Some Peculiarities of Metabolism of the Myocardium Under Conditions of Experimental Disturbance of the Microcirculation

By A. M. Chernukh and G. V. Chernysheva

ABSTRACT

The functional state and energy exchange of rabbit myocardium were investigated during disturbances of the microcirculation by means of combined intravenous administration of high molecular weight dextran (500 mg/kg) and vasopressin (5 units/kg). Sinus bradycardia, arrhythmia, and increase in the height of the T wave with its subsequent change to negative or biphasic were observed following administration of these substances. The respiration rate of mitochondria, determined by polarographic methods, decreased by 30 to 50% and the conjugate respiration and phosphorylation by 28 to 45% by use of pyruvic, glutamic, and α-ketoglutaric acids as oxygen substrates. Simultaneously, a changeover of respiration from the NAD-dependent substrates to the FAD-dependent one, succinic acid, took place. The ATP concentration in myocardial tissues decreased. Glycogen concentration and phosphorylase a activity remained unchanged.

KEY WORDS

ATP dextran arrhythmias myocardial glycogen mitochondrial respiration phosphorylase a

The increased attention directed to problems of the rheology of blood in recent years appears to be related to the fact that disturbances of the rheological properties of blood, leading to disorders of the capillary circulation, are observed in pathological conditions and, in particular, in myocardial infarction. In the latter a special place is occupied by intravascular aggregation of formed elements of the blood, which takes place in various types of tissue damage: after surgical operations, during artificial blood circulation and intravenous administration of large doses of X-ray contrast preparations, etc.

In 1945 Knisely designated the condition in which an increase in viscosity of the blood is accompanied by aggregations of the formed elements by the term "sludge." It is known that sludge is encountered in diverse conditions which are the result of extreme stimuli, for example, in shock of various etiologies, including cardiogenic shock and cardiac necrosis. Under experimental conditions the combination of sludge and restriction of coronary flow lead to changes in the myocardium.

According to the data of Swank and Nakamura, the formation of sludge is accompanied by a considerable reduction in oxygen consumption by the brain tissues. However, we have not found data in the literature on the effect of sludge on the metabolic processes in the heart. Accordingly, we attempted in this study to explain the effects on myocardial energy processes of disturbances of the microcirculation as a result of changes in the rheological properties of the blood. For this purpose the content of high energy phosphorus compounds and glycogen, respiration, and oxidative phosphorylation in the mitochondria were determined.

Material and Methods

Disturbance of the rheological properties of the blood was produced by means of a combination of intravenous administration of high molecular weight dextran and lysine-vasopressin (L4-vasopressin). The action of the latter is directed toward change in the size of the coronary vessel lumina. These disturbances were induced by Bicher and Beemer in approximately the same manner.

The tests were set up with 103 chinchilla rabbits weighing between 3.0 and 3.5 kg. High-polymer dextran (500,000) was administered intravenously (at a dose of 0.5 to 1 g/kg) 15 minutes before vasopressin administration (5 pressor units/kg weight).

The ECG and respiration of all animals were recorded for a period of one hour before and after administration of the substances. The aggregations of formed elements of the blood were examined in smears, and the erythrocyte sedimentation rate was also determined. In a special series of studies, in vivo observation of the blood flow in the microvessels of rat mesentery, after administration of the substances indicated above, was carried out with an ML-2 luminescence microscope. Although there may be functional differences between individual vascular regions and organs, Knisely et al. and Heimbecker and Bigelow consider that changes observed in any organ

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reflect changes in blood flow in capillaries of the entire body.

The rabbits were killed after an hour. The hearts were perfused with ice-cold 0.15 M KCl. A portion of the tissue was taken for determination of phosphorylase by the method of Cori. The mitochondria were separated from the remaining myocardium in 0.25 M saccharose, pH 7.4. Respiration and oxidative phosphorylation of the mitochondria were determined by the polarographic method, using a fixed platinum electrode. Pyruvic, succinic, α-ketoglutaric, and glutamic acids at a concentration of 10 m per sample were used as oxidation substrates. Incubation medium composition was as follows: 0.03 M K₂HPO₄, 0.05 M KCl, and 0.12 M saccharose; 0.24 m of ADP was added per sample. The suspension of mitochondria contained 1 to 2 mg/ml. The mitochondrial respiration rate was expressed in micromoles of oxygen per second per gram of protein (μM/sec/g protein) in the mitochondria. Proteins were determined according to Lowry. The mitochondrial metabolic state characteristics corresponded to those accepted in the literature.

The rate of active mitochondrial respiration connected with phosphorylation of the added ADP was designated V₄. Oxygen consumption by the mitochondria after utilization of the ADP characterizes the monitored fourth state, V₄. The ratio V₄/V₄ expresses the magnitude of the respiratory control and characterizes the degree of phosphorylation. The efficiency of phosphorylation is represented by the ratio ADP/O.

The concentration of high energy phosphate compounds and glycogen were studied in the heart frozen with liquid nitrogen. Adenylc nucleotides were determined by the method of L. A. Zeitlin, creatine phosphate according to A. M. Alekseeva, inorganic phosphorus according to Lowry, and glycogen by the method of Seifert. Malate dehydrogenase [1.1.1.37], glutamate dehydrogenase [1.4.1.2], and NADH-cytochrome C reductase [1.6.2.1] activities were determined in mitochondria previously treated with 1% deoxycholate. Statistical treatment of the data was according to Student’s table.

Results

Administration of the substances induced an increase in erythrocyte sedimentation rate to 30 to 50 mm/hr. Microscopic observation of the blood flow in microvessels of the mesentery revealed aggregates of various sizes consisting of erythrocytes. With slowing of the blood flow, splitting of the large aggregates was hampered, which facilitated earlier development of stasis in the microvessels.

After combined administration of dextran and vasopressin, a peaked T wave was noted on the electrocardiogram. After two minutes the T wave became negative or biphasic, and the ST segment became depressed. A prolonged cardiac arrhythmia occurred on a background of marked sinus bradycardia. An hour after administration of the substances, when the animals were killed, the T wave was below control, heart rate did not reach the initial level of 80 to 140 beats/min, and the respiratory rate ranged from 40 to 100/min.

Determination of the high energy phosphorus compound concentration in rabbit heart tissue after the action of high molecular weight dextran and vasopressin disclosed a 42% reduction from normal of ATP with a 38% increase in ADP. The AMP level remained unchanged. The creatine phosphate concentration increased by 60% (Table 1). These data are evidence, first, of a reduction in the energy necessary for insuring plasticity of the myocardium and, second, of changes in the regulating potentialities of the cells since the ATP/ADP ratio decreased, which is a key indicator of change in energy supply and energy consumption.

Proceeding from these observations, we might expect the more active switching on of glycolytic processes for replenishing the supply of energy-rich phosphorus compounds. However, the studies which we carried out indicated only a small reduction in glycogen concentration. In this case phosphorylase a activity, determining the rate of glycokysis, remained unchanged (Table 2).

### Table 1

Concentrations* of High Energy Phosphorus Compounds in Rabbit Myocardium with Intravenous Administration of Vasopressin and Dextran

<table>
<thead>
<tr>
<th></th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>Creatine phosphate</th>
<th>Creatine</th>
<th>Inorganic phosphorus</th>
<th>No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.52</td>
<td>0.88</td>
<td>0.61</td>
<td>3.07</td>
<td>13.7</td>
<td>5.8</td>
<td>10</td>
</tr>
<tr>
<td>SD</td>
<td>±0.08</td>
<td>±0.1</td>
<td>±0.03</td>
<td>±0.01</td>
<td>±0.56</td>
<td>±0.24</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vasopressin</td>
<td>1.45</td>
<td>1.15</td>
<td>0.6</td>
<td>5.4</td>
<td>14.4</td>
<td>5.7</td>
<td>7</td>
</tr>
<tr>
<td>SD</td>
<td>±0.06</td>
<td>±0.08</td>
<td>±0.02</td>
<td>±0.1</td>
<td>±0.47</td>
<td>±0.38</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* In μmole/g tissue.

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The combination of data obtained in these experiments leads to the conclusion that the reduction in ATP concentration in the tissues is determined by disturbances of oxidative phosphorylation in the mitochondria. Determination of respiration and the phosphorylation coupled with it in mitochondria isolated from the heart an hour after the combined effect of vasopressin and high molecular weight dextran disclosed a reduction in these indicators.

The oxygen absorption rate in state V, i.e., in the presence of oxidation substrates but without phosphate acceptors, decreased by 18% for pyruvic acid and by 53% for α-ketoglutarate. Respiration with glutamic acid remained unchanged, and it increased by 34% with succinate. The phosphorylative respiration rate, i.e., respiration of the mitochondria in the presence of a phosphate acceptor, was reduced with all substrates used except succinic acid: by 39% with pyruvate, 57% with α-ketoglutarate, and by 20% for glutamate. With all substrates used, the respiration coupled with phosphorylation was reduced, as reflected in the reduction of respiratory control and phosphorylation efficiency coefficient (Table 3). The reduction in mitochondrial respiration rate in oxidation of NAD-dependent substrates, such as pyruvate and α-ketoglutarate, indicates disruption of an important energy exchange link, the nicotinamide coenzyme system.

In cardiac mitochondria isolated after the action of NADH, the cytochrome C reductase activity was reduced by 30%. This finding indicates that the process of transfer of hydrogen formed during biological oxidation to cytochrome C is hampered. Malate dehydrogenase activity decreased by 52%. Glutamate dehydrogenase activity in the mitochondria did not change (Table 4).

### Discussion

In analysis of the studies designed to determine respiration and oxidative phosphorylation, particular attention should be directed to those experiments in which succinic acid was used as the substrate for oxidation.

The respiration rate of mitochondria with succinate with addition of a phosphate acceptor rose approximately 1.5 times above control. The high respiration level apparently is connected, first,

### TABLE 2

<table>
<thead>
<tr>
<th>Glycogen Concentration and Phosphorylase Activity in Rabbit Myocardium with Intravenous Administration of High Molecular Weight Dextran and Vasopressin (M = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen (mg/100 mg tissue)</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Vasopressin-dextran</td>
</tr>
</tbody>
</table>

### TABLE 3

<table>
<thead>
<tr>
<th>Effect of Intravenous Administration of High Molecular Weight Dextran and Vasopressin on Oxygen Absorption Rate of Heart Mitochondria</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Normal</th>
<th>Dextran + vasopressin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate + malate</td>
<td>α-Ketoglutarate + malate</td>
<td>Glutamate + malate</td>
</tr>
<tr>
<td>Pyruvate + malate</td>
<td>α-Ketoglutarate + malate</td>
<td>Glutamate + malate</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>3.0</td>
<td>4.7</td>
<td>2.52</td>
</tr>
<tr>
<td>±0.08</td>
<td>±0.14</td>
<td>±0.12</td>
</tr>
<tr>
<td>(After use of ADP)</td>
<td>1.16</td>
<td>2.6</td>
</tr>
<tr>
<td>±0.04</td>
<td>±0.1</td>
<td>±0.06</td>
</tr>
<tr>
<td>Respiratory control (V/V)</td>
<td>2.51</td>
<td>2.17</td>
</tr>
<tr>
<td>±0.07</td>
<td>±0.08</td>
<td>±0.13</td>
</tr>
<tr>
<td>ADP/O</td>
<td>2.8</td>
<td>1.7</td>
</tr>
<tr>
<td>±0.15</td>
<td>±0.1</td>
<td>±0.2</td>
</tr>
<tr>
<td>Number of animals</td>
<td>19</td>
<td>17</td>
</tr>
</tbody>
</table>

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with an increase in succinic acid concentration and, second, with succinic dehydrogenase activity.

Both of these factors, as has previously been shown, increase sharply under hypoxic conditions, which apparently arise in our studies with changes in the rheological properties of the blood and disturbances of the microcirculation.

The advantage of succinic acid over NAD-dependent substrates in the respiratory chain of mitochondria is that the flavin-adenine nucleotide, which preserves the oxidized state longer under hypoxic conditions, is included in the composition of succinic dehydrogenase. However, despite the high mitochondrial respiration level with succinate, we discovered that the degree of stimulation of ADP and DNP respiration was one-half to one-third of normal (Fig. 1).

Considering the results of studies by A. D. Vinogradov and M. N. Kondrashova, the data obtained permit one to think of the presence of an inhibiting, protecting effect of oxaloacetic acid on succinic dehydrogenase activity. For evaluation of the data obtained, a series of tests was set up to determine the respiration rate with succinate, with addition of glutamic acid, which reduces the inhibiting effect of oxaloacetic acid. As is evident from Figure 1, the stimulating effect of ADP and DNP in the presence of glutamate increased to normal limits.

In summary, after the combined action of high molecular weight dextran and vasopressin, the concentration of high energy phosphate compounds in heart tissue is reduced below normal because of weakening of the conjugated respiration-phosphorylation processes in the mitochondria by use of NAD-dependent substrates. Simultaneously, a changeover takes place to respiration with the flavin-adenine-dependent substrate, succinic acid. The change to primary oxidation of succinic acid may play a role in the adaptation and compensation of oxidative metabolism to unfavorable conditions.

However, excessive oxidation of succinic acid, which occurs in hypoxia as a consequence of disruption of the capacity for regulation of its economical consumption, is limited by oxaloacetic acid, which inhibits succinic dehydrogenase. Inhibition of the latter becomes a significant factor in regulation of the respiration rate in an energy deficit in the respiratory chain.

In this manner changes in the rheological properties of blood, by way of the combined effect of high molecular weight dextran and vasopressin, lead to changes in the metabolic processes of the myocardium. These changes arise in a number of pathological states of the body. An understanding of all the energy exchange links in the heart may facilitate precise definition of the pathogenesis of a number of cardiovascular diseases.

Acknowledgment

We thank the Sandoz-AG Basel Company for providing the lysine-vasopressin for these studies.

### TABLE 4

<table>
<thead>
<tr>
<th></th>
<th>NAD-H₂-cytochrome C reductase</th>
<th>Malate dehydrogenase</th>
<th>Glutamate dehydrogenase</th>
<th>No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>24.6 ± 0.83</td>
<td>6.35 ± 0.77</td>
<td>9.9 ± 0.91</td>
<td>10</td>
</tr>
<tr>
<td>Vasopressin + dextran</td>
<td>17.22 ± 0.55</td>
<td>3.0 ± 0.41</td>
<td>10.5 ± 0.88</td>
<td>12</td>
</tr>
</tbody>
</table>

* Per mg protein per 60 min.

---

**FIGURE 1**

Effect of stimulating action of adenosine diphosphate and dinitrophenol on heart mitochondria oxygen absorption rate.

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Discussion

Dr. Robert B. Jennings, Chicago, Illinois: I would like to congratulate you on a successful study of a very neglected but important subject. It is clear that defects in the capillary circulation, such as those which might be induced by sludging, could produce extensive changes in myocardial function, and I think that you have confirmed this idea in your experiments. I think that your mitochondrial results might be secondary to persistent localized areas of hypoxia; the mitochondria may not be completely inoperational but rather may have lost critical cofactors, for example, magnesium.

Mela et al. have reported that mitochondria isolated from the liver of rats after five hours of shock showed similar functional defects. These defects could be repaired by adding magnesium to the medium. I suspect that this kind of a change may well be occurring here.

Dr. Steven E. Mayer, La Jolla, California: Dr. Chernukh, your comments about the resynthesis of glycogen in the heart were very interesting. Cardiac tissue appears to be capable of resynthesizing glycogen quite rapidly after depletion, and in fact, one can demonstrate overcompensation following
hypothesis in intact animals.

You stated that in these experiments dextran activated glycogen synthesis. One would expect a polysaccharide, such as dextran, to activate phosphorylase kinase since glycogen does. Do you have evidence that dextran with a molecular weight of 500,000 can penetrate into the myocardial cell?

Dr. Chernukh: We have good evidence that it does not enter the myocardial cell but remains in the capillaries. I cannot explain its mechanism.

Dr. Eugene Braunwald, Boston, Massachusetts: I think that this metabolic analysis of cellular changes which follow alterations in the microcirculation is of great interest and importance. I know Professor Chazov also believes there are important changes in the microcirculation which proceed over a matter of hours after coronary occlusion. Our task as clinicians is to try to limit the extent of damage. There are some interesting experiments being done in the United States which suggest that platelet aggregation may be as important as the sludging of erythrocytes. This possibility is interesting because one can probably do something about the aggregation of platelets. In experimental studies aspirin has been shown to decrease platelet aggregation when it occurs as a secondary effect of a major coronary occlusion. I wonder if you have given any consideration to the platelet as being the initiating problem in the extension of damage that may occur after a coronary occlusion.

Dr. Chernukh: Platelets represent a special problem. We have experience with platelet aggregation since it is the first stage in aggregation of erythrocytes, but it is very difficult to investigate in the cardiac microcirculation. As you know, Dr. Bing has made a movie of superficial cardiac vessels which is very interesting. We would like very much to do collaborative work with scientists in this part of the world. For example, we would like to have contact with Melvin Knisely at South Carolina, the father of the sludge problem.

Dr. E. I. Chazov, Moscow, U.S.S.R.: From the clinical viewpoint we must remember (1) the problem of thrombus formation, (2) the disturbances in the zones adjacent to the areas of infarction, and (3) cardiogenic shock. I think that the work from groups such as that of Professor Chernukh, one of the leading specialists in the field of microcirculation, will clarify many mechanisms which are responsible for these pathological conditions. I would like to say, and Professor Braunwald will probably agree with me, that today we must strive to change the disturbed microcirculation of the heart since there are now means to do so.

Regarding platelets, Dr. Corveloia in our country has studied the electric potential between the normal blood vessel wall and the platelet. It is very interesting that this potential is different from that which exists between the wall of an atherosclerotic vessel and platelets.

There are so many factors which influence the microcirculation that it is virtually a never-ending process. Research in this area will undoubtedly provide us with interesting information to be used in clinical practice.

Dr. Robert M. Berne, Charlottesville, Virginia: To follow up Dr. Braunwald’s question, I wonder to what extent the agglutination of red blood cells is caused by liberation of ADP from the platelets. Have you added adenosine to the blood of your animals to see whether you could reverse this agglutination when you reverse the ADP-induced agglutination?

Dr. Chernukh: This question is interesting, and we are working on it, but it is very difficult. I agree that in the future we must be concerned with both aggregation and disaggregation.

Reference

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