Energy Metabolism in Myocardium

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As the biochemical studies of metabolic pathways advance, it has come to the fore to clarify the organ-specific metabolism. From this standpoint, one of the specific characters of myocardial metabolism may lie in acquisition of a large amount of free energy to maintain the cardiac beating.

Energy metabolism has three phases. They are phases of energy liberation, of energy conservation and of energy utilization, respectively. Energy liberation means the mode of ATP production, coupling with oxidation of the cardiac fuels, such as glucose, pyruvate, lactate and fatty acids. Energy conservation means the storage of energy in high energy intermediates, such as creatine phosphate. Energy utilization means the formation of actomyosin using liberated free energy, in myocardium.

In this symposium, I will emphasize some functions and characteristics on myocardial energy liberation on the ground of the data in our experiments.

Methods of Estimation of Myocardial Energy Metabolism

The mechanism of energy liberation is oxidative phosphorylation and related reactions. It is well known that the enzyme system of these reactions is localized in mitochondrion.

Mongrel dogs were anesthetized with pentobarbital soda, and treated as described in each article in Results. Several pieces of myocardium were removed from the extirpated hearts and were immediately cooled down in ice-cold, isotonic mannitol-sucrose solution. The pieces of myocardium were sliced and homogenated, and mitochondrial fraction was isolated by centrifugation at 0°C. Mitochondria were suspended in phosphate buffer of pH 7.4 at 25°C.

Oxygen consumption was measured polarographically using Oxygen Consumption Recorder PO100 which was equipped with a rotating platinum electrode.

Oxygen consumption rate in state 3 (QO₂₃), that in state 4 (QO₂₄), respiratory control ratio (RC) and ADP-to-Oxygen atom ratio (ADP/O) were estimated from the polarograms. State 3 is a respiratory state in which oxygen, substrate, inorganic phosphate and ADP are adequately present, hence oxidative phosphorylation believed to proceed. On the contrary, state 4 is a state in which ADP is absent and oxidative phosphorylation does not occur. RC represents a regulating function of the mitochondrial respiration, and is calculated by the formula QO₂₃/QO₂₄. ADP/O is equalling to P/O, theoretically, and is calculated by the formula Amount of added ADP/Amount of consumed oxygen in state 3.

Protein concentration of the mitochondrial suspension was measured by the method of biuret reaction.

Energy Metabolism in Intact Heart Mitochondria

The chests of untreated, anesthetized dogs

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were opened under artificial respiration, and the beating hearts were removed. For a comparison, a piece of the tissue was removed from the liver.

When succinate, glutamate, α-ketoglutarate or pyruvate was used as a substrate, mitochondrial $QO_2$ was higher from heart than from liver tissue (Fig. 1). This result is concordant with the morphological observations by other investigators that heart mitochondria are larger in size and have more dense cristae than liver mitochondria.

**Myocardial Energy Metabolism in Pathologic States**

Experimental Myocardial Ischemia (Table I)

Experimental animals were divided into three groups. In the first, myocardial infarction group, the circumflex branch of the left coronary artery was ligated. Beating hearts were removed 15, 30 or 60 minutes after coronary ligation. Two pieces of the myocardium were obtained from infarcted area and non-infarcted area in each dog. In the second group, blood was removed through the femoral artery for 60 minutes until some ischemic changes were detected on electrocardiogram. In the last group, intact hearts were removed and stored in saline for 60 minutes with or without forced beating.

Whenever above mentioned substrates were used, $QO_2$ increased in infarcted area of the infarction group, the cold-letting group and the group of storage with forced beating, but $QO_2$ and ADP/O were within normal range. Therefore, RC of these groups were markedly deteriorated. It is considered that the uncoupling of oxidative phosphorylation is induced in these groups. On the other hand, mitochondrial respiration was quite normal in non-infarcted area of the infarction group and the group of storage without beating.

Hence, it is concluded that 1) the uncoupling of oxidative phosphorylation occurs in the early stage of experimental myocardial ischemia and 2) the change is induced from a state of cardiac beating under severe ischemia.

**Experimental Heart Failure (Table II)**

Many investigators reported on the energy metabolism in congestive heart failure. The results, however, did not necessary coincide on oxidative phosphorylation. The reason of the discordance may be due to the complexity of pathophysiology on the mechanism of the development of congestive heart failure. Furthermore, secondary injury of mitochondria, such as anoxia, may be induced during the experimental procedures.

In this consideration, the effect of the left ventricular pressure loading on the oxidative phosphorylation was studied. Ascending aortae of dogs were constricted about 40 per cent in diameter. After 60 minutes, when the left

<table>
<thead>
<tr>
<th>Condition of Myocardium</th>
<th>No. of Cases</th>
<th>Oxygen Consumption Rate (μl atoms O/mg protein/min) State 3</th>
<th>Respiratory ADP/O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>10</td>
<td>83.0±18.0</td>
<td>5.05±2.17</td>
</tr>
<tr>
<td>Noninfarcted</td>
<td>21</td>
<td>91.0±18.8</td>
<td>5.16±2.40</td>
</tr>
<tr>
<td>Infarcted, 60 min</td>
<td>11</td>
<td>87.8±9.03</td>
<td>2.03±0.40</td>
</tr>
<tr>
<td>Ischemic, 60 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without beating</td>
<td>8</td>
<td>97.6±22.3</td>
<td>5.26±1.69</td>
</tr>
<tr>
<td>With beating</td>
<td>5</td>
<td>82.8±11.0</td>
<td>3.04±1.64</td>
</tr>
<tr>
<td>Blood-letting</td>
<td>5</td>
<td>90.4±8.20</td>
<td>2.76±0.54</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Condition of Myocardium</th>
<th>No. of Cases</th>
<th>Oxygen Consumption Rate (μl atoms O/mg protein/min) State 3</th>
<th>Respiratory ADP/O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>10</td>
<td>83.0±18.0</td>
<td>5.65±2.17</td>
</tr>
<tr>
<td>60 min after aortic stenosis</td>
<td>6</td>
<td>79.4±12.2</td>
<td>6.46±0.74</td>
</tr>
</tbody>
</table>

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ventricular end-diastolic pressure was apparently elevated and maximal systolic pressure was decreased after initial high pressure period, the heart was extirpated and mitochondria were isolated from the left ventricular muscle. No remarkable change was detected on \( QO_2 \), RC and ADP/O.

It is considered that the left ventricular pressure loading per se does not affect to mitochondrial respiration, although it is possible that there is the impairment of energy production in the clinical cases of failing hearts. Pathologic States of the Heart Muscle Induced by Drugs (Table III)

There are few reports on the change of myocardial energy metabolism in the “damaged” heart muscle. To affect the function of myo-cardium, some drugs such as sympathomimetic-, sympathetic blocking agents or cardiac glycoside were given to dogs, and mitochondria were isolated from the left ventricles. Doses of the drugs given were quite a large quantity, as described in Table III.

Norepinephrine: Norepinephrine is known as an \( \alpha \)-adrenergic stimulator. A large dose of 10–20 mg/kg of norepinephrine was infused intravenously. Slight shortening of RR interval, ST elevation and in later abnormal Q waves were detected on electrocardiogram. Dog was sacrificed by extirpation of the beating heart, after 60 minutes.

\( QO_3 \) of mitochondria from the left ventricle was decreased and \( QO_4 \) was somewhat increased. RC was deteriorated about 45 per cent, although ADP/O was remained in the normal range.

Isoproterenol: Intravenous droplet infusion of 20–30 mg/kg 1-isoproterenol, whose action was recognized as \( \beta \)-adrenergic stimulant, was performed in dogs. Marked tachycardia and premature beats were recorded on electrocardiogram. Accordingly, ST elevation and abnormal Q waves were also detected at several leads. Blood pressure was dropped and the left ventricular end-diastolic pressure was increased. Moderate or severe hemorrhage was found on the epicardium and in the myocardium when the animal was sacrificed after 60 minutes infusion.

Mitochondrial respiration revealed somewhat decrease of \( QO_3 \) and increase of \( QO_4 \). As the result of these changes, RC was markedly deteriorated. But ADP/O showed a normal value.

Propranolol: There are many interesting studies on propranolol, especially as a blocking agent of \( \beta \)-adrenergic receptor. Beyond a common pharmacological conception, a large quantity of 4–6 mg/kg of propranolol was injected intravenously in dogs. Also in these cases, apparent ST elevation was confirmed in several leads of electrocardiogram.

Mitochondrial respiration showed a little depression of \( QO_3 \) in both state 3 and state 4. Then, RC was normal and ADP/O remained in normal range.

Strospeside: It is well known classically that an overdose of cardiac glycoside impairs cardiac functions, as called digitalis intoxication. Then, 0.3–0.4 mg/kg of strospeside was rapidly injected into dogs. Acute digitalis intoxication was confirmed from electrocardiographic findings such as multifocal ventricular premature contractions and atrioventricular conduction disturbances.

In spite of electrocardiographic changes, respiration of mitochondria from intoxicated myocardium was quite normal; i.e. \( QO_3 \), \( QO_4 \), RC and ADP/O showed no significant alteration with intact controls.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>( \text{Oxygen Consumption Rate} ) (( \mu \text{ atoms O}/\text{mg protein/min}))</th>
<th>Respiratory Control</th>
<th>ADP/O</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>83.0 ± 18.0 state 3 16.7 ± 5.08 state 4</td>
<td>5.65 ± 1.67</td>
<td>2.77 ± 0.19</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>10–20</td>
<td>59.2 ± 8.67 state 3 20.1 ± 5.39 state 4</td>
<td>3.12 ± 0.66</td>
<td>2.86 ± 0.16</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>20–30</td>
<td>67.6 ± 8.21 state 3 33.4 ± 6.64 state 4</td>
<td>2.48 ± 1.18</td>
<td>2.89 ± 0.01</td>
</tr>
<tr>
<td>Propranolol</td>
<td>4–6</td>
<td>63.8 ± 13.0 state 3 13.8 ± 3.95 state 4</td>
<td>5.41 ± 1.89</td>
<td>2.90 ± 0.08</td>
</tr>
<tr>
<td>Strospeside</td>
<td>0.3–0.4</td>
<td>84.0 ± 12.7 state 3 16.2 ± 3.58 state 4</td>
<td>5.30 ± 0.79</td>
<td>2.82 ± 0.10</td>
</tr>
</tbody>
</table>

From these results, it is considered that the mode of affection in mitochondrial respiration is variable in compliance with the causes which induce "myocardial damage". Although much speculation will be capable, the real mechanisms of these metabolic changes remain unknown. Details and discussions will appear in elsewhere.

**Conclusion**

It is considered that the studies on myocardial energy metabolism have just started for exploitation as far as they are proceeded from the standpoint of physiological and pathological specificity of the myocardium. So, it is difficult to state systematically a general view of myocardial energy production and/or utilization in its true meanings.

Such being the case, I may show a scheme on this problem (Fig. 2). Experimental myocardial ischemia is easily induced by coronary ligation or arterial blood-letting. If cardiac beating still continues in that situation, or positive chronotropic load becomes severe by infusion of isoproterenol etc., aerobic pathway of myocardial metabolism will be affected soon. Thus, mitochondrial respiration will be altered and oxidative phosphorylation may be uncoupled, probably associated with the disturbances of transmembranous electrolyte distribution and/or free fatty acid mobilization.

It is considered that the mechanism of oxidative phosphorylation is not affected by ventricular pressure load per se, or by a large dose of cardiac glycoside.

Anyway, the purpose of this paper is to show a brisk energy liberation mechanism and its changes in some experimentally induced pathologic states of myocardium. The details of the mechanism will be elucidated in the near future.

**References**

1. Olson, R. E.: Medicine 30: 21, 1941.