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VENTRICULAR FIBRILLATION AND SUDDEN CORONARY DEATH

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Printed on acid-free paper.

... to the memory of my father,
Eugene Rajskin

Contents

Foreword

The publication embodied here represents the life work of a premier Russian scientist studying Sudden Cardiac Death. As one can gather from more than 35 first authored publications cited in the References, Dr. Rajskina has been involved with the investigation of mechanisms responsible for Sudden Cardiac Death for over 30 years. She has brought a classical approach to the subject, considering the effects of blood supply disturbances, electrophysiological changes that occur after regional ischemia, metabolic alterations, and the role of the autonomic nervous system in modulating these changes.

These studies naturally lead to a consideration of interventions, based on her research, to prevent ventricular fibrillation after coronary artery occlusion. This is a wide ranging treatise indicative of a lifetime of study of the problem and filled with the richness of scientific experiments generated in its pursuit.

There is so much in here that will be of interest to the arrhythmologist interested in Sudden Cardiac Death, whether this is on a single channel level, in vitro study of hearts, in vivo investigation of intact animals, or at the bedside. And throughout it all, statements are copiously documented with more than 850 references. That alone is worth hours of computer searching.

I am very proud to have been asked by this outstanding scientist to write a brief Preface to her monumental contribution. All of us involved in the study of arrhythmic mechanisms responsible for Sudden Cardiac Death can hold Dr. Rajskina up as a role model and her book as a lifetime goal to which we all should strive.

Douglas P. Zipes, M.D.
Distinguished Professor of
Medicine, Pharmacology and Toxicology
Director, Division of Cardiology
and Krannert Institute of Cardiology

List of Abbreviations

General terminology (I)

AP Arterial Pressure
CAO Coronary Artery Occlusion
HR Heart Rate
HW Heart Work
IZ Ischemic Zone
LV Left Ventricle
MI Miocardial Infarction
MV Minute Volume
NIZ Non-Ischemic Zone
RV Right Ventricle
SV Stroke Volume
VF Ventricular Fibrillation
VFT Ventricular Fibrillation Threshold
VPB Ventricular Premature Beat
VT Ventricular Tachicardia

Electrophysiological terminology (II)

AP Action Potential
APA Action Potential Amplitude
APD Action Potential Duration
ARP Absolute Refractory Period
DCC Duration of Cardiac Cycle
DD Dispersion of Depolarization
DR Depolarization Rate
DR Dispersion of Repolarization
ECG Electrocardiogram
EG Electrogram
ERP Effective Refractory Period
ET Excitation Threshold
IU Intercellular Uncoupling
LP Latent Period
MP Membrane Potential
ρ "Relaxability"
R Circulation Radius of Excitation
Rest.P Rest Potential
Ri Input Resistance
RP Refractory Period
RRP Relative Refractory Period
SDD Slow Distolic Depolarization
SEP Speed of Excitation Propagation
T Circulation Excitation Period
$\check{T}$ Calculated Circulation Excitation Period
$\dot{V}$ Maximal Depolarization Rate

Biochemical terminology (III)

2 PGl 2 Phosphoglycerat
3 PGl 3 Phosphoglycerat
$\mathbf{a}_K^+$, $\mathbf{a}_{Na}^+$, $\mathbf{a}_{Ca}^+$, $\mathbf{a}_{Cl}^-$ activity of ions K,Na,Ca,Cl
ADP Adenosinediphosphat
Ald Aldolase
AMP Adenosinemonophosphat
ATP Adenosinetriphosphat
BSA bull serum albumin
$\mathbf{C}_K$, $\mathbf{C}_{Na}$, $\mathbf{C}_{Ca}$, $\mathbf{C}_{Cl}$ concentration of K, Na, Ca and Cl
cAMP Cyclic Adenosinemonophosphat
Cit Cytoplasm
DMO 5,5-Dimethyl-2,4-Oxazolidinedion
DNA Deoxyribonucleic acid
DOAP Dioxyacetonphosphate
Emch Membrane potential of mitochondrion
$\mathbf{E}_K$ Equilibrium potential for K
En Enolase
F-6-P Fructose-6-Phophate
FDP Fructose-1,6-Diphosphate
G-1-P Glucose-1-Phosphate
G-6-P Glucose-6-Phosphate
GlA-3-P Glyceroaldehide-3-Phosphate
GlA-3-PDG Glyceroaldehyde-3-Phosphate Dehydrogenase
Gly Glycogen
IM Intracellular Media
K, Na, Ca, Cl Potassium, Sodium, Calcium, Chlorine
$\mathbf{K^+}$, $\mathbf{Na^+}$, $\mathbf{Ca^{++}}$, $\mathbf{Cl^-}$ Potassium ion, Sodium ion, Calcium ion, Chlorine ion
$\mathbf{[K^+]i}$,$\mathbf{[Na^+]i}$,$\mathbf{[Ca^+]i}$,$\mathbf{[Cl^-]i}$ intracellular concentration K, Na, Ca, Cl
$\mathbf{[K^+]e}$,$\mathbf{[Na^+]e}$,$\mathbf{[Ca^{++}]e}$,$\mathbf{[Cl^+]e}$ extracellular concentration K, Na, Ca, Cl

$[K^+]_{in}$ K in the space inaccessible to saccharose of mitochondrion

$[K^+]_{ex}$ K in the space accesible to saccharose of mitochondrion

KP Kreatinphosphate

Lac Lactate

LDG Lactatdehydrogenase

MAR Mass Acting Ratio

Mch Mitochondrion

Mf Myofibrille

NAD^+ Nicotinamid adenin dinucleotid, oxydized form

NAD.H Nicotinamid Adenin Dinucleotid, reduced form

ORP Oxidation-Reduction Potential

pK, pNa, pCa, pCl -log[K^+], -log[Na^+], -log[Ca^{++}], -log[Cl^-]

P_{CO_2} Partial Pressure of CO_2

P_{O_2} Partial Pressure of Oxygen

P-ase Phosphorilase

PEP Phosphoenolpyruvate

PFK Phosphofructokinase

PGI Phosphoglucoisomerase

PGlK Phosphoglycerokinase

PGlM Phosphoglyceromutase

PGM Phosphoglucomutase

pHe extracellular pH

pHi intracellular pH

Pi Phosphate

PK Pyruvatkinase

PP Phosphate Potential

Pyr Pyruvate

Q_{init}, Q_{rest}, Q_{III}, Q_{IV}, Q_{DNF} Mch respiration in different conditions

Q_{O_2} Consumption of Oxygen

RC_{ChW} Respiratory Control according to Chance, Williams

RC_{LW} Respiratory Control according to Lardy, Wellman

RC Respiratory control

RNA Ribonucleic acid

SDG Succinic dehydrogenase

SPR Sarcoplasmic Reticulum

TPI Triosephosphatisomerase

Neurophysiological terminology (IV,V)

(a-cs) arterio-coronary sinus difference

(a-cv) arterio-coronary vein difference

$(a\text{-}v)_A$ arterio-venous difference of adrenline

$(-a\text{-}v)_{NA}$ arterio-venous difference of noradrenaline

A_a concentration of A in arterial blood

AAAN Afferent Activity of Aortal Nerve

AACN Afferent Activity of Cardiac Nerves

A Adrenaline

AI Afferent Impulsation

CA Catecholamines

CBF coronary blood flow

ChE Cholinesterase

COMT Catechol O-methyltransferase

CR Chick Rectum

DA Diastolic Activity

DOPA Dihydroxyphenylalanine

DP Diastolic Pressure

EI Efferent Impulsation

$I_{A,NA}$ Index uptake (release) of A and NA

MAO Monoamineoxydase

NA_v concentration of NA in venous blood

NA Noradrenaline

PPr. Pulse Pressure

R Release of CA

re-U reuptake of CA

RSS Rat Stomach Strip

S Storage of CA

SD Systolic Discharge

SDD Systolic Discharge Duration

SP Systolic Pressure

U Uptake of CA

U-I Neuronal Uptake of CA

U-II Extraneuronal Uptake of CA

Pharmacological terminology (VI)

CG Corglycon

CoQ_{10} Ubiquinone

CoQ_4 Hexahydroubiquinone-4

DNP Dinitrophenol

EDTA Ethylenediamine tetra-acetic acid

MIA Monoiodacetat

MN Metanephrine

Prop. Propranolol

TRIS Trihydroxymethyl aminomethan

INTRODUCTION

The prevention of sudden death caused by ischemic heart disease is one of the most important and challenging problems of modern medicine. Every year, over half a million people in the world die suddenly [37].

According to a World Health Organization study, sudden death is prevailing in the cases of mortality due to myocardial infarction. It constitutes 65 to 70% of the total mortality during the first 28 days of the disease [188]. So, without a doubt, the prevention of sudden death is the most effective way for decreasing general mortality due to myocardial infarction.

Cardiac sudden death is generally considered the easiest form of death for the patient. However, in the vast majority of cases such a death is really premature. Usually, after sudden death, the anatomic changes in the coronary vessels are found to have been relatively small, and the condition of the heart is good . The patient is usually in the prime of life, and his or her death is a great loss to the society.

The social significance of sudden death is aggravated by the fact that it most frequently strikes relatively young people. Sudden death in cases of acute coronary insufficiency with a lethal outcome constitutes 96% among the patients in age under 40 years in comparison to 61.9% of patients in age between 60 to 69 [334].

The solution of the sudden death problem is complicated due to the fact that death usually occurs outside of the hospital. Framingham's study [143] showed that two thirds of sudden deaths due to coronary disease are observed outside the hospital.

For a long time sudden death only attracted the attention of ambulance physicians and medico-legal examiners. Neither of them studied the mechanism of sudden death development. Physicians are primarily interested in timely resuscitation, while the task of the medico-legal examinators is to disprove the possibility of violent death. Therefore, it is not surprising that the direct cause of sudden death has not been studied until a few decades ago.

Meanwhile as early as 1889, it was demonstrated that ventricular fibrillation (VF) is the cause of sudden cardiac death [180]. This conclusion was proven

in 1915 by electrocardiographic methods [120]. German authors also related sudden death ("sekunden Herztod") to ventricular fibrillation [123].

On the basis of experimental data, the author in 1964 convincingly showed that ventricular fibrillation is in fact the main cause of sudden death in acute phase of myocardial infarction [230].

The formation of special ambulance teams supplied with electrocardiographs and the introduction of monitoring of ECG in coronary care units confirmed the experimental data, leaving no doubt that ventricular fibrillation is the main cause of sudden death in cases of myocardial infarction and acute coronary insufficiency [173].

It could have been expected that scientific interest in the problem of ventricular fibrillation onset after myocardial infarction would have increased after this discovery. However, that did not occurs . Clinical investigations continued to focus on the dependence of the appearance of ventricular fibrillation on the preceding disturbances of heart rhythm and on improvement the methods for defibrillation and reanimation in coronary care units. The dependence of ventricular fibrillation on the preceding heart rhythm distortion was confirmed, however, only for a small group of patients with recurrent malignant arrhythmia. These patients represent only a small minority of the persons exposed to the risk of sudden death [175].

So, the most effective way to decrease sudden death caused by myocardial infarction is to find the basic mechanisms of VF onset, and to develop appropriate preventive means based on this finding.

In order to attract scientists' attention to this problem the author organized a symposium on sudden death in 1968 [271]. This symposium was attended by pathophysiologists, pathologists, medico-legal experts, pharmacologists and clinicians.

It seems strange that no extensive investigations of cardiac sudden death were published until the 1980s, with a single exception: the Surawicz and Pellegrino's monograph published in 1964 [300]. Only in the 1980s, were a number of symposia and conferences on sudden death held. Their results were summarized in a number of monographs [185, 198, 332, 333, 331, 174, 358, 112].

In the last decade numerous studies, among them the multicenter randomized clinical trials of sudden cardiac death, were performed [9]. In these trials, the prophylactory effectiveness of implantable cardioverter defibrillator (ICD) was studied and compared with drug therapy (amiodaron, beta blockers, and sotalol). The effectiveness of implantantable defibrillator was shown in [43].

It was shown, by comparing the effects of beta-blockers and calcium antagonists, that beta-blockers remain the only agents which, being given profilactically, reduce incidence of sudden death [290].

A question about individual susceptibility (possibly of genetic origin) to sudden cardiac death [200] was raised in clinical and epidemiological studies.

The necessity to test the young athletes to determine who among them are at risk of sudden death was discussed in [101]. This test should help to divide athlets on those who can participate in competitive sports and who should not.

A Consensus Conference was convened by the Steering Committees of the European and North American Registries on VF under the auspices of the Working Group on Arrhythmias of the European Society of Cardiology [2].

Numerous epidemiological and clinical studies [89, 8, 139, 37] did not answer the questions regarding the mechanisms of VF. In this respect, experimental investigations are more promising.

Recently, experimental investigations of the mechanism of ventricular fibrillation onset after coronary artery occlusion have been intensified recently. Very interesting material is included in monographs [354, 164], "D-671","D-668".

The majority of experimental investigations were dedicated, however, to the study of isolated processes, were not systematic, and did not answer the question: "Why do similar ischemic changes in the heart after coronary occlusion not cause VF in some cases and cause VF in the others"?

This monograph presents the results[1] of systematic investigations of the mechanisms of ventricular fibrillation onset and the development of the appropriate preventive means. Here we use the systemic approach which requires to consider the heart as a complex object consisting of several interacting systems.

The investigations were carried out both on experimental animals and using mathematical models and computer simulations . In experiments with dogs, the occlusion of the anterior descending left coronary artery (CAO) was performed in its upper third, as is typical for human pathology. Some of dogs (60ventricular fibrillation in 3 to 5 minutes after CAO. The investigations were focused on ascertaining the specificity of those cases of myocardial infarction which resulted in ventricular fibrillation.

In order to study the many of biochemical and biophysical processes in the heart after CAO and preceding to the VF(which onsets suddenly), it is necessary to have at one's disposal the corresponding continuous methods and means for registration.

New methods and sensors developed for the in situ study of the heart metabolism [248] are described briefly in this monograph.

The changes in the heart processes, preceding VF were studied according to a sequence of events, presented in fig.1.

CAO causes both local distortion of the heart's blood supply, which leads to a P_{O_2} decrease in the ischemic zone, and general disturbances of the heart's sympatho-adrenal control. The latter provoke changes in the adrenaline and noradrenaline balance. The decrease of P_{O_2} and the changes in the balance of adrenaline and noradrenaline in the heart produce metabolism changes, which, in turn, provoke changes in the heart electrophysiological processes.

In order to find what changes in these processes are responsible for the appearance of VF, we compare each of them in experiments with and without VF.

[1]The experimental results presented in this monograph were obtained by the author and staff of his Laboratory of Experimental Myocardial Infarction, Cardiological Center, Academy of Medical Science, Moscow.

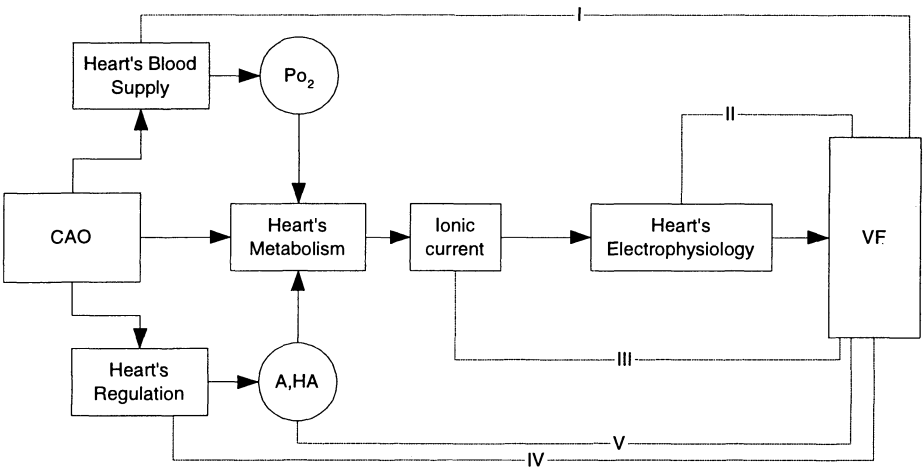

Figure I.1. The consequence of the processes in the heart which disturbances after CAO contribute to VF onset. CAO is coronary artery occlusion, VF is ventricular fibrillation, A, NA are adrenaline, noradrenaline, P_{O_2} is oxygen pressure. Roman numerals show the chapters number in which these processes are investigated and discussed.

It may be supposed that one of the consequences of CAO resulting in VF, may be a sharper disturbance of the heart blood supply. This hypothesis was studied using: 1)postmortem coronarography, 2)hemodynamics investigations, 3)investigation of the role of ischemic zone size and 4)P_{O_2} measurements in ischemic and non-ischemic zones. These studies formed the first line of our investigations, and the results are presented in Chapter I.

A second contributory factor to the VF onset after coronary artery occlusion may be some specific changes in the heart's electrophysiological processes. To find these changes we studied: 1) prefibrillatory rhythm disturbances, 2) membrane potential of the heart using intracellular microelectrodes and suction-electrodes, 3) heart's excitability and 4) heart's conductivity. Since the study of excitation propagation in a real myocardium represents essential difficulties, we additionally used mathematical modeling and computer simulation. These studies formed the second line of our investigations, described in Chapter II.

A third possible consequence of CAO, which may leads to the onset of VF, may be some specific changes in heart metabolism. To check this supposition,we investigated at the organ, cellular and subcellular levels the changes of: 1) redox equilibrium, 2) acid-base equilibrium and, 3) ion equilibrium. These studies formed the third line of our investigations, presented in Chapter III.

Finally, a fourth effect of CAO which may contribute to the onset of VF can be dstinctions in the heart's control, especially in the sympathoadrenal control. We investigated these distinctions in 1) changes of afferent activity of the heart's nerves, 2) changes of heart adrenaline and noradrenaline metabolism, 3)

changes in adrenaline and noradrenaline transfer between blood and the heart. These studies formed the fourth line of our investigations, presented in Chapter IV.

Adrenaline uptake in the heart was found more pronounced in the experiments that ended with VF in contrast to those without VF. It is interesting to determine what changes in heart metabolism and electrical activity are the consequence of increasde adrenaline concentration in the heart. For this purpose we studied the effect of adrenaline on the heart's metabolism and electrical activity and compared them with the effect of ischemia alone. These studies formed the fifth line of our investigations, presented in Chapter V.

The peculiarities that we observed in metabolism, electrophysiology and sympathoadrenal control changes after CAO complicated with VF allow us to propose and develop several possible approaches to prevent VF. They include: 1) elaboration of a method for VF onset prediction, 2) search of antifibrillatory substances, 3) elaboration the methods for antifibrillatory drugs introduction, and 4) development a method for postmortem diagnosis of VF.

A method was developed fo predict the possibility of VF onset after CAO for individuals with normal heart conditions at present time. The method is based on insulin test, which allows to determine the reactivity of the adrenal gland. This method, is simple and non-invasive, and can be used for the identification of a "risk-group" among: patients with coronary insufficiencies, healthy people on population, and atletes.

A search for antifibrillatory substances was conducted among the substances which: 1) stabilize redox equilibrium (ubiquinone, cytochrome), 2) prevent a shift of myocardial pH (TRIS), 3) normalize ion equilibrium (cardioplegic cocktail), 4) decrease adrenaline uptake by heart (metanephrine), and 5) block adrenaline's effect on the heart (propranolol).

A method of on-line computer control of drug introduction was developed. Feedback control was used here to adjust the parameters of the real action potential in such a manner (introducing drugs) that the onset of VF is prevented. For this purpose, the data obtained in course of computer simulations are used.

A method of postmortem diagnosis of ventricular fibrillation was developed on the basis of K distribution in the myocardium. The method serves for evaluation on population the efficiency of antifibrillatory influences , when VF had been developed in the absence of medical personal and a diagnosis should be given postmortem.

The development of all of these methods and the results obtained on VF prophylaxis formed the sixth line of our investigations, presented in Chapter VI.

Chapter VII concludes the monograph. On the basis of a systematic study the author formulated a hypothesis that the appearance of VF after CAO is a result of adrenal overcompensation of noradrenaline loss by the heart and presented proofs.

The author's first results were presented at the IV European Cardiological Congress in 1964 [229] and were published in [231]. The latter results in generalized form were published in [242, 236, 240, 241].

In getting the results, described in this book great contribution was made by the staff of author's former research laboratory: Drs.B.Shargorodsky, B.Feld, N.Onitchenko, K.Chalimova, D.Akelene, V.Dolgov, N.Dolgova, V.Vexler, A.Chatkevich, L.Podolsky, L.Machotina, V.Melnitchenko, V.Lakomkin, O.Morozova, A.Alexandry. The invaluable technical support was done by the engineers: B.Rastorguev, V.Titov, M.Yakunin and secretary G.Zalkind.

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This cooperation led to many joint publications.

* * *

This work could never have been completed without the invaluable help of my dear husband, Boris Kogan, and my beloved grandson, Eugene Grayver.

1 The Ventricular Fibrillation and Heart's Blood Supply

The disturbance of cardiac blood supply is the primary cause of VF occurrence after CAO. The greater the restriction of the coronary blood flow, i.e. the higher the level of CAO, is the higher the probability that VF occurs. However, there is no answer to the question of why after a similar level of CAO some animals (and patients) develop VF but others do not.

The simplest explanation of the above phenomenon could be this: different animals may have a different degree of cardiac blood supply disturbances after CAO at the same level.

The main factors which determine the cardiac blood supply are: 1) types of blood supply (left, right, mixed) 2) hemodynamic disturbances (blood pressure and cardiac output) induced by CAO, 3) the extent of the development of collateral vessels. The size of ischemic zone and P_{O_2} in it, is the resultant effect of all these factors. We studied the role of the above-mentioned factors in the development of VF after CAO.

1.1 The types of cardiac blood supply

There are three types cardiac blood supply: 1) the most frequent is the mixed type, also known as normal, usual, uniform, medium, balanced, 2) the right type, and 3) the left type.

The state of blood supply of the heart after CAO was studied in experiments on 47 dogs, 27 of which developed VF [150]. Data on the frequency of

Response to the ligation of the anterior descending coronary artery	Types of heart blood supply			
	Right	Left	Mixed	Total
No VF	1	9	10	20
VF within 5 min.	3	12	12	27
Total	4	21	22	47
Percent	75	60	54	57

Table 1.1. Relationship between the types of heart's blood supply and the development of VF after CAO in dogs

different blood supply patterns in dogs in experiments with and without VF are presented in table 1.

As can be seen from the table 1, the mixed type of blood supply is the most frequently observed one in dogs. The second most frequent type of blood supply is the left one. The dependence between the occurrence of VF after CAO and the type of heart's blood supply was found using χ^2-method ($p<0.01$). The greatest frequency of VF (75%) after occlusion of the left descending coronary artery was observed, in dogs with the right type of cardiac blood supply, when partial vascularization of the left ventricle is provided by the right coronary artery. This fact provides evidence against the dependence of VF development on the state of blood supply of the heart.

1.2 Hemodynamic changes

The primary hemodynamic disturbances after CAO consist of the decrease in minute volume and stroke volume of the heart, left ventricular work, blood pressure, and elevation of the left atrial pressure, left ventricular end systolic pressure and venous pressure.

The characteristics of hemodynamic changes in cases of CAO with VF were studied in our laboratory in collaborative investigations with Hungarian scientists [155]. Cardiac output was measured using thermodilution method every 30-60 seconds; stroke volume and cardiac work were determined by the calculation method. The obtained data are presented in fig.1.1.

As can be seen from fig.1.1-A, arterial blood pressure and heart rate decrease by the 3-rd minute after CAO. Two-phase changes in the cardiac output, stroke volume and cardiac work were observed: after a transient rise, by the 3-rd minute after CAO they decreased. 16 out of 25 dogs developed VF on average by the 30 th minute, while in 9 experiments VF was not observed for 1 hour. Fig.1.1-B shows averaged data of the changes after CAO in experiments with and without VF separately.

Decrease in the arterial blood pressure in experiments with VF was more pronounced whereas changes in heart rate, cardiac output and stroke volume in both groups of experiments were practically the same. However, great differences in the *rate* of development of these changes were observed. In experiments

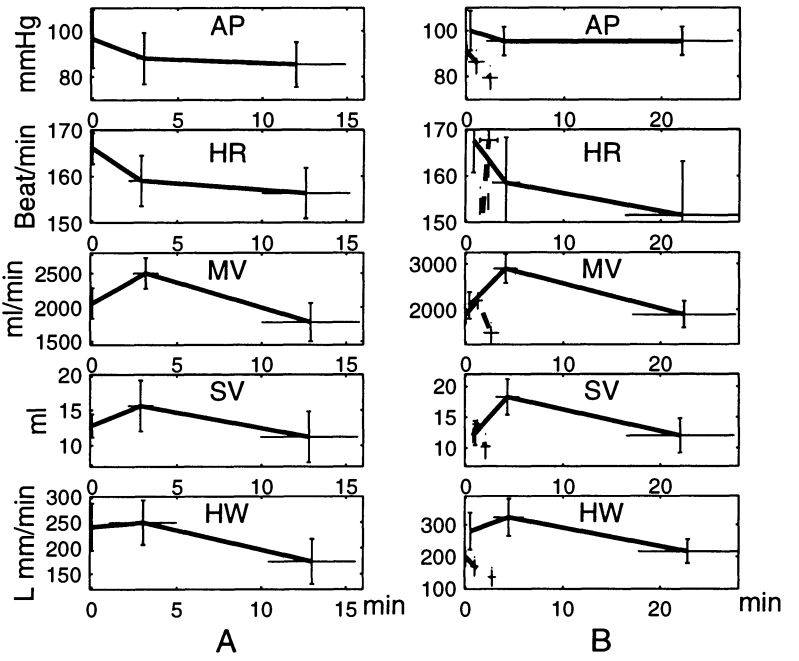

Figure 1.1. The hemodynamics changes after CAO in dogs. A - average data of all the experiments (n=25); B - average data in experiments without VF(n=9, solid line) and with VF (n=16, dotted line). On abscissa: minutes after CAO. On ordinate: AP, HR-heart's rate, MO-minute volume, SV -stroke volume, HW -heart's work

with VF, peak rise of cardiac output and stroke volume of the heart were observed 1.3 minutes after CAO (average value), and the maximum decrease 2.4 minutes after CAO. In experiments without VF, the time of development of these changes was 4.4 ($p<0.01$) and 22.4 ($p<0.001$) minutes respectively.

The changes in cardiac work in experiments with VF and without VF were quite different: in experiments with VF, it abruptly decreased, while in experiments without VF a primary, insignificant rise and a subsequent decrease of this index were observed. Differences in the change in cardiac work between experiments with and without VF can be attributed to a more abrupt drop in arterial blood pressure and a less pronounced elevation of cardiac output and stroke volume of the heart in experiments with VF.

The analysis of separate experiments has shown that the development of VF is not associated with any specific phase of changes in cardiac output and stroke volume.

Thus, the analysis of hemodynamic data does not reveal a distinct relationship between changes in hemodynamic values and the development of VF after CAO.

1.3 Collateral blood supply of the heart

Collateral circulation is provided by either opening of previously existing but inactive capillaries or by creation of new ones. The role played by the intercoronary anastomoses differs significantly in different animals: they are very developed in dogs, insignificantly developed in pigs and vary widely in humans. Schaper et al."D-656" believes that from the viewpoint of myocardial resistance to ischemia following acute coronary occlusion, the canine heart is more like the human heart.

Blood flow into the occlusion zone after CAO decreases but never stops completely. This is due to a pressure gradient that develops between the intact and occluded artery. The pressure in the latter achieves approximately 1/3 of the systemic pressure. The developing pressure gradient promotes blood flow into the ischemic zone through the collaterals. The collateral blood flow after CAO makes up 20% of the anterograde blood flow and is equivalent to the retrograde blood flow.

The resting collateral blood flow can provide only 40% of the myocardial oxygen requirement, but collateral blood flow does not change for 8-10 hours after CAO and increases later. Thus, the presence of collaterals before CAO is very important. In experimental studies by Meesmann et all., summarized in [189], the preexisting spontaneous collaterals were evaluated by observing retrograde filling of occluded circumflex coronary artery after the preliminary injection of contrast mass into the left and right descendent coronary arteries. The evaluation of retrograde filling was performed by means of coronarography on a 5-grade scale. 96.5% of animals which survived the occlusion of circumflex branch of the left coronary artery, had highly pronounced spontaneous collaterally, whereas VF arose in animals with weakly pronounced spontaneous collaterals.

In the clinical practice, coronary atherosclerosis is the main cause of augmentation of collateral circulation. In cases of coronary atherosclerosis, anastomoses between the coronary arteries become potent and abundant. The main coronary arteries are replaced by the abundant network of anastomoses. We investigated changes in the oxygen balance of the heart during CAO in an experimental study on dogs with preliminarily induced atherosclerosis [268]. Atherosclerosis was induced using a daily supplement of cholesterol (1-1$\frac{1}{2}$ g/kg) with methylthiouracil (1 g) to the food of animals for 21-23 month. Microscopically the damage of coronary vessels was pronounced either in the form of separate small plaques or in the form of total lesion. These changes occasionally induced severe narrowing of the vessel. The oxygen partial pressure was measured on 19 normal dogs and on 4 dogs with atherosclerosis in IZ and NIZ (table 2).

As can be seen from table 2, no significant changes in P_{O_2} were observed in NIZ in both groups of animals, whereas changes in P_{O_2} in IZ were very different. In normal dogs, P_{O_2} decreased to 62% of the initial level whereas in dogs with atherosclerosis it practically did not change. This can be attributed to the fact that due to the development of atherosclerosis, P_{O_2} in IZ was already lowered

Experiment Conditions	IZ		NIZ	
	n	M±m	n	M±m
Normal Dogs	19	62±5.1	13	95±7.0
Dogs with atherosclerosis	4	97±1.3	8	107±5.5
p		0.01		0.2

Table 1.2. Changes of oxygen partial pressure in the IZ and NIZ after CAO in normal dogs and in dogs with atherosclerosis (in % of initial level)

before CAO and due to preliminary opening of anastomoses, only insignificant changes in P_{O_2} took place after CAO. In accordance with that, in dogs with atherosclerosis ECG changes in IZ consisted of a small shift of S-T interval, whereas in normal dogs a pronounced monophase curve was observed.

Therefore, young people with unpronounced atherosclerosis and poor vascular anastomosis suffer of SD more frequently than elderly people with atherosclerosis and pronounced anastomosis. This fact indirectly testifies for the role of preexisting vascular anastomosis in the appearance of VF.

In the past few years a so-called ischemic preconditioning was developed. It has been observed that short periods of ischemia render myocardium more resistant to a subsequent prolonged CAO, resulting in a reduction of infarction size [299]. The ischemic preconditioning is considered to be just one of the components of adaptation [327].

Many authors connected ischemic preconditioning with myocardial catecholamines [327, 308, 95, 255, 342, 62, 14, 288, 166], but their results are contradictory. Research demonstrated that the antiarrhythmic effects of preconditioning is modified by the blockade of K-ATP channels. Experiments on dogs [326] showed that glibenamide reduces the antiarrhythmic effect of preconditioning, which suggests the involvement of K+ATP channels. However, glibenamide does not reduce the frequency of VF. Experiments on pigs, however, did not reveal a relationship between the time to VF and K-ATP channel opening by nicorandil and K-ATP channel inhibitor glibenclamide [262]. The evidence for an involvement of K-ATP channels in ischemic preconditioning is considered equivocal by other authors [114].

The results of Yamaguchi at all [350] suggest a possible contribution of muscarinic receptor stimulation to preconditioning. The antiarrhythmic effect of preconditioning was attenuated by blockade of bradykinin B^2 receptors [325], but was not mediated by prostanoids [171]. The role of the adenosine A_1 receptor in ischemic preconditioning was demonstrated in [335]. A negative result was obtained in [209]. The preconditioning decreased time of VF onset and did not limit the frequency of VF.

The another method of heart's protection during ischemia and infarction is reperfusion. However, at the time of reperfusion further injury occurs in

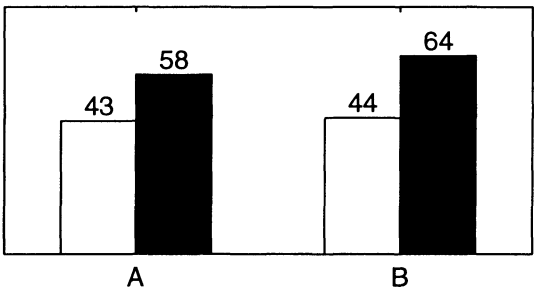

Figure 1.2. A - The number of visible vessels and B - retrograde filling of the distal part of occluding artery (average data) in experiments without VF (n=20, black column) and with VF (n=27, light column).

the myocardium. The reperfusion arrhythmias represent one of the types of reperfusion injury. They include ventricular tachycardia and VF [152].

We have studied [150] retrograde blood flow and the development of vessel system in the heart after CAO in the experiments with spontaneous VF and without VF by the method of posthumous coronarography. The development of the vessel system and the retrograde filling of the distal end of the occluded artery was expressed in % with such gradation: 0, 20, 40, 60, 80, and 100%. Total absence of vessels in the ischemic zone and no retrograde filling of the distal end of the occluded artery were evaluated as 0%. A pronounced vessel system and full retrograde filling of occluded artery were evaluated as 100%.

The obtained data are shown in fig.1.2. The differences in experiments with and without VF were statistically significant ($p<0.01$).

Posthumous coronary grams of dogs, which reflect the average data are given in fig.1.3-A, the exceptions are given in fig. 1.3-B.

Fig. 1.3-A shows cases, in which fibrillation appeared, as foretold by a weak development of the vessel system and total absence of retrograde filling of the distal region of the occluded artery, and did not appear in cases of well developed vessel system and high retrograde filling of the distal region of the left coronary artery. The fig.1.3-B shows coronary grams of the two dogs which do not demonstrate the dependance between VF development and collateral circulation. One of them developed of VF in spite of the complete retrograde filling of the distal region of the left descending artery and a good development of the vessel system in the field of the occluded artery. The second dog did not develop VF in spite of a low filling of the distal end of the occluded artery and a weak development of the vessels in ischemic zone.

The obtained data indicate that the probability of VF appearance with the same CAO level is not exclusively dependent on the collateral circulation.

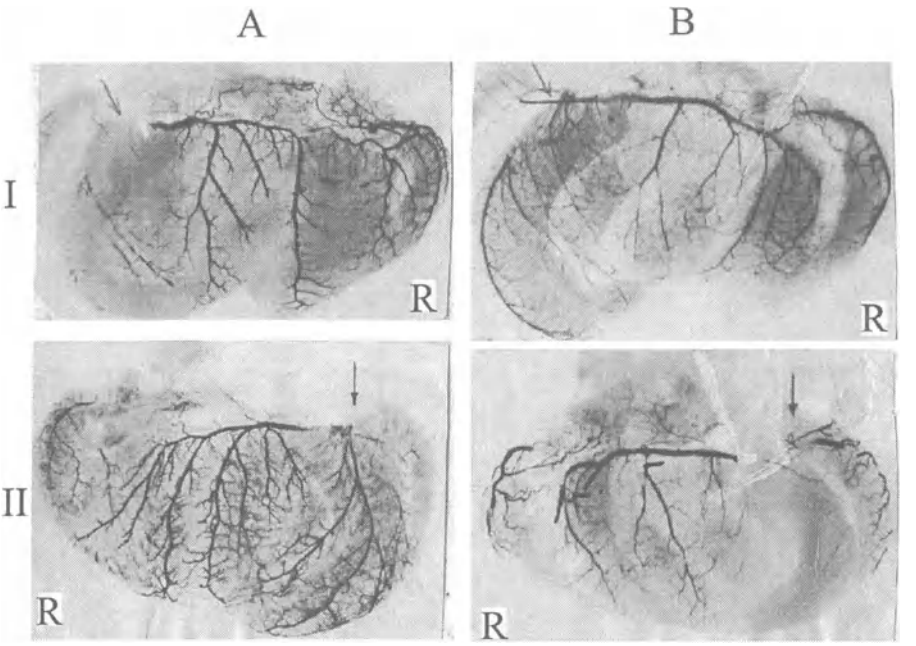

Figure 1.3. Angiogram of canine heart vessels (heart is unfolded by Rodreiguez in experiments with (I) and without VF (II). Typical changes - A, the exception -B. Arrow shows the place of occlusion.

1.4 The size of the ischemic zone

The final result of the data presented above is the size of ischemic zone. The size of the ischemic zone was determined as the ratio of the ischemic zone to the total heart weight. In experiments with and without VF after CAO, this ratio was 0.318 and 0.234 respectively. The difference in these ratio was statistically not significant [302]. However, an interdependency between the size of infarction and the frequency of arrhythmia was demonstrated in experiments on rats [207].

The role of the size of the ischemic zone in onset of VF was studied in our laboratory in experiments with ischemic perfusion of the bounded heart region. Unlike the studies which compared frequency of VF with the size ischemic zone, in different animals, in our experiments the size of ischemic zone during ischemic perfusion remained constant (in the same animal), but frequency of VF varied under the influence of pH and buffer capacity of the perfusate. During perfusion with a buffer-free solution having pH 7.4, frequency of VF was 44%, but in perfusion with buffer TRIS solution having the same pH, the frequency decreased to 0% for the same ischemic zone size. VF frequency also varied for

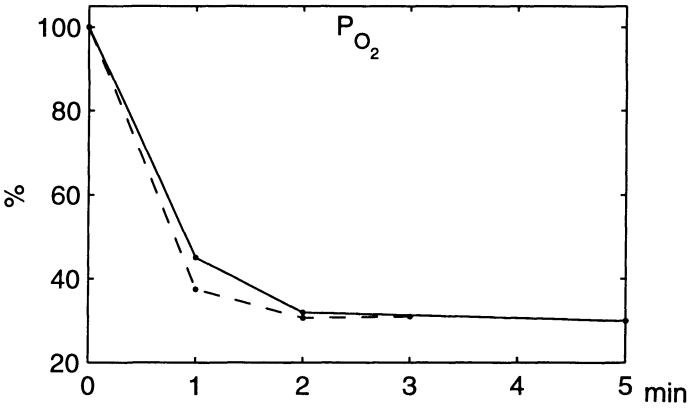

Figure 1.4. Changes in P_{O_2} in the ischemic zone after CAO in experiments without VF(n=12,solid line) and with VF (n=18,broken line).

different pH values of buffer solution averaging 7.7% for pH 7.6, 14.3% for pH 7.7-7.8 and 30% for acidic pH of 6.8 [287]. See chapter III.

The above data provide evidence that susceptibility of the heart to VF development can be different for the same size of ischemic focus but with different degrees of metabolic disturbances within this focus.

The other factor, which can determine the probability that VF develops is the level of hypoxia in the ischemic zone. The best evaluation of the level of hypoxia is obtained by measurement of P_{O_2} in the ischemic zone.

1.5 P_{O_2} in the ischemic zone

We studied changes in P_{O_2} in the ischemic and non-ischemic zones of the heart [236] using polarographic method simultaneously with EG recording in the same regions [248] (fig.1.4).

The decrease in P_{O_2} in the ischemic zone was the same in experiments with and without VF. By the third minute after CAO (the usual time that VF appeared), P_{O_2} decreased to 30% of the initial level in experiments both with and without VF. The obtained results indicate that appearance of VF after CAO does not depend on hypoxia level in the ischemic and non-ischemic zones [236].

1.6 Conclusion

All data presented in this chapter permit us to conclude that the degree of the decrease of the coronary blood flow during the CAO is an important, but not the only factor, which determines of the probability of VF development. This is testified by: 1) the possibility of fibrillation arising in cases with the

pronounced inter- and intracoronary anastomosis in the heart; 2) the absence of differences between the degree of P_{O_2} decrease in the ischemic zone in the experiments with and without VF; 3) the dependence of VF arise from buffer property of perfusion solution in cases of ischemic perfusion the same zones.

We consider the ischemia as a trigger-mechanism which leads to the development of metabolic, electrophysiological, and neurohumoral changes in the heart. The experimental data about these changes will be presented in the following chapters.

2 Ventricular Fibrillation and Electrophysiological Changes

Many books [345, 354, 301, 96, 217, 183, 273] have been recently published that deal with electrophysiological mechanisms of arrhythmia in local ischemia. Many books have been published about sudden cardiac death [89, 8, 139, 1]. Considerably fewer publications are concerned with the electrophysiological mechanisms of ventricular fibrillation (VF) [12, 354].

The relationship between the onset of VF and the ventricular premature beat (VPB) was investigated in numerous studies. However, it has not been determined why VPB develops into VF in some cases, and into sinus rhythm in other cases.

2.1 Role of the VPB in the onset of VF after CAO

Continuous measurement of cardiac rhythm from the moment of occlusion to the time of VF appearance in experiments on 40 dogs and 28 cats has allowed us to demonstrate a relationship between VPB and VF and the characteristic features of VPB in cases that resulted in VF after CAO [242].

In 70% of the experiments VF occurred after CAO. VPB was observed in 100% of these cases (fig. 2.1.A). In 10% of the experiments without VPB, VF did not occurred (fig. 2.1.B).

Thus, VF was always preceded by VPB, but VPB did not always result in VF. The question is: in which cases VPB transforms in VF, and in which cases it does not. This question was studied at our laboratory [92]. For this purpose

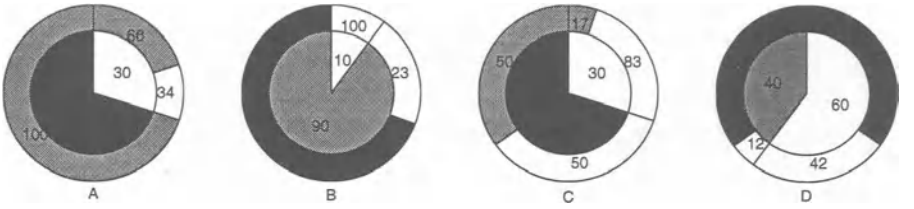

Figure 2.1. Dependence of the appearance VF on VPB. A - frequency of VF (inside part of circle) and VPB (outside part of circle) in experiments with and without VF. B - frequency of VPB (inside part of circle) and VF (outside part of circle) in experiments with and without VPB. Dependence of the onset VF from VT. C - the rise's frequency of VF (inside part of circle) and VT (outside part of circle) in experiments with and without VF. D - the rise's frequency of VT (inside part of circle) and VF (outside part of circle) in experiments with and without VT. The experiments with VF are represented by black, with VPB or with VT - by grey, and without VF, VPB and VT - by white.

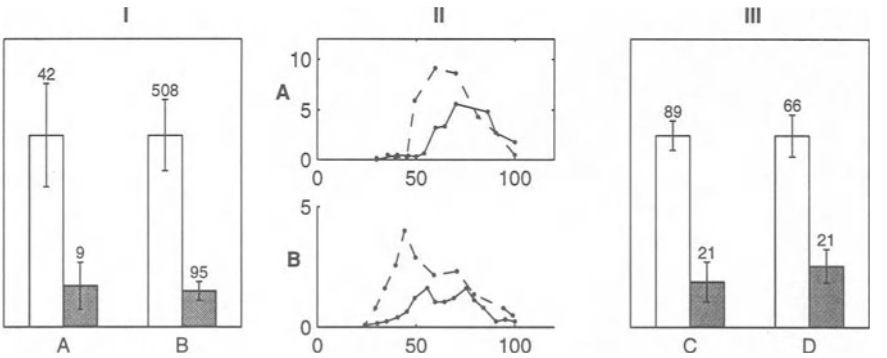

Figure 2.2. I - the number of VPB in the prefibrillatory period (light column) and during the average time of the occurrence of VF in experiments without VF (grey columns). II - VPB's frequency in different intervals of cardiac cycle in experiments with VF (broken line) and without VF (solid line). III - the number of VPB's groups in experiments with VF (light columns) and without VF (grey columns). A, C - experiments on dogs (n=40), B, D - experiments on cats (n=28).

the following parameters were compared in experiments with and without VF: 1) frequency of VPB , 2) frequency of VPB appearing in various parts of the cardiac cycle and 3) susceptibility to multiple VPB (fig.2.2).

The number of VPB's (fig.2.2-I) during the time period from CAO to the appearance of VF was 5 times greater in experiments with VF than in experiments without VF (during average time until VF appeared). In some cases with a greater number of VPB (43-54) VF, however, did not developed.

Frequency of VPB in earlier intervals of cardiac cycle (expressed in percent of the cardiac cycle length) was also higher in experiments with VF than in experiments without VF (Fig2.2-II). VPB in dogs, which are more susceptible to VF, occur earlier than in cats.

Susceptibility to multiple VPB (fig.2.2-III) was 3-4 times more pronounced in experiments with VF than in experiments without VF (in experiments on dogs and cats).

Our data indicate that premature ventricular excitations (VPB and VT) always precedes fibrillation. However it does not indicate the cause and relationship between them. Predisposition to VPB, VT and VF after CAO seem to develop simultaneously but independently from each other. This allows us to suppose that VF appears after CAO in cases which are characterized by certain qualitative and quantitative peculiarities in changes of the heart cell bioelectrical properties.

2.2 Role of ionic current, membrane potentials, automatism, excitability, and conductivity changes in the appearance of VF after CAO

The possible consequences of CAO (in changing of ionic current, membrane potentials, automatism, excitability and conductivity) which lead to the onset of VPB and VF are shown schematically in fig 2.3.

The changes in ionic currents and membrane potential include: an increase in K^+ outward current, a decrease of rapid Na^+ inward current, slow Na^+ and Ca^{2+} inward currents [339, 63].

The increase of the K outward current results in the decrease of the rest potential (RestP) of ischemic cells. The decrease of the rapid Na^+ inward current causes the decrease of the action potential amplitude (APA) and the slope of its depolarization phase ($\dot{V}_{max}$). Under normal conditions the L-type Ca^{2+} current determines the length of flat segment of repolarization phase [66, 356]. As a result of that, a rapid reaction is replaced by a slow reaction. The slow down of V_{max} causes conductivity changes.

The conductivity changes are manifested in the increase of the dispersion of the depolarization (DD) and repolarization (DR) times, in decreasing the speed of excitation propagation (SEP), in the appearance of conductivity blocking. The decrease in the slow Ca^{2+} inward current and the increase in the K outward current causes the shortening of the phase repolarization's flat segment ("plateau") and acceleration of the fast repolarization phase. It leads to the shortening of the action potential duration (APD).

Changes in automatism were connected with variations in outward velocity of Na and inward velocity of K during diastolic phase of MP. A slow diastolic depolarization (SDD) was observed in the myocardial fibers and an increase in SDD velocity was observed in the automatic cells. The heterotopic automaticity is considered to be the cause of arrhythmia in the late stage of myocardial infarction.

The excitability changes were connected with changes in RestP, APD and DPD. These changes are manifested in changes in the excitability threshold

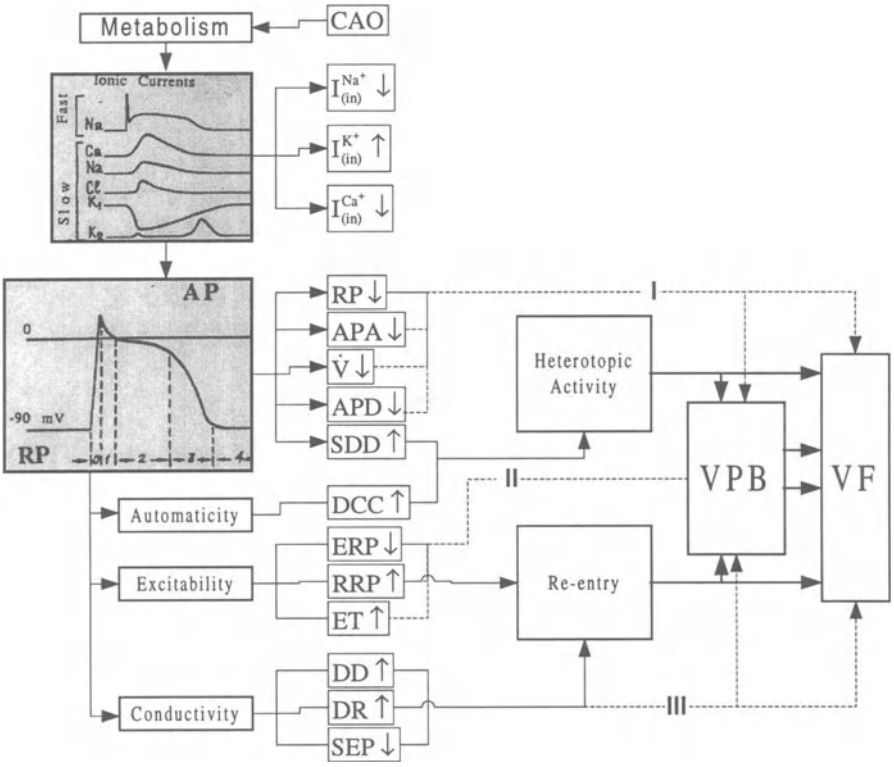

Figure 2.3. The sequence of changes in the heart after CAO which lead to VPB and VF. I^{Na}_{in} - inflow of Na ion current, I^{K}_{out} - efflux of K ion current, I^{Ca}_{in} - inflow of Ca ion current. Other abbreviations - see list of abbreviation. The Roman numerals show the possible methods of analysis of these dependencies.

(ET) and of the duration of the refractory periods (RP); effective (ERP) and relative (RRP). The shortening of the refractory period in a limited section of the heart,slowing down of the excitation wave propagation and the appearance of a low excitability zone in the heart - promote the return of excitation of myocardial cells - reentry, which is the most probable mechanism of VPB and VF.

In accordance with this data, investigations of VPB's and VF's electrophysiological mechanism consisted in the study of: 1) ionic currents and the heart's membrane potentials, 2) automatism of the heart 3) excitability of the heart and 4) excitation wave propagation in the heart before and after CAO and in studying the specific changes in these processes in cases of CAO followed by VF [242].

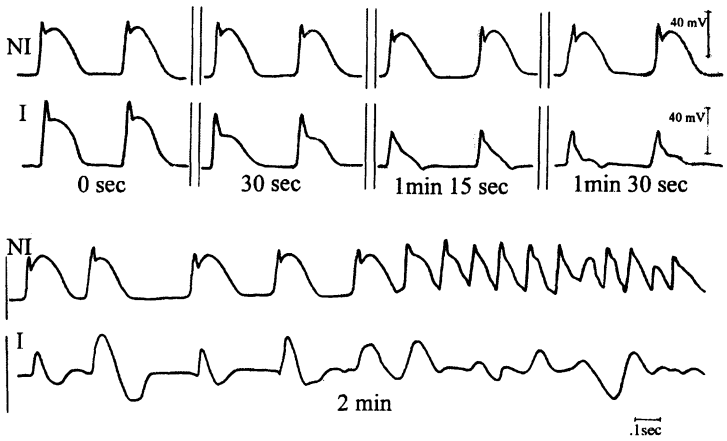

Figure 2.4. The time course of monophasic AP dog's heart in ischemic (I) and nonischemic (NI) zones after CAO

2.2.1 Ischemic changes in the membrane potentials of the heart

In our laboratory monophasic potential was continuously recorded on 17 dogs using suction electrodes before and after CAO until the appearance of VF [242]. VF occurred in 8 of 17 dogs. The dynamics of the monophasic AP changes in non-ischemic right and the ischemic left ventricle dog's heart in the experiment with VF is shown in fig. 2.4.

The monophasic AP in the ischemic zone already began to change 30 sec.after CAO and was completely deformed at the moment of VF. Average data of DR, APA and APD are shown in fig.2.5.

As shown fig.2.5-A, the changes DR, APA and APD were more pronounced in IZ. Continuous AP recordings in the ischemic zone during 10 min. after CAO allowed us to demonstrate that the phase of more drastic changes in the AP coincides with appearance of VPB and stops after their stabilization [231].

AP changes after CAO were observed both in cases resulting in VF and without VF. To clarify the role of the AP changes in VF onset we compare them in experiments with and without VF (fig.2.5-B). The DR, APA and APD during the first 2 min. after CAO decreased considerably more rapidly in experiments with VF than without VF.

Accordingly, VPB began earlier in the experiments with VF. Our data agrees with the results of the experiments [267] obtained using floating microelectrodes. The authors showed that changes of AP after CAO are more marked when CAO results in VF. These changes can due to metabolic factors of ischemia such as acidosis, K^+, lactate, fatty acids changes [270].

The influence of ischemic metabolism products on membrane potential was studied in our laboratory. The effect of "hypoxic" blood and K^+ concentration on the AP were investigated using microelectrode leads [242]. A stripe of a

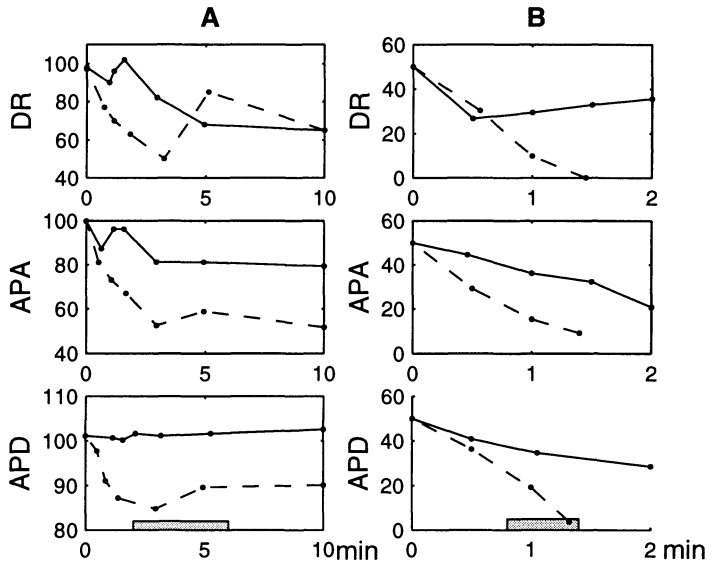

Figure 2.5. A - the changes of DR, APA and APD in IZ (dotted line) and in NIZ (solid line (n=17, dogs). B - the time course of DR, APA and APD in the experiments with VF (broken line, n=8, dogs) and without VF (solid line, n=9, dogs). The period of VPB in experiments without VF (A) and with VF (B) is shown shaded

rabbit's heart was perfused with the blood of a dog-donor with a different level of hypoxia. A strict dependence of changes in APA, APD, RestP and $\dot{V}$ from the level of hypoxia was shown (fig.2.6-A).

Addition of K^+ to the perfused cat papillary muscle caused a considerable decrease of APA and APD (fig.2.6.B), while addition of lactate was accompanied only by negligible changes in the above mentioned parameters (fig.2.6-C). So, potassium is probably the main metabolic factor which is responsible for membrane potential changes during ischemia.

2.2.2 Ischemic changes of the heart's automatism

Both the latent pacemaker and the contractile atrial and ventricular myocardial cells, which do not possess automatism under normal condition, can acquire automatic properties in ischemic conditions.

The effect of lactate on the heart's automatism was investigated on a culture of cardiac cells in order to study its effect on contractile and automatic cells separately (fig.2.6-E) [1]

[1]These investigations were done in the Physiological Institute of the Medical Academy (Magdeburg, Germany) and in the Department of Cells and Molecular Cardiology Institute of heart and circulation (Berlin, Germany). The results were not published

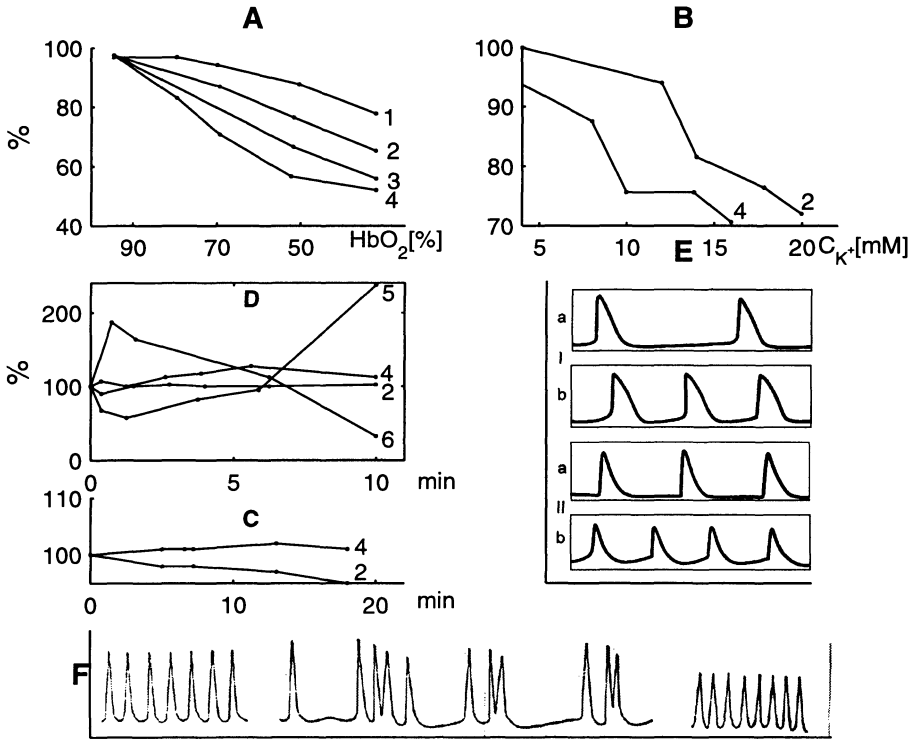

Figure 2.6. The action of ischemic metabolism's product on AP registered by intracellular microelectrode. A - AP changes (% to initial level) in the perfusion of the papillary muscle of rabbit's heart by hypoxic blood. B - AP changes of papillar muscle cat's heart by increase of K concentration in the perfusate. C - AP changes of papillar muscle cat's heart by addition of lactate (20 mM) in the perfusate. D - AP changes automatic cells in the cell culture by the addition of lactate Na (8 mM) in medium. E - AP of automatic (I) and contractile (II)cells in the cell culture before (a) and after (b) the addition lactate Na in the medium. F - appearance of arrhythmia in cardiac cell culture caused by the addition of lactate Na and cessation arrhythmia caused by washing the lactate Na. Designation of curve: 1- RestP, 2-APA, 3- $\dot{V}_{max}$.,4- APD, 5- DCC, 6- SDD.

As shown in fig 2.6-D, the lactate did not exert a substantial influence on the APA and APD of the automatic cell in the first 2-3 minutes of pH decrease, but substantially accelerated the cell SDD; this was accompanied by a decrease of the cardiac cycle length. In the next few minutes, the SDD velocity decreased while the cardiac cycle length increased. Fig. 2.6-E shows that lactate not only accelerated the SDD of automatic cells (fig.2.6-E,I,b), but also caused appearance of SDD in the cells of the contractile myocardium. When lactate is added, the RestP does not remain at a constant level as it did before lactate addition (fig.2.6-E,II,a) but increases continuously until it reaches the threshold

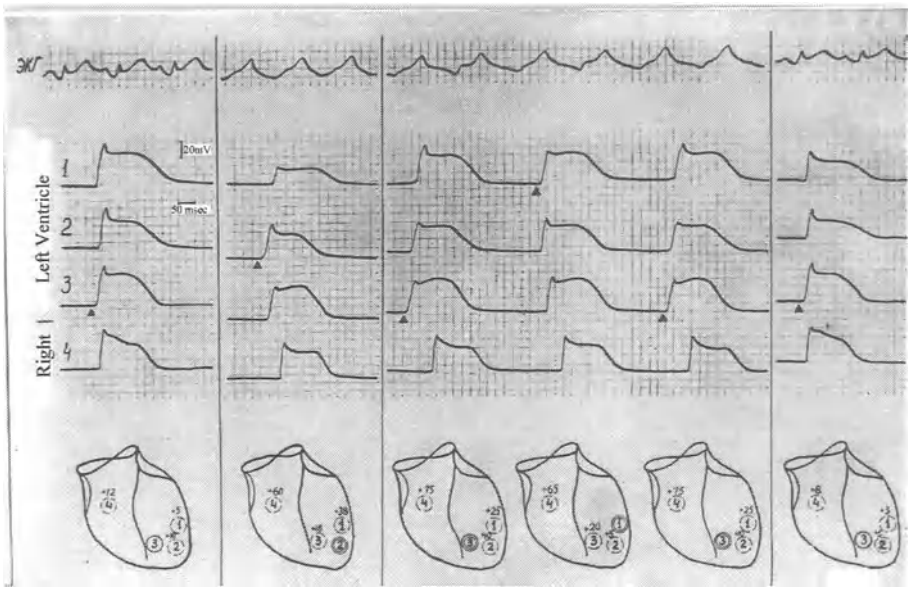

Figure 2.7. Changes in the path of excitation propagation of VPB, induced by adrenaline (10 μg). I - ECG, II - AP registered by suction electrodes and lead from 4 points of the heart. Numbers of leads correspond to numbers in circles on the diagram of the heart(III). The points excited first are shown by solid circle on the diagram and by triangle on AP. The points excited after a delay are shown by broken circle. The delay value is indicated next to the circle.

level at which rapid depolarization develops (fig.2.6-E,II,b). This behavior allows us to suppose that the arrhythmia observed under the effect of lactate (fig.2.6-F) is a consequence of the heterotopic automaticity.

The catecholamines accumulated in the ischemic zone can be another factor which accelerates the SDD and activates the latent pacemakers in the ischemic zone. The concentration of adrenaline in the ischemic zone becomes two times greater 5 min after CAO (see chapter IV).

The effect of adrenaline on the heart's AP was investigated in our laboratory in experiments on dogs using suction electrodes. Monophasic AP was measured at 4 areas of the heart. The dynamics of action potential changes after intravenous injection of adrenaline (dose 10 mg/kg) is shown in fig.2.7.

The area which was excited first was considered the "pacemaker." It was shown that the location of the "pacemaker" within the left ventricle changes after adrenaline administration. Before the adrenaline injection the "pacemaker" was located in the area of the 3-d electrode. After the injection of adrenaline ventricular arrhythmia started. During this time the excitation's succession of the heart changed. The area of the 2nd electrode became the "pacemaker." Then, the position of "pacemaker" changed from one cycle to the next; from

the 3rd to the 1st. A similar situation exists after CAO as will be shown in chapter V.

2.2.3 Ischemic changes of heart's excitability

The diastolic excitation threshold (ET) characterizes heart's excitability in the diastole, while the values of the absolute or effective (ERP) and relative refractory periods (RRP) characterize heart's excitability in the systole. ET was studied in parallel with latent periods of excitation (LP) and speed of excitation propagation (SEP) in various phases of cardiac cycle by natural rhythm [242] using a specially developed method [248]. We used 1 ms duration stimuli applied at intervals of 10-15 ms.

The data were averaged as follows. The moments of pacing were expressed in percent of the AP duration because the AP duration changed in various experiments. The data were averaged within four intervals of the cardiac cycle: I -the interval of the first responses; (82% APD): II - the end of the third AP phase (98.6% APD): III -the beginning of the fourth AP phase (104.4% APD): IV -the beginning of the diastole (125% APD). We considered the selection of these period necessary to completely characterize the excitability's cycle of the heart because the RRP is an extremely inhomogeneous period of the cardiac cycle.

Fig.2.8 shows the time course of AP, ET, LP, and SEP after CAO [242]. The plot insets show the averaged values for four selected periods. The values of ERP and RRP are shown below the x-axis.

As shown in figure 2.8, the time course of ET and LP within the selected intervals of initial AP, after CAO was similar to that observed before CAO. ET and LP within the I, II, III and IV intervals were permanently lowered down to the diastolic level. SEP increased in these intervals before CAO. After CAO, SEP decreased and subsequently increased.

Fig 2.9-A presents the dynamics of changes of ET, LP and SEP in the four interval of the cardiac cycle after CAO. Fig 2.9-B presents these changes and changes in RP in interval IV.

2.2.3.1 The excitation threshold. In intervals II, III and IV, the excitation threshold had a biphase shape (fig.2.9). It rose until the 30th minute, after which it decreased but did not reach the initial level. The maximum ET increase occurs in interval II; the latter is lesser in interval III and is lowest in interval IV. Consequently, maximum excitability depression occurs at the beginning of the RRP. Statistical analysis of the obtained data in the cats, using the sign rule, demonstrated the statistical significance of the direction of the changes of the ET.

Similar data were also obtained in our experiments on dogs. In single experiments, however, the ET in the interval I lowered for a short time immediately after CAO. Similar observations are reported by Brooks at al [40].

Significant differences in ET changes were discovered in experiments with and without VF [242] (fig.2.10-I).

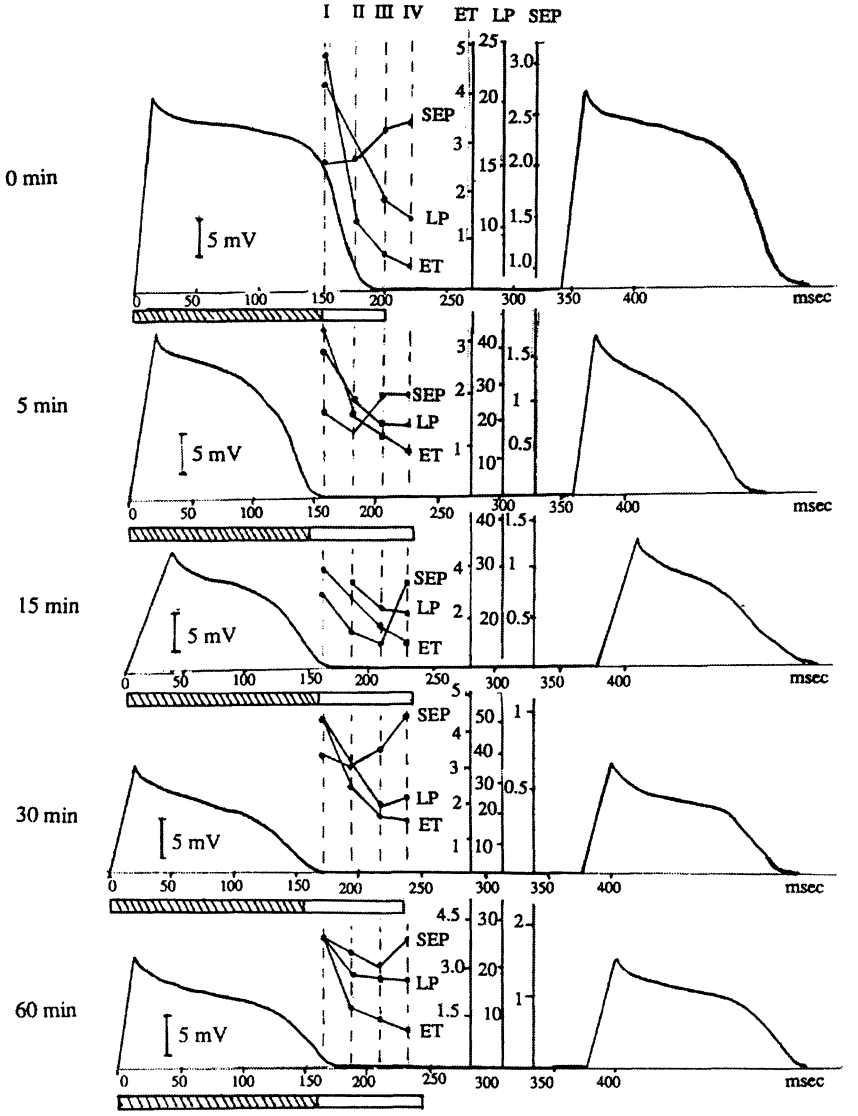

Figure 2.8. Dynamics of changes in cardiac cycle; ET (n-0=16, n-5=10 n-15=10 n-30=6 n-60=10); LP (n-0=7, n-5=4, n-15=5, n-30=6, n-60=6); SEP (n-016, n-5=5, n-15=13, n-30=6, n-60=6), shape of AP n-0=16, n-5=10, n-15=13, n-30=13, n-60=11) and effective refractory periods (ERP, shaded), relative refractory period (RRP, white). and total refractory period (RP, sum of ERP and ORP) before and after CAO (average data, cats, n=24).

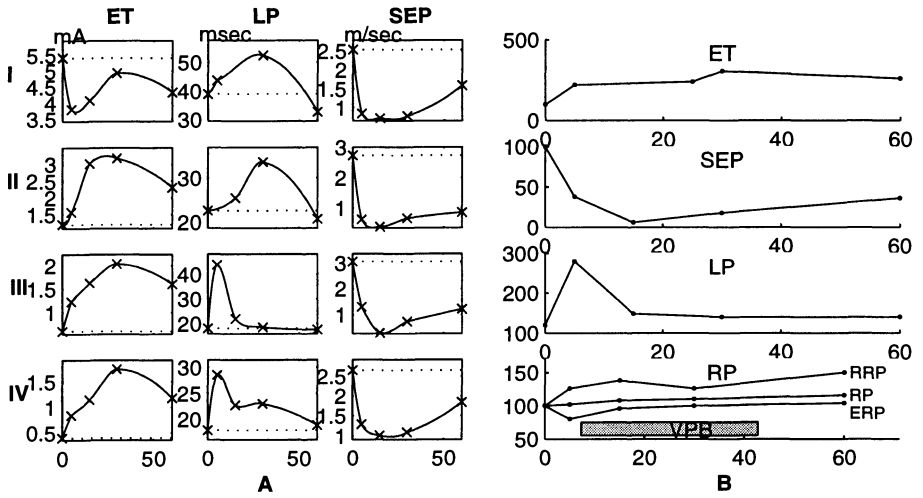

Figure 2.9. A - the dynamics of changes after CAO: ET, LP and SEP in fourth interval of the cardiac cycle (Roman numerals). B - the dynamics of changes after CAO ET, LP, SEP in III interval of cardiac cycle and RP, ERP, and RRP in percent of initial level. Average data, cats, n-ET=33, n-LP,SEP=17, n-RP=24. Period of VPB is shown by shade On abscissa - time after CAO in minutes.

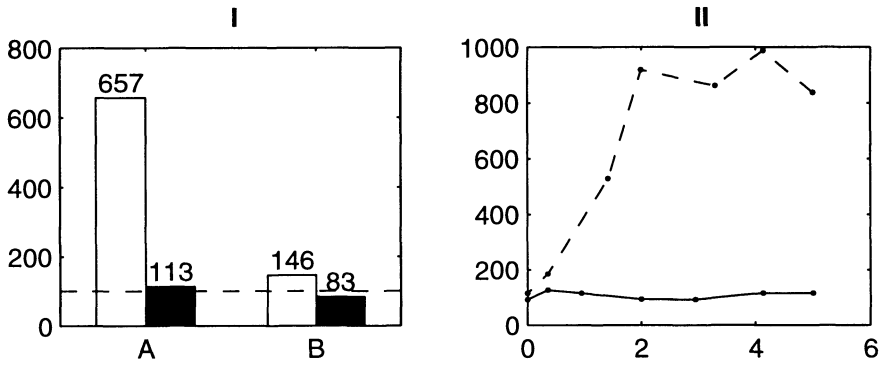

Figure 2.10. ET changes in experiments on dogs. I - ET changes in ischemic (light columns) and nonischemic (black columns) zones of the heart in experiments with (A, n=10) and without (B, n=7) VF (average data in % of the initial level)). II - time course of ET in experiments on dogs with (broken line, n=13) and without (solid line, n=10) VF.

In experiments with VF, the ET in the ischemic zone just before VF reached on average 657% of the initial level, while at the same time in experiments without VF the ET reached only 146%. In the nonischemic zones no essential changes of the ET were observed. Typical time course of ET changes in ischemic

and non-ischemic zones during 5 min. after CAO in experiments with VF are shown in fig.2.10-II.

Thus, the peculiarities of the experiments with VF after CAO consist of considerably greater velocity and level of the ET increase in the ischemic zone in comparison with experiments without VF.

2.2.3.2 The duration of refractory periods. Data of excitability recovery were obtained by applying multiple testing stimuli at 10-15sec intervals during different phases of the cardiac cycle and registering AP using suction electrodes. The time course of the refractory period changes after CAO in percent of the initial level is shown in fig.2.9-B.

The total refractory period duration (RP) increases rapidly until the 5th min. after CAO and then increases slower after a short stabilization. The ERP shortens after 5 min. of CAO and then gradually become longer and reaches a maximum value at the end of the experiment. The RRP, which corresponds to the difference between the RP and the ERP, increases to a maximum value in the first 5 min. of ischemia, due to as shortening of ERP so as lengthening of RP. At the 30th min. of CAO the RRP decreases slightly and increases again after a hour of ischemia. No dependence was observed between the changes in ERP and either RRP or APD (fig.2.8). In the first 5-15 min. of ischemia the most marked shortening both of the APD duration and of the ERP were observed. However the APD shortens to a greater degree than the ERP, and the ERP became approximately equal to the APD. The difference between APD and RP under normal conditions, according to our data, is 23 ms (APD is 195ms, RP is 218 ms). This difference increases considerably in ischemia and reaches 75 ms (183 and 258 ms respectively) at the 60th min. of ischemia. Analogous data were obtained by Brooks et all [40]. It was also shown that the end of the repolarization does not coincide with the excitability recovery. The RP duration shortens but to a lesser degree than the repolarization phase of AP.

In [167] the term *post-repolarizational refractoriness* was used to characterize this phenomenon. This term means that in a certain phase of ischemia the membrane could be unexcited in spite of complete repolarization.

The development of MAP recording allows to investigate drug-induced postrepolarization refractoriness and also early and delayed afterdepolarizations [99].

It was shown on the people [192] that interval between local activation during VF is correlated with local refractoriness and may be used as an index of local refractoriness.

The therapeutic approach should be based on drugs able to prolong the effective refractory period (K-channel blockers, such as class III antiarrhythmic drugs) [88].

It was shown in perfused, isolated, working rabbit hearts that APD and ERP in buffer-perfused hearts are significantly shorter than in blood-perfused hearts [106].

The peculiarity of experiments with VF were studied with an epicardial patch electrode containing 47 electrodes. The dispersion of refractoriness was significantly greater in 20 dogs with VF and VT than in control dogs [203]. A close linear inverse relationship between the duration of the ERP and VF frequency was discovered. RRP remained unchanged in the intact zones of a dog's heart, shortened and then increased sharply in ischemic focus 5 min after CAO [266]. Under these conditions, a sharp increase of the dispersion of the RRP duration between the center and the peripheral zone of the ischemic focus and between the intact parts of the heart and various parts of the ischemic focus occurred. When dispersion of the RRP increases, zone vulnerable to VF enlarges.

2.2.3.3 The latent period. The duration of the latent period during the development of ischemia increases in all the intervals of the cardiac cycle (fig.2.8 and 2.9). In the beginning of the RRP (intervals I and II) this increase reaches a maximum value at the 30th min. after CAO. At the end of the RRP and in the beginning of the diastole the LP increases reaches a maximum at the 5th min. of ischemia.

The increase in the LP duration in ischemia is probably connected with the slowing of the membrane depolarization which is the basis of the latency mechanism according to [129]. The effect of the membrane capacitance characteristics on the LP duration should also not be excluded.

2.2.4 Ischemic changes of conductivity

The velocity of depolarization ($\dot{V}_{max}$) is the most important factor which determines the speed of excitation propagation (SEP) in the heart. The slowing down of conductivity after CAO can be connected not only with the decrease of depolarization velocity but also with changes in the passive properties of muscle fibers. Cell disconnection due to cell contact damage can be one of the possible results of the changes in the passive properties and of the slowing of conduction in ischemia.

The changes in conductivity after CAO can be estimated based on SEP between two fixed points on the heart or based on the time dispersion, i.e. the sequence of excitation of various parts of the heart.

2.2.4.1 Speed of excitation propagation in the heart. In our laboratory SEP and LP duration were measured separately [242] in the four intervals of the cardiac cycle during 1 hour after CAO. As shown in fig.2.9 the changes of SEP in the four intervals of the cardiac cycle were similar in various stages of ischemia: the SEP decreased during RRP and increased during the beginning of the diastole. The most sharp decrease of the SEP occurs after 2 min. of ischemia, what coincides with the appearance of VPB and VF.

The SEP in the heart, which is defined as the ratio of the distance between two registration points and the conduction time, must be considered as a conditional characteristic because it does not take into account the three-dimensional spread of the excitation wave in the heart. Therefore, besides data

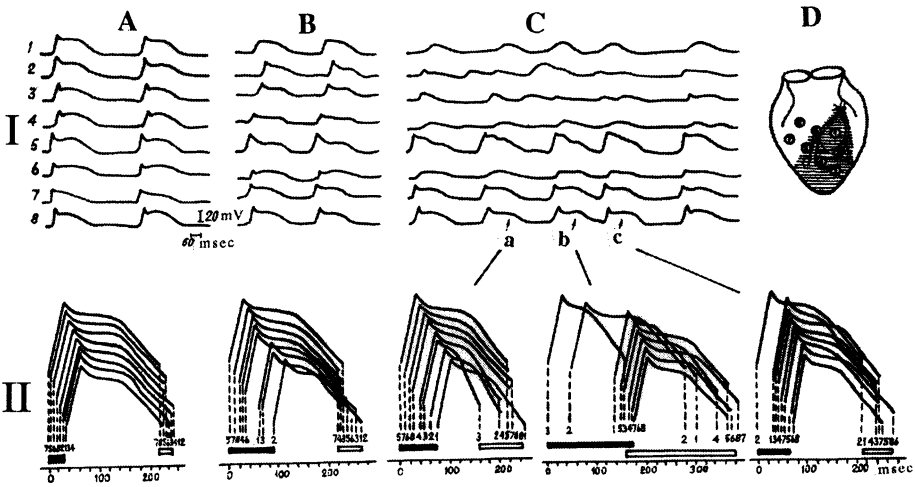

Figure 2.11. The distortion of excitation propagation in the heart after CAO. I - AP from 8 areas of a dog's heart (registered simultaneously using suction electrodes): A -before CAO, B - after CAO without VPB, C - after CAO with group VPB (a-normal excitation, b,c-VPB). D - schematics of the electrodes' location on the heart. II - schematic picture of sequence and dispersion excitation on the 8 areas of the heart. Numerals indicate the number of an area. Black rectangle - depolarization's dispersion, light rectangle - repolarization's dispersion.

about SEP changes, data about time dispersion and sequence of excitation of the heart's various parts are also necessary for understanding of the mechanism of ventricular arrhythmias.

2.2.4.2 Sequence and time dispersion of heart's excitation. The sequence and timing of heart's excitation were studied on humans using multiple electrographic leads from the heart. A computerized three-dimensional mapping system capable of recording simultaneously from 232 intramural sites shows that most of the conduction delay occurred in the subendocardial and midmyocardial regions. The development of VF is due to the intramural reentry and rapid recovery of excitability [222].

The acute ischemia induced by an 80% reduction of coronary flow caused an increase in dispersion of activation and dispersion of repolarization. As a result, the width of the vulnerable window increased [28]. It is not clear, however, why in some cases rhythm disturbances caused by excitation desynchronization are limited to VPB only, and result in VF in other cases.

We studied the changes in excitation's time dispersion in 8 areas of the heart after CAO and the role of these changes in appearance of VPB and VF [242]. These studies were done using suction electrodes (fig.2.11).

A diagram of AP leads from 8 areas of a dog's heart and a representative recorded curve are given in fig.2.11-I. The sequence of de- and repolarization

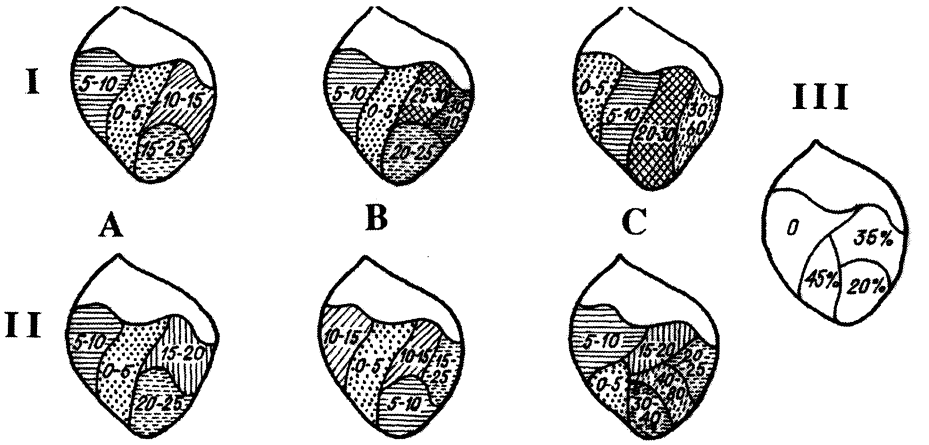

Figure 2.12. The delay (msec) in depolarization (I) and repolarization (II) relative to earliest de/re-polarized part. A - before CAO, B - after CAO without VPB, C - before VPB. III - the frequency of the origin of VPB in different part of the heart (dogs, n=18).

in those areas is shown in a schematic form and on a larger scale in fig.2.11-II. After CAO, time dispersion of the heart's excitation increases: dispersion of depolarization increases from 30 ms to 90 ms, dispersion of APD increases from 20 to 45 ms and dispersion of repolarization increases from 28 to 58 ms.

The sinus rhythm in these experiments was not disturbed. VPB occurred when the value of DR reached 95 ms (fig.2.11-I,C,b). The VPB began at the area 3 (b), in which the repolarization after the preceding sinus excitation completed the earliest (a). Slow spread of VPB in the heart caused a sharp increase the dispersion of depolarization (DD) from 82 to 187 ms and DR from 95 to 245 ms. A condition arose in which excitation was already complete in some parts of the heart, while it only began in other parts. A second VPB (b) occurred in the part that repolarization earliest after the first VPB (2). In the second VPB DD and DR decreased when it spread through the heart. DR became smaller (c) than DR of the sinus excitation before first VPB (a). The compensatory pause arose and the sinus rhythm recovered.

The data of the beginning of depolarization in the 8 areas of the heart (fig.2.12) allow us to trace the path of the excitation spread across these areas and to locate the area which was nearest to the source of the VPB after CAO [242].

Before CAO depolarization occurs first in the septum zone, then after a small interval depolarizes the right ventricle, the middle part and the top of the left ventricle. The repolarization occurs in the same sequence and during approximately the same amount of time. After CAO the sequence of depolarization does not change but occurs with a delay. The sequence of repolarization of the heart's ventricles changes: the top of the left ventricle repolarization finish earlier than the right ventricle. The repolarization of the middle part of the

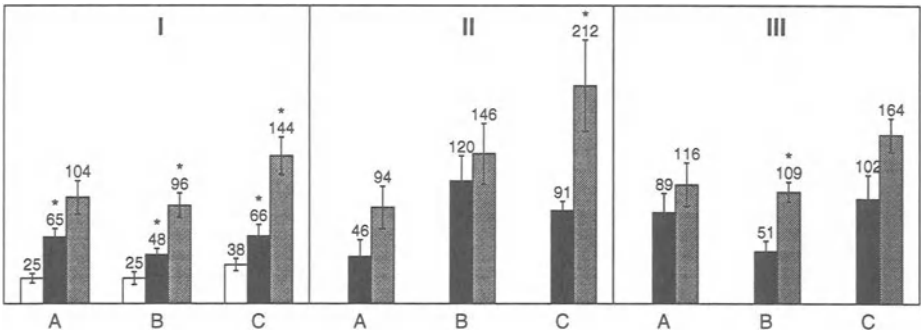

Figure 2.13. I - dispersion of sinus excitation (msec) before CAO (light columns), after CAO without VPB (black columns) and before VPB (grey columns, dogs, n=18). II - dispersion of VPB (msec) after the sinus rhythm (black columns) and after second VPB (grey columns). III - the dispersion of sinus excitation (msec) after CAO with VPB in experiments without VF (black columns, dogs, n=7) and with VF (grey columns, dogs, n=11). * show statistically significant data. A - dispersion of the APD, B - dispersion of the depolarization, C - dispersion of the repolarization.

left ventricle occurs earlier than it can be expected, considering that it was depolarized late.

The phenomena described above can be explained by the inhomogeneous decrease of the APD in the ischemic zone. In cases with VPB, all the areas in the ischemic zone,including the septum zone, depolarized later than in cases without VPB. The changes of DR were especially essential: DR increased considerably in areas adjacent to the ischemic zone. The repeated VPB's arose in one of these areas. The frequency of the VPB's origin was greatest (45%) in those areas of ischemic zone in which repolarization finished relatively earlier and were located near the area with the latest repolarization (fig.2.12-III). VPB never originated from the non-ischemic zone. The our data testifies to the fact that the most essential changes before VPB concern the DR. The relationship between desynchronization of the excitation and VPB occurrence are shown on the fig.2.13-I.

Fig.2.13-I demonstrates that while dispersion of APD, DD and DR in sinus excitation after CAO increases under a normal rhythm, this increase became especially great before VPB. The dispersion of the APD, DD and DR at the time of VPB determines whether the VPB develops into second VPB or into sinus rhythm (fig.2.13-II). If spread of VPB through the heart leads to a sharp increase in dispersion of the APD, DD and especially DR, (fig.2.11-II,C,b) a second VPB arises (c).

The changes of dispersion of depolarization and repolarization in experiments with VF differ quantitatively from changes in experiments without VF. A comparison of the values DR and DD and dispersion of APD of the sinus

excitation after CAO with VPB in experiments with and without VF is given in fig.2.13-III. The dispersion of DD and DR is considerably greater in experiments with VF than in those without VF. The fact that the DD in the experiments with VF is two times greater than in the experiments without VF, indicates a greater degree of conduction slowing down in experiments with VF, which is a necessary condition for reentry.

Thus, the electrophysiological data obtained in our laboratory show that changes which occur in a heart in local ischemia create the necessary conditions of VF appearance through the reentry mechanism. However, in a living heart it is difficult, if not impossible, to determine the effects of the AP parameters on the onset of reentry during ischemia. Mathematical modeling and computer simulation were used for this purpose.

2.3 Study of mechanisms of ventricular arrhythmia using mathematical modeling and computer simulation

Mathematical modeling and computer simulation have been used in numerous investigations of the mechanisms of occurrence of arrhythmias. A mathematical/computer model of cardiac tissue based on measurements of extracellular potential was used to study transmembrane currents [169].

A computer simulation was used to study the initiation of reentry mechanism. A study of reentry as a function of the size of ischemic zones and rate of dispersion of refractory periods showed that the latter parameter is of primary importance in triggering cardiac reentry [22].

A computer model was used to study the dependence between the size of the vulnerable window (portion of the cardiac cycle during which ectopic stimuli can induce VF) and the regional conduction depression. Using a programmed electrical stimulation protocol, vulnerability was quantified as the number of ectopic stimuli necessary to induce VF [97].

Chaos theory was applied in the point-correlation dimension analysis of heart beat variability [292].

Computerized mapping techniques showed that reentrant wave fronts (spiral waves) are present during the VT stage as well as during VF. When a spiral wave begins to meander, periodic activity is replaced by an unstable oscillation which results in chaotic behavior. Authors conclude that VF is a form of deterministic chaos [60]. In [145], propagation of excitation during VF was to studied under various conditions.

2.3.1 Mathematical model of a cardiac cell

The first mathematical ionic model of a cardiac cell was developed by Noble [202] for reconstruction of the AP generated by Purquinje fiber. This model is based on the method and formalism proposed earlier in application to nerve fibers by Hodgkin and Huxley [357]. After small changes, Noble's ionic model also reproduces the AP of a myocardial cell.

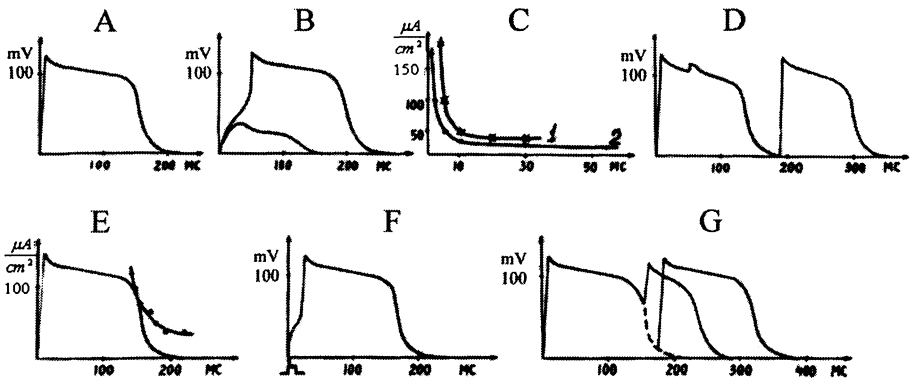

Figure 2.14. Reproduction of basic properties of myocardial cells by the mathematical model. A - reproduction of AP. B - cell response to subliminal and liminal stimulations. C - curves of current force-duration for anode (1) and cathode (2) stimulation. D - response to stimulation in ERP and after that. E - curve of current force-interval cardiac cycle. F - LP after liminal stimulation. G - AP shortening after stimulation in early and late phase of repolarization. Ordinate; in A,B,D,F,G - APA, in C and E - liminal stimulus. Abscissa; in A,D,E,G - time from beginning AP, in B and F - time from the beginning of stimulation, in C - stimulus duration.

Our model was developed in a join effort of the scientists in our laboratory and the Laboratory of Computer Simulation, Institute of Control Science Problems, USSR Academy of Sciences (Moscow). The model was implemented on a Hybrid Computer for investigation of electrical wave propagation along the heart muscle [115, 242]. Two interconnected problem had to be solved: 1) mathematical modelling and computer simulation of AP generation by a cardiac cell dependant on ionic currents and 2) 2D AP propagation along the heart muscle. The model is distinguished from the contemporary simplified models by two fundamental features. It properly reproduces the cell recovery processes [116] and diffusion interaction between the cells in 2D tissue [153].

Verification of the mathematical model for AP generation by a cardiac cell showed that the model correctly reproduced the main physiological properties of a real myocardial cell (fig.2.14)

The model correctly reproduced: 1) the shape of the AP (fig.2.14-A), 2) the AP generation in response to the over threshold stimuli and the local response to subthreshold stimulation (fig.2.14-B), 3) the relationship between the strength and duration of the overthreshold stimulation (fig.2.14-C), 4) the duration of the effective and the relative refractory periods (fig.2.14-D), 5) the "strength-interval" curve (fig.2.14-E), 6)the latent period (fig.2.14-F), 7) the relationship between the AP duration and the duration of the preceding cycle - APD restitution (fig.2.14-G).

Verification of the hypothesis that considers myocardium as continuous medium was carried out in computational experiments consisted of comparison between

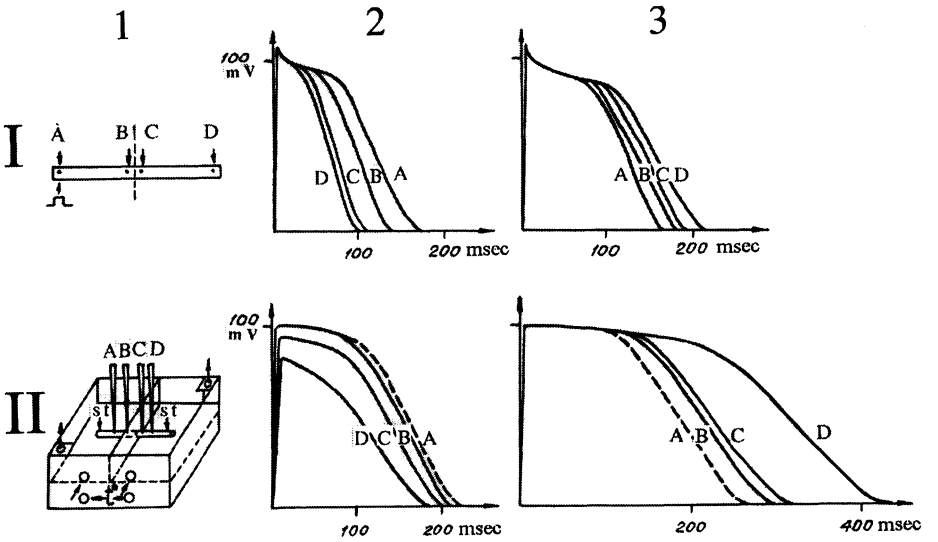

Figure 2.15. The interdependence between electrical influences in myocardial cells. I - computer experiment on a mathematical model of a muscle fiber. 1 -schematic picture of a fiber and lead's points (A-D) of AP. 2 and 3 - AP changes in lead's points by shortening (2) and lengthening (3) AP in right part of chamber. II - electrophysiological experiment on papillary muscle cat's heart. 1 - perfused thermostat chamber with screen. A,B,C,D - points of disposition of microelectrodes for AP lead. st - points of disposition of stimulated electrodes. Places of inward and outward of perfusate are shown by arrows. 2 and 3 - AP changes in lead's points by shortening (2) and lengthening (3) AP in right part of chamber.

the properties of continuous medium and a discrete structure, of the syncytium types. The shape of the propagated AP and characteristics of the plane excitation wave propagation are similar for the continuous medium model and for the syncytium. The propagation velocity in a hexagonal isotropic syncytium and in a continuous medium are almost the same [243].

Verification of the cell interactions in the mathematical model of the cardiac tissue

In the computer and electrophysiological experiments (fig.2.15) AP propagation along the fiber is considered [94].

In computer experiment, the parameters of the "cells" in the left half of the fiber were adjusted to generate AP with APD=153 ms, while the parameters of the "cells" in the right half generate AP with APD=90 ms. We observed (fig.2.15-I,2) that APD corresponded to these values only at the points A and D, while APD at point B decreased (relative to point A) but at point C increased (relative to point D). So, the equalization of APD occurs along the fiber. This equalization is caused by local currents. The effect of local currents decreases with the distance from the boundary between two media. A similar effect was observed by modeling AP propagation along the fiber, which "cells" on the

right side (in point D) generate AP with prolonged APD=206 ms, while APD of the left half remains at the same value - 153 ms (fig.2.15-I,3). APD at point C decreased (relative to point D) and increased at point B (relative to point A).

The electrophysiological verification (fig.2.15-II,1) was realized on a small myocardium strip placed into a chamber with a partition, which permitted us to realize changes of the APD in one side of the partition and to measure the changes of the APD in the other side, not submitted to external influences. In the beginning of the experiment the APD was 220 ms in both sides of the partition. After substitution of the solution with Na concentration 140 Mm for a solution with Na concentration 12 Mm in right side of chamber (fig.2.15-II,2), the APD in the right half of the chamber decreased to 185 ms (point D). APD closer to the boundary (point C) decreased less than at point D, evidently due to effect of the neighboring part, where the APD was greater. In the left half of the chamber, changes of the medium composition did not occur. However, the APD also decreased somewhat, to 210 ms (point A) and less at point B evidently due to effects of the neighboring part, where the APD was smaller.

Similar results were obtained after addition of EDTA into the right half causing an increase of the APD (fig.2.15-II,3). Before the EDTA addition, the APD was the same in both parts of the chamber, equal to 265 ms. EDTA addition increased the APD at point D to 420 ms, while at point C it increased only to 310 ms due to the effect of the APD in the left side of the chamber. At point B, where no changes in the medium composition occurred the APD also increased but to a lesser extent (to 295 ms).

So, the electrophysiological data have demonstrated that the model correctly reflects the electrical interaction of the cells in the myocardium. The equalization action of local current makes sharp changes in the APD of the neighboring cells impossible.

2.3.2 Computer simulation study of the initiation of arrhythmia and its sustaining

The propagation of AP along a 2D piece of cardiac tissue is studied under conditions completely excluding heterotopic automaticity [117, 353, 216]. Computer simulation reproduces the excitation propagation under these conditions, typical for local myocardial ischemia (fig.2.19).

More than 30 years ago Moe and Abildskov [109] formulated the multiple wavelet hypothesis. Allessie at all. [179] were first to provide experimental support for this hypothesis. Janse [136], however, supposed that the question "Is VF always due to multiple wavelet reentry?" required the further study.

Experiments on an isolated canine ventricle using 56-480 bipolar electrodes permitted the authors to hypothesize that cellular responses induced by a premature stimulus depend on the tissue vulnerability to a greater extent than on the effective refractory period. Wave front circulation and subsequent reentry were demonstrated in [111].

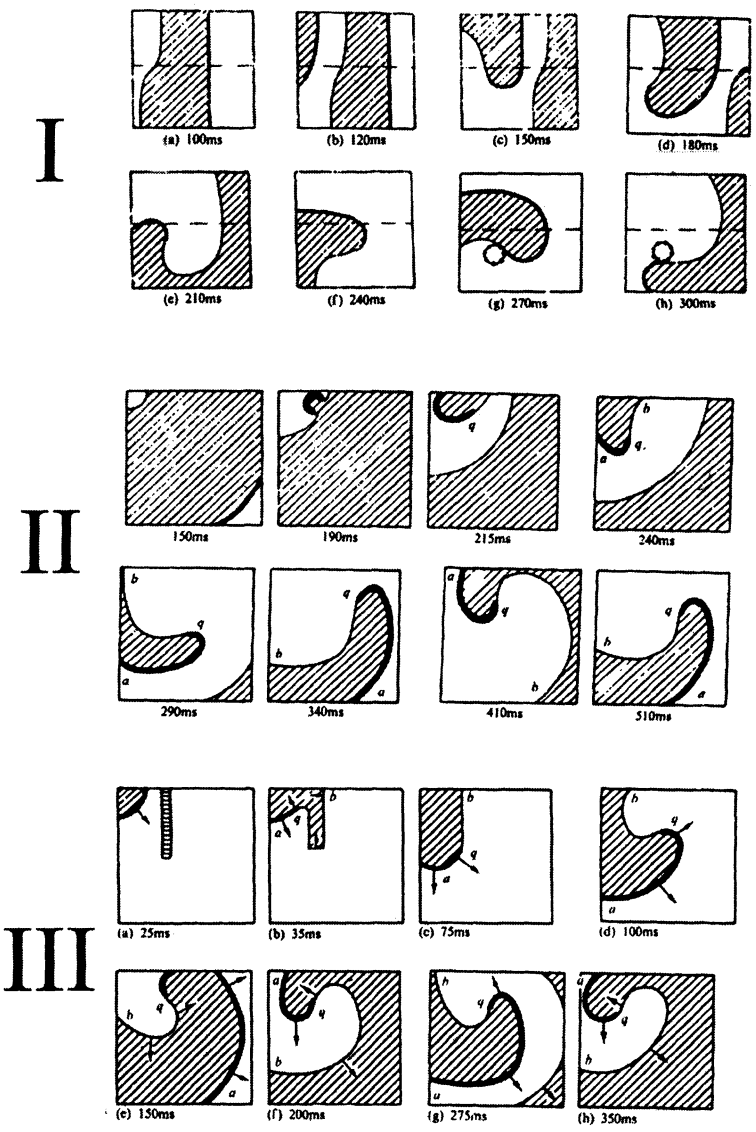

Figure 2.16. Different situation of spiral wave formation. The wave front is denoted by a thick line, the tail by a thin line and direction of wave front propagation by arrows. The excited area is shaded. "q"-point of phase transition on the tip of a spiral wave. I - formation of a spiral wave after a premature beat in a tissue with nonuniform in APD. II - spiral wave formation after a premature beat at the tail of a previous wave in originally uniform tissue. III - spiral wave formation when normal wave front meets temporary unexcited zone (obstacle)

Jalife and Gray [135] present results that strongly support the hypothesis that at least some cases of VF in a structurally normal heart may be the result of a single 3-D electrical scroll wave [135].

The critical medium parameters that defined condition for wave front-obstacle separation were determined analytically in [297].

In our experiments the initiation of spiral waves was obtained under the following conditions:

1. Excitation wave propagation in tissue with a non-uniform distribution APD is shown in fig.2.16-I. The APD below the broken line is greater than above it. The plane wave activated at the left side of the tissue acquires curved tail after some time. The wave length in the upper half of the tissue is shorter then in the lower half. Repeated rectilinear form excitation applied at the left side of the tissue propagates from the beginning only in the domain where APD is small. The propagation is blocked in the region of large APD due to the more pronounced cells recovery processes in that half of the tissue. As a result, the second wave front is curved and appears at two neighboring points in which cells respectively go in and out of excitation. This causes the creation of a spiral wave.

2. The spiral waves can be initiated in homogenous medium by application of a premature beat at the tail of a normal excitation wave. The sequence of stages in the development of spiral wave are shown in fig.2.16-II. The excitation is originally applied to a small region in the left upper corner. As a result, the circular wave propagates along the diagonal of the cell matrix. After application of a premature stimulus at the region located close to the upper border of the tissue and at the tail of first circular wave, the new excited wave can propagate only in direction opposite to the tail of the first wave where the cells recovery processes are not very pronounced. Thus, conditions are created for the appearance of a zone where cells going in and out of excitation are located close to each other (point "q"). The latter leads to formation of a spiral wave (fig.2.16-II).

3. The third case covers wave propagation in the presence of an ischemic zone in the myocardium. The latter is characterized by a dramatic decrease in excitability. In a computer simulation this zone is introduced as an temporary unexcitable zone (fig.2.16-III). The wave excited at the upper left corner of the stimulated area can not pass through the unexcitable zone and moves down. Part of the wave front close to the unexcitable zone soon comes out of excitation due to the local currents and thus the junction point "q" between front and tail of the wave is formed. The wave begins to twist around the unexcitable zone. The excitable properties of this zone are restored just at the moment when the wave is close to the other side of the zone. In this case the wave passes this zone without obstacles and reaches the area where cells were previously excited. If the time of wave circulation is more than the cell's relative refractory period, the repeated excitation of these cells will occur without an external excitation source. Under this condition, the spiral wave can form which leads to undamped activity in the cell matrix.

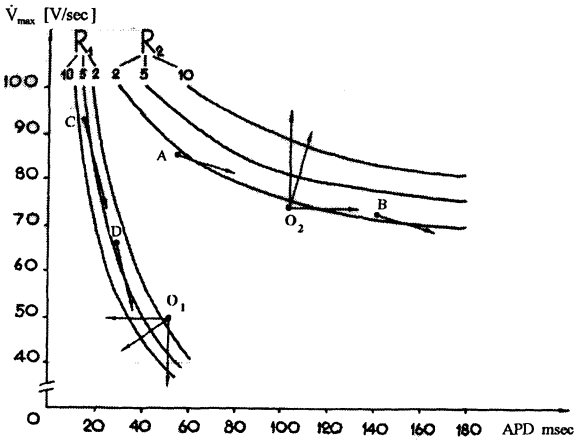

Figure 2.17. The dependence of the spiral wave radius (R) on $\dot{V}$ and APD. R_1 - radius of a stationary spiral wave, R_2 - radius of a nonstationary spiral wave in mm. O_1, O_2, A-D - different positions represent points in the space of AP parameters (explanation in the text)

Thus, computer simulations show that in case of local ischemia appearance of a heterogeneity of medium, premature excitation, or an unexcitable zone can all lead to formation of spiral wave.

2.3.3 Spiral waves and action potential characteristics

In the course of computer simulations, one of the most important characteristics of the spiral wave propagation was found to be the trajectory of the point "q". In case of stationary spiral waves, this trajectory has the form of a circle which is called the core of a spiral wave. The probability of a self-sustained stationary spiral wave depends on the radius of the core. The radius of the core, can be easily determined in the model, but is difficult to measure in physiological experiments.

APD, APA and $\dot{V}_{max}$. are considered as the primary parameters of AP. These parameters can be measured directly on the heart. The relationship between them turns out to be different for nonstable spiral waves and stationary spiral waves (stable arrhythmia).

Two families of curves representing the relationship between the core radius R and $\dot{V}_{max}$ for different APD and between the core radius and APD for different values of $\dot{V}_{max}$ are shown in fig.2.17.

The curves of both families are divided onto two branches. The left branch with the radius R_1 characterizes the stable stationary spiral wave, while the right one (R_2) characterizes the nonstationary circulation. When the initial AP parameters correspond to the point O_1 on the branch, the circulation can be stopped (i.e. R_1 can be increased) by decreasing APD, $\dot{V}_{max}$, or both simultaneously. In the case of a nonstationary spiral wave, when the initial AP

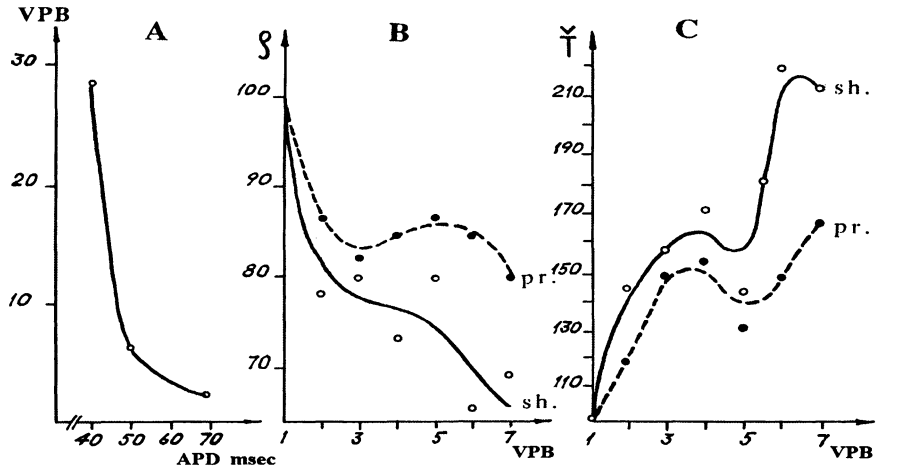

Figure 2.18. A - the dependence of appearance of arrhythmia (VPB) on APD (n=3). B and C - the dependence of sustained arrhythmia on changes in calculated parameters - ρ (B) and T (C) during arrhythmia. Time course of changes in ρ and T (relative to values at the time of the first VPB) for short duration arrhythmia (solid line, sh.,n=6) and prolonged (broken line, pr.,n=8) arrhythmia. The arrhythmia was induced by stimulation of isolated, perfused rabbit hearts during the vulnerable period.

parameters correspond to the point O_2 its transition into stable arrhythmia can be avoided (i.e. R_2 can be increased) by increasing APD, $\dot{V}_{max}$. or both.

The relationship between the arrhythmia characteristics and the AP parameters found in computer simulations, was confirmed in electrophysiological experiments [244]. Arrhythmia was induced in an isolated rabbit atrium perfused with Tirode solution by applying a premature stimulus during the "vulnerable period". The arrhythmia is characterized as *short-term* if the arrhythmic period contains fewer than 10 VPB's, and as *long-term* or *stable* if the arrhythmic period contains 10 to 100 VPB's. Stable arrhythmia occurred only in preparations in which APD was less than 80-100 ms. When APD was greater than 100 ms, it was impossible to induce stable arrhythmia (fig.2.18-A).

The estimates of changes in AP parameters were calculated based on the theoretically obtained generalized characteristics of spiral waves: relaxation - ρ and the spiral wave rotation period - $\check{T}$. The relationship between ρ, $\check{T}$ and AP parameters was found using the following formula:

$$\rho = \frac{APD \times \dot{V}}{APA}; \qquad \check{T} = \frac{APA}{\rho \times \dot{V}} \tag{2.1}$$

Computer simulations showed that in order to stop the stationary spiral wave, i.e. stable arrhythmia, ρ should be decreased and $\check{T}$ increased. Changes in ρ and $\check{T}$ obtained in electrophysiological experiments for short-term and long-term arrhythmias are shown in fig.2.18-B,C. As can be seen from the figure, ρ

decreased and $\check{T}$ already increased after the first 7 VPB's. Arrhythmia stopped when the increase of ρ and the decrease of $\check{T}$ occurred more rapidly. Indeed, the arrhythmia stopped under the effect of quinidin which caused $\check{T}$ to increase drastically and VPB returned again after removal of quinidin which caused $\check{T}$ to decrease.

2.3.4 Modifying action potential parameters to support or termination spiral waves

The effect of changes in AP parameters on the character of spiral wave propagation is not single-valued. This is due to the fact that the antiarrhythmic actions should be different depending on the initial AP parameters. Thus, in the case of unstable spiral waves, application of an agent which increases the AP duration to tissue with AP parameters corresponding to point A on fig.2.17, produces an antiarrhythmic effect. The same agent produces an arrhythmogenic effect when applied to tissue with AP parameters corresponding to point B. For the case of a stationary spiral wave an agent which decreases $\dot{V}_{max}$ is arrhythmogenic at point C and antiarrhythmic in point D. Under these conditions only an in-line computer control system can provide the selection and dosage of antiarrhythmic drug which would affect the corresponding parameters of AP to suppress or prevent the propagation of spiral waves in the heart. One of the approaches for creating of such a computerized control system was developed in our laboratory in collaboration with scientists at the Control Problem Institute, USSR Academy of Science [162, 161]. A detailed description of the experiments is given in chapter VI.

2.4 Conclusions

Occlusion of the anterior descending left coronary artery at the level of 1cm from auricular margin caused VF in 70% of all dogs and in 11% of cats within an hour after CAO. Electrophysiological changes observed in experiments with and without VF were qualitatively similar. A comparison of these changes, however, reveals a quantitative difference between these changes in the experiments with and without VF.

- In the experiments with VF, VPB occurred in 100% of all animals (fig.2.1). and was characterized by a higher frequency, by earlier occurrence of VPB's in the cardiac cycle and by a greater percentage of multiple VPB (fig.2.2). We showed that predisposition to VPB and VF after CAO develops in parallel but independently of each other.
- Qualitative differences in changes of the AP parameters were found in the experiments with and without VF. The APD, APA and DR decreased more rapidly in experiments with VF than in experiments without it (fig.2.5).
- Pronounced changes in excitability (increase of ET and LP, and a decrease of SEP) in cat hearts coincide with the appearance of VPB. The inverse of these changes coincides with the disappearance of VPB (fig.2.5). The ET

increased with at a considerably higher rate in experiments with VF, than in experiments without VF (fig.2.10).

- The dispersion of time depolarization (DD) and repolarization times (DR) increased in experiments with VF to a greater extent than in cases without VF (fig.2.13). Spatial analysis of changes in DD and DR shows that VPB appears in those areas of the ischemic zone which repolarize first and are located close to areas with the latest repolarization. VPB never occurs in nonischemic zones (fig.2.12).
- Thus, VF appears after CAO in cases when ischemic changes developed dramatically sharply and powerfully. Experiments with VF are characterized by a pronounced decrease in APD, APA, $\dot{V}_{max}$. and a pronounced increase in ET, DD and DR. All of these changes after CAO create the conditions for reentry, a possible mechanism of VF.
- The possibility of formation of spiral waves in the heart with local ischemia was shown using computer simulation (fig.2.16). The most important parameter which characterizes the spiral wave is the core radius R. When the core radius increases, probability of spiral wave termination increases.
- Simulation experiments enabled us to find a relationship between the radius of the spiral wave core and the AP parameters: $\dot{V}$ and APD (fig.2.17). The aptitude to relaxation, ρ, and the circulation period, $\check{T}$, can be used as overall characteristics determined by these parameters. We showed that ρ should decrease and $\check{T}$ should increase in order to terminate the arrhythmia. The results of computer simulation experiments were confirmed by experiments with arrhythmia induced in an isolated rabbit atrium. Termination of arrhythmia was connected with a decrease in ρ and an increase in $\check{T}$ (fig.2.18).

3 Ventricular Fibrillation and Changes in Metabolism of the Heart after Local Ischemia

As shown in the preceding chapter, the chain of electrophysiological processes that induces VF during local ischemia, includes changes in heart conductivity, automaticity and excitability. These changes are caused by changes in rest and action membrane potentials, which, in turn, are related to changes in K^+, Na^+ and Ca^{++} ionic currents across the cell membrane.

This chapter will discuss metabolic changes in the heart during local ischemia, which could be connected with changes in ionic currents. The K^+, Na^+ and Ca^{++} ionic equilibrium in the heart is closely connected with proton movement, which, in turn, is a consequence of the electron transfer processes. For that reason we will analyze not only the state of ionic exchange, but also changes in acid-base and oxidation-reduction equilibrium in the heart

A review of local myocardial biochemical changes during myocardial ischemia was made by Gettes at all [105]. The arrhythmogenic role of different biochemical factors during ischemia, such as hypoxia, acidosis, free fatty acids and fatty acid esters, phosphoglycerides, lysophosphoglycerides and cyclic AMP was discussed by Corr [65].

The sequence of metabolic changes which develop after CAO and possible ways of investigation of their role in appearance of VF are presented in fig.3.1.

Hypoxia, a decrease in oxygen partial pressure (P_{O_2}), is a primary change in the ischemic zone. The consequences of hypoxia are depression of respiration and decrease in phosphate potential in mitochondria and a compensatory increase in the glycogenolysis in the cytoplasm. The combined result of the res-

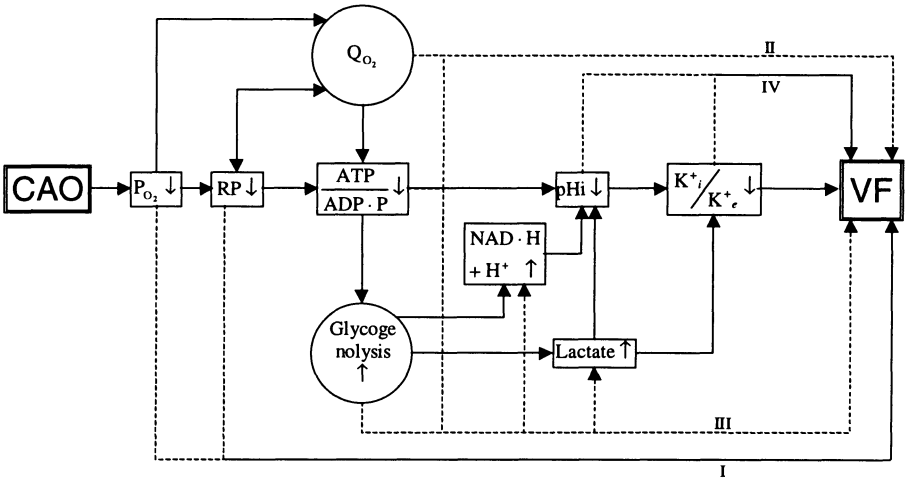

Figure 3.1. The sequence of metabolic changes after CAO, which contribute to appearance of VF. The ways of the study are indicated by Roman numerals. See list of abbreviations

piration depression and glycogenolysis increase is a decrease in the myocardial ox-red potential (ORP). The intensification of glycogenolysis causes accumulation of lactate and of reduced NAD with a free H^+ ion. Because of this and the prevalence of the ATP decomposition over the synthesis, intracellular Ph decreases. This causes changes in the intra-extracellular concentration gradients of K^+, Na^+ , Ca^{++}, Cl^-, which in turn, cause changes in the membrane potentials and eventually cause the appearance of VF.

Given the chain of events in the ischemic zone, the probability of VF after CAO can be determined by:

1. the degree of redox equilibrium disturbances in the myocardium, which, in turn, is determined by:
   (a) decrease in oxygen delivery,
   (b) respiration and phosphorylation alteration in the mitochondria
   (c) glycogenolysis activation which causes NAD·H and lactate accumulation;
2. the degree of acid-base equilibrium disturbances and
3. the degree of ionic homeostasis changes.

A review of experimental data obtained in our laboratory is presented in [236]. Investigations of the characteristics of the above mentioned processes in experiments with CAO resulting in VF, were carried out by means of specially elaborated methods;

1. The method of continuous simultaneous registration of numerous parameters, such as PO_2, ORP, Ph, Pna, pCl in the ischemic and non-ischemic

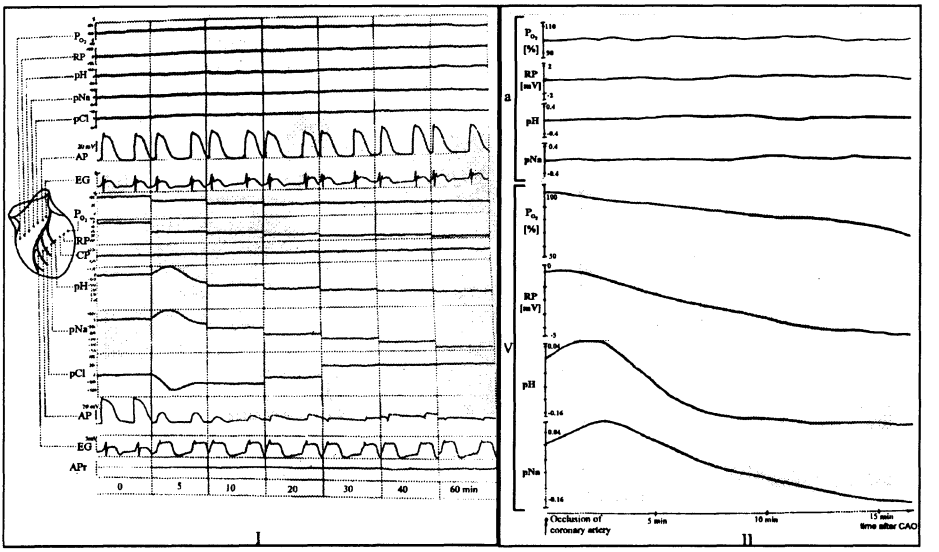

Figure 3.2. I - the continuous record of P_{O_2}, ORP, pH, pNa, pCl,AP, EG and arterial pressure (Apr) changes in ischemic and non- ischemic zones of the dog's heart before and after CAO II - continuous record of P_{O_2}, ORP, pH and pNa in arterial blood (a) and in the coronary sinus blood (V) before and after CAO

zones of the heart by means of special selective electrodes together with physiological characteristics, such as ECG, EG, AP,Apr.[248]

2. the method of continuous simultaneous measurements and registration of P_{O_2}, ORP, Ph and Pna in the blood flow by means of a manycomponent flowing chamber [248].

These methods made possible permanent recordings of the above mentioned parameters in situ before and after CAO until the appearance of VF. An example of synchronous registration of all the above mentioned parameters in the ischemic and non-ischemic zone of the heart is given in fig.3.2-I, in the arterial and coronary sinus blood is given in fig.3.2-II.

As follows from these figures there are substantial changes in all of the recorded parameters in the ischemic zone and in the coronary sinus blood while no changes were observed in the non-ischemic zone and in the arterial blood. A more detailed description of these changes is given in the corresponding sections.

3.1 Ischemic changes in the ox-red equilibrium

The ox-red equilibrium in the heart can be evaluated by measuring the general ORP of the tissue and by measuring the ORP of the main redox systems.

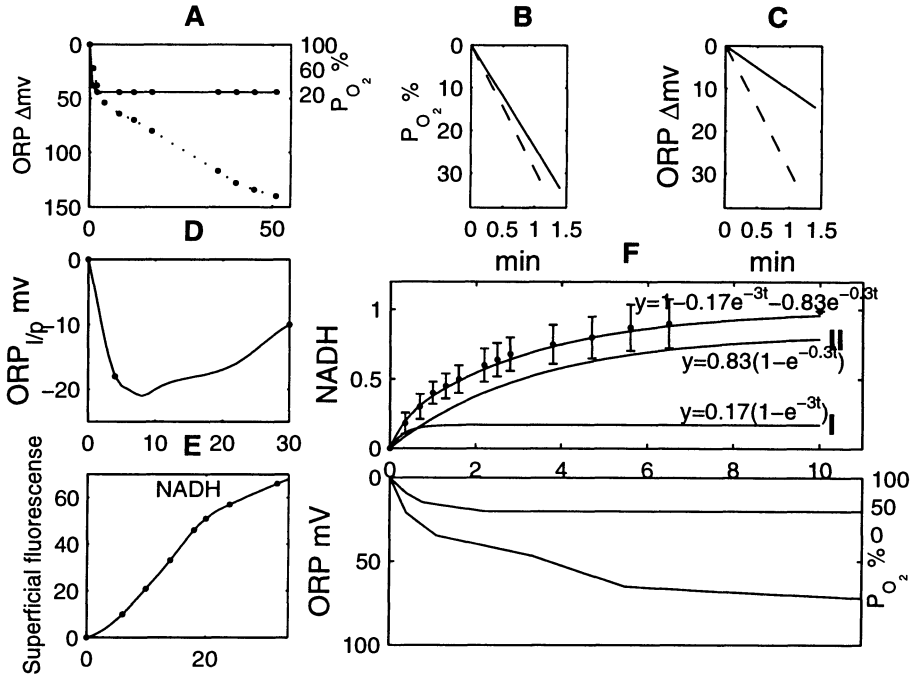

Figure 3.3. Ischemic changes in the ox-red equilibrium. A - changes in the ischemic zone of a canine heart P_{O_2} and ORP (n=29). B, C - the initial rate of their changes in experiments with VF (n=18, broken line) and without VF (n=11, solid line, D - ischemic changes in ORP system of lactat/pyruvate (RP l/p), E - ischemic changes in NAD/NAD·H system, F - the empirical curve showing the changes in NAD.H fluorescence in ischemic zone. The theoretical curve made up of I and II. Simultaneous changes in P_{O_2} and ORP

3.1.1 General oxred potential of myocardium

The permanent recording of the general ORP in the myocardium was performed by means of potentiometric method. P_{O_2} was simultaneous recorded using the polarographic method [248]. The average data of changes in the ORP and P_{O_2} during the first hour after CAO in dogs are shown in fig.3.3-A.

As can be seen from this figure, the rate of the ORP decrease maximal during the first 2-5 min and then slows down.

3.1.2 Ox-red potential of Lactate/Pyruvate system

The components of this system have high diffusion capacity, which allows them to be present not only in the tissue, but also in the blood. We have shown (fig.3.3-D) that the changes in this system have two phases. The decrease occurs until the 5^{th} min after CAO, and it continues to increase up to the 30^{th} min, but does not reach the initial level. These changes may be the result

of a sharp activation of glycolysis in the first few minutes of ischemia and its consequent inhibition [235].

3.1.3 Oxred potential of the NAD/NAD·H system

The method, developed by Chance [56] allowed us to record NAD·H in experiments in situ by means of measuring the intensity of fluorescence. The changes in NAD·H in ischemic zone 30 min after CAO are presented in fig.3.3-E. Changes in NAD.H during the first 10 min after CAO are presented in fig.3.3-F.

The empirical data were approximated via the least square method, a curve which is described by the following equation

$$y = 1 - 0.17e^{-3t} - 0.83e^{0.3t} \tag{3.1}$$

where:
y - the NAD·H fluorescence in relative units of fluorescence intensity (I/Imax);
t - the time in min;
e - the natural logarithm base.

Analysis of this equation shows that the changes in NAD·H fluorescence intensity are described by two exponential functions; therefore they are determined by two processes. During the first 2 min after CAO the NAD·H fluorescence intensity increases at the expense of the processes described both by the first and the second exponential functions. The first function

$$y = 0.17(1 - e^{-3t}) \tag{3.2}$$

exerts a considerably greater influence on the changes in NAD·H than the second one

$$y = 0.83(1 - e^{-0.3t} \tag{3.3}$$

2-3 min after the occlusion the influence of the first function on the changes in NAD·H decreases, approaching 0, while the influence of the second function gradually increases becoming significant at the 3^{rd}-4^{th} min after the onset of ischemia.

A comparison of exponential functions I and II with the graphs of P_{O_2} and ORP shows that function I is a mirror image of the P_{O_2} curve, while function II a mirror image of the ORP curve. Taking into account that P_{O_2} is one of the most important limiting factors in the mitochondria respiration, it can be supposed that function I reflects mitochondrial NAD·H. The slower NAD·H changes described by function II probably reflect the cytoplasmic NAD·H which is accumulated as a result of activation of glycogenolysis.

3.1.4 Partial pressure of oxygen in the heart

Average data of P_{O_2} changes are presented in fig 3.3-A. A drastic decrease in P_{O_2} to 20% of the initial level occurs during the first 3 min after CAO. Then P_{O_2} stabilizes, while the redox potential continues to decrease and NAD·H increases at the same time.

We have calculated the rate of P_{O_2} and ORP changes during the first few minutes after CAO (fig.3.3-B,C) in experiments with and without VF separately. ORP decreases faster in experiments with VF than in the experiments without VF, but the rate of P_{O_2} decrease is the same. The drastic changes in the redox potential in experiments with VF may be due to specific changes in respiration, oxidative phosphorylation and glycogenolysis.

3.1.5 Respiration and oxidative phosphorylation in mitochondria

Respiration and the coupling of respiration with phosphorylation in mitochondria from the ischemic and non-ischemic zones was studied 5 and 30 min after of CAO. In experiments with VF the measurements were made just after its onset [78, 236].

Mitochondrial respiration rate was registered using the polarographic method in five states [57]: 1) Q_{start} - just after the introduction of mitochondria into the incubation medium; this state reflects respiration on endogenic substrates; 2) Q_{rest} - in a quiet state which was recorded after superfluity addition of the oxidation substrate; 3)Q_{III} - after addition of 100 Mm ADP; 4) Q_{IV} - after exhausting ADP; 5) Q_{DNP} in the presence of $5 \cdot 10^{-5}$ M 2.4 -dinitrophenol (fig.3.4 demonstrates the average data of changes.

The rate of decrease of Q_{III} in the ischemic zone 5 and 30 min after CAO was significantly greater in cases with VF than in cases without VF. No differences were observed in non-ischemic zone (fig.3.4-I).

The activity of the succinate dehydrogenase, of the succinate cytochrome-C-oxidoreductase and of the cytochrome system enzymes was studied in our laboratory in order to clarify the role of the respiratory chain enzymes in changes in respiration. We have shown that a statistically insignificant decrease in succinate dehydrogenase activity and an increase in succinate cytochrome -C-oxidoreductase in the ischemic zone take place fig.3.4-III). The difference in the activity of these enzymes in experiments with and without VF also proved to be not statistically significant.

The comparison of differential spectra of cytochrome a,b and c of the mitochondria from the intact and ischemic zones 20 min after CAO [233, 236] (fig.3.4-V) revealed no difference in their redox capacities. [1].

So, the depression of the respiration discovered in various stages of ischemia is not connected with changes in catalytic activity of enzymes of the respiratory chain.

The coupling of respiration with phosphorylation was measured using the values of: 1) respiratory control according to Chance, Williams [57] - $RC_{ChW} = Q_{III}/Q_{IV}$; 2) respiratory control according to Lardy, Wellman [165]- $RC_{LW} = Q_{III}/Q_{rest}$. and 3) phosphate potential (fig.3.4-IV).

[1]These investigations were carried out with the contribution of Mrs. Mokhova E.N., and we are sincerely grateful to her.

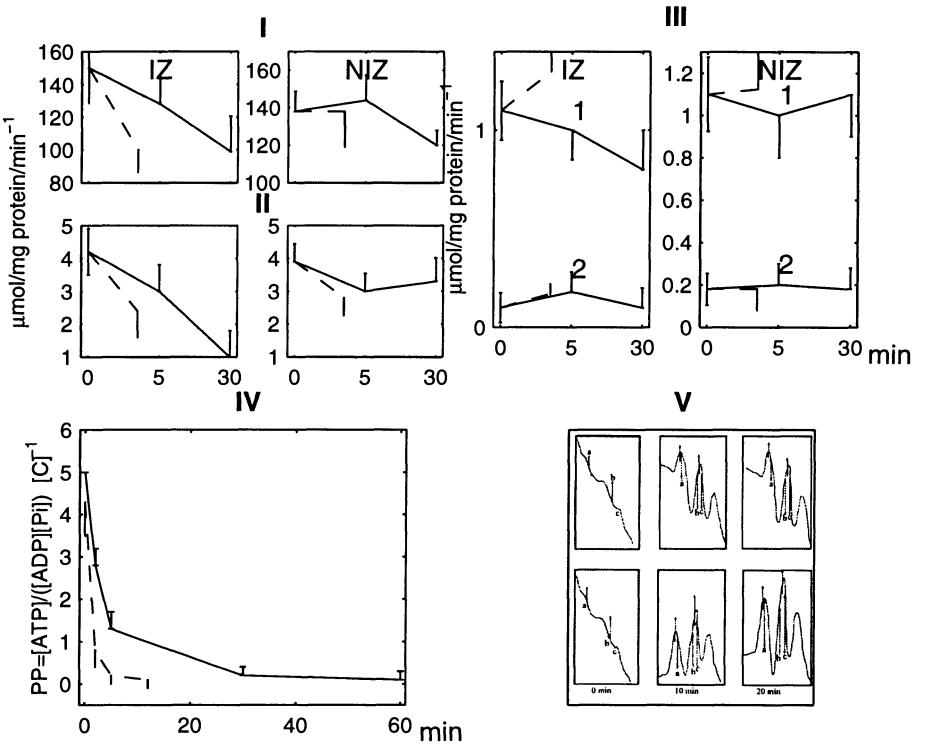

Figure 3.4. Changes in respiration and oxidative phosphorylation in the mitochondria after CAO. I - mitochondrial respiration - Q_{III}, II - mitochondrial oxidative phosphorylation - RC_{ChW}, III - activity succinic dehydrogenase(1) and succinic cytochrome oxidase-reductase (2) in IZ and NIZ before CAO (n=8), 5 min. (n=8), 30 min. (n=9) after CAO and at the time of VF (n=13) in experiments with VF (broken line) and without VF (solid line). Incubation media: 150 mMole KCl, 90 Mmole saccharose, 2 mMole TRIS -phosphate (pH 7.4), temperature 37°C, protein - 2-3 mg/ml. Addition: 100 mole ADP, $5 \cdot 10^{-5}$ mole 2.4 DNF, 4 Mmole TRIS-succinate (pH 7.4). IV - phosphate potential (PP) in IZ after CAO in dogs without VF (n=10) and with VF (n=15), V - spectra cytochrome a, b, c in mitochondria from IZ and NIZ of the heart in control group and after 10 and 20 min. after addition of reducing agent.

A more pronounced uncoupling between respiration and phosphorylation was observed in cases with VF. The rate of decrease in RC_{ChW} (fig.3.4-II) and phosphate potential (fig.3.4-IV) was statistically greater in cases with VF.

Thus, the high degree of respiration depression and uncoupling of respiration and phosphorylation in the mitochondria of the ischemic zone is one of the characteristics of cases with VF.

One of the ways of carrying out the search for antifibrillatory agents is by determining drugs that influence the oxidation-reduction processes. Addition of electron acceptors, i.e. substances with a high redox potential (cytochrome C, coenzyme Q), was used for respiration activation (see chapter VI). Respiration depression and uncoupling of the respiration and phosphorylation stimulate glycogenolysis activation in the ischemic zone.

3.1.6 Glycogenolysis changes in CAO

Serial sampling from the ischemic zone of dog hearts was carried out in our laboratory by means of special tongs, which provide momentary freezing of the tissue in situ at the temperature of liquid nitrogen, in order to observe the changes in glycogenolysis immediately preceding the VF.

Concentration of almost all glycogenolysis metabolites were measured in the samples taken from the myocardium at the 2, 5, 30 and 60 min after CAO (in experiments with VF the last sample was taken immediately before VF) [84, 236]. The activities of the respective glycogenolysis enzymes were determined by means of mass action ratio [264].

Data describing the main changes in metabolite contents and in the main glycogenolytic enzyme activity in the ischemic zone after CAO in experiments with and without VF are presented in fig.3.5.

The rate of change in the content of metabolites (fig.3.5-I) was highest during the first 5 min of ischemia and decreased considerably afterwards. After 30 minutes of ischemia the concentrations of most metabolites became constant.

The mass action ratio (MAR) of the respective glycogenolysis reactions (fig.3.5-II) increased during the whole period of observation for P-ase, PFK, TPI, and LDG ($p<0.05$). By assuming that the rate of the MAR changes for P-ase, PFK and LDG during the first 5 min of ischemia can be described by a linear function, we have approximated the data obtained by straight lines (fig.3.5-III). The differences between the regression coefficients in experiments with and without VF for P-ase and LDG were for found to be statistically significant.

At the same time a decrease in ATP content and an increase in ADP and Pi was observed (fig.3.5-IV). The rate of ATP, ADP, Pi changes was also higher in experiments with VF.

The changes in the content of the glycogenolysis metabolites (A) and of the MAR values for the respective enzymes (B) in their natural succession are presented in fig.3.6 in order to give a complete picture of the changes in glycogenolysis process.

Fig.3.6 shows that the activation of various glycogenolysis enzymes occurs at a different rate. The considerable increase in the activity of P-ase and PFK^{+} is not accompanied by a comparable increase in Ald and GlA-3-PDG activity. The inadequate increase in the Ald activity is testified to by the fact that only a negligible increase in the main reaction product (DOAP) is observed in spite of a considerable increase in substrate of the reaction (FDP). The inadequate increase in GlA-3-PDG activity is testified to by the increase in GlA-3-P.

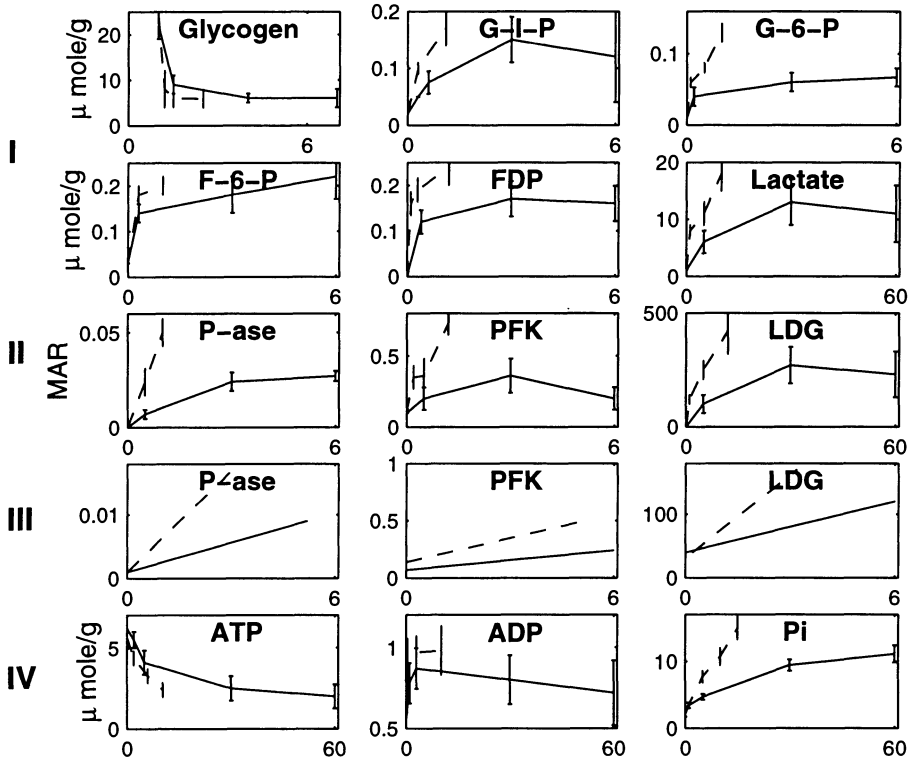

Figure 3.5. The changes in glycogenolysis in ischemic zone of a dog's heart after CAO. I - the changes in the main content of metabolites, II - the changes in mass action ratio (MAR) principal glycogenolysis enzymes, III - the regression line of the same enzymes changes,IV - the changes in the content of ATP, ADP, and PP

Thus, the central link of glycogenolysis - Ald and apparently GlA-3-PDG become the velocity limiting factors after 5 minutes of ischemia. This is also evidenced by the fact that a considerable increase in the intermediate metabolites G-1-P → GlA-3-P is accompanied by an insignificant increase in metabolites 3-PGl → pyruvate. The existence of velocity limiting links and decrease in MAR of PK and of GlA-3-PDG & PGlK reactions, i.e. the reactions related to generation of ATP, is the cause of insufficient ATP generation during the glycogen decomposition.

The comparison the data obtained in experiments with and without VF was shown that the changes in metabolite content (fig.3.5-1) and the MAR of the glycogenolysis reactions (fig.3.5-II,III) in experiments with and without VF occurred in the same direction, however a great quantitative difference was

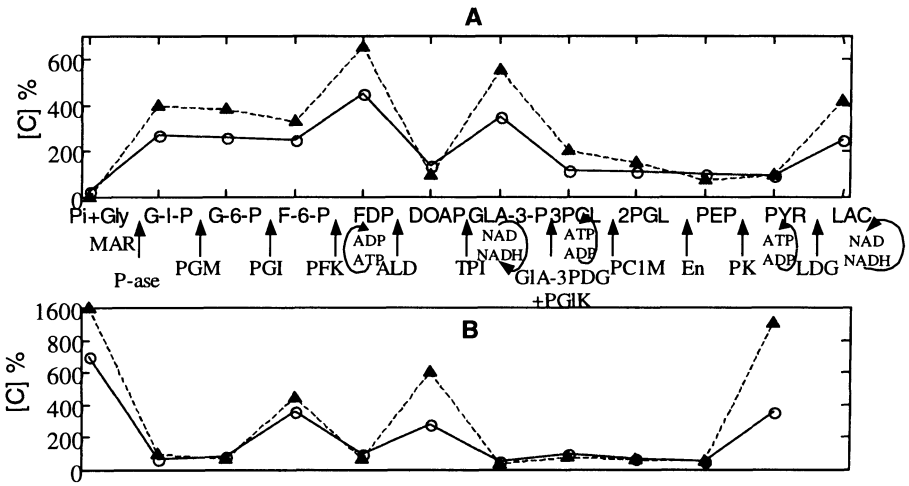

Figure 3.6. The sequential changes in glycogenolysis metabolites content (A) and mass action ratio (B) in 5 min. after CAO (% of initial level). Experiments with VF (n=15, broken line) and without VF (n=10,solid line). Abbreviation - see list abbreviation

observed between them. The changes in glycogen, G-6-P FDP, GlA-3-P and lactate contents, were more statistically significant in experiments with VF. The statistically significant increase in the MAR for P-ase, PFK, TPI, LDG and a greater decrease in MAR for PGlM and Ald were also observed in experiments with VF (only the main changes are presented in figure 3.5).

The analysis of changes in the glycogenolysis process as a whole showed (fig.3.6) that the velocity limiting role of the central glycogenolysis link i.e. Ald and possibly GlA-3-PDG is more pronounced in experiments with VF. As a result, there is a greater disparity between generation of ATP during the glycogenolysis and glycogen decomposition in experiments with VF than in experiments without VF.

So, when respiration suppress and glycogenolysis activation come, the decrease in general redox potential will be determined not only by the fact that respiration is slowing down, but also by the activation of the glycogenolysis. The activation of glycogenolysis which is designed to compensate for energetic deficiency in ischemic zone, does not prevent asystolic of this zone. The activated glycogenolysis, however, increases the disturbances in oxygen-reduction homeostasis. The ox/red ratio decreases not only as a result of a decrease in "ox" value but also as a result of an increase in "red" value.

In our opinion, it is one of examples of the imperfect compensatory mechanisms in pathology (other examples will be described in other chapters). The slowing down of the respiration in ischemic zone not only not accompanied by an adequate glycogenolysis inhibition, but, on the contrary, the glycogenolysis activation stimulates the decrease in the ox-red potential in ischemic zone.

Thus, inhibition of glycogenolysis could provide a second approach to the search for antifibrillatory agents . Monoiodacetat can be used for Inhibition of glycogenolysis (see chapter VI).

The decrease in ox-red potential is connected to changes in acid-base equilibrium.

3.2 Ischemic disturbances in acid-base equilibrium

According to the generally accepted opinion, acidosis develops in the ischemic zone immediately after CAO. Numerous phenomena underlie acidosis during local ischemia: 1) decrease in the ox-red potential in the mitochondria because of oxygen deficiency; 2) intensification of the glycogenolysis with lactate formation; 3) an increase in H^+ ion concentration occurs in the decomposition reaction of ATP into ADP and Pi:

$$MgATP^{--} \rightleftharpoons MgADP^{-} + Pi^{--} + H^{+} \tag{3.4}$$

with H^+ entrance into the cytoplasm from the mitochondria; 4) the acidosis is also facilitated by CO_2 generation due to the residual oxidative metabolism; 5) increase in quantity of fatty acids due to both the increase in lipolysis and to acceleration of their synthesis; 6) is it possible involvement of oxygen free radicals in the development of myocardial acidosis during ischemia.

It has been shown that systemic metabolic acidosis caused by the infusion of lactic acid causes a decrease in VF threshold in dogs [103], whereas systemic metabolic alkalosis, caused by infusion of $NaHCO_3$ and of trioximethyl aminomethane or by inhalation of 30% O_2 in N_2, increases the VF threshold.

The importance of development of acidosis in the appearance of VF during local ischemia promoted attempts to carry out both extra- and intracellular pH measurements.

3.2.1 The ischemic changes in extracellular pH

The extracellular pH changes were evaluated via:

1. pH changes in the coronary sinus blood
2. pH changes in the coronary vein blood which flows from the ischemic zone of the heart
3. pH changes in coronary effluate during ischemic perfusion of an isolated heart's area and
4. pH changes directly in the myocardium.

3.2.1.1 pH changes in the blood of coronary sinus. pH changes in the blood flowing out of an ischemic heart reflect the pH in the extracellular medium of the myocardium because the electrolyte exchange between the blood plasma and the interstitial liquid occurs at a very high rate. Measurements and recordings of pH simultaneously with other parameters (P_{O_2}, ORP, pNa) in the

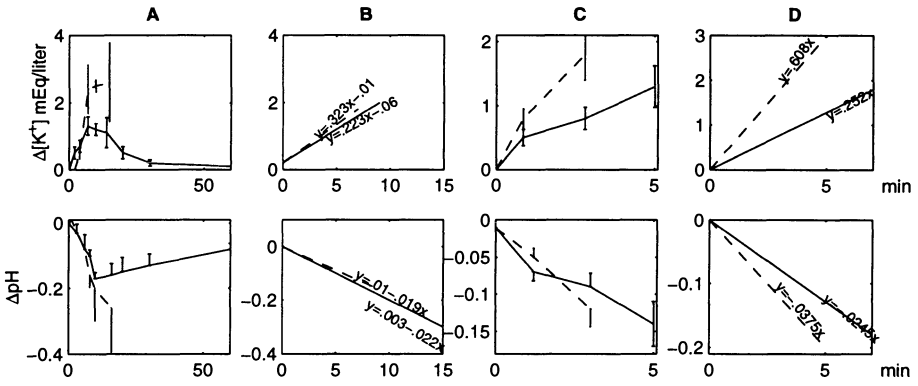

Figure 3.7. A - The of $[K^+]$ and pH in coronary vein blood in experiments with (n=11, broken line) and without (n=6,solid line) VF. B - regression lines those changes. C - The changes of $[K^+]$ and pH in coronary effluate during ischemic perfusion of a part of the heart in experiments with (n=8,broken line) and without (n=7,solid line) VF. Perfusate's composition:19 mmole NaCl, 3 mmole Kcl, 2.5 mmole $CaCl_2$, pH 7.4, non-buffer solution. D - regression lines those changes

blood flowing out of the coronary sinus were carried out by means of many-component flowing chamber (fig.3.2-II). The continuous recordings allowed us to find [248] two phases of pH changes in coronary sinus blood after CAO. The first phase is characterized by a small increase in pH by the 3rd min. The second phase is characterized by a considerable decrease in pH in coronary sinus blood, without any changes in the arterial blood.

The pH changes in the coronary sinus blood, however, do not completely reflect the changes in the ischemic focus because blood from the ischemic focus in coronary sinus is diluted by a great quantity of blood from the non-ischemic myocardium.

3.2.1.2 Changes in pH in the coronary vein blood. Ischemic changes in coronary vein blood, which drains the ischemic zone, were studied in 17 experiments on dogs. VF developed in 11 experiments. [328]. The averaged data of the pH changes in coronary vein blood after CAO in experiments with and without VF are presented in fig.3.7-A.

The initial pH of blood plasma in experiments with and without fibrillation was almost the same. In experiments without VF, pH decreased and returns to initial level. In experiments with VF the pH of coronary vein blood after a small but statistically significant ($p<0.01$) alkalization decreased. By assuming that the pH lowering process is nearly linear, regression coefficients for experiments with and without VF were calculated. The regression curves beginning 1 min after CAO until the average time of maximum decrease in pH are shown in

fig 3.7-B. The differences in rate of decrease in pH in experiments with and without fibrillation proved to be not statistically significant.

3.2.1.3 Changes in pH of coronary effluate in the time of ischemic perfusion of an isolated heart's area. The ischemic changes in extracellular pH of myocardium were also studied using the model of ischemic perfusion of an isolated heart area in 18 experiments on dogs [287]. After 5 min of ischemic perfusion with a non-buffering solution (pH 7.4), pH effluate (solution, flowing out of the ischemic area) decreased by 0.13 units at the 5th min of perfusion. During the perfusion period VF occurred in 8 cases. The development of the effluate pH changes in experiments with and without VF is presented in fig.3.7-C, regression lines of these changes is given in fig.3.7-D. The rate of decrease of pH in experiments with VF was somewhat higher but the difference in the rates was not statistically significant.

3.2.1.4 Changes in myocardial pH. Measurement of the myocardial pH by means of a glass electrode is another approach to the study of extracellular pH in the heart. A method of measurement and continuous recording of the myocardial pH in vivo was developed in our laboratory [248]. Direct myocardial pH measurement by means of an implanted glass electrode faces many difficulties and limitations. First of all, the implantation of electrode causes alterations of the myocardial cells and the medium surrounding the electrode becomes a mixture of the intra- and extracellular medium. This difficulty can be overcome by increasing the interval of time between the electrode implantation and the beginning of the measurement. During this interval the intracellular contents are washed out and the electrode becomes surrounded by the extracellular medium.

The second difficulty consists of the influence of the electric field of the heart on the electrochemical potential of the electrode couple. This difficulty can be avoided by decreasing the electrode sizes, bringing the pH electrode and the reference electrode closer together, and continuous measurements of the control potential (CP) between the two reference electrodes near the measuring electrode.

The changes in myocardial pH after CAO were studied [236] in 42 experiments on dogs, taking into account these requirements. Spontaneous VF occured in 22 experiments. Direct recordings of changes in pH after CAO simultaneous with other parameters in experiments without VF are shown in fig.3.2-I, in experiments with VF in fig.3.8-A.

As can be seen from the figure 3.2-I there is no change in the non-ischemic zone, while a small transitory alkalization with concomitant sharp acidification was observed in the ischemic zone (fig.3.2-I and fig.3.9-A).

The alkalosis phase could be explained by the influence of the depolarization phase of AP on the potential at the measuring electrode. However this assumption was rejected because alkalosis phase was also observed in coronary sinus blood (fig.3.2-II) and most importantly in coronary vein blood (fig.3.7-

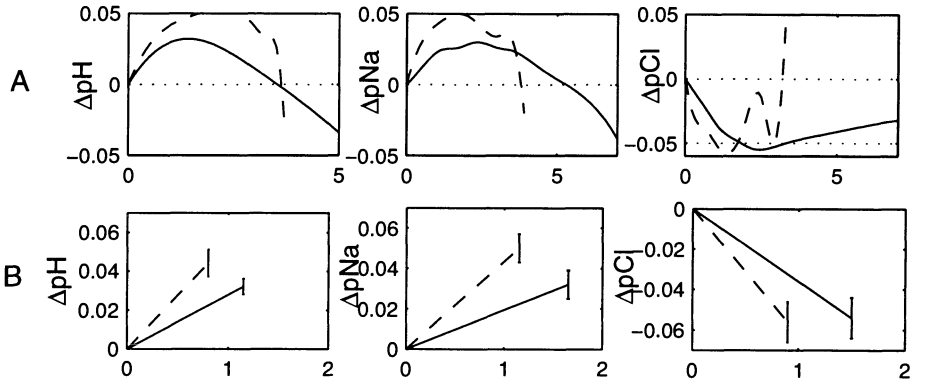

Figure 3.8. A - The changes in pH, pNa and pCl in ischemic zone of a dog's heart in experiments with (n=22, broken line) and without (n=20, solid line) VF. B - Time until the maximum of the changes

A), where true pH was determined, because the pH was determined in blood samples, absolutely disconnected from the electric field of the heart. In our experiments VF occurred in alkalosis phase in 82% of all experiments with VF.

As can be seen from fig.3.8 the changes in pH in the ischemic zone in experiments with and without VF are similar - the increase in pH is followed by its decrease. However, statistically significant differences exist between experiments with and without VF. The changes in pH are more pronounced during both acidosis and alkalosis phases, and pH reaches its maximum level faster in experiments with VF than in experiments without VF. In experiments without VF pH returned to the initial level. This did not occur in experiments with VF and fibrillation developed when the pH levels either considerably increased (more frequently) or decreased.

Our observations about shifts in pH in the ischemic focus shed light on the relationship between pH changes and occurrence of the heterotopic contractions and VF (fig.3.9).

The appearance of three extrasystole after CAO (fig.3.9-1) is accompanied by a three-step drop in pH. The more premature is the extrasystole, the greater is the magnitude of each step. When the compensatory pause takes place, pH level returns to the initial level. A new group of 3 extrasystole again developed as pH decreased (fig.3.9-2). In the subsequent normal cycles pH remained at this level but did not return to the initial level without a compensatory pause. A new group of extrasystole causes a further drop in pH, until it shifts to a level at which fibrillation begins (fig.3,9-3). This figure demonstrates the importance of the compensatory pause in the return of ischemic pH to the initial level. If the compensatory pause is absent, the pH does not return to the initial level and VF develops.

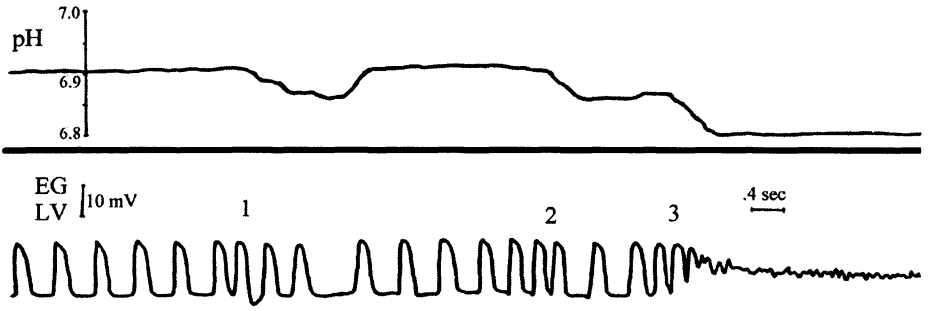

Figure 3.9. The role of compensatory pause in changes in myocardial pH during VPB (explanation in the text)

An important feature of the acid-base equilibrium disturbances in ischemic zone is their primarily intracellular origin. Taking into consideration the high rate of ischemic changes in the myocardial cells after CAO, it is evident that the extracellular medium does not reflect the intracellular pH changes at each given moment. Because of this fact, the intracellular pH (pHi) changes after CAO are of great interest.

3.2.2 The ischemic changes in intracellular pH of the myocardium

Many methods are used to measure the intracellular pH: acid-base indicator dye, tissue homogenate, glass microelectrodes, NMR and pH calculation base on a weak acid and a weak base distribution. A detailed analysis of the limitations and the difficulties arising in measuring pHi through various methods is given in the review [223].

pHi was measured in our laboratory using the methods of pH calculation based on distribution of weak acid and weak base. This method is based on the fact that the cell membrane is easily permeable by non-dissociated weak acids and bases and is non-permeable for the ionized ones. When the pH of the extra- intracellular medium changes, the content of these partly dissociated forms changes too. If the extracellular pH and the extracellular space volume is known, the intracellular pH can be also be calculated if this partially dissociated substance can be analytically determined. It was suggested [337] that 5,5-dimethyl-2,4 - oxazolidinedion (DMO) be used for this purpose.

Values of pHi in dogs were measured by means of DMO in 18 experiments with CAO, where VF developed in 10 experiments. 13 experiments were a control group [329, 236]. The results of these experiments are illustrated in fig.3.10.

After 5 min of local ischemia the myocardial intracellular pHi in the experiments without VF decreased insignificantly. It is quite possible that the true pHi values somewhat exceed the values obtained in our laboratory. In ischemia we are faced with a transient process and not with a stationary one. Therefore

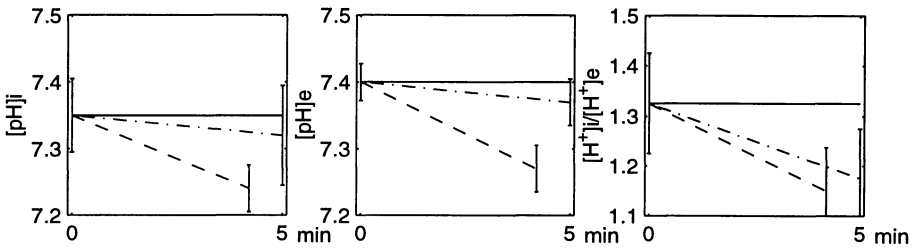

Figure 3.10. The changes in intracellular pH (pHi), extracellular pH (pHe) and the ratio of [pHi]:[pHe] of dog's heart in control group (n=13,solid line), in ischemic zone 5 min after CAO in experiments without VF (n=8, broken with dot line) and in ischemic zone at the time VF developed (n=10,broken line).

the exact equilibrium of DMO on both sides of the membrane at each moment in time cannot be established due to the continuously changing transmembrane pH gradient during ischemia. Hence, the pHi values we obtained may not correspond to the sampling moment but to an earlier one.

This error can not be considered as an obstacle in clarifying the features of pHi changes after CAO with VF. In these cases pHi dropped considerably more during a shorter time interval (average 4 min). In experiments with VF the extracellular pH (pHe) also decreased considerably more than in experiments without VF.

Despite the fact that acidification primarily occurs in the intracellular medium during ischemia, the difference in H^+ ion concentration between the intra- and extracellular media not only increased but even somewhat decreased in experiments both with and without VF. This can probably be connected with a greater buffer capacity of the intracellular medium on the one hand and with a greater velocity of the outward flux of the acidic products from the intracellular to the extracellular medium.

The above mentioned results demonstrate that the data of extracellular pH changes contain sufficient information for the evaluation of the changes in intracellular acid-base equilibrium in an ischemic myocardium

The stabilization of pH in ischemia is third way of looking for antifibrillatory substances. The buffer solutions of TRIS with various pH were used to increase the buffer capacity of the tissue (see chapter VI). The result of acid-base changes during ischemia should be the disturbances in ion equilibrium on the cellular membrane.

3.3 Ischemic disturbances in ion equilibrium

Numerous experimental investigations were performed to analyze the ionic changes during ischemia. The specific changes characteristic of VF during ischemia, were given much less attention, however. The review of data regard-

ing the electrolyte abnormalities underlying lethal ventricular arrhythmias is contained in work [104].

The methods of continuous recording of Na and Cl activity in blood and in the heart by means of selective electrodes [248] allowed to study the specifics of changes in the ionic equilibrium in CAO complicated by VF. The measurements were performed in the ischemic zone, in coronary vein blood, which drained the ischemic zone, in the coronary sinus blood, coronary effluate in experiments with ischemic perfusion of part of the heart, in the extra- and intracellular space and in the mitochondria isolated from the ischemic zone.

3.3.1 The changes in Na and Cl in the myocardium after CAO

The continuous registration of changes in pNa and pCl simultaneous with changes ?[] myocardial pH and other parameters in ischemic and nonischemic zones was performed by means of a potentiometric method using selective electrodes on 42 dog's heart. 22 developed VF (fig.3.2-1) [236].

The CAO caused two-phase changes in pNa and pCl: short-term increase in pNa and decrease in pCl followed by decrease in pNa and increase in pCl which continued until the 60th min after CAO. Given that the increase in pNa corresponds to a decrease in Na concentration, while a decrease in pCl indicates an increase in Cl in the extracellular medium, it seems likely that in phase I, Na enters the cell, while Cl leaves the cell.

These changes occurred in the experiments both with and without VF. However the latter were characterized by greater rates of pNa increase and pCl decrease during the first phase of changes and by a sharper decrease in pNa and an increase in pCl in the second phase just before VF. In experiments without VF, on the other hand, pNa and pCl became close to the initial level at the same time.

3.3.2 The changes of pNa in the coronary sinus blood

The coronary sinus blood flowed across a special camera with selective electrodes [248]. We discovered a two-phase change in pNa followed exactly by the changes in pH. After a short phase of pNa increase that reached the maximum value after 2.5 min of CAO it began to decrease. At the same time no changes occurred in the arterial blood.

3.3.3 The changes in content of K^+ in blood of coronary vein

In 17 dogs with CAO VF developed in 11 (65%) on average after 7.7±1.9 min of CAO [328, 330, 236]. Measurements of K^+ and Na concentration in the coronary vein blood plasma of were performed after 1, 3, 5, 7, 10, 15, 20, 30, 60 min of CAO by means of flame photometry. The average data describing these changes are presented on fig.3.7-A, the rate of the changes in fig.3.7-B.

The concentration of K^+ in the coronary vein plasma in experiments without VF increased and reached a maximum after 7 min of CAO then slowly decreased and at the 60th min returned to the initial level.

In the experiments with CAO complicated by VF the concentration of K^+ in the ischemic blood after a small and unstable decrease also increased and reached greater value than in experiments without VF. No substantial changes occurred in this case in the arterial blood. The fibrillation developed as a rule during the period when $[K^+]$ was rising in the ischemic blood. When $[K^+]$ stops rising, it is an indication that VF would not occur. The rate of $[K^+]$ increase in experiments with and without VF proved to be almost 1.5 times greater than in experiments without VF ($p<0.01$)(fig.3.7-B).

The changes in Na concentration in the blood plasma from the coronary vein were negligible, however the differences between the changes in Na after 3 min of CAO in experiments with and without VF were statistically significant ($p<0.02$).

Acidosis and K concentration 8-13.5 Mmole provide the conditions necessary for the onset of VF in an isolated perfused porcine heart. Heterogeneities of K concentration in this domain are associated with high inducibility of VF [64].The decrease in heterogeneity of K in ischemic, non-ischemic and border zones was realized by partial coronary sinus occlusion As a result of equalization of [K]o VF was not observed [156]. m-nifedipine improves the K balance together with lactate balance [254].

Superphysiologic magnesium was administered after myocardial infarction [29]. Administration of magnesium decreased the spontaneous depolarization of the myocardial cell and ventricular arrhythmias.

Na+/H+ exchange inhibitors, amiloride, or EIPA markedly decreased the incidence and duration of VF and even suppressed fibrillation completely. Both inhibitors diminished the activities of lactate dehydrogenase and creatine kinase in the venous effluent of the heart [275].

3.3.4 The changes of K content in coronary effluate

Changes in extracellular K^+ were also studied in experiments with ischemic perfusion of myocardial area by a non-buffer solution with pH of 7.4 for 5 min [287]. Fibrillation developed during the perfusion in 8 experiments out of 18. Changes in $[K^+]$ in the solution flowing out through the great cardiac vein which drained the perfused area (effluate) are given in fig.3.7-C. The rate of $[K^+]$ increase in the effluate was 2.4 times higher in experiments with VF than in experiments without VF ($p<0.001$), while there was no statistically significant differences in the pH decrease in experiments with and without VF (fig.3.7-D).

Thus, the high rate and magnitude of the increase are the characteristics of changes in K^+, and Na^+ concentration in the coronary vein blood and in the coronary effluate

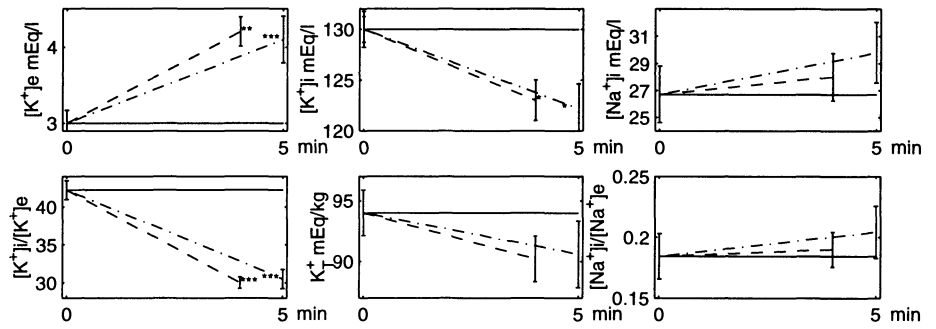

Figure 3.11. Extracellular concentration of K^+ [K^+]e, intracellular concentration of K^+ [K^+]i, intra/extracellular ratio K^+ concentration of ([K^+]i/[K^+]e and total K^+ concentration [K^+]T. Intracellular concentration of Na [Na^+]i and intra/extracellular ratio of Na ([Na^+]i/[Na^+]e) of the heart in the control group (solid line), in ischemic zone 5 min after CAO (broken with dot line) and at the time of VF occurrence (broken line). x - $p<0.05$, xx -$p<0.01$, xxx -$p<0.001$

The data describing the changes in electrolyte contents made it possible to imagine how the extracellular concentrations of the electrolytes change after CAO. It was of interest to know whether they reflect the intracellular changes.

3.3.5 Changes in intra- and extracellular K^+ contents in the myocardium

Determination of intracellular concentrations of K^+ and Na in the ischemic zone after CAO was carried out in our laboratory in 18 dogs [329, 236]. 13 experiments were performed as a reference group. The hearts were analyzed after 5 min of CAO. VF developed in 10 experiments out of 18 (56%), mainly after 4 min of CAO. The respective data are given in fig.3.11.

The intracellular concentration of K^+ in the left ventricle was 130.5±1.7 meq/l of intracellular water. In spite of a very short duration of the ischemia we discovered a statistically significant decrease in the intracellular potassium content in the cases both complicated and not complicated with VF. In these experiments the concentration of [K^+]e increased and the ratio of [K^+]i/[K^+]e decreased. The rate of changes in [K^+]i , in [Ke^+]e and in [K^+]i/ [K^+]e proved to be higher in experiments with VF.

The transmembrane transition of the main potential generating ion, K^+ provoked a drastic drop in the intra-/extracellular gradient which decreased by one third of the initial level. Although the quantitative differences between experiments with and without VF were not statistically significant, the qualitative differences were unidirectional for all the parameters and thus can be considered reliable.

Changes in the balance of Na after 4-5 min of ischemia were less pronounced than changes in the balance of K^+ . The normal intracellular concentration

of Na in the left ventricle proved to be 26.3+2.0 meq/l of intracellular water (fig.3.11). After 5 min of CAO $[Na^+]i$ and the ratio of $[Na^+]i$ /$[Na^+]e$ registered a statistically insignificant increase.

So, a higher rate of decrease in $[K^+]i$ and increase in K intra/extracellular ratio and also a higher rate of increase in $[K^+]e$ are the characteristics of changes in the intra-extracellular concentrations of K in experiments with VF after CAO.

Taking into account the unequal distribution of ions in the cell, the study of changes in the content of K^+ in the mitochondria was of great interest because mitochondria can lose K^+ in cytoplasma as a result of deenergization.

3.3.6 The changes K^+ in mitochondria

The content of K^+ in the cardiac mitochondria was measured by means of a K-selective valinomicine electrode based on the changes in K^+ activity in the incubation medium: 1) after introducing the mitochondria into the medium - the external K^+, $[K^+]ex$; 2) after destruction of the mitochondrial membrane by means of triton - the internal $[K^+]in$, and 3) K^+ total – $[K^+]t= [K^+]ex+[K^+]in$. In the control experiments $[K^+]ex$ was 110±9 nMole/mg of proteins, $[K^+]in$ – 110±4 Nmole/mg of proteins [78].

A statistically signficant decrease in K+ ion content is observed in mitochondria separated after 5 and 30 min after CAO. Q-III, RC, the succinate dehydrogenase activity both in ischemic and nonischemic zones (fig.3.12-I,A,B) decrease in parallel.

As seen from the figure the changes in Q_{III}, RC and $[K^+]in$ the mitochondria of the ischemic zone are substantially more pronounced in the experiments with VF than without it.

So, the peculiarity of the mitochondrial metabolism disturbances in CAO complicated with VF are the greater degree deenergization of mitochondrion: more sharper inhibition of respiration and uncoupling respiration and phosphorylation. The result of this is redistribution of K^+ in the cell: exit K^+ from mitochondrion and increase K^+ activity in the cytoplasm, increase in intra-extracellular K^+ gradient and greater velocity of K^+ exit from cell in accordance with more gradient of K^+ activity in experiments with VF then in experiments without VF.

3.3.7 Some mechanisms of ischemic ionic balance disturbances

The connection between the loss of K by ischemic cells and arrhythmias arise after CAO was firstly demonstrated by Harris at al. in 1954 [122]. However, until now the mechanism the loss of K do not clear. Same hypothesis try to explain this mechanism.

3.3.7.1 The role of ion channels in the loss of K by ischemic cells. In accordance with the more spread now hypothesis of ion channels, K^+ outflow during the ischemia is a result of the activating specific type of K^+ channels

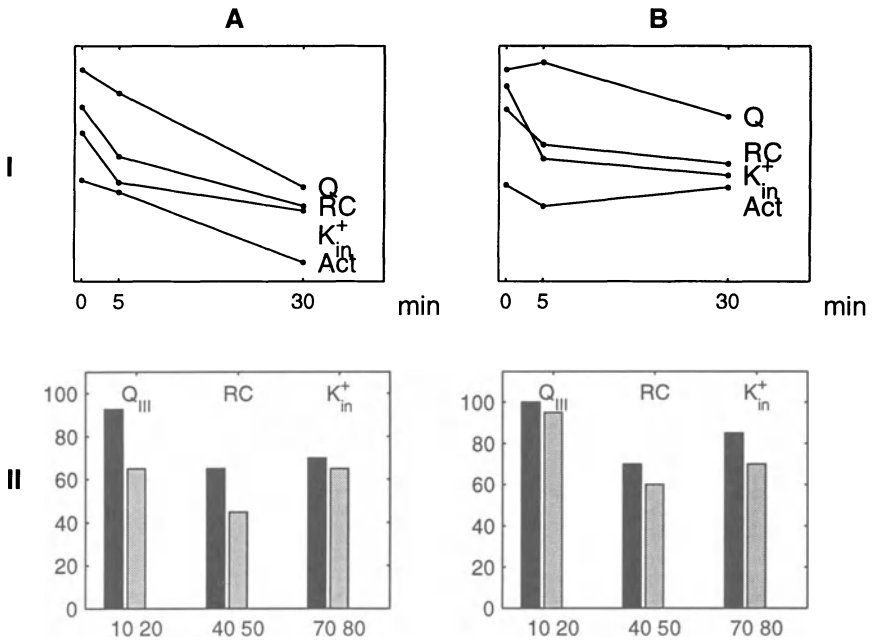

Figure 3.12. I - rate of decrease Q-III, RC, K^+in and activity succinic dehydrogenase in mitochondria ischemic (A) and nonischemic (B) zones after CAO. II - comparison of the changes of Q_{III}, RC and $[K^+]$in mitochondria of ischemic (A) and nonischemic (B) zones 5 min after CAO in experiments without VF (black columns) and with VF (shaded columns)

which is regulated by intracellular ATP-($I_{K(ATP)}$). The review about cardiac ion channel presented Weiss [340], about regulation those channels in ischemia presented Goldhaber [110].

These channels are closed under normal conditions, but are activated during of ischemia in vivo. It has been shown that activation of only a small percentage of channels (<0.5%) is sufficient for increased K efflux during ischemia [137].

Gasser et al [102] find that extracellular K^+ accumulation during ischemia can be completely blocked by the sulphonurea compounds tolbutamide and glibenclamide, a specific blockers of cardiac $I_{K(ATP)}$. The data Kanter et al [144] was shown that glyburide (glibenclamide) has a ability to prevent ischemia-induced fibrillation in Langendorff-perfused rat hearts. Consistent with this data, Wolleben at al [347] show that activators of $I_{K(ATP)}$, BPL 34915 and pinacidil decreased the time required for the development of fibrillation during low-flow ischemia. These responses to BRL 34915 and pinacidil were prevented by treatment with glyburide or tolbutamide, blockers of $I_{K(ATP)}$.

The ineffectiveness of traditional antiarrhythmic agents in preventing VF has increased the interest in potassium channel blocking agents. Many authors

assume that the pharmacological modulation of the ATP sensitive potassium channels offer a novel approach for the management of cardiac arrhythmias and prevention of VF [32]. In another work [38] effects observed with BRL-32872 was shown that novel antiarrhythmic agents should be a compound with a combination of potassium (class III) and calcium (class IV) channel antagonistic properties. The antifibrillatory effect of ambasilide [298], of UK66,914 [256],of RP58866 [258], of tacrine [257], of RP 49356 [259], of glibenclamide (glyburide) and pinacidil [313, 61, 170] was discovered in majority studies. However, in another study the present findings do not favor the idea of an antiarrhythmic effect of glibenclamide [30]. It was shown also that glibenclamide failed to reduce K loss in early ischaemia without reducing lactate release as would be expected for a selective K+ATP channel blocker [119].

These data point out on the role of glycogenolysis in K loss. In ischemia K transform from linked form in free one as a result transition of oxidized metabolites in reducing one (lactate, NAD.H) in processes of glycogenolysis activation and inhibition of respiration, i.e. in processes of metabolism.

3.3.7.2 The role of metabolism in the loss of K by ischemic cells. There are the dependence between the ox-red, acid-base and ion equilibrium as on the mitochondrial, so and on the cell membrane. The regulation of homeostasis in the mitochondria includes three elements:

$$\frac{[ox]}{[red]} \rightarrow \frac{H^+}{OH^-} \rightarrow \frac{K^+{}_{mch}, Na^+{}_{mch}, Ca^{2+}{}_{mch}}{K^+{}_{cyt}, Na^+{}_{cyt}, Ca^{++}{}_{cyt}} \tag{3.5}$$

where mch - mitochondria, cyt - cytoplasm. So, we have supposed that there is dependence between the redistribution K^+ in the cell in ischemia and the shift of the ox-red and acid-base equilibrium, which is connected in its turn with the glycogenolysis activation [231, 235]. The another factors promoted the ionic redistribution in ischemic cells are adrenalin and cAMP, which accumulate in the ischemic zone [234, 237].

In further investigations of the laboratory the dependance of the ischemic disturbances of ionic balance from the following parameters was studied: 1)respiration and oxidative phosphorylation 2)pH; 3) glycogenolysis; 4) adrenalin and cAMP.

3.3.7.3 Role of respiration and oxidative phosphorylation in redistribution K in the cell. Strict stoichiometric correlations exist between K accumulation, electron transfer in the respiratory chain and the number of phosphorylation points in the latter. A passage of each pair of electrons through such phosphorylation point is accompanied by binding of almost 4 K^+ ions [225] and 1,7 Ca^{++} ions [168]. A direct causes decrease of the $[K^+]$in content in mitochondrion can be: 1) decrease in mitochondrial membrane potential, which determines, according to the Mitchel's chemiosmotic coupling theory [193], the distribution of ions between the mitochondria and the medium and 2) the increase in the passive permeability of the mitochondrial membrane what can cause different loss of K^+ during the process of the mitochondria separation in

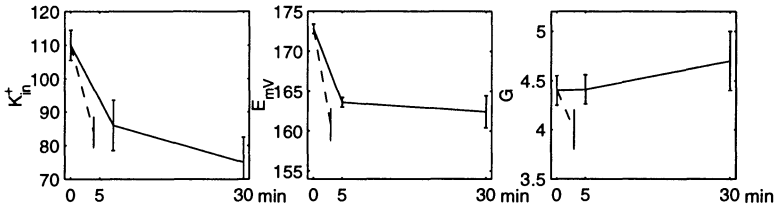

Figure 3.13. $[K^+]$in, membrane potential (E) and passive membrane conductivity (G) of mitochondria in control (n=19), 5 and 30 min (n=7 and n=8 accordingly) after CAO (solid line) and in the time VF rise (n=13, broken line). Incubation media for analysis of K mitochondria: 300 mmole saccharose,4 mmole TRIS-succinate (pH 7.4), 2 mmole TRIS-phosphate (pH 7,4), 0.01 Mmole KCl,temperature 37°C. Protein content 2-3 mg/ml. For determination of E valinomycin 10-8 mole added to incubation media. The determination of G performed in anaerobic condition

the control and experimental group. We were shown [236] that the decrease contents of $[K^+]$in in the mitochondria after CAO was associated with the decrease in the mitochondrial membrane potential [194], which value reflects level of their energization, while the permeability of the mitochondrial membrane for K^+ after CAO did not change (fig.3.13).

A close correlation was discovered between K^+ internal content in the mitochondria and respiration control of Chance-Williams ($r = 0.85$, $p<0.05$) what testifies about dependance between decrease in K^+ content in the mitochondria and uncoupling of respiration and phosphorylation.

3.3.7.4 The role of acid-base disturbance in the redistribution K^+ in the cell. in our laboratory [330]. The dynamic of changes in pH and $[K^+]$ in the ischemic coronary blood in the experiments with and without VF was studied in our laboratory [330]. As seen from the fig.3.7-A the changes in $[K^+]$ and pH in the experiments with and without VF are oppositely directed. The dynamics both of pH and of $[K^+]$ have the same characteristic peculiarities. In the experiments without VF the changes in pH and $[K^+]$ firstly increases, reaches a maximum value then after 10-15 min of ischemia returns to the initial level. In the experiments with VF these parameters increase and at the moment of maximal its increasing fibrillation arises. The comparison of the changes in pH and $[K^+]$ in the coronary ischemic blood discover a close correlation between these parameters ($r=-0.66$, $p<0.01$). The negative correlation was discovered also between K^+ concentration and pH as in the center of ischemic zones, so as border zones. However, the comparison between the changes in pH and K^+ shows that in spite of close correlation between them, exit of K^+ begins already at the alkalization stage and continues during the following acidification. The decreasing of pH continuing also when $[K^+]$ begins to return to its normal level. Therefore the changes in the extracellular pH can not be a cause of K^+ exit from the cell. It was naturally to suppose that the changes in the intracellular

pH, but not in the extracellular one could be the cause of the transmembrane transfer of K^+ while the changes in the extracellular pH reflect with a certain delay the changes in the intracellular pH.

Kline et al [151] studied pKi, pNai and pHi using double-barrel K^+, Na^+ and pH selective microelectrodes in subendocardial Purkinje fibers, surviving 24-hour infarcts and removed from hearts at earlier times. They concluded that reduced pHi can be implicated as a mechanism of activity Ki reduction. The complex approach to study mechanisms of ion changes in ischemia was developed in work [59]. Authors constructed a computer model that integrates internal pH, ion concentration, and cardiac energetics with electrophysiological changes during myocardial ischemia.

In our laboratory in experiments on the dogs [329] was established the pronounced negative correlation between K^+ and pHi ($r=-0.59$; $p<0.02$). These results show that K^+ exit from cell in extracellular medium controls by intracellular pH.

3.3.7.5 The role of the glycogenolysis activation in redistribution K in the cell. The role of the glycogenolysis activation in K loss in ischemia is indirectly confirmed by the fact that so called blocker of K(ATP) canals glibenclamide can not to reduce K loss in early ischemia without reducing lactate release,[119], i.e without glycogenolysis inhibition The results of Weiss et al [341] also suggests that K^+ efflux linked to lactate efflux during ischemia. In rabbit septa exposed to iodacetate there are marked decrease intracellular K^+ loss and decrease in venous lactate. Authors have been suggested that increased K^+ efflux during ischemia may be result of efflux of intracellular generated anions, such as lactate, for the maintenance of electroneutrality.

3.3.7.6 The role of the adrenaline accumulation in an ischemic heart in K redistribution. The role of adrenalin in the action of ischemia on the ionic balance of the myocardium was clarified by means both of direct studies of its influence on the contents of electrolytes in the ischemic zone and the change of its effect by means a blockade of the β-adrenoreceptors with propranolol. It was shown that the K^+ exit from the mitochondria caused by adrenalin is mediated through proteinkinase activated by cAMP.

The studies of the adrenalin action on the total concentration of K^+ in the cell, on the activity K^+ in cytoplasm and on its content in the mitochondria showed that the changes in the intra-extracellular K^+ gradient in ischemia could occur at the expense of K^+ decompartmentation from the mitochondria and obviously from other intracellular compartments (see chapter V).

So, influence on myocardial ionic equilibrium is the fourth way for the search of antifibrillatory agents. In our laboratory for a direct influence on the ion equilibrium a modified cardioplegic cocktail was used.

3.3.7.7 The role of the compartmentation and decompartmentation of ions. At present time, the concept that the cell membrane is determinant

factor in the ion transfer is the most widespread and experimentally confirmed. Nevertheless, not only the cell membrane must be taken into consideration for the comprehension of ion exchange disturbance mechanisms of the cell. In our opinion it is necessary to take into consideration the role of the other intracellular structures and of the processes occurring in them for the ion distribution.

In any metabolic processes in the intracellular structures, disturbances of the electrochemical, osmotic and acid-base equilibrium occur. These disturbances can be compensated by movements of highly mobile mono- and bivalent cations and anions between the internal medium of the respective organoid and the cytoplasm with their following redistribution between the cytoplasm and the extracellular medium.

In spite of the exclusive interest in the problem of the connection of the ion flows through the cell membrane with the binding and releasing of the ions by the intracellular structures, the published data of the problem are very scanty. Proceeding from these data and from hypothetical ideas we have prepared a speculative scheme of the intra- and extracellular cation transfer confronted with the data of ion flows through the cardiac cell membranes [63] and of energetic processes dynamics during the cardiac cycle [20] as well as of ECG and AP. (fig.3.14).

During the systole in the Mch hydrolysis of ATP occurs. The ionized groups of the cell macromolecule to which ATP belongs, selectively bind the $K^+$and Na^+ ions being the Na linkage stronger than that of K^+[296]. Due to that, during the ATP decomposition K^+ ions are preferentially released and transferred from the Mch into the Cyt. In the Mf $K^+$and Na^+ bind the muscle proteins which keep the ions between the carboxyl and the amine groups of the adjacent lateral chains. During the systole when the myoplasma acidity increases due to the glycogenolysis activation the ions bounded with the muscle proteins can be released and transferred from the Mf into the Cyt. Activity of the K in the Cyt increases and the increase in gK^+, i.e. of the K exit from the cell at the expense of the increase in the difference between the K^+activity in the intra- and extracellular media.

The SPR, the Mf and the Mch participate in the Ca transfer during the systole. The depolarization wave provokes the exit of Ca not only from the SPR cisterns but also from the Mch. The exit of Ca from the Mch is favored by the Na^+ ions which enters into the cell during the depolarization and by cAMP which accumulates during the systole in the heart [39].

A small amount of Ca enters into the cell from the extracellular medium what is testified by the gCa increase. The entering of Ca from the above mentioned three sources provokes the increase in free Ca in the Cyt to 10^{-5} M. Ca combines with the troponine of the Mf and provokes their contraction. During this phase the superfluity Ca moves of from the Cyt into the extracellular medium via the Ca^{++}-Na^+ exchange.

During the diastole the ATP synthesis in the Mch accompanied by the K^+ binding by the Mch. As a result, the decrease of K^+ in the Cit occurs and

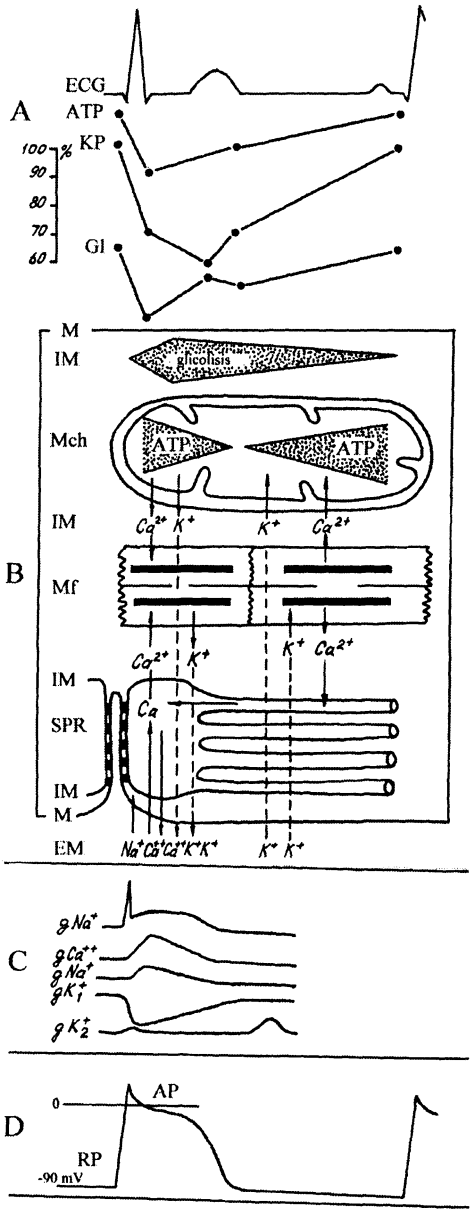

Figure 3.14. Schematic picture ionic transport between subcellular structures, cytoplasm and extracellular medium in comparison with ECG, AP, membrane ionic currents and dynamics of energetic processes in cardiac cycle. A - ECG and dynamics of ATP, KP and glycogen changes in cardiac cycle B - ionic transport between subcellular structures. M - membrane, IM - intracellular media, Mch - mitochondrion, Mf - myofibril, SPR - sarcoplasmatic reticulum. C - ionic currents on cellular membrane . D - RestP and AP.

K^+ entry into the cell at the expense of the decrease in intra-extracellular K^+activity. This is expressed in the increase in gK.

During the diastole Ca^{++} enters into the Cyt from the Mf. The moving away of Ca^{++} from the Cyt against the to the concentration gradient into the cavity of the longitudinal canals of the SPR occurs at the expense of the ATP energy and is provided by the Ca^{++}-Na^+ -ATP ase. From the longitudinal canals Ca^{++} is transferred back into the cisterns. The Mch also participate parallel with the SPR in the Ca moving away from the cytoplasm. So, the binding and release of ions in metabolic processes during the systole and diastole provoke changes in ion activity in the cytoplasm, which can determine inward and outward ion flows through cell's membrane.

Numerous data of the connection of the ion transfer between the organoid and cytoplasm with the metabolism confirm the idea on this subject of Mitchel, made already in 1961 [195]. He wrote that the terms "passive diffusion" and "active transfer" focuses the attention on the moving ions or molecules and not on the original causes of this movent on the chemical or the molecular level. Moreover, the transfer is referee to as a process which is separated in time and in space from the metabolic processes. Meanwhile on each phase of the metabolism the molecule, ions or functional groups pass from one place to another. The metabolic process which has vectorial component in Mitchell's opinion is "active transport".

It seems very probably that the compartmentation and decompartmentation of the ions which are connected with the metabolism and which cause changes in ion activity in the cytoplasm and in their intra- extracellular ion gradient can provoke intra-extracellular ion transfer.

It is especially important to take into account in pathologic circumstances because this allows to correlate the disturbances of the intra-extracellular ion currents not with some hypothetical "activation" and "inactivation" of the ion canals, but with definite metabolic processes which can be corrected by means pharmacological drugs.

3.4 Conclusion

1. Occlusion of the anterior descending branch of the left coronary arteria at the upper third in dogs results in VF in 60-70% of the experiments. The probability of fibrillation arising in this occlusion level do not determined by the state of blood supply and the degree of hypoxia in the ischemic zone (see chapter I).
2. The probability of fibrillation arising is determined by the extent of the redox equilibrium disturbances in the ischemic zone, what manifest in the cases with VF in greater degree of:
   (a) decrease myocardial ox-red potential,
   (b) decrease in the myocardial phosphate potential,
   (c) decrease in oxidative phosphorylation and the respiratory control in mitochondrion,

(d) increase in the glycogenolysis velocity in the cytoplasm and
(e) more pronounced increase in NAD.H myocardial concentration.

3. The consequence of greater degree of the ox-red equilibrium disturbances in cases of CAO with VF are the acid-base and ionic homeostasis disturbances also in greater degree. The measurements of pH, K^+ , Na^+, Cl^- contents in various media of the heart: directly in the myocardium, in the coronary sinus blood, in the blood of coronary vein, which drains the ischemic zone, in the coronary effluate in experiments with ischemic perfusion of part of the heart, in the intra-extracellular spaces and in the Mch, already during the first minutes of ischemia discovered more considerable changes in the experiments with VF.
4. It was demonstrated the dependence of the ion balance disturbances in the ischemic zone and vulnerability to the fibrillation from:

   (a) uncoupling respiration with phosphorylation
   (b) pH. It was discovered by a close correlation between the changes of intracellular pH and the loss velocity of K^+ as well as the possibility of diminishing of K^+ loss from the cell and the prevention of fibrillation via increase in the cell buffer capacity by means of buffer TRIS;
   (c) glycogenolysis activation. The inhibition of glycogenolysis by dibenclamide and by monoiodacetate allows to avoid VF:
   (d) adrenalin. Adrenalin infusion permitted to reproduce ischemic disturbances of the ionic balance, while block of the β-adrenoreceptors by means of propranolol permitted to diminish these disturbances and to reduce sharply the percentage of VF in CAO. It was shown that decompartmentation of K^+ from the intracellular organelle is responsible for transfer of K^+ between cell and extracellular medium and for changes in the cell membrane potential by the action of adrenaline.

5. The our investigations (fig.3.1) have showed that there was substantial quantitative differences in the studied parameters in experiments with and without VF, but there was no qualitative differences. In the experiments with VF the dynamics of changes the heart's metabolism parameters is characterized by a greater velocity of the initial changes and have exponential character, whereas in the experiments without VF they are described by a curve with an maximal point. This seems likely to reflects the inadequacy of the compensatory mechanisms by the sharp growth of the pathologic changes in experiments with VF and the prevalence of the compensatory changes over the pathologic ones in experiments without VF.

   We suggest that sharp growth of pathological changes in experiments complicated by VF occurs in expense of adrenalin. The role of adrenaline in the arising of VF will be discussed in chapter IV and V.

4 Role of the Sympathoadrenal System in the Appearance of Ventricular Fibrillation after Coronary Artery Occlusion.

In the preceding chapter we showed that VF appears after CAO in cases where disturbances of metabolism in the ischemic zone occur at a higher rate and reach greater values than in cases without VF. A hypothesis was formed that this is due to more dramatic disturbances of the sympathoadrenal heart control in cases with VF.

The role of the sympathoadrenal system in the development of arrhythmia and in particular of VF after CAO is now being studied very intensively. Numerous studies on this subject are summarized in monographs and reviews [36, 125, 283, 125, 282]. During acute myocardial ischemia the responsiveness of adrenergic receptors to stimulation by catecholamines is enhanced [280]. On the other hand, exclusion of sympathetic effects in CAO by means of beta-blockers is extremely effective against VF [75, 69]. Exclusion of sympathetic innervation, realized by removal of the stellate ganglia [157] significantly reduced the frequency of VF. Chemical exclusion by means of phenol prevented the onset of ischemic electrophysiological effects that are responsible for the onset of arrhythmia [186].

During recent years, two noninvasion tests for quantitative assessment of cardiac autonomic tone have been used: analysis of heart rate variability and determination of baroreflex-sensitivity using the phenylephrine method. Preliminary results of the largest study - ATRAMI indicate that the use of the baroreflex-sensitivity test allows prognosis of arrhythmias after a myocardial infarction [130].

Meanwhile, other authors believe that it is difficult to ascertain whether sympathetic hyperactivity represents an independent risk factor [98]. Studies have shown that catecholamines markedly potentiated the three mechanisms of rhythm abnormalities, which may be related to enhanced automaticity, triggered automaticity, and reentrant mechanisms [218]. Correlation between scintigraphic evidence of sympathetic neuronal dysfunction and ventricular refractoriness was obtained in experiments on human hearts [45]. On the other hand, the protective effect of vagal activity was demonstrated in an experimental study [46]. The electrical stimulation of the vagus can prevent VF [321, 323]. Despite the increase in evidence of vagal activity, VF recurred during the exercise-and-ischemia test [133].

Autonomic dysfunction, as detected by a decrease in heart rate variability, suggests that sympatho-vagotonic imbalance may trigger fatal arrhythmias during acute myocardial ischemia [224]. Even the proarrhythmic effect of flecainide, discovered in Cardiac Arrhythmia Suppression Trial (CAST) is explained by the authors as adrenergic modulation of drug binding [211].

So, the reported data demonstrate firstly, that activation of the sympathetic nervous system occurs in local myocardial ischemia, and secondly that its activation increases the tendency towards VF, while its elimination by surgical or pharmacological methods prevents appearance of VF after CAO.

Numerous studies confirm the proarrhythmic action of the increase of sympathoadrenal activity in ischemia. Those studies, however, have not answered the following questions: 1) why does VF appear after CAO in one group of animals and people and does not appear in another group, and 2) what is the mechanism of the fibrillatory action of the sympathoadrenal system.

To clarify these questions, our laboratory studied the contribution of the afferent and efferent links of sympathoadrenal system to the proarrhythmic effect.

The *afferent link* was studied by means of registration of afferent activity of cardiac nerve in CAO.

The *efferent link* was evaluated by studying the balance of the catecholamines - adrenaline and noradrenaline after CAO. This balance is informative because the efferent sympathetic nerves release noradrenaline when excited, while the chromaffin cells, contained in the heart and in the adrenal glands, release adrenaline.

The changes in the afferent activity of cardiac nerves and in the balance of adrenaline and noradrenaline in the heart were compared in the cases that did and did not result in VF after CAO.

4.1 Afferent cardiac nerve activity and ventricular fibrillation after coronary artery occlusion

The study of the afferent cardiac nerve activity after CAO was first made by Kaindle et al [140]. The authors observed an increase in the rate and length of discharges in non-splinted branches of the vagus nerve after CAO.

Afferent sympathetic cardiac nerve activity during coronary occlusion were first recorded by Brown in 1967 [41]. In five experiments on cats, manyfiber nerve preparations dissected from thoracic sympathetic nerve trunk showed large increases in afferent discharges two seconds after CAO. The effect of CAO on sympathetic afferent fibers was later reported by Brown and Malliani [42]. The authors recorded the electrical activity of single afferent cardiac fibers isolated from the sympathetic rami communicants T3 and T4 in anesthetized cats. The cessation of coronary pump flow in experiments with perfusion of the left coronary artery increased the discharges from these fibers.

Ushida & Murao [317] have examined the effect of CAO on afferent sympathetic nerve fibers of rami communicants, either the T2 or T3 of the left side in anesthetized dogs. After CAO, excitation occurred in both myelinated and unmyelinated "C" fibers. Authors think that nonphysiological motion of the ischemic left ventricular wall contributes to excitation of myelinated fibers, whereas chemical substances contribute to excitation of unmyelinated fibers. There is some recent evidence that activation of sympathetic afferent is realized by release of adenosine from the ischemic myocardium [305].

In addition to stimulating the mechanosensitive and chemosensitive sensory nerve endings, according to Zipes, ischemia might impair neurotransmission and inhibit cardiac reflexes [355]. The analysis of reflexes mediated by vagal and by sympathetic afferent fibers was done by Malliani [181].

The role of the excitation of afferent nerves in development of arrhythmia and especially ventricular fibrillation after CAO is of particular interest.

Our laboratory carried out recording of the afferent activity of cardiac nerves in 46 cats for 30-120 min before and after CAO [54] to clarify the peculiarities in changes of afferent activity of cardiac nerves in cases that resulted in VF after CAO. CAO induced VF in 25 experiment (54%) and did not in 21 (46%). The changes in afferent activity in experiments with and without VF were compared.

The recording of afferent activity was performed in the middle cardiac sympathetic nerve, in the heart's nerves which ramify both on the anterior and posterior heart surface in the coronary sinus region (known as "coronary" nerves), and in the aortal nerve.

The middle cardiac sympathetic nerve has small admixture vagus fibers, but is predominantly a sympathetic nerve [227]. Therefore, we consider the afferent activity of this nerve to be a sympathetic activity. The right stellate ganglion, the caudal cervical ganglion, the thoracic sympathetic trunk and vagus nerve were exposed. The rostral and caudal limbs of the ansa subclavian were dissected free of the surrounding tissue. The middle cardiac sympathetic nerve and the recurrent nerve were separated from the caudal cervical ganglion. These nerves frequently go away as a common trunk, making the identification of middle cardiac sympathetic nerve easier. The latter was separated up to the pericardium, where it divides into many branches. The vagosympathetic trunk under the caudal cervical ganglion and all prepared nerves were intersected.

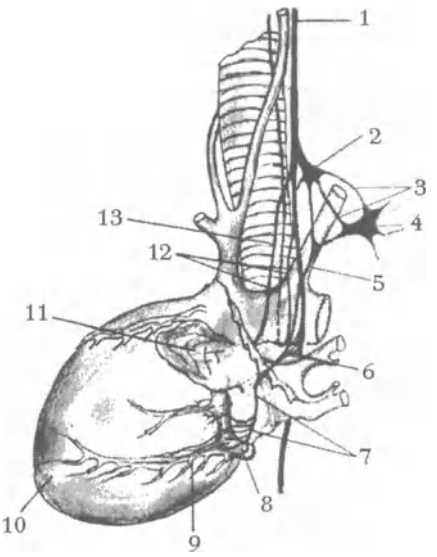

Figure 4.1. Nerves in a dog heart. 1 - n.vagus, 2 - ganglion cervical inferior, 3 - ansa subclavian, 4 - ganglion stellatum, 5 - n.cardiacus sympathicus inferior, 6 - rami coronary, 7 - plica nervina Vorobyev, 8 - coronary sinus, 9 - left coronary artery, 10 - left ventricular, 11 - left auriculum, 12 - n.recurrent, 13 - n.cardiacus sympathicus medius

A typical picture of nerves distribution in one of our experiments on a dog is presented in fig.4.1.

The preparation of cardiac nerves in cats was performed in the same fashion.

Measurement of afferent activity of cardiac nerves (AACN) was made from the non-splinted trunks concurrently with the arterial pressure (in separate experiments also with coronary sinus pressure) and with the electrocardiogram.

AACN had two components:1) high-amplitude systolic discharges which were not analyzed and 2) low-amplitude diastolic activity which we analyzed. The "power" of afferent cardiac low-amplitude activity was determined using the following formula:

$$P = \frac{nl^2}{t}, \tag{4.1}$$

where P is the power of afferent activity, n is the number of peaks, measured in the time interval t, l-amplitude of peaks.

Under normal conditions, low-amplitude activity varied from 10-13 to 30-40 mkV with an average of 17±1.6 mkV. The changes in AACN differed greatly in experiments with and without VF. In 21 *experiments without VF* no characteristic changes were observed. As a rule, 3-5 min after CAO, when ventricular arrhythmia developed the intensity of AACN increased. After normalization of the rhythm it returned to normal level. In 25 *experiments with VF* consistent

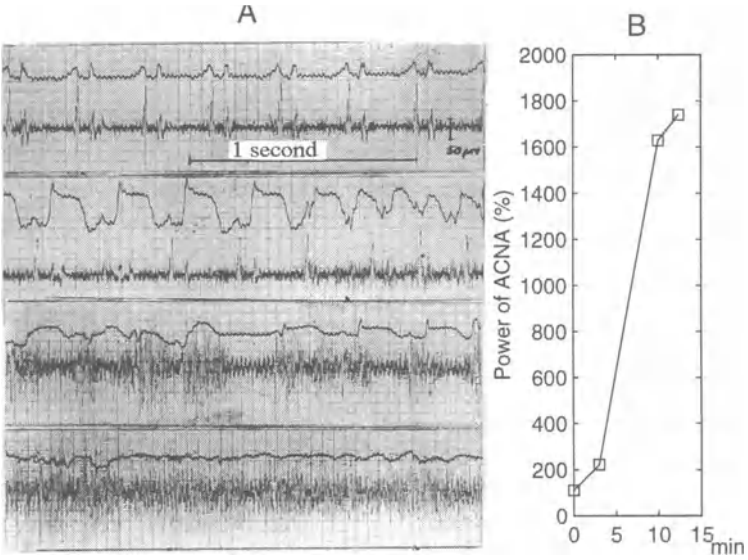

Figure 4.2. A. Increase in intensity of AACN after CAO in an experiment with VF. From top to bottom:ECG-II, AACN. B. Curve of changes in AACN power after CAO (% of the initial level).

changes in AACN were observed. A typical example of AACN changes in an experiment with VF is shown in fig.4.2.

A small constant activity was recorded before CAO. The activity almost did not grow for 3 min after CAO in spite of sharp disturbances in the myocardium, marked by monophasicity of the ECG. The AACN power began to grow in parallel with developing polytopic ventricular arrhythmia and reached a maximum level when VF appeared. At that time only continuous high-amplitude activity without systolic discharges was recorded. In other cases the AACN power grew considerably slower. In accordance with the slow growth of the AACN power, VF developed later in such experiments.

The characteristic differences in AACN changes between experiments with and without VF become apparent when the rate of increase in AACN (fig.4.3-I) and the average value of AACN are compared(fig.4.3-II).

A relationship between the rate of increase of AACN power and the time of VF appearance was found (fig.4.3-I) ($r=-0.61$, $p<0.01$). When the power of AACN increased rapidly, VF appeared early after CAO (on average after 2 minutes). When the increase was slow, VF appeared on average 9 minutes after CAO. If the power of AACN increased very slowly, VF did not appear.

After CAO, when the rhythm was normal, power of AACN doubled in experiments both with and without VF (fig.3-II). However, when arrhythmias appeared after CAO, the power of AACN nearly tripled in experiments with VF in comparison to the experiments without VF (to 706 and 272% respec-

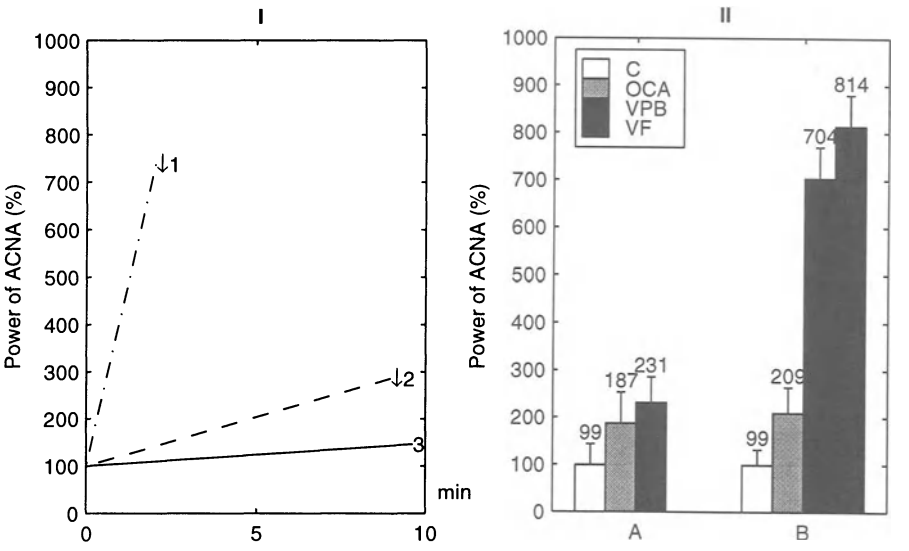

Figure 4.3. I - The dependence between the rate of AACN increase and time of VF appearance (average data). The arrow shows the moment of VF appearance. 1 - experiments with early (average of 2 min after CAO) and late VF (average of 9 min after CAO (n=25), 3 - experiments without VF (n=21). II - The increase of AACN power after CAO in experiments without (A, n=8) and with (B, n=5) VF (average data). Before CAO (white columns), after CAO , when the rhythm is normal (gray columns), when arrhythmias appear after CAO (black columns), and when VF appears (dark gray columns)

tively, $p<0.05$) and reached 800% when VF appeared. So, the final outcome of local myocardial ischemia – whether it will quietly develop into myocardial infarction or into VF and sudden death – depends on the intensity of the signals which the heart sends through cardiac nerves to the center. Transforming the AACN into audio signals, we literally heard how the heart "cried" for help. When the "cry" became especially powerful, we knew that VF would now arise and usually we were not wrong.

In our experiments the increase of AACN usually preceded VF. Therefore, it is possible to affirm that the increase in AACN is not a consequence of VF, but may contribute to the susceptibility of the heart to VF after CAO.

The increase in AACN power is the first link in a chain of processes which increase vulnerability to VF after CAO. Efferent activity of cardiac sympathetic fibers may increase due to an increase in afferent sympathetic activity. Lombardi et al.[172] found that the maximum increase of the firing rate of the preganglionic sympathetic neurons coincided with the greatest reduction of VF threshold (from 25±1.3 to 16±2.3 mA ($p<0.05$)). Gillis [107] also found an increase in sympathetic cardiac efferent neural activity during experiments with

myocardial ischemia. The increased activity of efferent fiber coincided with the onset of ventricular arrhythmias.

In addition to studying the AACN, we studied the afferent activity from the aortal nerve.

4.2 Afferent activity of the aortal nerve after CAO

Afferent activity of the aortal nerve after CAO was studied in experiments on 22 cats and 4 dogs [53]. The left aortal nerve was separated below the lower cervical sympathetic ganglion, where it passes as an independent trunk. It was cut and a pair of platinum electrodes were placed on the distal part of the aortal nerve. The afferent activity of the aortal nerve (AAAN), ECG-II and blood pressure were registered simultaneously.

The afferent activity of aortal nerve is a high-amplitude systolic discharge (SD), that occurs during ventricular isometrical contraction and maximal expulsion of blood. The length of discharges is 80-200 msec. The amplitude of discharges is 50-150 mkV, rate is 300-500 imp/sec. The number of impulses in the discharges is proportional to the aortal blood pressure. The frequency of impulses is proportional to the rate of increase in the aortal blood pressure.

In addition to SD, low-amplitude non-discharge diastolic activity (DA) was observed in set conditions (e.g., hypoxic conditions).

The changes in AAAN after CAO consist of two phenomena: 1) the changes in SD and 2)appearance or a gradual increase in low-amplitude, non-discharge DA.

4.2.1 Changes in systolic discharges

In experiments without arrhythmias the changes in SDD after CAO were in strict conformity with the changes in arterial pressure. A sharp, but short decrease in blood pressure immediately after CAO was observed (fig.4.4-A).

The blood pressure normalizes seven minutes after CAO and decreases slowly afterwards. The changes of SDD - a sharp decrease, an increase followed by a slow decrease, proceed in parallel with changes in the arterial pressure.

In experiments with arrhythmias the dynamics of changes of arterial pressure and SDD were the same for the first minutes after CAO as in experiments without arrhythmias. However, when arrhythmia starts (2-14 min after CAO) the power of SD was chanced considerably. An example of changes in SD is shown in fig.4.5.

The duration and amplitude of SD before arrhythmia were typical, but after the appearance of arrhythmia there were both a very large(a,b,e) and a small (c,d) SD were observed. Small amplitude and duration of SD was noted at the time VPB, which was non effective, but large amplitude and duration of SD were observed at the time of the compensatory pause.

An explanation of this variability is given in fig.4.4-B. Changes in SP, DP and PP and SDD are shown here for different cardiac cycle: before CAO (a), after CAO with a normal rhythm (b), after CAO with VPB (c), and after a

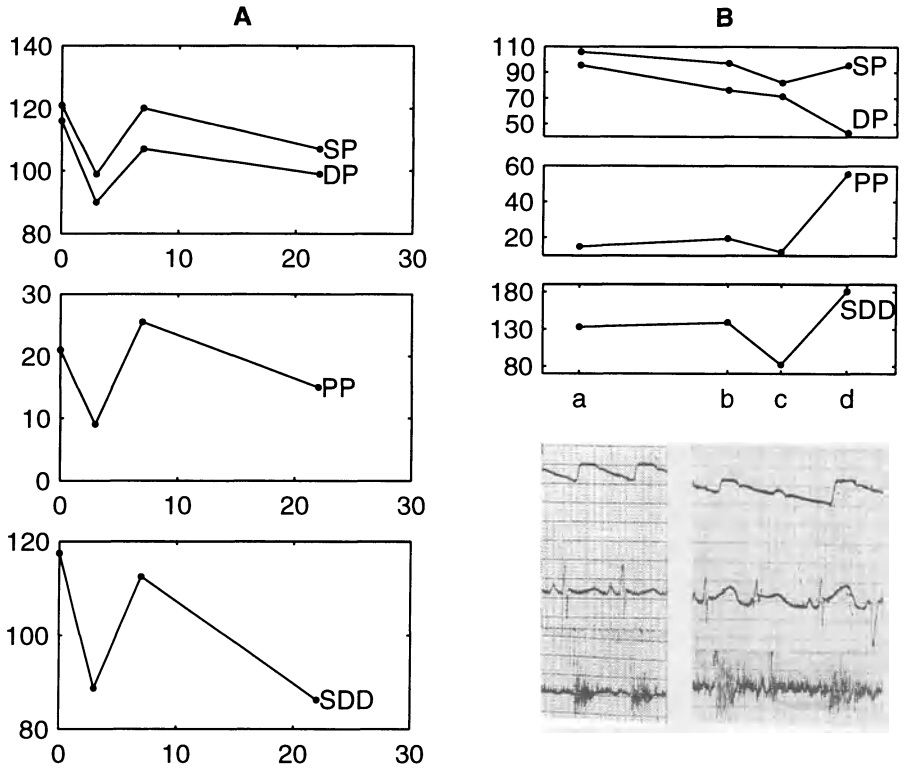

Figure 4.4. A. The dependence of duration of systolic discharges (SDD) in aortal nerve on SP, DP and PP after CAO in experiments without arrhythmias. B. The dependence of SDD in aortal nerve on SP, DP and PP in individual cardiac cycle after CAO in experiment with VPB. a - before CAO, b - after CAO in normal cardiac cycle, c - in extrasystolic and d - in postextrasystole cardiac cycle.

compensatory pause (d). The shortening of SDD (c) occurred in parallel with decrease in SP,DP and PP. This was a normal reaction of aortal receptors to the decrease in SP. However, the lengthening of SDD at the end of compensatory pause (d) was an abnormal reaction of these receptors. At that time SP increases, but remains below its initial level. The increase in SDD was in correlated only with the increase in PP. The latter increased from 15 to 40-50 mmHg due not only to an increase in SP, but also to a decrease in DP.

This data shows that aortal baroreceptor react to the increase in pulse pressure by increasing SDD independent of whether the SDD increases at the expense of a rise in the systolic pressure or at the expense of a decrease in the diastolic pressure. In the first case, the increase of SDD is expedient because it results is normalization of blood pressure. In the second case, when SP does not increase but DP decreases and PP rises during the compensatory pause

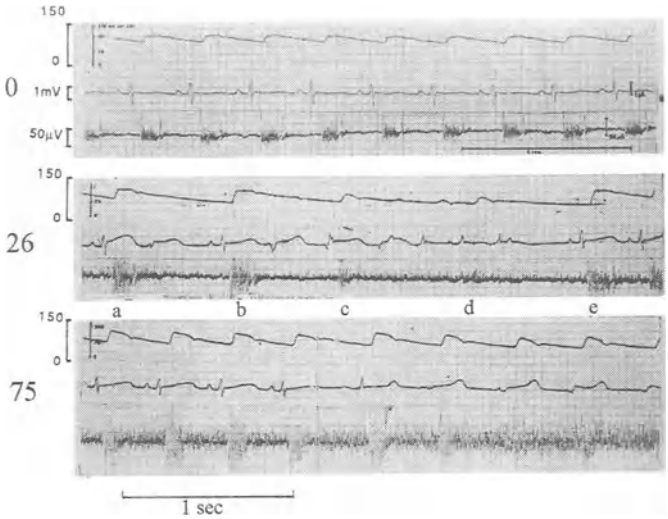

Figure 4.5. Different changes in the systolic discharges duration (SDD) in aortal nerve after CAO in experiments with arrhythmia (increase at a,b,c, decrease at c,d). Gradual increase in diastolic activity. From top to bottom: arterial pressure, ECG-II, AAAN. Numbers to the left of the plots denote time after CAO.

after extrasystole. the increase in SDD and baroreceptor reflex can be a cause an additional decrease in SP. As a result, this "compensatory" reaction, may be the cause of hypotension and the so-called cardiogenic shock. In this case ordinary "compensation" is erroneous and harmful [64]. It is second example of erroneous ordinary "compensation" in myocardial infarction (the first example is the activation of glycogenolysis in the zone of ischemia, see chapter III)

The connection between cardiogenic shock and arrhythmias was established in clinical works. It is sometimes referred to as "arrhythmical collapse". Intra-aortic balloon pumping had very beneficial clinical effects for patients with myocardial infarction and cardiogenic shock [265, 25]. It appears that the favorable effect of intra-aortic balloon pumping is attained by preventing a decrease in PP and an increase in SDD in myocardial infarction with arrhythmias.

4.2.2 Changes in diastolic activity

A second phenomenon in the changes in afferent activity of the aortal nerve is the appearance (or a gradual increase in the amplitude) of low-amplitude, non-discharged diastolic activity (DA). As can be seen in fig 4.5, changes begin 26th minutes after CAO and strengthen sharply after 75 minutes. This occurs in parallel with a decrease in the arterial pressure.

The power of DA after CAO was especially pronounced during arrhythmias. DA appears when DP decreases as a result of noneffective extrasystole (fig.4.4-B). During the compensatory pause, DA increased monotonously. Sometimes

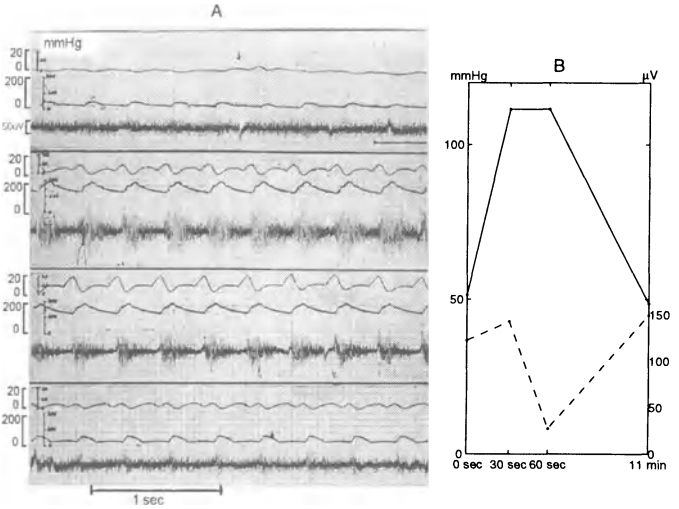

Figure 4.6. The influence of adrenaline (0.005%, moment of injection shown with an arrow) on the afferent activity in the aortal nerve. A. From top to bottom: left auricle pressure, arterial pressure, AAAN. B. The curve of APr (solid line), diastolic activity amplitude (broken line). Abscissa - time after CAO

DA does not appear after CAO, but appears only after VF, increases gradually and then decreases during cessation of electrical activity in the heart. Unlike the changes in systolic discharges, increase and decrease in DA are not directly connected with changes in arterial pressure. An increase in DA was observed during development of multiple extrasystole without substantial changes in diastolic and pulse pressure (fig.4.5-75min.).

We speculate that the increase of DA, which we observed in both cardiac and aortal nerves, is not associated with mechanical changes, but depends on metabolic changes.

4.2.2.1 The role of the arterial pressure in the increase of diastolic activity. This dependence was studied in our laboratory in 39 experiments on cats and dogs [55]. Substances were infused into one of the pulmonic veins, enabling them to act on the aortal receptor in high concentration. Various relationships were observed between the changes in the arterial pressure, the coronary sinus pressure and diastolic activity. In the majority of the experiments an inverse relationship was observed between the changes in arterial pressure caused by these substances and the increase in diastolic activity. An inverse relationship between the blood pressure and the diastolic activity was observed in adrenaline injection at a low initial pressure (fig.4.6).

When adrenaline was administered against this background, a sharp increase in pressure during thirty seconds after the infusion did not affect the amplitude of diastolic activity. After one minute the diastolic activity decreased, although

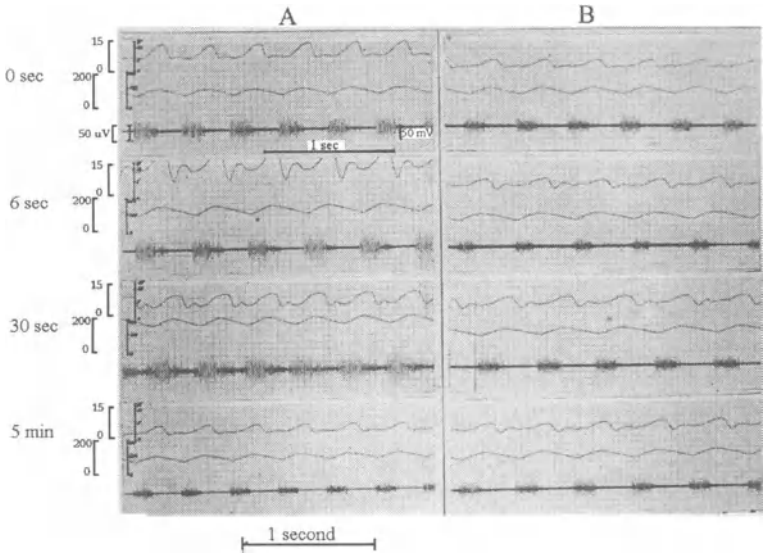

Figure 4.7. The effect of lobeline (1% -0.3 ml) injected before (A) and after (B) the acetic acid (0.5N - 0.4ml). From top to bottom: left auricle pressure, APr, AAAN. On the left - time after injection of lobeline

the pressure level did not change. Finally, when blood pressure decreased to its initial level, the diastolic activity increased again. The changes in the amplitude of diastolic activity after infusion of adrenaline seem to be caused not by the changes in the arterial pressure but by the excitation of chemoreceptor in the aortic arch.

4.2.2.2 The role of metabolic changes in the increase of diastolic activity. To verify the hypothesis that diastolic activity depends on the aortal chemoreceptor, we took advantage of the ability of acetic acid to block the chemoreceptor and the ability of lobeline to induce diastolic activity (fig.4.7).

Diastolic activity appears 30 seconds after the infusion of lobeline (fig.4.7-A) and stops after 5 min. After the recovery of the initial diastolic activity, a concentrated solution of acetic acid was infused, and then lobeline was administered again (fig.4.7-B). The latter did not induce diastolic activity against the background of acetic acid. This experiment confirms the dependence of the diastolic activity on the chemoreceptor.

The appearance of diastolic activity after CAO, revealed in our experiments in the absence of considerable changes in the arterial pressure, is probably connected with the penetration of the metabolic products from the ischemic myocardium into the blood. Considering the significance of K accumulation in the onset of VF and arrhythmia, we studied changes in the afferent activity in coronary nerves when KCl was infused into the coronary sinus (fig.4.8).

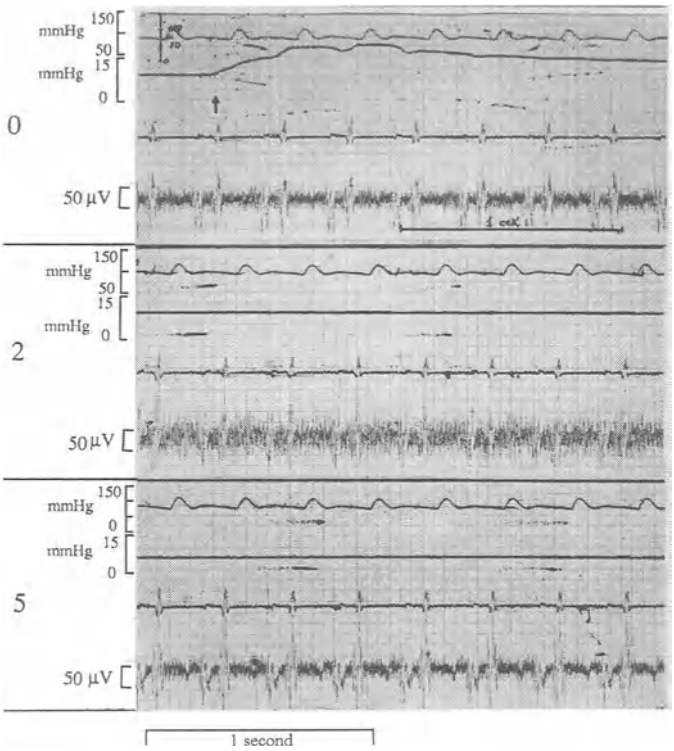

Figure 4.8. Example of AACN changes after injection KCl (1.15% - 0.5ml in coronary sinus). From top to bottom: APr, coronary sinus pressure, ECG-II, AACN. The arrow shows the time of injection of KCl. The solution of KCl stimulated an increase in AACN power two minutes after injection. Stimulation did not occur at the time of injection, when coronary sinus pressure increased.

As shown in fig.4.8, an increase in diastolic activity was observed 1.5-2 minutes after infusion of KCl (0.5 ml, 1.15% solution). In the meantime, no activation was observed at the time of the infusion, when the pressure in the coronary sinus increased sharply.

In cases described above, the changes in ischemic metabolism were created artificially. It was of interest to compare the changes in afferent activity in coronary nerves with changes in the myocardial metabolism that occur naturally after CAO. These changes are reflected in the composition of coronary sinus blood. The sharpest increase in the diastolic activity in the heart nerves occurred simultaneously with a sharp decrease in P_{O_2}, redox potential and biphase changes of pH and pNa in the coronary sinus blood (fig.3.2-II) [248].

The metabolic nature of the diastolic activity is additionally testified to by the fact that during VF, when there are no changes in arterial pressure, a further increase of diastolic activity occurs.

The following facts allow us to draw a connection between the increase in the diastolic activity in the heart nerves and the metabolic factors of ischemia:1) the fact that there is no relationship between the afferent activity in the coronary nerves and the changes in the arterial pressure, 2) the fact that diastolic activity increases simultaneously with changes in the coronary sinus blood composition, 3) the possibility of inducing this activity by means of artificially reproducing the "ischemic" changes in the metabolism.

The result of pathological afferent activity should be changes in the efferent activity. The result of the increase of the efferent activity in the sympathetic heart nerves should be changes in the balance of mediators of the nervous excitation - noradrenaline and adrenaline in the heart.

4.3 Catecholamine balance in the heart and ventricular fibrillation after coronary artery occlusion

The balance of CA in the heart after CAO is determined 1) by their content in the heart, 2) their metabolism and 3) by their transport between heart and blood.

4.3.1 Catecholamine content in the heart in CAO

The content of CA in the heart is composed of: 1) release of NA during excitation of sympathetic nerves; 2)A and NA synthesized in the chromaffin heart cells; 3) A and NA uptake by the heart from the blood flowing passing through it.

All these processes and their role in ventricular arrhythmias have been intensively studied in the last two decades. A review of the data is given in [36, 125, 212, 276, 303]. The content of CA in the heart can increase as a result of metabolic changes - activation of synthesis or inhibition of decomposition and as a result of transport changes - activation of release of CA by nerve endings, increased uptake from blood flowing into the heart or decreased release into blood flowing from the heart.

Data of A and NA content in an ischemic and non-ischemic myocardium are given in monograph [124]. A normal heart contains relatively little A (from 0 to 0.23 μg/g) and considerably more NA (from 0.06 to 1.3 μg/g of the tissue) [312].

The available data on the changes in A and NA content in the heart after CAO are 1) very contradictory, 2) often contain only the data of changes in the total CA (A + NA) content, and 3) very frequently mainly describe the later stages of ischemia. There is scarce information about the earlier stages, when VF usually appears.

The content of A and NA was studied in ischemic and non-ischemic zones of canine hearts before CAO, 5 and 30 minutes after CAO, and at the instant of VF onset using the fluorometry method [214]. Data obtained in these experiments are shown in fig.4.9.

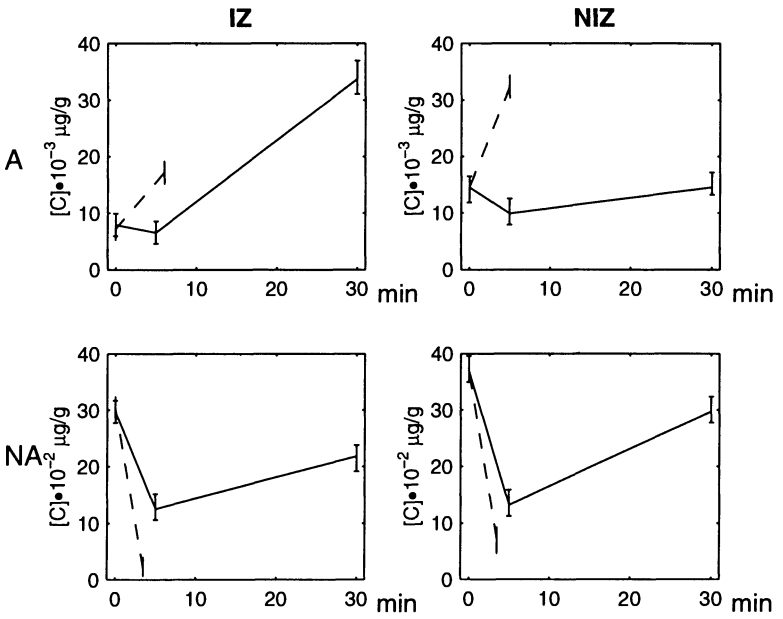

Figure 4.9. The dynamics of changes in concentration of A and NA in IZ and NIZ of canine hearts after CAO in experiments with (broken line,n=18) and without (solid line, n_0=13, n_5=15, n_{30}=21) VF

As can be seen from fig.4.9, in experiments with the VF just after the development of VF, the concentration of A in the ischemic and non-ischemic zones increased, but the content of NA in these zones decreased.

The peculiarities of experiments with VF after CAO consist of a more rapid increase in adrenaline and decrease in noradrenaline in both the ischemic and non-ischemic zones of the heart.

The role of disturbances of CA metabolism and transport in the changes in NA and A content in the heart will be discussed below.

4.3.2 Catecholamine metabolism in the heart after CAO

4.3.2.1 Catecholamine synthesis. The synthesis of NA in the sympathetic nerves occurs in the varicose diverticula which exist both in the nervous endings and in the pathway on the nervous fiber. NA is synthesized from L-tyrosine. L-tyrosine is transferred from the blood through the membrane of the sympathetic nerve by means of a special mechanism. Under the action of tyrosine hydroxylase, L-tyrosine is oxidized and transformed into L-dihydroxyphenylalanine (DOPA). DOPA is rapidly decarboxylate in the non's cytoplasm by the enzyme DOPA-decarboxylase and transforms in dopamine.

Dopamine enters into the granulated vesicles where it transforms into l-noradrenaline. Accumulation of NA line occurs in the vesicles, but the latter remain inactive until the excitation of the nerve.

The synthesis of NA in the chromaffin cells occurs in the same way as in the sympathetic nerves. However in the chromaffin cells of the heart, in the adrenal glands and in the brain stem l-noradrenaline transforms in l-adrenaline. Human adrenal glands contain 80-85% of A and only 15-20% of noradrenaline.

4.3.2.2 Catecholamine catabolism. Inactivation of CA occurs mainly due to oxidative deamination under the influence of MAO and due to catalysis of O-methylation by catechol O-methyltransferase (COMT). The free noradrenaline in the cytoplasm of the sympathetic nerves is deaminated in the mitochondria by MAO. The NA which enters in circulation undergoes O-methylation under the influence of COMT and is transformed into normetanephrine, while A is transformed in metanephrine. MAO causes further degradation of normetanephrine and metanephrine.

4.3.2.3 Ischemic changes in the catecholamine metabolism in the heart and their peculiarities in cases with ventricular fibrillation. In order to evaluate the changes in CA catabolism after CAO, the activity of MAO was studied in ischemic and non-ischemic zones of the heart. The study was conducted in our laboratory on 49 cats [52].

The activity of MAO was determined based on the quantity of ammoniac released during incubation of tyramine in the presence of mitochondria from the right and left heart ventricles. The activity of ChE was studied by means of potentiometric titration. Taking into account that changes in tissue pH during a myocardial infarction can cause disturbances in the activity of the enzyme, the determination of the ChE activity was performed at the pH of the tissue using a method developed in our laboratory [248]. The results this study are shown in fig.4.10.

The initial decrease in MAO activity after CAO in ischemic zone can be connected with the acute hypoxia which results in depression of the oxidative deamination. The second phase is probably causes an increase in decomposition of release of noradrenaline from nerve endings.

In the experiments with VF after CAO, the increase in MAO activity and the decrease in the activity of cholinesterase, both in the ischemic and non-ischemic zones, took place during earlier stages, and were more pronounced than in experiments without VF.

The increase in MAO activity was confirmed by Lamontagne et al

in experiments on an isolated perfused rat heart [163]. Authors concluded that the increase in MAO activity may contribute to the elimination of the NA lost by the cardiac tissue during ischemia.

Further experiments made in our laboratory were designed to study the dependence of changes in MAO and cholinesterase activity in the ischemic focus on the changes of conditions for the enzyme action [238]. For this purpose the

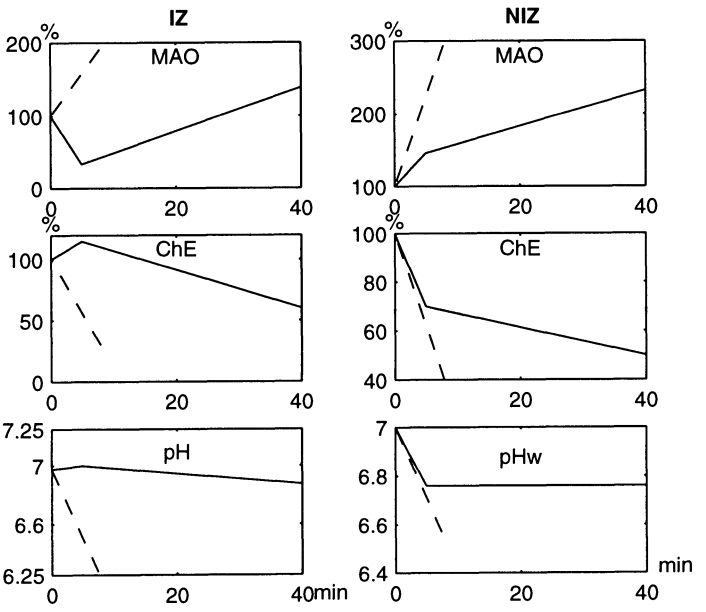

Figure 4.10. Changes in activity of MAO, ChE and pH in IZ and NIZ of feline hearts after CAO in experiments with VF (broken line, $n_{MAO,ChE}$=5) and without VF (for MAO n_0=14, n_5=15, n_{40}=14; for ChE n_0=8, n_{40}=7,solid line)

activity of cholinesterase under optimal pH (8.5) and under tissue pH were studied in parallel with the activity of MAO in a medium saturated with 100% oxygen and with a gas mixture containing 50% oxygen and 50% nitrogen.

The experiments revealed that conditions of relative hypoxia lowered the activity of MAO by 54% ($p<0.001$), while acidosis conditions lowered the activity of cholinesterase by 57% ($p<0.001$).

The increase in the activity of MAO seemed likely to be one of the factors in the decrease of the noradrenaline content in the heart after CAO. The other, perhaps more powerful factor which changes the concentration of noradrenaline and, especially of adrenaline, in the heart after CAO may be disturbances in transport of CA between the heart and blood.

4.3.3 Transport of catecholamines between the heart and blood

The transport of CA in tissues includes the following processes (fig.4.11): 1) storage (S) of CA in the intraneuronal vesicles; 2) release (R) of CA (mainly noradrenaline) by the nerve endings into the synaptic fissure, into the extra cellular medium and into the blood; 3) release of CA (mainly of adrenaline) into the blood by the chromaffin cells of the adrenal glands; 4) re-uptake(re-U) of release of noradrenaline from these structures by the nerve endings and

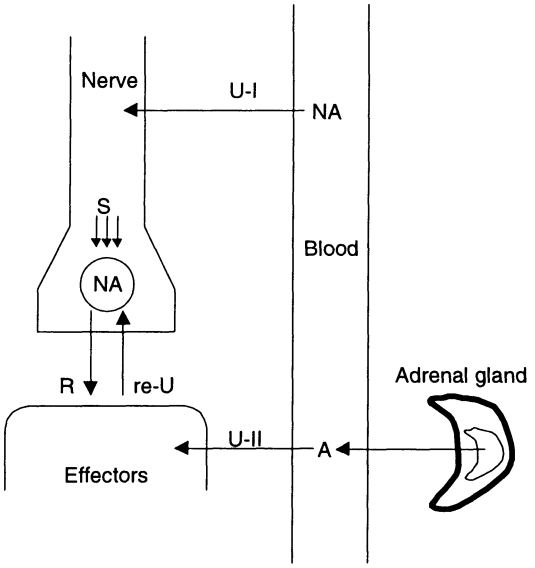

Figure 4.11. Schematics of catecholamine transport. U-I- uptake I, U-II- uptake II, R - Release, re-U - re-Uptake, S - storage

vesicles; 5) uptake (U) of CA from the blood: neuronal uptake I (U-I) by the nerve endings and extra neuronal uptake II (U-II) (mainly of adrenaline) by the effector cells.

A series of factors that develop during ischemia exert a substantial influence on the transport of CA [213]. The study of CA transport in the heart in vivo requires simultaneous and separate measurement of A and NA concentration in the blood going into and out of the heart. Changes in the concentration of A and NA in the arterial and in the venous coronary blood (in the coronary vein and coronary sinus) were studied using fluorometry and radio- enzymatic methods. To study the time relationship between the changes in CA concentration and the appearance of VF we used a semi-quantitative biological method.

4.3.3.1 Continuous determination of release of adrenaline and noradrenaline from the heart via the biological method. Biological method of continuous determination of the A and NA [58] was based on the use of two test objects: rat stomach strip (RSS) (which reacts equally to both A and NA) and chicken rectum (CR) which essentially reacts only to A because its sensitivity to the latter is 25-100 times higher than to noradrenaline. When both test-objects are used simultaneously, the reaction of CR indicates the A concentration. The concentration of NA is evaluated based on the difference between the reactions of RSS and CR. Both test-objects react to the CA by relaxation. The mechanical changes in the tonicity of both test-objects are transformed to electrical signals recorded by a recorder.

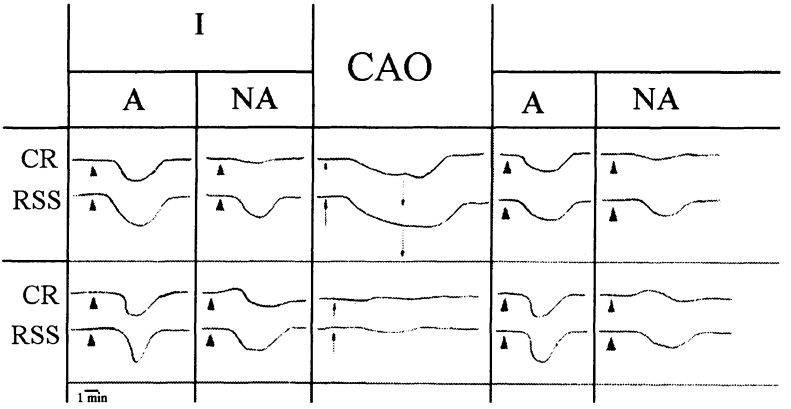

Figure 4.12. Typical examples of reactions of the chicken rectum (CR) and the rat stomach strip (RSS) superfused with coronary blood to application of A and NA (both given in the dosage of 20 ng/min) before (I) and after CAO (II) and during CAO in experiments with VF (a) and without VF (b). Time of A and NA administration is shown by a triangle. The upward arrows shows time of CAO, downward - the moment VF appeared

VF developed in 19 out of 25 experiments. The increase in CA concentration in the coronary sinus blood was observed in 14 out of 19 experiments with VF, while it was observed in only one out of 6 cases without VF. Typical examples of reactions of the test objects to the calibrated introduction of CA and to CAO in experiments with and without VF are given in fig. 4.12.

Continuous recordings of CA concentration in the coronary sinus blood allowed us to establish a temporal relation between the increase in CA and appearance of VF. The CA content begins to increase on average 3.5 min. after CAO, and reaches its maximum value 6 min after CAO. VF develops on average after 6 min. against the background of a sharp increase in CA content in the coronary sinus blood. So, the release of CA into the coronary sinus blood precede the appearance of VF.

4.3.3.2 Concentration of adrenaline and noradrenaline in arterial and venous coronary blood. The dynamics of changes in A and NA content in arterial blood, coronary vein blood, and coronary sinus blood is presented in fig.4.13.

The increase in A and NA concentration (in percent of the initial level) at the time of appearance of VF (VF developed on average at the 6th min, between the 3d and the 10th min) and in the experiments without VF (averaged from the 3rd to the 10th min) is presented in fig.4.14).

As it is apparent from fig.4.13 and fig.4.14, changes in A and NA contents in the arterial and coronary venous blood are substantially different in experiments with and without VF. In experiments with VF, A and NA concentrations in the arterial and coronary venous blood increased sharply (practically

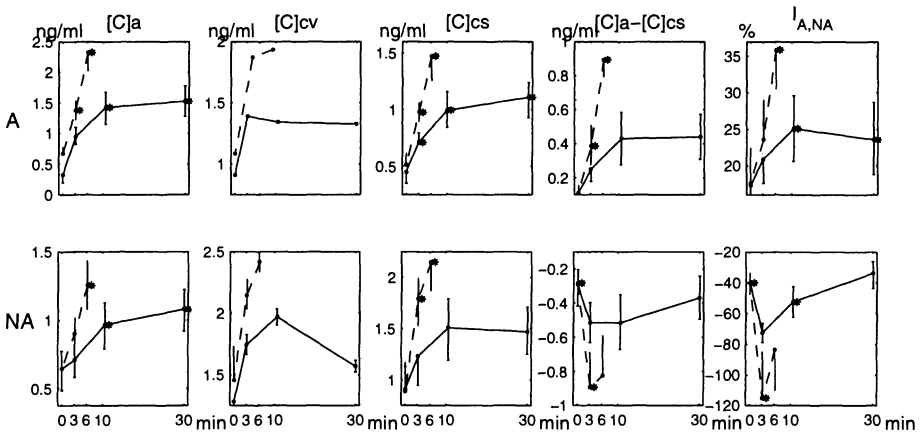

Figure 4.13. The time dynamics of changes in A and NA concentration in the arterial blood ([C]a), in the coronary vein blood ([C]cv and in the blood of coronary sinus ([C]cs). Arterio-venous difference (a-v) and index of uptake/release (I) of A and NA after CAO in experiments with VF (n=10,broken line) and without VF (n=5, solid line). The measurements were made using both fluorometry (FM) and radioenzymatic methods (RF). Statistically significant changes are marked with a star

linearly), reaching the maximum value at the moment of the VF appeared. In the experiments without VF, CA concentration increased considerably slower and after reaching a maximum value (which was lower than in experiments with VF) stabilized or even decreased somewhat.

Fig 4.14 shows that the concentration of A (determined by both fluorometry and radioenzymatic methods) increased more in the arterial blood than in venous blood. The increase in A concentration in the coronary sinus and coronary vein blood was practically the same.

The concentration of NA increases in coronary sinus blood more than in arterial blood. The increase in concentration of NA is almost the same in coronary sinus and coronary vein blood. The analysis of changes in A and NA contents in the arterial and venous coronary blood permits us to make the following conclusions:

1. The fact that the concentration of adrenaline increases more in arterial than in venous blood demonstrates that this increase is determined by extracardial processes, first and foremost by the increase in adrenaline secretion by the adrenal glands;

2. The fact that the noradrenaline concentration increases more in the venous blood than in the arterial blood shows that this increase is determined by release of NA from the heart;

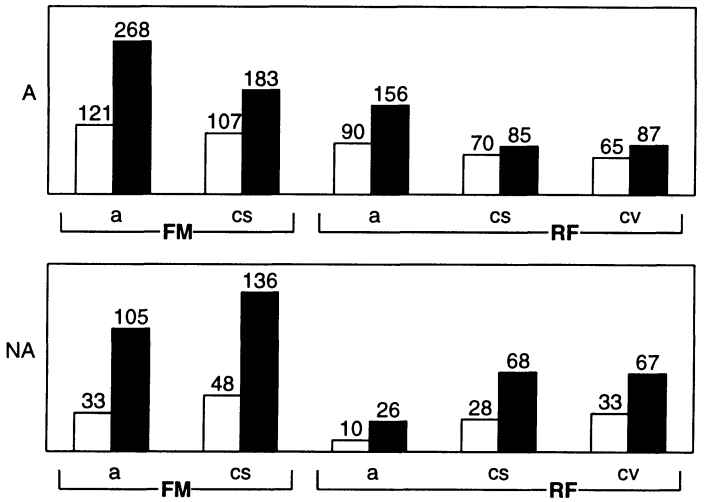

Figure 4.14. The highest increase in A and NA concentration (in % of the initial level) in arterial blood (a), in the blood of coronary sinus (cs) and coronary vein (cv) after CAO in experiments with VF (blood was taken at the time of VF appearance, black columns, n=10) and without VF (average from blood samples taken between 3 and 10 min after CAO, white columns, n=5). The measurements were made using both fluorometry (FM) and radioenzymatic methods (RF)

3. The fact that the A and NA concentrations increase to the same degree in coronary sinus and coronary vein blood shows that they reflect changes in the CA balance in the whole heart, and not only in the ischemic zone;
4. The fact that the increase in A concentration in the arterial blood and of NA concentration in the coronary sinus blood is greater in experiments with VF than in experiments without VF, 2.2 and 2.8 times, respectively, shows that VF develops in cases those of CAO that have more sharply expressed disturbances in CA balance.

Changes in CA content in the blood that enters and leaves the heart in experiments with and without VF allowed us to study the characteristics of changes in the processes of CA uptake and release by the heart.

4.3.3.3 Uptake of adrenaline and release of noradrenaline by the heart. The arterio-venous difference (a-v) of CA concentration can be used to evaluate the changes in their uptake and release by the heart after CAO assuming that no significant changes in the velocity of the coronary blood flow occur at the sampling instant. The measurements of blood flow from the coronary sinus showed that blood flow through the whole heart did not change significantly at the sampling instant. This allowed [240] the value of (a-v) to

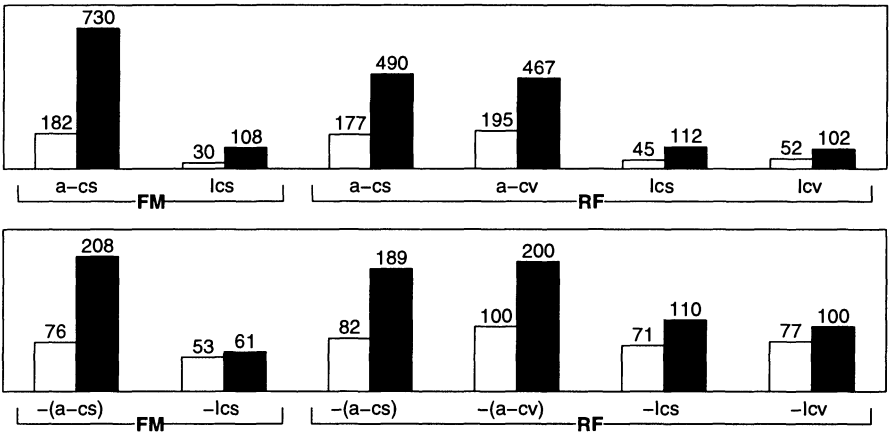

Figure 4.15. A and NA arterio-venous difference and uptake/release index calculated based on coronary sinus blood (a-cs, lcs) and coronary vein blood (a-cv, lcv) after CAO in experiments with (black columns) and without (white columns) VF. The measurements were made using both fluorometry method (FM) and radioenzymatic method (RF)

be used to measure the CA uptake and (v-a) to measure CA release by the heart. In order to evaluate the role of the heart itself in the changes in CA uptake and release and to exclude the influence of changes in CA concentration in the arterial blood flowing into the heart, uptake and release indexes were calculated using the following equation:

$$I_{A,NA} = \frac{[C_a] - [C_{cs,cv}]}{[C_a]} * 100\% \tag{4.2}$$

where $[C_a]$ is the concentration of A or NA in the arterial blood, $[C_{cs,cv}]$ is the concentration of A or NA in the coronary sinus or coronary vein blood. Positive values of this index signify uptake of A or NA, negative values signify release of A or NA.

The increase in (a-cs), (a-cv) and $I_{A,NA}$ relative to the initial level, measured at the time of VF occurred and the average of measurements taken 3 and 10 minutes after of CAO are shown in fig.4.15 for experiments with and without VF.

The values of (a-cs), (a-cv) and I are initially positive for A and negative for NA. This indicates that the uptake of A from the arterial blood and release of NA into the venous blood by the heart occurs even before CAO (fig.4.14). After CAO, (a-v) for A increased 4 times greater in experiments with VF than in experiments without VF according to the fluorometry method and 2.4 times according to the radioenzymatic method. The decrease in (a-v) for NA was almost linear. In experiments without VF these changes were expressed less noticeably; they reach a maximum at the 3rd-10th min and then stabilize or

even decrease somewhat. The decrease in release of NA was less pronounced than the increase in uptake of A. Release of NA during myocardial ischemia occurs via nonexostosis mechanisms [278].

The index of uptake of A I_A increased considerably less than (a-v) but the increase was also substantially greater (3.6 and 2.5 times) in experiments with VF than in experiments without VF. The increase in the uptake of A index was essentially the same when calculated using the coronary sinus blood or the coronary vein blood.

The index of release of NA exhibited a considerably smaller increase than the decrease in -(a-cs) and -(a-cv), but it also was more pronounced in experiments with VF than in experiments without VF. The increase of the index of release of NA was practically the same when calculated using either coronary sinus or coronary vein blood.

The analysis of changes in $(a\text{-}v)_{A,NA}$ and $I_{A,NA}$ allows us to make the following conclusions:

1. The fact that (a-v) is positive for adrenaline and is negative for noradrenaline in the initial state, shows that before CAO the heart uptakes adrenaline from the arterial blood and releases noradrenaline into the venous blood.
2. The fact that the positive (a-v) for A and the negative (a-v) for NA increase after CAO shows that local ischemia induces an increase in uptake of A and release of NA by the heart.
3. The fact that the increase in (a-v) is practically the same in coronary sinus and coronary vein blood, for both A and NA, indicates that the uptake of A and release of NA increase in the whole heart and not only in the ischemic zone.
4. The fact that the increase in the positive (a-v) for A and the negative (a-v) for NA considerably exceeds the increase in the corresponding indexes of I_A and I_{NA} indicates that the release of NA and especially the uptake of A by the heart strongly depends on their concentration in the arterial blood, i.e. ischemia stimulates the extraneuronal uptake of A.
5. The fact that the increase in (a-v) for A and increase in the negative (a-v) for NA is greater in experiments with VF, indicates that VF appears after CAO in cases with an especially sharp extraneuronal uptake of A and release of NA by the heart.

The represented data, however, do not answer on the question about the mechanisms of the increase in uptake of A and the increase in release of NA by the heart after CAO.

4.3.3.4 Possible mechanisms of increases in uptake of A and release of NA by the heart after coronary artery occlusion. The succession of events which results in the above disturbances in the CA balance in the heart can be the following: The ischemic disturbances in metabolism activate the afferent and efferent (arising by reflex) nervous activity. This results in the

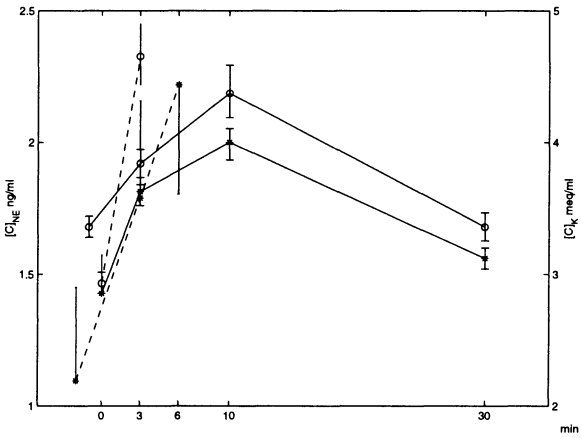

Figure 4.16. The dynamics of changes in concentration of NA ($[C]_{NA}$, stars) and K^+ ($[C]_{K+}$, circles) in coronary vein blood after CAO in experiments on dogs with VF (n=10, broken line) and without VF (n=5, solid line)

release of NA by nerve endings. Under ischemic conditions the NA re-uptake becomes impossible and its content in the heart decreases, but increases in the venous coronary blood. This serves as a signal for the stimulation of compensatory strengthening of A secretion by the adrenal glands. The concentration of A in the arterial blood rises and its uptake by the heart increases.

Based on the above succession, we believe that answering of the following three questions is of great importance:

1. whether the release of NA by the heart corresponds to the degree of ischemic metabolism disturbances in the ischemic zone;
2. whether the uptake of A by the heart corresponds to the release of NA;
3. whether the inadequacy of the strengthening of uptake of A and release of NA by heart is connected with changes in the properties of the proper heart or with the increase in A concentration in arterial blood.

To answer the first question, we chose to study the release of K into the coronary vein blood which drained the ischemic zone. This choice was made because the outward flux of K from the ischemic myocardium is one of the earliest manifestation of ischemia. Secondly, we chose it because the increase in the extracellular K concentration can be a direct cause of the local depolarization of the neuronal membrane and of release of NA from the vesicles [128, 213].

To clarify the relationship between the concentration of K in coronary vein blood and the release of NA into it, simultaneous measurements of the concentration of NA and K ions in the same sample of coronary vein blood were made (fig.4.16).

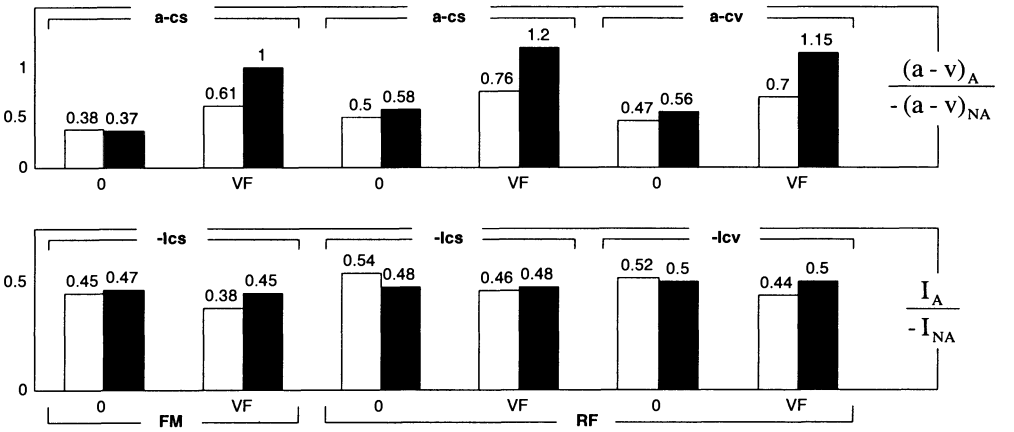

Figure 4.17. The ratio of uptake of A to the release of NA by a canine heart $(a\text{-}v)_A/\text{-}(a\text{-}v)_{NA}$ and $I_A/\text{-}I_{NA}$, calculated on coronary sinus blood (a-cs,Ics) and coronary vein blood (a-cv,Icv) before CAO (0), after CAO without VF (white columns) and at the time of VF (VF) (black columns)

NA and K in coronary vein blood increased in parallel with a correlation coefficient of r=0.62 ($p<0.05$). These data confirm that there exists a clear connection between the exit of K from the ischemic zone and the release of NA. A dependance between the degree of ischemia and release of noradrenaline was also observed in [126, 260].

To answer the second question, "Is there a relationship between the release of NA and uptake of A", the ratios of $\frac{(a-v)_A}{-(a-v)_{NA}}$ and $\frac{I_A}{-I_{NA}}$ were calculated both in the initial state and at the moment VF appeared. In experiments without VF the ratios were calculated for the corresponding time interval, i.e. the average of values 3 and 10 min after CAO. The data are presented in fig.4.17.

In the initial state, both the ratio of arterio-venous differences for A and NA and the ratio of uptake (release) indexes were close to 0.5. This signifies that only one of the two parts of NA that are released is replaced by A. These values are practically the same in experiments both with and without VF. The ratio $\frac{(a-v)_A}{-(a-v)_{NA}}$ increases after CAO.

In experiments without VF it increases to 0.76 in the coronary sinus and to 0.70 in the coronary vein blood at the average time of VF appearance. In experiments with VF at the time of VF appearance the above ratios increase to 1.20 and 1.15 respectively (radioenzymatic analysis). At the same time the ratio $\frac{I_A}{-I_{NA}}$ remains practically the same as the initial value in all the cases.

So, the release of NA after CAO is overcompensated by the uptake of A. An analogous phenomenon was observed in mice after trauma of extremities [138]. The sympatho-adrenal reaction to injuries included depression of the

sympathetic nervous system and stimulation of the adrenal gland medullar substance.

In our experiments VF did not appear in cases where the ratio of the uptake of A to the release of NA increased from 0.47-0.50 to 0.70-0.76, but VF did appear when this ratio increased to 1.15-1.20. This could be connected to the functional inadequacy of the replacement of NA by A because the arrhythmogenic action of A is considerably higher than that of NA. In light of these data, VF could be a direct consequence of the inadequate uptake of A by the heart in response to the release of NA.

The question whether the inadequate increase in the uptake of A and the release of NA is connected with the changes in the properties of the heart after CAO (for example, the increase in the affinity of ischemic tissue to A) or with the increase in A concentration in the arterial blood can be answered based on the changes in ratios: $\frac{(a-v)_A}{-(a-v)_{NA}}$ and $\frac{I_A}{-I_{NA}}$ (fig.4.17).

The ratios of the indexes of the uptake of A and release of NA, which are calculated without taking into account the changes in concentration of A and NA in the blood, remain practically unchanged in experiments both with and without VF. Meanwhile, the ratios of arterio-venous differences in A and NA content, which do take into account the changes in concentration of A and NA in the blood, increase considerably, primarily in experiments with VF.

We conclude from these data that the inadequacy that appears in the uptake of A and release of NA by the heart after CAO and especially in the experiments with VF is determined by the increased concentration of A in the arterial blood. The hyperadrenalemia, is apparently a result of compensatory release of A from adrenal gland in response to the loss of NA. This is the third example of an imperfection in compensatory mechanisms during myocardial ischemia. Dry et al [87] have shown that the ability of chromaffin cells isolated from bovine adrenal glands to react to the metabolic conditions through loss of CA is similar to that found during stop-flow cardiac ischemia.

The analysis of data about the mechanism of uptake of A and release of NA by the heart in CAO leads to the following conclusions:

1. The fact that the release of NA and K into the blood of coronary vein, which drains the ischemic zone, after CAO are closely correlated, testifies that the release of NA is connected with disturbances in metabolism in the ischemic zone;
2. The fact that the ratio of uptake of A to release of NA by the heart increases after CAO indicates that the uptake of A by the heart as a response to release of NA occurs with overcompensation;
3. The fact that in experiments with VF the uptake of adrenaline exceeds the release of noradrenaline to a greater degree than in experiments without VF, shows that VF arises as a result of replacing the heart's NA by A, which possesses stronger arrhythmogenic properties than NA;
4. The ratio of indexes A uptake to NA release, which does not depend on concentration A and NA in arterial blood, does not change after CAO in

experiments both with and without VF. This fact testifies that the excess uptake of A over the release of NA is due to the increase in the A concentration in the arterial blood. The more pronounced arterial hyperadrenalemia in dogs with VF can be a manifestation of a greater reactivity of their chromaffin system of adrenal glands.

4.3.3.5 Measuring the reactivity of the adrenal gland in experiments with ventricular fibrillation after coronary artery occlusion. The analysis of initial concentration of A and NA in the arterial blood measured after the anesthesia and thoracotomy but before CAO shows that in some experiments [214] the A concentration in dogs with VF was higher, while the NA concentration was the same as in dogs without VF. This allows us to suppose that dogs with VF are either permanently characterized by a higher activity of the adrenal gland medullar substance or by a higher reaction of this apparatus to stress conditions such as the anesthesia and the thoracotomy before CAO. If this is indeed so, the probability of VF onset must be predetermined by the A secretory function of the adrenal glands before CAO. To verify this supposition it was necessary to determine capacity of a dog's adrenal glands for secretion of A in response to a certain standard action before CAO.

It is well known that insulin contributes to the release of A from the medullar substances of the adrenal glands [44]. Therefore, insulin was used to evaluate the potential effects of the A secretion by the adrenal glands in response to a certain influence [146, 147].

We have shown that A secretion in response to insulin before CAO was greater in dogs which developed VF after CAO than in those which did not [118]. The secretion of A in response to insulin administration and in response to CAO may be determined by the individual characteristics of the chromaffin cells of the adrenal glands, by its intrinsic higher activity, or by its higher sensitivity to nervous influences,or by stronger nervous influences which arrive to the adrenal gland. The more powerful activity of the afferent heart nerves in CAO in experiments with VF allows to suppose that this activity is a cause of the higher A secretion. The greater initial level of A in arterial blood in narcotized dogs before CAO can be a sign of greater reactivity adrenal apparatus against stress in the dogs predisposed to VF. Finally, in ten time greater release of A in arterial blood in answer on insulin testifies to individual particularity of chromaffin cells of adrenal gland dogs predisposed to VF. Proceeding from these results we decided [118] to use the insulin test for prediction VF after CAO (see chapter VI).

4.3.3.6 Pharmacological control of uptake of adrenaline by the heart. Proceeding from the fact that VF appearance is connected with an especially sharp increase in secretion of adrenaline by the adrenal glands and with its uptake by the heart, we was attempted to influence the extraneuronal uptake of A by the heart with help of pharmacological means.

An O-methylated derivative of adrenaline - metanephrin[1] was used to block the extraneuronal uptake of adrenaline. Metanephrin manifest its main effect after 10 min of CAO by decrease of A content in the arterial blood and decrease of the A uptake by the heart. As a result of this effect, the frequency of VF in the group medicated with metanephrin decreased from 65% to 25% (see chapter VI).

4.4 Conclusions

The experimental data referred to in this chapter show that the probability of VF appearance is determined not only (and perhaps not at all) by local changes in the metabolic and electrical processes in the ischemic zone, but by the general reaction of the organism to local myocardial ischemia. The reaction is manifested by changes in the control of the heart by the sympatho-adrenal system.

The changing afferent activity in cardiac nerves is the source of these changes, but the disturbances of the adrenaline-noradrenaline ratio in the heart are their result (fig.4.18).

The afferent activity measured on non-splitting heart branches on its anterior and posterior surfaces in the coronary sinus region had a constant low amplitude activity. CAO caused a gradual increase in the power of this activity, which strengthened in extrasystolic arrhythmia and reached a maximum value at the instant VF appeared.

The characteristics of the afferent activity changes in experiments that resulted in VF after CAO were the following:

1. Whereas in experiments without VF an increase in afferent activity was usually observed during extrasystolic arrhythmia, in experiments with VF this increase was also observed during a normal rhythm.
2. The power of afferent activity during extrasystole arrhythmia increase on average to 706% of the initial level in experiments with VF, while in experiments without VF this increase was only 273%.
3. The rate of the afferent activity power increase was considerably higher in experiments with VF than in the experiments without VF; a tight correlation was shown between the rate of afferent activity power increase and the instant VF appeared ($r=-0.61$, $p<0.01$).
4. The changes in the afferent activity in cardiac nerves after CAO are not connected with changes in arterial and venous pressure or with changes in coronary sinus pressure, but they do occur simultaneously with ischemic changes in metabolism of the heart: with decrease in P_{O_2}, redox potential, pH, pNa and an increase in K in the coronary sinus blood.

[1]We sincerely thank the prof.L.Szekeresh (Hungary) for amiably supplying us with metanephrin

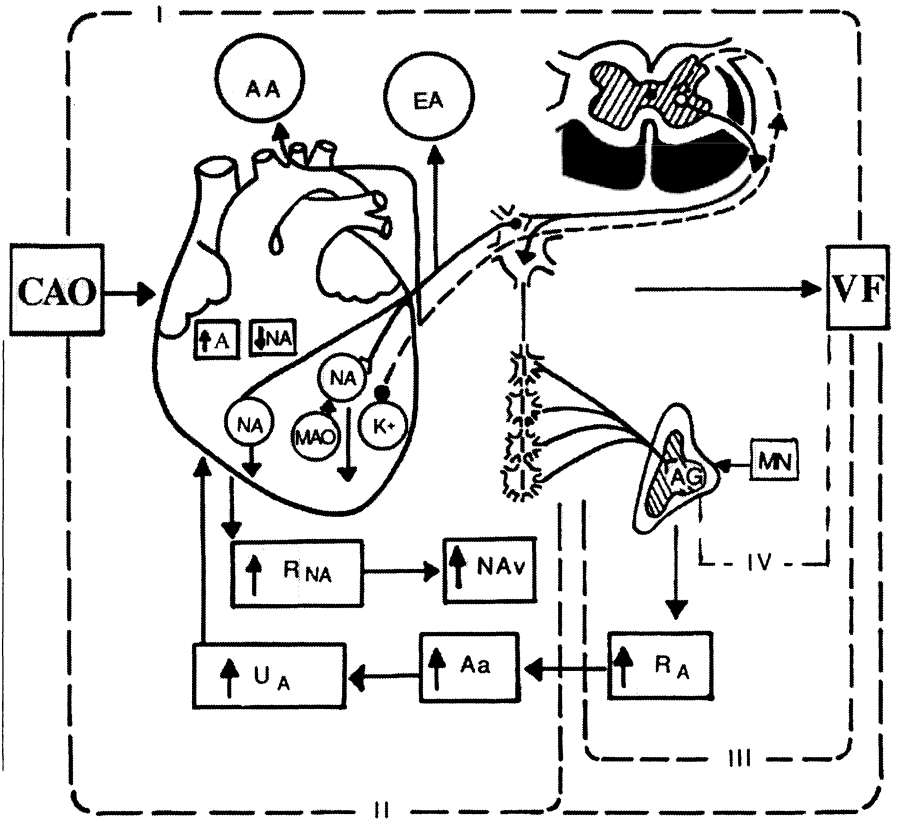

Figure 4.18. The schematic of disturbances of the sympathoadrenal heart regulation during CAO. AA - afferent activity, EA - efferent activity, R_A - release of A, R_{NA} - release of NA, U_A - uptake of A, NAv - NA concentration in coronary venous blood, Aa - A concentration in arterial blood, MN - methanephrin, MAO - monoamineoxidase. Roman numerals show directions of our study

5. The increase in the power of afferent activity in cardiac nerves results in disturbances of CA balance in the heart: in an increase in of A and a decrease in contents of NA both inside and outside the ischemic zone. A higher magnitude and rate of development of these processes are characteristic features of experiments with VF.

6. The increase in MAO activity observed in both the ischemic and non-ischemic zones can be one of the factors which stimulate the decrease in NA in the heart, particularly in the experiments with VF

7. Disturbances in the transport of CA between the heart and blood can be another, more powerful factor, which changes the NA and especially A concentration in the heart under ischemic conditions.
8. Continuous measurement of CA using the biological method shows that entry of CA into the coronary sinus blood reaches a maximum value at the instant of VF and always precede VF onset.

Separate quantitative measurements of adrenaline and noradrenaline contents in the arterial and venous blood (using the fluorometry and radioenzymatic methods) showed the following:

1. Before CAO the heart uptakes A from the arterial blood and releases NA into the venous blood.
2. After CAO the uptake of A and release of NA increase not only in the ischemic zone but in the heart as a whole.
3. The increase in A uptake by the heart after CAO is connected with the increase of A content in the arterial blood.
4. Release of NA from the ischemic zone is closely correlated with the outflow of K from it.
5. After CAO the uptake of A by the heart exceeds the release of NA.

The following characteristics distinguish the cases that resulted in VF after CAO from those that did not result in VF:

1. Greater increase of A concentration in arterial blood and greater rate of its increase.
2. Greater increase and greater rate of increase of uptake of A and release of NA by the heart.
3. Greater growth of the ratio of uptake of A to release of NA by the heart.

Metanephrin was used for blocking the extraneuronal uptake of A. Metanephrin decreases the A and NA concentration in the arterial blood, to lower the uptake of A and release of NA by the heart, to normalize the ratio of the uptake of A to the release of NA, and to decrease the VF frequency after CAO from 58% to 25%.

The insulin-test before CAO showed that individual characteristics of the reactivity of the medullar substances of the adrenal glands determine the value of hyperadrenalemia and A uptake that arises after CAO and the probability of the development VF. Insulin test before CAO can be used for prediction of VF after CAO.

The obtained data permit us to conclude that there is a dependence between the release of A by the adrenal glands, its uptake by the heart and appearance of VF after CAO. The possible mechanisms of the fibrillatory action of adrenaline after CAO will be discussed in the following chapter.

5 Role of Adrenaline in the Mechanisms of Ventricular Fibrillation Onset after Occlusion of Coronary Artery

In the previous chapter the assumption was made that adrenaline (A) is a casual factor in onset of VF after CAO. The role of adrenergic system in ventricular arrhythmias during myocardial infarction is discussed in monographs [36, 125] and review [277].

The numerous experimental and clinical results show that CA increase the tendency of ischemic myocardium to ectopic activity, but failed to illuminate the underlying mechanisms. We assume that ischemia and A both act in the same direction on those heart metabolic processes which are responsible of membrane potential changes. Thus, synergic effects of ischemia and A may cause such changes of the membrane potential which facilitate the onset of VF.

In order to verify this hypothesis, we have studied the influence of A on the ox-red, acid-base and ion equilibrium as well as on the heart membrane potentials (fig.5.1).

5.1 Effect of adrenaline on the ox-red equilibrium of the heart

Adrenaline affects on the changes of the ox-red equilibrium of the heart through the changes of the heart's blood supply, respiration and oxidative phosphorylation in mitochondrion, and glycogenolysis in cytoplasm.

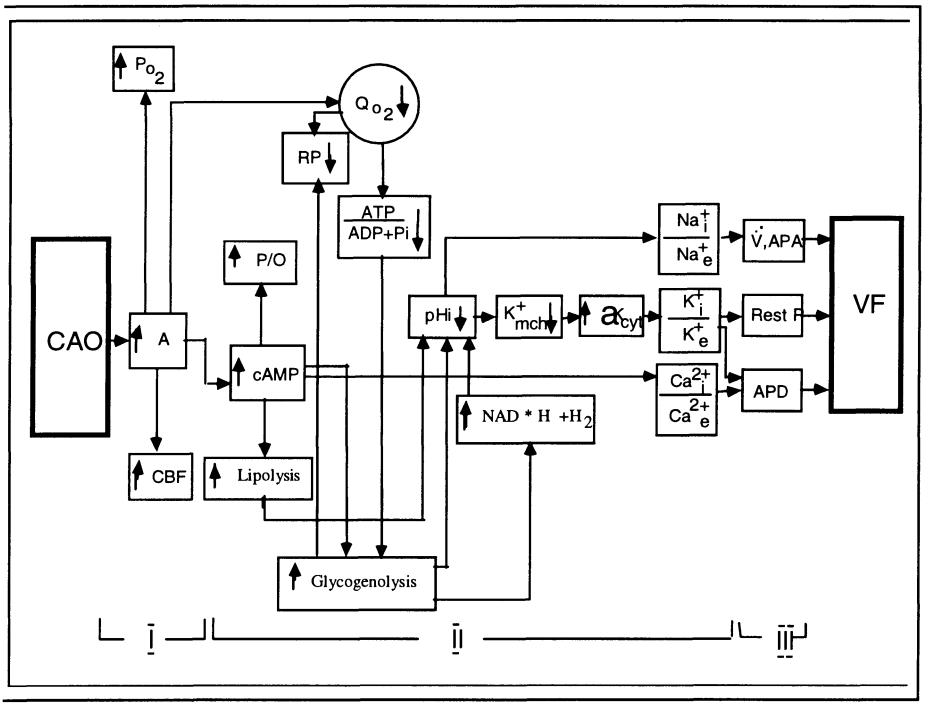

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Figure 5.1. The supposed sequence of adrenaline actions on heart's blood supply, metabolism and membrane potentials. Roman numerals show the reasonable directions of study. Abbreviations - see list of abbreviations

5.1.1 Effect of adrenaline on the heart's blood supply

According to our data [234], the adrenaline dose of 30 μg/kg leads to 86increase of the blood flow from the coronary sinus (fig.5.2).

In accordance to the increase of the coronary blood flow, the A causes an increase of the oxygen consumption by the heart. The oxygen consumption is calculated as the product of the coronary blood flow by the arterio-venous difference of oxygen content in the coronary blood (fig.5.2).

Such an apparently favorable effect of A on the oxygen supply of the heart allows to expect that it will produce a positive influence on the heart. In reality, administration of A provokes such pathologic phenomena as: coronary insufficiency, arrhythmia, VF, myocardial necrosis.

To explain this contradiction we studied the distinctions between the changes of myocardium oxygen content, the ox-red potential, and NAD.H under influence of adrenaline.

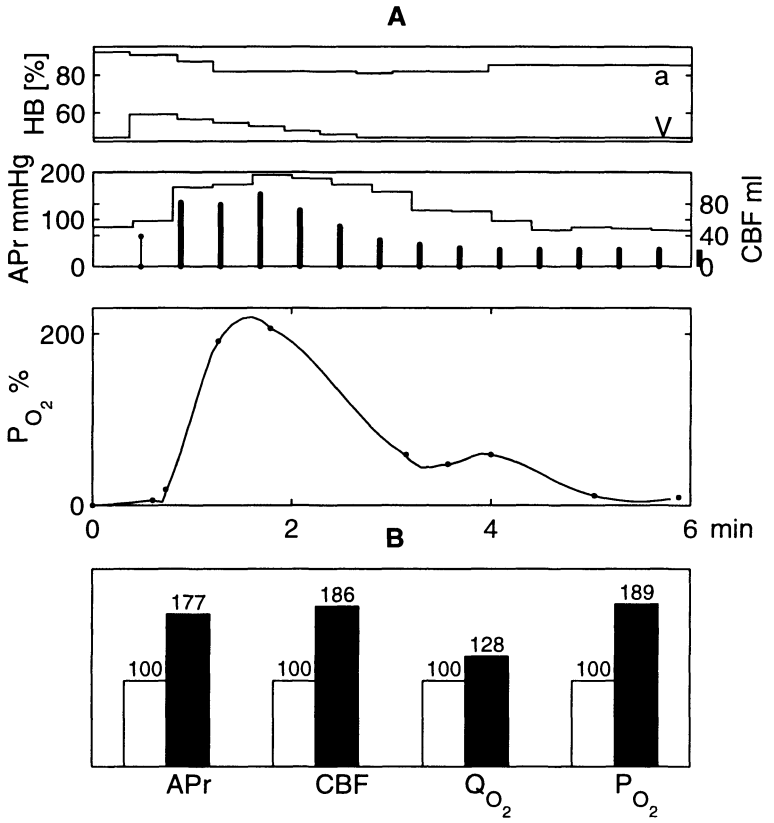

Figure 5.2. A. The dynamics of: oxygen saturation in arterial blood (a) and coronary sinus blood (V), arterial pressure (Apr), coronary blood flow (CBF) and myocardial P_{O_2} under intravenous administration of adrenaline (30 μg/kg). B. Average changes (n=17) of APr, CBF, oxygen consumption by heart (Q_{O_2}) and P_{O_2} at the moment of maximal action of adrenaline (black columns), in control (light columns

5.1.2 Effect of adrenaline on the oxygen content, oxred potential and NAD.H of the myocardium

As far as in 1963 we simultaneously recorded the oxygen saturation of the arterial and venous blood, the coronary blood flow and the oxygen partial pressure (P_{O_2}) in the myocardium (fig.5.2-A) under the influence of A. It was shown that P_{O_2} in the heart increases in a greater degree than the consumption of oxygen from the blood did (fig.5.2-B). Proceeding from these observations, we supposed that under the effect of adrenaline the oxygen which intensely diffuses from the blood, due to the increased coronary blood flow, is not utilizes by the heart [250, 228, 234]. To evaluate the utilization of the oxygen by the heart we recorded simultaneously with P_{O_2} the ORP [252] and NAD.H of the

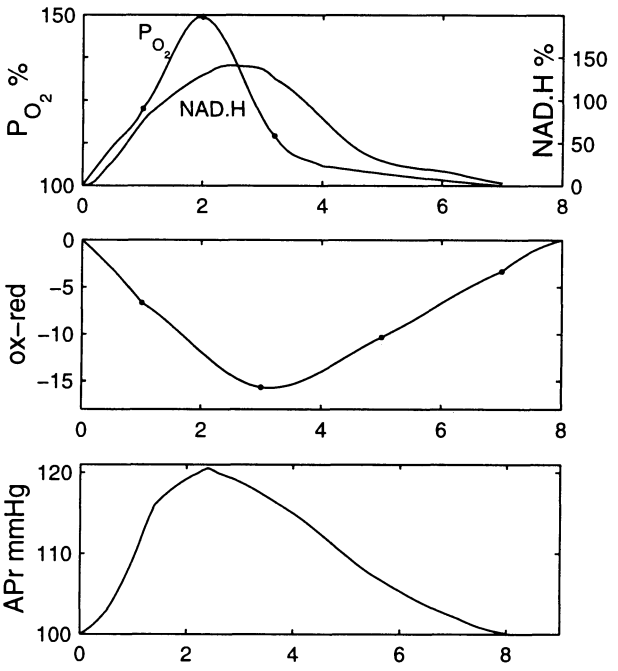

Figure 5.3. The synchronous changes of P_{O_2}, ox-red potential, NAD.H, and APr in cat heart under action of A in dose 20 μg/kg

myocardium [284]. It was shown that A in arrhythmogenic dose (20 μg/kg), despite of a sharp increase of P_{O_2}, provokes at the same time the decrease of the ORP and the accumulation of NAD.H in the heart (fig.5.3).

We supposed that A increases P_{O_2} as a result of the hemodynamics changes, i.e. increase of arterial pressure and coronary blood flow. The ox-red potential drop and the NAD.H increase are connected with A influence on the heart metabolism, and first of all on the respiration and phosphorylation in the mitochondrion.

5.1.3 Effect of adrenaline on the respiration and phosphorylation in mitochondria.

The effect of adrenaline on respiration and phosphorylation in the mitochondria we studied on 56 dogs. Among them 19 formed a control group. Ten dogs were administered intravenously with 2 mg/kg of adrenaline, while the resting 27 dogs were administered with 20 mg/kg of A [80]

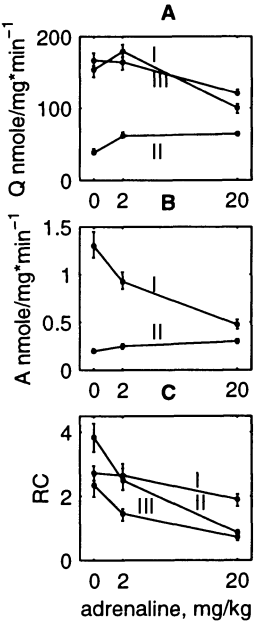

Figure 5.4. The effect of adrenaline administrated in vivo on respiration (A, I - Q_{III}, II - Q_{IV}, III - Q_{rest}; ferments activity (B, I - activity of succinic dehydrogenase, II - activity of cytochrome-C- oxidoreductase;and coupling oxidation and phosphorylation (C, I - Q_{III}/Q_{rest}, II - Q_{III}/Q_{IV}, III - Q_{DNF}/Q_{IV}. Average data (n_0=19, $n_{2\mu g/kg}$=10, $n_{20\mu g/kg}$=27). The incubation media: 300 Mm saccharose, 4 mM TRIS-succinate (pH-7.4), 2 mM TRIS-phosphate (pH-7.4), 0.1 mmole KCl. Protein - 2.5 mg/ml, temperature 37°C

Respiration and oxidative phosphorylation were studied using polarographic measurements of P_{O_2} in a nutrient medium for mitochondria. As compared with the control group the non-arrhythmogenic dose increases Q_{III} and Q_{IV} ($p<0.05$), while the arrhythmogenic dose increases Q_{IV} ($p<0.02$) and lowers the ratio Q_{rest}/Q_{III} ($p<0.02$) (fig.5.4-A).

Adrenaline in arrhythmogenic dose (20 mg/kg) decreases the velocity of phosphorylation oxidation and increases the velocity of non-phosphorylation oxidation. i.e. provokes an energy deficiency due to uncoupling effect. Probably, the inhibition of the succinatedehydrogenase activity (fig.5.4-B) participates in the slowing down the respiration velocity under effect of A.

All parameters of the oxidative phosphorylation: DK_{ChW} and Q_{DNF}/Q_{IV} ($p<0.05$) (fig.5.4-C) were lower in the group with non-arrhythmogenic doses of A ($p<0.05$) than in the control group. A in arrhythmogenic dose causes more strong uncoupling effect and disturbances of energy generation in the myocardium .

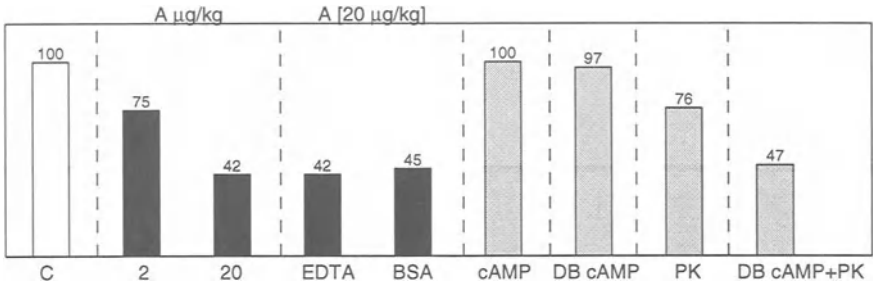

Figure 5.5. The role of Ca (n=11), fatty acid (n=11), and proteinkinase with cAMP (n=10) in adrenaline uncoupling effect. The study was done on dog's heart mitochondria. The values of respiratory control in % to control (C, light column); under in vivo administration adrenaline (black column) in dose 2 and 20 μg/kg, under addition to adrenaline treatment mitochondria EDTA and bull serum albumin (BSA), under addition to control mitochondria: cAMP, dibutyryl cAMP (DB cAMP), proteinkinase (PK) and dibutyryl cAMP with proteinkinase (DB cAMP + PK)

While A, introduced in vivo, causes a marked uncoupling effect, addition of adrenaline in vitro in doses 5.10^{-4} - 5.10^{-5}M practically does not affect the RC_{LV}, RC_{ChW} and Q_{DNF}/Q_{IV} in the heart mitochondria. No influence of adrenaline on these parameters was revealed in the oxidation of both succinic and glutamic acids.

Taking into consideration that the direct effect of adrenaline on the mitochondria is not associated with the uncoupling of the respiration and the phosphorylation, it is admissible to think that when adrenaline is infused in vivo its effect is realized via the cAMP. The latter is accumulated in the cells and causes the proteinkinase activation. The effect of adrenaline can also be manifested in the lipolysis activation and accumulation of fatty acids in the cytoplasm, as well as in the increase of Ca^{++} content in the cytoplasm and in the mitochondria.

The role of the fatty acids and Ca^{++} in the uncoupling effect of A was studied on the mitochondria from a canine heart which were medicated in vivo with 20 μg/kg of A (fig.5.5). For this purpose the mitochondria were separated in media added with albumin from ox blood serum (to link the fatty acids) or with EDTA (to link Ca^{++}) [83]. The ability of cAMP and proteinkinase reproduce the effect of A was studied on the mitochondria, taken from the control group of animals. For this purpose the nutrient medium was added with : 1) cAMP or dibutiryl cAMP with concentration 10^{-7} M; 2) proteinkinase 0.1 ml (activity-0.01 ml includes 1,3 nM of phosphate in histone during 1 min); 3) cAMP (dibutyryl cAMP) + proteinkinase with the same concentrations.

Addition of albumin from ox serum into the nutrient medium practically did not change the respiration and the coupling of respiration with phosphorylation. Addition of EDTA when mitochondria were separated, also did not affect the oxidation phosphorylation indexes (respiratory control). The addition of cAMP

and dibutiryl cAMP did not influence the coupling of respiration and phosphorylation. Therefore cAMP can not be responsible for the uncoupling of the respiration and phosphorylation. Addition of proteinkinase also did not affects this coupling. Only the simultaneous action of cAMP or dibutiryl cAMP and proteinkinase caused a sharp decrease (almost twice) of the phosphorylation and respiration coupling index. The uncoupling effect of proteinkinase when activated by cAMP is qualitatively similar to the effect revealed in intravenous infusion of adrenaline. It seems likely that the proteinkinase activated form participates directly in the uncoupling of the respiration and phosphorylation which is provoked in the mitochondria by A.

The sole possibility to compensate the disturbance in energy generation under effect of A is to intensify the glycogenolysis.

5.1.4 Influence of adrenaline on glycogenolysis

In order to verify the assumption that the glycogenolysis activation and the associated intracellular acidosis are very important factors of the CA arrhythmogenic effect, we compared the changes in glycogenolysis caused by arrhythmogenic (20 μg/kg) and non-arrhythmogenic (2 μg/kg) doses of A [85].

The comparison of the effect of the arrhythmogenic and non-arrhythmogenic A doses shows that they are qualitatively similar but differ quantitatively. The A in dose 20 μg/kg provokes more marked changes in the metabolite contents and in action mass ratio (AMR) in glycogenolytic reactions then in the dose 2 μg/kg.

Under activation of P-ase, PFK and TPI, after administration of A, the limiting links of the glycogenolysis became Ald and possibly GlA-3-PDG, as it is well seen in fig. 5.6.

The inadequate intensification of various glycogenolysis links under the effect of A disturbs the correspondence between the ATP synthesis in the glycogenolysis and the glycogen decomposition. This inadequacy more pronounced by effect of 20 μg/kg, than 2 μg/kg of A.

The increased accumulation of lactate, as a result of the A action in arrhythmogenic dose, can provoke great disturbances in the acid-base equilibrium.

5.2 Effect of adrenaline on the acid-base equilibrium.

The state of the acid-base equilibrium was evaluated in according to the pH changes of the myocardium and of the coronary sinus blood. These changes reflect the composition of the myocardial extracellular medium.

5.2.1 Effect of adrenaline on pH of coronary sinus blood

The recordings of pH in the blood in vivo are complicated by the electric interferences of the heart's biopotential and potential of pH electrode. In our laboratory was developed the special device eliminating these interferences [191]. Recordings of pH and P_{O_2} in the coronary sinus blood were done using this

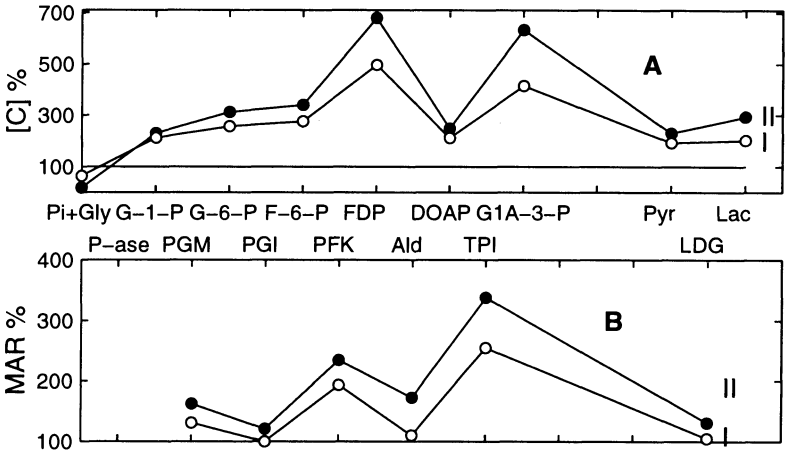

Figure 5.6. The consecutive changes of glycogenolysis metabolite content (A) and mass action ratio (MAR) of glycogenolysis ferments (B) in dog's heart under A administration in dose 2 μg/kg (I) and 20 μg/kg (II)

device [246]. The average data obtained for the flexion points are presented in fig.5.7.

As seen from fig.5.7, a transitory pH decrease is followed by some increase. At a moment of a sharp arterial pressure increase a posterior pH decrease was observed both in arterial and in the coronary sinus blood. After the arterial pressure nears the initial level, the arterial blood pH is stabilized, but continue to decrease in the coronary sinus blood.

The lack of correspondence between the changes of pH and P_{O_2} in the coronary sinus blood shows that the pH decrease is connected not only with the oxygen deficiency, but also with changes in the myocardial metabolism and first of all with the glycogenolysis and lipolysis activation. As a result, the decrease of pH was observed during the phase of maximal effect of A. This occurs despite the increase of the oxygen concentration in the heart.

5.2.2 Effect of adrenaline on myocardial pH

The typical changes of pH, together with pNa, pK, pCl and control potential (CP) in the dog's left ventricle are presented in fig 5.8. The data were obtained after intravenous continuous infusion of A in dose 10 μg/kg during 3 min.

As seen from fig.5.8, in the phase of maximal A effect pH increases and then decreases.

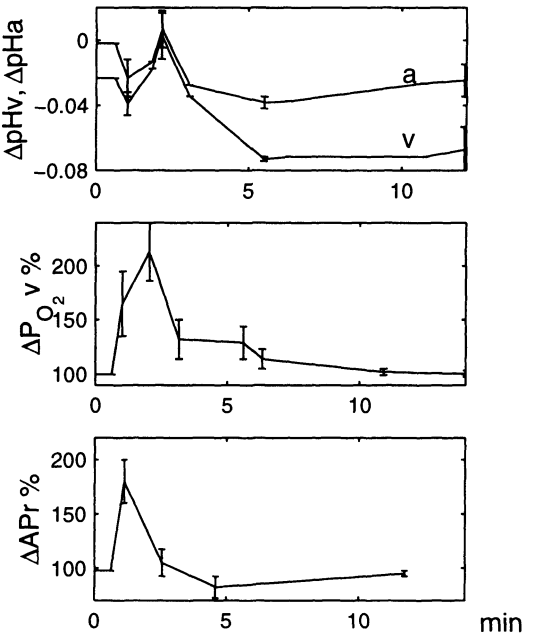

Figure 5.7. The average changes of pH(a), pH(v), $P_{O_{2(v)}}$, and APr under influence of A (10μg/kg). The curves are constructed according to point of flexion

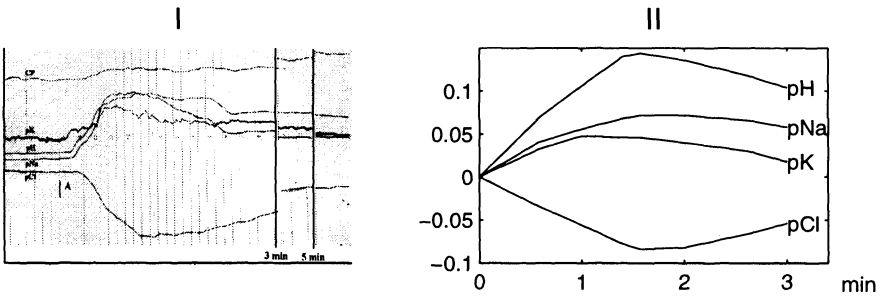

Figure 5.8. I - The dynamics of pH, pK, pNa, pCl and CP changes in the dog's myocardium after intravenous administration of A (10μg/kg) obtained in one of experiments. II - The average data (n=11) of heart's pH, pNa,pK and pCl changes during the interval of maximal effect of the A

5.3 Effect of adrenaline on ionic equilibrium

The review of the effect of CA on the potassium concentration in blood is presented in [141]. We studied the effect of A on ionic equilibrium in the heart on organ, cellular and subcellular levels.

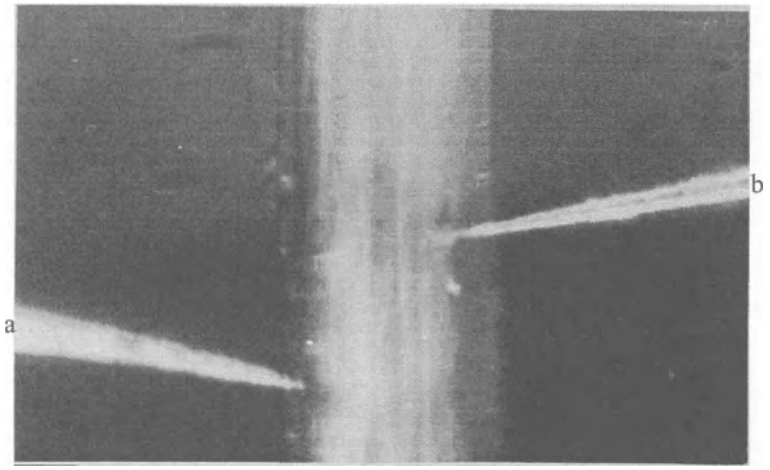

Figure 5.9. The isolated rat's muscle fiber with K-selective microelectrode (a) and electrode for membrane potential measuring (b)

5.3.1 Effect of adrenaline on pK, pNa and pCl in the myocardium

The changes of ion equilibrium in response to A were evaluated using the continuous simultaneous registration of the myocardial pK, pNa and pCl [232]. The figure 5.8-I shows an increase of pNa, pK and a decrease of pCl immediately after the A infusion in one of the experiments. The averaged data are presented in fig. 5.8-II. These data were calculated from the results of 11 experiments performed according to the same scheme. During the phase of maximal effect of A, the changes of pNa, pK and pCl were in accordance with the changes of myocardial pH and have similar character in all experiments (fig.5.8). In the first phase of the A effect pH, pNa, pK increase while pCl decreases. In the post-action phase, the decrease of pK and prolong increase of pNa were observed in one group of animals with pH shifted to the basic direction. In the second group the increase of pK and decrease of pNa were observed with pH shifted to the acid direction.

5.3.2 Effect of adrenaline on K and Na content in the cytoplasma cells

In order to find the effect of adrenaline on K content, we measured the K activity in cytoplasm (a_K^+) and K concentration in the cell. Measurements of (a_K^+) were performed using K-selective microelectrode. The total K concentration in the cell ($C_K t$) was determined by means of flame photometer. The membrane potential (RestP) was measured by glass microelectrode. The study was performed on a frog biceps muscle. The K-selective microelectrode and glass microelectrode were introduced into the muscle fiber as near as possible (fig.5.9).

The measurements of a_K^+ and RestP in each of the muscle fibers were repeated many times before and during 30 min after the fiber was incubated in a Ringer's solution added with 1.1×10^{-5} M of A [4]. The $[C_K]t$ in the cell was determined after 10 and 30 min of incubation of the fiber in a Ringer solution with the same A concentration [6].

The averaged data of the total, intra- and extracellular K concentration and total, intra- and extracellular water content in the control experiments and in series with 10 and 30 min incubation in the Ringer's solution with A are presented in fig.5.10 .

As seen from fig.5.10, after addition of A, the ($[C_K]_t$ in the muscle practically does not change. Neither does the K concentration in the solution ($[C_K]_e$). The total water content in the muscle ($[H_2O]_t$) remains unaffected by A addition, but redistributed between the intra- and extracellular medium: $[H_2O]_i$ decreases ($p<0.001$), while $[H_2O]_e$ increases ($p<0.001$).

The K concentration in the cell, calculated in meq/kg intracellular water ($[C_K]_i$) statistically significant increases from 136.5 to 152 meq/kg after 10 min and to 160.7 meq/kg $[H_2O]_i$ after 30 min ($p<0.01$ and >0.001 respectively). This corresponds to the decrease of water content in the cell after A addition. The a_K^+ in the cytoplasm of an isolated rat's muscle increases under effect of A on the average from 154 to 301 meg/l at the 16th min ($p<0.05$) and decrease to 133 meq/l at the 27th min after addition of A. Thus, a_K^+ in normal conditions, being almost equal to concentration K in the cell, increases on the average to 196% of the initial level after 16 min of adrenaline addition, while K concentration increases only to 112% after 10 min and to 117.5% after 30 min.

So, increase of a_K^+ in the cell's cytoplasm, observed in the first phase of adrenaline action, can not be explained via the increase of the $[C_K]t$ in the cell. It may be explained by the redistribution of K between the cytoplasm and the intracellular organella. In the second phase when the concentration of K remains almost unchanged, but its activity lowers to 86.7% of the initial level, opposite redistribution probably occurs.

The RestP increases on the average at the 9th min after adrenaline addition from -80.3 mV to -99.2 mV ($p<0.001$) and then decreases to -70.6 mV ($p<0.01$) at the 31 min after addition of adrenaline. The RestP values measured before and at various moments after addition of A were compared with the values of potassium equilibrium potential, calculated according Goldman's equation using ratios $[C_K]_i/[(C_K]_e)$ and $[a_K]_i/(a_K]_e$.

It was shown that K equilibrium potential, calculated from a_K was very close to the experimental values (-83.5 and -80.3 mV respectively). At the same time, K equilibrium potential, calculated from C_K did not absolutely coincide with experimental RestP value.

Thus, the changes in the total intracellular K concentration fail to explain the RestP increase and following decrease, observed after A addition. The changes can be explained by the increase of a_K^+ in the medium directly adjacent to the cell's membrane i.e. in the cytoplasm [5, 237].

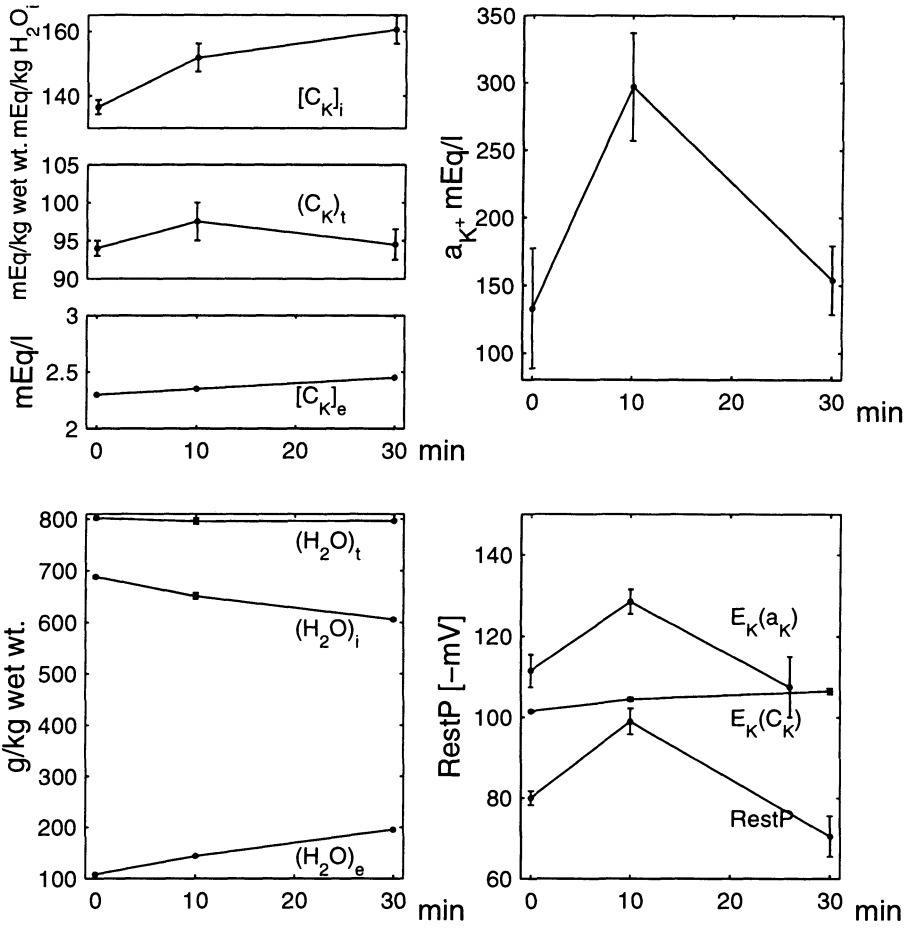

Figure 5.10. The effect of adrenaline (1.1×10^{-5}mole) on $[C_K]$i, $[C_K]$t, $[C_K]$e (averaged data, n_0=31, n_{5-10}=13, n_{20-30}=17), a_{K+} (n_0=7, n_{5-10}=7, n_{20-30}=5),RestP (n_0=12. n_{5-10}=7, n_{20-30}=10), and equilibrium K potential, calculated by Nernst formula using a_K^+ and $[C_K^+]$ of rat's isolated muscle fiber

The increase of a_K^+ in the cytoplasm is occurred probably due to K exit from mitochondrion into the cytoplasm as a result of the respiration depression and the uncoupling of oxidation and phosphorylation.

5.3.3 Effect of adrenaline on potassium content in the mitochondria.

As it is known, the mitochondria can accumulate and release the considerable amount of K. It was mentioned above,that this depends on the level of mito-

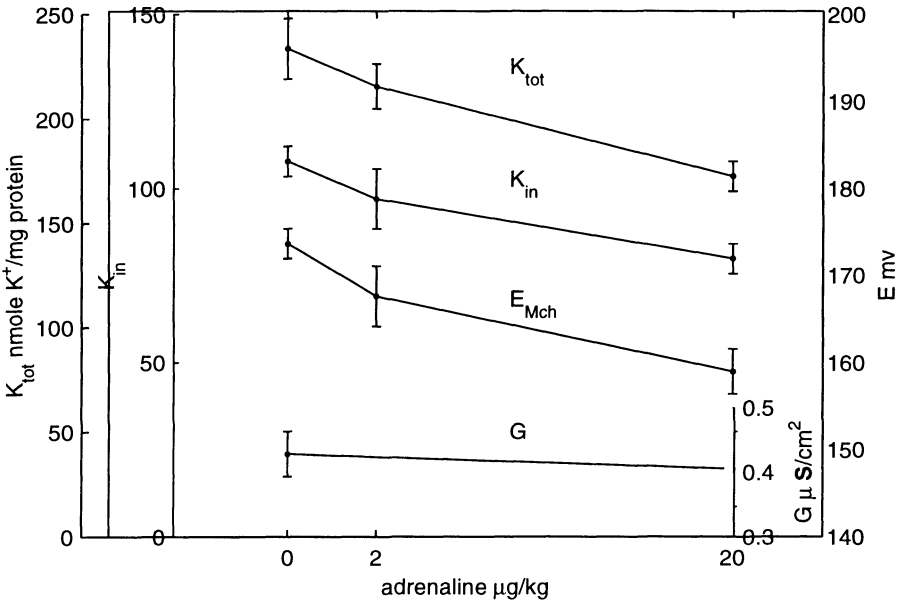

Figure 5.11. The effect of adrenaline (administrated in vivo) on the K total (K_{tot}), K inner (K_{in}), mitochondrial membrane potential (E mch) and K-conductivity (G) of mitochondria dog's heart. Incubation media for measuring K_{tot} and K_{in}:300mmole saccharose, 4 mmole TRIS-succinate (pH 7.4), 2 mmole TRIS-phosphate (pH 7.4), 0.1 mmole KCl, temperature 37^{o}C, protein content 2-3 mg/ml. The measuring of mitochondrial potential E was performed in anaerobic condition. For this purpose, the 10 $^{-8}$mole of valinomycin was added into the incubation media (average data, n_0=19, $n_{2\mu g/kg}$=9 $n_{20\mu g/kg}$=23

chondria energization, which changes due to adrenaline effect. Therefore it is possible to suppose that mitochondria is responsible for the increase of a_K^+ in the cytoplasm due to A action.

The effect of A on the K content in mitochondria was investigated in 19 dogs [80]. The intravenously infusion of A in doses 2 and 20 mg/kg was performed 10 dogs 10-20 min before the heart excision. The control group was composed of 9 dogs. The K content in the mitochondria was determined by the amount of K exit from the mitochondria into the nutrient medium after their destruction by means of the non-ionic detergent triton X-100. K content in the media is determined by K-selective electrode (fig.5.11).

A in non-arrhythmogenic dose lowers the K content in the mitochondria from 110±4 to 95±7 nmol/mg of protein ($p>0.05$); arrhythmogenic doses lower the K content from 110±4 to 75±5 nmol/mg of protein (statistically significant, $p<0.05$). The K exit from the mitochondria into the cytoplasm can occur as a result of decrease of the mitochondrial membrane potential or as a result of the increase of it membrane passive permeability.

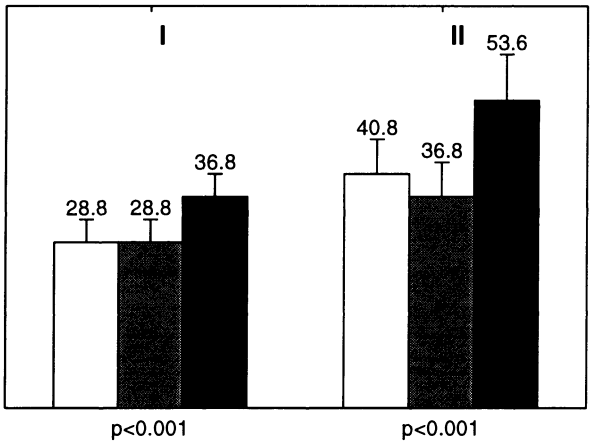

Figure 5.12. To the study of K exit from mitochondria due to adrenaline. The changes of a_K^+ in the incubation media when mitochondria is in the media (I) and after addition to the media the valinomycin and DNF (II): in control (n=13, light columns). after addition cAMP (n=7, grey columns) and after addition proteinkinase activated by cAMP (n=8, black columns). Incubation media: 0.25 mmole saccharose, 40mmole TRIS, 10 mmole $MgCl_2$, 5 mmole succinate, 2 mmole EDTA, 2 mmole theophylline, 1 mmole ATP, pH 7.4, temperature 37_oC

As it is shown in fig.5.11, A in a dose of 2 mg/kg provokes a tendency of the mitochondrial membrane potential to decrease, while in a dose of 20 mg/kg the decrease becomes substantial. The changes in E_{mch} and K_{in} have a similar character and that indicate some interconnection between them ($r_{K_{in}}$,K_{mch} = 0.83, $p<0.05$). The changes were not observed in the conductivity of the mitochondrial membrane for the K ions.

So, decrease of K content in the mitochondria is connected with decrease of the mitochondrial membrane potential, which reflects deenergization of mitochondrion due to the uncoupling of respiration and phosphorylation.

The uncoupling of the respiration and the phosphorylation in the mitochondrion is realized via the proteinkinase activated by the accumulation of cAMP (fig.5.5). The effect of the proteinkinase, activated by 10^{-6} cAMP and cAMP in concentration 10^{-6} M, on the K ions activity in the medium is presented in fig.5.12. These data were obtained after placing the mitochondria into medium and addition the valinomycin and DNP.

As follows from the figure, cAMP without proteinkinase does not affect on the K content in the mitochondria. The cAMP activated proteinkinase causes a statistically significant increase of K output from the mitochondria as a result of the uncoupling of respiration and phosphorylation.

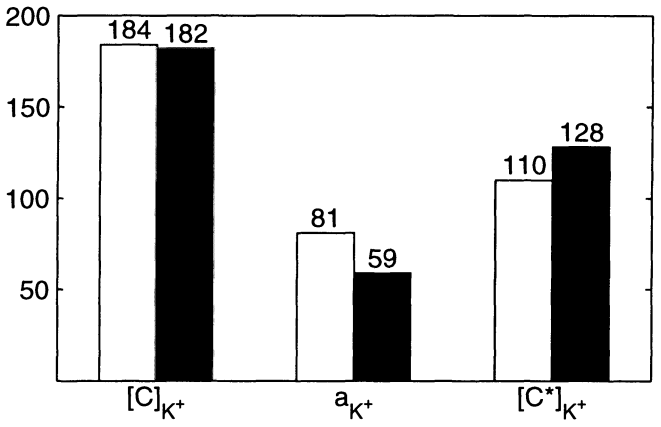

Figure 5.13. The changes of K concentration (mmole/mg protein) in fresh isolated rat heart's nuclei $[C]_K$, of K increment in the incubation media after 40 min incubation (a_K) and of K concentration in the nuclei after 40 min incubation ($C^*_K = [C]_K - a_K$) during intravenous introduction of adrenaline (20μg/kg). Control - light columns, n=11; adrenaline - black columns, n=9

5.3.4 *Effect of adrenaline on the K content in the nuclei.*

Determination of K content in the nuclei of the heart's cells is presented in fig.5.13.

The K concentration was determined using a flame spectrophotometer.

The concentration of K was 184±19 mM/mg of proteins or 37±4 mM/kg of wet weight. The (a_K^+) was measured using valinomycin K-selective electrode and was evaluated through the increase of a_K^+ in the nutrient medium during 40 min (after 40 min a_K^+ practically did not change) in respect to its activity measured before the introduction of nuclei. The a_K^+ in the nucleic fraction was 81±16 mM/mg of proteins and the residual K content in the nuclei was 110±20 mM/mg of proteins.

Treatment of rats with 20 μg/kg of A did not change the K concentration and unreliable lowered a_K^+. So, A administered in vivo does not influence the K content in the nuclei of heart cells. Consequently, the nuclei's K does not participate in the increase of K in cytoplasm.

5.3.5 *Possible mechanisms of adrenaline effect on the ionic exchange in the myocardium.*

The lack of A effect on K concentration in the cell shows that A does not change the K transport across the cell membrane. The increase of a_K^+ in cytoplasm in this case may be a result of it translocation from the intracellular organoid into the cytoplasm, i.e. decompartmentation.

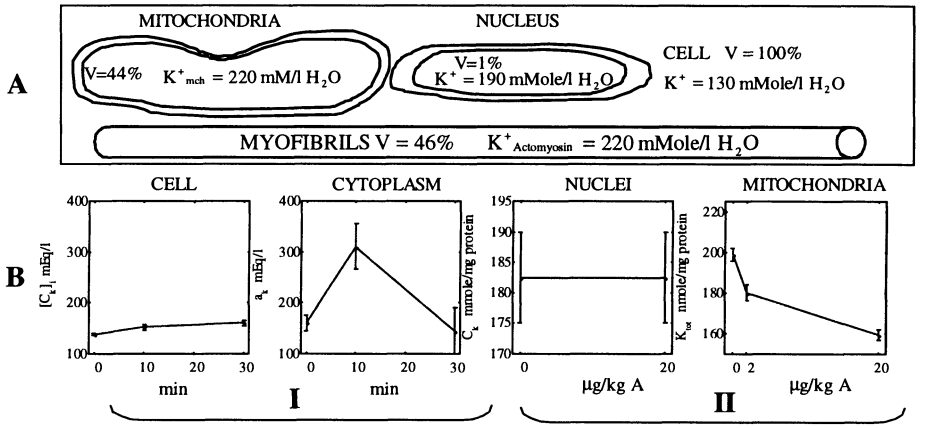

Figure 5.14. The K compartmentation in the cell. A - schematic picture of muscle fiber. V - volume of mitochondria, nuclei, myofibril. K^+ - K content in appropriate compartments. B - adrenaline effect on K content in the cell, cytoplasm,nuclei and mitochondria. Abscissa in I - time after A introduction, abscissa in II - dose of adrenaline

It was known that different enzymes and enzyme systems are located in particular organelle. For example, the glycolytic enzyme system is located in the cytosol, whereas enzymes of the tricarboxylic acid cycle are located in the mitochondria. So, intracellular compartmentation of enzymes, metabolites and K, Na, Cl, and H ions bounded with them may facilitate the nonuniform distribution of ions in the cells. In normal state, when the $[C_K]t$ in the cell is 130 mM/l water (fig.5,14-A), its concentration is almost twice as high in the mitochondria (220 mM/l water) and 1,5 times greater in the nuclei (190 mM/l water).

This means that there is a real possibility of considerable increase of K content in the cytoplasm at the expense of decompartmentation from mitochondria, nuclei and may be other organella, without K influx from extracellular medium [79].

To determine the possible contribution of K decompartmentation to the a_K changes in the cytoplasm, it is necessary to take into consideration its content in the main cell organelles (see table 5.1).

As a result of the decompartmentation, the K content in the cytoplasm can increase on 71 mM/l of cell water. That leads, after recalculation of membrane potential, to the 12mV of hyperpolarization.

In response to A (fig 5.14-B), intracellular K concentration $[C_K]_i$ increases from 136.5 to 152 at the 10th min and to 160.7 mM/l intracellular water at the 30th min. This comparatively small increase (occurring in parallel with a decrease of the intracellular water) is undoubtedly not connected with K influx into the cell. Meanwhile a_K in the cytoplasm is doubled from 154 to 301

Table 5.1. Concentration K in cell's compartments

Organoid	K^+ content	Concentration of decompartmentation K^+ on 1 liter of intracellular water
Mitochondria	220 nMol/mg protein	25 mM
Nuclei	184 nMol/mg protein	6 mM
Actomyosin	200 nMol/mg protein	40 mM
Hyaloplasm	130 nMol/mg protein	0
Total		71 mM

mM/l on 16th min after A addition. We have calculated the contribution of the mitochondrial K into the changes of K content in the cytoplasm [79].

The K content in the heart cell mitochondria decreases under effect of adrenaline from 220 mM/l (that correspond to 220 nMol/mg protein) to 160 mM/l of mitochondrial water. The volume of the mitochondria is 34% of the cell volume, while the mannitol-impermeable compartment of the mitochondrion is 44% of the mitochondrial volume [134]. Taking into account these data, the output of 60 mM K/l of the mitochondrial water corresponds to an increase of a_K in the cytoplasm by 18 mM/l cytoplasmic water and to an increase of the membrane potential by 4 mV. The latter value can be somewhat greater, because A provokes a decrease of the intracellular water [5, 6].

The release of K from the mitochondria probably plays a minor role in the hyperpolarization of the cell in response to A. a_K^+ in the cytoplasm increase also at the expense of its release from other cell compartments. Probably the myofibril and the sarcoplasmic reticulum also participate in the a_K increase during the A action.

The changes of a_K in cytoplasm shifts the K intra- extracellular gradient and stimulate transmembrane K transfer under impact of A. As a result, the cell membrane potential and electrophysiological properties heart's cells are changed.

5.4 Influence of adrenaline on the heart's electrophysiological processes

The A significantly reduces action potential duration and refractoriness by shortening the cycle lengths, typical for VT/VF [314]. It was shown that catecholamines are able to induced early and delayed afterdepolarization [226].

The second messenger of CA cAMP, which increases in case of acute myocardial ischemia [306, 348], causes an increase of intracellular calcium. That produces three adverse electrophysiology effects: it invokes delayed afterdepolarization, so that triggered automaticity can develop; can invoke calcium-dependent slow responses, so the possibilities for reentry are favored; excess intracellular calcium can cause intercellular uncoupling which promotes the de-

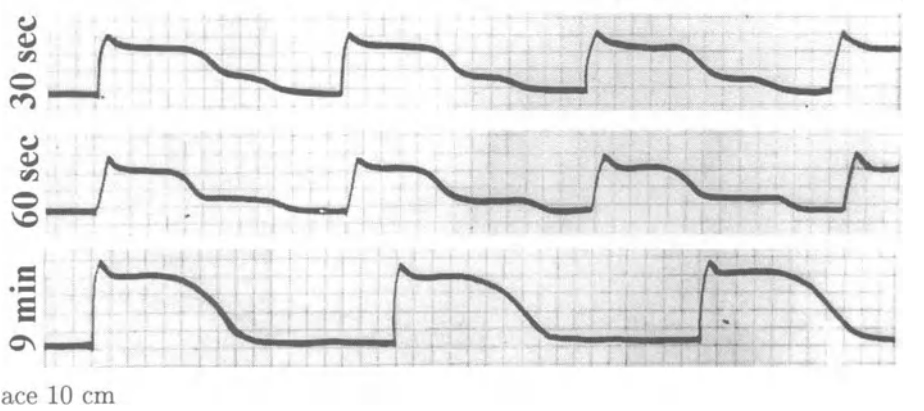

Figure 5.15. The changes of phase plato and terminal repolarization of AP after A infusion (10 μg/kg). Left - time after adrenaline introduction

velopment of VF [176]. However, the intervention that increases cAMP did not stimulate VF(see [18]). Authors of this paper concluded that changes in cAMP may not be responsible for VF in their model of sudden cardiac death. Moreover, it was shown that increase of intracellular cAMP level are involved in the intercellular synchronization by increasing intercellular coupling [196]. The low susceptibility to VF is connected [210] also with the increase of cyclic GMP. The role of CA in intercellular coupling and myocardial cell synchronization was studied in [184]. The CA alter the ionic channel responsible for the slow conduction. The effect depends on the local concentrations of CA [127]. The data about influence of A on the membrane potential and ions equilibrium are contradictory in current literature.

The changes of monophasic action potential under influence of A was studied in our laboratory (fig.5,15). The data were recorded continuously using suction electrode.

The scheme and averaged data of the monophasic AP changes after A administration are shown in fig. 5.16.

The APD firstly prolongs and then shortens. The initial increase of APD occurs at the expense of the phase plateau, In some cases a "second plateau" was formed (fig. 5.15). The subsequent shortening of AP occurs at the expense of the final repolarization phase. In some of experiments, however, the repolarization phase considerably lengthens and occupied almost the whole diastole. The amplitude and the depolarization rate of AP increase during the initial 30 s of A action and then decrease.

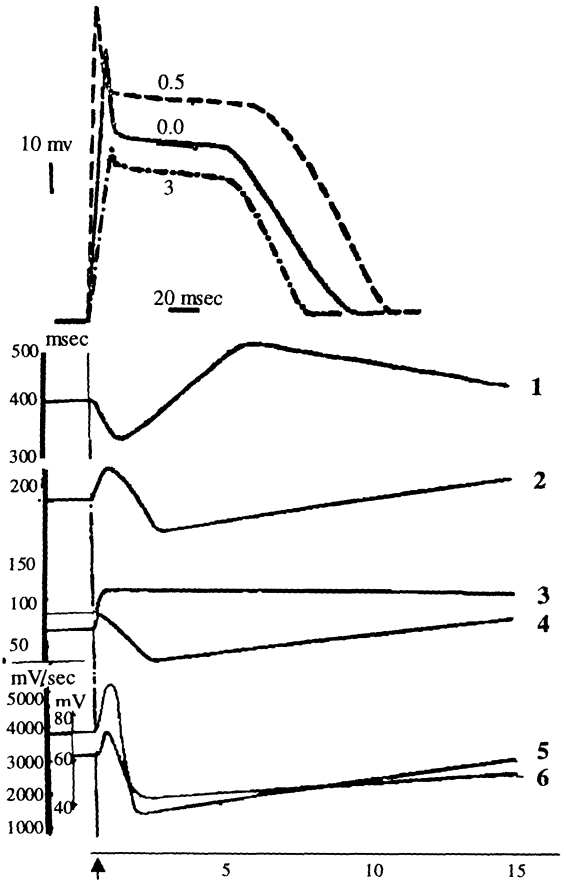

Figure 5.16. The changes of AP in left ventricle of the dog's heart during intravenous infusion of adrenaline (10 μg/kg, average data). Numbers on AP denote time after adrenaline introduction. The numbers on right: 1 - cardiac cycle duration, 2 - APD, 3 - phase plato duration, 4 - phase terminal repolarization duration, 5 - depolarization rate, 6 - depolarization amplitude. Abscissa - is the time after adrenaline infusion. Moment of infusion is shown by the arrow

5.5 The comparison of the effects of arrhythmic and non- arrhythmic doses of adrenaline on a metabolism

The data showing the effect of arrhythmogenic (20 μg/kg) and non-arrhythmogenic (2 μg/kg) adrenaline doses on the heart metabolism are summarized in fig. 5.17 [234, 251].

The adrenaline concentration in blood more than doubles after infusion of 2 μg/kg of adrenaline and almost 5 times after infusion of 20 μg/kg. The increase is comparable with those we revealed in the arterial blood after CAO.

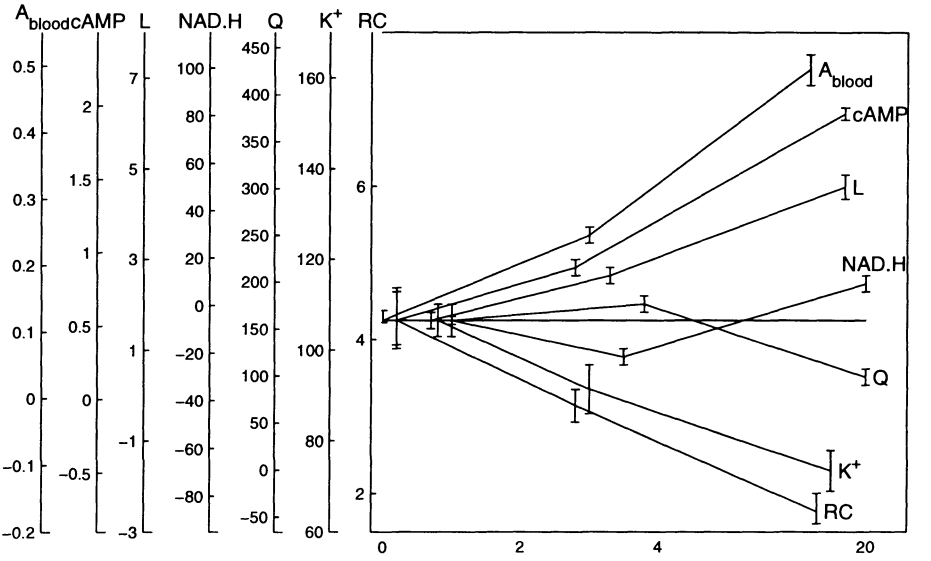

Figure 5.17. The adrenaline action in arrhythmic (20 μg/kg) and nonarrhythmical (2μg/kg) doses (average data) on the cAMP concentration (nmole/g tissue, n_0=5, $n_{2\mu g/kg}$=6, $n_{20\mu g/kg}$=8), on the lactate (L,mmole /g tissue, n_0=8, n_2=9, n_{20}=8), on the tissue respiration rate (Q, nMole/mg protein /min) and respiration control in mitochondria dog's heart (RC, nMole/mg protein, n_0=19, n_2=10,n_{20}=23), on the NAD.H (maximal increase in % to initial level, n_2=8,n_{20}=10). A_{blood} - adrenaline concentration in the blood

Directly opposite changes in the myocardial oxidation (Q) and concentration of NAD.H were shown by non- arrhythmic (increase of Q and decrease of NAD.H) and arrhythmic doses of A (decrease of Q and increase of NAD.H).

The A in non-arrhythmogenic dose lowers the NAD.H probably via respiration activation. The adrenaline in arrhythmogenic dose increases the NAD.H concentration, in myocardium via respiration depression which slow down the oxidation of NAD.H in the mitochondria.

All other parameters presented in fig.5.17 are changing similarly under effect of arrhythmic and non-arrhythmic A doses. However, the changes are quantitatively different. The respiration control and K content in the mitochondria lowered, while the cAMP and lactate concentration increased more considerably in response to arrhythmic dose of A. The sequence of changes in the myocardial metabolism under effect of A can be summarized as follows. The primary effect is probably the increase of cAMP in the myocardium. The proteinkinase activated by cAMP provokes uncoupling of respiration and phosphorylation in the mitochondria. The uncoupling effect of A is the basic one and determines all other effects. In the case of a moderate uncoupling, caused by a non-arrhythmic dose of adrenaline, the partial deenergization of the mito-

chondria arises. Under these conditions K exits from the mitochondria to the cytoplasm. K content in the mitochondria slightly decreases (from 110 to 95 nmol/mg of protein), while it increases correspondingly in the cytoplasm [4]. This causes small hyperpolarization of the cell membrane [5, 6]. Arrhythmia does not develop under these conditions.

The arrhythmic doses of adrenaline provokes considerably more marked accumulation of cAMP in the myocardium, more pronounced depression of respiration and uncoupling of respiration and phosphorylation in the mitochondria and activation of glycogenolysis in cytoplasm. As a result of the marked deenergization of the mitochondria, K exits from the mitochondrion in the cytoplasm in a considerable amount. Decompartmentation of K probably occurs also from other intracellular organelles. The hyperpolarization, which arises in response to the increase of a_K^+ in cytoplasm, then is replaced by depolarization due to K exit from the cell according to the increase of the intra-extracellular K gradient. The depolarization of a cell membrane is a necessary condition for the onset of arrhythmia [5, 6].

Under CAO when the A concentration in the blood increases in 3-4 times and become comparable with the concentration after administration of A in dose 20 μg/kg, probably the A effect is summarized with the effect of ischemia provoked by CAO.

5.6 Comparison of the effects of coronary artery occlusion and adrenaline on metabolism and electrophysiological processes in the heart.

5.6.1 The effect of adrenaline and CAO on oxidation-reduction equilibrium

To evaluate effect of A and CAO on oxidation-reduction equilibrium, we compare (see fig.5.18) the data of the changes in ischemic zone after CAO (presented in chapters II and III) and that after A infusion in an arrhythmic dose presented above.

The effect of A and CAO on the heart's blood supply and oxygen content is quite opposite. A provokes a sharp increase of the coronary blood flow and P_{O_2} in the heart, while CAO provokes the decrease of the coronary blood flow and the P_{O_2} in the ischemic zone.

In spite of increase of heart blood supply and P_{O_2}, A decreases ox-red equilibrium in the heart, i.e. acts on metabolism of the heart not opposite, but in the same direction as the ischemia. The ORP lowers, while the NAD.H content increases both under CAO and A infusion. The CAO and A provoked the changes in respiration and phosphorylation in the mitochondrion in the same direction (fig.5.18). Both the A and ischemia depress the mitochondrial phosphorylation oxidation (Q_{III}) probably via depression of the ox-red enzymes. The depression of mitochondrial succinic dehydrogenase (SDG) was observed both under CAO and A infusion.

Both ischemia and A provoke uncoupling of the respiration and the phosphorylation. This is testified by lowering of the respiratory control (RC) of the

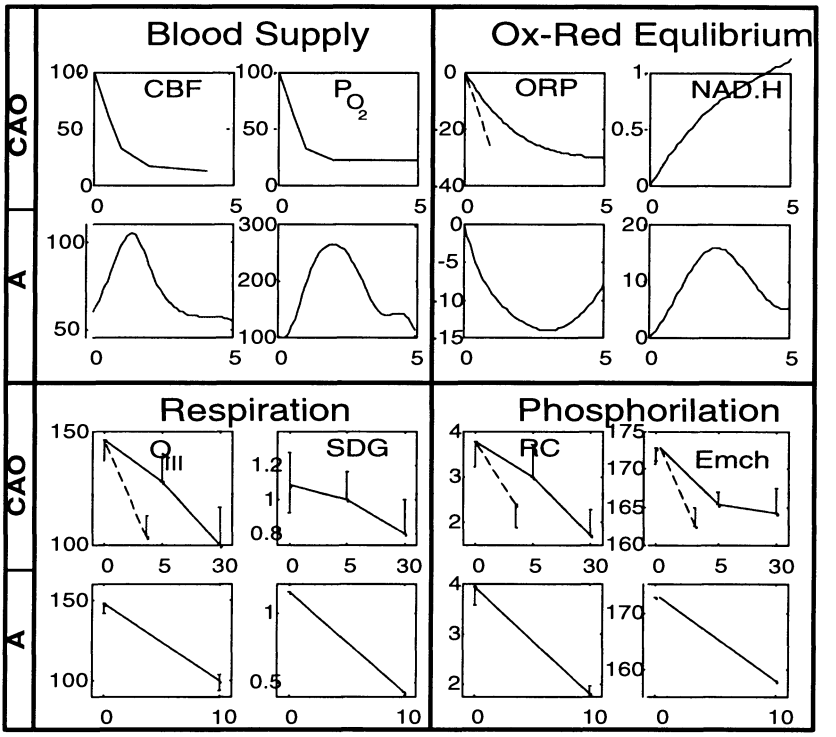

Figure 5.18. The comparison of the effects of CAO and adrenaline (A) on the oxidation-reduction equilibrium: coronary blood flow (CBF, during CAO % retrograde to anterograde blood flow , during adrenaline action ml/min), P_{O_2} (% to initial level) ORP (mv), NAD.H (fluorescence intensiveness), mitochondrial respiration in III state (Q_{III}, nmole/mg protein/min $^{-1}$), activity of succinic dehydrogenase (SDG, mkM/mg protein/min^{-1}), mitochondrial respiratory control (RS) and membrane potential of mitochondria, E_{mch},mV).On abscissa everywhere - time (min) after CAO, or A introduction. CAO experiments with VF are shown with broken line, without VF - with solid line

mitochondria. The mitochondrial membrane potential lowers in both cases due to mitochondrial deenergization.

5.6.2 The effect of adrenaline and CAO on the glycogenolysis

As a result of respiration and phosphorylation depression, the glycogenolysis is activated (fig.5.19) both in case of CAO and under effect of A.

This is manifested by the decrease of glycogen and by the increase of all intermediate metabolites of the glycogenolysis and including the final one i.e. of lactate. The aldolase reaction becomes the limiting link of the glycogenolysis instead of the phosphofructokinase reaction which is the limiting link of the

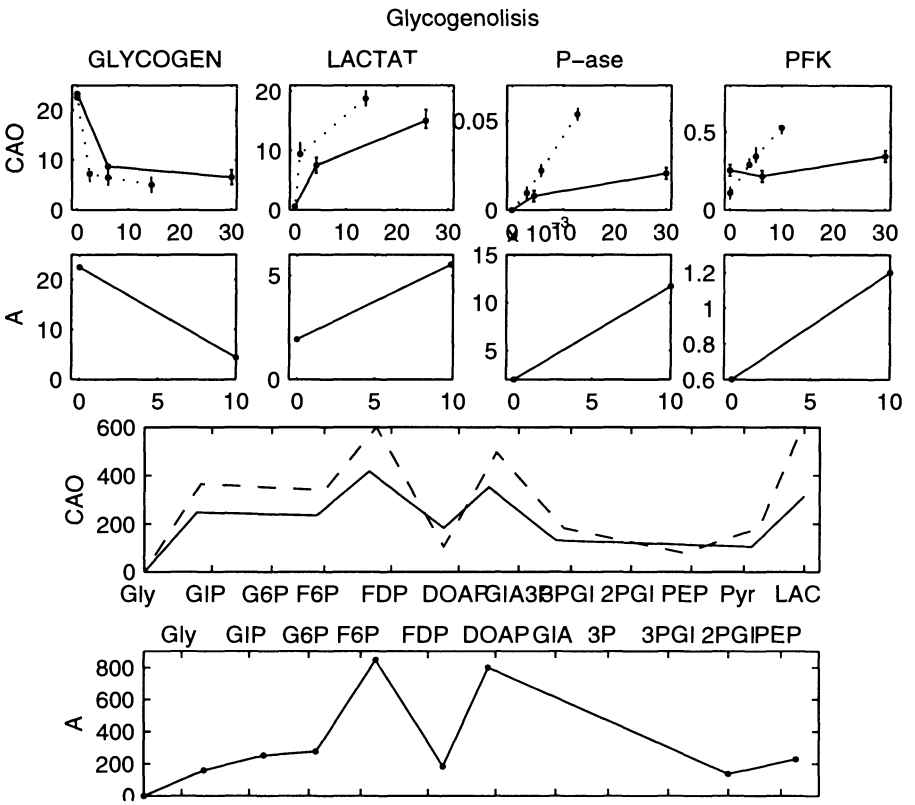

Figure 5.19. The comparison of the effects of CAO and A on the glycogenolysis: concentration of glycogen and lactate (mkM/g tissue), concentration of all glycogenolysis metabolites, (% to initial level), mass action relation for P-ase and PFK. Other designation - see fig. 5.19 and abbreviation list

glycogenolysis under normal conditions. This is evident from the considerable increase of FDP by a negligible increase of DOAP. In both cases P-ase and PFK are activated.

5.6.3 The action of adrenaline and CAO on acid-base and ionic equilibrium

The changes in pH and ions equilibrium have the same character as in the case of CAO so in response to A infusion (fig.5.20).

The increase and following decrease pH and pNa and decrease and following increase pCl in the ischemic zone are observed both under CAO and in response to A. The K content in the mitochondria decreases also in both cases, that leads to the increase of a_K^+ in the cytoplasm.

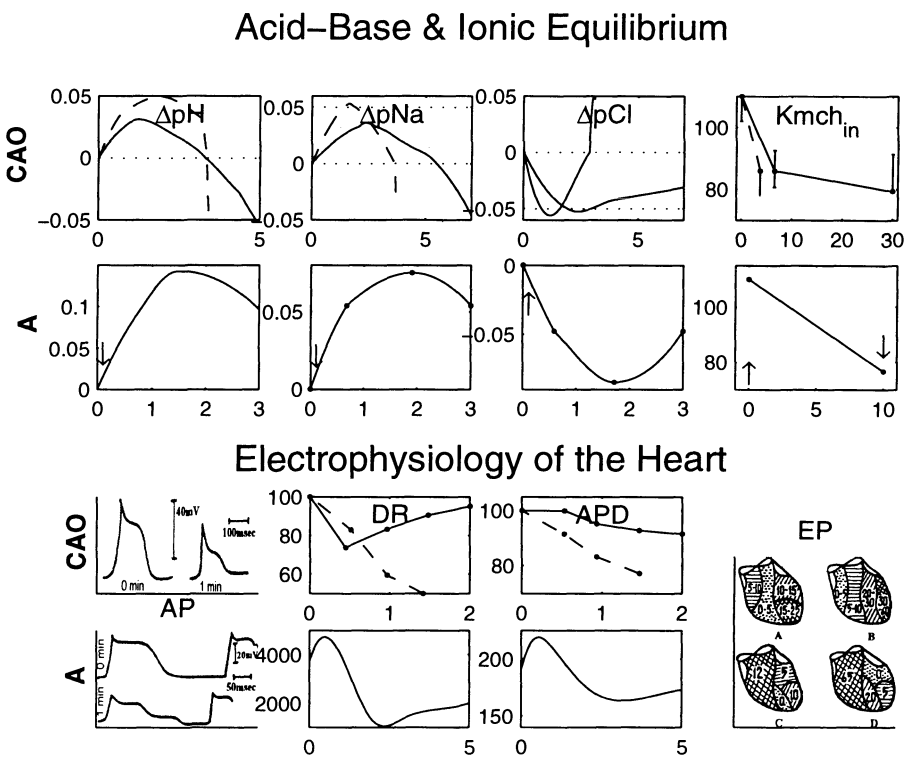

Figure 5.20. The comparison of the effects of CAO and A on the acid-base (ΔpH) and ionic equilibrium (Δward,ΔpCl)in myocardium and K content in mitochondria ($K_{mch.in}$,nmole/mg protein)

5.6.4 *The effect of adrenaline and CAO on the electrophysiological processes in the heart*

The unidirectional changes in the ionic equilibrium produced similarly changes in electrophysiological properties due to CAO and A (fig.5.20).

The DR and APD decrease in ischemic zone in CAO and sharply decrease after a transient increase under effect of A. As a result of the simultaneous action of ischemia and adrenaline a sharp decrease of DR and of APD occurs in cases with VF, which are characterized by a more marked accumulation of adrenaline in the zone of ischemia.

As a result of AP changes, the excitation propagation in the heart is disturbed. The velocity of the excitation propagation slows down and its direction changes. Using the multiple AP recordings from various heart areas, it was shown that a delay of the excitation propagation in the heart occurs under CAO just before the extrasystolic arrhythmia onset. The maximal delay of ex-

citation before CAO was 25 msec, it increase to 60 msec after CAO. Similarly, the delay of excitation at the maximal of adrenaline effect is increased from the initial value 12 to 65 msec. The sequence of heart involvement into excitation was also sharply disturbed both in case of CAO and A. These conditions facilitate the onset of VF in both cases.

Thus, both factors - ischemia which sharply decreases and adrenaline which sharply increases the blood supply and P_{O_2} of the heart - manifest a striking similarity in their effect on the energetic and ionic metabolism of the heart, as well as on the electrophysiological processes. This is not the first case when hormone, which at physiological concentrations activates the oxidative processes, depresses them at higher concentrations, arising under pathologic conditions. For instance, it is known that thyroxine, which is a powerful activator of the respiration, uncouples the respiration and phosphorylation, which make ineffective the intensified respiration. High adrenaline concentration acts even more dangerously: it also uncouples the respiration and the phosphorylation. The ineffective respiration not only is not compensated by its intensification (as in effect of thyroxine), but, on the contrary,is depressed.

In summary, the reaction of the organism against the NA release by the nerve endings in the ischemic zone, consist of intense secretion of A by the adrenal glands and its uptake by the heart. This reaction is a compensatory one, because its result is in replacing of noradrenaline lost by the heart with A and in sustaining the heart sympathetic activity. However, as it often happens in the organism, the compensation occurs with overshooting and noradrenaline is replaced by more active A not in a smaller amount, as it should be expected, but in a greater amount.

The A accumulated in high concentrations and influences the energetic and the ion exchange in the same direction with the ischemia. Both effects are summarized. The changes in ox-red equilibrium, in respiration and in phosphorylation (fig.5.18), in glycogenolysis (fig.5.19), in acid-base and ion equilibrium (fig.5.20) reach higher level (indicated by broken lines)) in experiments with VF. As a result , more considerable changes in AP and in excitation propagation through the heart occurs (fig.5.20) and these lead to the onset of VF.

Thus, adrenaline which accumulates in the heart as a result of ischemia, amplifies the effect of the ischemia itself. This leads to a significant increase of the speed and amount of disturbances appeared in the heart metabolism and electrophysiological processes. These disturbances became incompatible with the sustaining of a normal rhythm and provoke VF.

6 The Prevention of Ventricular Fibrillation after Coronary Artery Occlusion

The prevention of VF is a very complicated problem that has not yet been solved. The prophylaxis of VF is especially difficult because it appears unexpectedly, usually not in a hospital, but on the street, at work, at home, all in absence of medical personnel.

The realization of the VF prevention requires solving at least three problems.

1. Search of antifibrillatory substances.
2. Search of methods for using these antifibrillatory substances.
3. Search of methods for evaluation of the effectiveness of the antifibrillatory substances among the population.

6.1 The main directions of the antifibrillatory influences

The search for antifibrillatory substances was carried out in our laboratory by attempting to correct disturbances in metabolism and heart sympathoadrenal control that take place after CAO in experiments that result in VF. The correction of metabolic disturbances was realized using prescription drugs and chemicals such as metabolites, enzymes, hormones and so on [253]. We studied the possibility of preventing VF by means of: 1) activation of respiration, 2) depression of glycogenolysis, 3) stabilization of pH, 4) stabilization of the intra-extracellular ionic equilibrium, and 5) control of sympathoadrenal regulation.

6.1.1 Activation of respiration - by cytochrome C and hexahydroubiquinone

During ischemia, the tissue respiration (i.e. the electron transfer) is disturbed due to deficiency of electron acceptors, namely, oxygen. Under these conditions addition of electron acceptors (i.e. of substances with a high ox-red potential) can be used for respiration activation. We first used cytochrome C, with the redox potential of +260mV. Cytochrome C somewhat decreased the rate of ORP decrease in the ischemic zone, but it lowered the frequency of VF occurrence by only 10%, from 60 to 50%.

Coenzyme Q, ubiquinone (redox potential - 122 mV) was the second substance we studied. CoQ_{10} which functions between the flavoprotein and the cytochrome system, play a role of a universal electron "assembler". The antifibrillatory activity of hexahydroubiquinone-4 was we studied in experiments on dogs and cats. In experiments on cats, the antifibrillatory activity was evaluated via the fibrillation threshold value before and 15-20 min after CAO. In experiments on dogs the percentage of spontaneous VF was determined during intravenous infusion of the same ubiquinone doses 15 min before the occlusion. Animals administered with TVIN-90 without hexahydroubiquinone were used as a reference. The results obtained are presented in fig.6.1 and fig.6.2.

As seen from fig.6.1, hexahydroubiquinone-4 in a dose of 5-10 mg/kg causes a 60% ($p<0.01$) increase in VFT in cats and decreases the VFF in dogs after CAO from 60% in the reference group to 12% ($p<0.01$). In larger doses hexahydroubiquinone-4 did not have an appreciable effect or even had a toxic effect.

The antifibrillatory activity of hexahydroubiquinone-4 is evidently connected with a decrease in the ox-red equilibrium disturbances after CAO. This is testified to by smaller accumulation of NAD.H in cat myocardium in experiments with hexahydroubiquinone-4 as compared to the control group (fig.6.2). As can be seen from this figure, the accumulation of NAD.H was lowest for the dose of 5 mg/kg and it increased respectively when doses of 10 and 20 mg/kg were administered.

Thus, activation of respiration by administration of substances with high ox-red potential is the first of the possible methods for prevention of VF during local ischemia.

6.1.2 Depression of glycogenolysis by monoiodacetate

We assume that a higher value of glycogenolysis and a greater rate of its growth in experiments with VF is one of the links in the chain of processes which result in VF. We have tried to proceed from this assumption to prevent VF via glycogenolysis inhibition [236]. The glycogenolysis inhibition was realized in experiments on dogs using intravenous injection of 20 mg of monoiodacetate. Four series of experiments were carried out. In the first series, CAO was produced without monoiodacetate injection. In the second series, monoiodacetate was injected to animals without CAO. In the third series, monoiodacetate was injected 15 min before CAO. In the fourth series, monoiodacetate was injected

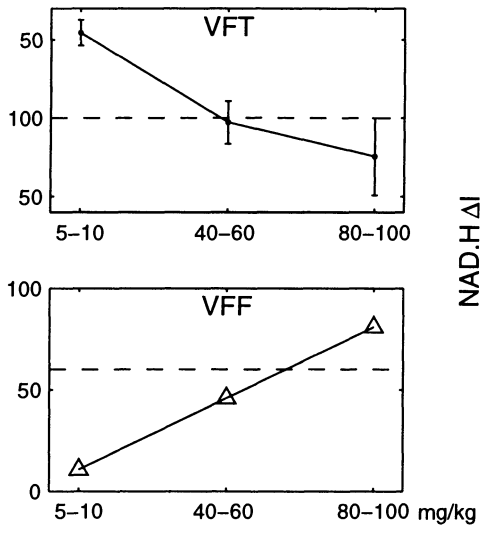

Figure 6.1. The effect of hexahydroubiquinone-4 emulsion with twin-80 in different doses on the ventricular fibrillation threshold (VFT,cats, n=9; control, n=4) and on the VF frequency (VFF, dogs, n=20; control n=10, broken line)

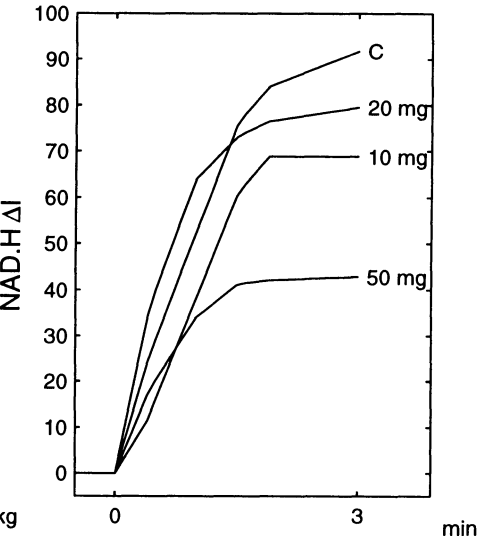

Figure 6.2. The effect of preliminary introduction of hexahydroubiquinone-4 in different doses (C - control) on the NAD.H in cat hearts after CAO.

immediately after CAO. In all of the above-mentioned series the time course of P_{O_2} and ORP was measured during 1 hour after CAO (except in experiments in which VF appeared or the heart stopped). After the experiment was over, histochemical investigation of glycogen in the ischemic and intact zones were carried out (fig.6.3).

Monoiodacetate injection both before and after CAO had practically no influence on P_{O_2} changes in the ischemic zone (fig.6.3-I), but substantially slowed the decrease in ORP (fig.6.3-II)

Monoiodacetate injection both before and after CAO had practically no influence on P_{O_2} changes in the ischemic zone (fig.6.3-I), but substantially slowed the decrease in ORP (fig.6.3-II) and prevent of accumulation of cytoplasmic NAD.H. As expected, injection of monoiodacetate before CAO assists in the conservation of glycogen in muscle fibers of the ischemic focus (fig.6.4-IV). The ischemic changes in action potential, recorded by means of a suction electrode, develop in opposite direction if MIA is administrated just after CAO (fig.6.4-V).

Data on dynamics of changes in pH, pNa and pCl during CAO in experiments without monoiodacetate are given in fig.6.4-A, the same data for dogs

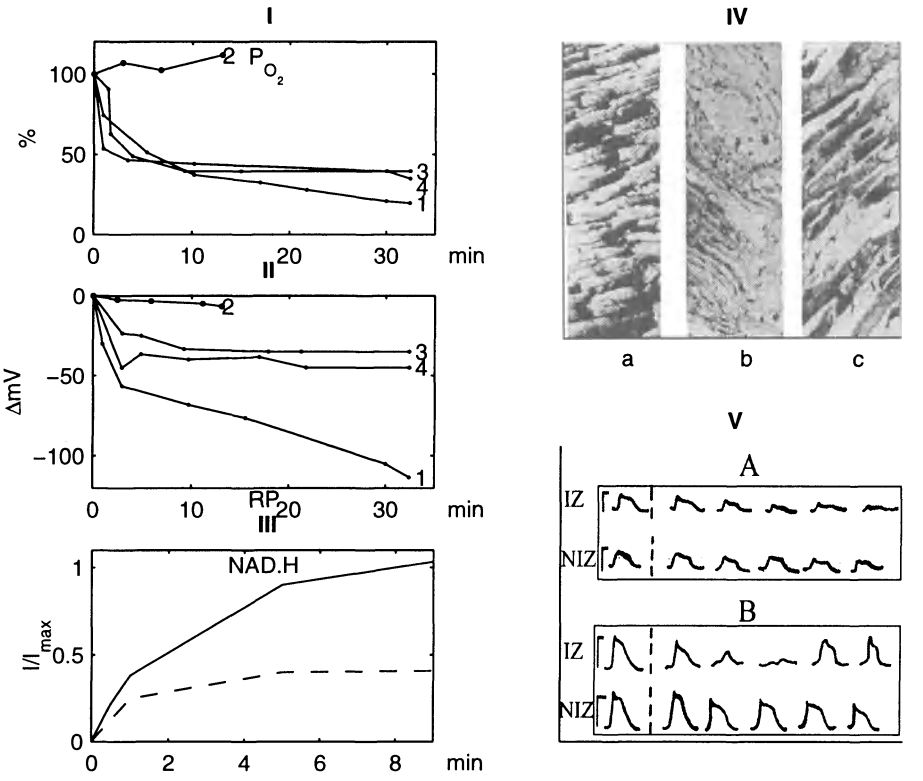

Figure 6.3. I - the influence of monoiodacetat (MIA) on P_{O_2} and II - on ORP in the ischemic zone dogs heart. 1 - CAO without MIA (n=10), 2 - after MIA (n=5), 3 - CAO 15 min after MIA (n=5), 4 - MIA right after CAO (n=12). III - the influence of MIA on NAD.H in ischemic zone cat's heart. CAO without MIA (solid line), CAO during action of MIA (broken line). IV - the influence of MIA on glycogen content in ischemic zone: a - before CAO, b - after CAO without MIA, c - after CAO and MIA. V - action potential in IZ and NIZ after CAO without MIA (A) and during infusion MIA right after CAO (B)

administered with monoiodacetate(20 mg/kg of body weight) 15 min before the occlusion are shown in fig.6.4-B.

As can be seen from the figure, monoiodacetate causes changes in pH, pNa and pCl in the same direction with those observed in the first phase of ischemia, i.e. increase in pH and pNa and decrease in pCl. CAO causes a further increase in pNa, decrease in pCl and maintenance of pH on the level above the initial. So, monoiodacetate prevents decrease in pH and pNa and increase in pCl which appear in second phase of ischemia and directly precede the appearance of VF. As a result of the action of monoiodacetate on the redox, acid-base and ionic equilibrium, VF frequency decreases from 60% to 0%.

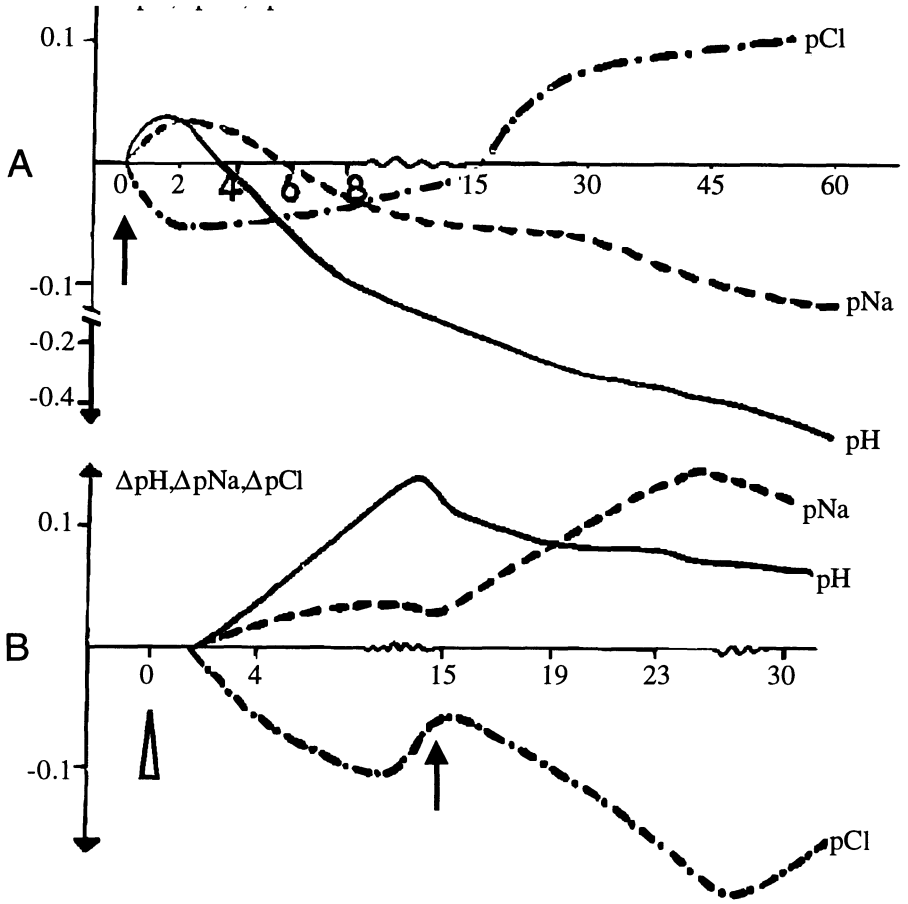

Figure 6.4. The influence of MIA on the dynamics of changes in pH, pNa and pCl in ischemic zone of a dog's heart after CAO. A - CAO (shown by an arrow) without MIA (n=20). B - Intravenous infusion of MIA (shown by a triangle) 15 min before CAO (shown by an arrow, n=19)

Thus, inhibition of glycogenolysis is the second of the possible way for prevention of VF during local ischemia.

6.1.3 Control of the acid-base equilibrium by buffer TRIS

The acid-base equilibrium during ischemia can be stabilized both indirectly, via the redox equilibrium (see above), and directly, by increasing of the buffer capacity of the heart.

Buffer solutions of TRIS (trihydroxymethyl aminomethan) with various pH were used for increasing the tissue buffer capacity [287]. TRIS was also used

in [159, 343]. The investigation was run using a model of a heart area perfused with ischemic blood. The carotid artery blood was replaced by corresponding solutions and the perfusion velocity was decreased to 30% of the initial level. TRIS solutions (0.05 M) with pH 6.8, 7.4, 7.6 and 7.7-7.8 were used to modify the acid-base state of the perfused area. The solution contained K^+ 3.0 meq/l, Na^+ 140 meq/l and Ca^{2+} 2.5 meq/l. In the control series of experiments a non-buffer solution with pH 7.4 was used. The control and the investigated solutions were pumped into the coronary artery using a second pump. Each solution was used for 5 minutes. Samples of the effluate from the great coronary vein were taken before, after 1.3 min, and after 5 min the beginning of perfusion. pH (by Astrup method) and K^+ content (by flame photometric method) were determined in effluate.

If spontaneous VF occurred, defibrillation was performed and perfusion was continued with a normal blood volume. 30 min later, after a complete recovery of heart functions, the same ischemia was induced again and perfusion of the investigated area was performed with another solution. 3-4 different solutions were studied in one experiment. If spontaneous VF did not occur during the ischemic perfusion, tendency towards VF was evaluated based on the value of VF thresholds. The data obtained in these experiments are presented in fig.6.5.

As can be concluded from figure 6.5-A, efflux of K^+ into the extracellular medium was substantially less for perfusion with a buffer solutions for all pH levels than for perfusion with non-buffer solutions. The decrease in pH level in the effluate (fig.6.5-B was similar for perfusion with buffer TRIS solution and for non-buffer solution with the same pH. A hypothesis can be formed that decreased efflux of K^+ after perfusion with TRIS is due to increased buffer capacity of the myocardium. The frequency of spontaneous VF after 5 min of perfusion with TRIS solution with pH=7.4 decreased from 44.4% to 0% and smaller decrease was observed for other pH values (fig.6.5-C).

After 5 min of ischemic perfusion, the VF threshold was highest for effluate with pH 7.3-7.4 and the lowest for effluate with acidic pH. The K concentration in effluate was lowest in experiments with highest values of VFT (fig.6.5-D). So, the antifibrillatory activity of TRIS is due to its ability to decrease the loss of potassium by the ischemic myocardium.

Thus, data obtained in these experiments show that the increase in myocardial buffer capacity by administration of a buffer solution is the third possible method for prevention of VF during local ischemia.

6.1.4 Influences on disturbances in ionic equilibrium by cardioplegic cocktail

6.1.4.1 Effects on loss of K^+ by myocardial cells. A modified cardioplegic cocktail was used in our laboratory for direct influence on the ionic equilibrium. After some modifications the composition of the solution became the following: KCl 520 mg, $MgCl_2$ 200 mg, novocaine 2 g, glucose 1.1 g, mannose 73 mg, EDTA 600 mg; distilled water 1 liter. Just before the experiment, the pH was changed from 4.0-5.0 to 7.4 using the TRIS buffer.

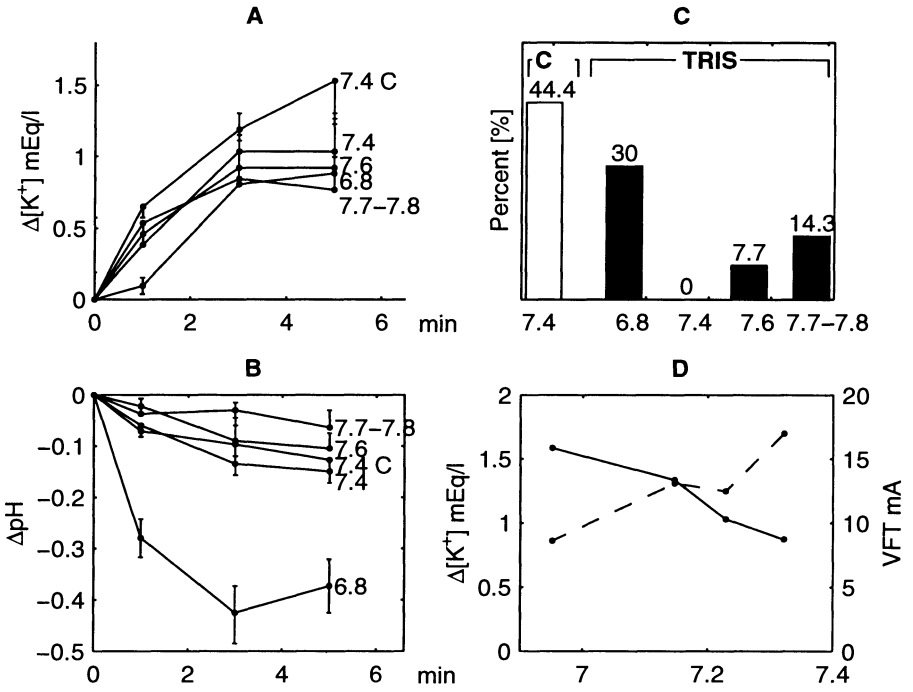

Figure 6.5. A - changes in K concentration ($\Delta[K^+]$) and pH (ΔpH) - B - in the effluate during ischemic perfusion with a non-buffer solution (C) and with TRIS with different pH (dogs, n=20). C - frequency of VF (%) after 5 min of ischemic perfusion with a nonbuffer solution pH 7.4 (C-control) and with a buffer solution TRIS different pH (TRIS). D - changes in K concentration in the effluate ($\Delta[K^+]$, solid line) and VF threshold (VFT, broken line) after 5 min of ischemic perfusion with a buffer solution TRIS (abscissa - pH in the effluate.

The antifibrillatory properties of the modified solution were studied in experiments on cats and dogs. In experiments on cats, the solution was introduced into the left atrium (dose 2-3ml/kg) at the moment of CAO [286]. In experiments on dogs, the solution was introduced intravenously 20-30 seconds after CAO in doses 8-10 ml each 3-5 min. The results of these experiments are shown in fig.6.6.

The data reported above show that both intracardial and intravenous introduction of the cardioplegic solution significantly lowers of the tendency towards VF after CAO (increase in VFT and decrease in frequency of spontaneous VF). However, the solution does not influence the tendency towards VPB.

The pronounced decrease of vulnerability to fibrillation in experiments with infusion of the solution is obviously due to its influence on myocardial excitability. Typical changes in the ET during the cardiac cycle (strength-interval curves) observed in one of the experiments before and after the intravenous infusion of the solution are shown in fig.6.7-A.

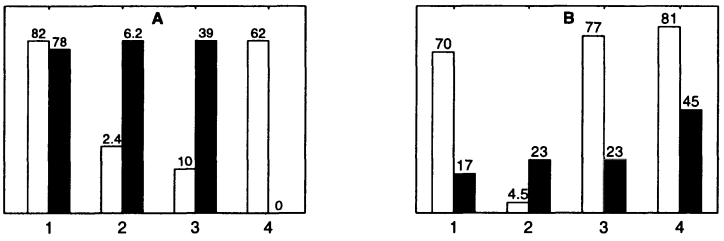

Figure 6.6. A - the effect of intracardial introduction of cardioplegic solution on the frequency of VPB (1,%), time of appearance of VPB (2, min.), VF threshold (3, mA) and frequency of spontaneous VF (4,%) after CAO. Light columns - CAO without cardioplegic solution (in 1,2.3.4 n=28), black columns - CAO with cardioplegic solution (in 1,2,3, n=14, in 4 n=8) B - the action intravenous introduction of cardioplegic solution on 1 - VF frequency (% of total number of experiments), 2 - time of the arise VF after CAO (min), 3 - frequency of early VPB transition in VF (% of total number experiments with VPB), 4 - frequency of transition VT in VF (in % of total number experiments with VF). Light columns - CAO without cardioplegic solution (n=26 dogs), black columns - CAO with cardioplegic solution (n=25 dogs)

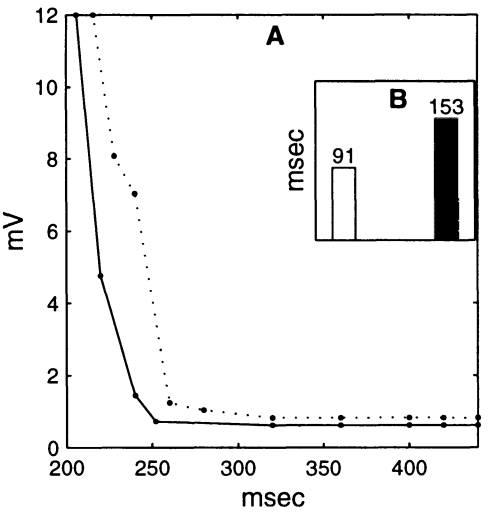

Figure 6.7. The influence of intravenous introduction of cardioplegic solution on the excitability of the heart. A - dependance between stimulus force (mv) and cardiac cycle interval (msec). B - dispersion of repolarization (msec). Solid lines and light columns - before introduction of cardioplegic solution, broken lines and black columns - after its introduction. n=4.

As can be seen from the figure, the strength-interval curve shifts up and to the right, which testifies to the increase in the effective refractory period, the total refractory period durations, and the diastolic ET (fig.6.7-A). The dispersion of time repolarization increases($p<0.01$) in experiments with infusion of the solution (fig.6.7-B). The increase in dispersion of time repolarization, which promotes VF (see chapter II), prevents VF in experiments with antifibrillatory solution.

The antifibrillatory activity of the solution could be due to its ability to: decrease the acidosis due to the presence of TRIS buffer in the solution; prevent potassium loss by the cells and decrease of the heart excitability due to the presence of novocaine and absence of Na in it; promote decrease in the myocardial edema at the expense of hyperosmotic. Electrophysiological effect of the cardioplegic solution is probably related to the increase duration of the effective refractory period and the ET value.

Thus, the introduction of solutions, which changes ionic content of myocardium, is the fourth possible method for prevention of VF during local ischemia.

6.1.4.2 Effects on retention of Ca^{2+} by the myocardial cells. Use of Ca^{2+} blockers, which prevent accumulation of Ca^{2+} in the cell, is another method for correcting disturbances in the ionic balance after CAO. Calcium antagonists reduce and calcium agonist enhance the susceptibility to VF induced by the combination of exercise and acute myocardial ischemia [31]. Verapamil, ineffective during normal coronary circulation, raises fibrillation threshold lowered by ischemia. The beneficial effect of verapamil for prevention sudden death in humans was demonstrated when myocardial contractility not affected too much [311]. However, no real benefit could be expected from verapamil when ischemia is persistent since it only delays the onset of VF [310].

There exists a hypothesis that the mechanism of antifibrillatory action of verapamil consist in the inhibition the electrophysiological changes induced by ischemia, such as shortening of the effective refractory period and intramyocardial conduction time [351]. However, Schomig at al. [279] believes that mechanism of calcium-blockers, such as gallopamil, verapamil, diltiazem, felodipine, and nifedipine is the suppression of ischemia-induced release of noradrenaline. Amlodipine reduces the frequency of sudden death by lengthening time to onset of VF, but has the least negative inotropic effect [309]. Diltiazem also prevents sudden death and does not reduce left ventricular dP/dt max by more than 6.8% [17]. The calcium-channel agonist Bay-K-8644 reduces VFT by acting in part through enhanced recycling of calcium through the sarcoplasmic reticulum [349].

6.1.4.3 Effects on different mechanisms of antifibrillatory action. In the last decade, a number studies were dedicated to the prevention of VF during myocardial ischemia. The defibrillatory action of dibenzepin led to a decreased intercellular resistance and to improved conduction [13]. Flecainide,

lignocaine and disopiramide were observed to have antifibrillatory properties [16]. Leu-enkephalin prevents a decrease in VFT during experimental coronary occlusion[187]. Amiodarone and sotalol were shown to be antifibrillatory agents[291, 77]. A multiple-dose regimen of MS-551 provides protection against ischemia-induced VF in postinfarction hearts [100]. Infusion of free n-3 polyunsaturated fatty acids and omega 3 fatty acids prevented VF in dogs [34, 33].

Acidic reperfusion is required for protection against VF. Protective mechanism may involve enhanced recovery of Na-K-ATP-ase activity as well as inhibition of Na^+ influx [19]. The selective Na^+/H^+ exchange inhibitor, HOE 642, has pronounced cardioprotective and antiarrhythmic effect in ischemic rat hearts [274].

The modulation of anion homeostasis by substitution of extracellular chloride with nitrate, prevents ischemia-induced VF. The author supposes that this may be a focus for future search of antiarrhythmic drugs [67].

There exists evidence that histamine may function as one of many endogenous biochemical mediators of ischemia-induced VF. Based on this evidence, many attempts were recently made to use angiotensin-converting enzyme (ACE) inhibitors for prevention of VF. Perfusion of isolated perfused rat hearts with cimetidine reduced the frequency of ischemia-induced VF, but ranitidin did not have the same effect [21]. Captopril and lisinopril prevented the occurrence of serious ventricular arrhythmias [76, 35]. A review of this research is presented in [27]. Most authors agree that ACE-inhibitors should be used by patients with coronary artery disease and with left ventricular dysfunction [27, 320, 215]. There exists evidence that ACE-inhibitors - captopril, enalapril and trandolapril, improve of cardiac function by increasing concentrations of ATP, KP and increasing consumption of oxygen by mitochondria [269].

Research shows that exogenous adenosine is an antiarrhythmic agent[304]. There also exists evidence that adenosine is a factor in antiarrhythmic effect of preconditioning in dogs [76].

6.1.5 Influences on cardiac sympathoadrenal control by reserpine, metanephrine and propranolol

We consider the following sequence of evens for potential sources of influence on disturbances in catecholamine balance in the heart after CAO (see chapters IV and V): noradrenalin release by the heart (in response to CAO) $\longrightarrow$ adrenaline uptake by the heart (in response to loss of noradrenaline) $\longrightarrow$ effect of adrenalin on the so-called β-adrenergic receptors.

We made the attempt to influences on sympatho-adrenal regulation of the heart in CAO by means 1) reserpine, which exhausted stores of noradrenalin in the granules and to prevent noradrenalin release by nerve endings in response to CAO; 2) metanephrine, an ortho-methylated adrenalin derivative, which depress the adrenalin uptake by the heart; and 3) propranolol, β-blocker (inderal) which blocks the effect of adrenaline on the heart receptor system.

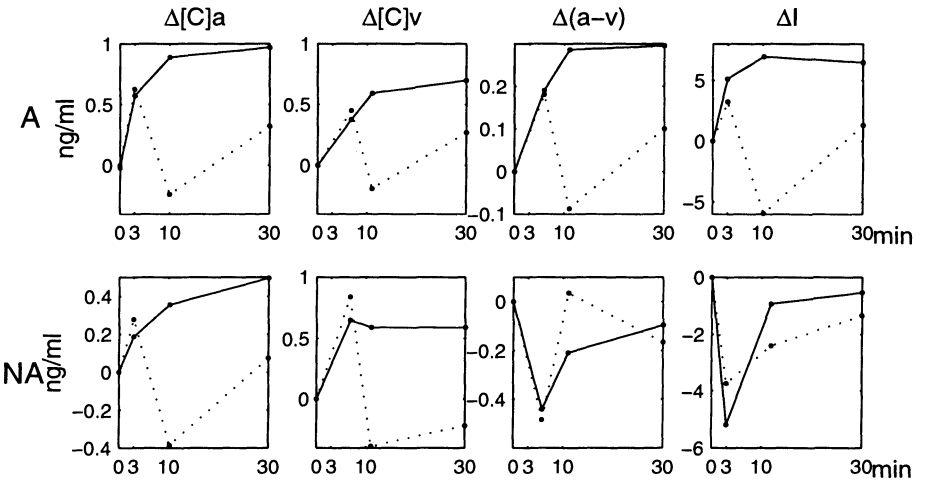

Figure 6.8. The dynamics of changes in A and NA concentration in arterial blood (Δ[C]a), in coronary sinus blood (Δ[C]v), in arterio-venous difference (Δ(a-v)) and in indexes of A uptake and NA release by a dog heart (ΔI) after CAO without treatment (n=12, solid line) and after a methanephrine (50 μg/kg) infusion into coronary artery just before CAO (n=12, broken line)

6.1.5.1 Exhaustion of noradrenaline stores in the granules by reserpine. Reserpine was used to exhaust the noradrenalin stores in the granules. The effect of reserpine was studied in experiments on 20 dogs [86]. Reserpine ("Rau-sed") was administered subcutaneous 24 hours before the experiment (dosage 0.5 mg/kg). The effectiveness of preliminary reserpine action was validated by the absence of changes in heart rhythm and arterial pressure during electric stimulation of the stellate ganglion. 24 hours after administration, reserpine caused a decrease in arterial pressure and heart beat frequency. Frequency of VF after CAO decreased to only 50% in animals administered with reserpine compared to 60% in the control group.

6.1.5.2 Depression of adrenalin uptake by the heart with metanephrin. Proceeding from the observation that cases resulting in VF after CAO are characterized by a more pronounced uptake of A by the heart, in our laboratory a blockade of extraneuronal A uptake by the heart was performed using a O-methylated derivative of A - metanephrin (MN) [240]. 12 dogs were medicated with MN, by injection into the descending branch of left coronary artery at the level of the subsequent ligation (dosage 50 μg/kg of weight). CAO was produced at a standard level immediately after the medication. The results obtained in these experiments are presented in fig.6.8.

As can be seen from fig.6.8, no substantial difference was observed in the increase in A concentration in arterial blood between the control and MN med-

icated groups within 3 minutes of CAO. However, 10 minutes after CAO, the A concentration not only did not increase, as in the reference group, but even decreased relative to the initial level and increased somewhat at the 30th minute. This seems to indicate that secretion of A by the adrenal glands decreases under the influence of MN. The arterio-venous difference in A concentration increased at the 3^{rd} min, decreased at the 10^{th} min to below the initial level and increased somewhat at the 30^{th} min. Similar changes were observed in the index of A uptake by the heart, which takes into account the changes in A concentration in the arterial blood. This indicates that the decrease in A uptake by the heart at the 10^{th} min resulted not only from the decrease in A concentration in the arterial blood but also from depression of the extraneuronal A uptake by heart itself.

The NA concentration in arterial blood 3 min after CAO did not substantially differ control and MN. It decreased sharply at the 10^{th} min and increased at the 30^{th} min. Changes in the arterio-venous difference in NA content were characterized by a more marked biphase in experiments with MN, while no substantial differences in the index of NA release were observed between the control and MN group.

The effect of MN is maximal 10 minutes after CAO. The effect is manifested by a decrease in A content in the arterial blood and a decrease in A uptake by the heart. Comparison of changes in (a-v) and I allows us to conclude that decrease in A uptake by the heart is not only due to the decrease in arterial hyperadrenalemia, but also to the decrease in the ability of the heart itself to uptake A.

Based on the fact that the A uptake by the heart compensates for the loss of NA, inhibition of A uptake by MN can be considered to be a result of the decrease in the NA loss by the heart. The dependence of A uptake on NA loss is demonstrated by the directly opposite of curves $(a\text{-}v)_A$ and $(a\text{-}v)_{NA}$ (fig.6.9). To show the influence of MN on the relationship between A uptake and NA loss, we calculated the ratios $(a\text{-}v)_A/\text{-}(a\text{-}v)_{NA}$ and $I_A/\text{-}I_{NA}$ (fig.6.9).

Infusion of MN increased $(a\text{-}v)_A$ from 0.10 to 0.15 and $(a\text{-}v)_{NA}$ from -0.28 to -0.19 even before CAO. This resulted in an increase in their ratio from 0.36 in the control group to 0.79 in the group medicated with MN. 10 min after CAO, when this ratio in the control group increased from 0.36 to 0.78, MN lowered it to an almost normal level 0.47. I.e. A replaced only one of two parts of released NA.

As far as the ratio of A uptake and NA release indexes are concerned, MN increased it even before CAO but this ratio decreased twice as much 10 min after CAO as in the control group.

Thus, the undesirable effect of MN just after its administration changes to a very desirable effect 10 min after CAO. MN recovered the normal relationship between A uptake and NA release by not only decreasing A concentration in the arterial blood but also by decreasing the capability of the heart itself to uptake A. As a result, the effect of A on the heart was decreased and the VF frequency decreased from 65% to 25% in experiments with MN.

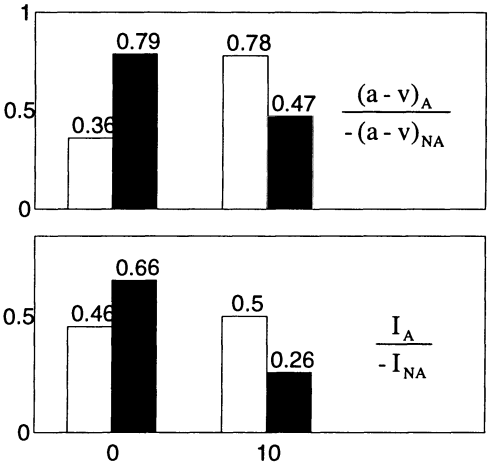

Figure 6.9. The ratio of A uptake to NA release by a canine heart $(a\text{-}v)_A/-(a\text{-}v)_{NA}$ and $I_A/-I_{NA}$ in control (CAO both with and without VF,n=12, white columns) and after methanephrine (50 μg/kg) infusion into coronary artery just before CAO (both with and without VF, n=12, black columns) before CAO - 0 and 10 min after CAO

6.1.5.3 Blockade of β-adrenoreceptors by propranolol. The antifibrillatory effect of beta-blockers has been demonstrated in numerous clinical as well as experimental studies on animals. However, the question about the actions mechanism of beta-blockers remained not clear.

In 1966 we got from firm ICI (Great Britan) inderal (propranolol) for study in experiments on the animals. In 1967 we presented a report of our results at the symposium of the clinical use of inderal [247]. To clarify the mechanism of the propranolol antifibrillatory activity we studied its effect on the blood supply, metabolism, and electrophysiological characteristics of the heart.

Effect of propranolol on cardiac blood supply. The blood supply of the heart was studied using the postmortem coronarography method (chapter I). Inderal was administered intravenously in doses of 1 mg/kg of animal weight. Its effect was studied for 15 min after administration. Then ligation of the anterior descending branch of the coronary artery at the upper third was performed. Measurements were taken for 1 hour. Frequency of VF in this series of experiments decreased from 60% in the control group to 0% [285, 245]. The quantity of vessels below the ligature filled with contrast substance and the degree of retrograde filling of the ligated artery was considerably higher in experiments in which inderal administration preceded coronary artery ligation than in the experiments without inderal. An example which illustrates almost complete filling of the distal part of the coronary arteria and a well developed vessel net in the ischemic zone in an experiment with inderal is shown in fig.6.10.

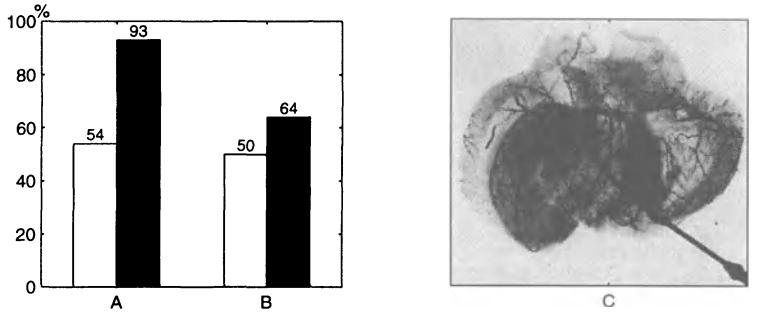

Figure 6.10. The influence of inderal on the opening intercoronary anastomosis in a canine heart after CAO (n=12). A - retrograde filling of the distal part of the occluded artery. B - number of vessels in the ischemic zone. Light columns - CAO without inderal, black columns - with inderal. C - coronary gram of the unfolded canine heart. Left coronary artery occluded 15 min. after preliminary introduction of inderal

So, inderal promotes opening of inter- and intra-coronary anastomosis. The mechanism could be as follows: inderal decreases the contractile aptitude of the heart. This results in a decrease in pressure exerted by the myocardium on the intramural coronary arteries during the systole. The decrease in pressure allows the artery lumen to not decrease.

Effect of propranolol on the heart ox-red potential. The effect of inderal on the partial oxygen pressure and on the ox-red potential in a normal heart and in a heart with inderal introduction 15 min before CAO is illustrated in fig.6.11.

As can be seen from figure 6.11-A, inderal causes a drop in the myocardial P_{O_2} to 40% of the initial level in normal dogs. This is probably connected with its hypotensive effect and with the associated decrease in the coronary blood flow. At the same time the myocardial ORP does not change substantially. The absence of changes in ORP in spite of a decrease in P_{O_2} could be connected with a decrease in the heart's work and its energy requirements.

CAO performed 15 min after inderal administration, causes a smaller drop in myocardial P_{O_2} and ORP than in experiments without inderal administration (fig.6.11-B). The smaller decrease in P_{O_2} after CAO could be connected with the preceding opening of intracoronary anastomosis and decrease in P_{O_2} due to administration of inderal before CAO.

The smaller decrease in the ORP in response to CAO can be connected with influence of inderal on respiration coupled with phosphorilation and the glycogenolysis in the ischemic zone.

Effect of propranolol on respiration and phosphorylation. The effect of propranolol on respiration and phosphorylation was studied in experiments on 68 dogs using intravenous administration in a dose of 1 mg/kg of weight

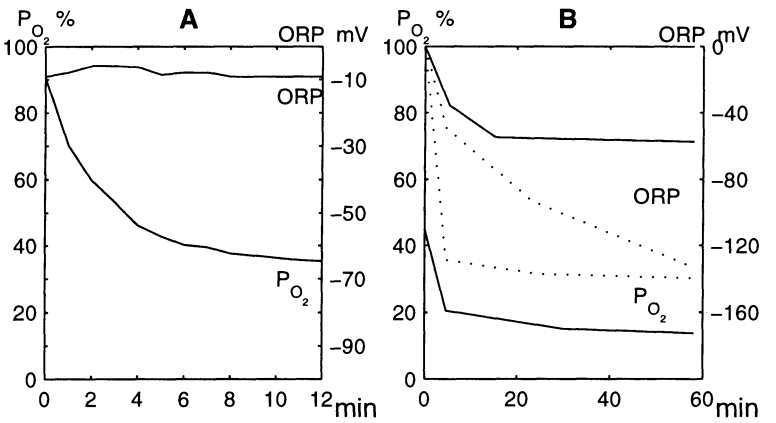

Figure 6.11. A - The dynamics of changes in P_{O_2} and ORP during introduction of inderal (1 mg/kg) to normal dogs. B - CAO 15 min. after inderal introduction (n=12,solid line) and CAO without inderal (n=12,broken line)

[81, 82]. Heart samples were taken at the 15th min after the beginning of propranolol administration – the time of maximal effect on the arterial pressure and heart rhythm. The observed data are presented in fig.6.12.

Propranolol was shown to prevent decrease in phosphorylated respiration rate (Q) almost completely, prevent decrease in respiratory control (RC) in mitochondria of the ischemic zone and in a considerable degree and to a lesser degree in mitochondria in the nonischemic zone. In this series of experiments, propranolol lowered the frequency of VF from 60% to 24% ($p<0.05$).

Effect of propranolol on glycogenolysis. Changes in glycogenolysis in the heart after CAO were studied after preliminary propranolol administration in experiments on 9 dogs [86, 85]. Propranolol (obsidan) was continuously infused in a dose of 1mg/kg of weight. The control group was made up of 25 dogs. A heart sample was taken with special pincers (see chapter III) at the 15th min after propranolol administration when the arterial pressure decreased from 110 to 90 mm Hg (on average) and heart rhythm slowed from 78 to 60 beats/min. CAO was produced simultaneously with the first sampling. None of the 9 dogs administered with propranolol developed VF within 1 hour after CAO. The observed data are presented in fig.6.13.

There was a statistically significant increase in the mass action ratios of P-ase, PFK, and LDG ($p<0.05$) during the entire period of observation in experiments both with and without propranolol administration(fig.6.13-A). Changes in the metabolite content were the most pronounced during the first few minutes of ischemia. Decomposition of glycogen and increase in lactate during the first 2 min of ischemia were smaller in animals that were preliminary adminis-

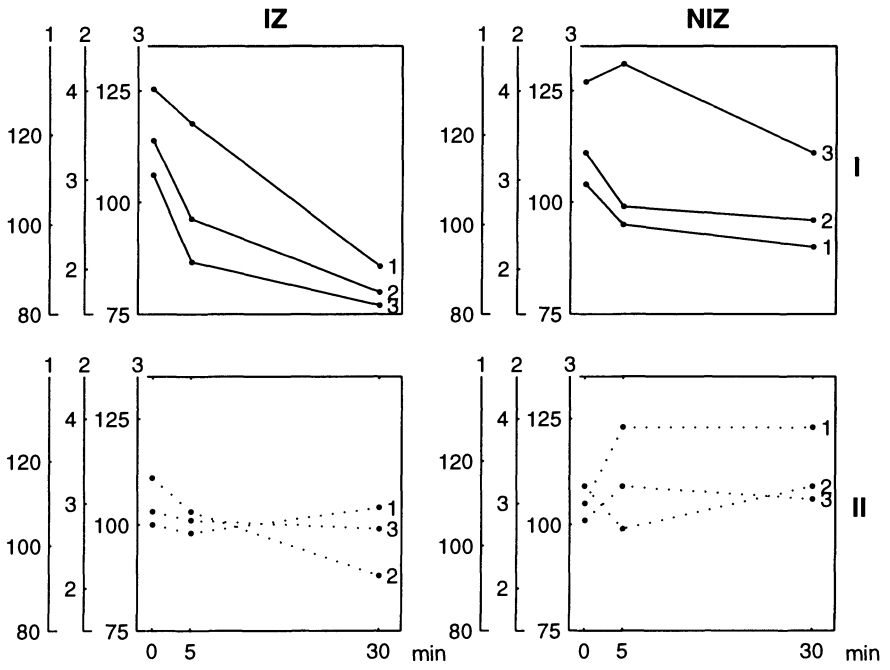

Figure 6.12. The influence of propranolol on the rate of oxidative phosphorylation (Q), respiratory control (RC), internal K content (K^+in) in mitochondria IZ and NIZ zones after CAO without propranolol (I, solid line) and after CAO with preliminary introduction of propranolol (dose 1mg/kg) (II, broken line)

tered with propranolol compared to the control group (fig.6.13-B). The decrease in glycogenolysis and prevention of the decrease in phosphorylated respiration could be the cause of the smaller decrease in ORP in response to propranolol administration.

Effect of propranolol on acid-base and ionic equilibrium. Changes in acid-base and ionic balance caused by administration of inderal 15 min before CAO very similar in various experiments. The dynamics of changes in pH, pK, pNa and pCl activity after CAO are presented in fig.6.14.

As can be seen from this figure, inderal causes a marked decrease in the myocardial pH, associated with a decrease in pNa and pCl, and an increase in pK. CAO after inderal administration induces further decrease in pH, pNa and pCl and increase in pK. In this case, the initial increase in pH and pNa which is associated with appearance of VF (see chapter III) does not occur.

The increase in pK^+ (i.e. decrease in K^+ concentration in the extracellular medium) observed after CAO in experiments with inderal probably proves that

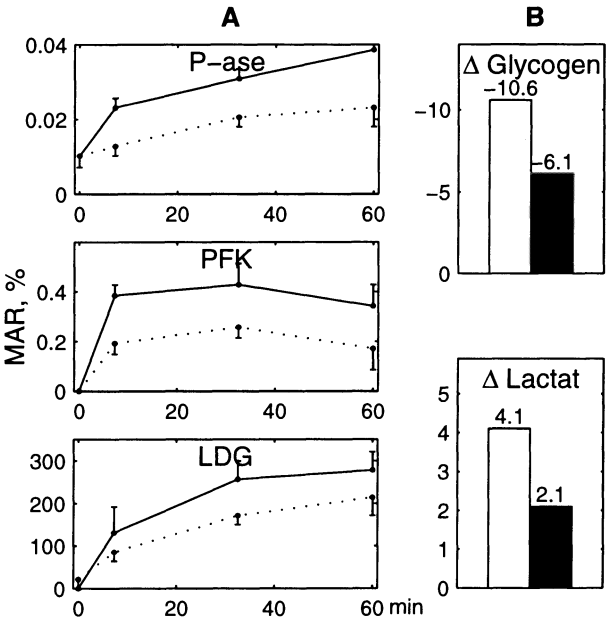

Figure 6.13. The dynamics of changes in mass action ratio (MAR), glycogenolysis ferments (A), and increments in glycogen and lactate content (mkM/g tissue) at the second min after CAO (B) in ischemic zone of a canine heart with preliminary introduction of propranolol (n=9, broken line, black columns) and without propranolol (n=25, solid line, light columns)

K^+ enters the cell, while in experiments without inderal K^+ leaves the cell. We consider this to be the main feature of the antifibrillatory effect of inderal. The normalizing effect of inderal on the ionic exchange can explain why VF was not observed in any experiments with inderal administration, but was observed in 52% of the control experiments.

The effect of intravenously administered propranolol (the same dose of 1 mg/kg of the weight) on the ionic exchange of heart mitochondria was studied in another series of experiments on dogs [81]. Heart samples were taken 15 min after the beginning of propranolol administration and 50-60 min after CAO. CAO was made in 27 experiments. 5 of 21 experiments resulted in VF. Thus propranolol lowered the frequency of VF in these experiments from 53 to 24% ($p<0.05$).

The K^+ content in the mitochondria in the ischemic zone 5 min after CAO is higher in experiments with propranolol than in experiments without it ($p<0.05$); the difference becomes greater after 30 min ($p<0.02$) (fig 6.15-A). Similar changes in K^+ content were revealed in the mitochondria in the intact zone (fig.6.15-B). The use of propranolol after CAO almost prevents the loss of K^+

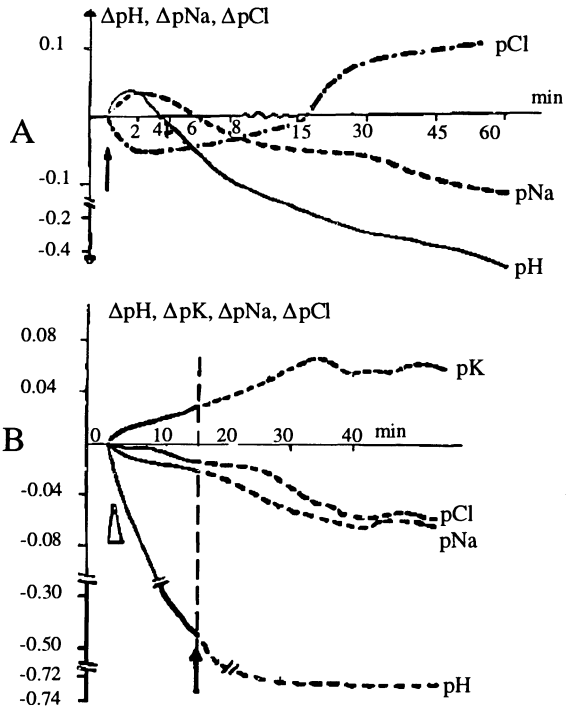

Figure 6.14. Changes in pH, pNa and pCl recorded with selective subepicardial electrodes in ischemic zone of a canine heart after CAO without inderal - A, after inderal introduction (1 mg/kg) and following CAO - B. Triangle - moment inderal introduction, arrow - moment of CAO.

and prevents a decline in E_{mch} in both the intact and ischemic zones. The stabilizing effect of propranolol on the mitochondrial membrane potential probably underlies its influence on K^+ content in the heart mitochondria. The changes in the membrane passive permeability of the mitochondria (G) of ischemic and nonischemic zones did not observed.

A comparison of data on the main parameters of energetic and ionic exchange obtained during CAO in experiments with and without preliminary propranolol administration, as well as with A administration are presented in fig.6.16. These data permits us to conclude that, on one hand, CAO and A cause changes in these parameters in the same direction, and, on the other hand, that the ischemic changes can be prevented by blocking the effect of A with propranolol.

Thus, the antifibrillatory effect of propranolol during myocardial infarction is related to its ability to prevent additional changes in the energetic and ionic exchange in the mitochondria in the ischemic zone. These changes are caused by A which is intensively uptake by the heart.

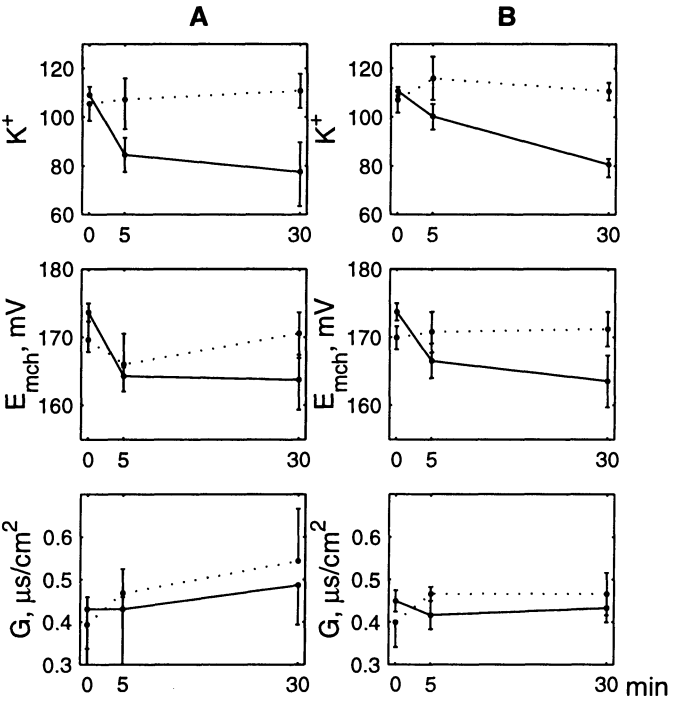

Figure 6.15. Dynamics of changes in K content in mitochondria (K^+), membrane potential of mitochondria (E mch) and passive membrane conductivity of mitochondria (G) in the ischemic zone (A) and nonischemic (B) zones after CAO without propranolol (solid line, n_0=19, n_5=8, n_{30}=9) and after CAO with preliminary introduction of propranolol (broken line, n_0=8, n_5=8, n_{30}=8).

The effect of propranolol on the heart membrane potential is probably related to prevention of those additional changes.

Effect of propranolol on the heart membrane potential. Propranolol administration increases the APD and decreases the APA in the left ventricles [245] (fig.6.17).

CAO, made 15 min. after inderal administration, lowered the AP duration and amplitude just like in experiments without inderal. However, since the initial APD in experiments with inderal was considerably greater then in experiments without inderal the decrease did not lead to AP as short as in experiments without inderal. At the same time, due to the decrease in initial APA, the decrease in APA after CAO was more considerable in experiments with inderal administration.

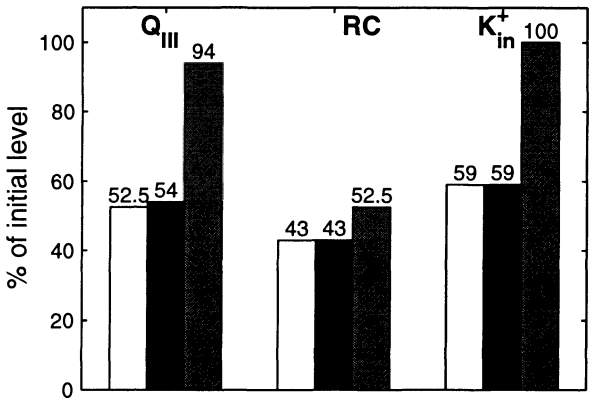

Figure 6.16. Comparative data on changes in Q_{III}, RC and internal K content (K^+in) in mitochondria 30 min after CAO (n=9, white columns), after intravenous introduction A (20mkg/kg,n=27, grey columns) and after CAO with preliminary introduction propranolol (n=8, black columns)

Effect of propranolol on the heart excitability. As can be seen from fig.6.18, the strength-interval curve shifts to right and upward due to the effect of inderal. This testifies to the increase in diastolic ET, in the ERP and RRP.

Effect of propranolol on the excitation propagation along the heart. The dispersion of depolarization and repolarization increase after CAO and reach a maximum value just before VPB appear [93]. The increase in de- and repolarization dispersion is also observed when propranolol is administered after CAO, but VPB appear in this case when the increase in dispersion is considerably greater (fig.6.19) [245].

Effect of propranolol after removal of its negative inotropic action. The considerable cardiac depressive and hypotensive effects of propranolol limit its use during acute myocardial infarction. In collaboration with Hungarian scientists we studied whether is it possible to maintain the antifibrillatory effect of propranolol and to remove its side effect [319]. For the study we used corglycon, which has a considerably smaller toxic effect on the heart than other heart glycosides.

Experiments were conducted on dogs. In the first series of experiments, the initial values of VF threshold and local contractility were measured after thoracotomy and attachment of electrodes and transducers to the heart for the corresponding measurements. Then, propranolol (obsidan) was administered to the animals (dose 1 mg/kg) and the same parameters were measured after 15 min. After that dogs were administered with corglycon in a dose of 0.02

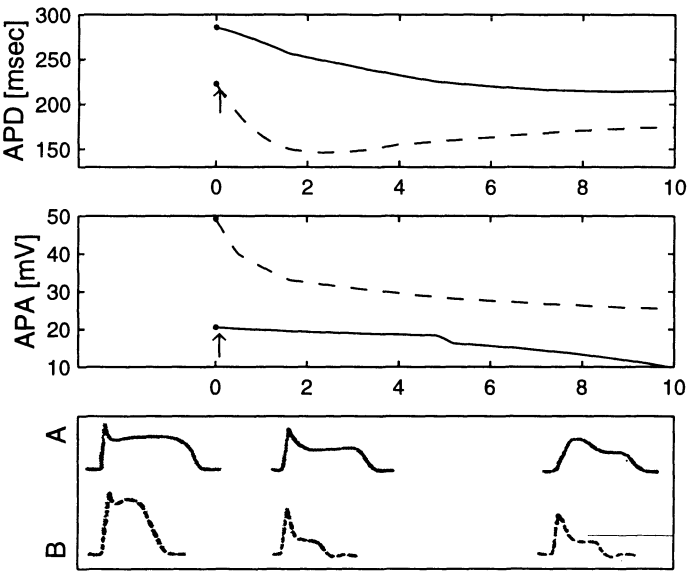

Figure 6.17. Changes in APD and APA in left ventricle of a canine heart after CAO with preliminary introduction of inderal (solid line) and without inderal (broken line). The shape of AP after CAP without inderal (A) and CAO with inderal (B). Arrow shows the time of CAO.

mg/kg and VF threshold and the local contractility were measured again. In the second series of experiments the antifibrillatory effect of these drugs was evaluated based on the frequency of spontaneous VF after CAO. The heart function was evaluated based on the changes in the cardiac output, arterial pressure and cardiac rhythm. The results of these experiments are presented in fig.6.20.

As can be seen from fig.6.20, addition of corglycon to propranolol almost eliminated the hypotensive effect of propranolol and considerably improved the cardiac output and local contractility of the intact myocardium. At the same time the antifibrillatory effect of propranolol was maintained after the addition of corglycon. VF threshold increased and frequency of spontaneous VF after CAO in this series decreased from 60% to 23%.

Thus, the antifibrillatory effect of propranolol is not dependant on its cardiac depressive and hypotensive effects.

6.1.6 Comparative characteristic of the studied antifibrillatory influences

Data on the antifibrillatory effects of substances we studied are summarized in fig.6.21.

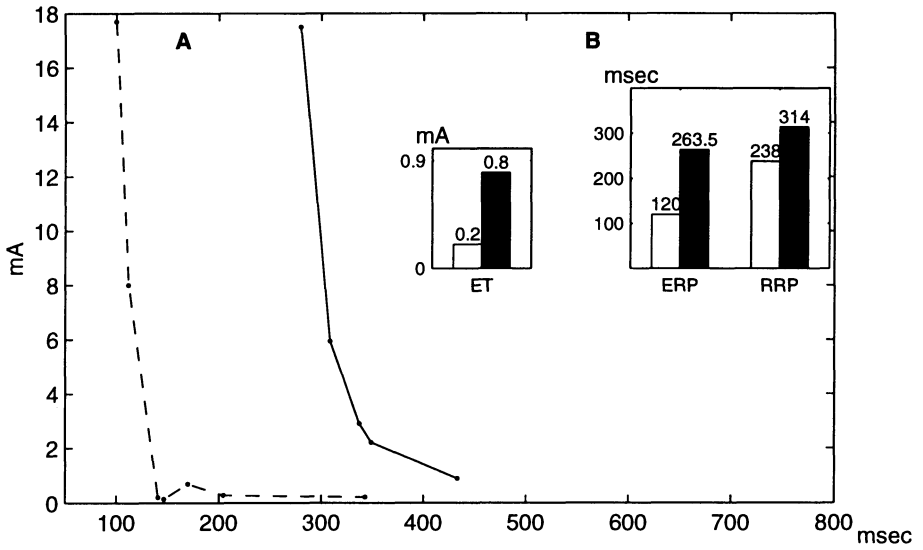

Figure 6.18. The curves of current force-interval of cardiac cycle left ventricle dog's heart before (broken line) and 15 min after (solid line) intravenous introduction of inderal (A). The excitability threshold (ET), effective refractory period (ERP) and relative refractory period (RRP) before (light columns) and after (black columns) introduction of inderal (B).

As can be seen from fig.6.21 the antifibrillatory effect can be obtained both by acting on various stages of metabolism and on the sympathoadrenal control of the heart.

The influences on metabolism (fig.6.21-A) was directed towards:

- the normalization of ox-red equilibrium through the activation of the respiration by means of hexahydroubiquinone-4 (1) and through glycogenolysis inhibition by means of monoiodacetate (2),
- pH stabilization by TRIS administration (3) and
- normalization of the ion equilibrium by means of the cardioplegic cocktail (4,5).

Thus, the chain of disturbances in the metabolic processes was interrupted at various levels and an antifibrillatory effect occurred in all of the cases.

The influences on *the sympathoadrenal system* (fig.6.22-B) were directed towards :

- the exhaustion of the noradrenaline stores from the granules by means of preliminary reserpine infusion (6),
- the inhibition of the uptake of adrenaline by the heart by administration of metanephrine (7) and

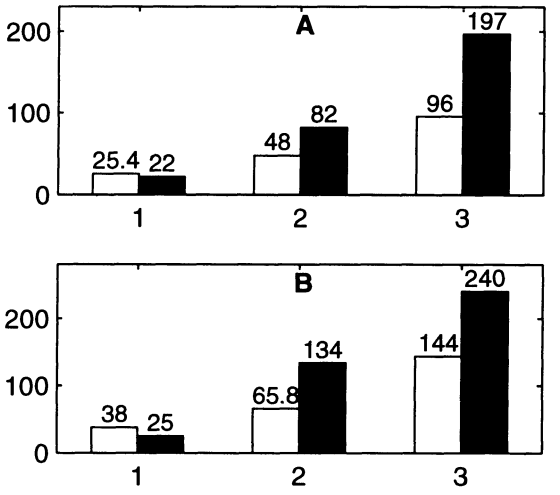

Figure 6.19. The dispersion of depolarization (A) and repolarization (B) after CAO without inderal (light columns) and with inderal (black columns): before CAO (1), after CAO without VPB (2) and before VPB (3).

- the blockade of the β-adrenoreceptors by means of propranolol both separately (8) and in combination with corglycon (9).

The above data indicate that there are very promising possibilities of pharmacological prophylaxis of VF after CAO by using drugs to prevent the disturbances in metabolism and sympathoadrenal regulation of the heart.

However, the use of antifibrillatory substances meets with serious difficulties and requires the development of special methods.

6.2 Method of computer control of the use of antifibrillatory substances

The main difficulties in using antifibrillatory drugs consist in the absence of VF precursors , in quickness and unexpectedness of VF appearance in acute stage of myocardial infarction. Therefore it is expedient to organize the automatic control for introduction the antifibrillatory drugs. Taking into consideration that changes occurring in the heart under influence of antifibrillatory substances are reflected in changes of the AP, we decided to control the introduction of antifibrillatory substances use the AP parameters.

In collaboration with the Institute Control Problem (Moscow) [154, 162, 161] we studied the possibilities of automatic control of intracellular AP parameters by antifibrillatory substances on the base of current information about changes in these parameters, i.e. in real time computerized experiments with feed-back. The elaboration of the method of the automatic control of AP parameters using of antiarrhythmic substances included:1) the search of the control criterion ;2)

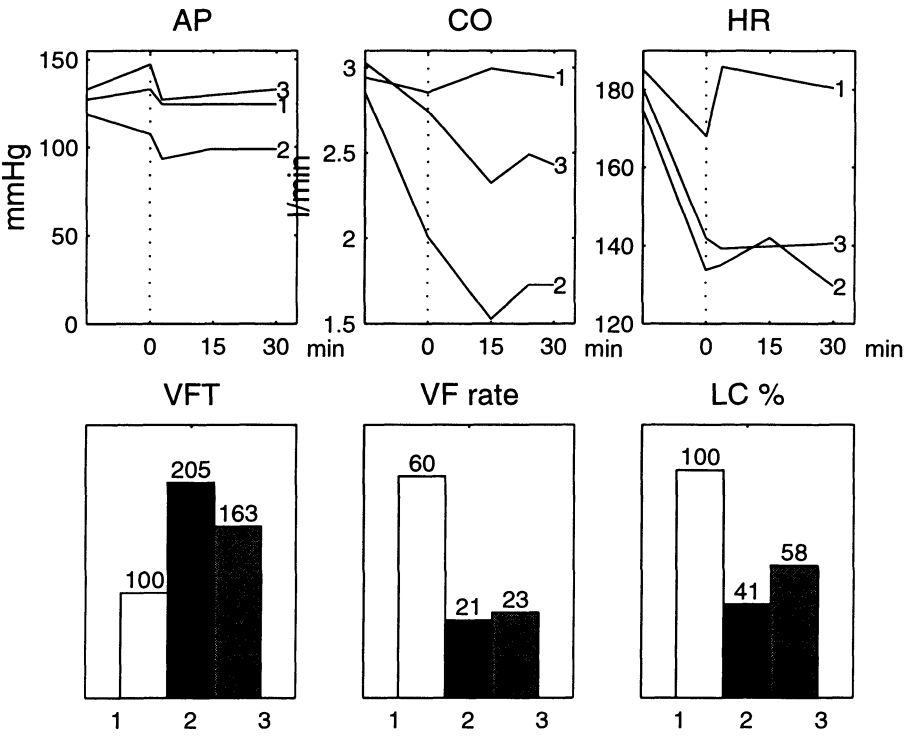

Figure 6.20. The influence of propranolol and propranolol (1mg/kg) with corglycon (0.02mg/kg) on the arterial pressure (AP), cardiac output (CO), heart's rate (HR), local contractility (LC) in an intact canine myocardium, VFT and VF rate before and after CAO. CAO without medication (n=12, white columns), CAO with propranolol (n=10, black columns), CAO with propranolol and corglycon (n=12, gray columns)

clarification of the quantitative relationship between this criterion and appearance of VF; 3) obtaining the data on the quantitative relationship between the dosage of antiarrhythmic substances and the changes in control criterion; 4) elaboration of the control system.

6.2.1 Control criterion of AP parameters - relaxation

This criterion was found in course of mathematical modeling (see chapter II). A mathematical model of a part of the myocardium was used to show that the probability of the development of excitation circulation (analogous to VF) and maintenance of circulation is determined by the excitation circulation radius (R). The increase in R makes the development and maintenance of circulation of excitation impossible. The radius of circulation of excitation can be determined through the period T of excitation circulation due to the direct proportional

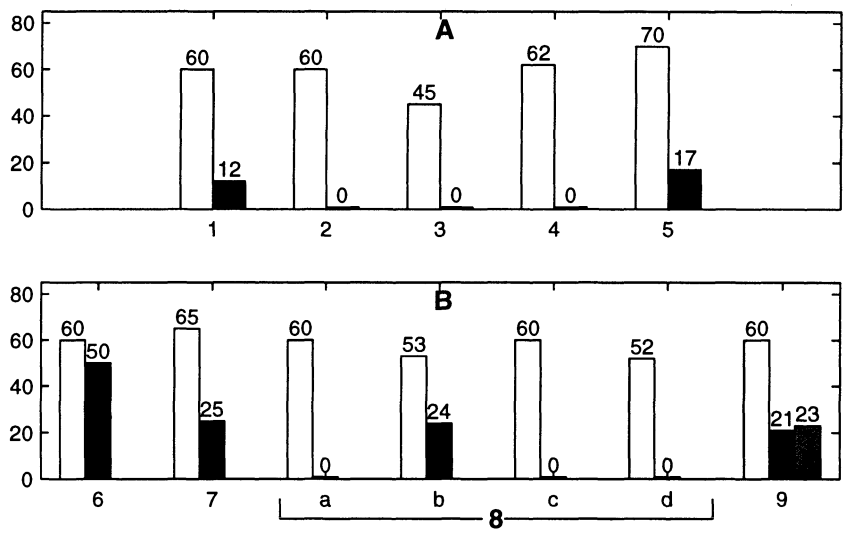

Figure 6.21. The comparative data on the influence of substances on the frequency of VF after CAO (% of total number experiments with CAO in different series). A - influences on metabolism, B - influences on the sympathoadrenal system. CAO without substances - light columns, CAO with substances - black columns. 1. Hexahydroubiquinone-4 with twin-80 (HU), 5-10 mg/kg intravenous, 15 min before CAO, $n_{control}$=10, n_{HU}=8, dogs. 2. Monoiodacetat (M), 20 mg intravenous 15 min before CAO, $n_{control}$=10, n_M=5, dogs. 3, TRIS (T), pH 7.4, introduction in perfusate on the model of ischemic perfusion of the heart's part, $n_{control}$=18, n_T=18,,dogs. 4. Cardioplegic solution (CS), 10-15 ml/kg intravenous, 20-30 sec after CAO, $n_{control}$=26, n_{CS}=25, dogs. 5. Cardioplegic solution, 8-10 ml/kg intracardial introduction, 20-30 sec after CAO, $n_{control}$=28, n_{CS}=14, dogs. 6. Reserpine ("Raucedil") (R), 0.5 mg/kg subcutaneous 24 hours before CAO, $n_{control}$=25, n_R=20, dogs. 7. Metanephrin (M), 50 μg/kg into coronary artery just before CAO, $n_{control}$=12, n_M=12, dogs. 8. Propranolol (Pr), 1 mg/kg intravenous 15 min before CAO: a/ series (study blood supply) $n_{control}$=29, n_{Pr}=12, dogs. b/ series (study mitochondrial metabolism),$n_{control}$=27,n_{Pr}=21,dogs. c/ series (study glycogenolysis), $n_{control}$=25, n_{Pr}=9,dogs. d/ series (study of pH,pNa,pCl,pK), $n_{control}$=42, n_{Pr}=42,dogs. 9. Propranolol (1 mg/kg) and corglycon (0.02mg/kg) (Pr+C) intravenous 15 min before CAO, $n_{control}$=12, white columns, n_{Pr}=10, black columns, n_{Pr+C}=12 (gray columns)

dependence between the them. The experiments with the mathematical models showed that T can be approximately determined by the AP parameters even before the circulation of excitation development. The period of circulation of excitation ($\check{T}$) can be approximately determined from the formula:

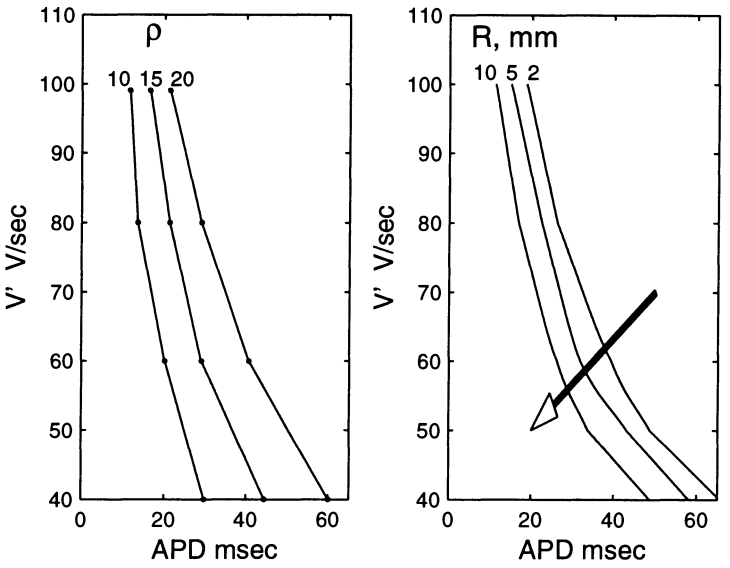

Figure 6.22. The dependance of relaxation (ρ) and radius circulation of excitation (R) on APD and $\dot{V}$. The family of curves ρ and R is given in stationary regime corresponding with the data of mathematical modelling.

$$\check{T} = \frac{APA}{\rho \times \dot{V}}, \quad where \quad \rho = \frac{APD \times \dot{V}}{APA} \tag{6.1}$$

The parameter ρ is called relaxation. The term "relaxation" was introduced in theory of nonlinear generators where it defines ratio of whole pulse duration to the duration of it upper front. By analogy with this theory the relaxation it was, it was suggested to use the relaxation coefficient as a generalized parameter of the AP [153].

The dependence of the relaxation coefficient and the radius of excitation on $\dot{V}$ and APD in stationary circulation (i.e. during stable arrhythmia) is given in fig.6.22.

As seen from this figure the dependance of R and ρ on APD are similar. Taking into account that the relaxation coefficient reflects the inclination of the myocardium to sustained activity, we chosen it as a control parameter.

The second task is consisted in clarifying how relaxation coefficient must be changed to prevent and to stop the circulation of excitation.

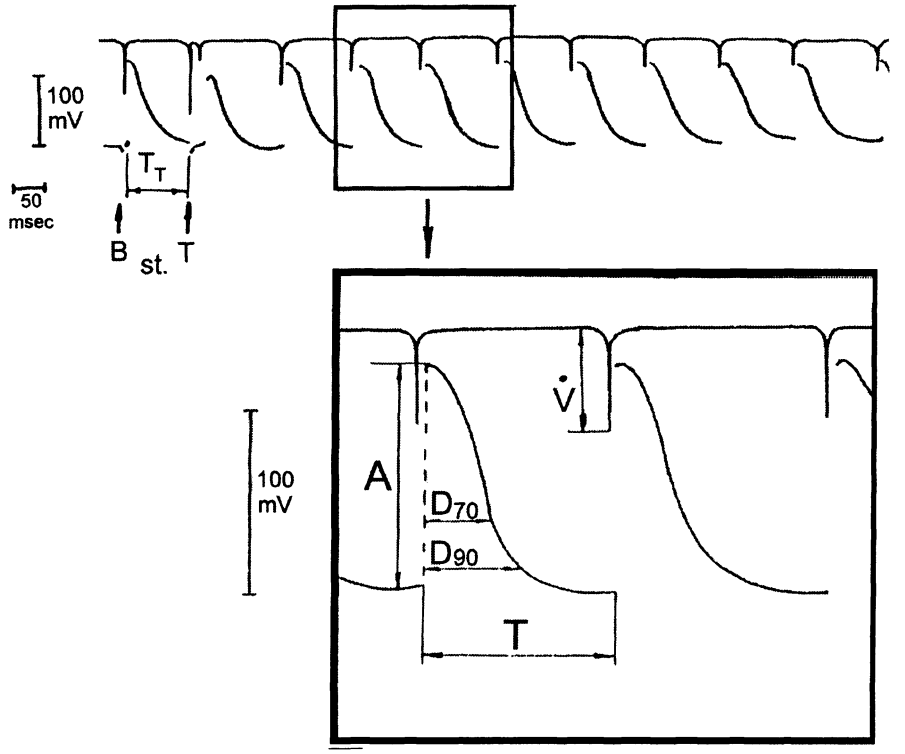

Figure 6.23. The example of provoked arrhythmia in left atrium of a rabbit heart. B and T st - basic and testing stimulation, Tt - testing interval, T - period of arrhythmias; A - amplitude of action potential; D_{90} and D_{70} - AP duration on 90% and 70% of repolarization, $\dot{V}$ - depolarization rate.

6.2.2 *The dependence between relaxation coefficient and susceptibility to circulation of excitation*

This dependence was studied on an isolated left atrium of a rabbit. We succeeded in reproducing the multiple activity of the re-entry type by means of pacing at the vulnerable period of AP and by measuring the parameters necessary for the control. Arrhythmia was caused by the method developed in [11]. A 2 Hz rectangular wave form with a duty cycle of 3 ms was used for main pacing. The testing stimuli were applied after 12-24 main stimuli at the vulnerable phase of AP. The parameter $\dot{V}$ was measured by the height the peek of the differentiated signal of the second oscillograph ray. An example of artificially provoked arrhythmia and a schematic illustration of the measured parameters is given in fig.6.23.

When the stimulus was applied, both short (less than 10 extrasystole) and long (from 10 to 100 and more extrasystole) arrhythmias developed. To clarify the dependence between the susceptibility to VF and AP parameters, we

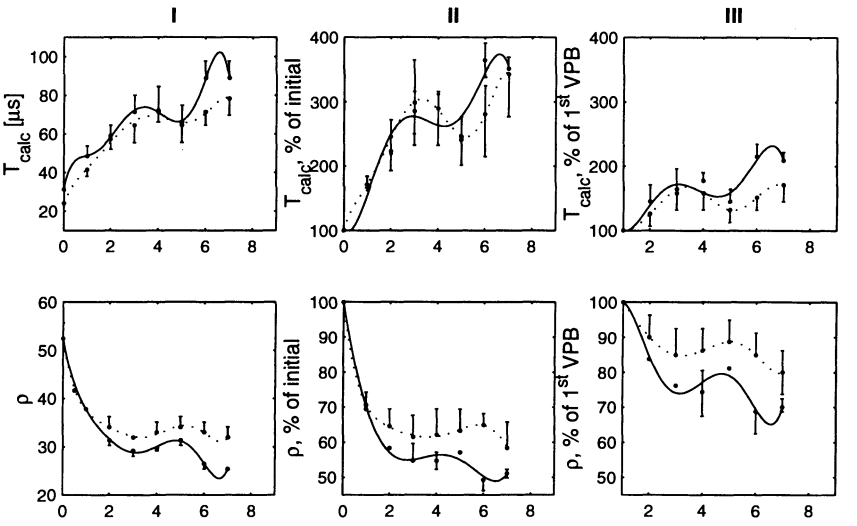

Figure 6.24. I. The changes in relaxation (ρ) and calculated circulation period ($\check{T}$) in seven first VPB in cases with prolonged (broken line) and short (solid line) arrhythmia. II. Parameters relative to those during the initial VPB. III. Parameters relative to those during the first VPB.

analyzed the differences between the AP parameters and their generalized characteristics (ρ and $\check{T}$) in the short and long arrhythmias, given the consideration that the susceptibility to VF is greater in cases with long arrhythmias than with short.

An analysis of calculated ρ and $\check{T}$ in short and long arrhythmias was performed. The results of this analysis for the first 7 extrasystole are presented in fig.6.24. Considerable differences in short and long arrhythmias were revealed in the parameters ρ and $\check{T}$.

In short arrhythmias a sharper increase in $\check{T}$ and especially sharp decrease in ρ were observed. The data obtained allows to deduce that the pharmacological substances used to decrease the susceptibility to circulation of excitation must lower the relaxation coefficient.

6.2.3 Effect of antiarrhythmic substances on relaxation coefficient

Two substances, the membrane depressant quinidine and β-adrenoblocker propranolol were used for the control of the AP parameters of the myocardial cells. The changes in the values of relaxation coefficient together with the changes in AP parameters (average data) are presented in fig.6.25.

As is apparent from the figure, ρ decreases in response to the action of both quinidine and propranolol, i.e. it changes in the direction required for the

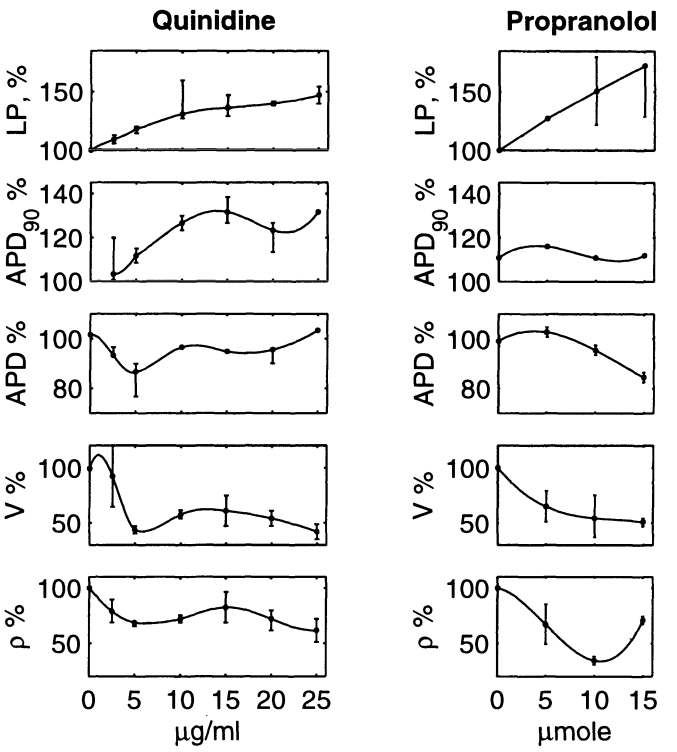

Figure 6.25. The influence of quinidine and propranolol in different doses on relaxation coefficient and on AP parameters of papillary muscle of a cat 10 min after introduction of the drug in % of the initial level (average data), $n_{quin.}$=49, $n_{propr.}$=13.

antifibrillatory effect. Quinidine was chosen for the control because it had a more pronounced effect on AP parameters - $\dot{V}$ and APD.

Thus, the development of the control criterion, the information obtained about the direction it needs to change for the realization of the antiarrhythmic effect, and the data of the influence of antiarrhythmic substances on the control criterion allowed us to develop a scheme of automatic control of AP parameters in such a way as to prevent or stop the arrhythmia.

6.2.4 *Computer control of myocardial action potential based on the values of relaxation coefficient*

Computer control of the AP parameters of the isolated papillary muscle of a cat included the following stages:

1. Continuous observation of the intracellular AP in real time, translation the results through the communication network to the computer system, calculation of the control criterion based on the AP parameters.

2. Choice of ρ values which must be reached at each determinate moment to avoid and to depress circulation of excitation on the base of the data obtained on the mathematical model of a myocardial part:
3. Choice of the quinidine dose that is necessary in order to reach the required level of AP parameters and ρ; this choice was made based on the data about the effects of various quinidine doses on the AP parameters and ρ stored in the computer memory.
4. The computation of the control signal in real time is based on:
   (a) analysis of current changes in AP parameters and value of ρ, calculated on the base of measured AP parameters;
   (b) chosen of ρ value, which has to be reached in process of drug introduction and
   (c) data about the effects of various quinidine doses on the value of ρ.
5. Sending the control signal through the network to the special dose making device;
6. Introduction of the chosen quinidine doses into the perfusion medium and their correction every 10 min.

An example of an automatic control of the AP parameters based of ρ criterion is illustrated in fig.6.26.

In this example the value of ρ had to be lowered by 50% of the initial level in order to increase circulation of excitation radius and to reach an antiarrhythmic effect. During the first ten minutes of the retracing the AP parameters, the level ρ=75%was chosen as the initial one (the point-hatch line), while the level to which it had to be lowered was ρ=37% what made up 50% of the initial level(pointed line).

The control by means of computer began with a small dose (1.5 μg/ml). This dose provoked a marked increase in APD, a slight increase in APA and a slight decrease in $\dot{V}$. As a result of these changes the value of ρ decreased somewhat. After the next dose of 11 μg/ml the APA remained unchanged, while $\dot{V}$ went down substantially and the APD increased. Due to these changes, the value of ρ reached the chosen level (50% of the initial) and was sustained at the chosen limits by a quinidine dose of 12-13 μg/ml. Under these conditions the APD was sustained at a considerably heightened level, the APA at a somewhat lower and $\dot{V}$ at a considerably lower levels. The chosen level of ρ was reached without overcontrol.

The method of computer control of the intracellular AP parameters is still very far away from clinical use. However, further investigations of this subject could result in development of a method suitable for use in the hospitals. This will open possibilities of an non-invasive method in choosing antifibrillatory drugs and their adequate doses based on the dynamic information taking from the heart. So, there is a real possibility for VF prophylaxis in acute myocardial infarction in the hospitals.

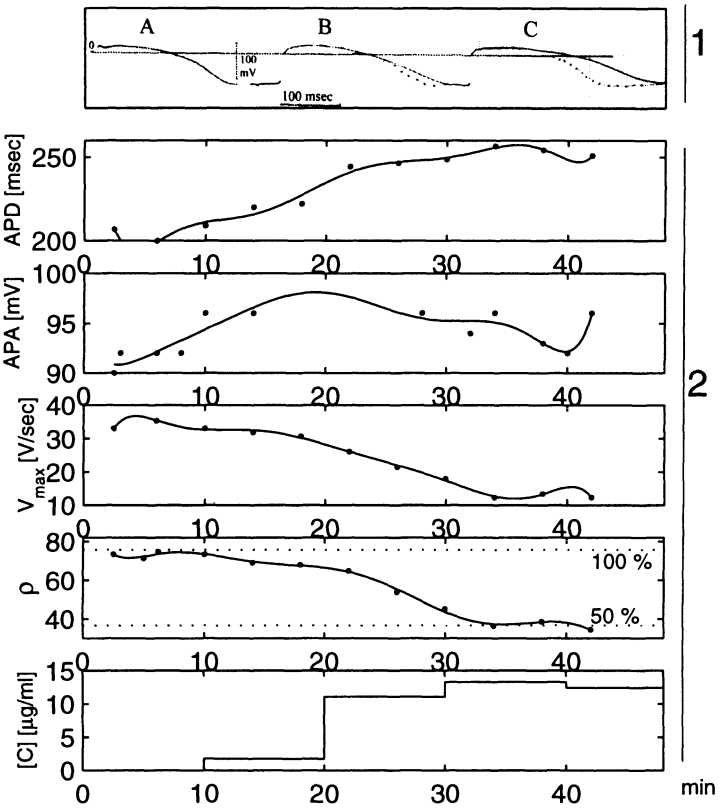

Figure 6.26. The computer control of AP parameters using quinidine. The parameter ρ is calculated in real time. 1. The dynamics of AP changes in course of control. A - is initial AP, B - is AP in process of achievement the chosen value of ρ, C - is AP when chosen value of ρ is achieved (in B and C initial value AP is indicated by dots. 2. The dynamics of APD,APA, $\dot{V}$, ρ changes in the course of control. 100% - is taken as initial value of ρ, 50% - is a chosen value of ρ. [C]- is quinidine concentration in the perfusion camera.

The problem of VF prophylaxis out-of-hospital is considerably more complicated and important. It is more complicated because VF often develops suddenly in practically healthy people, not undergoing medical care. VF prophylaxis in these cases is more important because the majority of cases of VF occurs out-of-hospitals. Among people younger than 65 years only 18% died from VF in the hospital, while 82% died, at home, at work, and in other places [352].

Therefore, the VF prophylaxis must be performed first of all among the people out-of-hospitals. Thus, requires the determination of VF risk group and the development of VF prognostication methods.

6.3 The methods of prognostication of ventricular fibrillation

The hypotheses about VPB as a risk-factor of sudden cardiac death (VF) were not confirmed by the special CAST investigation (Cardiac Arrhythmias Suppression Trial).

The decisive role of the autonomic nervous system in the genesis of sudden death and VF was shown in numerous experimental and clinical investigations [24, 316, 190, 7, 208]. Therefore, quantitative characteristics of the autonomic activity were used as methods of prediction of VF and sudden death.

6.3.1 The methods of prognostication of ventricular fibrillation by means of determination of the heart rate variability and baroreflex-sensitivity

During recent years, two non-invasive tests for quantitative assessment of cardiac autonomic tone have become available: analysis of heart rate variability (HRV) from 24-h ambulatory recordings and determination of baroreflex-sensitivity (BRS) by means of the phenilephrine method [160, 130].

The multicenter prospective study ATRAMI has shown that both HRV and BRS are strong and independent risk factors for post- infarction mortality [322]. The pharmacological modulation of autonomic control of vagal activity was able to reduce risk for lethal arrhythmias after myocardial infarction [321]. Vulnerability to VF was shown to increase and BRS to decrease in experiments on dogs. The administration of a benzodiazepine and a powerful analgesic (analgosedation) augmented both BS and VF threshold [336]. The methodology of baroreflex testing in man is described [158]. The daily physical training provoked the increase in the baroreflex test and prevented VF [70]. It was shown that vagal reflex may also identify high risk of death before myocardial infarction [71]. The protective effect of vagal activity was confirmed in experimental study with muscarinic stimulation, both electrically and pharmacologically induced [324]. On the other hand, sympathetic hyperactivity was demonstrated to be a coronary risk factor. A significant relationship was observed between the circadian pattern of plasma catecholamine level and the onset of coronary events [98].

The HRV may now be measured from 24-hour ECG recordings (Holter monitoring), in the time domain (by statistical indexes: mean RR interval, standard deviation and the coefficient of variance) and in the frequency domain (spectral analysis). This non-invasive method was used in numerous mainly clinical studies [132, 113, 199, 293, 91]. The reduced 24-hour heart rate variability is an independent predictor of sudden cardiac death. The attenuation of HRV indicates a reduced vagal and concomitantly high sympathetic activity [197].

The depression in HRV produced by myocardial infarction in dogs, was clearly different between low- and high risk of VF animals [3]. The abnormal

HRV provides evidence that altered neurohumoral regulation is an important trigger mechanism for the spontaneous onset of life-threatening arrhythmia [131]. It has been shown that HRV of patients who have had an myocardial infarction is very similar to that of a heart transplant recipient [263].

The comparison of the methods BRS and HRV has shown that BRS is more valuable than HRV in prediction of sudden cardiac death and VF [159]. However, the clinical utility of these tests has been limited by their low sensitivity [24].

6.3.2 *Method of prognostication of ventricular fibrillation by means of insulin test*

In chapter IV we shown that VF develops in animals with a heightened adrenaline secretory function of the adrenal glands. This feature of animals with a greater susceptibility to VF after CAO was taken to be a starting-point for the development of a method of prognostication of VF. The essence of the method for VF prediction in CAO consists in measurement of A secretion by the adrenal glands of normal animals before CAO in response to a standard stimulus. Insulin administration was used as a standard stimulus.

The use of the insulin test is based on the properties of insulin to activate the parasympathetic elements of the vegetative nervous system. The reaction of the organism to this activation is an increase in sympatho-adrenal activity which behaves so as to avoid shifts in the internal medium of the organism. The insulin test was used under various conditions: during hypertension, diencephalic syndrome, in an altitude chamber [148, 149].

We used the insulin test to clarify the dependence of VF appearance on the A secretory function of the adrenal glands after CAO [240]. For this purpose insulin was administered to 12 dogs two days before CAO in doses of 0.2 U/kg of animal's weight. Arterial blood samples were taken for A and NA measured at the 10th and 30th min after insulin infusion. CAO was triggered in all the dogs two days later. VF developed in 7 out of 12 dogs. The results of the experiment were separated into two groups of animals: with and without VF. The data obtained are given in fig.6.27 As can be seen from fig.6.27-I the concentration of A increased during the first 10 min after insulin administration and at the 30^{th} min returned to the initial level in both groups of dogs.

The changes in concentrations of A and NA after CAO, two days following the insulin administration are given in fig.6.27-II. As can be seen from the figure, the dogs that react to insulin administration by a considerable increase in A secretion, developed a considerable hyperadrenalemia in response to CAO, and at its peak VF developed. On the contrary, in dogs which responded to insulin only with negligible A secretion, the CAO caused only a moderate hyperadrenalemia, the level of which became almost stable at the 10^{th} min and no VF developed. The increase in concentration of A in response to insulin in experiments with VF was 10 times greater than in experiments without VF and measured 200%, but without VF only a 20% increase was observed($p<0.001$).

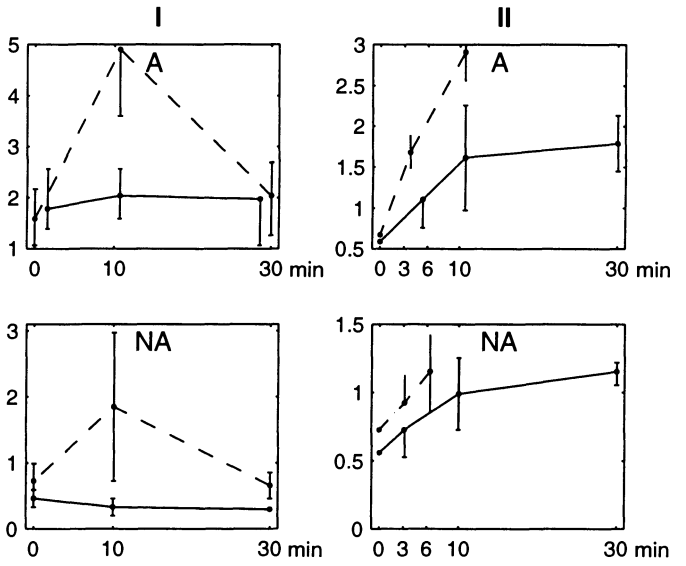

Figure 6.27. I - the time course of changes in concentration of A and NA in arterial blood of normal dogs after intravenous administration of insulin (0.2 U/kg). II - the time course of changes in concentrations of A and NA after CAO following 2 days after insulin administration in the same dogs in experiments with VF (n=7, broken line) and without VF (n=5, solid line)

We offered the insulin test as a means of selection of a group of animals, and probably of people out-of-hospital with a high risk of VF after CAO [239].

6.4 Evaluation of effectiveness of antifibrillatory influences

The effectiveness of antifibrillatory substances can be evaluated only directly by their antifibrillatory activity. However, its evaluation in hospitals is difficult because the low VF frequency does not allow to obtain the statistically significant data. Its evaluation out-of-hospital is needed to determine the rate of VF occurrence in the two groups of the population that use and do not use antifibrillatory drugs. The sudden death from VF occurs very often in the absence of medical personal and the diagnosis of VF when the person is alive can not be established. Thus, VF must be diagnosed postmortem.

6.4.1 Method of postmortem diagnosis of ventricular fibrillation in cases of sudden death from acute coronary insufficiency in the population

Such method was developed by us in collaboration with the Krasnoyarsk Medical Institute and with medicolegal bureau in Moscow, Krasnoyarsk and Kiev

[23, 249, 51, 205, 10]. Sudden death as a result of VF from acute coronary insufficiency occurs in the majority of cases out-of-hospital. Persons who have died from VF most often are the subjects of the medicolegal examination.

The morphologic changes in the hearts of these persons, specific to myocardial infarction have not had the time to develop. On the other hand, the histochemical changes specific to ischemia are masked by autolytic changes [23]. In these cases the medicolegal examination does not dispose of objective criteria to ascertain not only the direct cause of death - VF, but even to reveal some signs of local ischemia, which provoked VF. So, we developed methods of postmortem diagnosis of local ischemia and VF in cases of sudden death. They are presented separately below.

6.4.1.1 Postmortem diagnosis of acute coronary insufficiency. It is not surprising that the initiative in the development of a method of postmortem detection of local ischemia in cases of sudden death was taken by medicolegal examiners [49, 50, 359]. The first of these works was carried out by Chait [49].

The distribution of K and Na in the heart was studied by means of flame photometry in the hearts of 79 corpses of persons who had suddenly died of acute coronary insufficiency (according to the documents) and in hearts of 65 corpses of persons who had died from trauma. These hearts are considered as control.

The significance of the time elapsed from death until the autopsy was studied in special investigations. No substantial postmortem changes in K and Na contents were shown to occur after 6,12,24,32 and 48h after death because ions only transpose between intra- and extracellular media, but do not leave the body after circulation stops.

Since the location of the ischemic zone was unknown, in all cases samples of myocardium were taken from 8-10 areas of the heart and a sample from musculus rectus abdominous. The diagram of the sampling presented in fig.6.28.

No statistically significant differences in content of K were observed in "practically healthy" people independent of sex and age (between 20 and 85 years). These people died during every-day activities due to external causes. The K content in their hearts was taken to be "normal". The content of K in the left ventricle of the control group proved to be higher than in the right one. The content of K in left and right ventricles, on average, is 247 and 176 mg% in all the age groups, respectively.

The average values of content of K in the left ventricle (in the areas of maximum decrease of K), in the right ventricle and in skeletal muscle of persons who died from acute coronary insufficiency compared with the respective areas in hearts of members of the control group are shown in fig.6.29.

As seen from this figure the K content in persons who died from acute coronary insufficiency dropped most sharply in the left ventricle, less sharply in the right ventricle and still less sharply in musculus rectus abdominous.

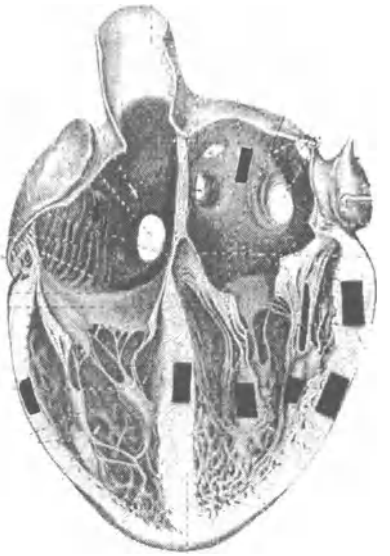

Figure 6.28. The parts of the heart where the content of K was analyzed

The decrease in K content in some zones of the left ventricle after sudden death and the data of catamnesis, makes it possible to diagnose postmortem the acute coronary insufficiency as the cause of sudden death.

This method, based on our suggestion, was recommended by the World Health Organization in 1969 [23] for postmortem diagnosis of acute coronary insufficiency. In subsequent investigations the method received further confirmation [205]. In 1982 the Health Ministry of the Ukrainian SSR issued special methodic recommendations. The local decrease in K concentration in the left ventricle and in the interventricular septum to below 200 mg% (162 mg% on average) in cases of sudden death is regarded as a sign of acute coronary insufficiency.

However, the postmortem diagnosis of acute coronary insufficiency in cases of sudden death is not yet sufficient for the affirmation that the death resulted from VF.

There are at least five main causes of death in the acute period of myocardial infraction: cardiogenic shock (collapse),heart failure, thromboembolism, heart rupture and, finally, VF. Two of these causes: heart rupture and thromboembolism can be determined postmortem during autopsy. The remaining three causes: heart failure, cardiogenic chock and VF, if they develop in the first hours of the disease, can not be identified during autopsy because of the absence of specific changes in the heart.

However, as it is well known, death due to VF differs from death due to heart failure and of cardiogenic shock because it happens suddenly and unexpectedly, while death from heart failure and from cardiogenic shock occurs slowly with an agonal period. We supposed that the determination of how fast the death

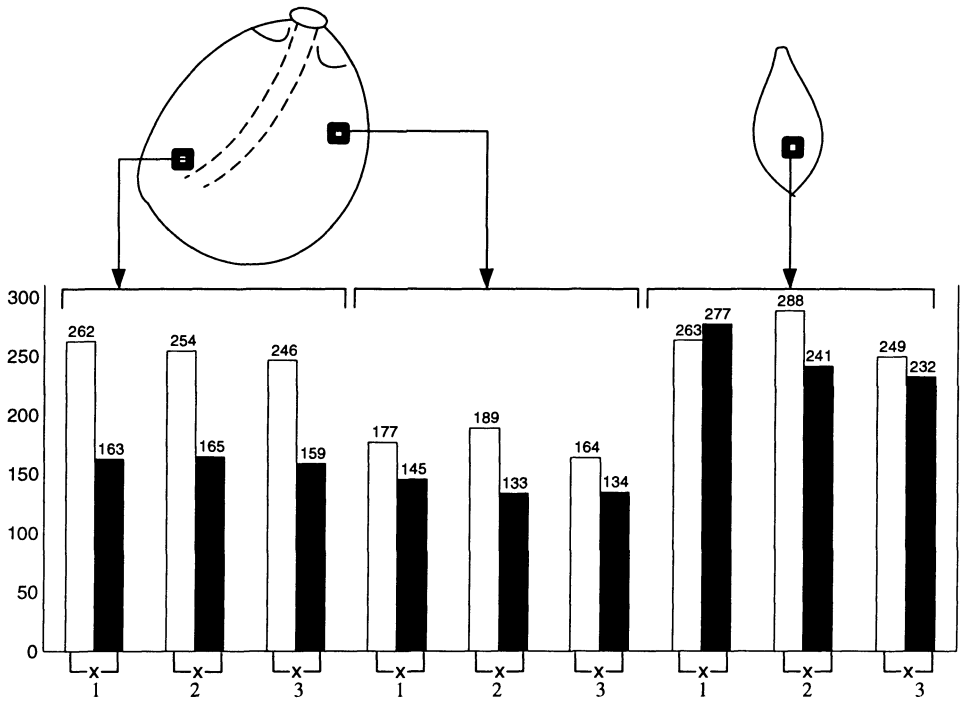

Figure 6.29. The K content in parts of left ventricle with the most (maximum) decrease of K (LV), in the right ventricle (RV) and in musculus rectus abdominous (MRA) of persons dying suddenly from acute coronary insufficiency (ACI, black columns) compared with traumatic death, "normal"(N, light columns) in three group different age: 1 - 20-39 (n_{ACI}=14, n_{N-LV}=34, n_{N-RV}=29, n_{N-MRA}=22); 2 - 40-59 (n_{ACI}=29, n_{N-LV}=12, n_{N-RV}=13, n_{N-MRA}=10); 3 - 60-85 (n_{ACI}=36, n_{N-LV}=16, n_{N-RV}=15, n_{N-MRA}=14. x -shows statistically significant difference

occurs can be used for postmortem diagnosis of VF. Based on these premises, a method of postmortem VF diagnosis was developed.

6.4.1.2 Postmortem diagnosis of ventricular fibrillation. There is an opinion that VF is a "killer" that does not leave any traces after himself. We made an attempt to discover these traces by studying the distribution of K in the heart.

From the analysis of Chait's data (fig.6.29) we notice that K concentration in the left ventricle in cases of sudden death sharply decreased, while in the right one and especially in the skeletal muscle the decrease was completely negligible. Meanwhile the postmortem hypoxia had to provoke the loss of K by all the tissues of the organism. It did not happen, however, because as a result the sudden ceasation in blood circulation K was transferred from the cell

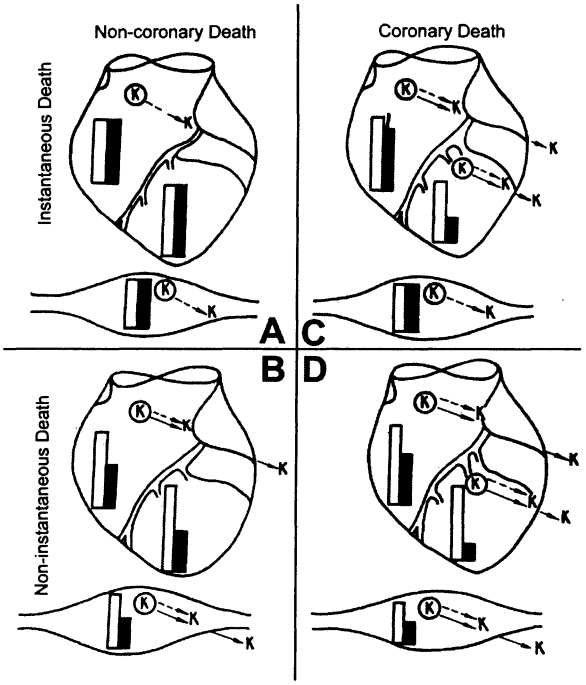

Figure 6.30. The schematic presentation of distribution of K in the heart and skeletal muscle in cases of instantaneous and non- instantaneous coronary and non-coronary death. Light columns - normal K content in the cardiac and skeletal muscle, black columns - K content in the cardiac and skeletal muscle in respective types of death. Outflow of K, that occurs when the person is alive is indicated by a solid arrow, posthumous outflow of K is indicated by a broken arrow (explanation in text).

into the extracellular medium, but was not washed out by the blood flow and remained in the tissue.

Thus, K concentration in right ventricle and in skeletal muscle can indicate if the death occurred instantaneously or slowly and if hypoxia in the heart developed in life or postmortem. The distribution of K into the heart and into the skeletal muscle may occur in four ways (fig.6.30).

In absence of local myocardial ischemia if the death takes place instantaneously *"instantaneous, non- coronary death"*, K is transferred postmortem from the cell into the extracellular medium and is retained there because the blood circulation stops abruptly. In this case K content in the heart and the skeletal muscle remains unchanged (fig.6.30-A).

However, if the non-coronary death does not occur instantaneously and is followed by the agonal period *"non-instantaneous non-coronary death"* K which moves from the cell into the extracellular medium when a person is alive, is washed out by the blood because of the sustained blood circulation during the

agonal period. As a result, concentration of K in all parts of the heart and in skeletal muscle decreases (fig.6.30-B).

During local myocardial ischemia if the death occurs instantaneously *"instantaneous, coronary death"* distribution of K is very non-uniform because the loss of K by the cells in ischemic zones occurs in continuing blood circulation and K is washed out from tissue. Meanwhile in the intact areas and in the skeletal muscle the content of K remains almost unchanged because its postmortem transfer from the cells into the extracellular medium is not accompanied by it being washed out from the tissue by blood (fig.6.30-C).

In cases of *"non-instantaneous coronary death"* the non-uniformity of distribution K is less pronounced. The content of K in areas of ischemia that takes place when the person is alive is somewhat lower than in cases of instantaneous coronary death because in addition to being washed out as the result of local ischemia, K is also washed out during the agonal period. Substantial decrease in the concentration of K is also observed in the intact heart areas and in the skeletal muscle (fig.6.30-D).

If these premises are correct, the K content in non-ischemic zones of the heart and in skeletal muscle of persons who died of VF must be less pronounced than in persons who died of heart failure and cardiogenic shock.

Experimental investigations were run in our laboratory [10] in experiments on 35 dogs with CAO in order to verify the premises above. CAO was induced through ligation of the left anterior descending arteria at the upper third. Three series of experiments was carried out to show the differences in the distribution of K in the heart and in the skeletal muscle in cases of instantaneous death from VF and in cases of death from acute heart failure.

In the first series of experiments (15 dogs) VF developed spontaneously on average 8.8 min after CAO; in the second series (14 dogs) general hypoxia was provoked by decreasing the respiratory volume by 2/3 practically simultaneously with the occlusion; this led to a gradual decrease in the arterial pressure and resulted in death, on average, 9 min after CAO. In the third series(control, 6 dogs) all stages of the experiment were reproduced, except CAO and hypoxia. The data obtained are illustrated in fig.6.31.

As can be seen from the figure 6.31 the content of K in the left ventricle and in the interventricular septum was statistically significantly higher in experiments with CAO that ended with VF, than in experiment with a gradual development of heart failure, although the time elapsed in the series was the same 8.8 and 9 min).

Similar results were obtained in analysis of human corpses. The samples were obtained from the 5th section of Medicolegal Bureau of Moscow 24 h after the autopsy, which, in turn, was performed within 48 h of the death. Samples (heart, muscles rectus abdominous) of 41 corpses were studied [10]. The experimental material was classified into three groups according to catamnesis data (information obtained from eye-witnesses of the death, from ambulance personnel), macroscopic examination (presence and expressiveness of pulmonary and brain edema, blood supply of the myocardium and other organs) and mi-

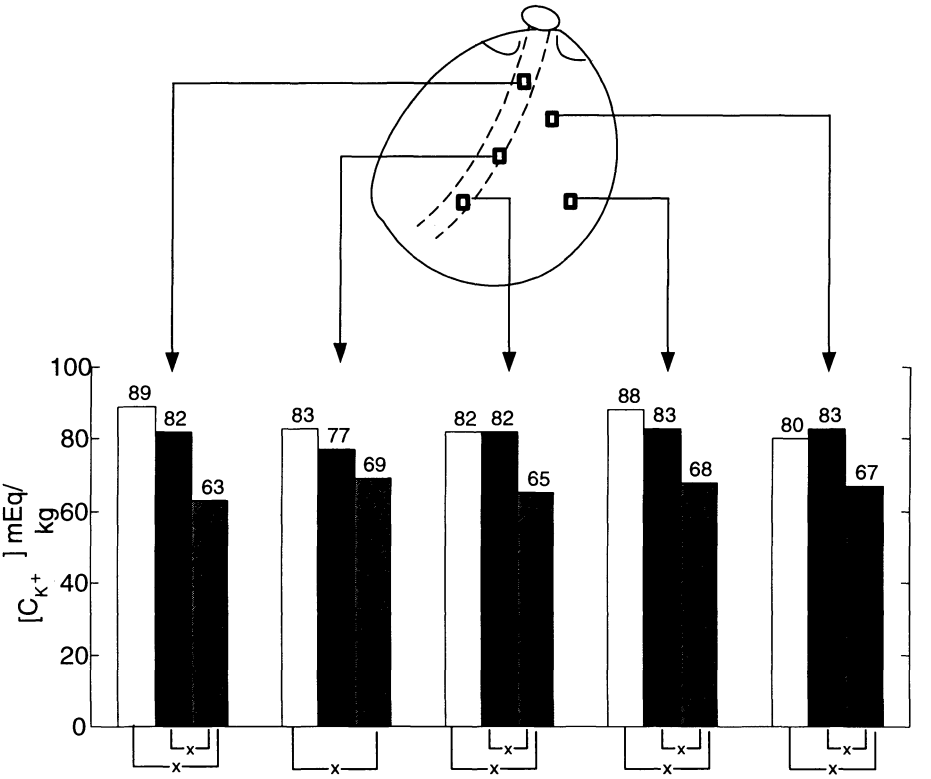

Figure 6.31. Content of K in left ventricle and interventricular septum of a dog's heart after CAO with VF (black columns, n=15) and with acute coronary insufficiency, provoked simultaneously by CAO and general hypoxia (gray columns, n=14) compared with control (without CAO and hypoxia,light columns, n=6). Average data. x - shows statistically significant difference.

croscopic examination of the liver which allows to determine reliably how fast the death occurred.

The first group (16 persons) consisted of persons who died instantly of acute coronary insufficiency in urban transport, in stores, on the street, at work, during skiing, walking, on rail platform and other places before the ambulance arrived, as well as in a out-patient clinic, in a pharmacy before medical help arrived. The second group (16 person) consisted of a person who died from acute coronary insufficiency with a short agonal period. The third (control)

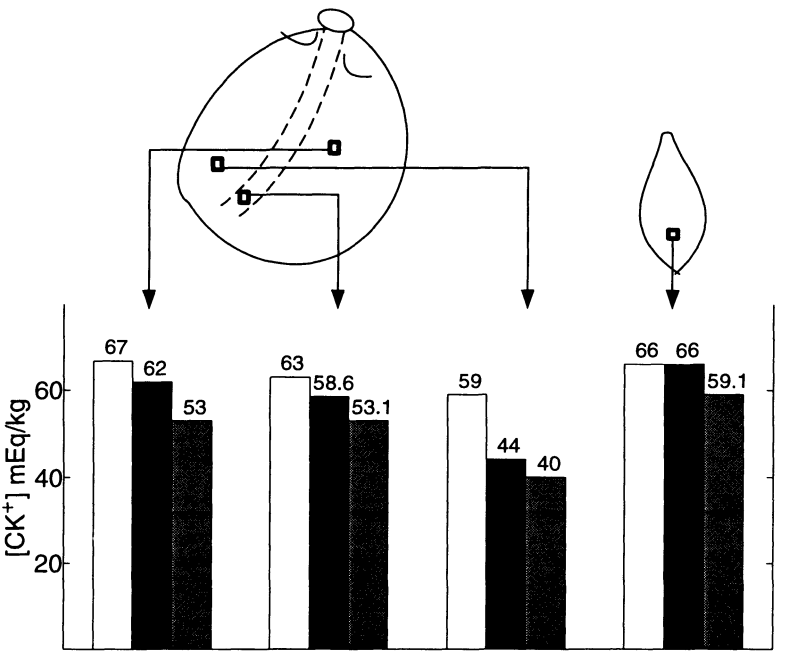

Figure 6.32. The content of K in the left and right ventricles, interventricular septum of the heart and in skeletal muscle corpses of persons who died from acute coronary insufficiency instantaneously (black columns, n=16) and non-instantaneous with agonal period (gray columns, n=16) compared with persons who died instantaneously from non-coronary diseases (light columns,n=9). Average data. x - shows statistically significant difference.

group consisted of 9 persons who died instantly of non-coronary pathology (fatal railroad accidents). The results obtained are illustrated in fig.6.32.

As was expected, the results based on corpse samples were less expressed than in the experimental study. However the content of K in the heart and in the skeletal muscle was higher in cases of instantaneous death than in cases of death with an agonal period. These differences were statistically significant in the left ventricle and in the skeletal muscle.

Distinct data were obtained on samples of the Krasnoyarsk Medicolegal Bureau [249, 272]. To determine the dependence of K distribution in the heart on how long it took for the death to, the 67 cases were divided into four groups.

The first group consisted of 34 persons who died out-of-hospital. The study of catamnesis, of death circumstances effectuated by the ambulance physician, allowed to ascertain that death came instantaneously accompanied by phenomena of acute coronary insufficiency.

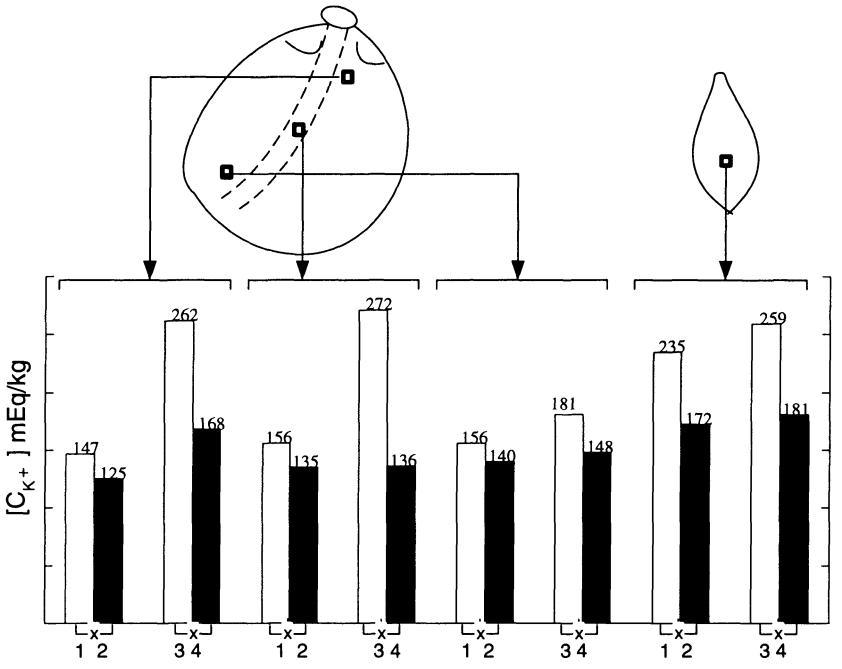

Figure 6.33. The K content in left and right ventricles, interventricular septum of heart and skeletal muscle of corpses of persons who died instantaneously from coronary insufficiency (1,light columns, n=34) and non-instantaneous (2, black columns n=11); of persons who died instantaneous from non-coronary diseases (3, light columns, n=10) and non-instantaneous (4, black columns, n=12). x - shows statistically significant difference.

The second group consisted of 11 persons who died in the hospital with the diagnosis of myocardial infarction, complicated with a shock and with death occurring after several hours.

The third group was a reference group in relation to the first group. It consisted of 10 persons who died instantaneously from the non-coronary pathology: craniocerebral trauma, gunshots or stabbing wounds to the head.

The fourth group was used as reference for the second one; it consisted of 12 persons who died with an agonal period of non-coronary diseases.

The obtained data are shown in fig.6.33.

The majority of deaths were due to the left coronary insufficiency; therefore the left ventricle and the intraventricular septum was considered as the ischemic zone, while the right ventricle was regarded as the non-ischemic zone. The K

content found in cases of instantaneous non-coronary death (3d group) was taken as "normal"; the K content in other cases was expressed in percentage of "normal" level.

The dependence of K distribution in the myocardium and in the skeletal muscle on coronary changes is made apparent by comparison of the first with the third and the second with the fourth groups.

The differences in K content in coronary and non-coronary deaths are expressed most sharply in the left ventricle and in the interventricular septum, i.e. in the ischemic zone in cases of instantaneous death. The K content in these areas decreased respectively to 56 and 57% of the "normal," while in the right ventricle and in the skeletal muscle it decreased only to 86 and 91%.

The dependence of K distribution in the myocardium and in skeletal muscle on the time it takes for death to occur is revealed by the comparison of the first with the second and the third with the fourth groups, which differ from each other only by how fast death occurred. The difference between instantaneous and non-instantaneous non-coronary death consisted of the fact that in case of instantaneous non-coronary death (group 3) the content of K in left ventricle remains unchanged, while in case of non-instantaneous non-coronary death (group 4) it decreases, but it is not clear that K content in the various areas of the heart and in skeletal muscle is non-uniform.

The differences in the K content between instantaneous and non- instantaneous death are of most interest. The K content in the left ventricle and in the interventricular septum goes down almost by the same degree both in cases of instantaneous (group 1) and non-instantaneous (group 2) deaths, while in the right ventricle and in the skeletal muscle considerable differences are revealed: small decrease in cases of instantaneous and a considerable decrease in cases of non-instantaneous coronary death.

A different relationship between the content of K in the left ventricle and in the skeletal muscle exists for each type of death. If the content of K in the left ventricle and in the skeletal muscle is close to normal and the difference between the two is close to 0, this indicates an *instantaneous, non-coronary death.*

If the content of K in the left ventricle is lower than normal and the K content in the skeletal muscle is close to normal, but the difference between the two is small, this indicates an *non-instantaneous, non-coronary death.*

If the content of K in the left ventricle is considerably lower than normal, while in the skeletal muscle it is close to normal, this indicates on *instantaneous, coronary death.*

If the content of K in the left ventricle is much lower than normal, but it is also lower in the skeletal muscle, in result of what not very great difference between the two is observed, this indicates an *non-instantaneous, coronary death.*

Thus, two main signs exist for the determination of the character of death: 1) the absence of substantial changes in the content of K in the skeletal muscle

	Death due to AIHD				Percentage of VF among patients who:	
	n	%	due to VF		died of AIHD (n=671)	survived AIHD (n=1515)
			n	%		
Out of hospital	508	75.7	373	94	73.7	24.5
In hospital	163	24.3	24	6	14.7	1.6
Total	671	100	397	100	59.1	26.2

Table 6.1. VF frequency due to acute ischemic heart disease (AIHD) and myocardial infarction in and outside of the hospital

is a sign of instantaneous death; 2) a substantial difference in the content of K between the left ventricle and the skeletal muscle is a sign of coronary death.

Therefore, the decrease in K content in the left ventricle in absence of substantial changes in the skeletal muscle allows to suppose that death resulted from VF. A sharp decrease in K content in the left ventricle and a substantial decrease in the skeletal muscle shows that the death was preceded by an agonal period and that it came from heart failure or from cardiogenic shock (naturally if heart rupture and thromboembolism are excluded beforehand).

The study of K distribution in the heart and in the skeletal muscle was used for postmortem determination of the frequency of sudden death from VF in the city of Krasnoyarsk.

6.4.2 Postmortem study of the frequency of sudden death from ventricular fibrillation out-of-hospital and in hospital

1515 cases of acute ischemic heart disease were analyzed during the 3 years of observations in the city of Krasnoyarsk: 671 of the patients died; among them 508 died out-of-hospital and 163 died in hospital [272].

By researching the witness accounts given to the special group of physicians on how the death occurred, by conducting a postmortem medico-legal examination and selective postmortem study of K distribution in the heart and in the skeletal muscle allowed us to determine the frequency of sudden death as a result of VF in out-of-hospital cases and to compare it with the frequency of sudden death due to VF that was observed in hospital (table).

The data obtained show that only 6% of deaths due to VF occur in the hospital, while the remaining 94% of all cases take place out-of-hospital. It is not surprising that only 14.7% of the total number of deaths in hospital from acute ischemic heart disease happen as a result of VF, while the main causes of death in hospital are heart failure and cardiogenic shock.

Until recently, the above relationship between the causes of death from acute ischemic heart disease in hospital were extrapolated to the overall mortality from ischemic heart disease. This is obviously wrong, because mortality in the

hospital makes up only 24.3% of the overall mortality, whereas out-of-hospital mortality makes up 75.7% of all deaths, mostly sudden deaths.

If only instantaneous death is considered as sudden death it could be almost completely attributed to VF. Taking into account that all cases of death that happened during the sixth hour after the first signs of acute ischemic heart disease are recognized as cases of sudden death, the frequency of VF, then, proves to be lower and makes up 73.3% of the total number of cases of sudden death and 59.1% of total number of persons who had died of acute ischemic heart disease.

Meanwhile if the number of cases of VF is related to the total number of persons who have fallen ill of acute ischemic heart disease, than the frequency of cases of VF that take place in the hospital and out-of-hospital makes up only 1.6% and 24.5%, respectively, while the total number of cases of VF makes up 26.2% of persons fallen ill of acute ischemic heart disease.

The data above show that one fourth of persons fallen ill of acute ischemic heart disease face a threat of death from VF and almost 2/3 of deaths of acute ischemic heart disease are due to VF.

The data obtained could serve as a starting point for the study of effectiveness of various methods of VF prophylaxis.

6.5 Conclusions

As mentioned in the beginning of this chapter the problem of VF prevention includes four main tasks: 1) search for antifibrillatory substances; 2) development of methods for their use; 3) elaboration of VF prediction method; 4) elaboration of the method evaluation of the effectiveness of antifibrillatory factors.

1. In the *search for antifibrillatory substances* we tried to influence

   (a) the metabolism's "hot points" which are related to the development of VF, as shown in the preceding chapters. We discovered substantial decrease in the frequency of VF during high coronary artery occlusion via by influencing on the disturbed: ox-red equilibrium by means of hexahydroubiquinone and monoiodacetate; acid-base equilibrium by means of TRIS buffer solution; ionic equilibrium by means of cardioplegic cocktail;

   (b) sympathoadrenal control of the heart by means of both β-adrenoreceptors blockade using propranolol and inhibition of adrenaline uptake by the heart by using metanephrine.

2. *method of computer control administration of antifibrillatory substances* was developed. The method is based on computer analysis of the information about current changes in AP of myocardial cells. In the future the method could be suitable for use in care units by means replacing the AP parameters with ECG, transformed in an especial manner. The use of this method gives the possibility choice of the antifibrillatory substances and of the dose on the base of current changes in the heart, but not on the base of past experience.

3. *method of prediction of possibility VF onset in acute coronary insufficiency* was developed for the selection of risk groups of VF appearance in acute coronary insufficiency. We used insulin test for the selection of dogs (and evidently people) with high activity sympatho-adrenal system, which is the main reason of VF rise.
4. The evaluation of the effectiveness of antifibrillatory means needs in determination of VF percentage, what is a very difficult task, because it arises as a rule in absence of medical personnel. So, VF must be diagnosed postmortem. In collaboration with medico-legal experts of Kiev, Moscow and Krasnoyarsk we developed *method of VF postmortem diagnosis* with help study of K distribution in the heart and in the skeletal muscle.

The use of this method showed that 2/3 of all dying people and 1/4 of all patients with the acute coronary insufficiency and myocardial infarction are dying from VF. So, VF prophylaxis is one of the most urgent problem of modern medicine. Our investigations did not resolve this problem, but only shows the approaches to the search of antifibrillatory substances, to the developing of the methods their application and to of evaluation of their prophylactic effectiveness

7 Mechanism of Ventricular Fibrillation Onset After Coronary Artery Occlusion

The electrophysiological hypotheses prevailed for a long time in the explanation of mechanism of VF. Heart metabolism study resulted in formulation of numerous metabolic hypotheses of the VF onset after CAO. The development of neuro-humoral hypotheses is based on new data about the role of the sympathetic and parasympathetic nervous system and their mediators (noradrenaline, adrenaline and acetylcholine) in the appearance of VF. A short review of these hypotheses is given below.

7.1 Electrophysiological hypotheses of ventricular fibrillation mechanism

Electrophysiological studies led to two hypotheses about the VF onset. Namely, the hypothesis of mono- or polyfocus heterotopic automaticity and the hypothesis of a reentrant excitation.

7.1.1 Heterotopic automaticity hypotheses

The hypothesis of monofocus heterotopic automaticity suggests formation of a focus in the myocardium which generates high frequency impulses. According to the hypothesis of polyfocus heterotopic automaticity, several foci of automaticity with independent rhythms arise simultaneously in the myocardium. Both hypotheses assume that contractile myocardial cells can acquire the automaticity properties or automaticity properties of Purkinje's cells are reinforced. Trigger automaticity was regarded as a possible mechanism of tachyarrhythmia

in case of myocardial infarction. This automaticity appears in the boundary zone, where injury current serves as the trigger.

Triggered activity in Purkinje fibers caused by early afterdepolarization is responsible for the initiation of ventricular arrhythmia in German shepherd dogs, which have the inherited predisposition to sudden death [108].

7.1.2 Re-entry hypothesis

The reentry hypothesis is based on the disturbance of the AP propagation in the heart in contrast to heterotopic automaticity hypothesis, which is based on abnormality in AP generation. Under normal physiological conditions, impulses propagate from the sinus node and after consecutive activation of the auricles and ventricles disappear because they encounter newly excited and, therefore, refractory tissue. The concept of reentry assumes that the propagated impulse is not extinguished after a complete activation of the heart, but repeatedly returns to the already excited cells after the refractory period is over. Hence, either the whole conduction time in the reentry cycle must be sufficiently long or the refractory period in the initially depolarized fiber must be sufficiently short.

During the last decade only few publications were dedicated to electrophysiological hypothesis. It was shown that sotalol contributes to a more homogenous distribution of refractoriness and due to this effect has an antifibrillatory effect [178]. In experiments on dogs, during induced VT, data were obtained which characterize the spectrum of epicardial reentrant circuits [142]. It was demonstrated that proarrhythmic effect of flecainide is due to significant rate-dependent slowing of conduction preferentially in ischemic myocardium [261]. The study of termination mechanism of reentrant activity in VF was done in [48]. It was shown that the refractory period in cases with VF is much shorter than the VF cycle length. The presence of a large excitable gap contributes to reentrant wave- front termination.

For a long time reentry and heterotopic automaticity were regarded as alternative mechanisms of post-infarction arrhythmias. However the latest findings recognize the role of both mechanisms in the genesis of post-infarction arrhythmia. Early arrhythmia is attributed to the reentry mechanism which is associated with the slow down of the conduction in the ischemic cells [344]. Arrhythmias occurring several hours after CAO are attributed to the increase of the automatic properties of the subendocardial Purkinje's fibers [289]. This point of view is supported in the experimental studies. Three-dimensional mapping from 232 simultaneous sites in the feline heart in vivo revealed that non-reentrant or focal mechanisms were responsible for initiation of VT (except the cases when VF was initiated by reentry) and often support it continuation [221].

7.1.3 Intercellular uncoupling hypothesis

The intercellular uncoupling hypothesis of appearance of VF in ischemia attributes an independent role to the disturbances in intercellular contacts and transfer of excitation from one cell to another [294, 15]. Due to considerable changes in metabolism and ionic current, CAO creates conditions for changes in the intercellular contacts state. The respiration inhibition which occurs in the ischemic zone blocks the Na-pump on the cell membrane, which results in Na^+ entry into the cell according to the concentration gradient, and an increase in $[Na^+]i$. The increase in free $[Na^+]i$ activates the Na-Ca exchange which promotes an increase in Ca concentration in the cell. On the other hand, glycolysis (and glycogenolysis) activation and lactate accumulation decrease the intracellular pH, which, in turn, increases the intracellular Ca concentration. The accumulation of free Ca in the cell increases the resistance (Ri) of the intercellular contacts and leads to intercellular uncoupling. The cAMP has further negative effect on the intercellular contacts [90, 74]. On the other hand, it was shown, that sotalol, a class III antiarrhythmic agent, helps spontaneous ventricular defibrillation due to enhancement of cellular synchronization [318].

Under the conditions of local ischemia, an increase in the intercellular resistance can ensue as a defensive mechanism which inhibits the injury current and prevents the diffusion of the metabolites from pathological cells into normal ones. However the same mechanism can facilitate VF, as it disrupts the synchronism of the excitation process [73].

7.2 Metabolic hypotheses of ventricular fibrillation

7.2.1 Role of the oxygen differential

Beck [26] showed that electrical instability of the heart can be induced by 1) coronary artery ligation in a well oxygenated heart and 2) by infusion of oxygenated blood into the coronary artery of a cyanotic heart. VF arises in both cases. The hypothesis of the role of oxygen differential in electric instability of the heart was based on these data. This hypothesis was additionally developed in [338].

7.2.2 Role of K exit from the cardiac cells

Harris [122] showed that a time relation exists between the loss of K by the myocardial cells and appearance of VF . He also showed that VF arises when K is locally infused into the myocardium. These data enabled the author to formulate the hypothesis that loss of K is the main cause of VF onset. This was later confirmed in a number of studies. These data were summarized by the author in [121].

7.2.3 Role of fatty acids

Oliver and Kurien [204] suggested a hypothesis that the main role in arrhythmia after myocardial infarction belongs to the fatty acids. The hypothesis was based on the observation that ventricular arrhythmias develop in patients with a higher content of fatty acids in the blood. The fatty acids either directly influence the cell membrane as detergents, increasing its permeability, or have an indirect effect, inhibiting oxidative processes in the tissue.

7.2.4 Role of pH and lactate

The data accumulated in cardiac surgery with artificially provoked VF showed that acidosis favors, while alkalosis prevents the appearance of VF. Metabolic acidosis after CAO is associated with a rapid lactate accumulation, but the formation of lactate is proportional to the loss of K. Each of the lactate molecules leaving the cell is followed by one ion of K^+ and one ion of H^+. Taking this into consideration, the primary importance in the development of ventricular arrhythmias was attributed to glycogenolysis activation associated with accumulation of lactate and development of metabolic acidosis [201].

7.3 Neurohumoral hypotheses of ventricular fibrillation

7.3.1 Role of sympathoadrenal system

The well established facts that the susceptibility to VF is increased by stimulation of the sympathetic nervous system, on one hand [281], and that sympathetic activity is increased after CAO [182], on the other hand, (see chapter IV) confirm the role of the sympathoadrenal system in the development of VF. This hypothesis was corroborated by reproducing ischemic damage by means of catecholamines administered in doses simulating their spontaneous secretion after CAO [47]. The mechanism of fibrillatory effect of sympathoadrenal system, however, remains unknown.

7.3.2 Role of parasympathetic system

Potential antiarrhythmic efficacy of parasympathetic activation were shown in many works. The muscarinic agonist, oxotremorin, significantly reduced malignant arrhythmias during acute myocardial ischemia [72]. Activation of muscarinic receptors (methacholine and oxotremorine) caused much less antifibrillatory effect than propranolol, but authors of [69] assume that activation of muscarinic receptors may represent a new approach to prevention of sudden cardiac death.

7.3.3 The role of cAMP

It is known that cAMP is an intracellular transmitter of β-adrenergic stimulation. This allows us to suppose that cAMP has an arrhythmogenic effect. A group of researchers [206, 306] developed a hypothesis that cAMP plays a key

role in the development of VF via intensification of the slow inward Ca current [219]. Direct evidence of the dibutyryl cAMP influence on the development VF showed that [177]: 1) dibutyryl cAMP lowers the VF threshold; 2) addition of theophylline, which inhibits the phosphodiesterase and increases the cAMP content, causes a greater decrease in the VF threshold; A supposition was made in [220] that cAMP causes formation of Ca dependent slow responses which participate in the generation of reentry arrhythmia. The fact that high concentrations of Ca-antagonist have an antifibrillatory effect also testifies in favor of the Ca mediatory effect on cAMP [307].

However, other mechanisms can account for this effect. cAMP can act: 1) through changes in K permeability and can control the effect of K on the pacemaker and on the tissue conductivity [315]; 2) by stimulation of intracellular lipolysis, as lysophosphoglycerides released in phospholipides decomposition by lipase possess detergent properties and can be responsible for the malignant arrhythmia in ischemia [295]. Another possible mechanism is that cAMP can induce afterpotential, and can activate the automaticity in normal ventricular fibers due to these potentials [68]. cAMP can also promote arrhythmia due to the electric uncoupling of myocardial cells [346].

7.4 Author's hypothesis based on the systemic approach to study of ventricular fibrillation mechanisms

It is clear from this short review that most of the hypotheses consider the role of isolated factors related to the appearance of VF: the disturbance in the generation of normal impulses and their propagation through the heart, intercellular contact disturbance, oxygen differential in the heart, K exit from myocardial cell, accumulation of fatty acids, decrease in pH and lactat formation, accumulation of cAMP, activation of sympathoadrenal system, or inhibition of vagus activity and others. Meanwhile, VF is the final result of many complicated and interconnected processes.

7.4.1 Sequence and interaction of ischemic changes in the heart preceding ventricular fibrillation

A systemic approach to the study of VF mechanism, which was developed in our laboratory during 20 years, allows us to present all ischemic changes related to VF, in their proper sequence and interaction (fig.7.1).

Separate links of this chain include:

1. Changes in *metabolism*, which consist of: inhibition of respiration, coupling respiration and phosphorylation, glycogenolysis activation and decrease in intracellular pH, which plays a key role in the ischemic changes of the cellular compartments;

2. *Decompartmentation* of ions, which is the result of metabolic changes in intracellular compartments and causes the changes of ionic activity in the

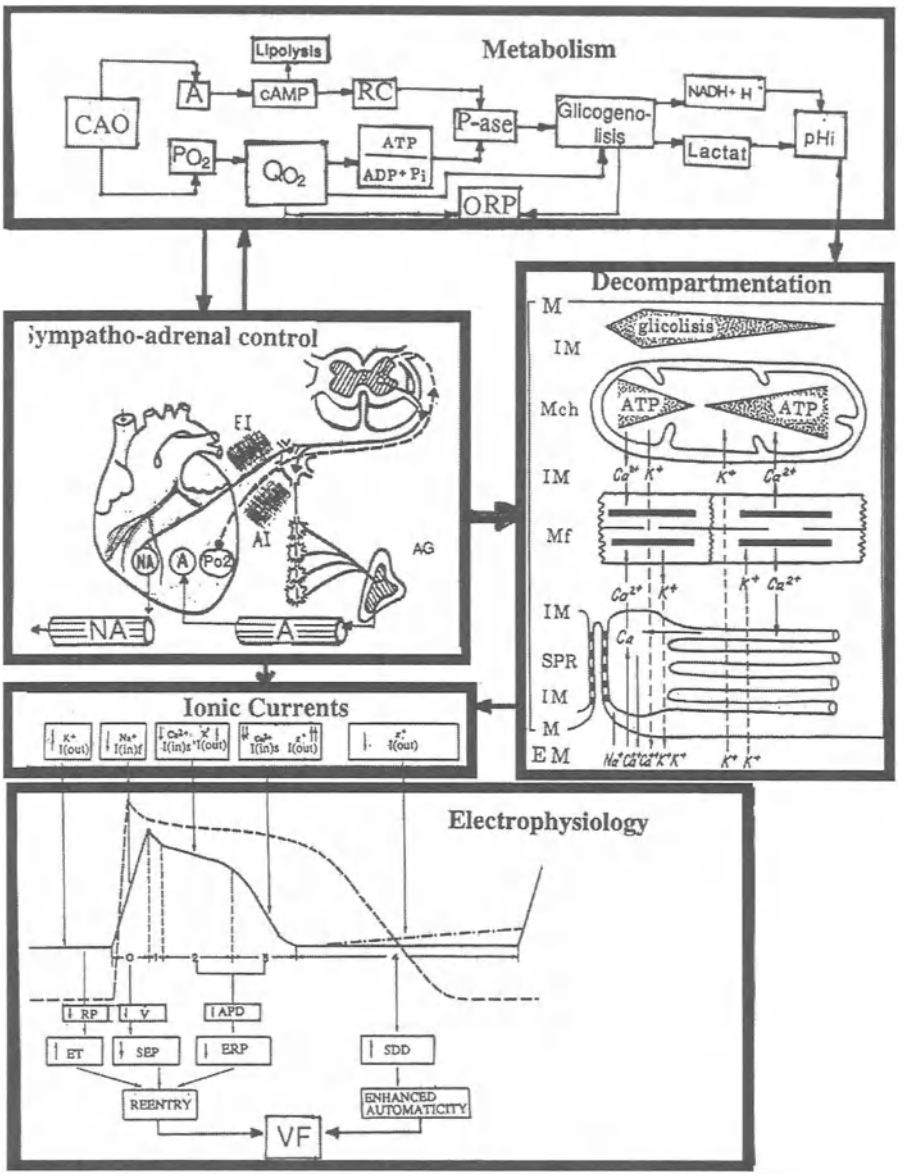

Figure 7.1. The chain of processes related to the appearance of VF. Metabolism, decompartmentation and ionic current - see chapter III. Sympatho-adrenal control - see chapter IV. Electrophysiology - see chapter II.

cytoplasm, of ionic intra/extracellular ratios and ionic translocation between cells and extracellular medium;

3. Changes in *ionic currents* through the cell membrane which causes MP changes of the cells;
4. Changes in *electrophysiological processes* which are the result of changes in ionic current and are a direct cause of VF;
5. Activation of the *sympatho-adrenal system* is the result of all previously mentioned changes and causes intensification of all ischemic changes in the metabolism, ionic currents and electrophysiological processes, which lead to VF.

The sequence and interconnection of the processes which develop during local ischemia are similar in individual animals. It remains unclear why VF develops in some animals and does not develop in others.

7.4.2 The specifics in metabolic and electrophysiological changes preceding ventricular fibrillation after coronary artery occlusion

The specific metabolic and electrophysiological ischemic changes preceding VF were discovered by comparing the following parameters in experiments with and without VF:

1. blood supply of the heart,
2. oxidation and phosphorylation,
3. glycogenolysis,
4. acid-base and ionic equilibrium,
5. the electrophysiological processes.

To visually represent the difference in temporal changes of these processes in experiments with and without VF, all typical experimental curves and a generalized curve are presented in fig.7.2.

Fig.7.2 shows first of all that the probability of VF onset after CAO performed at the same level, does not depend on the degree of decrease in *blood supply* of the ischemic zone (see chapt.I). This is evident in the same decrease in P_{O_2} at the center of ischemic zone in experiments with and without VF.

The metabolic (see chapt.III) and electrophysiological parameters (see chapt.II) as opposed to parameters describing the blood supply, have essential quantitative difference in experiments with and without VF. The index of *oxidation-reduction processes* - ORP and the indicator of respiration state - Q_{III} decrease faster in experiments with VF than in experiments without VF. In cases resulting in VF a more marked uncoupling between respiration and phosphorylation was observed, as manifested by a sharper decrease in respiration control (RC). *The activation of glycogenolysis*, shown by an increase in lactate and in MAR for phosphorilase and other enzymes, was notably greater in experiments with VF (see chapt.III) than in experiments without VF.

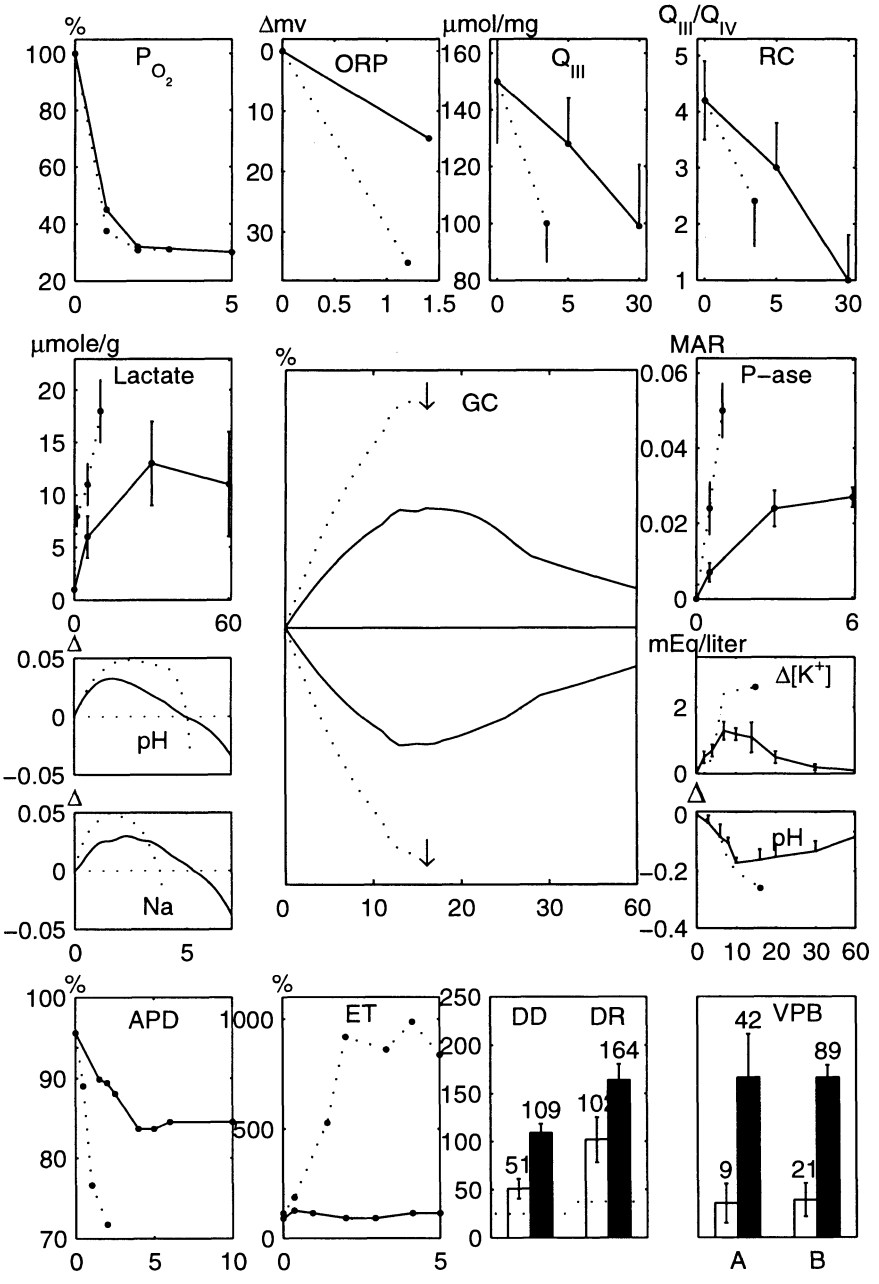

Figure 7.2. The comparison of basic parameters that characterize metabolic and electrophysiological changes in ischemic zone together with generalized curves (GC), which reflect the qualitative changes in these parameters after CAO in experiments with (broken line) and without (solid line) VF

The changes in pH in the ischemic zone of myocardium and the decrease in pH of coronary vein blood which drains the ischemic zone were more pronounced in experiments with VF (see chapt.III). As a result of more pronounced pH changes, the *ionic changes* were also larger in experiments with VF (see chapt.III). The period of increase and decrease in pNa in the ischemic zone of myocardium and the increase in the concentration of K in the blood of coronary vein was more pronounced in experiments with VF and VF developed at the maximal increase of K (see chapt.III).

The electrophysiological changes were observed both in experiments with and without VF, but the value and rate of decrease in APD during the first 2 min after CAO were greater in experiments with VF than in experiments without VF (see chapt.II). ET increased sharply to ten times its initial level at the 4th minute in experiments with VF, while in the experiments without VF the increase in ET was insignificant. The increase in DD and DR in 8 areas of the heart were also markedly greater in experiments with VF. This indicates a greater slowing down of conductivity, which is a necessary condition of re-entry. Consistent with more pronounced electrophysiological changes in experiments with VF, VPB began earlier, the frequency of VPB in prefibrillatory period was 5 time higher, particularly in earlier phases of cardiac cycle in experiments with VF. Moreover, a tendency towards multiple VPB, which are precursors of VF, was 3-4 times more pronounced in experiments with VF.

Thus, experiments in which CAO resulted in VF can be distinguished from experiments without VF by:

1. greater speed of the initial changes, which have exponential character and
2. higher magnitude of these changes, while in experiments without VF, on the contrary, changes grow slowly, are described by curves with a maximum point and reach a smaller value.

VF appears after CAO when metabolic and electrophysiological changes undergo a dramatically sharp and powerful development. We suggest that the sharp growth of pathological changes in experiments resulting in VF may occur due to inadequate changes in the sympatho-adrenal control of the heart.

7.4.3 The specific changes of heart's sympathoadrenal control, preceding ventricular fibrillation after coronary artery occlusion

We consider the afferent activity of cardiac nerves (AACN) as an indicator of afferent link of *sympathoadrenal control* state. The balance of catecholamines - adrenaline and noradrenaline is an indicator of state of efferent link (see chapt.IV). The basic changes in sympathoadrenal control of heart during CAO are presented in fig.7.3.

The changes in AACN differed greatly in experiments with and without VF An increase in the magnitude of AACN after CAO when arrhythmia occurs is nearly three times greater in experiments with VF than in experiments without VF and reaches 800% when VF appears.

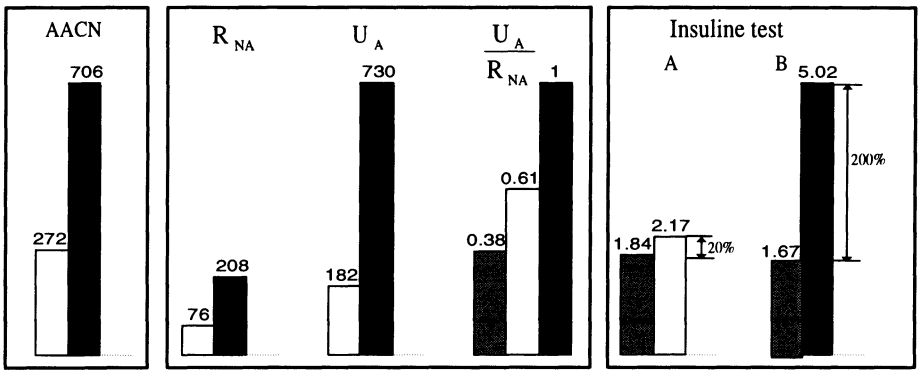

Figure 7.3. The comparison changes in AACN, release of NA (R_{NA}), uptake of A (U_A), ratio of U_A/R_{NA} in experiments without VF (white columns) and with VF (black columns). The comparison changes in A concentration in arterial blood after administration of insulin, CAO without VF (white column), CAO with VF (black column), control (gray column). Average data.

The balance of catecholamines - release of NA and uptake of A after CAO increase both in experiments with and without VF. In experiments with VF however, the uptake A increase is four times greater than in experiments without VF.

The ratio between the uptake A and release of NA by heart in the initial state is 0.38. This signifies that only one of three parts of the released NA is replaced by adrenaline. After CAO in experiments without VF this ratio increases to 0.61 i.e., almost two of three parts of noradrenaline are replaced by adrenaline and only when this ratio increased to 1, i.e. when all of the released noradrenaline is replaced by adrenaline, does VF arise. Thus, the release of noradrenaline after CAO in experiments with VF is overcompensated by the uptake of adrenaline.

The bigger uptake A due to greater arterial hyperadrenalemia in dogs with VF, can be a manifestation of a greater reactivity of their adrenal glands medullar substance. If this is indeed so, the probability of VF can be predetermined by the release of A from the adrenal glands prior to CAO in response to a certain standard action.

For this purpose insulin was administered two days before CAO. The increment increase in A concentration in arterial blood 10 minutes after administration of insulin was 10 times higher in dogs which reacted to subsequent CAO by VF, than in dogs that did not. Thus, the probability of VF appearance after CAO can be determined prior to CAO using the insulin-test, which we proposed for prediction of VF (sudden death) after CAO (see chapter IV).

The adrenal overcompensation is the most important feature of experiments with VF because A has a greater influence on the metabolic and electrophysi-

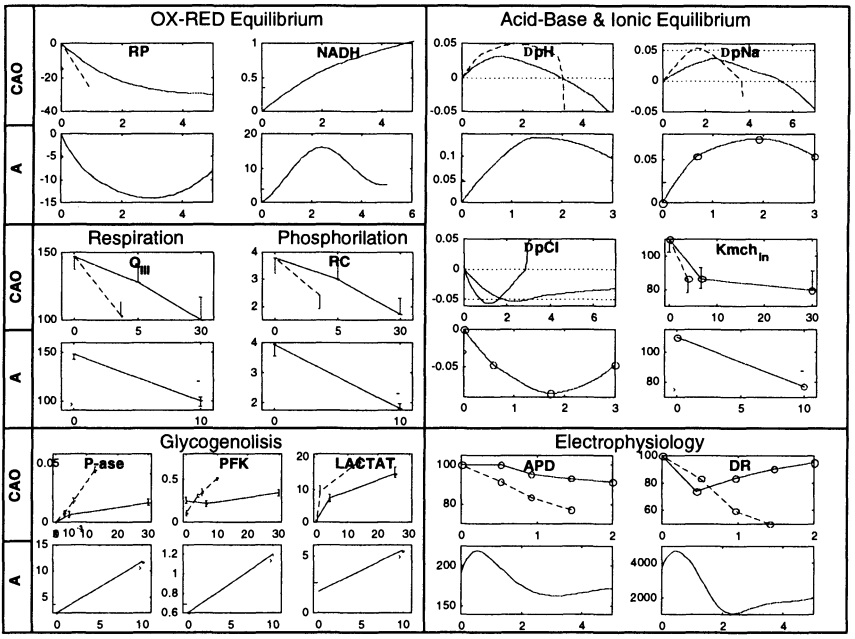

Figure 7.4. The comparison of the effect of CAO with effect of adrenaline on ox-red equilibrium, respiration and phosphorylation, glycogenolysis, acid-base and ionic equilibrium and electrophysiology of the heart in experiments with VF (broken line) and without VF (solid line)

ological processes in the heart than NA and its effect is similar to the effect of hypoxia (see chapter V).

7.4.4 *Comparison of the effects of coronary artery occlusion and adrenaline on the metabolic and electrophysiological processes in the heart*

The effects of adrenaline and CAO on the metabolic and electrophysiological processes (see chapter V) are shown on fig.7.4.

Although CAO decreases the blood supply and P_{O_2}, but A increases the blood supply and P_{O_2}, both of them decrease the ox-red equilibrium in the heart. Administration of A in a dose of 20 μg/kg causes a 3-4 fold increase in A concentration in ischemic zone, which is comparable to the effect of CAO. Adrenaline causes the same decrease in ox-red potential and increase in NAD.H as CAO.

Both CAO and adrenaline suppress respiration (Q_{III}) and induce uncoupling of respiration and phosphorylation, i.e. the decrease of respiratory control (RC) and call deenergization of mitochondrion. As a result, both CAO and A activate P-ase, PFK and increase glycogenolysis, causing a decrease in glycogen and an increase in lactate.

Both CAO and A cause an increase and a subsequent decrease in pH and pNa and a decrease and a subsequent increase in pCl in the ischemic zone. The K content in the mitochondria also decreases in both cases. This leads to an increase in K activity in the cytoplasm and to an increase in intra/extracellular K gradient. This causes K outflow from cell and a decrease of the RestP.

The depolarization rate (DR) and the action potential duration (APD) decrease both in action of A, so and in ischemic zone after CAO. The speed of the excitation propagation decreases and its direction changes as in action of A so and after CAO. Thus, the conditions favoring VF appear in both cases.

Thus, both factors - ischemia and A manifest a striking similarity in their effect on the energetic and ionic metabolism, as well as on the electrophysiological processes in the heart. The effect of ischemia is due to inhibition of respiration while the effect of adrenaline is due to the uncoupling of respiration and phosphorylation. The deenergization of mitochondrion arises in both cases.

We can speculate that greater changes in all parameters in experiments with VF is due to the additive effects of adrenaline and ischemia.

So, the greater release of A by adrenal gland and its uptake by the heart is one more example of "mistaken compensation". Release of A is supposed to replace NA released from the heart, but occurs at an excessive degree and rate. It appears that nature did not create a special compensatory mechanism for the local ischemia and uses "standard" mechanisms.

In order to clarify what links of the metabolism and the sympathoadrenal control of the heart are disturbed during ischemia, we affect these links with different substances.

7.4.5 The prophylaxis of ventricular fibrillation

The search for antifibrillatory substances was carried out in our laboratory by correcting the disturbances in metabolism and cardiac sympathoadrenal control which occur when CAO results in VF (see chapt.VI). The results of these corrections using medicamentous and non-medicamentous substances (metabolites, enzymes, hormones and so on) are presented in fig.7.5[253].

We studied the possibility of VF prevention by means of: 1) respiration activation, 2) glycogenolysis depression, 3) pH stabilization, 4) stabilization of the intra-extracellular ionic equilibrium, and 5) control of sympathoadrenal regulation.

The respiration activation was performed using cytochrome C, which decreased the VF frequency from 60 to 50% and using hexahydroubiquinone (coenzyme Q), which decreased VF frequency from 60 to 12%. So, *activation of respiration* administering substances with high ox-red potential is the first of the possible routes for prevention of VF in local ischemia.

The glycogenolysis inhibition was realized using monoiodacetate. The effect of monoiodacetate on the redox, acid-base and ionic equilibrium decreased VF frequency from 60 to 0%. So, the *inhibition of glycogenolysis* is the second of the possible routes for prevention of VF in local ischemia.

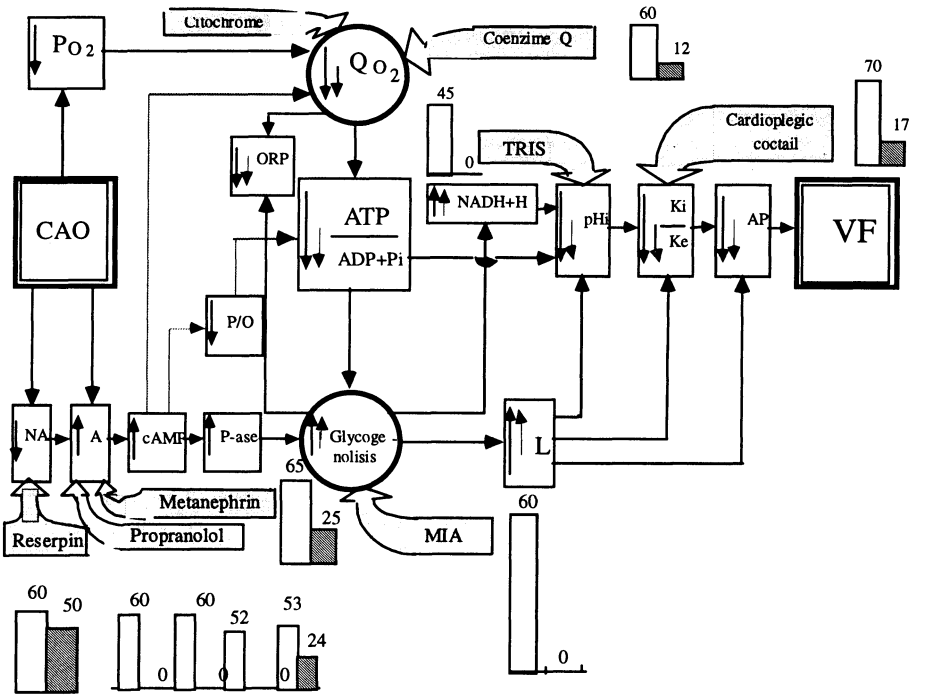

Figure 7.5. The schematics of processes that start after CAO and lead to VF. Shaded arrows show the possible influences upon these processes. Numbers over columns show VF frequency in %. Light columns in experiments without influences, gray columns - with appropriate influences.

Acid-base equilibrium was stabilized using buffer solution TRIS with different pH values. The frequency of spontaneous VF after 5 min perfusion with TRIS solutions with pH 7.4 decreased from 44.4% to 0. So, the obtained data show that the *increase of the myocardial buffer capacity* by means of buffer solution is the third of the possible routes for prevention of VF in local ischemia.

Ionic equilibrium was stabilized using a modified cardioplegic solution. The VF frequency decreased from 70 to 17%. So, influence on the *ionic content of myocardium* is the fourth of the possible routes for prevention of VF in local ischemia.

Thus, presented data show that the chain of disturbances of the metabolic processes can be corrected at various levels, obtaining the antifibrillatory effect.

The influences on the *sympathoadrenal system* were directed on 1) the NA release by the heart, 2) the depression of A uptake by the heart and 3) the depression of β-receptors in the myocardium.

The preliminary exhaustion of NA reserve in the granules decreased VF frequency from 60 only to 50%. The *influences on the A uptake* by the heart using metanephrine were considerably more effective. Metanephrine decreased

VF frequency from 65 to 25%. The influences directed toward the *blockade of the β-receptors* were the most effective. The propranolol decreased VF frequency from 53 to 24% in one of the set of experiments and to 0% in 3 others.

So, a great potential exists for pharmacological prophylaxis of VF after CAO, directed toward correction of disturbances in metabolism and sympathoadrenal regulation of the heart.

7.5 Ventricular fibrillation as a result of adrenal overcompensation

The facts described above were analyzed in order to illuminate the mechanisms of VF appearance after CAO.

On one hand, dogs with VF respond to CAO by: 1) more powerful afferent and efferent impulsation of cardiac nerves and 2) bigger loss of NA by heart than dogs without VF. These distinctions can be explained by more pronounced hypoxia in cases with VF. Indeed, the correlation between concentration NA and K (ischemic agent) in coronary sinus blood after CAO confirm this suggestion. However, material in chapter I shows that probability of VF onset after occlusion below a certain level is not determined by the degree of hypoxia.

On the other hand, dogs with VF response after CAO show: 1) bigger release of A by adrenal gland, 2) bigger increase in A concentration in arterial blood and 3) bigger increase in A uptake by the heart. More powerful reaction of the dogs to CAO observed in dogs with VF can be the due to: 1) more intensive loss of NA or 2) greater reactivity of chromaffine cells of adrenal gland. The facts that the uptake of A exceeds the loss of NA and that this is more pronounced in cases with VF, allow us to suppose that probability of VF onset is determined by greater reactivity of adrenal gland. The most important fact confirming this suggestion is that A secretion in response to a standard influence (insulin test) increases more (both before and after CAO) in dogs which react to CAO with VF, even if the test was performed two days before CAO. The other indicator of greater reactivity of adrenal gland is the higher initial level of A in the arterial blood in narcotized dogs before CAO as a reaction to stress.

The analysis of all these facts allowed us to propose following sequence of phenomena which arise after CAO and lead to VF (fig.7.6)

The intensive afferent nerve activity after CAO leads to the release of NA by nerve endings from vesicles, washing of NA away from the heart and to a decrease in NA concentration in the heart.

In response to this, the efferent activity in preganglionic sympathetic nerves, affect the adrenal gland and thus increases the secretion of A. Concentration of A in arterial blood, the uptake of A by the heart and of its concentration in the heart all are increased.

So, the loss of NA by the heart is replaced by the uptake of A. In the cases with VF the uptake of A sharply exceeds of the release of NA in response to CAO.

Adrenaline has a stronger effect on metabolic and electrophysiological processes than NA and acts in the same direction as hypoxia. The effects of hypoxia

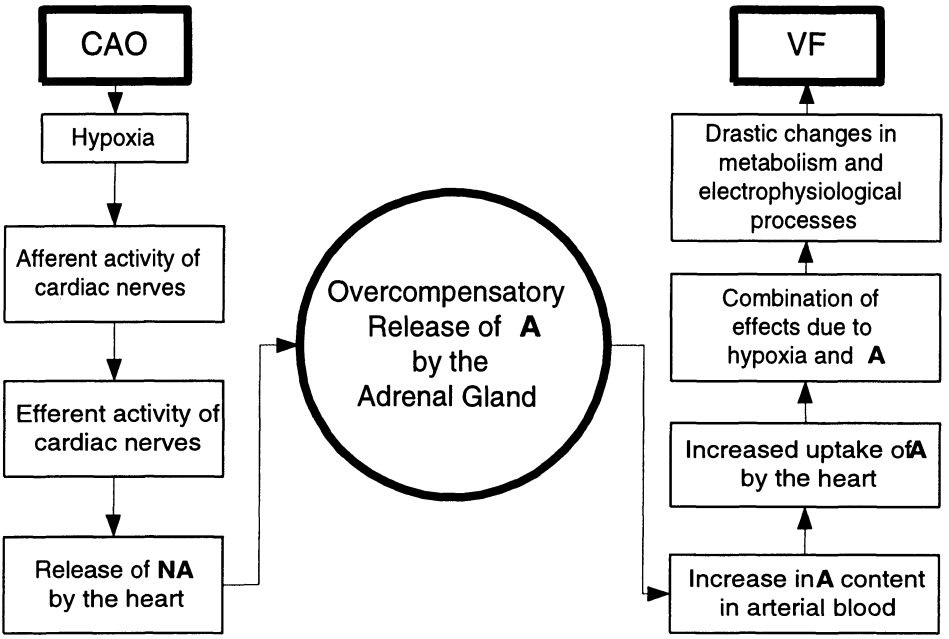

Figure 7.6. Schematics of processes which lead to VF after CAO

and adrenaline are combined. As a result, drastic changes in all metabolic and electrophysiological processes arise in cases resulting in VF.

Thus according to our hypothesis, VF occurs after CAO in animals (and it seems in people) with higher reactivity of chromaffin cells of adrenal gland. The greater reactivity of the adrenal gland is the main individual feature (which can be of genetic origin) of animals which developed VF after CAO. The genetirc origin does not exclude the possibility VF prevention.

The determination of the reactivity of the adrenal gland using the insulin test allows us to separate the people with high risk of VF onset after coronary artery occlusion long before the occlusion.

In our time, when pharmaceutical industry produces a great variety of drugs, we can hope that drugs can be developed which decrease the reactivity of adrenal gland's chromaffine cells. Such a drug makes the prevention of the VF and sudden death after myocardial infarction a reality.

Implications

- - *Pharmaceutical firms* could use our experimental data that antifibrillatory substances should have the following effects:
  - stabilize ox-red equilibrium (ubiquinone, cytochrome),
  - prevent shift of myocardial pH (TRIS),
  - normalize ionic equilibrium (cardioplegic cocktail),
  - decrease uptake of adrenaline by the heart (metanephrine), and
  - block the effect of adrenaline on the heart (propranolol)
- VF after occlusion could be predicted based on the reactivity of adrenal gland using the insulin-test. This - *could be used by cardiologists* for identification of a "risk-group" for VF
  - among patients with coronary insufficiency,
  - among healthy people out-of-hospital, and
  - among young athletes to determine who can participate in competitive sports and who should not (see chapt.VI).
- A method of real-time computer control of drug administration *could be used by cardiologists in coronary care units* for administration of medications based on the current information about membrane potential changes (see chapt.VI).
- A method postmortem diagnosis of acute coronary insufficiency and VF on the basis of K distribution in the myocardium *could be used in medicolegal services* for postmortem determination of cause of death. This information allows to separate all cases of sudden death occurred out-of-hospital into violent and those due to coronary insufficiency and VF. It can be used to evaluate the effectiveness of antifibrillatory influences (see chapt.VI).

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