from experimental myocardial infarction in cats. *Circ. Res.* 41:73–84.
25. Karaguezian, H.S., Fenoglio, J.J. Jr., Weiss, M.B., and Wit, A.L. 1979. Protracted ventricular tachycardia induced by premature stimulation of the canine heart after coronary artery occlusion and reperfusion. *Circ. Res.* 44:883–846.
26. Michelson, E.L., Spear, J.R., and Moore, E.N. 1980. Electrophysiologic and anatomic correlates of sustained ventricular tachyarrhythmias in a model of chronic myocardial infarction. *Am. J. Cardiol.* 45:583–590.
27. Krenzle, M.G., Tan, R.C., and Ramza, B.M., et al. 1987. Alterations in endocardial activation of the canine papillary muscle early and late after myocardial infarction. *Circulation.* 76:860–874.
28. Saltman, A., Dillon, S., Ursell, P.C., et al. 1987. Influence of anisotropic structure of the infarct epicardial border zone on conduction. *Fed. Proc.* 46(4):1438.
29. Spach, M.S., Miller, W.T. III, Geselowitz, D.B., et al. 1981. The discontinuous nature of propagation in normal canine cardiac muscle. Evidence for recurrent discontinuities of intracellular resistance that affect membrane currents. *Circ. Res.* 48:39–54.

30. Spach, M.S., Miller, W.T. III, Dolber, P.C., et al. 1982. The functional role of structural complexities in the propagation of depolarization in the atrium of the dog. Cardiac conduction disturbances due to discontinuities of effective axial resistivity. *Circ. Res.* 50:175–191.
31. Spach, M.S. and Dolber, P.C. 1986. Relating extracellular potentials and their derivatives to anisotropic propagation at a microscopic level in human cardiac muscle. Evidence for electrical uncoupling of side-to-side fiber connections with increasing age. *Circ. Res.* 58:356–371.
32. Dillon, S., Allessie, M.A., Ursell, P.C., and Wit, A.L. 1988. Influences of anisotropic tissue structure on reentrant circuits in the epicardial border zone of subacute canine infarcts. *Cir. Res.* 63:182–206.
33. Allessie, M.A., Bonke, F.I.M., and Schopman, F.J.G. 1977. Circus movement in rabbit atrial muscle as a mechanism of tachycardia. III. The "leading circle" concept: a new model of circus movement in cardiac tissue without the involvement of an anatomical obstacle. *Circ. Res.* 41:9–18.
34. Antzelivitch, C., Davidenko, J.M., Shen, X., and Moe, G.K. 1985. Reflected reentry: electrophysiology and pharmacology. in *Cardiac Electrophysiology and Arrhythmias,* D.P. Zipes and J. Jalife, eds., pp. 253–244, New York, Grune & Stratton.
35. Mines, G.R. 1914. On circulating excitations in heart muscles and their possible relations to tachycardia and fibrillation. *Trans. Roy. Soc. Can.* Ser 3 Sect IV 8:43–52.
36. Smeets, J.L.R.M., Allessie, M.A., Lammers, W.J.E.P., et al. 1986. The wavelength of the cardiac impulse and reentrant arrhythmias in isolated rabbit atrium. *Circ. Res.* 58:96–108.
37. Lammers, W.J.E.P., Wit, A.L., and Allessie, M.A. 1987. Effects of anisotropy on functional reentrant circuits: Preliminary results of computer simulation studies. in *Activation, Metabolism and Perfusion of the Heart,* pp. 133–149, S.Sideman and R. Beyar, eds., The Nether-lands, Martinus Nijhoff.
38. Boyden, P.A. and Wit, A.L. 1983. Pharmacology of the antiarrhythmic drugs. in *Cardiac Therapy,* pp, 171–234, M.R. Rosen, and B.F. Hoffman, Eds., pp. 171–234, The Netherlands, Martinus Nijhoff.
39. Spach, M.S., Dolber, P.C., Heidlage, F., et al. 1987. Propagating depolarization in anisotropic human and canine cardiac muscle: apparent directional differences in membrane capacitance. *Circ. Res.* 60:206–219

16. ARRHYTHMIAS IN THE EARLY ISCHEMIC PERIOD

MICHIEL J. JANSE

It has been known for more than a century that in experimental animals acute ischemia produced by occlusion of a major coronary artery frequently results in the occurrence of ventricular fibrillation within several minutes after the onset of ischemia [1]. The same study also states that sudden release of a coronary artery occlusion may induce fibrillation. It is of interest to note that the reason for undertaking this experimental study was the clinical observation that the only pathologic finding in humans who died suddenly ("strong, well-fed individuals in the prime of their life, who during a walk, at the opera or during a meal, as if struck by lightning, suddenly collapsed and within a few minutes stopped living" [1]) was sclerosis of the coronary arteries. The hypothesis put forward to explain these dramatic events was that cardiac metabolism produces substances that are harmful; normally these substances are washed away by the coronary circulation, and only on their accumulation when coronary flow stops are they able to exert a noxious influence.

It has taken the medical profession a rather long time to become aware of the impact of sudden cardiac death on mortality in the industrialized western world [2, 3]. Only in the last decade has the number of experimental studies designed to unravel the electrophysiologic changes underlying ischemia-induced arrhythmias markedly increased.

The purpose of this chapter is to describe briefly the various experimental models used to study ischemia-induced arrhythmias and to examine in some

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detail the electrophysiologic alterations caused by the sudden arrest of coronary flow. As will be seen, the concept of Cohnheim and Von Schulthess-Rechberg [1], that accumulation of metabolites is responsible for these alterations, is largely correct.

A VARIETY OF ANIMAL MODELS

It is impossible to provide precise figures on the incidence of ventricular arrhythmias caused by myocardial ischemia, owing to the variety of experimental models. To give just one example, when coronary artery occlusion was performed in series of ten dogs in different laboratories, the incidence of ventricular fibrillation varied from 0 to 70 percent. Only when a series of 100 dogs was used, were stable values reached, the range for occurrence of fibrillation being 14 to 36 percent [4].

Many factors determine whether and how frequently arrhythmias do occur following occlusion of a coronary artery. They include size of the ischemic area, size of the heart, presence of preexisting collaterals, use of anesthetics, stress in conscious animals, activity of the autonomic nervous system, the mode of coronary artery occlusion, heart rate, and presence of a previous infarction.

Thus, the larger the heart and the larger the size of the ischemic area, the more frequent the arrhythmias [4, 5]. If no collaterals are present, coronary artery occlusion in dogs may result in an incidence of ventricular fibrillation of 100 percent, whereas in the presence of an abundant collateral network, fibrillation may never occur [6]. Anesthesia has been reported to have little effect [7] to decrease the incidence of ischemia-induced arrhythmias [8] and to exacerbate reperfusion arrhythmias [9].

In conscious pigs, coronary artery occlusion resulted more often in ventricular fibrillation in the presence of stressor-events (for example unexpected cutaneous shocks, audible tones forewarning such a shock etc.). This was related to a learning-dependent noradrenergic process in the frontocortical regions [10, 11].

Enhanced activity of the sympathetic nervous system is considered to be arrhythmogenic in the setting of acute ischemia [12], and chronic denervation in experimental animals has been shown to reduce the incidence of ischemia-induced arrhythmias [13].

It is not certain to what extent the arrhythmogenic effects of enhanced sympathetic activity are due to their indirect effects (such as an increase in heart rate or an increase in the size of the ischemic area) or to direct electrophysiologic effects on the ischemic myocardium (the nature of which remains to be elucidated).

Since the pioneering studies of Harris [14] it has been shown that a 30-minute period of coronary artery stenosis would reduce the incidence of ventricular fibrillation following a subsequent complete occlusion of the same artery. In animals with an old, healed infarct, a new ischemic event results in

a higher incidence of lethal arrhythmias than in hearts without an infarction [15]. There is no doubt that heart rate is an important factor determining the occurrence of arrhythmias in the presence of acute ischemia, but there is some dispute as to whether bradycardia or tachycardia is the more arrhythmogenic factor.

This brief survey is meant to convey the message that experimental studies of interventions that influence the incidence of ischemia-induced arrhymias have to be interpreted with caution. When the size of the ischemic area, extent of collateral circulation, and heart rate are unknown or uncontrolled, only very large series can provide reliable data.

ELECTROPHYSIOLOGIC ALTERATIONS DURING ISCHEMIA

Changes in transmembrane potential characteristics

Changes in transmembrane potential characteristics have been described in detail elsewhere [16] and will be summarized only briefly here. Within minutes after coronary artery occlusion, cells in the center of the ischemic zone (total absence of coronary flow) depolarize, until after some 5 to 7 minutes levels of resting membrane potential around −60 to −65 mV are reached. Concomitantly, action potential amplitude, upstroke velocity and duration decrease. Often, the action potentials alternate with respect to amplitude and duration and may even show 2:1 responses during normal sinus rhythm. During alternation the upstroke of the larger action potential consists of two components, the upstroke of the smaller action potential shows only the first component. Within 5 to 10 minutes cells within the center of the ischemic zone become unresponsive. There is evidence that they are at that time not completely inexcitable, but that the excitatory wave invading the central ischemic zone has insufficient "strength" to provide an adequate stimulus [17].

Following a phase of unresponsiveness, ischemic cells (despite complete absence of blood flow) temporarily regain their electrical activity and, during a period extending from about 15 to 30 minutes following the onset of ischemia, produce action potentials that are able to propagate. Finally, after some 30 to 40 minutes of complete ischemia, the cells become permanently inexcitable.

By far the most important factor causing the decrease in resting membrane potential is the net loss of K^+ ions from ischemic cells, and, due to the lack of washout, their accumulation in the extracellular space [18]. The decrease in action potential amplitude and upstroke velocity cannot be attributed solely to the increase in extracellular K^+ concentration. When porcine hearts are regionally perfused with normoxic high K^+ solutions, action potentials have larger amplitudes and faster upstrokes than action potentials at the same site during coronary occlusion [19]. Ischemic action potentials can be imitated in vitro by a combination of high K^+, hypoxia, and acidosis. Probably other

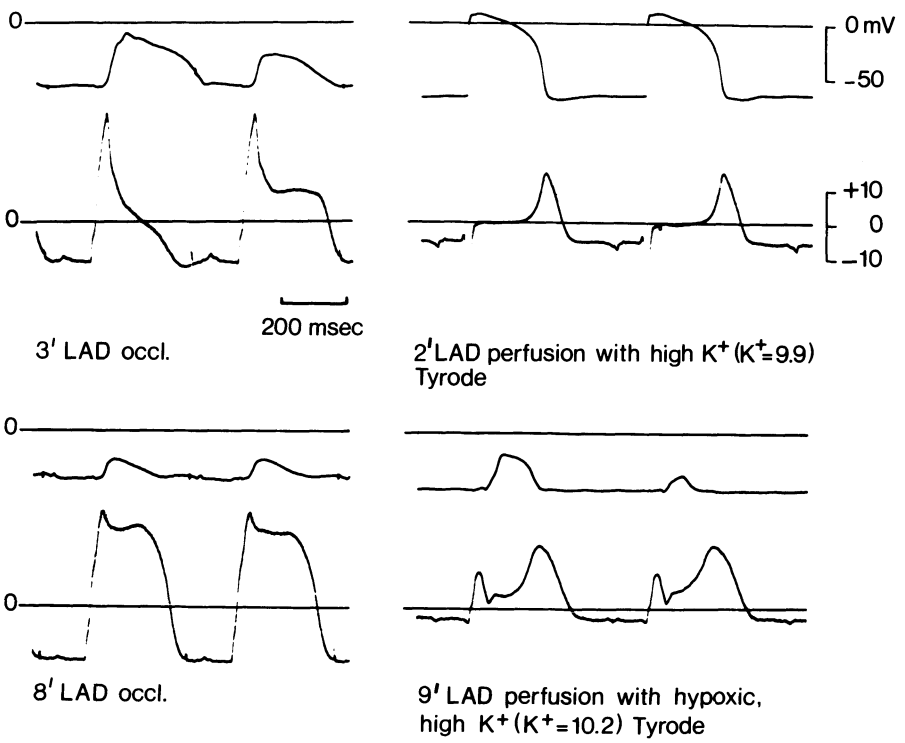

Figure 16-1. Subepicardial transmembrane potentials (upper tracings) and local direct current extracellular electrograms (lower tracings) from the left ventricle of an isolated pig heart. Left panels show potentials three and eight minutes after occlusion of the left anterior descending coronary artery. Note alternation (3 minutes) and almost complete absence of action potentials after 8 minutes. Right panels show potentials at the same site when, after a long reperfusion period, the left anterior descending coronary artery was selectively perfused with a normoxic high K^+ solution (upper panel) and with a hypoxic high K^+ solution. Note that K^+, alone, does not produce ischemic potentials, whereas the combination of high K^+ and hypoxia does. Modified from Morrena et al. [19].

components of ischemia, such as catecholamines and lysophosphoglycerides, also play a role. Figure 16-1 shows transmembrane potentials and local direct current extracellular electrograms from the anterior surface of the left ventricle of an isolated, Langendorff-perfused pig heart. In the left colums, potentials are shown 3 and 8 minutes after occlusion of the left anterior descending coronary branch. Note alternation (three-minutes) and almost complete absence of regenerative action potentials after eight minutes. The upper right panel shows how at the same site, after a long reperfusion period, selective perfusion of the left anterior descending coronary artery with a normoxic solution containing 9.9 mM K^+ fails to reproduce the changes brought about by ischemia. When a high K^+ solution is made hypoxic however, action potentials very similar to those seen during ischemia are

recorded (lower right panel). When comparing the lower two panels, it is of interest to note that although the transmembrane potentials are very similar, the extracellular complexes are very different. This may be attributed to the different effects of ischemia and hypoxia on cellular coupling resistance. Despite similar intracellular potential differences between ischemic and nonischemic cells, hypoxic perfusion apparently leads to less pronounced changes in extracellular potentials because of an increased coupling resistance.

The reasons for the temporal improvement in electrical activity after some 15 minutes of ischemia have not been completely elucidated. One factor may be a gradual "diffusion" of K^+ out of the ischemic region [17]; the release of endogenous catecholamines may be another [20].

Changes in Passive Electrical Properties

Only recently has it become possible to measure extracellular and intracellular longitudinal resistances accurately during acute ischemia [21]. This was made possible by the development of an arterially perfused papillary muscle preparation placed in a H_2O-saturated gaseous environment acting as an electrical insulator [22]. In such a preparation arrest of coronary flow (in addition to changing the gaseous environment to 94 percent N_2 and 6 percent CO_2) resulted in an immediate increase in extracellular resistance (related to the loss of intravascular volume), followed by a secondary slower increase (most likely as a result of osmotic cell swelling). Intracellular resistance remained constant during the first 10 to 15 minutes of ischemia; thereafter, ischemic cells rapidly uncoupled, and longitudinal intracellular resistance increasing by 400 percent within 5 minutes. These changes are illustrated in figure 16-2. Note the change in conduction velocity as intracellular longitudinal resistance increases (in this particular experiment between 10 and 15 minutes after onset of ischemia).

Changes in Conduction Velocity

Conduction velocity remains fairly constant during the first 2 minutes following onset of ischemia in isolated perfused hearts, but rapidly decreases thereafter. For conduction velocity along the longitudinal axis of myocardial fibers, the following values have been measured: control situation to 2 minutes after coronary occlusion: 50 cm/sec; after 3 minutes of ischemia: 40 cm/sec; after 4 to 5 minutes: 30 cm/sec. After 4 to 5 minutes, conduction velocity could no longer be determined accurately because of the development of conduction block, shortly followed by inexcitability [23]. These rapid changes in the thick-walled left ventricle contrast to the slower development of changes in conduction velocity observed in the perfused papillary muscle (figure 16-2). One reason for this discrepancy may be that in the very thin papillary muscle (diameter less than 0.8 mm), CO_2 produced by ischemic cells could diffuse rapidly out of the preparation, whereas it accumulates in the thick-walled left ventricle.

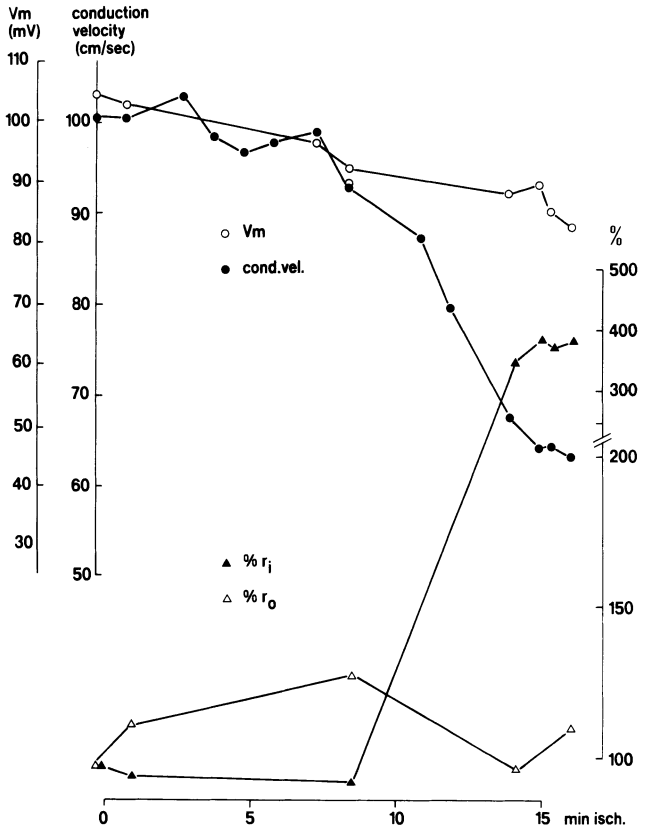

Figure 16-2. Changes in intracellular (ri) and extracellular (ro) resistance, resting membrane potential (Vm) and conduction velocity (cond. vel.) in an arterially perfused rabbit papillary muscle following arrest of coronary flow and a change of the surrounding gaseous atmosphere to 94% N_2 and 6% CO_2. Note early increase in extracellular resistance. Intracellular coupling resistance remains unchanged during the first ten minutes and increases markedly thereafter (A.G. Kleber, C. Riegger and M.J. Janse, unpublished results).

There are two major reasons for the decline in conduction velocity: the decrease in amplitude and upstroke velocity of the action potential and the increase in extracellular and intracellular resistance. The contribution of changes in resistances have been calculated according to linear cable theory [21]. The predicted overall effect was a decrease in conduction velocity by 12 percent after 10 minutes and by 23 percent after 20 minutes. This indicates that in intact hearts, where conduction velocity decreases to about 50 percent of the control value within four to five minutes, the contribution of the changes in action potential upstroke characteristics is more important.

It must be emphasized that the lowest values for conduction velocity observed in ischemic myocardium are significantly higher than the conduc-

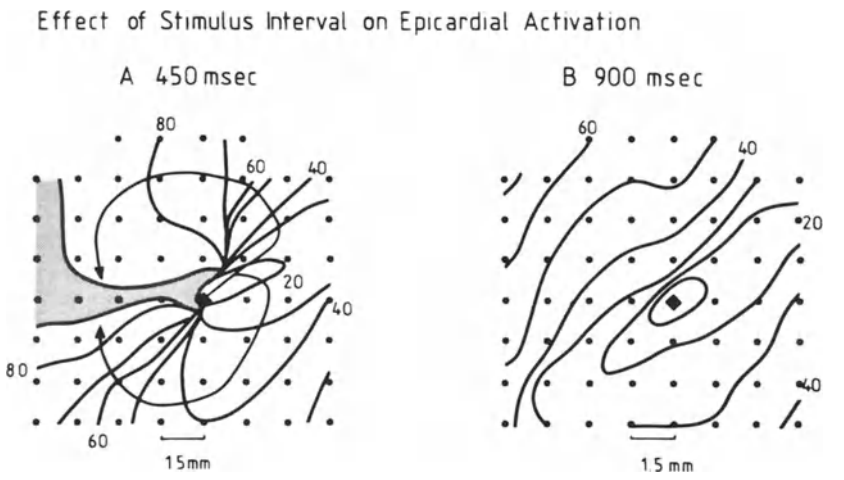

Figure 16-3. Effect of changes in cycle length on epicardial activation six minutes after onset of ischemia. Dots indicate individual recording electrodes. The heart was stimulated through a central electrode (diamond in the middle). Isochrones are drawn in increments of 10 msec. Shaded area is zone of block. In A, stimulus interval was 450 msec. Block occurs in the longitudinal and transverse fiber direction on left and upper part. Arrows indicate attempt at circus movement. In B, activation of the beat following the one in A is shown, when stimulus interval was suddenly changed to 900 msec. Doubling of cycle length causes immediate restoration of elliptical spread of excitation. Reproduced from Kléber et al. [23].

tion velocity of a so-called slow response, induced by exposure of the myocardium to high K^+ solutions containing adrenaline [23]. The depressed action potentials in acute ischemia are still initiated by the rapid Na^+ inward current: They are abolished at resting membrane potentials positive to −60 mV [23] and by lidocaine [24].

An important characteristic of the conduction block that occurs after four to five minutes of ischemia is that it is cycle length dependent. Zones that block the impulse at short cycle lengths can conduct the impulse upon a sudden increase in cycle length (figure 16-3). This indicates that delayed, time-dependent recovery of excitability is a major cause of conduction block, and one of the major determinants for the occurrence of reentrant arrhythmias.

RECOVERY OF EXCITABILITY

Because the electrophysiologic changes during the first minutes of ischemia occur very rapidly, it is difficult, if not impossible, to determine systematically the refractory period duration at multiple sites. Furthermore, not only must the shortest possible interval preceeding a successful test stimulus be taken into account when considering the recovery of excitability in acutely ischemic myocardium, but also the "quality" of the premature action poten-

tial. In cardiac cells partially depolarized by exposure to high K^+ concentrations, recovery from inactivation of both the slow and fast inward currents is markedly delayed and lags behind completion of repolarization [25]. As a consequence, premature stimuli delivered after completion of repolarization may elicit either no response, or when applied later, result in graded responses with small amplitudes and low upstroke velocities. This so-called postrepolarization refractoriness has been demonstrated in acutely ischemic myocardium [26]. In simulated ischemia, produced by exposing isolated papillary muscle preparations to hypoxic, acidotic solutions with elevated K^+ concentrations, postrepolarization refractoriness has been studied in more detail [20]. In control conditions, action potential duration was 180 ms, and within 20 ms following repolarization the maximal upstroke velocity of premature responses had reached maximum values. During simulated ischemia (12 mM K^+, $pO_2 < 5$ mm Hg, pH 6.8), action potential duration had shortened to 90 msec. After completion of repolarization there was a period of 110 msec during which premature stimulation did not result in a response, which in turn was followed by a 150 ms interval during which graded responses could be elicited. Thus, the passage of 350 ms was required following the action potential upstroke before inward current had recovered sufficiently to produce an action potential with an upstroke velocity almost equal to that of the basic action potential (despite the fact that action potential duration had shortened from 180 to 90 ms).

In the presence of hypoxia and acidosis, small changes in extracellular K^+ have a marked influence on the recovery of excitability. This indicates that within the central ischemic zone, where differences in extracellular K^+ are known to occur, considerable differences in refractory periods may exist. The dispersion in and around an ischemic zone is enhanced by the fact that in the border zone no postrepolarization refractoriness may occur, yet action potential duration (and thus refractory period duration) becomes shorter than in nonischemic myocardium [28] (figure 16-4). Thus, differences in refractory period duration between the central ischemic and border zones may be as great as 150 msec during a regularly driven rhythm with a basic cycle length of 450 ms.

ARRHYTHMOGENESIS

In my view, the most important factors for the occurrence of arrhythmias in the very acute stage of ischemia are the following:

1. The decrease in conduction velocity to about half its initial value.
2. The delayed, time-dependent recovery of excitability.
3. The dispersion of recovery of excitability both within the central ischemic zone, and between the central and border zones.

It is useful to employ the concept of wavelength to determine the arrhyth-

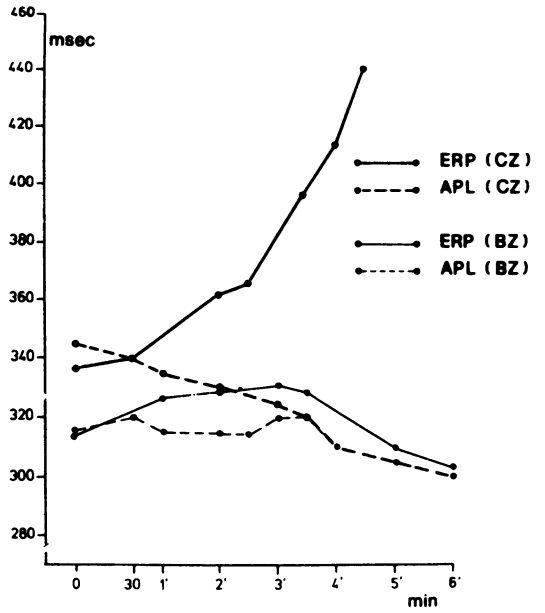

Figure 16-4. Changes in action potential duration (APL) and refractory period (ERP) of the central ischemic zone (CZ) and the border zone (BZ) during the first six minutes following coronary artery occlusion in an experiment that ended in spontaneously occurring ventricular tachycardia. Note large dispersion in refractoriness between BZ and CZ despite similar changes in action potential duration. Reproduced from Capucci et al. [28], by permission.

mogenic effects of interventions, wavelength being the product of conduction velocity and refractory period [29]. The shorter the wavelength, the greater the likelihood for reentrant arrhythmias. If we use previously identified values for conduction velocity and refractory periods before and 5 minutes after coronary occlusion in pig hearts [23, 28], we obtain the following: wavelength in control conditions—50 cm/sec × 0.320 sec = 16 cm; wavelength during ischemia—25 cm/sec × 0.410 sec = 10 cm. It should be obvious that this gives only a very rough indication of the propensity for reentry in ischemic myocardium. It can easily be appreciated that the substrate for reentry is present: short wavelengths and inhomogeneity of refractory periods. For reentry to occur, a trigger in the form of a premature depolarization is needed, as is an area of unidirectional block sufficiently large to allow slow conduction around it before its retrograde invasion leads to reexcitation of the tissue proximal to it. Mapping experiments, as shown in figure 16-5, have indicated that the ventricular premature beats that initiate a reentrant arrhythmia originate close to the border [30]. In that study, no evidence for reentry was found. Although in some experiments intramural mapping was performed, most experiments used epicardial mapping only. The accuracy of reconstructing activation patterns during spontaneously

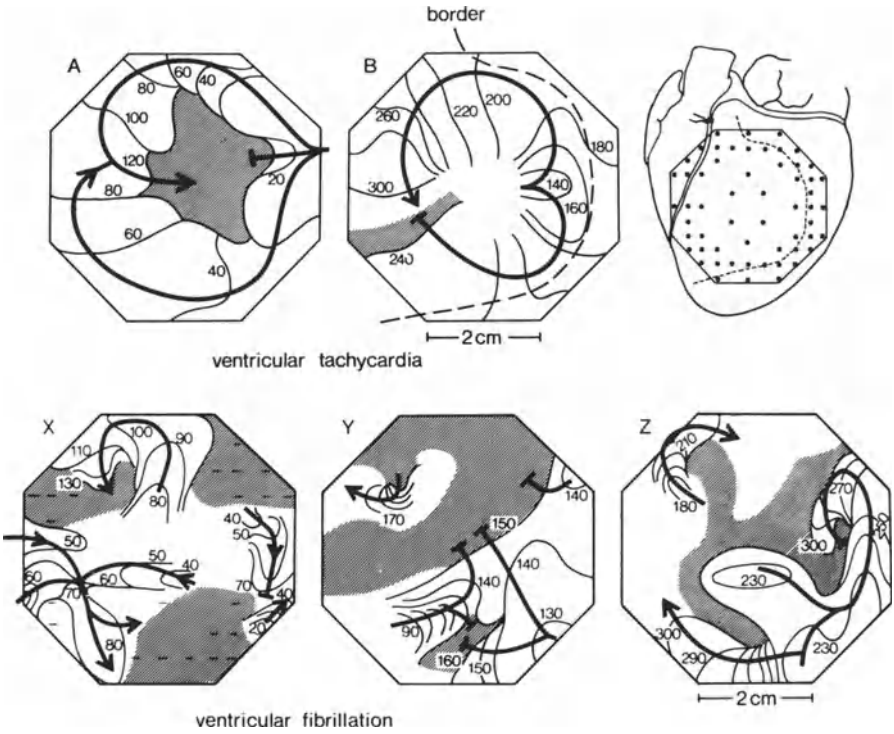

Figure 16-5. Upper panels: Pattern of excitation during the first two beats of a spontaneous ventricular tachycardia, after four minutes of ischemia in an isolated porcine heart. In the right panel the electrode configuration is shown: Each dot is a recording electrode. The broken line is the border of the ischemic zone after clamping the left anterior descending coronary artery. Isochronic lines in the area covered by the electrode are drawn in 20 msec increments. Time zero was arbitrarily chosen. Thick lines with arrows indicate the general direction of the wavefront; shaded areas are zones of block. In A, the earliest activity is found on the normal side of the border. Two wavefronts bypass an area of unidirectional block, which in B is retrogradely invaded after 140 msec, resulting in reentry. Lower panels: Activation patterns of three successive beats, when the tachycardia shown above had degenerated into ventricular fibrillation. Multiple wavefronts are present, and multiple reentry can be observed. The wavefront in the upper part of the area covered by the electrode describes an S-shaped pathway in three successive beats.

occurring arrhythmias was hampered by the fact that at that time simultaneous recordings could be made from only 64 sites. Later experiments [31] used a system capable of recording simultaneously from 232 intramural sites in the cat heart, thus allowing better spatial resolution. Pogwizd and Corr found that 76 percent of all ventricular premature beats occurred through intramural reentry, where a sinus beat was delayed in midmural and subendocardial areas, giving rise to reexcitation in the subendocardium. In 24 percent of cases, premature beats (or the first beat of a ventricular tachycar-

dia) were caused by a non-reentrant mechanism. Non-reentrant initiation of tachycardia could occur both in the subendocardium and in the subepicardium at the border between ischemic and nonischemic myocardium. The mechanism for this nonreentrant ectopic activity is unknown. We have proposed that injury currents flowing between ischemic and nonischemic myocardium could initiate premature beats, either by directly stimulating the nonischemic myocardium at the end of the refractory period, or by enhancing the amplitude of delayed afterdepolarizations [30, 32]. Whatever their mechanism, ventricular premature beats can initiate ventricular tachycardias, which in hearts of animals such as pig and dog terminate spontaneously, or ventricular fibrillation, which in these hearts does not end spontaneously. As shown in the upper panel of figure 16-5, the premature impulse is blocked in the central ischemic zone, but two semicircular wavefronts travel around this zone to invade it retrogradely after 120 ms, resulting in reexcitation of the tissue close to the border after 140 msec. In ventricular tachycardia, the arrhythmia continues as circus movement reentry, where the circuit changes its position and dimension from beat to beat. Usually, a single circus movement is present, but other configurations, such as a "figure of 8" reentry, may occur as well. The pattern of excitation during ventricular fibrillation is quite different, as shown in the lower panel of figure 16-5. The single reentrant circuit of tachycardia has broken up into multiple independent wavelets, traveling around multiple islets of conduction block.

The length of the circulating waves during ventricular tachycardia in our experiments on dog and pig hearts were in the order of 8 cm. In the cat heart, much smaller reentrant pathways were found, the smallest being 1.8 cm [31].

FUTURE RESEARCH

Whereas there can be no doubt that reentry within the ischemic zone plays a dominant role in the arrhythmias of early ischemia, several details still need clarification. The first of these is the nature of the non reentrant mechanism responsible for at least some of the ectopic beats that initiate a reentrant arrhythmia. The second is how reentry within the ischemic area is transferred to the nonischemic myocardium, so that there, also, multiple wavelet reentry occurs, and the entire heart fibrillates. There is some evidence that the subendocardium plays a role in this process because, following the destruction of the subendocardium, reentry remains confined to the ischemic myocardium and fibrillation no longer occurs [33]. Third, we do not know whether the mechanisms underlying the very early (phase 1A) arrhythmias are also responsible for the arrhythmias of phase 1B and for the arrhythmias following reperfusion.

Fourth, although a large body of evidence suggests that the combination of enhanced sympathetic activity and ischemia is particularly arrhythmogenic,

very little is known about the direct electrophysiologic effects of catecholamines on ischemic myocardium. It will not be easy to devise experiments that will give unambiguous answers. No doubt, mapping experiments using simultaneous recordings from multiple electrodes at short distances will have to continue. A possible approach to unraveling the mechanism of the nonreentrant ectopic beats might be simultaneous recording of DC extracellular potentials and local K^+ activity. Combined with knowledge about tissue anisotropy, this may provide more accurate information about the role of injury currents in eliciting ectopic beats. Mapping of both ischemic and nonischemic myocardium, including Purkinje fibers, combined with interventions that prolong refractoriness of nonischemic tissue could establish the role of nonischemic tissue in ischemia-induced fibrillation. Combinations of several experimental techniques (recording of extracellular K^+ concentration, determination or prevention of endogenous catecholamine release, stimulation and ablation of cardiac sympathetic nerves, measurements of changes in passive electrical properties, and mapping) should be used to elucidate the possible differences in mechanisms for 1A and 1B arrhythmias. The electrophysiologic effects of antiarrhythmic drugs, notably on conduction velocity and recovery of excitability, should be investigated on both ischemic and nonischemic tissues to arrive at a more rational approach to pharmacologic intervention. Also, the indirect effects on arrhythmogenesis of agents that influence heart rate, collateral flow, and size of the ischemic area should receive more attention. Finally, it should be remembered that planning of future research is best described by Berthold Brecht in the *Dreigroschenoper:*

Ja mach nur einen Plan
sei nur ein groszes Licht
und mach dann noch 'ne zweite Plan
gehen tun sie beide nicht.

REFERENCES

1. Cohnheim, J. and von Schulthess-Rechberg, A. 1881. Ueber die Folgen Kranzarterieinverschliessung fuer das Herz *Virchows Arch.* 85:503–537.
2. Kennel, W.B. and Thomas, H.E. 1982. Sudden coronary death: The Framingham Study. *Ann. NY Acad. Sci.* 382:3–21.
3. Lown, B. 1979. Sudden cardiac death: the major challenge confronting modern cardiology. *Am. J. Cardiol.* 43:313–329.
4. Trolese-Mongheal, Y., Duchesne-Marulluz, P., Trolese, J.F., et al. 1985. Sudden death and experimental acute myocardial infarction *Am. J. Cardiol.* 56:677–681.
5. Bolli, R., Fisher, D.J., and Entman, W.L. 1986. Factors that determine the occurrence of arrhythmias during acute myocardial ischemia. *Am. Heart J.* 111:261–270.
6. Meesmann, W. 1982. Early arrhythmias and primary ventricular fibrillation after acute myocardial ischemia in relation to pre-existing collaterals. In *Early Arrhythmias Resulting from Myocardial Ischemia,* J.R. Parratt, ed., pp. 93–112, London, MacMillan.
7. Curtis, M.J., MacLeod, B.A., and Walker, M.J.A. 1987. Models for the study of arrhythmias in myocardial ischemia and infarction: the use of the rat. *J. Mol. Cell. Cardiol.* 19:399–419.

8. Manning, G.W., McEachern, C.G., and Hall, G.E. 1939. Reflex coronary artery spasm following sudden occlusion of other coronary branches. *Arch. Intern. Med.* 64:662–674.
9. Bolli, R., Myers, M.L., Zhu, W-X, and Roberts, R. 1986. Disparity of reperfusion arrhythmias after reversible myocardial ischemia in open chest and conscious dogs *J. Am. Coll. Cardiol.* 7:1047–1056.
10. Skinner, J.E., Lie, J.T., and Entman, M.L. 1975. Modification of ventricular fibrillation latency following coronary artery occlusion in the conscious pig: the effects of psychological stress and beta-adrenergic blockade. *Circulation* 51:656–667.
11. Skinner, J.E. 1987. How the head rules the heart. in *Life Threatening Arrhythmias During Ischemia and Infarction,* D.J. Hearse, A.S. Manning, and M.J. Janse, eds., pp. 135–151, New York, Raven Press.
12. Schwartz, P.J. and Stone, H.L. 1982. The role of the autonomic nervous system in sudden coronary death. *Ann. NY Acad. Sci.* 382:162–180.
13. Martin, C. and Meesmann, W. 1985. Antiarrhythmic effect of regional myocardial chemical sympathectomy in the early phase of coronary artery occlusion in dogs. *J. Cardiovasc. Pharmacol.* 7:576–580.
14. Harris, A.S. 1950. Delayed development of ventricular ectopic rhythm following experimental coronary occlusion. *Circulation* 1:1381–1328.
15. Lynch, J.J. and Lucchesi, B.R. 1987. How are animal models best used for the study of antiarrhythmic drugs? in *Life Threatening Arrhythmias During Ischemia and Infarction,* D.J. Hearse, A.S. Manning, and M.J. Janse, eds., pp. 169–196, New York, Raven Press.
16. Janse, M.J. and Kléber, A.G. 1981. Electrophysiological changes and ventricular arrhythmias in the early phase of myocardial ischemia *Cir. Res.* 49:1064–1081.
17. Coronel, R., Fiolet, J.W.T., Wilms-Schopman, F.J.G., et al. in press. Distribution of extracellular potassium and its relation to electrophysiologic changes during acute myocardial ischemia in the isolated porcine heart *Circulation.*
18. Kléber, A.G. 1984. Extracellular potassium accumulation in acute myocaridal ischemia. *J. Mol. Cell. Cardiol.* 16:389–394.
19. Morena, H., Janse, M.J., Fiolet, J.W.T., et al. 1980. Comparison of the effects of regional ischemia, hypoxia, hyperkalemia and acidosis on intracellular and extracellular potentials and metabolism in the isolated porcine heart *Circ. Res.* 46:634–646.
20. Wilde, A.A.M. 1988. Myocardial Ischemia and Hypoxia. Effect on Cellular Ionic and Electrical Activity. Ph.D. dessertation. University of Amsterdam.
21. Kléber, A.G., Riegger, C.B., and Janse, M.J. 1987. Electrical uncoupling and increase of extracellular resistance after induction of ischemia in isolated, arterially perfused rabbit papillary muscle. *Circ. Res.* 61:271–279.
22. Kléber, A.G. and Riegger, C.B. 1987. Electrical constants of arterially perfused rabbit papillary muscle. *J. Physiol. (Lond.)* 385:307–324.
23. Kléber, A.G., Janse, M.J., Wilms-Schopman, F.J.G., et al. 1986. Changes in conduction velocity during acute ischemia in ventricular myocardium of the isolated porcine heart. *Circulation* 73:189–198.
24. Cardinal, R., Janse, M.J., van Eeden, I., et al. 1981. The effects of lidocaine on intracellular and extracellular potentials, activation and ventricular arrhythmias during acute regional ischemia in the isolated porcine heart. *Cir. Res.* 49:792–801.
25. Gettes, L.S. and Reuter, H. 1974. Slow recovery from inactivation of inward currents in mammalian myocardial fibers *J. Physiol. (Lond.)* 240:703–724.
26. Downar, E., Janse, M.J., and Durrer, D. 1977. The effect of acute coronary occlusion on subepicardial transmembrane potentials in the intact porcine heart *Circulation* 56:217–224.
27. Hill, J.L. and Gettes, L.S. 1980. Effect of acute coronary artery occlusion on local myocardial extracellular K^+ activity in swine *Circulation* 61:768–778.
28. Capucci, A., Coronel, R., Fabius, M., and Janse, M.J. 1985. Electrophysiologic mechanism of ventricular arrhythmias in acute ischemia: further observations. *New Trends in Arrhythmias* 1:41–56.
29. Smeets, J.L.R.M., Allessie, M.A., Lammers, W.J.E.P., et al. 1986. The wavelength of the cardiac impulse and re-entrant arrhythmias in isolated rabbit atrium. *Circ. Res.* 58:96–108.
30. Janse, M.J. and van Capelle, F.J.L. 1982. Electrotonic interactions across an inexcitable region as a cause of ectopic activity in acute regional myocardial ischemia. A study in intact porcine and canine hearts and computer models. *Circ. Res.* 50:527–537.

31. Pogwizd, S. and Corr, P.B. 1987. Re-entrant and non re-entrant mechanisms contribute to arrhythmogenesis during early myocardial ischemia: results using three-dimensional mapping. *Circ. Res.* 61:352–371.
32. Janse, M.J., van Capelle, F.J.L., Morsink, H., et al. 1980. Flow of "injury" current and patterns of excitation during early ventricular arrhythmias in acute regional myocardial ischemia in isolated porcine and canine hearts. *Circ. Res.* 47:151–165.
33. Janse, M.J., Kleber, A.G., Capucci, A., et al. 1986. Electrophysiological basis for arrhythmias caused by acute ischemia. Role of the subendocardium. *J. Mol. Cell. Cardiol.* 18:339–355.

17. ARRHYTHMIAS AND THE HEALED MYOCARDIAL INFARCTION

ROBERT J. MYERBURG, SHINICHI KIMURA, PATRICIA L. KOZLOVSKIS, ARTHUR L. BASSETT, HEIKKI HUIKURI, AND AGUSTIN CASTELLANOS

HYPOTHESIS

The major hypothesis guiding our studies of arrhythmias in experimental healed myocardial infarction is that during the evolution and subsequent healing of experimental myocardial infarction, some tissues exposed to ischemic injury heal, but persisting abnormalities in the healed tissues influence the characteristics of electrophysiologic responses to future events. A subordinate premise is that mechanisms for electrophysiologic disturbances in hearts with healed myocardial infarction extend beyond the structural and geometric abnormalities created by the presence of a scar with surrounding or intertwining bands of muscle. There are data demonstrating that long-term changes in patterns of repolarization and response to antiarrhythmic agents may result from either uncoupling or long-term changes in membrane properties; that persistent changes in adrenergic receptors and the adenylate cyclase-cyclic AMP axis are present in hearts with healed infarction; and that neurophysiologic abnormalities may result from nerve damage, membrane responses to sympathetic stimulation, or both. All of these changes are quantitatively and/or qualitatively *regional* in distribution. The consequence

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of regional alterations in properties of hearts with healed infarction becomes most obvious during subsequent acute ischemia and may explain, in part, why the heart with healed myocardial infarction is more susceptible to potentially lethal arrhythmias.

CLINICAL BACKGROUND AND CORRELATIONS

Patients who have survived myocardial infarction are subject to a broad array of ventricular arrhythmias, ranging from various forms of ventricular ectopic activity to sustained ventricular tachycardia and sudden cardiac death [1]. A distinction between arrhythmias occurring during the convalescent phase of myocardial infarction (up to six to eight weeks) and those that appear or persist in the chronic phase (beyond six to eight weeks) suggest that the first 1 to 2 months after myocardial infarction is a dynamic period of changing electrophysiologic characteristics. Recent data demonstrate that the prevalence of premature ventricular contractions identified by ambulatory monitor techniques increases from the first to the sixth week after myocardial infarction [2], with estimates as low as 6 percent in the early period and as high as 20 to 25 percent later. In the period beyond eight weeks, frequent or complex forms of ventricular arrhythmias may occur in as many as 25 percent of patients, and more than 50 percent of those identified early have persistence of the arrhythmias at six months [3]. Among patients who have no arrhythmias identified early after myocardial infarction, approximately 15 percent will demonstrate arrhythmias at 6 months. The occurrence of sustained ventricular tachycardia [4] or ventricular fibrillation [5] in the convalescent phase after myocardial infarction presages a very poor prognosis, although it is unclear whether the prognosis is based primarily on electrophysiologic changes or concomitant hemodynamic abnormalities. Ventricular tachycardia of later onset has a somewhat better prognosis, although follow-up data with aggressive interventions still suggest at least a 10 percent one-year mortality rate.

Another expression of the close correlation between healed myocardial infarction and ventricular arrhythmias is its association with sudden cardiac death. As many as 70 to 75 percent of patients who die suddenly due to coronary heart disease have had a prior myocardial infarction [6], many of which are unrecognized before death.

These clinical and epidemiologic observations point to prior myocardial infarction as a major factor contributing to risk for significant or potentially lethal ventricular arrhythmias. In addition, we have recently emphasized, on the basis of clinical observations, the need to make a distinction between causes and contributing factors (figure 17-1) for potentially lethal ventricular arrhythmias [7]. The identification of a specific underlying etiology does not describe the *mechanisms* by which the underlying pathologic physiology or pathologic anatomy establishes high risk or susceptibility to a potentially

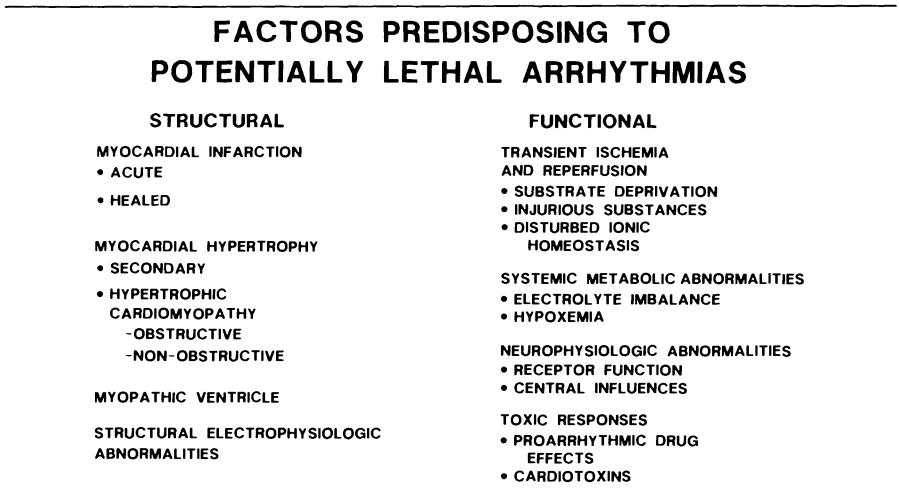

Figure 17-1. Causes and contributing factors to potentially lethal arrhythmias. Structural or functional abnormalities may predispose to potentially lethal arrhythmias. Most underlying etiologies (i.e., causes) have a structural basis, and contributing factors may be either structural or functional. In the case of ischemic heart disease, transient ischemia and reperfusion, systemic metabolic abnormalities, neurophysiologic abnormalities, and perhaps certain toxic responses may interact with the healed myocardial infarction in the genesis of arrhythmias. Secondary myocardial hypertrophy or myopathic changes in ventricular structure may also interact with the healed myocardial infarction.

lethal arrhythmia at a point in time. In the case of coronary artery disease as a cause of sudden cardiac death, specific events, such as acute transient hemodynamic dysfunction, transient ischemia, alterations in autonomic activity, and metabolic or electrolyte fluctuations, all may function as factors contributing to the risk of genesis of lethal arrhythmias in a heart made susceptible by a prior infarction, chronic LV hypertrophy, or other structural abnormalities [7, 8]. Because of these associations and concepts, an understanding of the electrophysiologic disturbances associated with healed myocardial infarction, and its interaction with other contributing factors, is an important field for future investigations. Experimental preparations that have a prior myocardial infarction more closely mimic the clinical circumstances in which acute or transient disturbances adversely alter cardiac electrophysiology.

HEALED MYOCARDIAL INFARCTION AND EXPERIMENTAL ARRHYTHMIAS

Experimental left ventricular myocardial infarction can be produced by a number of techniques of coronary occlusion, including coronary ligation and intravascular occlusions. We have used a technique in cat hearts in which a closed circle of multiple overlapping sutures through the anterior wall of the left ventricle near the apex results in transmural myocardial infarction pre-

dictably involving the base of the anterior papillary muscle and surrounding free wall, sometimes extending onto the apical end of the septum [9, 10]. A nontransmural infarct results if the circle of sutures is incomplete, with a nonligated arc of about 90° [11]. Anatomically, the transmural form of the infarction is characterized by dense scar tissue with an overlying layer of endocardium, three to ten cell layers deep [9], and a less consistent, overlying layer of epicardium of similar thickness. Complete healing requires at least one to two months.

Cellular Electrophysiology After Healed Myocardial Infarction

The cells overlying the healed myocardial infarct (HMI) scar, which are subjected to ischemic injury at the time of acute myocardial infarction, demonstrate persisting abnormalities of action potential duration and refractory periods after healing, with no changes in resting membrane potential, action potential amplitude, or upstroke velocity [12]. Compared to normal cells from the same hearts, or from the same areas of the hearts from control or sham-operated animals, action potential duration at 90 percent repolarization (APD_{90}) and local refractory periods (RP) in the HMI cells are prolonged (APD_{90} at drive cycle length of 630 msec = 178±21 msec [Mean ± SD] for HMI cells, versus 145 ± 13 msec for normal—$P<.01$). Local RP changes were quantitatively and qualitatively similar. Approximately one third of the 61 HMI hearts studied had inducible sustained ventricular arrhythmias in isolated tissue bath, and 17 percent had complex premature ventricular contractions (PVCs) or ventricular tachycardia during a period of continuous monitoring. An additional 38 cats were studied during the acute phase of myocardial infarction (AMI) to compare cellular electrophysiologic changes and spontaneous and induced arrhythmias to those in the HMI animals. The mean APD_{90} at a drive cycle length of 630 ms recorded from surviving cells in AMI zones, corresponding to the HMI zones of the chronic hearts, was 124 ± 18 ms, significantly shorter than the corresponding normal cells (151 ± 12 ms—$P<.01$). Normal cells from both the AMI and HMI preparations were not significantly different from each other in APD_{90} or local RPs, whereas the differences between injured cells in the AMI and HMI preparations were quantitatively similar, but directionally opposite [12]. Specifically, the APD_{90}'s and RPs of AMI cells were shortened to approximately the same extent that those of the HMI cells were lengthened (Δ versus control = −27 ms [AMI] and +33 ms [HMI], $P = p < .001$). Inducible sustained ventricular arrhythmias were observed in 34 percent of the AMI hearts, similar to the HMI hearts, and spontaneous ventricular tachycardia was recorded in 8 percent. However, when a 90-minute AMI was superimposed upon the HMI (n = 24), there was a higher frequency of both spontaneous and inducible arrhythmias [13]. Sustained ventricular arrhythmias in isolated tissues were observed in two thirds of the AMI/HMI hearts, and spon-

taneous complex PVCs, VT, or VF was recorded from 63 percent of the hearts in situ. The magnitude of dispersion of local RPs in AMI/HMI hearts exceeded that of normals, AMI alone, or HMI alone.

Sustained ventricular tachycardia or fibrillation was difficult to induce in our HMI model unless AMI was superimposed. However, other investigators, using HMI models histologically comparable to our *nontransmural* infarct model in cats, were able to induce sustained ventricular tachycardia [14] predictably. Surviving islands of myocardium, rather than discrete dense scars, may account for these differences.

The Electrophysiologic Characteristics of the Border Between HMI Scars and Normal Tissue

This region has electrophysiologic characteristics of special interest. Early studies demonstrated that transmembrane action potential characteristics across a very narrow border between ventricular muscle cells overlying the scar and the adjacent normal areas differed from the characteristics in either of these two zones [15]. Clusters of cells having reduced resting membrane potentials, upstroke amplitudes, and upstroke velocities, with more dramatic reductions in action potential durations, were identified in this region. In addition, there were data suggesting that these cells could be separated into two populations based on their response to verapamil, those that were only minimally affected by the compound and those that were suppressed [16]. These areas also had a limited margin of safety for responses to rapid drive rates and conduction and might not follow rapid rates initiated in other areas of ventricular preparations [15].

In a subsequent study, regional changes in intracellular K^+ and Na^+ activity were studied with ion-sensitive microelectrodes in normal, border, and infarct zone cells of the cat left ventricle 2 to 6 months after myocardial infarction [17]. In this study, the previously reported lengthening of APD_{90} in infarct zone cells was again observed, in addition to a reduction in APD_{90} and resting membrane potential in the border cells. The resting potential for normal and infarct zone cells was -79.3 ± 1.5 mV and -79.2 ± 1.6 mV respectively, whereas that of the border zone cells was -68.9 ± 2.2 mV ($P < .01$, $n = 11$). APD_{90} in the normal tissue was 130 ± 12 msec, was lengthened to 169 ± 20 msec in the infarct zone, and was reduced to 96 ± 15 msec at the border zone ($P < .01$). The intracellular K^+ activity (A^i_{K+} was 89.6 ± 12.3 mM and 91.2 ± 15.0 mM in the normal and infarct zone cells respectively, but was reduced to 71.4 ± 5.1 mM in the border zone cells ($P < .01$). In contrast, the intracellular Na^+ activity calculated for five preparations ($A^i_{Na^+}$) was 10.5 ± 2.2 mM and 10.5 ± 3.3 mM in normal and infarct zone cells respectively, whereas it was elevated to 19.1 ± 5.9 mM in the border zone cells ($P < .05$). The relationship between V_m and E_K in border zone cells also differed from that in normal or infarct zone cells. V_m was more positive to E_K in border

zone cells (16.3 ± 2.2 mV), than in normal zone cells (11.4 ± 3.7 mV) or in infarct zone cells (11.6 ± 4.8 mV). These data suggest that a decreased K^+ conductance and increased Na^+conductance, or both, is involved in the depolarization of the border zone cells. However, the mechanisms underlying shortening of action potential duration in border zone cells of HMI preparations is still unclear. Possibilities include, but are not limited to, increasing background K^+ currents, or intracellular Ca^{++} accumulation by Na^+-Ca^{++} exchange with activation of outward K^+ current during repolarization. Cytosolic Ca^{++} accumulation might also lead to cellular uncoupling, isolating border zone cells from adjacent cells overlying the infarcts, and thereby accounting for marked discrepencies in action potential durations between border and infarct zone cells.

CARDIAC AUTONOMIC STATUS AFTER HEALED MYOCARDIAL INFARCTION

A body of information on cardiac autonomic responses after healed myocardial infarction is being developed. Studies have included the response of the heart with a HMI to sympathetic nerve stimulation and to adrenergic agonists, receptor biochemical and pharmacologic studies, and tissue adenylate cyclase, cyclic AMP, and norepinephrine content. The common denominator in all observations to date is *regional variability of baseline properties and of responses to pertubations* in the heart with a HMI.

Effect of Sympathetic Stimulation on Electrical Properties of Hearts with HMIs

Observations reported to date have been influenced heavily by experimental models. Both the anatomic location and method of denervation will influence results. In the study by Gaide et al. [18], the response to sympathetic stimulation in preparations with apical healed transmural HMIs, (≥two months), measured as reductions in epicardial local refractory periods (RP) during bilateral sympathetic stimulation, was quantitatively similar to studies in control hearts in areas overlying the infarct zone. A moderately enhanced responsiveness (i.e., reduction of RPs) was recorded at the border zone, and a markedly increased response more basally. In contrast, the observations of Barber et al. [19], in which chronic infarcts were created in the mid-left ventricular free wall, and studied at one to three weeks, resulted in mixed responses apical to the infarct and normal responses basally. The apical mixed responses consisted of absent responses at denervated sites and normal-to-blunted responses at nondenervated sites. Sites that did not respond to sympathetic stimulation did respond to norepinephrine infusion. It was subsequently shown that epinephrine or isoproterenol infusions resulted in supersensitive responses at denervated sites apical to infarcts in five to ten-day preparations [20]. The supersensitive responses did not appear to be beta-receptor mediated.

Comparison and interpretation of data on sympathetic responses in HMI hearts requires careful analysis of differences in the anatomy and age of the healed infarcts.

Beta-Adrenergic Receptors in Healed Myocardial infarction

Measurements of beta-adrenergic receptor activity and affinity in HMI prepations have been carried out by Kozlovskis et al. [21]. Using ^{3}H-dihydroalprenelol (DHA), it was observed that surviving muscle in the scarred area of HMI hearts had reduced beta-adrenergic receptor numbers, with a less marked reduction in tissue adjacent to the scar and normal numbers in remote areas of the heart. Beta-receptor affinity (K_D), however, was increased in tissue from the scarred area ($K_D = 0.21 \pm 0.15$), compared to the remote tissues in HMI hearts ($K_D = 0.79 \pm 0.46$) or sites in control hearts comparable to scarred areas in HMI hearts ($K_D = 1.06 \pm 0.31$). Regional reduction in norepinephrine content was observed in a similar group of preparations [11], as it had been in apical sites of a different HMI mode previously [19]. When corrected for myosin content, norepinephrine content was significantly reduced in the area adjacent to the scar, but was normal in the scarred zone and in the free wall remote from the scar [11]. Kozlovskis et al. [21] subsequently observed that adenylate cyclase activity was normal under basal conditions in scarred tissue (53.0 ± 16.5 pmol cAMP/mg/min) and significantly elevated in tissue remote from the healed infarct scar (79.5 ± 12.6 pmol cAMP/mg/min). With isoproterenol and forskolin stimulation, adenylate cyclase activity significantly increased in the remote zone and insignificantly increased in adjacent tissue, as compared to the scarred areas and to control hearts. However, the response was appropriate to the basal conditions in the remote area, suggesting that the basal increase in adenylate cyclase activity in remote tissue accounted for the elevated absolute values during isoproterenol or forskolin stimulation. These preliminary data are under study in relation to other biochemical changes in the HMI preparation, to determine the potential implications of the beta-receptor—adenylate cyclase—cAMP axis in electrophysiologic instability of HMI hearts.

Alpha-Adrenergic Receptors in Healed Myocardial Infarction

Limited data are available on the characteristics of alpha-adrenergic receptor changes in experimental HMIs in cat hearts. Kimura et al. [22] reported reduced rates of automatic activity in isolated Purkinje fibers from areas of healed myocardial infarction during phenylephrine infusion in the presence of propranolol. The phenylephrine-induced reduction in rate of automaticity was specific for Purkinje fibers from HMI zones; noninfarcted areas from the same hearts showed changes similar to normal control hearts. In addition, an enhanced ability of phenylephrine to initiate afterdepolarizations and triggered activity was observed in isolated HMI preparations [22]. In contrast, in

normal feline Purkinje fiber preparations, phenylephrine could induce afterdepolarizations and triggered activity only in the presence of markedly increased Ca^{++} concentrations in the superfusate [23]. Both the phenylephrine-induced reduction in rates of automatic activity and afterdepolarizations could be blocked by phentolamine. These two observations suggest regional enhanced activity or responsiveness of alpha-adrenergic receptors in hearts with HMI.

TRANSIENT ISCHEMIA AND REPERFUSION

As a prelude to studies of the influence of transient acute ischemia on arrhythmogenesis in HMI preparations, Kimura et al. [24] studied the effects of ischemia and reperfusion on normal isolated cat ventricular myocardium. The basic preparation consists of isolated left ventricle, limited to the vascular distributions of the left anterior descending and/or left circumflex beds. The left anterior descending artery, or both the left anterior descending and left circumflex arteries, are cannulated and perfused with Tyrode solution oxygenated with 95 percent O_2–5 percent CO_2. The perfused preparations are maintained in a superfusion bath in which oxygen is displaced by bubbling Tyrode solution with 95 percent N_2–5 percent CO_2. The pO_2 of the perfusate is approximately 500 mm Hg, and in the superfusate 30 to 40 mm Hg. With this preparation, it is possible to carry out repeated runs of ischemia and reperfusion. Ischemic runs are continued for 30 minutes, followed by 45 to 60 minutes of perfusion before a second ischemic run is carried out. Ischemia causes a rapid reduction in resting membrane potential, action potential amplitude, APD_{50}, and APD_{90} in endocardial ventricular muscle cells. These changes reach a plateau after 30 minutes, and reperfusion results in return to baseline characteristics within five minutes. During ischemia in these preparations, spontaneous PVCs occur, but it is not possible to induce sustained ventricular arrhythemias, possibly due to the physical size of the preparation. During reperfusion, however, runs of spontaneous sustained ventricular activity ("ventricular tachycardia") are observed consistently. They abate spontaneously after a period of seconds to minutes. There is a tendency for the frequency of PVCs to be greater during regional ischemia than during global ischemia, but there is no difference in the incidence or characteristics of reperfusion arrhythmias following regional or global ischemia. The reperfusion arrhythmias are not influenced by procainamide pretreatment prior to ischemia runs, but they are prevented by pretreatment with verapamil and by the use of Ca^{++}-free Tyrode solution during ischemic runs.

In a modified preparation, designed to allow recording from epicardium and endocardium simultaneously during ischemia and reperfusion, Kimura et al. [25] observed distinct electrophysiologic differences between the response of epicardium and endocardium to ischemic runs. Specifically, endocardial APD_{90}s decrease to a lesser extent during the first 10 minutes of ischemia

than do epicardial APD_{90}s, but the endocardial APDs continue to decrease for up to 30 minutes of ischemia, whereas the epicardial durations tend to relengthen between 10 and 30 minutes of ischemia. Measurements of refractory periods (RP) revealed that endocardial RPs decrease linearly over 30 minutes, whereas epicardial RPs maximally reduced at 10 minutes and then lengthen significantly, exceeding endocardial RPs at 30 minutes. The greatest dispersions between endocardial and epicardial RPs are at 10 minutes and 30 minutes, with epicardial RPs significantly shorter than endocardial RPs at 10 minutes and significantly longer at 30 minutes. Quantitation of PVCs revealed peak frequencies at 10 and 30 minutes, and the ability to induce sustained ventricular tachyarrhythmia VT is observed only at 10 and 30 minutes, when disparity of RPs is maximum.

Pretreatment of these preparations with verapamil (1 μg/ml in the perfusate) prevented arrhythmias at 30 minutes of ischemia [26]. However, the mechanism by which this occurred does not appear to be related to an influence on afterdepolarizations or triggered activity. Rather, there were changes in the response of epicardial cells to ischemia characterized by a loss of the late prolongation of epicardial RPs relative to endocardial RPs and, therefore, abolishing the dispersion of refractoriness at 30 minutes of ischemia. These data demonstrate an effect of a Ca^{++}-entry blocking agent on properties of conduction and refractoriness rather than afterdepolarizations and triggered activity, and this effect appears to be "antiarrhythmic." Moreover, the response to verapamil in ischemic preparations is regionally different in epicardium and endocardium.

ACUTE ISCHEMIA AND HEALED MYOCARDIAL INFARCTION

The effects of transient ischemia and reperfusion on isolated perfused cat hearts have been studied in hearts with prior myocardial infarctions. The basic design is the same isolated left ventricular preparation in which the muscle mass served by the left coronary system is maintained and other tissue discarded. We have studied HMI hearts two to four months after healing of the acute myocardial infarction, recording transmembrane action potentials from the endocardial cells in normal areas and areas which have healed after infarction [27]. In this preparation, there were no significant differences in measured action potential variables and RPs between the normal and infarct zone cells prior to acute ischemia. There was a tendency for the cells overlying the HMI to have longer APD_{90}s and RPs, but they did not achieve the statistical significance seen in our superfused experimental model [12]. When coronary perfusion was discontinued, resting potentials, action potential amplitudes, and APD_{90}s of cells in normal zones all decreased, and the RPs shortened progressively. However, the changes in action potentials were less prominent and RPs were unchanged in cells from infarct zones. The RP discrepancies between normal and infarct zone areas had reached statistical significance within 10 minutes of ischemia, and the discrepency

continued to increase through 30 minutes of ischemia. During periods of ischemia, four of eight HMI preparations had spontaneous VT, while none of six normal preparations had spontaneous VT. In contrast, during reperfusion, six of seven normal and seven of eight HMI preparations had reperfusion VT. These observations lend further support to the concept that superimposition of ischemia on HMIs produces electrophysiologic inhomogeneities, which may enhance arrhythmogenesis, and that the presence of an HMI makes a heart more susceptible than a normal heart to ischemic arrhythmias. However, reperfusion arrhythmias may occur in either.

GOALS FOR THE FUTURE—THE ISCHEMIC ARRHYTHMIA CASCADE

Based on both clinical and epidemiologic observations and an array of recent experimental data, experimental HMI models appear to provide important avenues for future investigation. One of the more interesting aspects of the HMI preparation is its propensity to develop *regional* differences in baseline electrophysiologic characteristics and regional differences in responses to interventions. In addition, initial data suggest that a *transmural* HMI preparation has different characteristics than a *nontransmural* preparation. In view of these facts, the following areas of investigation hold promise for the future:

1. Comparison of the electrophysiologic properties of experimental transmural and nontransmural HMIs.
2. Characterization of the electrophysiologic significance of Na^+, K^+, and Ca^{++} ion activities in the border between healed infarction and surrounding normal tissue.
3. Elucidation of patterns of regional beta- and alpha-adrenergic receptor changes in hearts with healed myocardial infarction, including regional differences in responsiveness to agonists, antagonists, and sympathetic stimulation.
4. Studies of the effects of transient ischemia and reperfusion in transmural and nontransmural HMI preparations, including correlations between ischemia/reperfusion responses and HMI-related regional adrenergic changes.
5. Clarification of the electropharmacology of membrane-active drugs and Ca^{++}-entry blockers in HMI hearts, in contrast to previously normal tissue, under conditions of acute ischemia.

Cascade for the Initiation of Potentially Lethal Arrhythmias

The avenues of investigation outlined above will contribute to the understanding of differences between specific underlying etiologies for VT/VF (i.e., *causes*) and transient destabilizing factors involved in the actual initiation of VT/VF (i.e., *contributing events*) (figure 17-1). This distinction forms the basis of the mechanistic cascade responsible for the initiation of potentially lethal

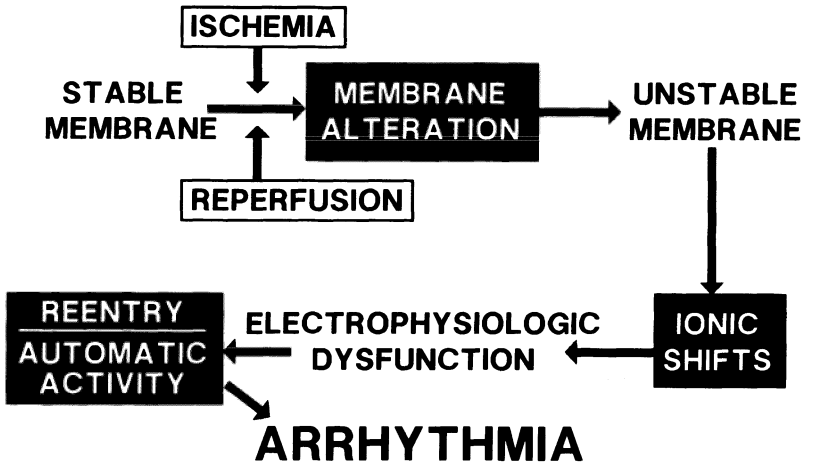

Figure 17-2. Cascade for the genesis of ventricular arrhythmias in ischemic heart disease. Transient ischemia or reperfusion may convert a stable membrane to an unstable one, resulting in ionic shifts that lead to electrophysiologic dysfunction, manifest as automatic activity or reentrant circuits, which form the electrophysiologic basis for arrhythmias (see text). The membrane alterations initiated by ischemia or reperfusion may be biochemical or receptor based and are modulated by other systemic and local influences. The consequent changes in ionic flux are the direct causes of electrophysiologic dysfunction.

arrhythmias in ischemic heart disease. Based on existing data, we currently view the cascade as shown in the simplified flow diagram in figure 17-2. It begins with a stable ventricular muscle mass, which may be structurally normal or abnormal. When a pertubation occurs (e.g., ischemia, reperfusion), metabolic alterations in myocardial cells (e.g., amphipathic metabolites [28], superoxide radical formation [29], loss of membrane integrity, and alteration in channels and receptors), which are simultaneously modulated by contributing factors, such as autonomic tone, electrolyte imbalances, and hemodynamc status, convert a stable muscle mass to an unstable mass. The destabilization of membrane biochemical and biophysical properties lead to ionic shifts and the development of electrophysiologic instability. The latter may take the form of reentrant pathways or automatic foci, which may coexist in unstable preparations [24, 30, 31] and lead to the common endpoint of ventricular arrhythmias.

REFERENCES

1. Myerburg, R.J., Zaman, L., Luceri, R., et al. 1983. Antiarrhythmic drug therapy after myocardial infarction. *The First Year After a Myocardial Infarction,* in H.E. Kulbertus and H.J.J. eds. Wellens, pp. 321–339, Mt. Kisco, NY, Futura Publishing Co.
2. The CAPS Investigators. 1986. The cardiac arrhythmia pilot study. *Am. J. Cardiol.* 57:91–95.
3. Ruberman, W., Weinblatt, E., Frank, C.W. et al. 1981. Repeated 1 hour electrocardiog-

raphic monitoring of survivors of myocardial infarction at 6 month intervals: arrhythmia detection and relation to prognosis. *Am. J. Cardiol.* 47:1197–1204.
4. Wellens, H.J.J., Bär, F.W.H.M., Vanagt, E.J.D.M., and Brugada, P. 1982. Medical treatment of ventricular tachycardia: Considerations in the selection of patients for surgical treatment. *Am. J. Cardiol.* 49:186–193.
5. Lie, K.L., Liem, K.L., Schuilenberg, R.M. et al. 1978. Early identification of patients developing late in-hospital ventricular fibrillation after discharge from the coronary care unit. *Am. J. Cardiol.* 41:674–677.
6. Myerburg, R.J. and Castellanos, A. 1988. Cardiac arrest and sudden cardiac death. In *Heart Disease: A Textbook of Cardiovascular Medicine,* E. Braunwald ed., pp. 742–747, 3rd Ed. Philadelphia, WB Saunders Co.
7. Myerburg, R.J., Kessler, K.M., Zaman, L., et al. 1987. Factors leading to decreasing mortality among patients resuscitated from out-of-hospital cardiac arrest. in *Cardiac Arrhythmias: Where Do We Go From Here?* P. Brugada and H.J.J. Wellens eds., pp. 505–525, Mt. Kisco, NY., Futura Publishing Co.
8. Myerburg, R.J., Kessler, K.M., Estes, D., et al. 1984. Long-term survival after prehospital cardiac arrest: analysis of outcome during an 8-year study. *Circulation* 70:538–546.
9. Myerburg,R.J., Gelband, H., Nilsson, K., et al. 1977. Long-term electrophsiologic abnormalities resulting from experimental myocardial infarction in cats. *Cir. Res.* 41:73–84.
10. Myerburg, R.J., Epstein, K., Gaide, M.S., et al. 1982. Cellular electrophysiology in acute and healed experimental myocardial infarction. *Ann. NY Acad.Sci.* 382:90–115.
11. Kozlovskis, P.L., Fieber, L.A., Bassett, A.L., et al. 1986. Regional reduction in ventricular norepinephrine after healing of experimental myocardial infarction in cats. *J. Mol. Cell. Cardiol.* 18:413–422.
12. Myerburg, R.J., Bassett, A.L., Epstein, K., et al. 1982. Electrophysiologic effects of procainamide in acute and healed experimental ischemic injury of cat myocardium. *Circ. Res,* 50:386–393.
13. Myerburg, R.J., Epstein, K., Gaide, M.S., et al. 1982. Electrophysiologic consequences of experimental acute ischemia superimposed upon healed myocardial infarction in cats. *Am. J. Cardiol.* 49:323–330.
14. Michelson, E.L., Spear, J.F., and Moore, E.N. 1980. Electrophysiologic and anatomic correlates of sustained ventricular tachyarrhythmias in a model of chronic myocardial infarction. *Am. J. Cardiol.* 45:583–590.
15. Wong, S.S., Bassett, A.L., Cameron, J.S., et al. 1982. Dissimilarities in the electrophysiological abnormalities of lateral border and central infarct zone cells after healing of myocardial infarction in cats. *Circ. Res.* 51:486–493.
16. Wong, S.S., Myerburg, R.J., Cameron, J.S., et al. 1982. Electrophysiologic effects of verapamil on cells bordering healed myocardial infarction in the cat. *Proc. Soc. Exper. Bid. Med.* 171:289–293.
17. Kimura, S., Bassett, A.L., Gaide M.S., et al. 1986. Regional changes in intracellular potassium and sodium activity after healing of experimental myocardial infarction in cats. *Circ. Res.* 58:202–208.
18. Gaide, M.S., Myerburg, R.J., Kozlovskis, P.L., and Bassett, A.L. 1983. Elevated sympathetic response of epicardium proximal to healed myocardial infarction. *Am. J. Physiol.* 14:646–652.
19. Barber, M.J., Mueller, T.M., Henry, D.P., et al. 1982. Transmural myocardial infarction in the dog produces sympathectomy in non-infarcted myocardium. *Circulation* 67:787–796.
20. Kammerling, J.J., Green, F.J., Watanabe, A.M., et al. 1987. Denervation supersensitivity of refractoriness in noninfarcted areas apical to transmural myocardial infarction. *Circulation* 76:383–393.
21. Kozlovskis, P.L., Smets, M.J.D., Bassett, A.L., et al. 1988. Regional beta-adrenergic receptors and adenylate cyclase activity after healing of myocardial infarction in cats. (abstr.) *J. Amer. Coll. Card.* 11:252.
22. Kimura, S., Bassett, A.L., Kohya, T., et al. 1987. Automaticity, triggered activity, and responses to adrenergic stimulation in cat subendocardial Purkinje fibers after healing of myocardial infarction. *Circulation* 75:651–660.
23. Kimura, S., Cameron, J.S., Kozlovskis, P.L., et al. 1984. Delayed afterdepolarizations and triggered activity induced in feline Purkinje fibers by alpha-adrenergic stimulation in the presence of elevated calcium levels. *Circulation* 70:1074–1082.

24. Kimura, S., Bassett, A.L., Saoudi, N.C., et al. 1986. Cellular electrophysiologic changes and "Arrhythmias" during experimental ischemia and reperfusion in isolated cat ventricular myocardium. *J. Am. Coll. Cardiol.* 7:833–842.
25. Kimura, S., Bassett, A.L., Kohya, T., et al. 1986. Simultaneous recording of action potentials from endocardium and epicardium during ischemia in the isolated cat ventricle: relation of temporal electrophysiological heterogeneities to arrhythmias. *Circulation* 74:401–409.
26. Kimura, S., Bassett, A.L., Kohya, T., et al. 1987. Regional effects of verapamil on recovery of excitability and conduction time in experimental ischemia. *Circulation* 76:1146–1154.
27. Kimura, S., Bassett, A.L., Kozlovskis, P.L., and Myerburg, R.J. 1987. Electrophysiologic changes during acute ischemia in coronary perfused cat ventricle with healed myocardial infarction (HMI). *Circulation* 76 (Suppl. IV):109.
28. Corr, R.B., Gross, R.W., and Sobel, B.E. 1984. Amphipathic metabolites and membrane dysfunction in ischemic myocardium. *Circ. Res.* 55:135–154.
29. Manning, A.S., Coltart, D.J. and Hearse, D.J. 1984. Ischemia and reperfusion-induced arrhythmias in the rat: Effect of xanthine oxides inhibition with allopurinol. *Circ. Res.* 55:544–548.
30. Pogwizd, S.M. and Corr, P.B. 1987. Electrophysiologic mechanisms underlying arrhythmias due to reperfusion of ischemic myocardium. *Circulation* 76:404–426.
31. Pogwizd, S.M. and Corr, P.B. 1987. Reentrant and nonreentrant mechanisms contribute to arrhythmogenesis during early myocardial ischemia: results using three-dimensional mapping. *Circ. Res.* 61:352–371.

V. STRATEGIES FOR PREDICTION, PREVENTION, AND THERAPY

18. CLINICAL SIGNIFICANCE AND LIMITATIONS OF VENTRICULAR LATE POTENTIALS

GÜNTER BREITHARDT, MARTIN BORGGREFE, ULRICH KARBENN AND ANTONI MARTINEZ-RUBIO

For almost ten years, signal averaging has been used for noninvasive recording of ventricular late potentials [1–10]. Delayed and fractionated potentials have been observed in regions of experimental myocardial infarction [11–13], as well as during intraoperative epicardial and endocardial mapping [1, 2, 14, 15] and endocardial catheter mapping in patients with ventricular tachycardia [16, 17].

Ventricular late potentials are related to the anatomic characteristics of myocardial infarction that may show islands of relatively viable muscle alternating with areas of necrosis and later fibrosis [18]. Such a tissue may result in fragmentation of the propagating electromotive forces with consequent development of high-frequency components [18–20]. Regional slow conduction alone is not sufficent to cause fragmented activity [21]. Highly fractionated electrograms occur only in preparations from chronic infarcts with interstitial fibrosis forming insulating boundaries between muscle bundles. The individual components of fragmented electrograms, therefore, most probably represent asynchronous electrical activity in each of the separate bundles of surviving muscle under the electrode. The intrinsic asym-

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metry of cardiac activation due to fiber orientation may be accentuated by infarction and may predispose to reentry [21, 22]. The slow activation might result from conduction over circuitous pathways caused by the separation and distortion of the myocardial fiber bundles. The low amplitude of the electrograms from these regions probably results from the paucity of surviving muscle fibers under the electrode because of the large amounts of connective tissue and not from depression of the action potentials. It is generally assumed that the anatomic substrate for reentry may be present in regions where fragmented electrograms can be recorded, which thus indicates slow inhomogenous conduction. However, fragmented electrograms are probably found wherever myocardial fibers are separated by connective tissue, even if reentry does not occur in the region [23].

Despite the extensive work done during the last ten years on ventricular late potentials, a number of problems remain unresolved. Therefore, the purpose of this chapter is to highlight some of the problems and limitations of ventricular late potentials in the clinical setting.

METHODOLOGY OF RECORDING LATE POTENTIALS

Since various groups started independently to develop their recording systems, it was unavoidable that different hardware configurations, as well as different software algorithms, were developed. The criteria proposed for characterization of ventricular late potentials include the duration and/or the voltage of low amplitude activity at the end of the QRS complex, the interval between the end of a given late potential and the point (retrogradely) when the downslope of the QRS complex falls below 40 μV, shape characteristics of the terminal portion of the QRS complex, the frequency content of the terminal QRS complex, and the total duration of the signal-averaged QRS complex.

In a recent multicenter study, different recording systems were used consecutively in the same patients [24]. Though there was some general uniformity with regard to the morphology of the signals recorded with the various systems, there were major differences in the incidence of late potentials, which resulted mostly from diverging definitions of late potentials. Therefore, more widely accepted and applied definitions and diagnostic criteria for late potentials irrespective of the recording system used are urgently needed to make data comparable from various groups. Though data in patients with documented ventricular tachycardia and/or fibrillation (outside the acute phase of myocardial infarction) may be useful in the definition phase, the ultimate solution to this problem can only be derived from large prospective studies in well-defined populations.

Another problem is related to the fact that not all patients with documented ventricular tachycardia and/or fibrillation exhibit late potentials on their body surface recordings. One explanation may be that their ventricular tachycardia is not of the reentry type but instead may be due to some form of

triggered activity. In the latter case, no regional slow conduction is required. How frequently this is the case has not yet been determined. However, several other reasons explain why late potentials may not be detectable on the body surface. First, the amount of tissue with regional slow conduction and thus late activation may be too small. In a recent study, we compared the results of signal averaging and intraoperative endocardial mapping [25]. The extent of regional slow conduction (i.e., the duration of local activation) did not correlate with the duration of late potentials on the body surface. However, the greater the number of sites with abnormal slow activation during endocardial mapping, the greater the chance of recording ventricular late potentials on the body surface. Thus, a critical mass of tissue having abnormal slow activation probably is necessary to be detected from the body surface.

Second, fragmented activation of an area of the myocardium may occur so early that it takes place at the time of activation of the remaining normal myocardium. Therefore, its activity will be hidden within the QRS complex [26]. The finding that various left ventricular sites are activated at different times [27] may also be of importance. The inferoposterobasal areas of the left ventricle are activated later than the other areas. Thus, an electrogram of a given duration from an inferoposterobasal area may clearly extend beyond the QRS complex as this area starts to be activated later than, for example, the anterior wall of the left ventricle.

Third, the signals may be too short and may occur immediately at the end of the QRS at a time when filter ringing occurs. This may have been a problem in earlier systems in which filters with steep characteristics were used. As a solution to this problem, M. Simson suggested analysis of the QRS complex retrogradely starting within the S-T segment [9]. To avoid filter ringing, we used single-pole filters with flat characteristics in our inital system, which showed a negligible degree of filter ringing [5].

Fourth, unstable triggering of the QRS complex may prevent the recording of late potentials, which may be cancelled by the continuously changing timing in relation to the trigger point. This seems to be of minor importance as the jitter for triggering is relatively small in available systems. However, there may be beat-to-beat variations in the configuration and timing of late potentials that may lead to cancellation during the averaging process. This may cause some attenuation in the high frequency components, thus acting as a low-pass filter. Finally, the level of baseline noise originating from muscular activity or from interference by alternating current may hide ventricular late potentials if it is too high [28].

Signal Averaging in the Time or Frequency Domain

The vast majority of studies have used signal averaging in the time domain. This means that a varying number of identical heart beats are averaged over time with regard to the timing and the voltage of the signals. In contrast,

recording in the frequency domain uses Fast Fourier Transform Analysis (FFTA) to analyze given portions of the signal for its frequency content [29–31]. Though this approach is promising, it still must be determined whether it offers any advantage over recording in the time domain with regard to sensitivity and specificity of identifying patients at risk of ventricular tachyarrhythmias (e.g., after myocardial infarction). One advantage of signal averaging in the frequency domain may be that there are no distortions of the signal by filters. Therefore, superiority of this approach has been claimed by some investigators [29–31].

EVALUATION OF THE EFFICACY OF MEDICAL AND SURGICAL THERAPY OF VENTRICULAR TACHYARRHYTHMIAS

Since late potentials represent electrical activity arising from the substrate that may be responsible for initiation and perpetuation of life-threatening ventricular arrhythmias, the question arises whether pharmacologic or surgical interventions might modify these low-amplitude signals to an extent permitting assessment of antiarrhythmic efficacy. Experimental studies in myocardial infarction using epicardial mapping have shown that late activity occurring at the end of the QRS complex was significantly altered if antiarrhythmic drugs were administered. In clinical studies, there has been no uniform antiarrhythmic response to pharmacologic interventions [32]. However, surgical treatment of ventricular tachycardia has a significant influence on the presence of late potentials, and abolition of late potentials after antitachycardia surgery correlates with a successful outcome [32–34].

These results suggest a promising role for identification of ventricular late potentials in the control of antitachycardia surgery. Moreover, recent analysis of our own data in 68 patients, as well as re-evaluation of the data reported by Marcus et al. [33], clearly showed a significant correlation between success of surgery and modification or abolition of late potentials. However, this applied only to group analysis. When the response of the individual patient was analyzed, it was much more difficult to predict the outcome after antitachycardia surgery on the basis of late potentials alone. One reason is that not all patients exhibit late potentials preoperatively. Another reason is that, even if ventricular late potentials disappear after antitachycardia surgery, there still is a proportion of false-negative results [34]. Even the chance that 10 to 20 percent of patients might be classified incorrectly seems to be unacceptable. Therefore, our present view is that programmed ventricular stimulation after antitachycardia surgery remains the gold standard for assessing the propensity to postsurgical ventricular tachyarrhythmias [35].

PROGNOSTIC SIGNIFICANCE OF VENTRICULAR LATE POTENTIALS

Although there is no doubt of the close association between ventricular late potentials and the propensity to sustained ventricular tachycardia, the signi-

ficance of the detection of late potentials in asymptomatic patients for prospective identification of those who might subsequently develop ventricular tachyarrhythmias is of utmost importance. There is now convincing evidence that detection of ventricular late potentials is significantly correlated to a subsequent development of sustained ventricular tachycardia or sudden cardiac death after recent myocardial infarction (for further information, Breithardt and Borggrefe [36]). Based on our own experience in a total of 778 patients that were studied with our first signal averaging system [5], the interval between myocardial infarction and the time the patient is studied is important. Obviously, if patients are included who have survived their myocardial infarctions for a long interval, they belong to a selected population with a reduced propensity to ventricular tachyarrhythmias. In addition, no study to date has shown any prognostic significance of ventricular late potentials in patients with other types of heart disease such as cardiomyopathies. Therefore, we believe signal averaging should be restricted to those patients who have recovered from their myocardial infarction and who then should be studied before discharge from the hospital. Other studies were able to demonstrate that the prevalance of late potentials increases during the first two or three weeks after myocardial infarction [37, 38], which may be due to ongoing morphologic changes. Thus, though it might be attractive to use signal averaging as early as possible during the course of myocardial infarction, its true value may be limited to those patients studied later.

Signal averaging alone is obviously not sufficient, as ventricular late potentials can be found in a high proportion of postmyocardial infarction patients. To increase the predictive value, other parameters, such as left ventricular function, time after myocardial infarction and results of long-term ECG recording were included. Though this obviously increased the predictive value and was able to identify a subgroup of patients at markedly higher risk of sudden death or sustained ventricular tachycardia, a substantial proportion of patients who subsequently developed one of these arrhythmic events was not identified by these findings.

Using a linear discriminant function analysis (unpublished results) the presence of ventricular late potentials and left ventricular contraction disturbances or reduced ejection fraction were of equal significance for predicting arrhythmic events after myocardial infarction. In addition, by combining the results of long-term ECG recording and signal averaging, subgroups of patients could be identified with markedly different prognoses. For instance, if both techniques did not yield an abnormal result (no late potentials; ventricular ectopic beats below 30 per hour, no pairs, no salvoes), the risk of sudden death or sustained VT was 1.8 percent, whereas it was 12.1 percent if both techniques were abnormal.

ADDITIVE PROGNOSTIC VALUE OF PROGRAMMED VENTRICULAR STIMULATION

Becaues of the high percentage of false-positive results of signal averaging and long-term ECG monitoring, other methods for identifying subgroups of patients with late potentials at high risk are mandatory. In addition to characteristics of myocardial infarction, such as site and size, our group has advocated the use of programmed ventricular stimulation as an approach to identifying a subgroup of patients at increased risk [39]. Patients at higher risk for developing symptomatic sustained ventricular tachycardia after myocardial infarction were characterized as follows: first, by the presence of late potentials; second, by an abnormal response to ventricular stimulation; and third, by a rate of the induced ventricular arrhythmia of less than 270 beats/min.

VENTRICULAR TACHYCARDIA VERSUS VENTRICULAR FIBRILLATION

The type of documented ventricular tachyarrhythmia has some influence on the incidence and duration of ventricular late potentials. Among patients with chronic recurrent ventricular tachycardia, 46 of 62 patients (74 percent) had late potentials, an incidence similar to that in patients with only one documented episode of ventricular tachycardia (21 of 26 patients; 80 percent). In contrast, patients with a history of ventricular fibrillation, but no acute myocardial infarction and no previous documentation of sustained ventricular tachycardia had late potentials in only 8 of 15 patients (53 percent) [27], in 6 of 27 patients (22 percent) [40], and in 1 of 14 patients (9 percent) [41]. Denniss et al. [42] detected late potentials in only 32 percent of patients with previous ventricular fibrillation but in 58 percent of those with sustained ventricular tachycardia with rates greater than 270 beats/min, and in 95 percent of those with rates of 270 beats/min or less.

Factors that govern the duration of late potentials have not yet been studied sufficiently. Data by Berbari et al. [43] suggest that an increase in atrial rate by pacing does not exert any influence on the duration of a late potential, whereas the high frequency components of the signal change. There was an inverse relationship between the duration of late potentials and the rate of sustained ventricular tachycardia; however, this relationship was not significant due to a large scatter of data [27]. Late potentials were significantly more frequent in those patients in whom the cycle length of induced ventricular tachycardia was greater than 250 ms (90 percent) compared to those with cycle lengths less than 250 ms (40 percent) [40]. A similar relation was reported by Spielman et al. [44] using left ventricular catheter mapping. A late potential of short duration may be due to a small area with slow fractionated conduction or to more rapid conduction in a larger area. However, as the wavefront of excitation probably travels along multiple pathways and in various directions, no calculations of conduction velocity

or of wave length can be performed. In experimental studies, the amplitude and duration of electrograms in dogs with inducible ventricular fibrillation were more normal than those recorded in animals with inducible ventricular tachycardia [22].

This inverse relation between the rate of ventricular tachycardia and the duration of late potentials points to one of the limitations of this technique. Especially in those patients at highest risk of sudden cardiac death, ventricular late potentials may be too short to be detected or to extend beyond the termination of the QRS complex. Therefore, techniques that induce a stress, causing a late potential prolongation and, thus, appearance of hidden late potentials, would be desirable.

CONCLUSIONS

The presently available information warrants further studies in larger groups of patients after recent myocardial infarction to evaluate the predictive value of noninvasive techniques, such as long-term electrocardiographic recording, exercise testing, and signal averaging, and of procedures, such as invasive programmed ventricular stimulation and angiographic and hemodynamic studies. It will also be necessary to establish the independent prognostic value of ventricular late potentials, the relation to spontaneously occurring ventricular arrythmias during long-term electrocardiographic recording, and the type of interventions required to prevent the occurrence of arrythmic events after myocardial infarction. Other studies will have to focus on specific topics, such as the need for standardization and optimization of recording technologies and algorithms for evaluation. Inclusion of late potentials as a screening variable in prospective intervention trials should be discussed to allow the design of adequate trials.

REFERENCES

1. Fontaine, G., Guiraudon, G., and Frank, R. 1978. Intramyocardial conduction defects in patients prone to ventricular tachycardia, III. The post-excitation syndrome in ventricular tachycardia, in *Management of Ventricular Tachycardia—Role of Mexiletine*, E. Sandøe, D.G. Julian, and J.W. Bell, eds., Amsterdam, pp.67–69. Excerpta Medica.
2. Fontaine, G., Frank, R., Gallais Hamonno, F., et al. 1978. Electrocardiographie des potentiels tardifs du syndrome de post-excitation. *Arch. Mal. Coeur.* 71:854–864.
3. Berbari, E.J., Scherlag, B.J., Hope, R.R., and Lazzara, R. 1978. Recording from the body surface of arrhythmogenic ventricular activity during the ST-segment. *Am. J. Cardiol.* 41:697–702.
4. Breithardt, G., Becker, R., and Seipel, L. 1980. Non-invasive recording of late ventricular activation in man. *Circulation* 62 (suppl. III):III–320.
5. Breithardt, G., Becker, R., Seipel, L., et al. 1981. Non-invasive detection of late potentials in man—a new marker for ventricular tachycardia. *Eur. Heart. J.* 2:1–11.
6. Hombach, V., Höpp, H.W., Braun,, V., et al. 1980. Die Bedeutung von Nachpotentialen innerhalb des ST-Segmentes im Oberflächen-EKG bei Patienten mit koronarer Herzkrankheit. *Dtsch. Med. Wochenschr.* 195:1457–1462.
7. Rozanski, J.J., Mortara, D., Myerburg, R.J., and Castellanos, A. 1981. Body surface detection of delayed depolarizations in patients with recurrent ventricular tachycardia and left ventricular aneurysm. *Circulation* 63:1172–1178.

8. Simon, M., Horowitz, L., Josephson, M., et al. 1980. A marker for ventricular tachycardia after myocardial infarction. *Circulation* 62:(Suppl. III):III–262.
9. Simson, M.B. 1981. Identification of patients with ventricular tachycardia after myocardial infarction from signals in the terminal QRS-complex. *Circulation* 64:235–42.
10. Uther, J.B., Dennett, C.J., and Tan, A. 1978. The detection of delayed activation signals of low amplitude in the vectorcardiogram of patients with recurrent ventricular tachycardia by signal averaging, in *Management of Ventricular Tachycardia—Role of Mexiletine*, E. Sandøe, D.G. Julian, J.W. Belleds., pp. 80–82, Amsterdam, Excerpta Meica.
11. El-Sherif, N., Scherlag, B.J., Lazzara, R., and Hope, R.R. 1977. Reentrant ventricular arrhythmias in the late myocardial infarction period. I. Conduction characteristics in the infarction zone. *Circulation* 55:686–702.
12. El-Sherif, N., Scherlag, B.J., Lazzara, R., and Hope, R.R. 1977. Reentrant ventricular arrhythmias in the late myocardial infarction period. II. Patterns of initiation and termination of reentry. *Circulation* 55:702–19.
13. El-Sherif, N., Scherlag, B.J., Lazzara, R., and Hope, R.R. 1977. Reentrant ventricular arrhythmias in the late myocardial infarction period. III. Manifest and concealed extrasystolic grouping. *Circulation* 56:225–34.
14. Klein, H., Karp, R.B., Kouchoukos, N.T., et al. 1982. Intraoperative electrophysiologic mapping of the ventricles during sinus rhythm in patients with a previous myocardial infarction. Identification of the electrophysiologic substrate of ventricular arrythmias. *Circulation* 66:847.
15. Ostermeyer, J., Breithardt, G., Kolvenbach, R., et al. 1979. Intraoperative electrophysiologic mapping during cardiac surgery. *Thorac. Cardiovasc. Surg.* 27:260–70.
16. Spielman, S.R., Untereker, W.J., Horowitz, L.N., et al. 1981. Fragmental electrical activity—relationship to ventricular tachycardia (abstr). *Am. J. Cardiol.* 47:448.
17. Josephson, M.E., Horowitz, L.N., Farshidi, A., et al. 1978. Sustained ventricular tachycardia: evidence for protected localized reentry. *Am. J. Cardiol.* 42:416.
18. Daniel, T., Boineau, J., and Sabiston, D. 1971. Comparison of human ventricular activation with canine model in chronic myocardial infarction. *Circulation* 44:74–89.
19. Langner, P.H. Jr., Greselowitz, D.B., and Briller, S.A. 1973. Wide band recording of the electrocardiogram and coronary heart disease. *Am. Heart J.* 86:308–17.
20. Flowers, N.C., Horan, L.G., Thomas, J.R., and Tolleson, W.J. 1969. The anatomic basis for high frequency components in the electrocardiogram. *Circulation* 39:531–9.
21. Gardner, P.I., Ursell, P.C., Pham, T.D., et al. 1984. Experimental chronic ventricular tachycardia: anatomic and electrophysiologic substrates, in *Tachycardias: Mechanisms, Diagnosis, Treatment,* Philadelphia, Lea & Febiger. M.E. Josephson, H.J.J. Wellens, eds, pp. 29–60.
22. Richards, D.A., Blake, G.J., Spear, J.F., and Moore, E.N. 1984. Electrophysiologic substrate for ventricular tachycardia: correlation of properties in vivo and in vitro. *Circulation* 69:369–81.
23. Kienzle, M.G., Miller, J., Falcone, R., et al. 1984. Intraoperative endocardial mapping during sinus rhythm: relationship to site of origin of ventricular tachycardia. *Circulation* 70:957–65.
24. Oeff, M., von Leitner, E.R., Sthapit, R., et al. 1986. Methods for non-invasive detection of ventricular late potentials—a comparative multicenter study. *Eur. Heart J.* 7:25.
25. Schwarzmaier, J., Karbenn, U., Borggrefe, M., et al. 1987. Quantitative relation between intraoperative registration of late endocardial activation and late potentials in signal-averaged electrocardiogram (abstr.). *Circulation* 76 (suppl. IV):1363.
26. Simson, M.B., Untereker, W.J., Spielman, S.R., et al. 1983. Relation between late potentials on the body surface an directly recorded fragmented electrocardiograms in patients with ventricular tachycardia. *Am. J. Cardiol.* 51:105.
27. Borggrefe, M., Karbenn, U., and Breithardt, G. 1982. Spätpotentiale und elektrophysiologische Befunde bei ventrikulären Tachykardien (abstr.). *Z.Kardiol.* 71:627.
28. Steinberg, J.S., Bigger, T. Jr., and Bernstein, F. 1987. Results of signal averaged ECG are dependent upon endpoint of noise reduction (abstr.). *Circulation* 76 (Suppl. IV):342.
29. Cain, M.E., Ambos, D., Witkowski, F.X., and Sobel, B.E. 1984. Fast-Fourier Transform analysis of signal-averaged electrocardiograms for identification of patients prone to sustained VT. *Circulation* 69:711–720.

30. Cain, M.E., Ambos, H.D., Markham, J., et al. 1985. Quantification of differences in frequency content of signal-averaged electrocardiograms in patients with compared to those without sustained VT. *Am. J. Cardiol.* 55:1500–1505.
31. Haberl, R., Hengstenberg, E., Pulter, R., and Steinbeck, G. 1986. Frequenzanalyse des Einzelschlag-Elektrokardiogrammes zur zur Diagnostik von Kammertachykardien. *Z. Kardiol.* 75:659–665.
32. Breithardt, G., Borggrefe, M., Karbenn, U., and Schwarzmaier, J. 1987. Effects of pharmacological and non-pharmacological interventions on late potentials. *Eur. Heart J.* 8 (suppl A):97–104.
33. Marcus, N.H., Falcone, R.A., Harken A.H., et al. 1984. Body surface late potentials: effects of endocardial resection in patients with ventricular tachycardia. *Circulation* 70:632–637.
34. Borggrefe, M., Karbenn, U., Podczeck, A., et al. in press. Effects of non-pharmacological interventions on ventricular late potentials. Herz—Kardiovaskuläre Erkrankungen. Rudolph, W. München, ed., Verlag Urban-Vogel.
35. Borggrefe, M., Breithardt, G., et al. Value of epicardial versus endocardial stimulation after map-guided antiachycardia surgery, in preparation.
36. Breithardt, G., and Borggrefe, M. 1987. Recent advances in the identification of patients at risk of ventricular tachyarrhythmias: role of ventricular late potentials. *Circulation* 75:1091–6.
37. Höpp, H.W., Hombach, V., Braun, V., et al. 1982. Kammerarrhythmien und ventrikuläre Spätpotentiale bei akutem Myokardinfarkt. *Herz-Kreislauf* 3:III–120.
38. Kertes, P.J., Pollak, S.J., and Walter, P.F. 1985. Programmed stimulation for ventricular tachycardia: responses predicted by signal averaging in patients with and without coronary disease. *Circulation* 72:III–433.
39. Breithardt, G., Borggrefe, M., and Haerten, K. 1985. Role of programmed ventricular stimulation and non-invasive recording of ventricular late potentials for the identification of patients at risk of ventricular tachyarrhythmias after acute myocardial infarction, pp. 53–61, in *Cardiac Electrophysiology* and *Arrhythmias*, O.P. Zipes and J. Jalife, eds., pp. 53–61, Grune & Straton.
40. Freedman, R.A., Gillis, A.M., Keren, A., et al. 1984. Signal averaged ECG late potentials correlate with clinical arrhythmia and electrophysiology study in patients with ventricular tachycardia or fibrillation. *Circulation* 70 (Suppl. II):II–252.
41. Kertes, P.J., Glabus, M., Murray, A., et al. 1984. Delayed ventricular depolarization—correlation with ventricular activation and relevance to ventricular fibrillation in acute myocardial infarction. *Eur. Heart J.* 5:974–83.
42. Denniss, A.R., Holley, L.K., Cody D.V., et al. 1983. Ventricular tachycardia and fibrillation: differences in ventricular activation times and ventricular function (abstr.). *J. Am. Coll. Cardiol.* 1:606.
43. Berbari, E.J., Friday, K.J., Jackmann, W.M., et al. 1984. Effects of atrial pacing on surface recorded late potentials in patients with ventricular tachycardia. *Circulation* 70 (suppl. II):II–373.
44. Spielman, S.R., Horowitz, L.N., Greenspan, A.M., et al. 1981. Activation mapping in sinus rhythm in patients with ventricular tachycardia—relationship to cycle length and site of origin (abstr.) *Am. J. Cardiol.* 47:497.

19. PACING TO PREDICT MECHANISM: TRANSIENT ENTRAINMENT AND REENTRY

ALBERT L. WALDO AND RICHARD W. HENTHORN

As summarized recently [1], until the last decade or so, it was generally thought that cardiac rhythms resulted either from an automatic mechanism or a reentrant mechanism. Nevertheless, apart from the early and classic experiments of Mayer [2] on reentry in the Medusa ring, and subsequent studies by Mines [3] on ring preparations cut from dogfish auricles or from canine right ventricles, it was only with the publications of the in vitro studies of depressed bundles and loops of canine Purkinje fibers in 1972 by Wit et al. [4, 5] that the occurrence of reentry actually was documented. Before the latter studies, reentrant mechanisms had been postulated as being present in many arrhythmias, but this postulate was based primarily on indirect evidence. In fact, for in vivo rhythms, it had been thought that one could distinguish reentrant arrhythmias from automatic rhythms by a few key characteristics. Simply stated, it was generally accepted that if the rhythm could be either initiated or terminated by premature beats or rapid pacing, the rhythm was due to a reentry mechanism [6].

The best proof of reentry had always been thought to be mapping the sequence of activation of the heart during the tachycardia, thereby demonstrating the reentrant circuit. Even so, the admonition of Mines [3] that "...the chief error to be guarded against is that of mistaking a series of

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automatic beats originating in one point of a ring [substitute apparent reentry circuit] and traveling around it in one direction only owing to a complete block close to the point of origin of the rhythm on one side of this point" must be remembered. The point, of course, is that even sequence of activation mapping may not provide proof of reentry per se, even though it appears to do just that. Mines suggested that severing the ring [again, substitute reentry circuit] or the like and then demonstrating that no further reentrant excitation could occur was required for proof of reentry. Nevertheless, sequence of activation mapping now has become quite possible to perform in vivo, but only with the use of recording techniques with the chest open and the heart exposed. Since pacing techniques were far less invasive and far easier to apply for virtually all clinical studies and most animal studies, investigators relied on the above responses to cardiac pacing to establish the presence of reentry. When it became recognized that triggered rhythms could be initiated and terminated with premature beats or rapid pacing [1, 7], it became necessary to reexamine the criteria that could distinguish reentrant tachyarrhythmias from other arrhythmias, in particular, triggered rhythms [7].

TRANSIENT ENTRAINMENT: A PACING TECHNIQUE TO IDENTIFY REENTRANT ARRHYTHMIAS WITH AN EXCITABLE GAP

On the basis of a series of studies during rapid pacing of atrial flutter, ventricular tachycardia, circus movement tachycardia involving an AV bypass pathway, AV nodal reentrant tachycardia, and intraatrial reentrant tachycardia, we described the phenomenon of transient entrainment of tachyarrhythmias. Transient entrainment of a tachycardia is an increase in the rate of all tissues responsible for sustaining the tachycardia to the faster pacing rate, with resumption of the intrinsic rate of the tachycardia on either abrupt cessation of pacing or slowing of the pacing rate below the intrinsic rate of the tachycardia [8]. On the basis of studies during the above rhythms in patients, and in an experimental model of atrial flutter, we suggested that the phenomena of transient entrainment are best explained by pacing capture of a reentry circuit from two directions. Thus, we propose that during transient entrainment of a tachycardia, the wave front from each pacing impulse enters into the exitable gap of a reentrant circuit and travels in two directions, antidromically, that is, in the opposite direction of the circulating wave front of the spontaneous tachycardia, where it collides with the orthodromic wave front of the preceding beat, and orthodromically, that is, in the same direction as the circulating wave front of the spontaneous tachycardia. The latter wave front continues the tachycardia, resetting it to the pacing rate (figure 19-1). On the basis of those and subsequent studies, we suggested that the ability to demonstrate transient entrainment of a tachycardia indicates that the tachycardia is due to a reentrant mechanism, with an excitable gap being present in the reentrant circuit.

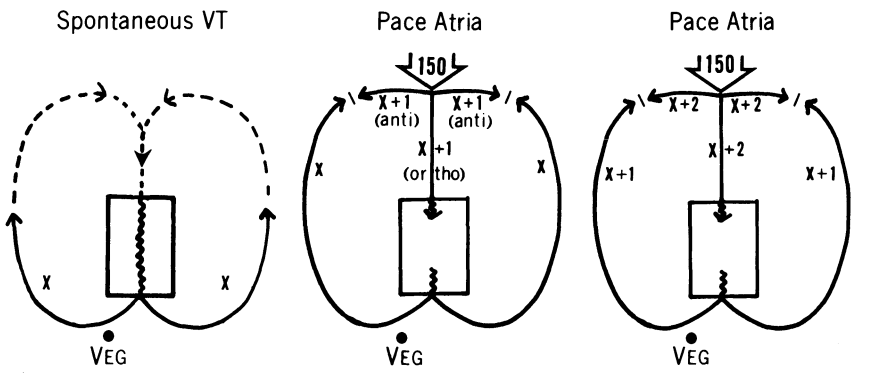

Figure 19-1. Left Panel: Schematic representation of transient entrainment, in this case, of a ventricular tachycardia by atrial pacing. The reentry circuit is represented by the figure of eight loop model of ventricular tachycardia (VT), in this example, at a rate of 141 bpm. The X represents the orthodromic wave fronts of the reentrant rhythm. In this and subsequent schema, the arrows indicate the direction of spread of the impulse, the box represents an area of slow conduction, the serpentine line indicates slow conduction of the impulse, the dashed lines indicate the excitable gap in the reentry circuit, the dot represents a right ventricular electrogram (VEG) recording site, and the large arrow (middle and right panels) indicates the wave front from the pacing impulse entering into the excitable gap of the ventricular tachycardia reentrant circuit, where it is conducted orthodromically (ORTHO) and antidromically (ANTI). Middle Panel: Schematic representation of the introduction of the first pacing impulse (X+1) during atrial pacing at a rate of 150 bpm during the spontaneous ventricular tachycardia. The antidromic wave fronts from the pacing impulse (X+1) collide with the orthodromic wave fronts from the previous spontaneous beat (X), resulting in fusion of ventricular activation which, in effect, interrupts the tachycardia. However, the orthodromic wave front from the pacing impulse (X+1) continues the ventricular tachycardia, resetting it to the pacing rate. Right Panel: Schematic representation of the introduction of the second pacing impulse (X+2) during atrial pacing at a rate of 150 beats/min during the spontaneous ventricular tachycardia. The antidromic wave fronts (X+2) collide with the orthodromic wave fronts from the previous paced beat (X+1), again resulting in ventricular fusion, which, again, in effect interrupts the ventricular tachycardia. However, once again, the orthodromic wave front (X+2) from the pacing impulse continues the ventricular tachycardia, resetting it to the pacing rate. Note that during the spontaneous rhythm and during the period of pacing, the right ventricular electrogram (VEG) recording site is always activated by an orthodromic wave front. Reprinted from Waldo et al. [15], by permission.

CRTERIA TO ESTABLISH THE PRESENCE OF TRANSIENT ENTRAINMENT

We have proposed four criteria (table 19-1), any one of which, if demonstrated, establishes the presence of transient entrainment, and thereby, we suggest, the presence of reentry with an excitable gap. Figures 19-1 to 19-7 illustrate the first three criteria in a single case of ventricular tachycardia [15]. Figures 19-8 to 19-10 demonstrate the fourth criterion recently described by Henthorn et al. [14], the example shown being a case of AV reentrant tachycardia involving a left sided accessory AV connection [8]. These four criteria have been demonstrated to one degree or another during five putative reentrant rhythms: classical (type I) atrial flutter [9–14], ventricular tachycar-

Table 19-1. Criteria to establish the presence of transient entrainment

1. The demonstration of constant fusion beats in the ECG during the period of rapid pacing at a constant rate except for the last captured beat, which is entrained but not fused (i.e., the last entrained beat demonstrates the ECG morphology of the spontaneous tachycardia).
2. The demonstration of progressive fusion, i.e., constant fusion beats in the ECG during rapid pacing at any constant rate, but different degrees of constant fusion at different rapid rates.
3. Interruption of the tachycardia associated with localized conduction block to a site(s) for one beat, followed by subsequent activation of that site(s) from a different direction, manifest by a change in morphology of the electrogram at the blocked site(s), and with a shorter conduction time.
4. A change in conduction time and electrogram morphology at one recording site when pacing from another site at two different constant pacing rates, each of which is faster than the spontaneous rate of the tachycardia but fails to interrupt it.

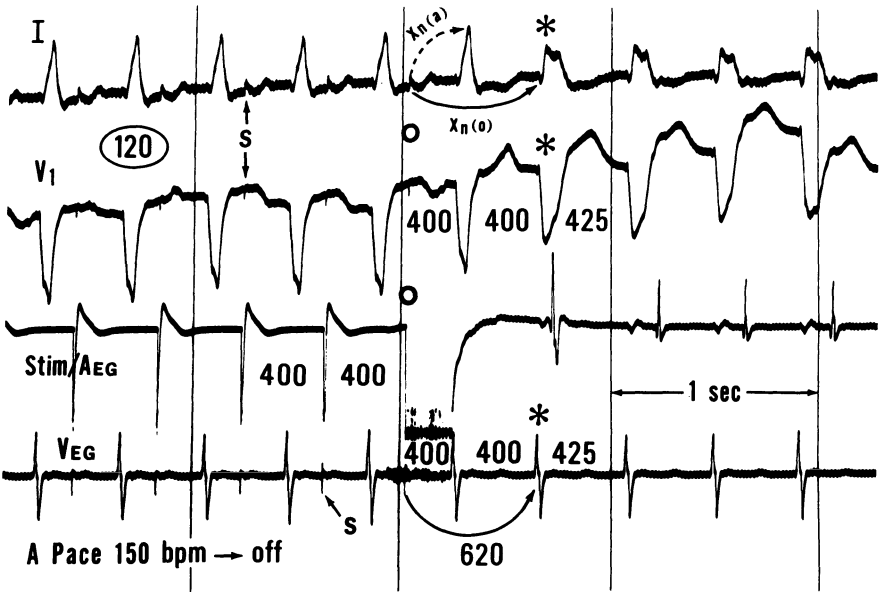

Figure 19-2. ECG leads I and V1 recorded simultaneously with the atrial pacing (A Pace) stimulus artifact (Stim) or atrial electrogram (AEG) and the unipolar ventricular electrogram (VEG) at the termination of atrial pacing at a rate of 150 bpm (400 ms). In this and subsequent figures, the open circle denotes the last stimulus artifact (S), the asterisk denotes the last entrained beat, the arrow from the stimulus artifact in the ventricular electrogram points to the resulting ventricular electrogram (with the stimulus-to-ventricular electrogram interval being indicated in milliseconds), the dashed arrow in lead I represents the last antidromic wave front ($X_n[a]$), the solid arrow the last orthodromic wave front ($X_n[o]$), from the last pacing impulse, and the circled number represents the duration of the QRS complex in the ECG. This figure illustrates the first criterion for transient entrainment (see table 19-1). From Waldo et al. [15], by permission.

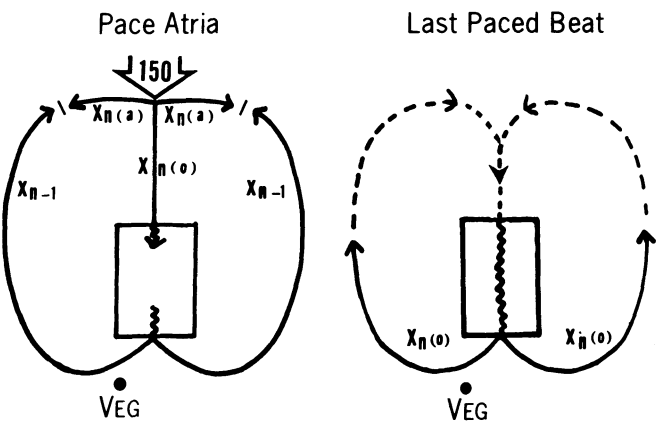

Figure 19-3. Schematic representation of the termination of atrial pacing illustrated in figure 19-2 Left Panel: the large arrow indicates the wave front from the last pacing impulse delivered at a rate of 150 bpm entering into the excitable gap of the reentrant circuit of the ventricular tachycardia, where it is conducted orthodromically and antidromically. The antidromic wave fronts (X_n[a]) collide with the orthodromic wave fronts (X_n[o]) of the previous beat (X_n-1), resulting in fusion of ventricular activation, which, in effect, interrupts the tachycardia, but the orthodromic wave front from the last pacing impulse continues the tachycardia, resetting it to the pacing rate. Right Panel: The orthodromic wave fronts from the last pacing impulse are now unopposed by antidromic wave fronts from a subsequent pacing impulse, so that no fusion of ventricular activation occurs despite the presence of transient entrainment. This last entrained beat continues the tachycardia (dashed lines), which then resumes at its previous spontaneous rate. This diagrammatically illustrates the first criterion of entrainment (see table 19-1) Reprinted from Waldo et al. [15], by permission.

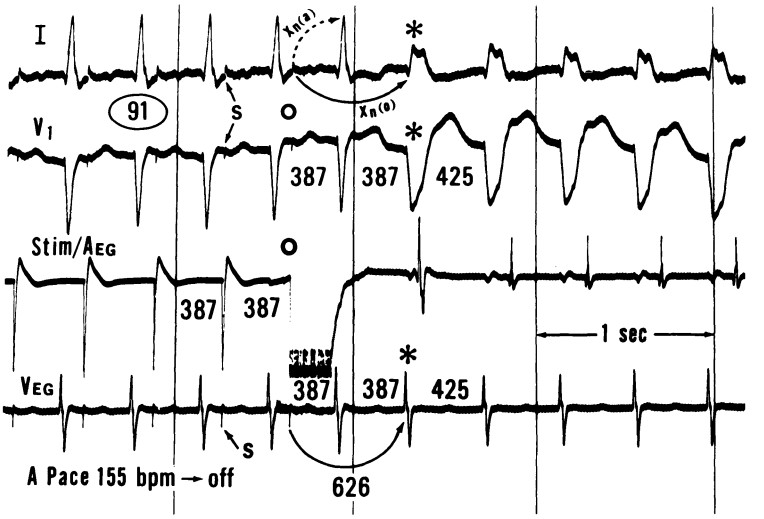

Figure 19-4. ECG leads I and V1 recorded simultaneously with either the atrial pacing stimulus (S) artifact (Stim) or atrial electrogram (AEG) and unipolar ventricular electrogram (VEG) at the termination of atrial pacing at a rate of 155 bpm (387 ms). All intervals are in milliseconds. Note the difference in the morphology of QRS complexes in the ECG leads during pacing at this rate compared with pacing at 150 bpm (see figure 19-2). This figure demonstrates the second criterion of transient entrainment, namely progressive fusion in the ECG leads (see table 19-1). Reprinted from Waldo et al. [15], by permission.

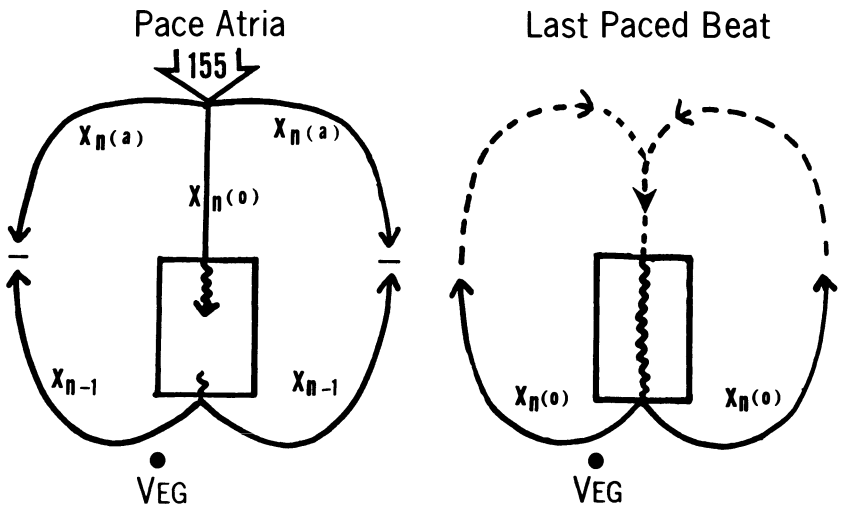

Figure 19-5. Schematic representation of the termination of atrial pacing illustrated in figure 19-4 Left Panel: Identical to the description of the left panel in figure 19-3, except that the pacing impulse was delivered at a rate of 155 bpm. Note also that the greater penetration by the antidromic wave front from the pacing impulse ($X_n(a)$) at this pacing rate when compared to pacing at 150 bpm explains the progressive fusion in the electrocardiogram. Atrial pacing at a rate of 160 bpm once again entrained the ventricular tachycardia, producing yet a different degree of fusion beats in the ECG (not shown) because of the still greater penetration of the reentrant circuit by the antidromic wave front from the pacing impulse. Right Panel: Identical to the description of the right panel in figure 19-3. Note that the right ventricular electrogram (VEG) recording site is activated only by an orthodromic wave front from the pacing impulse. Reprinted from Waldo et al. [15].

dia [14–21], AV reentrant tachycardia involving an accessory AV connection [8, 14, 22], AV nodal reentrant tachycardia [23, 24], and ectopic atrial tachycardia [14, 25]. In addition, several in vivo studies that used mapping techniques to document the presence of a reentrant circuit during ventricular tachycardia [26–28] and atrial flutter [29–31] have also demonstrated that the phenomena postulated to occur during transient entrainment and used as part of the entrainment criteria indeed do occur.

LIMITATIONS OF TRANSIENT ENTRAINMENT IN IDENTIFYING A REENTRANT MECHANISM

Are the Observations During Transient Entrainment Only Explained by the Presence of Reentry?

Although the pheonomena associated with transient entrainment of a tachycardia with or without its subsequent interruption are best explained by reentry, it still must be asked whether any or all of the criteria for the demonstration of transient entrainment can be explained by another mechanism. Present understanding of the response of automatic rhythms to rapid

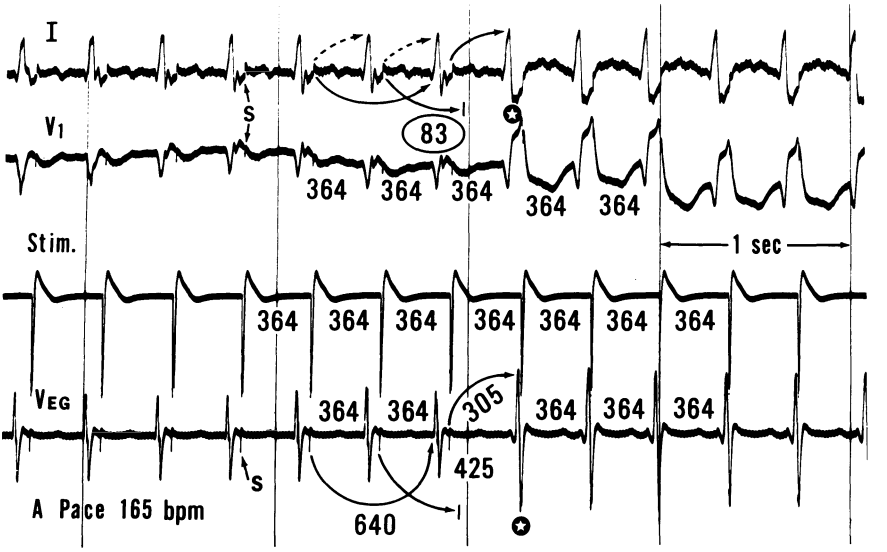

Figure 19-6. ECG leads I and V1 recorded simultaneously with the atrial pacing stimulus (S) artifact (Stim) or atrial electrogram (AEG) and the unipolar ventricular electrogram (VEG) during atrial pacing at a rate of 165 bpm (364 ms cycle length). Note the further progressive fusion of the QRS complexes in the ECG leads when compared with figures 19-2 and 19-4. Then, denoted by the circled stars, there is an abrupt change in the morphology of the recorded QRS complexes in both ECG leads and in the unipolar ventricular electrogram complex. In the ventricular electrogram tracing, each arrow points to the resulting ventricular electrogram. Before the change in configuration of the QRS complexes and ventricular electrogram, the stimulus-to-ventricular electrogram interval is 640 ms. Then, after the localized conduction block to the ventricular electrogram recording site, the stimulus-to-ventricular electrogram interval becomes 305 ms. Note that this localized conduction block is associated with an increase for one cycle only in the beat-to-beat cycle length at the ventricular electrogram recording site (from 364 ms to 425 ms and then back to 364 ms). In the ECG lead I tracing, the dashed arrows represent the antidromic wave fronts and the associated solid arrows represent the orthodromic wave fronts from the fifth and sixth pacing impulses shown in the figure. After the block of both the antidromic and orthodromic wave fronts of the sixth pacing impulse in the reentrant circuit of the ventricular tachycardia, the ventricles are activated by the seventh pacing impulse as expected during overdrive atrial pacing of a sinus rhythm because the ventricular tachycardia has been interrupted. Finally, had the pacing been terminated before achieving block of both the antidromic and orthodromic wave fronts of the same pacing impulse, the tachycardia would not have been interrupted, emphasizing the critical duration of pacing at the critical pacing rate required for interruption of the tachycardia. All intervals are in milliseconds. Reprinted from Waldo et al. [15], by permission.

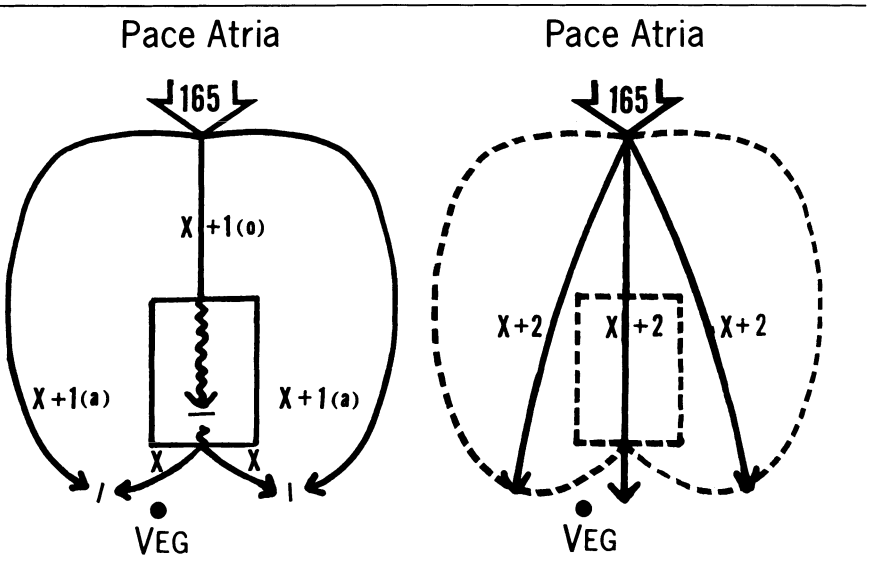

Figure 19-7. Schematic representation of the events recorded in figure 19-6. Left Panel: The large arrow indicates the wave front from the pacing impulse delivered at a rate of 165 bpm entering into the reentrant circuit of the ventricular tachycardia, where it is conducted orthodromically (X + 1[o]) and antidromically (X + 1[a]). The antidromic wave fronts collide with the orthodromic wave fronts from the previous beat (X) resulting in fusion of ventricular activation. Note the still greater antidromic penetration of the reentry circuit by the wave front from the pacing impulse, explaining the initial appearance of still more progressive fusion of the QRS complexes in the ECG leads. This time, however, the orthodromic wave front is also blocked (presumably in the area of slow conduction) during the same beat. Note that the right ventricular electrogram (VEG) recording site is still activated by the orthodromic wave front of the previous beat (X), but is not activated either by the antidromic or orthodromic wave front of the X + 1 beat, i.e., there is localized conduction block to that site. Right Panel: The large arrows indicate the next pacing impulse (X + 2) delivered at the same pacing rate (165 bpm) from the same atrial pacing site as in the left panel. The dashed lines indicate the reentrant circuit present during the previous periods of spontaneous ventricular tachycardia and transient entrainment of the ventricular tachycardia. Because the ventricular tachycardia has been interrupted by the previous pacing impulse (X + 1), the sequence of ventricular activation of the next pacing impulse (X + 2) is as one would expect during overdrive pacing of a sinus rhythm. Therefore, the right ventricular electrogram (VEG) recording site is activated from a different direction than during previous periods of transient entrainment from the same pacing site. In addition, because the presumed area of slow conduction is no longer functionally present, the stimulus-to-right ventricular electrogram conduction time will be shorter. This fulfills and explains the third criterion for transient entrainment (see table 19-1). Reprinted from Waldo et al. [15], by permission.

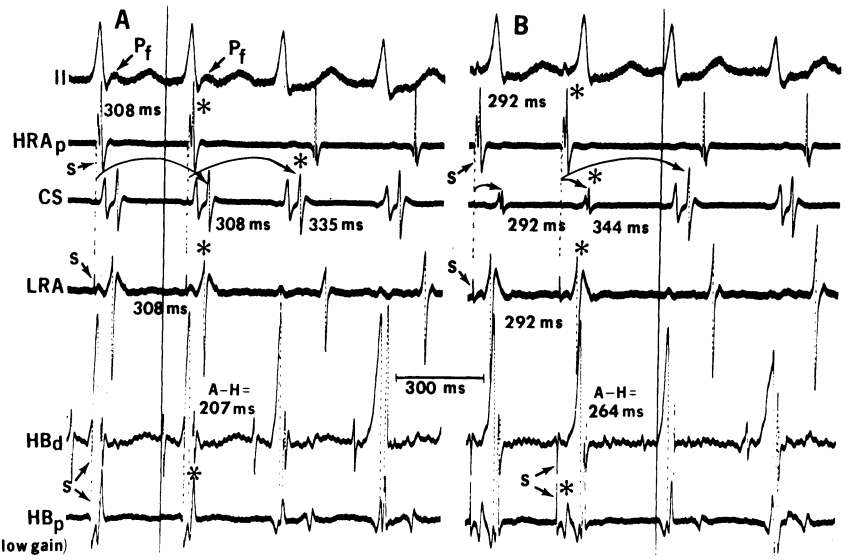

Figure 19-8. Illustration of the fourth criterion for the demonstration of the transient entrainment (see table 19-1) during atrial pacing from the high right atrium (HRA) during an AV junctional reentrant tachycardia (spontaneous cycle length 339 ms). In both panels, ECG lead II is recorded simultaneously with bipolar electrograms from the proximal pair of electrodes from a catheter electrode placed in the high right atrium (HRA_p), the coronary sinus (CS), the low right atrium (LRA), and the distal pair (HB_d) and proximal pair (HB_p) of electrodes of a tripolar catheter in the His bundle position during termination of pacing at a cycle length of 308 ms (panel A) and 292 ms (panel B). In the recordings from the coronary sinus site, the arrows from each stimulus artifact indicate the atrial complexes that result from that stimulus. The asterisk indicates the last transiently entrained beat at each recording site. S = stimulus artifact. P_f = fusion P wave. Note that the atrial electrogram at the coronary sinus site in panel A is identical to that during the spontaneous rhythm, and that, as indicated by the arrow from the stimulus artifact to the atrial complex at the coronary sinus recording site, conduction time during pacing is long. However, with pacing at the shorter cycle length in panel B, the atrial electrogram morphology changes at the coronary sinus site and the stimulus-to-atrial electrogram conduction time becomes much shorter. Note also that the tachycardia is not interrupted by pacing in either instance. This fulfills the fourth criterion for the demonstration of transient entrainment (see table 19-1). Reprinted from Waldo et al. [8], by permission.

pacing is completely inconsistent with the phenomena observed during transient entrainment. Thus, observations consistent with overdrive suppression and subsequent warm up of a pacemaker [32] or observations consistent with entrance block into an automatic focus have not been seen during transient entrainment. In addition, constant fusion beats in the ECG, progressive fusion beats in the ECG, interruption of the tachycardia, and all the observations of the fourth criterion have not been described during rapid pacing of an automatic rhythm and seem completely inconsistent with the presence of an underlying automatic mechanism. Also, although it has been demonstrated in the canine heart [32] that automatic foci tend to accelerate and

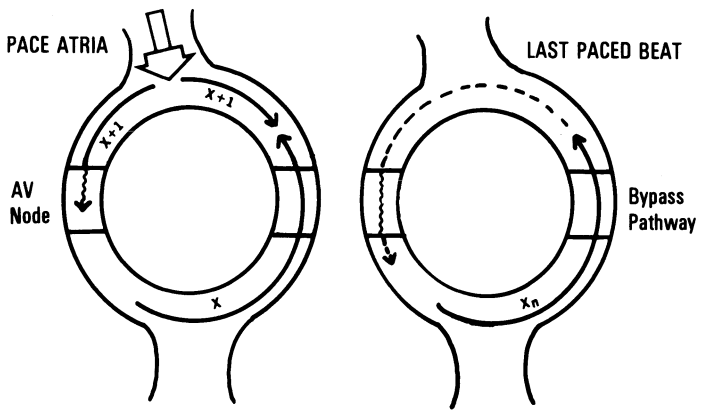

Figure 19-9. Schematic representation of the events recorded in panel A of figure 19-8. Left Panel: The large arrow indicates the pacing impulse from the high right atrium entering into the reentry circuit, whereupon it is conducted orthodromically (X + 1) and antidromically (X + 1). The antidromic wave front collides with the orthodromic wave front of the previous beat (X) resulting in intraatrial fusion, which, in effect, terminates the tachycardia; but the orthodromic wave front from the pacing impulse continues and, in fact, resets it. Right Panel: The orthodromic wave front of the last paced beat (X_n) is unopposed by an antidromic wave front, so that no atrial fusion occurs despite the presence of transient entrainment, and the tachycardia (dashed lines) then continues spontaneously. The serpentine line indicates slow conduction. Note that the coronary sinus recording site, that is, the area just on the atrial side of the AV bypass pathway, is activated orthodromically during pacing (left panel) and during the spontaneous tachycardia, so that the electrogram recorded at that site is the same during pacing as during the spontaneous rhythm. Also, conduction time from the stimulus artifact to the coronary sinus recording site is long (left panel) because the impulse must traverse the specialized AV conduction system and the ventricles before reaching that recording site. Reprinted from Waldo et al. [8], by permission.

compete with imposed drives that exceed the spontaneous rate by a relatively small percentage (10 to 15 percent), this still seems most unlikely to explain the phenomena observed during transient entrainment, as the automatic pacemaker tends to keep its accelerated rate for a period following termination or slowing of the pacing rate.

Whether rapid pacing in the presence of a triggered rhythm can produce any of the entrainment criteria is, to our knowledge, unknown, as this question has yet to be studied systematically. Relevant published data are not consistent with the observations made during entrainment [33]. Nevertheless, this is one fruitful area for further investigation to test the hypotheses that entrainment equals reentry with an excitable gap.

Finally, while we believe that entrainment of a tachycardia is best explained by the presence of an underlying reentrant mechanism, we recognize that, as with almost all complex phenomena, it is possible to invent alternative explanations, in this case explanations that have a basis other than reentry, regardless of how convoluted the explanations. Therefore, although the

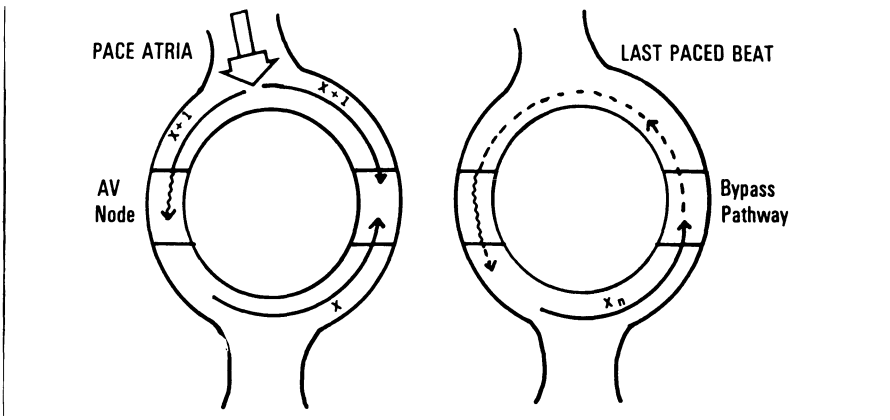

Figure 19-10. Schematic representation of the events recorded in Panel B of figure 19-8. Left Panel: The large arrow indicates the pacing impulse from the high right atrium entering into the reentry circuit, whereupon it is conducted orthodromically (X + 1) and antidromically (X + 1). Antegrade block of the antidromic wave front from the pacing impulse and retrograde block of the orthodromic wave front of the previous beat (X) occur in the AV bypass pathway, resulting in the absence of atrial fusion beats in the ECG, and apparent termination of the tachycardia. However, the tachycardia is continued and reset by the orthodromic wave front of the pacing impulse (X + 1). Note that the coronary sinus recording site, diagrammatically represented as just on the atrial side of the AV bypass pathway, is now activated by the antidromic wave front from the pacing impulse, so that the morphology of the atrial electrogram recorded at that site is different, and conduction time from the pacing stimulus to the atrial electrogram is now shorter. This fulfills and explains the fourth criterion for the demonstration of transient entrainment (see table 19-1). Right Panel: The orthodromic wave front of the last paced beat (X_n) is unopposed by an antidromic wave front, so that it is now conducted in the retrograde direction over the AV bypass pathway to the atria, so that the tachycardia (dashed lines) continues spontaneously. The serpentine line indicates an area of slow conduction. Note that with resumption of the spontaneous tachycardia, the coronary sinus recording site will again be activated orthodromically. Reprinted from Waldo et al. [8], by permission.

presently available data seem to support our hypotheses, continued systematic investigation of the hypotheses is in order.

Concealed Entrainment

An additional limitation concerning transient entrainment as a marker of reentry has been recognized. We have shown that a tachycardia may be transiently entrained and even interrupted without being able to demonstrate any of the entrainment criteria (i.e., there may be concealed entrainment [22]). This can result, we have shown, when pacing is performed from a site that is orthodromically distal to the area of slow conduction in the reentrant circuit. Thus, it is clear that unless one is able to pace from an appropriate site, a reentry circuit with an excitable gap may be present, but entrainment, though present, will not be demonstrable using pacing techniques.

Another form of concealed entrainment hypothetically ought to be present, and although not labeled as such, has probably now been described

[34–36]. In this form of concealed entrainment, pacing is performed from the area of slow conduction in the reentry circuit during the tachycardia at a pacing rate faster than that of the tachycardia, but fails to interrupt the tachycardia. During such pacing, the antidromic wave front from the pacing impulse is blocked (unidirectional block) in the area of slow conduction, but the orthodromic wave front from the pacing impulse exits from the area of slow conduction, entraining the tachycardia to the pacing rate. Because the antidromic wave front from the pacing impulse is blocked within the area of slow conduction, it does not collide with the orthodromic wave front of the previous beat, and thus there are no fusion beats. The result is that the tachycardia is entrained to the pacing rate, but the morphology of complexes in the ECG are the same as during the spontaneous tachycardia. Such pacing may be associated with a long stimulus-to-onset of the appropriate ECG complex (QRS or P wave) in the ECG, but does not allow demonstration of any of the proposed criteria for the demonstration of transient entrainment.

In any event, the presence of concealed entrainment becomes of particular importance in at least two ways. First, it is obvious that the failure to demonstrate transient entrainment does not mean per se that a reentrant mechanism is not present. Second, it naturally follows that studies that attempt to quantitate the incidence of demonstrability of transient entrainment during various putative reentrant rhythms will not be meaningful unless pacing from an adequate number of sites is performed.

FUTURE DIRECTIONS

Although we think the criteria we have proposed to demonstrate the presence of entrainment of tachycardias with cardiac pacing are best explained by the presence of a reentrant rhythm with an excitable gap, we would like to say that they can *only* be explained by the presence of a reentrant rhythm with an excitable gap. To this end, some obvious studies need to be performed. First, the entrainment criteria need to be tested in the presence of an ectopic automatic tachycardia, either in an animal model or in patients. In addition, the entrainment criteria need to be tested in the presence of a tachyarrhythmia due to a triggered rhythm, once again in an animal model or in patients. And, of course, the criteria should be tested in the presence of both spontaneous and drug induced triggered rhythms, due either to early or delayed afterdepolarizations.

SUMMARY AND CONCLUSIONS

Until recently, cardiac pacing techniques thought indicative of the presence of an underlying reentrant arrhythmia proved to be nonspecific. However, with the advent of the demonstration of transient entrainment, it is now at least reasonable to state that the demonstration of transient entrainment of a tachycardia is best explained by the presence of an underlying reentrant

rhythm with an excitable gap. Additional studies are needed to determine whether transient entrainment of a tachycardia by cardiac pacing can only be explained by an underlying reentrant mechanism.

REFERENCES

1. Cranefield, P.F. 1975. *The Conduction of the Cardiac Impulse. The Slow Response and Cardiac Arrhythmias.* Mt. Kisco, NY, Futura Publishing Co.
2. Mayer, A.G. 1908. Rhythmical pulsation in scyphomedusae. II. in *Papers from the Tortugas Laboratory of the Carnegie Institution of Washington,* 1:113.
3. Mines, G.R. 1914. On circulating excitations in heart muscles and their possible relation to tachycardia and fibrillation. Trans. R. Soc. Can. Ser 3, sec 4, 8:43.
4. Wit, A.L., Hoffman, B.F. and Cranefield, P.F.1972. Slow conduction and reentry in the ventricular conducting system. I. Return extrasystole in canine Purkinje fibers. *Circ. Res.* 30:1–100.
5. Wit, A.L., Cranefield, P.F., and Hoffman, B.F. 1972. Slow conduction and reentry in the ventricular conducting system. II. Single and sustained circus movement in networks of canine and bovine Purkinje fibers. *Circ. Res.* 30:11–22.
6. Wellens, H.J.J. 1978. Value and limitations of programmed electrical stimulation of the heart in the study and treatment of tachycardias. *Circulation* 57:845–853.
7. Rosen, M.R., and Reder, R.F. 1981. Does triggered activity have a role in the genesis of cardiac arrhythmias? *Ann. Intern. Med.* 94:794–801.
8. Waldo, A.L., Plumb, V.J., Arciniegas, J.G., et al. 1982. Transient entrainment and interruption of A-V bypass pathway type paroxysmal atrial tachycardia. A model for understanding and identifying reentrant arrhythmias in man. *Circulation* 67:73–83.
9. Waldo, A.L., MacLean, W.A.H., Karp, R.B., et al. 1977. Entrainment and interruption of atrial flutter with atrial pacing: Studies in man following open heart surgery. *Circulation* 56:737–745.
10. Waldo, A.L., Plumb, V.J., and Henthorn, R.W. 1984. Observations on the mechanism of atrial flutter. in *Tachycardias* p. 213–229, B. Surawicz, ed., Boston, Martinus Nijhoff.
11. Olshansky, B., Okumura, K., Henthorn, R.W., et al. 1986. Entrainment of human atrial flutter localizes the area of slow conduction in the inferior right atrium (abstr.) *J. Am. Coll. Cardiol.* 7:128A.
12. Inoue, H., Matsuo, H., Takayanag, K., and Murao, S. 1981. Clinical and experimental studies of the effects of atrial extrastimulation in rapid pacing on atrial flutter cycle length. Evidence of macroreentry with an excitable gap. *Am. J. Cardiol.* 48:623–631.
13. Beckman, K., Lin, H.T., Krafchek, J., and Wyndham, C.R.C. 1986. Classic and concealed entrainment of typical and atypical atrial flutter. *PACE* 9:826–835.
14. Henthorn, R.W., Okumura, K., Olshansky, B., et al. 1988. A fourth criterion for transient entrainment: The electrogram equivalent of progressive fusion. *Circulation* 77:1003–1012.
15. Waldo, A.L., Henthorn, R.W., Plumb, V.J., and MacLean, W.A.H. 1984. Demonstration of the mechanism of transient entrainment and interruption of ventricular tachycardia with rapid atrial pacing. *J. Am. Coll. Cardiol.* 3:422–430.
16. Plumb, V.J., Henthorn R.W., and Waldo, A.L. 1984. Characteristics of the transient entrainment of ventricular tachycardia by ventricular pacing (abstr.). *PACE* 7:463.
17. Okumura, K., Olshansky, B., Henthorn, R.W., et al. 1987. Demonstration of the presence of slow conduction during sustained ventricular tachycardia in man. *Circulation* 75:369–378.
18. MacLean, W.A.H., Plumb, V.J., and Waldo, A.L. 1981. Transient entrainment and interruption of ventricular tachycardia. *PACE* 4:358–366.
19. Anderson, K.P., Swerdlow, C.D., and Mason, J.W. 1984. Entrainment of ventricular tachycardia. *Am. J. Cardiol.* 53:335–340.
20. Mann, D.E., Lawrie, G.M., Luck, J.C., et al. 1985. Importance of pacing site in entrainment of ventricular tachycardia. *J. Am. Coll. Cardiol.* 5:781–787.
21. Almendral, J.M., Gottlieb, C., Marchlinski, F.E., et al. 1985. Entrainment of ventricular tachycardia by atrial depolarizations. *Am. J. Cardiol.* 56:298–304.
22. Okumura, K., Henthorn, R.W., Epstein, A.E., et al. 1985. Further observations on transient entrainment: Importance of pacing site and properties of the components of the reentry circuit. *Circulation* 72:1293–1307.

23. Brugada, P., Waldo, A.L., and Wellens, H.J.J. 1987. Transient entrainment and interruption of atrioventricular nodal tachycardia. *J. Am. Coll. Cardiol.* 9:769–775.
24. Portillo, B., Mejias, J., Leon-Portillo, N., et al. 1984. Entrainment of atrioventricular nodal reentrant tachycardias during overdrive pacing from high right atrium and coronary sinus: with special reference to atrioventricular dissociation and 2:1 retrograde block during tachycardias. *Am. J. Cardiol.* 53:1750–1756.
25. Henthorn, R.W., Plumb, V.J., Arciniegas, J.G., and Waldo, A.L. 1982. Entrainment of "ectopic atrial tachycardia:" Evidence for re-entry (abstr.). *Am. J. Cardiol.* 49:920.
26. Chen, P.S., Lowe, J.E., German, L.D., et al. 1986. Mapping ventricular fusion beats during entrainment. (abstr.) *Circulation* 74:II–484.
27. El-Sherif, N., Gough, W.B., and Restivo, M. 1986. The mechanism for "entrainment" in termination of reentrant ventricular tachycardia by overdrive pacing (abstr.). *J. Am. Coll. Cardiol.* 7:40A.
28. Dillon, S., and Wit, A.L. 1986. Mechanisms for effects of overdrive on reentrant ventricular tachycardia. (abstr.) *Circulation* 74:II–118.
29. Okumura, K., Plumb, V.J., and Waldo, A.L. 1985 Entrainment of experimental atrial flutter and its use to localize the slow conduction area in the reentry loop (abstr.). *Circulation* 72:III–382.
30. Frame, L.H., Page R.L., and Hoffman, B.F. 1986. Atrial reentry around an anatomic barrier with a partially refractory excitable gap. A canine model of atrial flutter. *Circ. Res.* 58:495–511.
31. Boyden, P.A., Frame, L.H., and Hoffman, B.H. 1986. Activation patterns during transient entrainment of reentrant excitation in isolated canine atrium (abstr.). *Circulation* 74:II–350.
32. Lange, G. 1965. Action of driving stimuli from intrinsic and extrinsic sources on *in situ* cardiac pacemaker tissues. *Circ. Res.* 17:449–459.
33. Wit, A.L., and Rosen, M.R. 1986. Afterdepolarizations and triggered activity. In *The Heart and Cardiovascular System. Scientific Foundations,* H.A. Fozzard, E. Haber, R.B. Jennings, et al., eds. p. 1449, New York, Raven Press.
34. Morady, F., Schienman, M.M., DiCarlo, L.A., Jr., et al. 1987. Results of transcatheter ablation of ventricular tachycardia in 33 patients (abstr.). *J. Am. Coll. Cardiol.* 9:250A.
35. Frank, R., Tonet, J.L., Kounde, S., et al. 1987. Localization of the area of slow conduction during ventricular tachycardia. In *Cardiac Arrhythmias: Where To Go From Here?* P. Brugada and H.J.J. Wellens eds. p. 191–268, Mt. Kisco, NY, Futura Publishing Co.
36. Stevenson, W.G., Weiss, J.N., Wiener, I., et al. 1988. Resetting of ventricular tachycardia: Implications for localizing the area of slow conduction. *J. Am. Coll. Cardiol.* 11:522.

20. THE VALUE OF PROGRAMMED ELECTRICAL STIMULATION IN TRIGGERED ACTIVITY-INDUCED ARRHYTHMIAS

ANTON P.M. GORGELS, MARC A. VOS, PEDRO BRUGADA,
AND HEIN J.J. WELLENS

Triggered activity induced by delayed afterdepolarizations has been investigated extensively in tissues isolated from different sites in the heart and from different species. In spite of this we still do not know the clinical relevance of this arrhythmogenic mechanism. An answer to this question is important because the ability to identify mechanisms in the clinical situation can improve our understanding of pathophysiology and provide new treatments. Presently, different methods are used to discriminate among mechanisms of arrhythmias, including electrical stimulation [1], antiarrhythmic drugs, and recording of monophasic action potentials. Unfortunately no method has proven to be sufficiently sensitive or specific [2, 3].

Programmed electrical stimulation also has been used to uncover the underlying mechanisms of arrhythmias. Most of these data have been derived from isolated tissue preparations. This model differs from the intact human heart in a number of respects. First, the preparation lacks the influence of the autonomic nervous system, which is likely to be important in arrhythmias based on triggered activity. Second, only a small number of pacemaker cells are investigated, whereas in the intact heart many ectopic foci may be active and mutually interfere. Third, whether the investigated preparation would be active in the intact heart is not known. Fourth, although a certain mechanism may indeed be occurring in the intact heart, it may be overridden by other, concomitantly active mechanisms. An example is arrhythmias occurring 24 hours after myocardial infarction [4]. Although delayed afterdepolarizations

have been shown to be present in this condition, the arrhythmias occurring in this setting have been generally attributed to abnormal automaticity.

Finally, in contrast to isolated tissue preparations, ectopic foci in the intact heart are continuously dominated by the sinus node or other sites of impulse formation, whether supraventricular or ventricular. This makes definitions of arrhythmogenic mechanisms used in isolated tissue preparations difficult to use in the intact heart. For instance, triggered activity has been defined as an arrhythmia that can never arise spontaneously, but is always preceded by another impulse [5]. In the intact heart, however, every arrhythmia will be preceded by an impulse.

Similar problems are encountered when considering modes of termination of arrhythmias. Whereas slowing down or termination can be accurately observed in the isolated tissue preparation, in the intact heart these phenomena may be obscured by sinus beats that capture the ventricles and may then reinitiate the arrhythmia.

Therefore, to establish whether results from isolated Purkinje fibers or from the coronary sinus are applicable to clinical arrythmias, the effect of similar stimulation protocols has to be evaluated in the intact heart. An animal model is necessary before it becomes possible to faithfully study mechanisms of clinical arrhythmias. Patients suspected of having automatic arrhythmias cannot always be studied thoroughly because the disease inducing the arrhythmia or the arrhythmia itself does not allow this (e.g., myocardial infarction, digitalis intoxication) or the arrhythmia does not last long enough for collection of sufficient interpretable data.

To study the effect of pacing on triggered activity, we prefer to use intact conscious dogs studied several weeks after induction of AV block with formalin 37 precent. In this model, unipolar epicardial electrodes are fixed to the apex of the left ventricle and to the base of the right ventricle. These sites are chosen because ouabain-induced ventricular tachycardias frequently arise from these areas [6]. As a model of triggered activity, we use ouabain-induced ventricular tachycardias because ouabain is easily administered, gives stable arrhythmias, and is frequently used in in vitro experiments.

We have used several pacing protocols depending on the questions we wanted to investigate:

1. Is a ouabain-induced arrhythmia in the intact heart likely to be due to triggered activity?
2. Do ouabain-induced triggered arrhythmias show specific characteristics that may distinguish them from abnormal automaticity and/or reentry?
3. Can ouabain-induced ventricular tachycardias be terminated by pacing and, if so, is the mode of termination suggestive of reentry, triggered activity or abnormal automaticity as the underlying mechanism?
4. Can a nonreentrant tachycardia show phenomena fulfilling one of the criteria for transient entrainment? If so, these criteria, may not be specific for reentry as the underlying mechanism of the arrhythmia.

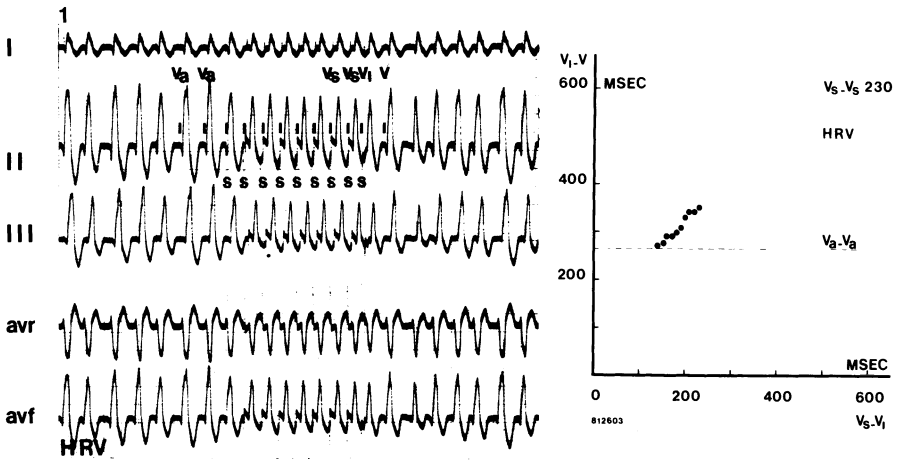

Figure 20-1. Overdrive stimulation during ouabain-induced ventricular tachycardia. Five electrocardiographic leads are simultaneously recorded. Pacing is accomplished from the basal part of the right ventricle (HRV). Eight stimuli are given with an interstimulus interval of 230 ms (Vs − Vs = 230 ms). The ninth interstimulus interval (Vs-V1) is decreased by steps of 10 ms. The right panel shows a direct linear relation between the last paced interval and the first postpacing interval (V1-V). See text for details. Va − Va = last tachycardia cycle length before pacing; S = stimulus artifact.

We will now present some characteristics of ouabain-induced ventricular tachycardias based on the results of these experiments. We will also discuss these characteristics in relation to the possibility of differentiating triggered arrhythmias from those based on other arrhythmogenic mechanisms.

OUABAIN-INDUCED VENTRICULAR TACHYCARDIAS AND EFFECTS OF PACING

The First Postpacing Interval

The behavior of the interval of the first QRS complex following pacing is quite typical in ouabain-induced ventricular tachycardia [7] (figures 20-1, 20-2).

1. Pacing induces a first postpacing interval longer than the tachycardia cycle length.
2. Decreasing the interstimulus interval results in shortening of the first postpacing interval.
3. This pattern is largely independent of the mode of stimulation, i.e., whether 1, 2, or more stimuli are used and whether only the last interstimulus interval or all interstimulus intervals are changed.
4. A biphasic response curve for the first postpacing interval is obtained during initiation of ouabain-induced ventricular tachycardia [8].

When pacing is performed with interstimulus intervals longer than 400 ms (figure 20-2, panels 1 through 3), the first postpacing interval approximately equals the interstimulus interval. At a critical cycle length, however, a sudden increase of the coupling interval is observed. A further shortening of the interstimulus interval results in a further reduction of the first postpacing interval (figure 20-2, panels 4 through 6). This pattern is explained by the in vitro observation that the first delayed afterdepolarization (DAD) has a high amplitude after long interstimulus intervals. However, at shorter interstimulus intervals the first DAD amplitude decreases and the second DAD increases and becomes more likely to induce an action potential [9, 10]. This biphasic response supports the triggered nature of ouabain-induced arrhythmias in the intact heart [11].

Overdrive Acceleration

Another interesting and consistent finding in ouabain-induced ventricular tachycardias is the occurrence of overdrive acceleration of the tachycardia after the first postpacing QRS complex. In contrast to the first postpacing interval, the subsequent tachycardia intervals become shorter than the QRS intervals before pacing. In this way trains of 20 stimuli accelerated tachycardias from a cycle length of 420 ± 60 ms to 350 ± 60 ms in seven of seven dogs (table 20-1). Even tachycardias with shorter cycle lengths that had been stable for at least ten minutes showed slight but significant acceleration following stimulation. This was found using one and two stimuli (given with a prematurity varying between 330 and 140 ms) but also after longer periods of stimulation, i.e., 15, 60, and 120 seconds (using interstimulus intervals between 330 and 200 ms) (table 20-1).

Although overdrive acceleration may occur in clinical reentrant arrhythmias, it is an exceptional phenomenon, and, when observed, it is frequently accompanied by a change in QRS configuration.

In our experiments overdrive acceleration was observed both with and without changes in QRS configuration. In abnormal automaticity only the first postpacing interval was studied in detail [12]. To our knowledge the question whether overdrive acceleration of the subsequent intervals can also be induced has not been answered.

FAILURE TO TERMINATE OUABAIN-INDUCED VENTRICULAR TACHYCARDIAS BY PACING

Both from pathophysiologic and therapeutic points of view it is important to know whether ouabain-induced ventricular tachycardias can be terminated by electrical stimuli. In isolated Purkinje fibers, termination has been found possible in more than 80 percent of the stimulation trains [10].

Therefore, we wanted to know whether termination was also possible in the intact heart. For this reason we chose a stimulation protocol similar to the protocol used to terminate in vitro arrhythmias. First, one and two extrasti-

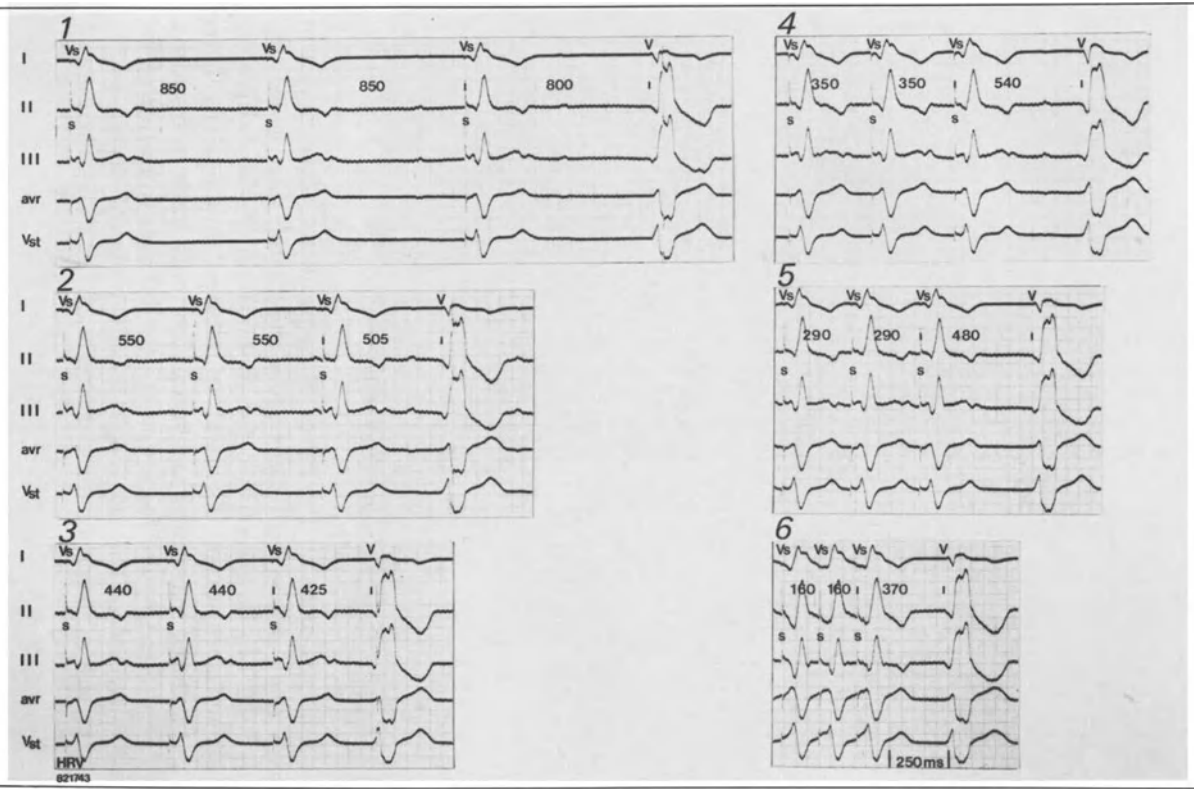

Figure 20-2. The biphasic behavior of the first postpacing interval during initiation of ouabain-induced ventricular tachycardia. Pacing is accomplished with four stimuli at the basal right ventricle (HRV). Four extremity leads and a chest lead (Vst) are displayed simultaneously. Only the last three stimulated QRS complexes (Vs) and the first postpacing QRS complex (V) are shown. From panel 1 to 6 the interstimulus intervals decrease from 850 to 160 ms. See text.

Table 20-1. Overdrive acceleration

NVS*	Duration(s) §	Acc/total‖	Cycle length	
			Vt pre†	Vt post‡
20	—	7/7	420 ± 60	350 ± 60 ¢
1	—	8/9	360 ± 30	340 ± 20 ¢
2	—	8/9	340 ± 10	320 ± 20 ¢
—	15	9/9	320 ± 20	300 ± 20 ¢
—	60	8/9	310 ± 20	290 ± 20 ¢
—	120	8/8	310 ± 20	300 ± 20 ¢

* NVS = number of stimuli
† Vt pre = tachycardia cycle length before pacing (mean ± SD)
‡ Vt post = tachycardia cycle length after pacing (mean ± SD)
§ s = seconds
‖ Acc/total = experiments showing overdrive acceleration in relation to total number of experiments.
$P < .01$ cf "VT pre".

muli were applied with increasing prematurity until the refractory period was reached. Such a protocol is also frequently used to terminate reentrant tachycardias. Thereafter, trains were given of 15, 60, and 120 seconds at different cycle lengths (from 330 to 200 ms). A comparable stimulation procedure has also been applied in vitro to suppress arhythmias based on abnormal automaticity [11]. Interestingly, only after 2 of 161 stimulation trains given in nine dogs did the tachycardia stop. This virtual inability to terminate ouabain-induced ventricular tachycardia is in contrast to the results obtained in isolated Purkinje fibers and in coronary sinus preparations [13]. Several mechanisms may be invoked to explain this difference. First, the degree of toxicity may have been different in both experimental situations. This argument is favored by the fact that the tachycardia cycle length in our experiments (310 ± 20 ms) was shorter than the cycle length of the tachycardias studied in vitro (669 ± 295 ms).

A second influence may be the effect of sympathetic nervous system activation as a result of the high tachycardia and pacing rate. Third, the pacing mode itself can be responsible for continuation of the tachycardia through different mechanisms. Electrical stimulation in the intact heart during ouabain-induced ventricular tachycardia not only leads to overdrive acceleration but also to impulse formation at sites different from that of the origin of the tachycardia. The latter is especially observed during pacing distant from the site of origin of the tachycardia. After pacing, temporary competition between the spontaneous tachycardia and the induced foci is observed until the original tachycardia regains dominance of the ventricles [7]. Another mode of perpetuation, which we have observed less frequently, is that electrical stimulation at the site of origin of the tachycardia leads to temporary suppression of the original tachycardia and to induction of a

tachycardia from a different site (figure 20-3, panels 1 through 3). It is not clear whether the pacing procedure leads to complete termination of the original tachycardia, which becomes reinitiated by the induced tachycardia. The other possibility is that pacing has only slowed down the rate of the original tachycardia (panel 4).

CAN OUABAIN-INDUCED VENTRICULAR TACHYCARDIA PHENOMENA BE SHOWN WHICH FULFILL CRITERIA FOR TRANSIENT ENTRAINMENT?

Transient entrainment is defined as an increase in the rate of all tissues responsible for sustaining a tachycardia to a faster pacing rate with resumption of the intrinsic rate of the tachycardia on either cessation of pacing or slowing of the pacing rate below the intrinsic rate of the tachycardia[14]. When a reentrant tachycardia becomes entrained, the surface electrocardiogram shows constant fusion between the configuration of the paced QRS complex and the ventricular tachycardia. Pacing at a faster rate causes progressive fusion (i.e., the paced ventricular activation pattern contributes more to the configuration of the fusion complexes). We have investigated whether it is possible to mimic entrainment in ouabain-induced ventricular tachycardias by demonstrating constant and progressive fusion. These experiments were done in seven dogs using trains of 20 stimuli starting with interstimulus intervals slightly shorter than the tachycardia cycle length. Thereafter, increasingly shorter interstimulus intervals were used until the ventricles were dominated completely by the pacing site. Pacing was always accomplished distant from the origin of the tachycardia.

Theoretically, constant fusion should be induced easily in a regular monomorphic tachycardia. However, in ouabain-induced ventricular tachycardia this was rarely, if ever, demonstrable. Fusion may have been variable because of beat to beat variation in ventricular activation when the paced impulse failed to enter the site of origin of the tachycardia. Another possibility is that capture of the site of origin of the ventricular tachycardia resulted in modification of the cycle length of the original tachycardia.

Pacing at cycle lengths 8 ms shorter than that of the tachycardia resulted in complete capture of the tachycardia focus and QRS complexes completely determined by the pacing site. This is in contrast to reentrant tachycardias, which show constant and progressive fusion when the reentry circuit is invaded from the pacing site.

Finally, invasion of the tachycardia focus never did result in termination as in reentry tachycardias, but as previously described, in a first postpacing interval longer than the tachycardia cycle length and acceleration of the tachycardia. These findings are illustrated in figure 20-4.

We consider these observations of importance because they support the specificity of the (surface) ECG criteria of entrainment as indicative of reentry tachycardias.

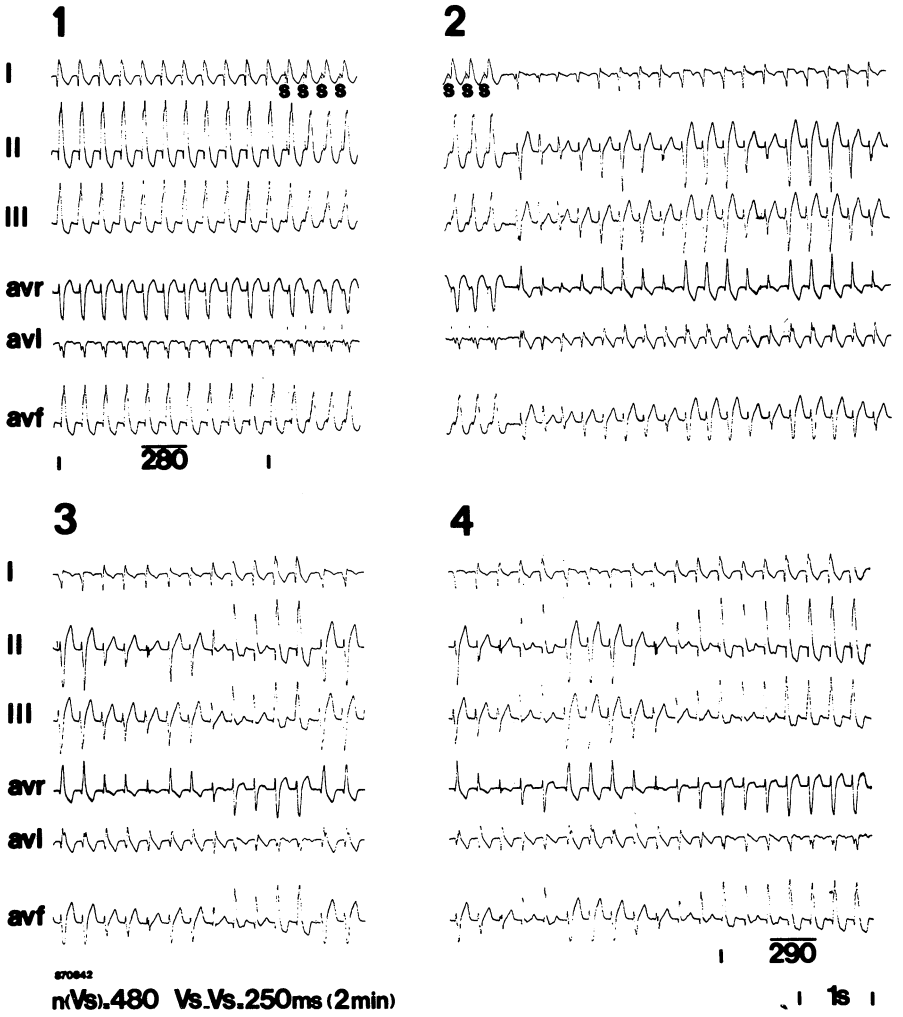

Figure 20-3. Unusual mode of perpetuation of ouabain-induced ventricular tachycardia. Panels 1 through 4 are a continuous recording. In all panels six extremity leads are simultaneously displayed. Panel 1: A monomorphic tachycardia from the basal right ventricle having a mean cycle length of 280 ms is overpaced from the same site with 480 stimuli (S) and interstimulus intervals (Vs-Vs) of 250 ms. Panel 2: The first postpacing QRS complex has a different configuration, suggesting suppression or termination of the original tachycardia. Thereafter, fusion complexes between this configuration and the original tachycardia configuration are observed and continue in panels 3 and 4. At the second part of panel 4, the original tachycardia regains dominance of the ventricles, but with a slightly longer mean cycle length (290 ms) than before pacing.

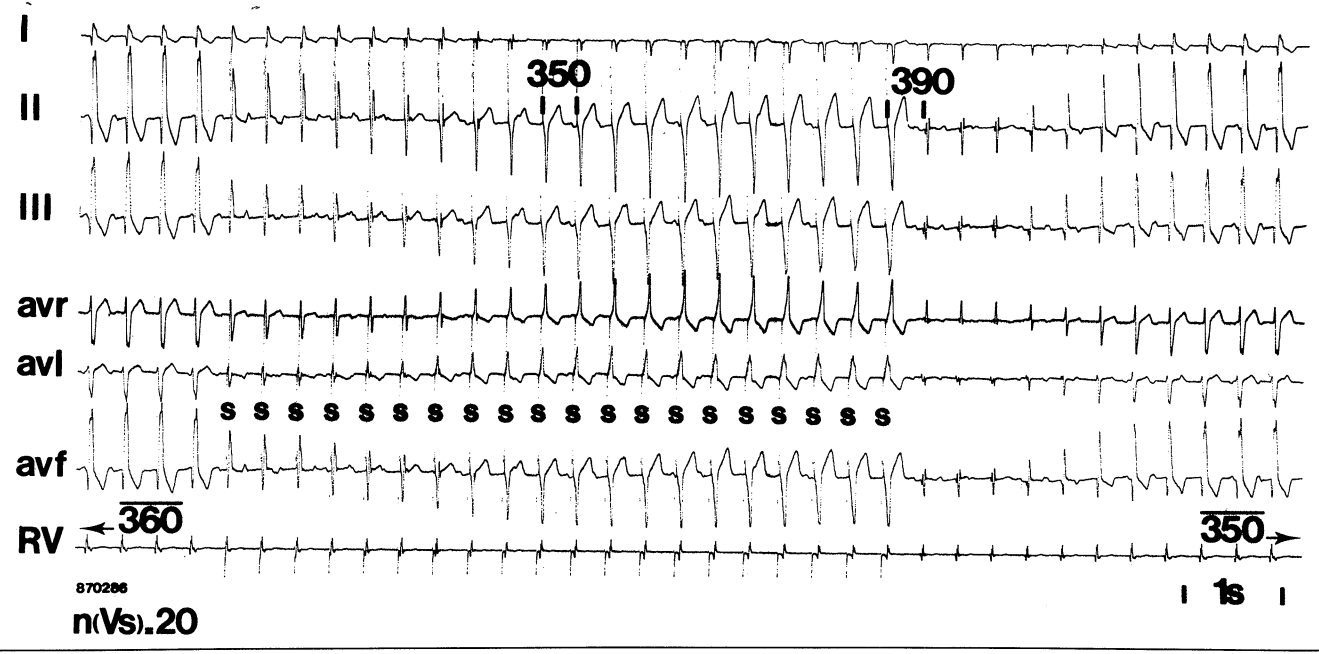

Figure 20-4. Effect of overdrive stimulation on ouabain-induced ventricular tachycardia. Six extremity leads and a local RV electrogram are recorded simultaneously. Pacing is accomplished from the apex of the left ventricle. The tachycardia originates at the basal right ventricle. The mean cycle length is 360 ms. During pacing at a cycle length of 350 ms, variable fusion is observed. The first postpacing interval is longer than the mean prepacing interval (390 ms). A tachycardia with a different QRS configuration is induced, which gradually changes to the original tachycardia configuration. The mean cycle length of the tachycardia after pacing is slightly shorter than before pacing (350 ms). S = stimulus artifact; NVS = number of stimuli.

FUTURE PROSPECTS

The presented data show several characteristics of ouabain-induced ventricular tachycardia that may be useful to differentiate triggered activity from other arrhythmogenic mechanisms in the human heart. The behavior of the first postpacing interval is different from that observed in reentry and in abnormal automaticity. In abnormal automaticity the first postpacing interval does not change on shortening the interstimulus interval [11]. In reentry an increase or no change of the first postpacing interval is the rule [15]. Also, overdrive acceleration and the inability to terminate the tachycardia may suggest that the tachycardia is based on triggered activity.

There are, however, limitations in applying these criteria to the clinical situation. We have used a model of triggered activity in which ouabain intoxication is global. It is conceivable that the effect of similar pacing protocols is different in focal triggered activity. This may especially be the case for the results in relation to termination of the tachycardia because in focal triggered activity, no impulse formation is likely to be induced close to the stimulation site. Therefore, reinitiation of the tachycardia is less likely to occur under these circumstances. Another problem in the investigation of clinically occurring arrhythmias by pacing is the lack of a "gold standard" for identifying a given arrhythmogenic mechanism. Drugs that specifically suppress mechanisms of arrhythmias should be more useful for this purpose.

Unfortunately no such drugs are presently clinically available or sufficiently specific. Doxorubicin has been shown experimentally to suppress DAD-induced triggered activity [16]. We have been investigating the calcium overload blocker flunarizine for this purpose because this drug has not been shown to influence the fast sodium and slow calcium channel [17] but instead to suppress ouabain-induced arrhythmias in anaesthetized guinea pigs [18].

This drug has been studied in ouabain-induced ventricular tachycardias in nine dogs. After intravenous injection of 2 to 3 mg/kg flunarizine, the tachycardia cycle length increased in all experiments from 310 ± 20 ms to 405 ± 60. Tachycardia terminated in seven dogs. Reproducibility was present in all six dogs in which this was tested. Because of the described electrophysiologic profile, this may become an example of a clinically useful drug, specific in suppressing triggered activity.

In conclusion, whereas programmed electrical stimulation of the heart has contributed to our understanding of clinical arrhythmias, until now it has had a limited role in differentiating between triggered activity and reentrant tachyardias. A few specific new findings for triggered activity are the occurrence of overdrive acceleration and the behavior of the first postpacing interval. Findings that may be specific for reentry arrhythmias include the demonstration of transient entrainment according to the criteria proposed by Waldo et al. [14]. A combination of effects of pacing and drugs specifically active on tachycardia mechanism should contribute further to our ability to uncover arrhythmogenic mechanisms.

REFERENCES

1. Wellens, H.J.J. 1978. Value and limitations of programmed electrical stimulation of the heart in the study and treatment of tachycardias. *Circulation* 57:845–853.
2. Brugada, P. and Wellens, H.J.J. 1984. The role of triggered activity in clinical ventricular arrhythmias. *PACE* 7:260–271.
3. Rosen, M.R. 1986. Is the response to programmed electrical stimulation diagnostic for mechanism of arrhythmias? *Circulation* 73 (Suppl. II):18–27.
4. Le Marec, H., Dangman, K.H., Danilo, P., and Rosen, M.R. 1985. An evaluation of automaticity and triggered activity in the canine heart one to four days after myocardial infarction. *Circulation* 77:1224–1236.
5. Cranefield, P.F. 1977. Action potentials, afterpotentials and arrhythmias. *Circ. Res.* 41:415–423.
6. Gorgels, A.P.M., de Wit, B., Beekman, H.D.M., et al. 1986. Effect of different modes of stimulation on the morphology of the first QRS-complex following pacing during digitalis induced ventricular tachycardia. *PACE* 9:842–859.
7. Gorgels, A.P.M., Beekman, H.D.M., Brugada, P., et al. 1983. Extrastimulus related shortening of the first postpacing interval in difitalis induced ventricular tachycardia. *J. Am. Coll. Card.* 1:840–857.
8. Gorgels, A.P.M., de Wit, B., Beekman, H.D.M., et al. 1987. Triggered activity induced by pacing during digitalis intoxication. Observations during programmed electrical stimulation in the conscious dog with chronic complete atrioventricular block. *PACE* 10:1309–1321.
9. Ferrier, G.R., Saunders, J.H., and Mendez, C. 1973. A cellular mechanism for the generation of ventricular arrhythmias by acetylstrophantidin. *Circ.Res.* 32:600–609.
10. Moak, J.P. and Rosen, M.R. 1984. Induction and termination of triggered activity by pacing in isolated canine Purkinje fibers. *Circulation* 69:149–162.
11. Rosen, M.R., and Reder, R.F. 1984. Does triggered activity have a role in the genesis of cardiac arrhythmias? *Ann. Int. Med.* 94:794–801.
12. Dangman, K.H. and Hoffman, B.F. 1983. Studies on overdrive stimulation of canine Purkinje fiber. Maximal diastolic potential as a determinant of the response. *J. Am. Coll. Card.* 2:1183–1179.
13. Johnson, N., Danilo, P., Jr., Wit, A.L., and Rosen, M.R. 1986. Characteristics of initation and termination of catecholamine induced triggered activity in atrial fibers of the coronary sinus. *Circulation* 74:1168–1179.
14. Waldo, A.L., Olshansky, B., Okumura, K., and Henthorn, R.W. 1987. Current perspective on entrainment of tachyarrhythmias. in *Cardiac Arrhythmias: Where to Go from Here* P. Brugada and H.J.J. Wellens, eds., pp. 171–189, Mount Kisco, NY, Futural Publishing Co.
15. Almendral, J.M., Stamato, J.M., Rosenthal, M.E., et al. 1986. Resetting response patterns during sustained ventricular tachycardia: relationship to the excitable gap. *Circulation* 74, 722–730.
16. Le Marec, H., Spinelli, W., and Rosen M.R. 1986. The effects of doxorubicin on ventricular tachycardia. *Circulation* 74, 881–889.
17. Borgers, M., de Clerck, F., van Reempts, J., et al. 1984. Selective blockade of cellular Ca-overload by flunarizine. *Int. J. Angiol.* D25–31.
18. Jonkman, F.A.M., Boddeke, H.W., and van Zwieten, P.A. 1986. Protective activity of calcium entry blockers against ouabain intoxication in anesthetized guinea pigs. *J. Cardiovasc. Pharmacol.* 8:1009–1013.

21. PHARMACOLOGIC MECHANISMS IN THE TREATMENT OF ARRHYTHMIAS DUE TO ISCHEMIA AND INFARCTION

BRIAN F. HOFFMAN AND WALTER SPINELLI

The general question of how drugs act to modify the arrhythmias caused by ischemia and infarction is far too broad to attempt an answer. Some drugs may act to modify the intensity of ischemia or the severity of infarction and thus alter the incidence or severity of associated arrhythmias. Thus, adrenergic blocking agents, by limiting the increase in sinus rate and attenuating the effects of norepinephrine on the ventricular myocardium, will decrease the severity of arrhythmias associated with acute ischemia [1] and diminish the likelihood of lethal arrhythmias after infarction. Arrhythmias of the ischemic or infarcted heart are more likely in the presence of failure, probably because of the elevated diastolic pressures, and so drugs that improve cardiac perfomance by decreasing preload and afterload probably will reduce the likelihood and severity of arrhythmias. What perhaps is possible is to restrict consideration to antiarrhythmic drugs of the two major classes, class I and class III.

One approach to evaluating mechanisms by which arrhythmic drugs can or do act to terminate arrhythmias caused by ischemia or infarction is first to select an arrhythmogenic mechanism likely to be operative in a heart damaged by ischemia or infarction, then to decide what modification or modifications of cardiac electrical activity most likely would make that

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Table 21-1. Mechanisms for reentrant excitation

I. Circus Movement
  A. Inexcitable Structural Barrier
    1. Normal fast response action potentials
    2. Depressed fast response action potentials
    3. Slow response action potentials
      [1 + 2, 1 + 3, 1 + 2 + 3]
      α) permanent unidirectional block
      β) activity-dependent unidirectional block
  B. Functional Barrier
    1. Depressed fast response action potentials
    2. Slow response action potentials
      [1 + 2]
      α) permanent unidirectional block
      β) activity-dependent unidirectional block
  C. Combined Barrier (A + B)

II. Reflection
  A. True Reflection
    1. Slow conduction
    2. Action potential prolongation
    3. Early or delayed afterdepolarizations
  B. Apparent Reflection

arrhythmogenic mechanism inoperative, then select one or more antiarrhythmic drugs able to bring about the necessary, and only the necessary, changes in electrical activity, and finally test these drugs against an arrhythmia known to result from the selected mechanism. Then, if the results are as expected and if it can be determined with certainty that in presence of ischemia or infarction a particular arrhythmia results from the selected mechanism, it is possible to make the final test to see if the selected drug or drugs are effective. If they are, the test is reasonably good; if they are not it may be that the actions of the drugs have been modified by the presence of ischemia or infarction. Also, in at least some cases, the arrhythmogenic mechanism will not actually be the assumed mechanism. In spite of these obvious limitations it is reasonable to attempt to follow this sequence if for no other reason than to identify other inadequacies in the proposed tests.

There is convincing evidence from many studies that quite frequently ventricular arrhythmias of infarcted hearts result from reentrant excitation [2–4]. Although reentrant excitation most often is assumed to result from circus movement, it also can be caused by reflection [5, 6]. Table 21-1 presents a simplified classification of the electrophysiologic bases for the two major types of reentrant mechanisms. Reentry due to circus movement has been studied much more thoroughly than that caused by reflection because it is easier to demonstrate circus movement than reflection and because the concept of reentry due to reflection is the more recent.

Reentrant excitation due to circus movement occurs when the impulse circulates in an adequately bounded path around a permanently inexcitable

obstacle of sufficient size [7, 8] or around a zone in which suitable alterations of excitability and/or conduction limit or block impulse propagation [9, 10]. The latter mechanism has been termed leading circle reentry [9]. The changes in electrical activity sufficient to terminate circus movement need not be the same for each type of circuit. For the first type, circus movement around a permanent barrier, Lewis [11] predicted that a drug able to prolong refractoriness more than it slowed conduction should be effective because, in the presence of such a drug, the circulating impulse would encounter tissue not sufficiently recovered from prior activity to generate a propagating response. The idea seems reasonable for circus movement in a uniform path. An adequate test of the idea has been difficult because of the need to select a path with spatially uniform properties and an excitable gap that was not unreasonably long. Also, some tests have been of limited value because most antiarrhythmic drugs exert a multitude of effects on cardiac electrical activity and even may modify the passive properties of the membrane [12], and thus it has not been clear that termination of circus movement resulted from the drug-induced prolongation of refractoriness.

We have attempted to test this idea through studies on circus movement in the supravalvular lamina of the canine right atrium [7, 13]. The impulse is induced to circulate around this structure by an atrial lesion, like one first employed by Rosenblueth and Garcia-Ramos [14] that interrupts alternate pathways. The tissues of the circus path generate fast responses throughout and the anatomical and electrophysiologic properties of the path are spatially reasonably uniform. During circus movement, the impulse spreads in incompletely recovered tissues and so the duration of the excitable gap is reasonable. The cycle length of the arrhythmia is quite constant among animals because conduction velocity varies as a function of path length. In the conscious, chronically prepared dog, circus movement in this structure results in a persistent arrhythmia resembling atrial flutter. The arrhythmia satisfies all electrophysiologic tests for reentry due to circus movement and maps of atrial excitation sequence prove that circus movement in the supravalvular lamina is the arrhythmogenic mechanism.

To test the proposed hypothesis, we selected drugs that had no significant effect, at suitable concentration, on any aspect of the canine atrial transmembrane action potential other than its duration and that did not cause postrepolarization refractoriness. These criteria indicated that N-acetylprocainamide [15, 16] and *d*-sotalol [17] were acceptable agents that could be expected to cause only a dose-dependent increase in the duration of the atrial action potential. We administered NAPA as a bolus and *d*-sotalol as a bolus followed by an intravenous infusion. Both drugs were reasonably effective in terminating the circus movement (table 21-2). Before termination, they caused only a modest increase (NAPA, +23 percent and *d*-sotalol, +10 percent) in cycle length of the reentrant rhythm. After termination of the arrhythmia, the duration of the effective refractory period, measured during

Table 21-2. Drug effects on cycle length and excitable gap during flutter, and effective refractory period and conduction time during pacing

	Flutter cycle length (msec)		ERP_{300} (msec)		Conduction time (msec)		Excitable gap (% of CL)	
Drug	C	D	C	D	C	D	C	D
N-acetylprocainamide (n = 7)	154 ± 8*	190 ± 17	105 ± 16*	157 ± 37			49 ± 9	48 ± 9
d-Sotalol (n = 5)	149 ± 5*	163 ± 4	110 ± 8*	128 ± 10	34 ± 6	34 ± 7	41 ± 5*	22 ± 7

C = Control; D = after drug
* = $P < .05$ between C and D
ERP_{300} = effective refractory period at a paced cycle length of 300 msec

Table 21-3. Drug effects on cycle length and excitable gap during flutter and effective refractory period and conduction time during pacing

	Flutter cycle length (msec)		ERP_{300} (msec)		Conduction time (msec)		Excitable gap (% of CL)	
Drug	C	D	C	D	C	D	C	D
Propafenone (n = 6)	147 ± 7*	262 ± 28	99 ± 6*	142 ± 10	36 ± 5*	59 ± 12	47 ± 3	47 ± 2
Flecainide (n = 6)	151 ± 6*	261 ± 19	102 ± 8*	137 ± 15	32 ± 4*	60 ± 11	46 ± 7	51 ± 6
Lidocaine (n = 5)	153 ± 7*	185 ± 8	98 ± 3	102 ± 2	42 ± 11	42 ± 11	47 ± 1	45 ± 6

C = Control; D = after drug
* = $P < .05$ between C and D
ERP_{300} = effective refractory period at a paced cycle length of 300 msec

atrial pacing, was found to have increased only moderately and there was no significant change in conduction velocity [16].

Although it is clear in this model that the drugs that prolong refractoriness without any major and direct effect on the action potential upstroke or conduction can and do terminate reentry due to circus movement, it is not clear why termination occurs. It would be reasonable to assume that since the action potential was prolonged, this forced the circulating impulse to propagate ever more slowly in progressively less fully repolarized tissues until a propagating impulse no longer could be generated in advance of the wavefront. If this were the course of events, the duration of the excitable gap would decrease progressively, and the gap would vanish at the moment of termination. Our measurements of the duration of the excitable gap indicate that it did not decrease in duration, measuring 49 and 48 percent of the cycle length (or 76 and 91 ms) with NAPA, but did decrease from 41 to 22 percent of the cycle length (61 to 36 ms) for *d*-sotalol. Unfortunately, these measurements were not made at the moment of termination, but only after the arrhythmia had been reinitiated. In addition, if the mechanism proposed were the cause of termination one would expect a progressive and probably large increase in the cycle length as the impulse propagated in tissues with ever decreasing responsiveness. In fact, the arrhythmia terminated after only a modest increase in cycle length. In conclusion, drugs that prolong the action potential and thus the duration of refractoriness can terminate reentry due to circus movement around an inexcitable barrier, but the mechanism by which they do so is uncertain.

It also is interesting to examine drugs that slow conduction more than they prolong refractoriness since Lewis' prediction was that they would be ineffective in terminating circus movement. To use the term "refractoriness" without further restriction can be very misleading, but in general, drugs with the properties described would cause a progressive increase in both the cycle length and also the duration of the excitable gap. We have studied a number of such drugs and data for two, propafenone and flecainide, are shown in table 21-3. Both caused marked slowing of conduction, a great increase in cycle length of the arrhythmia and an increase in the duration of the excitable gap in terms of its absolute duration. However, the fraction of the cycle length occupied by the gap was unchanged. After termination of the arrhythmia, conduction of paced impulses in the atrium was slowed significantly and to approximately the same extent as conduction during the arrhythmia. During pacing, the effective refractory period was prolonged but the percentage change was smaller than that in conduction (ERP, +43 percent, conduction time +63 percent for propafenone; ERP +34 percent, conduction time +87 percent for flecainide). In summary, contrary to prediction, drugs that slow conduction to a greater extent than they prolong refractoriness are quite effective in terminating circus movement in this model. However, once again the mechanism responsible for termination is unclear.

Obviously the intensity of block of fast channels is a function, among other variables, of both the characteristics of the drug and the drug concentration. For the agents we employed, some use dependence is anticipated, but it seems unlikely that the increase in cycle length caused any great decrease in the degree of fast channel blockade, since all three drugs dissociate slowly from blocked fast channels. Perhaps more important is the effect of the increase in cycle length on the level of membrane potential in advance of the circulating impulse. Under control conditions in this model, the impulse circulates in tissues that clearly are not fully repolarized. The increase in cycle length would, by itself, allow membrane potential to attain a more negative value in advance of the circulating impulse and this typically would permit greater removal of inactivation and greater recovery from drug-induced fast channel block. For drugs that dissociate only slowly from inactivated fast channels the change in cycle length in our experiments would not have a prominent effect. These drugs all do increase the duration of refractoriness, but since we are quite sure that the duration of the excitable gap increased quite markedly, slowing of repolarization and any increase of postrepolarization refractoriness were obviously outweighed by the depression of conduction.

In marked contrast are the data for lidocaine (table 21-3). One might anticipate that in spite of its rapid dissociation from blocked fast channels, lidocaine would act strongly against a reentrant arrhythmia with a cycle length of 140 to 150 msec [18]; also, the incomplete repolarization between action potentials might favor binding of drug to inactivated fast channels and promote antiarrhythmic action. We found that infusions of lidocaine that resulted in plasma concentrations of 4.3 to 11.2 μg/ml caused only minimal slowing of the arrhythmia (cycle length increasing from 153±7 to 185±8 ms) and generally were ineffective in terminating the circus movement.

Obviously, the conditions for our model and in particular the nature of the circus path do not represent those present when ischemia or infarction lead to reentrant excitation caused by circus movement. It may be possible, however, to suggest how the actions of antiarrhythmic drugs might be modified by the properties of the circus path. One reasonably simple change would be to insert in the path a segment in which the impulse propagated much more slowly than elsewhere in the reentrant circuit. The excessively slow conduction might result from either a positive shift in the threshold potential or an increase in gap junctional resistance and thus in internal longitudinal resistance. We can assume that action potential duration in this abnormal segment is somewhat less than in the remainder of the path. Because of the time that had elapsed while the impulse spread throught the abnormal segment, when the impulse entered the normal part of the path, it would encounter fully repolarized tissues and thus begin to propagate at normal velocity. Under these conditions, a drug that acted solely to prolong the action potential and refractoriness might be rendered relatively ineffective because of the presence

of a long excitable gap. On the other hand, the insertion of a slowly conducting segment into the path might cause action potential prolongation proximal to the slowly conducting segment [19]. In this case, when the impulse emerged from the slowly conducting segment, initially it would propagate at full velocity but then, as it encountered incompletely repolarized tissues, the conduction velocity would decrease and block might occur. The effectiveness of drugs that prolong the action potential in this type of circuit obviously would depend on the extent to which a slowly conducting segment had prolonged the action potential proximal to that segment.

For the same modified circuit, we can ask if the actions of drugs that both block fast channels and alter refractoriness would be changed. In other words, would the mechanism of action of a drug depend on the active and passive properties of the path? If conduction in the abnormal segment had been slowed by a positive shift in the threshold potential, the slowly conducting segment certainly would be sensitized to the fast channel blocking effect of the drug and this might have several consequences. In one case, propagation in the abnormal segment might slow and then block at a reasonable drug concentration, thus terminating circus movement. Another example well worth mentioning is a possibility suggested by Wit (personal communication). As a consequence of his studies on reentrant excitation due to circus movement in the epicardial border zone of the infarcted canine heart, he has suggested the following: If slow conduction results from partial uncoupling of cells and if that uncoupling results from an elevated intracellular calcium activity, the following mechanism of action of class I antiarrhythmic drugs should be entertained. As the drug reduces net inward sodium current during each action potential, sodium-for-calcium exchange will be enhanced, intracellular calcium activity will be lowered, and gap junctional conductance increased. In this way, a segment demonstrating abnormally slow conduction might change to one in which conduction is normal.

The main purpose of these speculations is to emphasize that lacking accurate and precise descriptions of the active and passive properties of all parts of the circus path and detailed information on its geometry, it probably is not fruitful to speculate on the mechanisms by which antiarrhythmic drugs of different types terminate reentrant excitation due to circus movement.

It is also possible that some arrhythmias associated with ischemia and infarction result from automatic impulse generation. Janse et al. [20] have provided evidence from studies on acutely ischemic isolated supported hearts that injury currents between the ischemic zone and the surrounding endocardium may enhance phase 4 depolarization of subendocardial Purkinje fibers. Measurements of transmembrane potentials in isolated preparations of subendocardial tissues from canine hearts with 24-hour infarcts have shown abnormal automaticity [21, 22] and data for the Harris dog from many sources support the concept that an automatic rhythm frequently underlies the ventricular arrhythmia typically present 24 hours after coronary occlusion [23].

This automatic rhythm can be readily captured by overdrive, but does not typically demonstrate either overdrive suppression or enhancement, and thus it most likely results from abnormal automaticity generated in partially depolarized fibers [24]. The problem here is similar to that for reentrant rhythms. First, a self-sustaining rhythm can result from either automatic or triggered activity [25]; the automatic activity may occur in normally polarized Purkinje fibers or in partially depolarized Purkinje or ventricular muscle fibers. Depending on the level of membrane potential, the characteristics of the automatic rhythm and its response to antiarrhythmic drugs will differ.

If the rhythm is generated by normally polarized Purkinje fibers, we currently assume that the phase 4 depolarization is caused by the pacemaker current designated i_f [26]. A number of antiarrhythmic drugs have been shown to block this current [27, 28]. Also, any drug that blocks fast channels by shifting the threshold potential toward zero will tend to slow the automatic rhythm; this effect can be demonstrated for agents like ethmozin that do not modify the initial slope of phase 4 [29]. Ordinarily, in Purkinje fiber bundles from canine hearts, there is a transient increase in K_o during the plateau due to net K efflux and a time-dependent decrease in K_o after repolarization due to the Na-K pump [30]. These changes in K_o will modify the conductance of potassium channel g_{K1}, and it is quite possible that a decrease in K_o early during phase 4 results in a decrease in g_{K1} and thereby contributes to the slow depolarization typical of the automatic cell. The decline in pump current during phase 4 also will cause an inward shift in net current and a slow depolarization. Antiarrhythmic drugs that modified the duration or potential level of the plateau would modify K efflux, and drugs that either blocked fast channels or changed rate would modify pump current. Finally, recent results suggest [31] that one of the sarcolemmal calcium currents, $i_{Ca,T}$ may contribute to the latter part of phase 4 depolarization in an automatic Purkinje fiber. Although not much is known about the effects of antiarrhythmic drugs on this channel, it at least provides an additional possible site of drug-induced modification of automatic activity.

It is clear that, lacking data from direct demonstrations, it is impossible to state the mechanism by which an antiarrhythmic drug might influence a rhythm due to enhanced normal automaticity in an ischemic or infarcted heart even if one could unambiguously prove that the arrhythmia did in fact result from enhanced normal automaticity and not from afterdepolarizations or abnormal automaticity.

As previously mentioned, abnormal automaticity is a more probable cause of arrhythmias in ischemic tissues than is enhanced normal automaticity. Abnormal automaticity generally has been assumed to result from a waning of outward K current and a waxing of inward current in the channels that carry $I_{Ca,L}$. If the maximum diastolic potential is sufficiently reduced (-55 to -60 mV) the automatic rhythm is not influenced appreciably by overdrive, and so one can assume that neither activity-dependent changes in K_o nor

activity-induced changes in Na-K pump current plays a prominent role in modulating rate. For an arrhythmia caused by this mechanism, drugs might cause slowing or termination by a variety of effects. The essential abnormality underlying the mechanism is failure to attain a normal resting or maximum diastolic potential, and thus any drug capable of significantly increasing net outward current might well shift the membrane potential from the low to high level [32–34] and abolish the arrhythmia. In general such a change in net current could result from a decrease in inward current, as would result from block of window current in fast channels, or from partial block of current in slow channels of the $I_{Ca,L}$ type. It also might result from augmented outward current due to drug effect on the delayed rectifier. Finally, a drug might modify the activation of $I_{Ca,L}$ so that the initial phase of slow depolarization failed to attain threshold with sufficient rapidity.

In terms of arrhythmias caused by ischemia and infarction, the greatest body of data on drug effects is available for the 24-hour arrhythmias in the Harris dog, those we judge to be caused most often by abnormal automaticity. Unfortunately, most drugs that have been tested in this model have been at least transiently effective in causing a restoration of sinus rhythm. This may reflect the multiplicity of actions of most of these drugs, the multiple way in which drugs can modify abnormal automaticity or, more likely both. Either way, it seems that for automatic as for reentrant rhythms, it would be most difficult if not impossible to ascribe antiarrhythmic efficacy to a specific drug action or to a specific set of actions.

REFERENCES

1. Rosenfeld, J., Rosen, M.R., and Hoffman, B.F. 1978. Pharmacologic and behavioral effects on arrhythmias that immediately follow abrupt coronary occlusion: a canine model of sudden coronary death. *Am. J. Cardiol.* 41:1075–1082.
2. Durrer, D., van Dam, R.T., Freud, G.E., and Janse, M.J. 1971. Reentry and ventricular arrhythmias in local ischemia and infarction of the intact dog heart. *Proc. K. Ned. Akad. Wet. (Biol. Med.)* 74:321–334.
3. Wit, A.L., Allessie, M.A., Bonke, F.I.M., et al. 1982. Electrophysiological mapping to determine the mechanism of experimental ventricular tachycardia initiated by premature impulses: experimental approaches and initial results demonstrating reentrant excitation. *Am. J. Cardiol.* 49:166–185.
4. El-Sherif, N., Hope, R.R., Scherlag, B.J., and Lazzara, R. 1977. Reentrant ventricular arrhythmias in the late myocardial infarction period. 1. Conduction characteristics in the infarction zone. *Circulation* 55:686–702.
5. Cranefield, P.F., 1975. *The Conduction of the Cardiac Impulse.* Mount Kisco, NY, Futura Publishing.
6. Antzlevitch, C., Davidenko, J.M., Shen, X., and Moe, G.K. 1985. Refected reentry: electrophysiology and pharmacology. In *Cardiac Electrophysiology and Arrhythmias*, D.P. Zipes and J. Jalife, eds. pp. 253–264, Orlando, FL, Grune & Stratton.
7. Frame, L.H., Page, R.L., and Hoffman, B.F. 1986. Atrial reentry around an anatomical barrier with a partially refractory excitable gap. A canine model of atrial flutter, *Circ. Res.* 58:495–511.
8. Wit, A.L., and Cranefield, P.F. 1978. Reentrant excitation as a cause of cardiac arrhythmias. *Am. J. Physiol.* 235:H1–H17.
9. Allessie, M.A., Bonke, F.I.M., and Schopman, F.J.G. 1977. Circus movement in rabbit

atrial muscle as a mechanism of tachycardia. III. The "leading circle" concept: a new model of circus movement in cardiac tissue without the involvement of an anatomical obstacle. *Circ. Res.* 41:9–18.
10. Allessie, M.A., Lammers, W.J.E.P., Bonke, F.I.M., and Hollen, J. 1984. Intraatrial reentry as a mechanism for atrial flutter induced by acetylcholine and rapid pacing in the dog. *Circulation* 70:123–135.
11. Lewis T. 1925. *The Mechanism and Graphic Registration of the Heart Beat*, 3rd Ed., London, Shaw Sons.
12. Arnsdorf, M.F. and Sawicki, G.J. 1987. Effects of quinidine sulfate on the balance among active and passive cellular properties that comprise the electrophysiological matrix and determine excitability in sheep Purkinje fibers. *Circ. Res.* 61:244–255.
13. Frame, L.H., Page, R.L., Boyden, P.A., et al. 1987. circus movement in the canine atrium around the tricuspid ring during experimental atrial flutter and during reentry in vitro. *Circulation* 76:1155–1175.
14. Rosenblueth, A., and Garcia-Ramos, J. 1947. Studies on flutter and fibrillation. II. The influence of artificial obstacles on experimental auricular flutter. *Am. Heart J.* 33:677–684.
15. Dangman, K.H. and Hoffman, B.F. 1981. *In vivo* and *in vitro* antiarrhythmic and arrhythmogenic effects of N-acetylprocainamide. *J. Pharmac. Exp. Ther.* 217:851–862.
16. Wu, K.M. and Hoffman, B.F. 1987. Effect of procainamide and N-acetyl-procainamide on atrial flutter: studies *in vivo* and *in vitro*. *Circulation* 76:1397–1408.
17. Campbell, T.J. 1987. Cellular electrophysiological effects of D- and DL-sotalol in guinea-pig sinoatrial node, atrium anf ventricle and human atrium: differential tissue sensitivity. *Br. J. Pharmacol.* 90:593–599.
18. Hondeghem, L.M. and Katzung, B.G. 1984. Antiarrhythmic agents: the modulated receptor mechanism of action of sodium and calcium channel-blocking drugs. *Ann. Rev. Pharmacol. Toxicol.* 24:387–423.
19. Wit, A.L., Hoffman, B.F., and Cranefield, P.F. 1972. Slow conduction and reentry in the ventricular conducting system. I. Return extrasystole in canine Purkinje fibers. *Circ. Res.* 30:1–10.
20. Janse, M.J.J., van Capelle, F.J.L., Morsink, H., et al. 1980. Flow of "injury" current and patterns of excitation during early ventricular arrhythmias in acute regional myocardial ischemia in isolated porcine and canine hearts: evidence for two different arrhythmogenic mechanisms. *Circ. Res.* 47:151–165.
21. Hauswirth, O., Noble, D., and Tsien, R.W. 1969. The mechanisms of oscillatory activity at low membrane potentials in cardiac Purkinje fibers. *J. Physiol.* 200:255–265.
22. Dangman, K.H. and Hoffman, B.F. 1985. Effects of single premature stimuli on automatic and triggered rhythms in isolated canine Purkinje fibers. *Circulation* 71:813–822.
23. LeMarec, H., Dangman, K.H., Danilo, P., and Rosen, M.R. 1985. An evaluation of automaticity and triggered activity in the canine heart one to four days after myocardial infarction. *Circulation* 71:1224–1236.
24. Dangman, K.H. and Hoffman, B.F. 1983. Studies on overdrive stimulation of canine cardiac Purkinje fibers: maximal diastolic potential as a determinant of the response. *J. Am. Coll. Card.* 2:1183–1190.
25. Cranefield, P.F. and Aronson, R. in press. *Cardiac Arrhythmias: The Role of Triggered Activity and Other Mechanisms*. Mt. Kisco, NY, Futura Publishing Co.
26. DiFrancesco, D. 1981. A new interpretation of the pacemaker current in calf Purkinje fibers. *J. Physiol.* 314:359–376.
27. Carmeliet, E., and Saikawa, T. 1982. Shortening of the action potential and reduction of pacemaker activity by lidocaine, quinidine and procainamide in sheep cardiac Purkinje fibers. An effect on Na or K currents? *Circ. Res.* 50:257–272.
28. Carmeliet, E., and Xhonneaux, R., 1971. Influence of lidoflazine on cardiac transmembrane potentials and experimental arrhythmias. *Naunyn Schmiedeberges Arch. Pharmacol.* 268:210–228.
29. Dangman, K.H. and Hoffman, B.F. 1983. Antiarrhythmic effects of ethmozin in cardiac Purkinje fibers: suppression of automaticity and abolition of triggering. *J. Pharmacol. Exp. Ther.* 227:578–586.
30. Kline, R.P., Cohen, I., Falk, R., and Kupersmith, J. 1980. Activity-dependent extracellular K^+ fluctuations in canine Purkinje fibres. *Nature* 286:68–71.

31. Hagiwara, N., Irisawa, H. and Kameyama, M. 1988. Contribution of two types of calcium currents to the pacemaker potentials of rabbit sino-atrial node cells. *J. Physiol.* 395:233–253.
32. Katzung, B.O. and Morgenstern, J.A. 1977. Effects of extracellular potassium on ventricular automaticity and evidence for a pacemaker current in mammalian ventricular myocardium. *Circ. Res.* 40:105–111.
33. Gadsby, D.C. and Cranefield, P.F. 1977. Two levels of resting potential in cardiac Purkinje fibers. *J. Gen. Physiol.* 70:725–746.
34. Wiggins, J.R. and Cranefield, P.F. 1976. Two levels of resting potential in canine cardiac Purkinje fibers exposed to sodium-free solutions. *Circ. Res.* 39:466–474.

22. CARDIAC INNERVATION AND SUDDEN DEATH: NEW STRATEGIES FOR PREVENTION

PETER J. SCHWARTZ

The last decade has witnessed the acceptance of the concept that the sympathetic nervous system plays a critical role in the genesis of life-threatening arrhythmias, particularly in the setting of ischemic heart disease [1–4]. The clinical counterpart of this concept has been the evaluation of pharmacologic blockade of beta-adrenergic receptors in the prevention of sudden death among patients with a myocardial infarction. The results have been largely favorable [5, 6] and have had a profound impact on the clinical management of these patients.

Of more recent origin, and still in a developing phase, is the concept that the presence of adequate vagal tone and reflexes during acute myocardial ischemia can exert an antifibrillatory role [4, 7–11]. A significant obstacle to this concept is represented by the uncritical acceptance of the profibrillatory role of profound bradycardia secondary to excessive vagal reflexes, particularly in the setting of acute inferior myocardial infarction, an important phenomenon correctly described by Pantridge [12]. As discussed later, there has been confusion between bradycardia and reduction in heart rate, two potentially quite different conditions.

In this chapter, I will briefly review the background suggesting that fresh and clinically viable approaches for the prevention of sudden cardiac death can be derived from experiments involving manipulation of the autonomic nervous system. I will then discuss the feasibility, the potential usefulness, and the limitations of some of these new strategies. This will be accomplished in sequence for the sympathetic and parasympathetic nervous systems.

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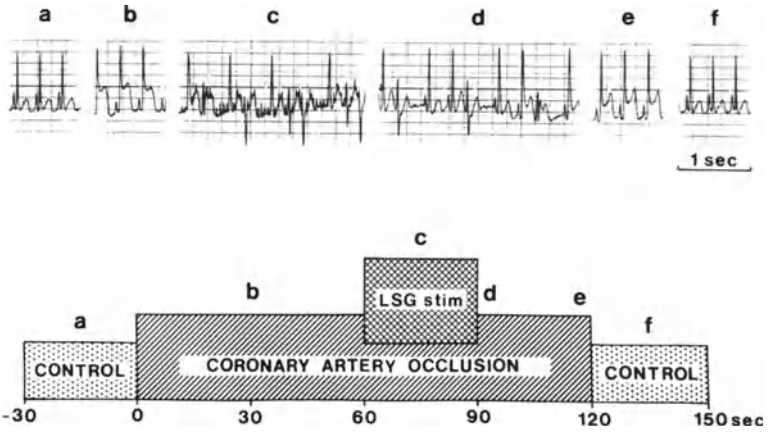

Figure 22-1. Diagram of experimental model with example of ECG changes. Letters indicate different phases of the occlusion-stimulation protocol during which the ECG was recorded. Ventricular arrhythmias appear during left stellate ganglion stimulation, they persist during the last part of coronary artery occlusion, and the ECG tracing returns to control a few seconds after release of occlusion. LSG stim = left stellate ganglion stimulation.

SYMPATHETIC NERVOUS SYSTEM

Experimental Background

The evidence for the different arrhythmogenic potential of right- and left-sided cardiac sympathetic nerves in inducing life-threatening arrhythmias has already been reviewed [13]. Here, I would like to stress that this difference is quantitative, not qualitative. The greater likelihood of inducing ventricular tachyarrhythmias by stimulation of the left stellate ganglion, particularly during a brief episode of acute myocardial ischemia, has indeed led to the development of a quite reproducible animal model (figure 22–1) [14]. The analysis of the response of this preparation to several drugs (figure 22–2) [15–17] indicates that ventricular tachycardia and fibrillation are precipitated by a combination of two effects of sympathetic activation: an increase of the area of ischemia and an electrophysiologic effect mediated by both alpha- and beta-adrenergic receptors.

Following the pioneering work by Feigl [18], much evidence has been presented to indicate that even in conscious animals not pretreated with beta-blockers, left-sided sympathetic nerves exert a tonic constraint on the capability of coronary arteries to dilate [19]. Extending this concept, Janse et al. [20] demonstrated that the activation or ablation of left-sided cardiac sympathetic nerves increases and decreases, respectively, the extent and severity of ischemia produced by a brief coronary occlusion in the same animal, independently of heart rate and blood pressure changes.

The electrophysiologic mechanism(s) by which sympathetic stimulation

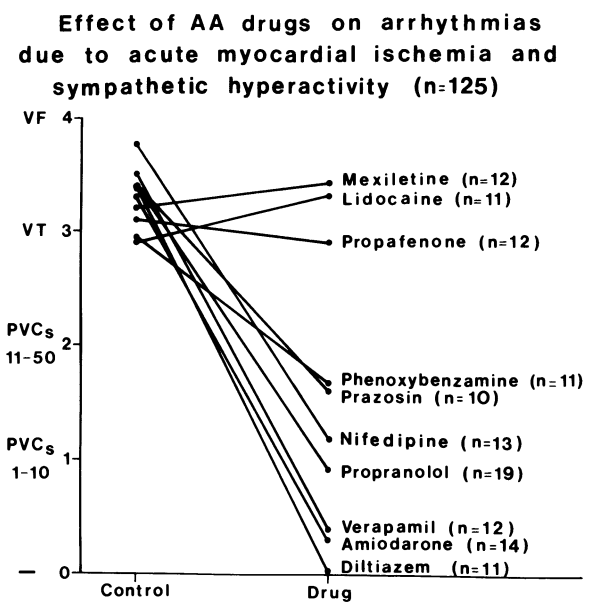

Figure 22-2. The antiarrhythmic effects of the drugs tested. The number of animals in each group appears in brackets next to drug name. An arbitrary grading for ventricular arrhythmias was established as follows: VF = 4, VT = 3, frequent (11-50) PVCs = 1 – 10 PVCs = 1. For each animal "control score" is the average result of three trials in control, and "drug score" is the average result of three trials after drug administration. The figure shows the mean score for each group studied before and after treatment. VF = ventricular fibrillation; VT = ventricular tachycardia; PVCs = premature ventricular contractions.

initiates ventricular arrhythmias is not yet fully elucidated [21, 22]. The rather unexpected finding [20] that left stellate stimulation during acute myocardial ischemia can improve conduction through areas in which it was blocked (figure 22–3) would perhaps make more likely an automatic, rather than reentrant, mechanism. These data were obtained by recording simultaneously DC extracellular electrograms from 60 left ventricular epicardial sites. The electrodes were arranged in five rows of 12, and interelectrode distance was 4 mm. Quite recently, evidence has also been provided that links cardiac sympathetic nerves to "triggered activity;" namely, stimulation of the left stellate ganglion in anesthetized cats was found capable of inducing delayed afterdepolarizations [23] (figure 22–4). This may represent another mechanism by which sympathetic stimulation may generate arrhythmias, independent of myocardial ischemia. Indeed, the origin of ventricular tachycardia and fibrillation often depends on the first initiating ventricular premature beat, and a delayed afterdepolarization, as well as an automatic beat, could play such a trigger role.

The likelihood of a reflex increase in sympathetic activity during acute myocardial ischemia and the importance of this cardiocardiac reflex in en-

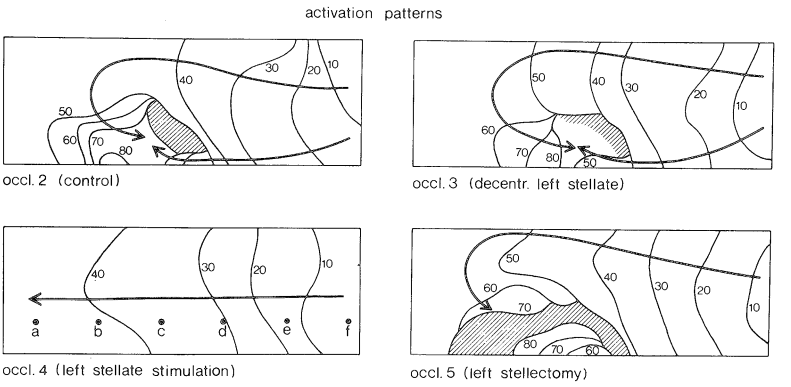

Figure 22-3. Effects of left stellate ganglion stimulation on conduction in ischemic myocardium. Isochrones separate areas activated within the same 10 msec interval; areas of conduction block are shaded. a to f indicate different sites of recording (1 of 12 rows). Activation patterns are shown five minutes after each of the following: control occlusion; the third occlusion, before which the left stellate ganglion was decentralized; the fourth occlusion, during which the left stellate ganglion was stimulated; and the fifth occlusion, after removal of the left stellate ganglion. Note improvement of conduction during stellate ganglion stimulation.

hancing ventricular arrhythmias was demonstrated almost 20 years ago [24–26].

The converse aspect of the potentially detrimental effects of left sympathetic activation during the course of acute myocardial ischemia, and probably also in hearts with chronically restricted coronary flow or with healed myocardial infarctions, is represented by the multiple evidence of the favorable effects that result from the surgical ablation of these nerves, without a significant impairment of myocardial contractility [27]. One specific advantage of a selective sympathetic denervation is the possibility of not entirely depriving the myocardium of the adrenergic support necessary for the maintenance of an adequate left ventricular function. Based on these notions it is logical to wonder whether these positive effects can be transposed to the clinical setting.

Clinical Studies

In the most typical example of neurally mediated noncoronary sudden death (i.e., the idiopathic long Q-T syndrome [28]), the surgical ablation of the lower part of the left stellate ganglion along with the first four thoracic ganglia (high thoracic left sympathectomy [HTLS]) has already proven to be the most effective treatment for those patients who continue to have syncopal attacks despite adequate beta-blockade. The most recent data of the International Prospective Registry [29, 30], which has already enrolled over 1,200 individuals, indicate that among this subgroup at especially high risk there have been 5 sudden deaths out of 64 patients (8 percent); the mean follow-up is five years (range 0.5 to 16) and the five-year survival is 91 percent. This is a very rewarding result; indeed, with the combination of beta-blockers and high thoracic left sympathectomy, the five-year mortality for symptomatic

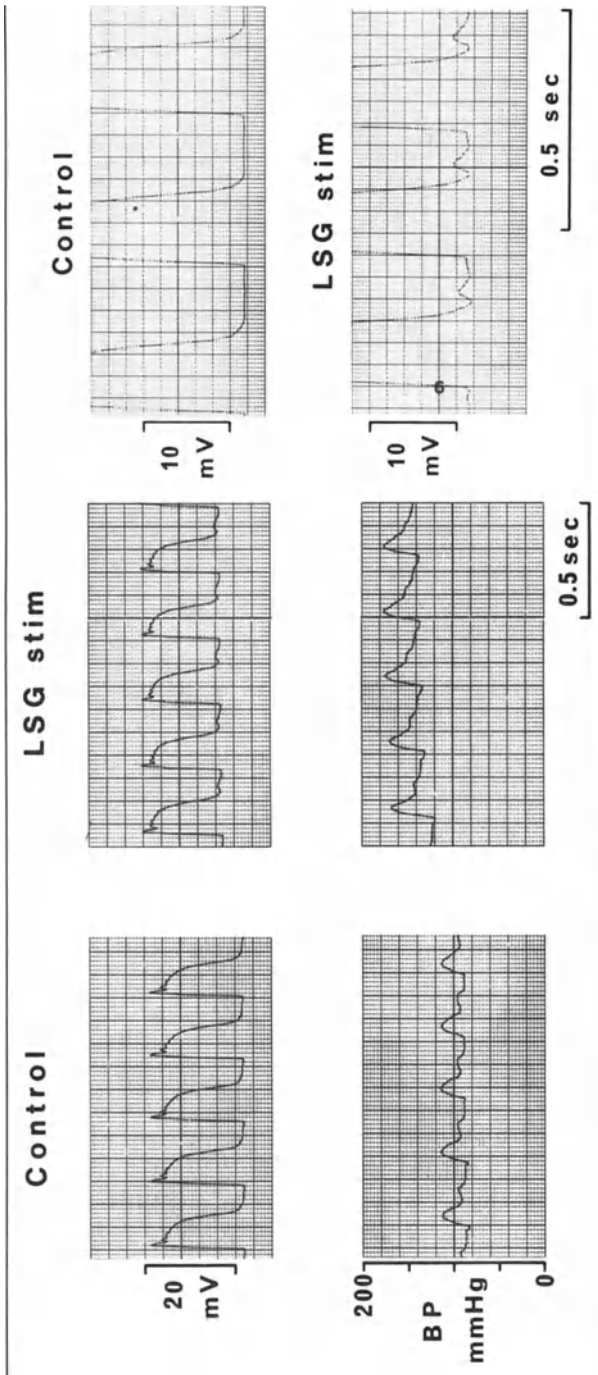

Figure 22-4. Simultaneous recording of monophasic action potential (MAP) (upper trace) and blood pressure (BP, lower trace) in control conditions (left) and during stimulation (center) of the left stellate ganglion (LSG stim). Note the rise in blood pressure and the presence of delayed after depolarizations (DAD) after LSG stim. On the right, high amplification of the MAP in control condition and after LSG stimulation when DAD are present.

patients has decreased from approximately 32 percent among untreated patients to approximately 3 percent among treated patients.

The situation is much more complex, however, when dealing with ischemic heart disease. Here, the primary objective of sympathetic denervation is to prevent or reduce the effect, potentially lethal in an acutely ischemic heart, of a sudden and nonhomogeneous release of noradrenaline. This nonhomogeneity is likely to be accentuated in hearts that have already suffered an infarction [31].

The clinical evaluation of a nontraditional intervention requires a specifically suited population; in the present case, it did seem necessary to deal with patients at high risk for sudden death but without a significant left ventricular dysfunction to allow a comparison with beta-adrenergic blocking agents. It should be kept in mind that when our study was initiated in 1979, the evidence for a protective effect of beta-blockers was growing but was far from fully established. A suitable population was identified through a targeted case-control epidemiologic study involving 250 postmyocardial infarction patients with a 5-year mean follow-up [32]. Patients below age 65 discharged from the hospital after an anterior myocardial infarction complicated by early and primary ventricular fibrillation were found to have a one-year mortality rate of 27 percent compared to 6 percent for their matched controls who had myocardial infarction without ventricular fibrillation. In this high risk group, the incidence of sudden death was 71 percent. Interestingly, the long-term prognosis for patients with an inferior myocardial infarction did not seem to be affected by the occurrence of early ventricular fibrillation (figure 22–5); an observation that explains in part the incorrect but widespread belief that early ventricular fibrillation has no adverse prognostic significance.

Based on this epidemiologic finding, it was decided to study two groups of postmyocardial infarction patients, one at higher and one at lower risk for sudden death [33]. The first group (n=144) included patients with an anterior myocardial infarction complicated by primary ventricular fibrillation or by documented ventricular tachycardia; the second group (n=869) included patients with an anterior myocardial infarction without evidence for these life-threatening arrhythmias. Patients in group 1 were randomized to either placebo, the beta-blocking agent oxprenolol (160 mg), or high thoracic left sympathectomy (HTLS). Patients in group 2 were randomized only to either placebo or oxprenolol. Randomization took place 30 days after the myocardial infarction, and the mean follow-up was 22 months (range 6 to 48 months). Thirty-two clinical centers located in northern Italy participated.

In group 1, the incidence of sudden death was indeed very high in the placebo group (21.3 percent) and was strikingly reduced to 2.7 percent and to 3.6 percent by oxprenolol and by HTLS respectively. In group 2, the incidence of sudden death in the placebo group was indeed lower (5.2 percent) and was still reduced by oxprenolol to 1.6 percent. The results were quite

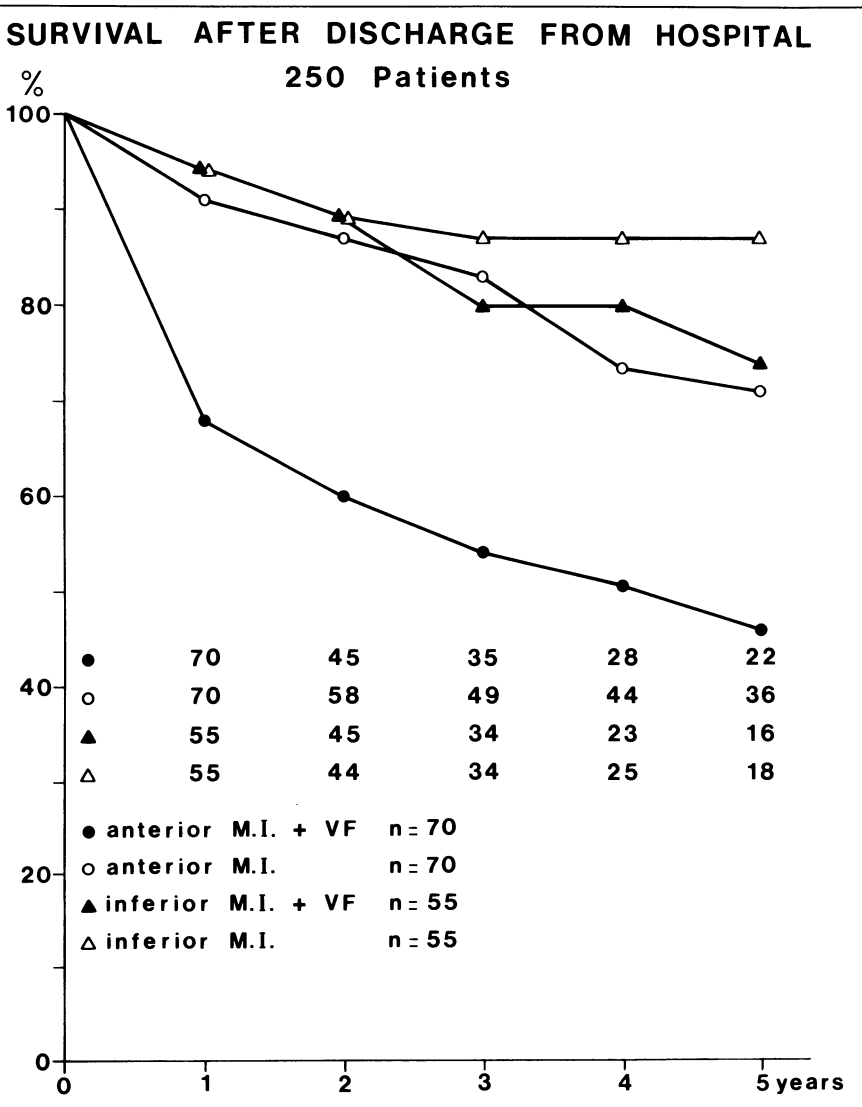

Figure 22-5. Survival curves for the four groups of patients (total 250) after hospital discharge. The numbers represent the number of patients studied at each interval. MI = myocardial infarction; VF = ventricular fibrillation.

similar for total mortality and the differences were significant according to both "intention to treat" and "drug efficacy" type of analysis.

This clinical trial was unique for the populations studied and for one of the treatments employed. It confirmed in a prospective study the validity of the preceding epidemiologic investigation, and it demonstrated that both pharmacologic and surgical antiadrenergic interventions can significantly reduce

the incidence of sudden cardiac death among postmyocardial infarction patients. These clinical results also concur with the experimental background that prompted this study.

Implications and Future Directions

Is there a place for unilateral cardiac sympathetic denervation in the clinical management of life-threatening arrhythmias? For the idiopathic long QT syndrome, the data clearly indicate that if a patient continues to have syncope despite full dose beta-blockade (an event that occurs in approximately 20 to 25 percent of treated patients), there should be no hesitation in proceeding with HTLS. Protection from sudden death can then be expected in 85 to 90 percent of these patients. The synergistic effect of beta-blockade and HTLS [30] makes any other form of therapy experimental and justifiable only after failure of the combined antiadrenergic treatment.

With respect to ischemic heart disease, the situation is more complex. At this time, most factual considerations have to be based on the only controlled study available, the multicenter clinical trial just described. Given the fact that HTLS was as effective as the beta-adrenergic blocking agent used, at first glance there would seem to be no reason to consider a surgical intervention when medical therapy is equally effective. This view does not take into account the fact that a considerable number of postmyocardial infarction patients with adequate left ventricular function, such as the participants in the Italian study, have contraindications or poor tolerance for beta-adrenergic blocking therapy. For these patients, HTLS may represent a reasonable alternative. An attractive characteristic of HTLS is that it can be associated with any pharmacologic therapy deemed useful for a given patient without the problems that often occur with multiple drug regimens.

One may be tempted to say that the most interesting role for HTLS might be in the management of those high risk patients who cannot be treated with beta-blockers because of significant left ventricular dysfunction. Although this might be true, the data available do not allow such an extrapolation. This important point could be settled only by a new multicenter study in which these patients would be randomized to HTLS as one treatment and to whatever else is viewed as logical and potentially useful.

Personally, I am rather skeptical about the future use of HTLS as a single modality of treatment, even for selected groups of postmyocardial infarction patients. This view does not at all reflect skepticism concerning the validity and efficacy of the intervention, which is supported by many converging pieces of evidence; rather, it reflects the perception of a basic difficulty in the adoption of nontraditional *surgical* interventions in *clinical* medicine.

PARASYMPATHETIC NERVOUS SYSTEM

Experimental Background

Several laboratories have provided evidence for an antifibrillatory effect of vagal stimulation [4, 7–11, 34–36], particularly in the presence of increased

sympathetic activity. Most of these studies were performed in open chest, anesthetized animals. So far, the concept of vagally mediated protection from sudden cardiac death has received little, if any, clinical consideration. This seems to be largely a consequence of results obtained either by electrical stimulation of the cervical vagus, viewed as not feasible or unacceptable in conscious individuals, or by administration of cholinergic drugs, such as physostigmine [37], metabolized too rapidly and/or having systemic effects.

Several pieces of relevant information, which may open new perspectives on the relationship between the parasympathetic nervous system and life-threatening arrhythmias with implications for novel preventive strategies, have been gathered by a series of experiments conducted in an animal model for sudden cardiac death that Lowell Stone and I had developed years ago [38, 39]. The main feature of this preparation is the reproducible induction of ventricular fibrillation by the combination of a brief ischemic episode in conscious animals with a one-month old myocardial infarction.

Briefly, with the animal under general anesthesia, an anterior myocardial infarction is produced, a balloon occluder is positioned around the circumflex coronary artery, and the dogs are chronically instrumented. One month later they perform a submaximal exercise stress test and, whenever heart rate reaches 210 to 220 beats/min, the circumflex coronary artery is occluded for two minutes; after one minute of occlusion the motor-driven treadmill stops and the occlusion continues for another minute. The animals perform this exercise and ischemia test with steel paddles applied to the chest so that, if necessary, cardioversion can be performed within seconds of onset of ventricular fibrillation. This clinically relevant combination of transient myocardial ischemia at the time of physiologically elevated sympathetic activity in conscious dogs with a healed myocardial infarction results in ventricular fibrillation in 60 percent of the animals. One important characteristic of this model is the fact that the outcome during the exercise and ischemia test (ventricular fibrillation in "susceptible" animals, but not in "resistant" animals) is highly reproducible for several months.

A critical observation made several years ago was that the heart rate response to myocardial ischemia is quite different in susceptible and resistant animals [8]. Whereas the former group tends to increase further the already elevated (by exercise) heart rate, the latter tends to show a decrease. This heart rate reduction, despite ischemia and continuation of exercise, is prevented by atropine and clearly indicates the presence of powerful vagal reflexes. This interested us in the assessment of a potential relationship between baroreflex sensitivity (BRS), largely a marker of vagal reflexes, and susceptibility to sudden death. BRS was assessed by correlating the heart rate response to a blood pressure increase produced by a bolus injection of phenylephrine, according to the method described by Smyth et al. [40].

The initial observations [41] were confirmed and extended by our most recent results in 192 dogs with a healed myocardial infarction [42]. BRS, evaluated a few days before the exercise and ischemia test, was lower among

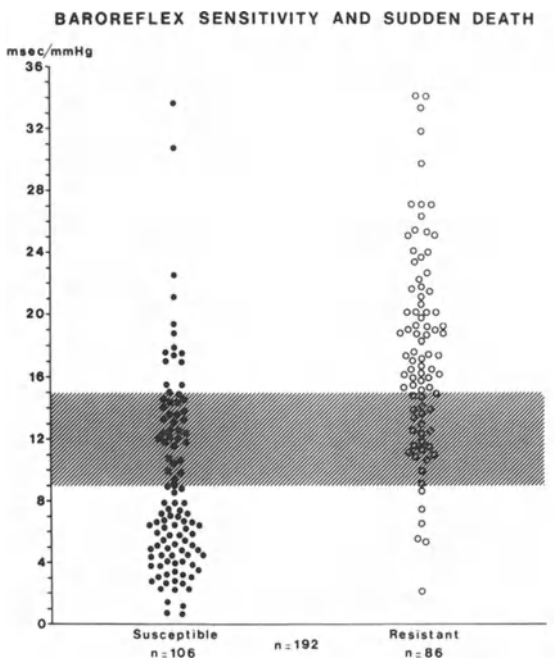

Figure 22-6. Baroreflex sensitivity in 151 postinfarction dogs and its relationship to susceptibility to sudden death. The darkened area is an arbitrary "gray zone." Below the limit of 9 ms/mm Hg, 95 percent of the dogs are susceptible to sudden death, whereas above 15 ms/mm Hg, the great majority prove to be resistant to sudden death during the exercise plus ischemia test.

susceptible dogs than resistant dogs (9.1 ± 6.0 vs. 17.7 ± 6.5 ms/mm Hg, P<.0001). Analysis of the individual data allows the calculation of risk for sudden death with respect to BRS measured four weeks post myocardial infarction (figure 22-6). The risk increased from 20 percent of dogs (15 of 73) having a BRS > 15 ms/mm Hg to 91 percent of dogs (62 of 68) having a BRS < 9 ms/mm Hg (P<.001). When the validity of a BRS < 9 ms/mm Hg in determining subsequent risk was assessed, sensitivity was found to be 58 percent, specificity 93 percent, and the predictive value 91 percent. Thus, analysis of BRS after a myocardial infarction allows the identification of a group of animals at very high risk for ventricular fibrillation during a new ischemic episode.

In 55 animals BRS was analyzed before and four weeks after myocardial infarction and was reduced from 17.8 ± 6.6 to 13.5 ± 6.7 ms/mm Hg (P<.001). When the changes in individuals included in this internal control study were examined, it was evident that a reduction in BRS (change > 3 ms/mm Hg) occurred in 73 percent of animals, whereas no change was present in 20 percent and an increase occurred in 7 percent.

We then analyzed the BRS values obtained before the creation of the myocardial infarction in relationship to the outcome during the exercise and ischemia test and also to the sudden deaths occurring during the first few days postmyocardial infarction. These data were available for 68 dogs. BRS in the premyocardial infarction condition was lower among the susceptible animals than the resistant (16.2 ± 5.9 vs. 22.2 ± 6.2 ms/mm Hg, P<.001). The individual data allow the calculation of risk for sudden death *after* a myocardial infarction with respect to BRS measured *before* myocardial infarction. The risk increased from 35 percent of dogs (9 of 26) having a BRS ≥ 20 ms/mm Hg to 85 percent of dogs (17 of 20) having a BRS < 14 ms/mm Hg (P<.001). Thus, the wide spectrum of autonomic nervous system responses seems to contain, even in basal conditions, information useful for identifying individuals with reduced cardiac electrical stability who may be highly vulnerable to acute ischemic events.

Is it possible to modify the autonomic state of an animal with depressed BRS by increasing its vagal responses? Physical exercise seemed, in theory, a potentially adequate means because it induces physiologic changes mimicking the effects of augmented vagal activity. Accordingly, two groups of susceptible dogs that had a markedly reduced BRS and had undergone ventricular fibrillation during the exercise and ischemia test were randomized to daily exercise for six weeks or to rest in a cage for the same period of time [43]. Among the cage-rested dogs, BRS did not vary appreciably (from 4.7 ± 1.3 to 6.1 ± 4.8, NS); a clear increase occurred among the dogs that were exercised (from 5.4 ± 1.2 to 16.3 ± 5.0, *P*<.01). Moreover, the exercised dogs all became resistant (i.e., they survived the exercise and ischemia test), whereas all but one of the cage-rested dogs remained susceptible to ventricular fibrillation. It seems of more than anecdotal interest that the only susceptible and cage-rested dog that survived the exercise and ischemia test was also the only one that, for unknown reasons, had a spontaneous increase in its BRS (from 4.4 to 16.7 ms/mm Hg), which reached the range typical of resistant animals. The precise mechanism by which daily exercise modifies baroreceptor reflexes and cardiac electrical stability requires further investigation; however, a combined effect on autonomic reflexes (increase in vagal and decrease in sympathetic reflexes) and on left ventricular function is likely to have contributed to these results.

Another means by which vagal activity can be increased is by direct electrical stimulation of the cervical vagus, a maneuver thought unsuitable in the nonanesthetized state. During the last few years, our group has developed a technique using chronically implanted electrodes to perform stimulation of the right or left cervical vagus for up to several months [11, 44]. The technique produces modest or no side effects, depending on both the stimulation parameters and individual variability. It was then possible to evaluate the potential value of vagal stimulation, performed a few seconds after the beginning of coronary occlusion, in reducing the incidence of sudden death during the exercise stress test.

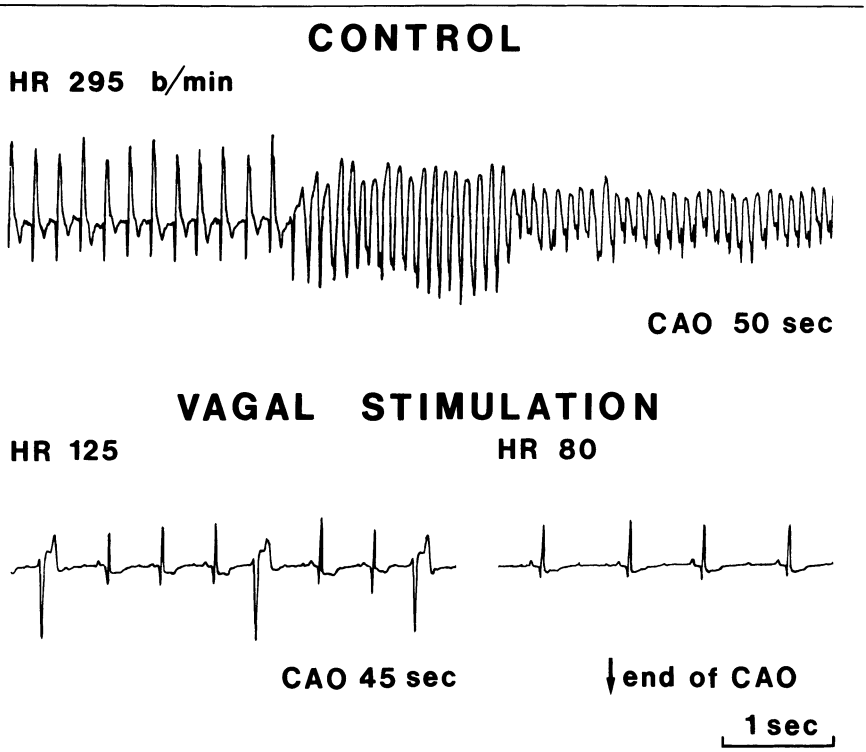

Figure 22-7. Electrocardiographic recording in a susceptible dog during an exercise plus ischemia test one month after myocardial infarction. In the upper tracing, the control test: 50 seconds after the coronary artery occlusion (CAO), onset of ventricular tachycardia that deteriorated into ventricular fibrillation. In the lower tracing, the same dog in which electrical stimulation of the right cervical vagus is performed during a subsequent exercise plus ischemia test: only a few premature ventricular beats are noted.

This study involved two groups of susceptible animals. After the first exercise and ischemia test had, by definition, resulted in ventricular fibrillation in 100 percent of dogs, they were randomized to either repeat the test in control conditions (n=25) or to repeat it with vagal stimulation [45]. During this second trial ventricular fibrillation occurred in 23 of 25 dogs (92 percent), whereas this occurred in only in 3 of 15 (20 percent) vagally stimulated dogs ($P<.001$). An example of vagally mediated protection is shown in figure 22–7. Vagal stimulation produced a mean reduction of 80 beats/min from 216 ± 33 to 136 ± 29 beats/min. The potent antifibrillatory action of vagal stimulation, observed in this study, was largely dependent on the heart rate reduction. Although heart rate was markedly reduced, bradycardia was not produced; this issue is relevant to what I mentioned above in regard to the important difference between bradycardia, which can indeed be arrhythmogenic, and the reduction from unduly and detrimentally high heart rates

that, particularly under ischemic conditions, can favor ventricular fibrillation. Thus, vagal stimulation performed in conscious animals with a healed myocardial infarction greatly decreases the susceptibility to life-threatening arrhythmias caused by the clinically relevant combination of transient myocardial ischemia and elevated sympathetic activity.

What can be expected by the converse operation, namely by interfering with the vagal outflow to the heart? The effect of atropine has been evaluated in 41 resistant dogs, and 9 of them (22 percent) developed ventricular fibrillation during the exercise and ischemia test [46]. When these trials were repeated in control conditions while at the same keeping heart rate level with atropine, most dogs still developed ventricular fibrillation. This suggests that vagal reflexes, with their protective effect mostly due to the attendant heart rate reduction, are the *sole* determinant of survival in approximately one fifth of the postmyocardial infarction resistant dogs. The majority of resistant animals seems to be characterized, not only by intact vagal reflexes, but by the absence of excessive sympathetic responses to acute myocardial ischemia.

Clinical Implications

Do these experimental data have any direct clinical correlation? Two recently completed studies are relevant to this issue. The first [47] has compared the BRS of 21 control individuals and 32 patients studied 18 days after a myocardial infarction (MI) and has also examined the evolution of BRS during the first year post-MI. Among the post-MI patients, BRS was lower when compared to that of control subjects (8.2 ± 3.7 vs. 12.3 ± 2.9 ms/mm Hg, p=.0001). Despite considerable overlap, 13 of 32 (41 percent) patients had a BRS less than two standard deviations below the mean of the control population. The internal control follow-up study showed that BRS increased three months post-MI to values quite similar to those of the control subjects; no further change was observed between 3 and 13 months post-MI.

These data indicate that, as in the experimental studies summarized here, BRS is reduced in a proportion of post-MI patients when compared to control subjects. This transient depression of BRS may relate to the reduced cardiac electrical stability present in the first few months post-MI.

The second study [48] examined the relationship between BRS, several clinical and hemodynamic variables, and mortality during a 24-month follow-up period of 78 patients who had suffered a first myocardial infarction 30 days before entering the study. The most important finding is that the BRS of the six patients who died because of a sudden (n=4) or a nonsudden (n=2) cardiac death was markedly lower when compared to that of the 72 survivors (2.4 ± 1.7 vs. 8.2 ± 4.8 ms/mm Hg, $P<.01$). A BRS<3.0 ms/mm Hg, which represents one standard deviation below the mean of the entire population, was defined as "markedly depressed". Whereas the mortality for patients with BRS greater than 3.0 ms/mm Hg (n=68) was 2.9 percent, it increased strikingly to 40 percent for those patients (n=10) with markedly

depressed BRS. In this study BRS was not correlated with left ventricular function [49].

These patients have also been randomized to exercise training or normal activity, and none of them was treated with beta-blockers. Although the sedentary patients had no BRS changes, the exercise-trained patients had an approximately 30 percent increase in BRS [50].

Thus, depressed BRS seems to be associated with a markedly increased risk for subsequent cardiovascular mortality in post-MI patients. Analysis of BRS may contribute to post-MI risk stratification, and it seems possible to increase a depressed BRS in humans, as well as in animals by exercise training.

Recently, Kleiger et al. [51] reported that another index of vagal tone, heart rate variability, is inversely correlated with mortality among post-myocardial infarction patients. Thus, there seem to be convergent data supporting the concept that derangements in the autonomic balance favoring a sympathetic dominance and largely secondary to impaired vagal activity (vagal tone in Kleiger's study and vagal reflexes in our own) are associated with increased mortality in patients after myocardial infarction [11].

Implications and Future Directions

It is probably too early for an objective evaluation of the practical implications of the notions mentioned here. Nonetheless, it is possible to outline some logical evolution of these data. Analysis of baroreflex sensitivity in postmyocardial infarction patients may become clinically useful if it is demonstrated that it identifies, as individuals at high risk, patients who would be missed by traditional risk stratification. This will require a large prospective study with multivariate analysis; such a study is currently in preparation. It is encouraging that the available data indicate that even among patients with depressed left ventricular ejection fractions, mortality is significantly influenced by the level of BRS [48].

Is it reasonable to explore the possibility of augmenting vagal tone and reflexes in patients with ischemic heart disease? Exercise training seems a very reasonable option, and both the experimental background and the rationale behind it are sound. The current skepticism about its value stems largely from the fact that several large trials have failed to show a statistically significant reduction in subsequent mortality [52]. However, in all these studies a favorable trend was not statistically significant, mostly because the number of events was too small; this, in turn, was secondary to the fact that these studies included, almost exclusively, low risk patients. In other words, most training programs seem to have been directed toward those who would benefit less from it and to have excluded, at times with valid reasons, those in greater need. It would be of interest to perform a trial with exercise training limited to high risk patients and BRS analysis may contribute to the patient

selection. The potential advantages of a nonpharmacologic, inexpensive, and easily available preventive approach are too large for us to disregard this possibility.

The potential applications of direct vagal stimulation are not easy to assess at the present time. On the other hand, the possibility of activating vagal activity when necessary is of interest. The combination of an implantable device with appropriate sensors is certainly within the reach of modern technology.

CONCLUSIONS

The data discussed here indicate that progress has been made in better understanding how the autonomic nervous system relates to the occurrence and prevention of sudden cardiac death. New therapeutic tools and new diagnostic techniques may result from our present knowledge. Careful manipulation of the autonomic nervous system may contribute usefully to the ongoing efforts to reduce mortality secondary to cardiovascular disease.

REFERENCES

1. Lown, B., and Verrier, R.L. 1986. Neural activity and ventricular fibrillation. *N. Eng. J. Med.* 294:1165–1170.
2. Schwartz, P.J., Brown, A.M., Malliani, A., and Zanchetti, A., eds. 1978. *Neural Mechanisms in Cardiac Arrhythmias.* New York, Raven Press.
3. Schwartz, P.J., and Stone, H.L. 1982. The role of the autonomic nervous system in Sudden Coronary Death. *Ann. NY Acad. Sci.* 382:162–181.
4. Corr, P.B., Yamada, K.A., and Witkowski, F.X. 1986. Mechanisms controlling cardiac autonomic function and their relation to arrhythmogenesis. In *The Heart and Cardiovascular System,* H.A. Fozzard, E. Haber, R.B. Jennings, et al. eds., pp. 1343–1404. New York, Raven Press.
5. Hjalmarson, A. 1984. Beta-blocker effectiveness post infarction: an antiarrhythmic or anti-ischemic effect. *Ann. NY Acad. Sci.* 427:101–111.
6. Frishman, W.H., Furberg, C.T., and Friedewald, W.T. 1984. Beta-adrenergic blockade for survivors of acute myocardial infarction. *N. Eng. J. Med.* 310:830–837.
7. Verrier, R.L. 1980. Neural factors and ventricular electrical instability. In *Sudden Death,* H.E. Kulbertus and H.J.J. Wellens, eds., pp. 137–155. Martinus Nijhoff, The Hague.
8. Schwartz, P.J. and Stone, H.L. 1985. The analysis and modulation of autonomic reflexes in the prediction and prevention of sudden death. In *Cardiac Arrhythmias: Mechanisms and Management.* D.P. Zipes and J. Jalife, eds., New York, Grune & Stratton, pp. 165–176.
9. Myers, R.W., Pearlman, A.S., Hyman, R.A., et al. 1974. Beneficial effects of vagal stimulation and bradycardia during experimental acute myocardial ischemia. *Circulation* 49:943–947.
10. Schwartz, P.J., and Stramba-Badiale, M. 1988. Parasympathetic nervous system and cardiac arrhythmias. In *Neurocardiology,* H.E. Kulbertus and G. Frank, eds. pp. 179–200, M. Kisco, NY, Futura Publishing Co.
11. Schwartz, P.J. 1987. Manipulation of the autonomic nervous system in the prevention of sudden cardiac death. In *Twenty Years of Cardiac Electrophysiology.* Where to go from here. P. Brugada and H.J.J. Wellens, eds., pp. 741–765, M. Kisco, NY, Futura Publishing Co.
12. Pantridge, J.F. 1978. Autonomic disturbance at the onset of acute myocardial infarction. In *Neural Mechanisms in Cardiac Arrhythmias,* P.J. Schwartz, A.M. Brown, A. Malliani, and A. Zanchetti, eds., pp. 7–17, New York, Raven Press.
13. Schwartz, P.J. 1984. Sympathetic imbalance and cardiac arrhythmias. In *Nervous Control of Cardiovascular Function,* W.C. Randall, ed., pp. 225–251, New York, Oxford University Press.

14. Schwartz, P.J. and Vanoli, E. 1981. Cardiac arrhythmias elicited by interaction between acute myocardial ischemia and sympathetic hyperactivity: a new experimental model for the study of antiarrhytmic drugs. *J. Cardiovasc. Pharmacol.* 3:1251–1259.
15. Schwartz, P.J., Vanoli, E., Zaza, A., and Zuanetti, G. 1985. The effect of antiarrhythmic drugs on life-threatening arrhythmias induced by the interaction between acute myocardial ischemia and sympathetic hyperactivity. *Am. Heart J.* 109:937–968.
16. Schwartz, P.J., Priori, S.G., Vanoli, E., et al. 1986. Efficacy of diltiazem in two experimental models of sudden death. *J. Am. Coll. Cardiol.* 8:661–668.
17. Priori, S.G., Zuanetti, G., and Schwartz, P.J. 1988. Ventricular fibrillation induced by the interaction between acute myocardial ischemia and sympathetic hyperactivity: effect of nifedipine. *Am. Heart J.* 116:37–43.
18. Feigl, E.O. 1983. Coronary physiology. *Physiol. Rev.* 63:1–205.
19. Schwartz, P.J. and Stone, H.L. 1977. Tonic influence of the sympathetic nervous system on myocardial reactive hyperemia and on coronary blood flow distribution. *Circ. Res.* 41:51–58.
20. Janse, M.J., Schwartz, P.J., Wilms-Chopman, F., et al. 1985. Effects of unilateral stellate ganglion stimulation and ablation on electrophysiological changes induced by acute myocardial ischemia in dogs. *Circulation* 72:585–595.
21. Gettes, L.S., Symanski, J.D., Fleet, W.F., et al. 1986. The intracellular and extracellular changes associated with ischemia—effects of catecholamines in arrhythmogenesis. *Eur. Heart J.* 7(A):77–84.
22. Schwartz, P.J. and Priori, S.G. in press. Adrenergic arrhythmogenesis and long QT syndrome. In *Handbook of Experimental Pharmacology* Vol. 89 *Antiarrhythmic Drugs*. E.M. Vaughan-Williams and T.J. Campbell eds., Heidelberg, Springer Verlag. 1988:519–543.
23. Priori, S.G., Mantica, M., and Schwartz, P.J. 1988. Delayed afterdepolarizations elicited *in vivo* by left stellate ganglion stimulation. *Circulation* 78:178–185.
24. Malliani, A., Schwartz, P.J., and Zanchetti, A. 1969. A sympathetic reflex elicited by experimental coronary occlusion. *Am. J. Physiol.* 217:703–709.
25. Schwartz, P.J., Foreman, R.D., Stone, H.L., and Brown, A.M. 1976. Effect of dorsal root section on the arrhythmias associated with coronary occlusion. *Am. J. Physiol.* 231:923–928.
26. Malliani, A., Recordati, G., and Schwartz, P.J. 1973. Nervous activity of afferent cardiac sympathetic fibers with atrial and ventricular endings. *J. Physiol. (Lond.)* 229:457–469.
27. Schwartz, P.J. 1984. The rationale and the role of left stellectomy for the prevention of malignant arrhythmias. *Ann. NY Acad. Sci.* 427:199–221.
28. Schwartz, P.J. 1985. The idiopathic long QT syndrome: Progress and questions. *Am. Heart J.* 109:399–411.
29. Schwartz, P.J. 1983. The idiopathic long QT syndrome: The need for a registry. *Eur. Heart J.* 4:529–531.
30. Moss, A.J., Schwartz, P.J., Crampton, R.S., et al. 1985. The long QT syndrome: a prospective international study. *Circulation* 71:17–21.
31. Barber, M.J., Mueller, T.M., Davies, B.G., et al. 1985. Interruption of sympathetic and vagally-mediated afferent responses by transmural myocardial infarction. *Circulation* 72:623–631.
32. Schwartz, P.J., Zaza, A., Grazi, S. et al. 1985. Effect of ventricular fibrillation complicating acute myocardial infarction on long term prognosis: importance of the site of infarction. *Am. J. Cardiol.* 56:384–389.
33. Schwartz, P.J., Motolese, M., Pollavini, G., et al. 1985. Surgical and pharmacological antiadrenergic interventions in the prevention of sudden death after a first myocardial infarction. *Circulation* 72:III 358.
34. Kent, K.M., Smith, E.R., Redwood, D.R., and Epstein, S.E. 1973. Electrical stability of acutely ischemic myocardium. Influence of heart rate and vagal stimulation. *Circulation* 47:291–298.
35. Corr, P.B. and Gillis, R.A. 1974. Role of the vagus nerves in the cardiovascular changes induced by coronary occlusion. *Circulation* 49:86–97.
36. Zuanetti, G., De Ferrari, G.M., Priori, S.G., and Schwartz, P.J. 1987. Protective effect of vagal stimulation on reperfusion arrhythmias in cats. *Circ. Res.* 61:429–435.
37. Das, P.K. and Bhattacharya, S.A. 1972. Studies on the effect of physostigmine on experimental cardiac arrhythmias in dogs. *Br. J. Pharmacol.* 44:397–403.

38. Schwartz, P.J. and Stone, H.L. 1978. Unilateral stellectomy and sudden death. In *Neural Mechanisms in Cardiac Arrhythmias*, P.J. Schwartz, A.M. Brown, A. Malliani, and A. Zanchetti, pp. 107–122, eds. New York, Raven Press.
39. Schwartz, P.J., Billman, G.E., and Stone, H.L. 1984. Autonomic mechanisms in ventricular fibrillation induced by myocardial ischemia during exercise in dogs with a healed myocardial infarction. An experimental preparation for sudden cardiac death. *Circulation* 69:780–790.
40. Smyth, H.S., Sleight, P., and Pickering, G.W. 1969. Reflex regulation of arterial pressure during sleep in man: A quantitative method of assessing baroreflex sensitivity. *Circ. Res.* 24:109–121.
41. Billman, G.E., Schwartz, P.J., and Stone, H.L. 1982. Baroreceptor reflex control of heart rate: A predictor of sudden cardiac death. *Circulation* 66:874–880.
42. Schwartz, P.J., Vanoli, E., Stramba-Badiale, M., et al. 1988. Autonomic mechanisms and sudden death. New insights from the analysis of baroreceptor reflexes in conscious dogs with and without a myocardial infarction. *Circulation* 78:in press.
43. Billman, G.E., Schwartz, P.J., and Stone, H.L. 1984. The effects of daily exercise on susceptibility to sudden cardiac death. *Circulation* 69:1182–1189.
44. Stramba-Badiale, M., Vanoli, E., De Ferrari, G.M., et al. 1987. Autonomic interactions and heart rate in unanesthetized dogs with a healed myocardial infarction. *Fed. Proc.* 46:1256.
45. De Ferrari, G.M., Vanoli, E., Stramba-Badiale, M., et al., 1987. Vagal stimulation and sudden death in conscious dogs with a healed myocardial infarction (abstr.) *Circulation* 76: IV–106.
46. Stramba-Badiale, M., De Ferrari, G.M., Vanoli, E., et al. 1988. Myocardial ischemia and ventricular fibrillation in conscious dogs. Atropine and the role of vagal reflexes. *Fed. Proc.* 47:A327.
47. Schwartz, P.J., Zaza, A., Pala, M., et al. 1988. Baroreflex sensitivity and its evolution during the first year after a myocardial infarction. *J. Am. Coll. Cardiol.* 12:in press.
48. La Rovere, M.T., Specchia, G., Mortara, A., and Schwartz, P.J. in press. Baroreflex sensitivity, clinical correlates and cardiovascular mortality among patients with a first myocardial infarction. A prospective study. *Circulation* 78:in press.
49. La Rovere, M.T., Mortara, A., Ghio, S., et al. 1987. Baroreflex sensitivity and left ventricular function in post-myocardial infarction patients. (abstr.). *Circulation* 76:IV–59.
50. La Rovere, M.T., Specchia, G., Mazzoleni, C., et al. 1986. Baroreflex sensitivity in post-myocaridal infarction patients. Correlation with physical training and prognosis (abstr.). *Circulation* 74:II–514.
51. Kleiger, R.E., Miller, J.P., Bigger, J.T. Jr., et al. 1987. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am. J. Cardiol.* 59:256–262.
52. Shephard, R.J., 1983. The value of exercise in ischemic heart disease: a cumulative analysis. *J.Cardiac Rehab.* 3:294–298.