Ischemic preconditioning suppresses the noradrenaline turnover in the rat heart

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Abstract

Objective: The mechanism by which ischemic preconditioning protects the heart is presumed to be related to the reduction of energy consumption during a subsequent myocardial infarction. Since the sympathetic nervous system enhances cardiac function and energy consumption, we investigated the relation between ischemic preconditioning and the turnover rate of noradrenaline (NA) in the rat heart.

Methods: The effect of 3 cycles of 5-min occlusions of the rat left coronary artery on changes in arterial blood pressure and heart rate provoked by a subsequent 30 min of ischemia were examined until 60 min after reperfusion. The effect of 3 cycles of occlusions on the infarct size was also evaluated 60 min after reperfusion by comparing the infarcted area with the area at risk in these animals (6 per preconditioned and sham-operated group). The tissue concentration of NA during sustained ischemia was determined in the left ventricle, the intraventricular septum, and the right ventricle in the preconditioned and sham-operated groups. Changes in the turnover rate of NA after 3 cycles of occlusions were also evaluated by assessing the alpha-methyl-p-tyrosine-induced depletion of NA (n=7 per group).

Results: A series of transient occlusions reduced the infarct size 60 min after a sustained ischemia for 30 min. Arterial pressure and heart rate were not affected. The concentration of NA was decreased in the left ventricle 60 min after the onset of sustained ischemia in both the preconditioned and sham-operated groups. The treatment with alpha-methyl-p-tyrosine decreased the NA concentration in all regions of the heart in the sham-operated group after 60 min. However, the treatment with alpha-methyl-p-tyrosine did not deplete the NA concentration in both the occluded and nonoccluded regions in the preconditioned group.

Conclusions: Transient ischemia ameliorated the heart injury induced by a subsequent sustained ischemia, as assessed histologically. The activity of the sympathetic nervous system in all regions of the heart was reduced by transient ischemia in the left coronary vascular bed. These findings suggest that the inhibition of the sympathetic nervous system by the treatment of ischemic preconditioning takes part in the cardiac protection.

Keywords: Ischemic preconditioning; Noradrenaline; Sympathetic nervous system; Heart; Rats

1. Introduction

Many investigators have shown that ischemic preconditioning reduces the extent of myocardial injury in a subsequent myocardial infarction in various animal species [1–5]. Although the mechanism underlying the protective effect remains unclear, ischemic preconditioning is speculated to protect the myocardium by reducing energy consumption in a subsequent myocardial infarction [6]. On the other hand, it has been shown that energy depletion in ischemia induces the release of noradrenaline (NA) from the sympathetic nerve endings in the heart [7,8], and ischemic preconditioning has been reported to reduce the NA release in a subsequent sustained ischemic period in isolated rat hearts [9,10]. However, the effect of ischemic preconditioning on the activity of the sympathetic nervous system in vivo hearts has not been clarified. Further, the actions of catecholamines and adrenergic receptors have been demonstrated to take part in the reduction of infarct size by ischemic preconditioning [11,12]. The depletion of...
NA in the sympathetic nerve endings in the rabbit heart by reserpine has also been reported to abolish the effect of ischemic preconditioning [13]. These findings indicate that the beneficial outcomes by ischemic preconditioning may be related to the alteration of the activity of the sympathetic nervous system. Because the sympathetic nervous system enhances cardiac contractile function and energy consumption, we investigated in the present study, the effect of transient ischemia on the changes in the activity of the noradrenergic (NAergic) system in the rat heart. We then determined the correlation of these changes with the functional and histologic outcomes.

2. Methods

2.1. Animals

This study was approved by the Committee on Animal Experimentation at Ehime University School of Medicine and conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985). Male Wistar rats weighing about 350 g (Charles River, Yokohama Japan) were housed in groups in a room controlled at 23±1°C and maintained in an alternating 12-h light/12-h dark cycle (lights on at 6:00 h). Food and water were provided ad libitum. Animals were killed between 12:00 and 17:00 h in all experiments in view of the diurnal variation of the activity of the sympathetic nervous system.

2.2. Experimental protocols

In experiment 1, the effects of transient ischemia on physiologic variables, plasma concentrations of myocardial enzymes, and infarct size were evaluated after a subsequent myocardial infarction (Fig. 1A). In experiment 2, the effect of transient ischemia on the concentration of NA in a sustained ischemia was examined (Fig. 1B). In experiment 3, the effect of transient ischemia on the turnover rate in the NAergic system was estimated (Fig. 1C).

2.3. Ischemic preconditioning

The animal was anaesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg). To maintain anesthesia for experimental periods, additional sodium pentobarbital (20 mg/kg) was given every 30 min. This anesthesia was chosen not to affect the release of NA [14]. The depth of anesthesia was controlled at an appropriate level for the comfort of the animal, because the physiologic variables were not changed by the operation. Through a ventral middle cervical incision, the trachea was exposed, and an endotracheal tube was inserted. The animal was kept artificially ventilated with oxygen throughout the experiments. In the preliminary experiment, the ventilation volume and frequency were determined to be 1.5 ml and 40/min, respectively, by analysis of the arterial blood gas. Esophageal and rectal temperatures were measured by thermocouple probes, and were maintained at 37–38°C with a heating lamp throughout the experiment.

A left thoracotomy was performed through the fourth intercostal space. After the dissection of the pericardium, a piece of nylon thread (4-0) was introduced through the ventricular tissue surrounding the left coronary artery and vein a few millimeters distal to the aortic root. Ten units of heparin was injected via the caudal vein. Following a stabilization period of 10 min, 3 cycles of 5-min occlusions, each separated by 5 min of reperfusion, were performed. In sham-operated control rats, transient ischemia was not performed, although a nylon thread was introduced surrounding the left coronary artery after a thoracotomy.

2.4. Physiologic variables and blood analysis (Experiment 1)

Twelve rats were prepared and evenly assigned to 2
2.6. Determination of the NA concentration in a sustained ischemia (Experiment 2)

Thirty rats were prepared and evenly assigned to 5 groups. Animals in one group served as intact controls. Animals in 2 other groups were subjected to transient ischemia by means of the procedure described above. The remaining 2 groups received the sham operation. Five min after the reperfusion following the last cycle of transient ischemia or the sham operation, sustained myocardial ischemia was induced. After 20 or 60 min of ischemia, the heart was quickly dissected and rinsed in saline, and myocardial samples were dissected from three regions: left ventricular free wall (LV), intraventricular septum (IS), and right ventricular free wall (RV).

Each tissue sample was homogenized in 1 ml of 0.4 mol/l perchloric acid. After being centrifuged and filtered through a membrane filter (0.22 μm), 10 μl of the filtrate was applied to a high-performance liquid chromatography (HPLC) system with electrochemical detection for determining the amount of NA, according to the method of Magnussen et al. with a modification [15]. The HPLC system was composed of a pump equipped with a damper (EP-300, Eicom, Kyoto, Japan), an electrochemical detector (ECD-300, Eicom) with a graphite working electrode operated at 450 mV vs. an Ag–AgCl reference electrode (RE-100, Eicom), and a reversed-phase column (C5ODS, 2.1×150 mm inside diameter, Eicom). The mobile phase was 0.1 mol/l sodium phosphate buffer containing 5% methanol, 1.85 mmol/l sodium 1-octanesulfonate and 10 μmol/l disodium EDTA, pH 6.0.

2.7. Determination of the NA turnover (Experiment 3)

The turnover rate was estimated from the depletion of NA induced by alpha-MT. Twenty-eight rats were prepared and evenly assigned to 4 groups. Two groups were subjected to transient ischemia by means of the procedure described above. The other 2 groups received the sham operation. Immediately after the reperfusion of the last cycle of transient ischemia or the sham operation, alpha-MT (200 mg/kg) or saline was injected intraperitoneally into each pair of preconditioned and sham-operated groups, respectively. Sixty min after the injection, the heart was quickly dissected, and myocardium samples were dissected from the three regions. The difference in the NA turnover rate among the groups was evaluated by comparing the alpha-MT-induced depletions of NA.

2.8. Drugs and chemicals

Sodium pentobarbital was obtained from Abbott Laboratories (North Chicago, IL, USA). Alpha-MT hydrochloride methyl ester was purchased from Sigma Chemical, (St. Louis, MO, USA). Zinc cadmium sulfide yellow fluorescing microbeads were obtained from Duke Scientific (Palo Alto, CA, USA). TTC-H2O was purchased from Research Organs (Cleveland, OH, USA). All chemicals used were of guaranteed reagent grade.

2.9. Statistical analysis

The data from blood pressure and heart rate were analyzed by repeated measures analysis of variance followed by Scheffé’s test. The results of the blood analysis were evaluated by analysis of variance (ANOVA) with Bonferroni’s adjustment. The data from the ischemic size in the heart was evaluated by the Mann-Whitney test. The data from the measurement of NA and the turnover rate
were evaluated by two-way analysis of variance followed by Scheffé’s test.

3. Results

3.1. Physiologic variables

There was no significant difference in the blood pressure and heart rate values before and after the sustained ischemia in the sham-operated rats (Table 1). The treatment of ischemic preconditioning did not alter these physiologic variables before or after the myocardial ischemia.

Table 1
Effects of 3 cycles of 5-min transient occlusions on mean arterial blood pressure and heart rate in rats

<table>
<thead>
<tr>
<th></th>
<th>Before ischemia</th>
<th>Immediately after reperfusion</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sham-operated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>78±10</td>
<td>80±23</td>
<td>72±21</td>
</tr>
<tr>
<td>HR (/min)</td>
<td>375±36</td>
<td>369±28</td>
<td>374±39</td>
</tr>
<tr>
<td><strong>Preconditioned</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>87±13</td>
<td>81±15</td>
<td>76±24</td>
</tr>
<tr>
<td>HR (/min)</td>
<td>387±49</td>
<td>384±51</td>
<td>390±51</td>
</tr>
</tbody>
</table>

A sham operation or 3 cycles of 5-min transient ischemia was performed, and mean arterial blood pressure (MABP) and heart rate (HR) were determined before sustained ischemia, immediately after reperfusion and 60 min after reperfusion. Each value is the mean±SD of 6 animals.

3.2. Blood analysis

There were also no differences in the values of arterial blood gas analysis between the groups (Table 2A). The sustained ischemia for 30 min produced marked increases in the plasma concentrations of GOT, LDH, and CPK 60 min after reperfusion (Table 2B). The treatment of ischemic preconditioning did not affect these elevations of plasma enzymes.

3.3. Infarct size

There was no difference in the area at risk between the two groups. However, the infarct size 60 min after the sustained ischemia was markedly smaller in the precon-

Table 2
Effects of 3 cycles of 5-min transient occlusions on arterial blood gas and plasma enzymes

(A) Arterial blood gas analysis

<table>
<thead>
<tr>
<th></th>
<th>Before ischemia</th>
<th>Immediately after reperfusion</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sham-operated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.426±0.063</td>
<td>7.383±0.053</td>
<td>7.436±0.052</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>35±4</td>
<td>37±7</td>
<td>33±6</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>247±40</td>
<td>261±101</td>
<td>295±57</td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/l)</td>
<td>23±4</td>
<td>22±3</td>
<td>22±5</td>
</tr>
<tr>
<td>BE (mmol/l)</td>
<td>−0.8±4.5</td>
<td>−2.6±2.8</td>
<td>−1.0±4.5</td>
</tr>
<tr>
<td><strong>Preconditioned</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.360±0.074</td>
<td>7.402±0.030</td>
<td>7.403±0.060</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>37±11</td>
<td>34±5</td>
<td>33±7</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>248±88</td>
<td>275±62</td>
<td>261±39</td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/l)</td>
<td>20±2</td>
<td>21±2</td>
<td>21±6</td>
</tr>
<tr>
<td>BE (mmol/l)</td>
<td>−4.8±1.6</td>
<td>−2.7±1.9</td>
<td>−2.9±5.5</td>
</tr>
</tbody>
</table>

(B) Plasma enzymes (I.U./l)

<table>
<thead>
<tr>
<th></th>
<th>GOT</th>
<th>GPT</th>
<th>LDH</th>
<th>CPK</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sham-operated</strong></td>
<td>543±282</td>
<td>106±84</td>
<td>4596±4942</td>
<td>902±437</td>
</tr>
<tr>
<td><strong>Preconditioned</strong></td>
<td>385±279</td>
<td>112±148</td>
<td>2761±2654</td>
<td>630±273</td>
</tr>
</tbody>
</table>

A sham-operation or 3 cycles of 5-min transient ischemia was performed. (A) Arterial blood gas was determined before sustained ischemia, immediately after reperfusion, and 60 min after reperfusion. (B) The plasma concentrations of GOT, GPT, LDH and CPK were determined 60 min after reperfusion. Each value is the mean±SD of 6 animals. BE, base excess; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; LDH, lactic dehydrogenase; CPK, creatine phosphokinas.
ditioned group than in the sham-operated group ($P<0.01$). The ratios of infarcts of the area at risk were $0.54\pm0.11$ (mean$\pm$SD, $n=6$) in the sham-operated group and $0.16\pm0.17$ in the preconditioned group (Fig. 2). The infarct size was reduced in most animals in the preconditioned group except one. The blood flow of the left coronary artery may not have been resumed after the transient occlusions in this animal.

3.4. Changes in the NA concentration in a sustained ischemia

The sustained ischemia gradually decreased the NA content in the LV region in the sham-operated animals, the effect being significant 60 min after the start of ischemia (Fig. 3). The NA content in the preconditioned group also decreased gradually. There was no significant difference in the decrease in the NA concentration between the two groups. On the other hand, the NA concentration in the IS and RV regions did not change as a result of sustained ischemia in both the sham-operated and preconditioned groups.

3.5. Changes in the NA turnover by ischemic preconditioning

There was no difference in the heart concentration of NA in each region examined between the saline-injected preconditioned and saline-injected sham-operated groups. The administration of alpha-MT decreased the concentration of NA in all regions in the sham-operated rats (Fig. 4). However, the alpha-MT injection did not deplete the tissue NA in any region in the animals subjected to transient ischemia.
4. Discussion

In the present study, a series of 3 cycles of 5-min occlusions of the left coronary artery markedly ameliorated the extent of necrosis provoked by a subsequent myocardial infarction in the rat heart. This is in good agreement with the previous result that 3 cycles of 3- or 5-min occlusions successfully preconditioned the rat heart [4,5].

Concerning the tissue concentration of NA, sustained ischemia by the left coronary artery occlusion gradually decreased the NA content in the LV region, and transient ischemia prior to sustained ischemia did not exert an influence on this change. Since the myocardium is innervated by the sympathetic nervous system, in which NA plays a neurotransmitter role, the amount of NA in the nerve endings does not seem to be influenced by transient ischemia.

To estimate the turnover of the heart NA, we applied the enzyme inhibition technique using alpha-MT, which inhibits the hydroxylation step from tyrosine to dopa, the rate-limiting step in the synthesis of catecholamines [16]. Although transient ischemia did not alter the NA content after 60 min, the alpha-MT-induced depletion of NA was markedly suppressed by transient ischemia in all three regions of the heart examined. This finding indicates that the treatment of ischemic preconditioning reduces the activity of the sympathetic nervous system in the heart. It is therefore likely that the activity of the sympathetic nervous system in preconditioned animals is also depressed during subsequent sustained ischemia. Since the turnover rate also represents the extent of the release of the neurotransmitter as well as the synthesis, transient ischemia may suppress the release of NA from the sympathetic nerve endings. This finding is in good agreement with previous reports that showed the reduction of the ischemic NA release by transient ischemia in isolated hearts [9,10].

Ischemic preconditioning prevents malignant arrhythmias such as ventricular fibrillation and ventricular tachycardia [4,17–21]. This action is speculated to be caused by the depletion of the NA store in the sympathetic nerve terminals, since the pharmacological depletion of endogenous catecholamines protects the heart against reperfusion-induced arrhythmias. In the present study, however, transient ischemia did not deplete the myocardial NA; it inhibited the release of NA, and thus afforded a marked protection against subsequent ischemia. The antiarrhythmic effect may be provided by the suppression of the NA release. It has recently been shown that a single cycle of 5 min of ischemia is sufficient to abolish ischemia-induced arrhythmias, but that 3 cycles are required against necrosis [22]. This difference implies that the mechanism by which ischemic preconditioning protects the heart against necrosis may be qualitatively different from that against arrhythmias.

The protective effect of ischemic preconditioning is presumed to be related to the balance between energy supply and demand during a subsequent ischemic event [6,23]. The amount of creatine phosphate, a high-energy compound, has been demonstrated to be increased by ischemic preconditioning. However, since this increase is counterbalanced by a depletion of ATP, the net amount of high-energy phosphate in preconditioned myocardium at the onset of myocardial ischemia is speculated to be no greater than that in the nonpreconditioned control myocardium [6,24]. In contrast, much less ATP has been shown to be utilized in the preconditioned myocardium during the first 10 min of ischemia [6]. For these reasons, slowed energy consumption in preconditioned myocardium may provide the benefit during ischemia and reperfusion. The NA release facilitates energy consumption through the beta-adrenergic action on ventricular myocytes. Further, an energy-sparing effect by ischemic preconditioning has been shown to be caused by the inhibition of the beta-adrenergic action [25,26]. Therefore, the inhibition of the NA release observed in the present study may be a mechanism by which ischemic preconditioning protects the heart.

As observed in the present and previous studies, ischemic preconditioning provides its beneficial effect in as short a time as a few minutes. This rapid adaptation suggests that the effect of ischemic preconditioning is caused by circulating ischemic metabolites or neuronal modulation. Ischemic preconditioning of the circumflex artery with 4 cycles of 5 min occlusions was reported to protect the left anterior descending coronary vascular bed as well as the circumflex vascular bed [27]. A similar phenomenon was observed in the present study. The reduced turnover rate of NA produced by transient ischemia was found in the RV region as well as in the LV and the IS regions, although the vasculature of the left coronary artery does not exist in the RV region. Although the mechanism of this distant effect of preconditioning is not clear, the effect implies the involvement of humoral or neuronal elements in the protection. Adenosine is released after the onset of myocardial ischemia [28], and the protective effect of preconditioning is thought to be closely related to the action of adenosine A1 receptors coupled with the Gi protein activation, which has an energy-sparing effect [29–34]. The released adenosine from the occluded area may exert a beneficial influence on the nonoccluded area as well as the occluded region. On the other hand, adenosine inhibits the exocytotic release of NA from nerve endings through presynaptic A1 receptors in a nonischemic condition. This action of adenosine may also take part in the distant effect by affecting the activity of the NAergic system in both the occluded and non-occluded regions.

In ischemia, NA is released from sympathetic nerve endings during the first few min of ischemia in an
exocytotic manner, whereas it is released in a nonexocytotic manner in the late phase of ischemia. Since the NA release in the early phase of ischemia is reduced by adenosine [35,36], ischemic preconditioning may preserve the transmitter NA by suppressing the activity in the early phase of ischemia. Thus, the preservation of the transmitter NA store by pretreatment with reserpine reportedly reversed the protective effect of ischemic preconditioning [13]. This indicates the importance of the NAergic system in the mechanism of the protective action of ischemic preconditioning.

In the present study, we observed the reduction of the NA turnover by transient ischemia in both preconditioned and nonpreconditioned areas of the heart. The decrease in the activity of the sympathetic nervous system may be involved in the beneficial effect of ischemic preconditioning.

References

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