Relationship between Regional Myocardial Blood Flow and Tissue ATP Content in Acute Ischemia

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SUMMARY

This study was undertaken to determine the relationships between myocardial mitochondrial function, regional myocardial blood flow (MBF), and tissue ATP content in acute ischemia. Fifty-one anesthetized dogs were used in the tests. MBF was measured by the H2 gas clearance method in order to define the ischemic area, during periods of coronary occlusion of 10, 20, 60, and 90 min.

The correlation between MBF and myocardial ATP content in the ischemic area was positive and significant in each group (r=0.54-0.82). The ATP content in the true ischemic area (where MBF was less than 20 ml/min/100 Gm) decreased significantly even after 10 min of occlusion, but mitochondrial function decreased only after 20 min of coronary occlusion when compared to the nonischemic area. Although ischemia induces mitochondrial dysfunction, a very short period of ischemia did not cause significant disturbance of mitochondrial function.

Moreover, in the areas with MBF of 20–40 ml/min/100 Gm, the ATP content began to decrease 60 min after occlusion, whereas 10 or 20 min of occlusion did not reduce the ATP content. These results suggest that normal maintenance of ATP levels depends not only on MBF itself but also on the duration of ischemia plus the degree of damage to mitochondrial function, and that critical blood flow, defined as the minimum flow necessary to maintain the level of myocardial ATP, varies with the duration of ischemia.

Additional Indexing Words:
Regional myocardial blood flow  Hydrogen gas clearance  Ischemia  ATP  Mitochondria

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OF various biochemical changes resulting from ischemia, the depletion of tissue ATP is particularly important, since ATP is required for all energy demanding processes in a living organism.1)-4) For example, ATP is the direct energy source for the contractile apparatus in the heart.5) ATP is produced mainly by the TCA cycle in mitochondria, although it is also produced in part by anaerobic glycolysis. Accordingly, preservation of both the supply of substrates and oxygen and the mitochondrial function are essential factors for the biosynthesis of ATP. It is well known that a decrease in ATP content in the myocardium is induced shortly after ischemia. Ischemia is defined as the reduction of regional myocardial blood flow, but, as Becker et al have described, the degree of the reduction of myocardial blood flow is never uniform in the ischemic area.6,7) Therefore, measurement of the regional myocardial blood flow (MBF) is necessary for the evaluation of the metabolic changes occurring in the ischemic area. Several methods (e.g., hydrogen gas clearance and microsphere methods) have been used to determine MBF.6,8-10)

In this experiment, using the hydrogen gas clearance method, we investigated the relationships, between MBF, myocardial ATP and mitochondrial function in acute myocardial ischemia and tried to determine whether there is a critical MBF for the maintenance of myocardial ATP stores in the ischemic canine heart.

MATERIALS AND METHODS

Adult mongrel dogs of either sex, weighing 7 to 16 Kg, were anesthetized by intraperitoneal administration of 50 mg/Kg pentobarbital sodium. The dogs were intubated and placed on controlled respiration, with ventilation set at a rate of about 300 ml of room air/Kg body weight/min. The peripheral blood pressure was monitored by means of a catheter introduced into the right femoral artery. A left thoracotomy was performed via the fourth intercostal space, and the heart was exposed and suspended in a pericardial cradle. The left anterior descending coronary artery was dissected free immediately before the first diagonal branch for ligation (Fig. 1). After an equilibration period of 30 min, 51 dogs were divided into 4 groups according to the duration of coronary occlusion: Group I (n=10), 10 min of coronary occlusion; Group II (n=14), 20 min; Group III (n=17), 60 min; and Group IV (n=10), 90 min.

MBF was measured by the hydrogen gas clearance method described by Aukland et al.11) Four or five H₂ gas electrodes, made of platinum wires 0.08 mm in diameter, were embedded in the heart muscle to a depth of 4 to 5 mm from the epicardial surface. These electrodes were connected to an
Fig. 1. Schematic diagram of the main coronary arteries in the canine heart. The site of ligation is indicated by an arrow immediately distal to the first diagonal branch. The dotted area shows the ischemic region. Platinum electrodes were inserted into the midmyocardium labeled I to V, and regional myocardial blood flow was measured; I is ischemic area, V non-ischemic area, and II, III, and IV the border at the visible ischemic edge, respectively. Heart muscle mitochondria were sampled from the shaded ischemic and nonischemic regions. Tissue samples were obtained by a dental drill biopsy method for ATP determination.

amplifier (UH Meter Model PHG 201, Unique Medical Co., Tokyo). The electrodes were attached to the ischemic and nonischemic areas of the myocardium, as shown in Fig. 1. The animals received about 8% hydrogen gas via tracheal intubation for 2 min prior to measurement of MBF. This concentration of hydrogen gas has been confirmed to produce no change in heart rate or blood pressure. The hydrogen gas clearance curve was plotted, and MBF was determined from it by the theory of Kety according to Aukland. At 10, 20, 60, and 90 min following the coronary occlusion, 4 or 5 biopsy specimens were taken from the Pt-H₂ electrode areas of the beating heart for use in the determination of tissue ATP. Tissue samples with a 3 mm diameter were obtained by the dental drill biopsy method of Pool et al and frozen instantaneously by liquid nitrogen. The entire procedure for sampling was finished within a second. Following the measurement of MBF and drill sampling, the heart was removed and mitochondria were prepared from the ischemic and nonischemic areas (indicated by hatched bars in Fig. 1).

Heart mitochondria were prepared as reported previously from the true ischemic and nonischemic areas (Fig. 1). The indexes of mitochondrial function examined were the respiratory control index (RCI), the rate of oxygen consumption in State III (St. III O₂), and ADP/O. In order to measure the mitochondrial function, 2.8 ml of mannitol reaction mixture (0.3 M mannitol, 10 mM phosphate, 2.5 mM MgCl₂, 10 mM KCl, and 0.25 mM EDTA,
pH 7.4) and 0.3 ml of the mitochondrial sample were added, together with 0.1 ml of potassium succinate (0.2 M) and 0.05 ml of ADP (0.01 M) as substrates, to a reaction cell equipped with an oxygen electrode. The RCI was taken as the ratio between the rate of oxygen consumption after and before addition of ADP. St. III $O_2$ was calculated from the mitochondrial oxygen consumption as n atoms of oxygen consumed per mg of mitochondrial protein per min during State III respiration. ADP/O was calculated from the ratio of μmoles of ADP phosphorylated to μatoms of oxygen consumed. These indexes were measured immediately after the preparation of mitochondria.

Each frozen myocardial tissue sample obtained by the drill biopsy was weighed and homogenized with the addition of 2.5 ml of 7% perchloric acid per 100 mg tissue at 0°C. After deproteinization, tris buffer containing 4 N KOH was added to the homogenate to neutralize the perchloric acid, and the sample was centrifuged at 3,000 rpm for 10 min. The ATP content of samples was determined by a modification of McElroy-Strehler's firefly luminescence method which uses a firefly lantern extract (FLE 50, Sigma Company, St. Louis, Mo.). The measurements were made with a bioluminescence reader (Model BLR-101 C, Aloka Co., Tokyo).

All data were assessed by Student's t-test for the significance of difference between groups of animals and reported as standard deviation.

**RESULTS**

Although the MBF in the ischemic area has been determined as not being uniform following coronary ligation, MBF in the central ischemic area (indicated by hatched bars in Fig. 1) was found in this study to be consistently less than 20 ml/min/100 Gm. We defined the central ischemic area as the true ischemic area. Average MBF in the true ischemic area of Groups I–IV was 14±8, 11±5, 13±5, 14±5 (ml/min/100 Gm), respectively. There were no statistically significant differences among the 4 groups. Table I shows the measurements of mitochondrial function in the true ischemic area as well as the nonischemic area. In Group I, RCI of mitochondria from the true ischemic area was decreased slightly to 3.78±0.41, but this was not significant compared with mitochondria from the nonischemic area. St. III $O_2$ and ADP/O of mitochondria from the true ischemic area did not differ significantly from those from the nonischemic area. In Groups II, III, and IV, three functional indexes of mitochondria in the true ischemic area were decreased significantly compared with those in nonischemic area. That is, although ischemia induced mitochondrial dysfunction, a very short period of ischemia did not cause significant disturbance of mitochondrial function. Fig. 2 shows the changes
Table I. Mitochondrial Function in the True Ischemic Area and Nonischemic Area

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ischemia</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>RCI</td>
<td>3.78±0.41</td>
<td>3.10±0.58*#</td>
<td>2.51±0.62$</td>
<td>2.37±0.58*</td>
</tr>
<tr>
<td>St. III $O_2$</td>
<td>295±61</td>
<td>236±58*#</td>
<td>215±52*</td>
<td>209±69*</td>
</tr>
<tr>
<td>ADP/O</td>
<td>1.97±0.23</td>
<td>1.84±0.30</td>
<td>1.35±0.41$</td>
<td>n.d.</td>
</tr>
<tr>
<td><strong>Nonischemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCI</td>
<td>4.12±0.34</td>
<td>4.22±0.70</td>
<td>3.82±0.58</td>
<td>4.00±0.73</td>
</tr>
<tr>
<td>St. III $O_2$</td>
<td>338±53</td>
<td>336±58</td>
<td>302±60</td>
<td>287±62</td>
</tr>
<tr>
<td>ADP/O</td>
<td>2.02±0.08</td>
<td>2.01±0.10</td>
<td>1.96±0.13</td>
<td>1.88±0.24</td>
</tr>
</tbody>
</table>

(values are mean±SD)

In Group I, RCI in the true ischemic area appeared to be decreased slightly but this proved not to be significant compared to mitochondria from the nonischemic area. St. III $O_2$ and ADP/O in the ischemic area did not differ significantly from the value for mitochondria prepared from the nonischemic area.

Group I: 10 min of coronary occlusion
Group II: 20 min of coronary occlusion
Group III: 60 min of coronary occlusion
Group IV: 90 min of coronary occlusion

*: $p<0.01$ versus nonischemic area of corresponding group,
#: $p<0.01$ Group I versus Group II,
$: p<0.01$ Group II versus Group III.
n.d. = not detectable.

Fig. 2. Effects of coronary occlusion on the ATP content of myocardium in the true ischemic area (MBF was less than 20 ml/min/100 Gm) and nonischemic area. The ATP contents in the true ischemic area decreased significantly even after 10 min of occlusion compared to that in the nonischemic area.
Fig. 3. The relationship between MBF and tissue ATP content in Groups I to IV. A significant correlation between MBF and tissue ATP was found for each group. 

(A) = Group I; $r = 0.54$, $p < 0.01$,  
(B) = Group II; $r = 0.55$, $p < 0.01$,  
(C) = Group III; $r = 0.71$, $p < 0.01$,  
(D) = Group IV; $r = 0.82$, $p < 0.01$.

in ATP content after ligation, both in the true ischemic area and in the non-ischemic area. The ATP content in the true ischemic area decreased significantly even after 10 min of occlusion compared to that in the nonischemic area. Twenty min of occlusion induced a significant decrease in ATP content in the ischemic area compared to that in the ischemic area 10 min after occlusion. Fig. 3A–D show the relationship between MBF and ATP content in Groups I–IV, respectively. The MBF in the nonischemic area was $118 \pm 30$ ml/min/100 Gm. When the ischemic area is defined as the area with MBF
Table II. Correlation between Myocardial Blood Flow (MBF) and Tissue ATP Content

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>True ischemia</td>
<td>3.38±0.80*</td>
<td>2.77±0.74*#</td>
<td>1.67±0.81*$</td>
<td>1.97±0.73*</td>
</tr>
<tr>
<td>(0≤MBF&lt;20)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Border area 1</td>
<td>4.51±0.79</td>
<td>4.28±0.63</td>
<td>3.47±0.79*</td>
<td>3.52±0.68*</td>
</tr>
<tr>
<td>(20≤MBF&lt;40)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Border area 2</td>
<td>4.91±0.75</td>
<td>4.51±0.68</td>
<td>4.39±0.51</td>
<td>4.21±0.58</td>
</tr>
<tr>
<td>(40≤MBF&lt;55)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Nonischemia</td>
<td>5.14±0.66</td>
<td>4.97±0.72</td>
<td>5.01±0.85</td>
<td>4.90±0.80</td>
</tr>
</tbody>
</table>

ATP content in the true ischemic area was significantly decreased compared to the nonischemic area ATP content in each group. In Groups III and IV, significant decreases in ATP content in the border area 1 were observed.

*: p<0.005 versus nonischemia,
#: p<0.05 10 min versus 20 min,
$: p<0.005 20 min versus 60 min.
MBF = myocardial blood flow (ml/min/100 Gm).
ATP: (μmoles/Gm wet).

below the mean MBF minus two standard deviations, the ATP content in ischemic area was found to depend on the MBF. That is, the following relationship was observed:

Group I \( f(\text{ATP}) = 0.0327 \times \text{MBF} + 3.33 \), \( r = 0.54, p<0.01 \)
Group II \( f(\text{ATP}) = 0.0402 \times \text{MBF} + 2.44 \), \( r = 0.55, p<0.01 \)
Group III \( f(\text{ATP}) = 0.0708 \times \text{MBF} + 1.06 \), \( r = 0.71, p<0.01 \)
Group IV \( f(\text{ATP}) = 0.0657 \times \text{MBF} + 1.26 \), \( r = 0.82, p<0.01 \)

A stronger correlation was observed in Groups III and IV than in Groups I and II. Table II shows the ATP content in the true ischemic, border and nonischemic areas in each group. The border area was defined as the area with MBF between the true ischemic and nonischemic area and this border area was further divided into 2 groups according to the MBF. Border area 1 is defined as the area with MBF (20≤MBF<40), border area 2 (40≤ MBF<55). As shown in Fig. 2, ATP content in the true ischemic area (MBF<20) was significantly decreased compared to the nonischemic area. ATP content of Group III was significantly lower than that of both Groups I and II. In Groups I and II, no significant difference in the ATP content in border area 1 was found compared to that in the nonischemic area, while in Groups III and IV, a significant decrease in ATP content in border area 1 was observed. ATP content in border area 2 did not differ significantly from that in the nonischemic area in any of the 4 groups.
DISCUSSION

While many studies have examined the biochemical changes caused by ischemia, the effect of the degree of MBF reduction on the metabolic changes in the ischemic myocardium has not been studied in detail.\textsuperscript{17)-21} It has been pointed out that MBF is markedly reduced in the area surrounding an ischemic center.\textsuperscript{7} As we have demonstrated in the present paper, blood supply was almost equal in each group in the nonischemic area, while in the ischemic area, the decrease in blood supply was not uniform. Is there a critical level of MBF which induces metabolic changes in the ischemic area? If so, what is the critical level and how much MBF is necessary to maintain the required rate of ATP formation? The answers to these questions would contribute a great deal to the clinical determination of the viability of an ischemic heart when non-invasive measurement of MBF in the ischemic heart muscle becomes possible in the near future.\textsuperscript{22),23} To answer these questions, it is necessary to clarify the relationship between the MBF and the myocardial ATP stores in ischemic areas. In order to measure MBF, several methods (including the microsphere and the gas clearance procedures) have been established. Becker, using the radioactive microsphere method, found MBF in the ischemic area to be 0–30 ml/min/100 Gm, and Lekven, using the hydrogen gas clearance method, reported that MBF was closely related to local ischemic ST-T changes in ECG.\textsuperscript{6),8} Isselhard, using the \textsuperscript{85}Kr clearance method, showed that 35 ml/min/100 Gm was the critical blood flow for the maintenance of myocardial ATP content.\textsuperscript{24} Jennings also reported that the myocardial level of ATP was correlated with the duration of ischemia, but he did not measure the MBF in his experiments.\textsuperscript{1} In the present paper, it was demonstrated that when MBF was below 20 ml/min/100 Gm, the myocardial level of ATP was lower in the ischemic area than in nonischemic area after just 10 min of ischemia. Moreover, in the areas with MBF of 20–40 ml/min/100 Gm, the level of ATP began to decrease 60 min after occlusion, although 10 or 20 min of occlusion had no effect on the level of ATP. These results suggest that ATP depends not only on MBF itself but also on the duration of ischemia, and critical blood flow, defined as the minimum flow to maintain the level of myocardial ATP, is a function of the duration of ischemia.

Mitochondrial function was damaged after 20 min of occlusion. On the other hand, myocardial tissue ATP was already decreased after 10 min of occlusion. This fact suggests that myocardial ATP levels were not reduced because of damage to mitochondria in the ischemic area, but rather because of decreased substrate supply, oxygen supply, or H+ elimination. However, after more than 20 min of coronary occlusion, the decrease in ATP content
seems to have depended upon both the reduction of MBF and the deterioration of mitochondrial function due to ischemia. Although myocardial mitochondrial function is compromised when the MBF is moderately decreased, the ATP content is reduced in proportion to the increase of the duration of ischemia. Therefore, mitochondrial function in border area 1 will probably deteriorate during 60 min of coronary occlusion because of the decrease in ATP content in that area. Consequently, the true ischemic area is enlarged over a period of 60 min ischemia. It is extremely important to learn how border area 1 can be protected or rescued clinically during the ischemic insult.

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