Ischemic Preconditioning Fails to Limit Infarct Size in Reserpinized Rabbit Myocardium

Implication of Norepinephrine Release in the Preconditioning Effect

Christopher F. Toombs, PhD; Ann L. Wiltse, BS; Ronald J. Shebuski, PhD

Background. Infarct size reduction by ischemic preconditioning is believed to be mediated by adenosine; however, whether adenosine is the factor responsible for the initiation of this protection remains unknown. It is possible that during preconditioning, adenosine stimulates receptors on presynaptic nerve terminals and retards the release of norepinephrine (NE) during the prolonged ischemia or that NE release during preconditioning augments adenosine production.

Methods and Results. To test whether the release of NE is involved in the preconditioning phenomenon, rabbits were pretreated with reserpine (5 mg/kg sc, 24 hours before) to deplete presynaptic nerve terminals of NE stores. On the day of the experiment, the rabbits were anesthetized with ketamine-xylazine and instrumented for coronary occlusion. Nonreserpinized animals were used as controls. The control group (n=7) was subjected to 30 minutes of coronary occlusion and 120 minutes of reperfusion (ischemia-reperfusion) only. The preconditioned group (n=10) received 5 minutes of preconditioning ischemia and 10 minutes of reperfusion before the prolonged ischemia-reperfusion. Of the reserpinized animals, half (n=7) received preconditioning before ischemia-reperfusion and the remaining animals (n=7) did not. At termination of the experiment, an intravenous tyramine challenge (1 mg/kg) was used to confirm NE depletion in reserpinized rabbits. The resulting infarcts were measured with tetrazolium and planimetry. With comparable hemodynamics and areas at risk, infarct size in control animals was 39.8±2.1% of the risk region. Preconditioned animals showed an expected reduction of infarct size to 14.8±2.2% of risk region (P<.05 vs control). Of the reserpinized animals, those that received reserpine alone had infarcts that were 38.5±4.5% of risk region, and those that were preconditioned had infarcts that were 41.4±3.6% of risk region, which was not significantly different than the control group.

Conclusions. We conclude that preconditioning fails to protect ischemic-reperfused myocardium in reserpinized rabbit myocardium, indicating that the release of NE during either preconditioning or prolonged ischemia is critical to preconditioning mediated protection. (Circulation. 1993;88[part 1]:2351-2358.)

Key Words • ischemia • infarction • preconditioning • norepinephrine • reserpine

The phenomenon of ischemic preconditioning first described by Murry\(^1\) has received much attention in scientific circles. Proposed mechanisms and contributors to the inducible tolerance of myocardium to a prolonged ischemic insult have included the involvement of adenosine at the A\(_1\)-receptor,\(^2,3\) inhibitory G-proteins,\(^4\) and the involvement of ATP-sensitive potassium channels of the ventricular myocyte.\(^5-8\) Regarding the involvement of adenosine, it is also true that adenosine receptors exist on presynaptic nerve terminals in the myocardium, where they are involved in the regulation of norepinephrine release.\(^9-11\) thereby providing a site where these two elements (adenosine and norepinephrine) may interact. Therefore, the potential involvement of norepinephrine release from the sympathetic nerve terminal in ischemic preconditioning should be evaluated.

We hypothesized that infarct size limitation by ischemic preconditioning is dependent on the presence of releasable stores of norepinephrine in sympathetic nerve terminals. In this report, we studied the ability of ischemic preconditioning to produce tolerance to subsequent ischemia in animals that were pretreated with reserpine to deplete myocardial norepinephrine stores. Our results indicate that when myocardium is depleted of its norepinephrine stores, ischemic preconditioning fails to produce tolerance to a subsequent ischemic event, thereby implicating the sympathetic nerve terminal in the initiation of the ischemic preconditioning's cardioprotective effect.

Methods

All animals used in this study were handled in compliance with the Guiding Principles in the Care and Use of Animals, DHEW Publication No. (NIH 80-23, revised 1978, reprinted 1980, Office of Science and Health Reports, DRR/NIH, Bethesda, Md). All experiments

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were performed in AAALAC-approved laboratories with protocols that were reviewed and approved by the Corporate Animal Welfare Committee.

**Effect of Reserpine (Pressor Response to Tyramine)**

Previous reports have shown that a 24- to 48-hour treatment with reserpine can deplete myocardial norepinephrine stores in the rabbit to less than 1% of normal values. In a separate series of studies (n=9), rabbits that had been treated with reserpine (5 mg/kg per day) were retrieved from their cages after treatment for either 24 or 48 hours. Animals that had not received reserpine were used as controls. The rabbits were anesthetized with ketamine (35 mg/kg) and xylazine (6 mg/kg) and instrumented for monitoring of arterial pressure through a cannula in the left common carotid artery. Venous access was gained through cannulation of a marginal ear vein. Acute changes in mean arterial pressure were measured in response to an intravenous challenge dose of tyramine (1 mg/kg), which is known to stimulate the release of norepinephrine from sympathetic nerve terminals. We have previously found that the 1 mg/kg dose of tyramine produces an immediate increase in mean arterial pressure of approximately 45 mm Hg, an effect that is sustained for nearly 1 minute and thereafter returns to baseline over the next 2 to 3 minutes. Mean arterial pressure responses were measured in rabbits that had not received reserpine (n=3) and those that had received either a 24-hour treatment (n=3) or a 48-hour treatment (n=3).

**Myocardial Norepinephrine Determination**

In another series of studies (n=6), rabbits that were treated with the aforementioned reserpinization protocol for 24 hours (n=3) were assessed for depletion of myocardial catecholamines using nonreserpinized rabbits as controls (n=3) and the high-performance liquid chromatography (HPLC) method of Mayer and Shoup. Briefly, reserpinized and nonreserpinized rabbits were anesthetized with ketamine (35 mg/kg) and xylazine (6 mg/kg), were systemically heparinized with 600 U sodium heparin, and then were killed shortly thereafter with an overdose of sodium pentobarbital. Samples of ventricular myocardium weighing at least 400 mg were removed from the chest, washed in three changes of phosphate-buffered saline (PBS), and then rapidly frozen in liquid nitrogen and stored at −80°C until needed for further analysis.

Frozen tissue samples were thawed and prepared for HPLC analysis by extraction using 0.1 N perchloric acid containing 2 μg/mL dihydroxybenzylamine (DHBA) as an internal standard. The tissue was homogenized and sonicated in extraction media and then centrifuged at 12,000 g for 3 minutes in a microcentrifuge. The resulting supernatant was used for determining norepinephrine content by HPLC. Supernatant samples (10 μL) were injected onto a 25-mm ODS column (Bioanalytical Systems; West Lafayette, Ind) and separated under isocratic conditions at a flow rate of 1 mL/min. Norepinephrine was detected using an electrochemical detector with glassy carbon electrode set at 850 mV. The mobile phase was composed of monochloroacetic acid (14.15 g/L), 10 N NaOH, sodium octyl sulfate (150 mg/mL), and disodium EDTA (250 mg/L). To this solution was added 35 mL acetonitrile, 13.5 mL tetrahydrofuran, and an additional 72 mL of distilled water.

A standard solution containing norepinephrine (1 μg/mL) was analyzed daily to establish column retention times and response factors for unknown samples that also contained norepinephrine. Retention times of sample peaks were typically within 1% of the retention time for standards. Accurate determination of norepinephrine levels by HPLC is based on the experimental assumptions that catecholamines are maximally extracted from tissue and that tissue levels of catecholamines fall within the linear portion of the electrochemical dose response. Tissue norepinephrine content was calculated according to the following equation:

\[ m_{ne} = \frac{S_{ne} \times A \times 1}{S_{DHBA} \times rf_{NE} \times m_{DHBA}} \]

where \( m_{ne} \) equals the mass of norepinephrine in μg/g of tissue; \( S_{ne} \times A \) equals sample peak area of norepinephrine; \( S_{DHBA} \times rf_{NE} \) equals standard peak area of DHBA, the internal standard; \( rf_{NE} \) equals response factor of norepinephrine; \( m_{DHBA} \) equals mass of DHBA in extraction media (2 μg/mL), and g equals grams of myocardial tissue used in extraction.

**Ischemia-Reperfusion Study**

New Zealand White rabbits (2 to 3 kg) of either sex were used for this study. Twenty-four hours before study, the rabbits were momentarily retrieved from their cages and given a subcutaneous injection of reserpine (5 mg/kg) dissolved in a minimal volume of DMSO and returned to their cages.

On the day of the experiment, rabbits were anesthetized with an intramuscular injection of ketamine HCl (35 mg/kg) and xylazine (6 mg/kg). Adequate depth of anesthesia was ensured before any surgical procedures by the absence of pedal and palpebral reflexes. Subsequent doses of ketamine-xylazine (10 mg/kg and 2 mg/kg, respectively) were administered using repeat intramuscular injections to maintain surgical anesthesia while the animals were instrumented for reversible occlusion-reperfusion of a marginal branch of the left coronary artery, as previously described. Positive-pressure ventilation was established via a tracheostomy with a 3.0-mm internal diameter tracheal tube connected to a volume-cycled respirator (Harvard Apparatus; Natick, Mass), which was supplied with oxygen-enriched room air. The tidal volume was set for approximately 14 mL, and the respiratory rate was adjusted between 30 and 40 cycles per minute to maintain the carbon dioxide tension between 35 and 40 mm Hg. Positive end-expiratory pressure (2 cm H2O) was applied to the expiratory limb of the respirator to reduce atelectasis. Venous access was gained using a 19-gauge butterfly cannula introduced into a prominent marginal ear vein. The left common carotid artery was cannulated with PE-90 tubing, which was advanced to the aortic arch for blood pressure monitoring (Gould Instruments; Oxnard, Calif). ECG leads were attached to subcutaneous electrodes to monitor limb lead II.

The chest was opened by left thoracotomy in the fourth intercostal space, and the pericardial sac was incised to expose the heart. A prominent marginal branch of the left circumflex coronary artery was iden-
tified, and a 4-0 prolene suture on a tapered needle was passed around the vessel twice to allow reversible occlusion of the coronary artery. The animals were then systemically heparinized with 600 U of sodium heparin.

After all surgical procedures had been performed, 15 minutes were allowed for stabilization, and the animals were divided into one of four groups. The first group (control group, n=7) consisted of nonreserpinized animals that were subjected to 30 minutes of coronary occlusion and 120 minutes of reperfusion (ischemia-reperfusion) and served as controls. The second group (PC group, n=10) consisted of nonreserpinized animals that received preconditioning before ischemia-reperfusion. The remaining two groups included animals that had received 5 mg/kg reserpine the previous day. The third group (RES group, n=7) of animals was reserpinized and subjected to ischemia-reperfusion only. The fourth group of reserpinized animals received preconditioning before ischemia-reperfusion (RES+PC group, n=7).

Experimental Protocol

Baseline hemodynamic measurements (blood pressure and heart rate) were taken before any experimental manipulations. In groups that were to be preconditioned before ischemia-reperfusion (PC and PC+RES groups), preconditioning was achieved using a single 5-minute coronary occlusion followed with 10 minutes of reperfusion. Hemodynamic measurements were taken during the last 30 seconds of the preconditioning occlusion (5' PC) and during the last 30 seconds of the intervening reperfusion period (10' post-PC). Animals that were not to be preconditioned (control and RES groups) were not studied during this time interval.

Animals in all groups were then subjected to 30 minutes of ischemia and 120 minutes of reperfusion. The suture around the coronary artery was tightened to produce a zone of regional left ventricular ischemia, which was confirmed by regional cyanosis, dyskinetic movement, and prominent ST segment elevation. The use of lidocaine or other antiarrhythmic agents, either prophylactically or as treatment for arrhythmias, was strictly avoided due to the beneficial effect that such agents may have on infarct size. At 5 and 25 minutes of ischemia, repeat hemodynamic measurements were taken. The snare was then released after 30 minutes of ischemia to begin reperfusion (120 minutes). An intravenous infusion of phenylephrine (range, 0.25 to 0.50 mg/min) was used during the reperfusion period to assist in the maintenance of arterial pressure and to ensure coronary perfusion pressure sufficient for washout of enzymes during reperfusion. Repeat hemodynamic measurements were taken at 15, 60, and 120 minutes of reperfusion.

After all measurements had been taken, an intravenous tyramine challenge (1 mg/kg) was used to confirm norepinephrine depletion in reserpinized animals (less than 3 mm Hg change in mean arterial pressure). In vivo demarcation of the myocardium at risk was accomplished by reocclusion of the coronary artery and injection of India ink into the venous cannula. The animals were then immediately killed with an intravenous bolus injection of 3 mL of saturated KCl solution. The hearts were quickly excised, and the atria and right ventricle were dissected away and discarded.

Determination of Infarct Size

The left ventricular mass was sliced transversely along the septal groove (breadloafed) into 6 to 7 slices of 3-mm thickness each. The slices were stained in 50 mL of phosphate-mannitol buffered 1% 3,5,5-triphenyl-tetrazolium chloride (TTC) at 37°C, pH=7.4. TTC staining has been shown to demarcate viable tissue by reacting with myocardial dehydrogenase enzymes to form a brick-red stain.19 Necrotic tissue (which has lost its dehydrogenase enzymes) does not form a red stain and shows as pale yellow. The apical and basal surface of each tissue slice was then traced onto acetate sheets, taking care to outline the nonrisk (India ink stain), risk (red stain), and necrotic (pale yellow) areas. The non-risk, risk, and necrotic areas were determined using computer-aided planimetry of the acetate tracings by an investigator who was unaware of the particular treatment group. The two-dimensional area of nonrisk, risk, and necrotic myocardium in each slice was multiplied by the weight of each slice to yield weights of nonrisk, risk, and necrotic myocardium. Myocardium at risk expressed as a percent of the entire left ventricle was calculated as [(weight of necrotic and nonnecrotic tissue/weight of the left ventricle) · 100]. The amount of necrosis expressed as a percent of the risk region was calculated as [(weight of necrotic region/weight of risk region) · 100].

Statistical Analysis

All data were analyzed using either STATISTICAL ANALYSIS SOFTWARE for the personal computer (SAS Institute; Cary, NC) or EZSTATS software for the CMS environment. Comparison of data regarding the effects of reserpine on the pressor response to tyramine and myocardial norepinephrine content were made by an unpaired t test. Hemodynamic data were compared using analysis of variance, corrected for multiple comparisons across time. Comparison of data used in calculating infarct size including left ventricular mass, mass of myocardium at risk, mass of necrotic myocardium, and necrotic myocardium as a percentage of left ventricular mass were made by analysis of variance. Comparison of necrotic myocardium as a percentage of risk area (infarct size) was performed by analysis of covariance using risk region as an independent covariate for infarct size. All data are reported as group means and standard errors of the mean (SEM) unless otherwise indicated and were considered statistically significant at a probability value less than or equal to .05.

Results

Effect of Reserpine (Pressor Response to Tyramine)

Intravenous tyramine bolus injection (1 mg/kg) was used to determine the magnitude of the norepinephrine-dependent pressor response as shown in Fig 1. In control animals (nonreserpinized), mean arterial pressure increased by 47.0±2.9 mm Hg in response to intravenous tyramine bolus. When tyramine challenge was used in reserpinized animals after either 24 or 48 hours of reserpinization, mean arterial pressure only changed by 1.3±1.0 and 2.6±3.2 mm Hg, respectively, which was significantly reduced (P<.01) in comparison to nonreserpinized (control) animals.
arterial pressure was significantly higher (32%) in the preconditioned animals relative to the control group animals. Pressure-rate product, an indirect index of myocardial oxygen demand, was comparable among all groups at all times during the experiment except for at 5 minutes of ischemia where pressure-rate product was significantly lower (24%) in preconditioned animals relative to control animals but was not different from reserpine only or preconditioning with reserpine groups.

**Infarct Size**

The weights of global left ventricular myocardium, risk region, and tissue that became acutely necrotic are presented in Table 4. Myocardium in the risk region expressed as a percentage of the left ventricle is also presented in Table 4. With comparable risk regions among groups, necrosis in the risk region was 39.8±2.1% in control group animals (Fig 3). In animals that were preconditioned, infarct size was reduced by approximately 68% to 14.8±2.2% of the risk region (P<.05 vs control group). In animals that were treated with reserpine and not preconditioned, infarct size was 38.5±4.5% of the risk area, which was not different from the control group (P=NS). When animals were preconditioned in the presence of reserpine, the resulting infarct was 41.4±3.6% of the risk region, which was not different from either the control or control with reserpine groups (P=NS).

**Discussion**

By demonstrating that ischemic preconditioning fails to limit infarct size in reserpinized rabbit myocardium, we have provided important evidence that implicates the sympathetic nerve terminal in the evolution of preconditioning's myoprotective effects. We have demonstrated this effect in a model where it can be presumed that interstitial adenosine builds up under ischemic conditions and that ATP-sensitive potassium channels are responsive to metabolic conditions during ischemia, yet no protection was observed.

**Effects of Reserpine**

These data indicate that norepinephrine is effectively depleted in this study, as demonstrated by both the lack of a pressor response to tyramine and by the depletion of myocardial norepinephrine as measured by HPLC. However, it is a bit surprising that, given the clinical indications for reserpine, we did not detect an effect of reserpine on the hemodynamic parameters that we measured (ie, slower heart rate and lower blood pressure). It is possible that a slower heart rate and/or lower blood pressure may become manifest with chronic administration of reserpine as opposed to the acute administration of reserpine used in the present study. It is also possible that other mediators of arterial pressure regulation such as vasopressin or the renin-angiotensin-aldosterone system may have had a significant impact on the maintenance of systemic blood pressure in this study. Moreover, the selection of ketamine-xylazine as the anesthetic agent in this study results in heart rate that is significantly lower than other rabbit ischemia-reperfusion studies where pentobarbital is used as anesthesia.20 Perhaps the lower heart rate as a result of
ketamine-xylazine anesthesia masks an effect that reserpine may otherwise have had on hemodynamics.

In this study we have demonstrated that the release of norepinephrine from the sympathetic nerve terminal is critical to preconditioning mediated protection and have used a norepinephrine-depleted model to support our claim. It may be considered problematic then, that phenylephrine is used as a pressor agent during the reperfusion period. One could argue that the use of phenylephrine affects the ultimate degree of tissue necrosis. However, we have provided data in the control group that is comparable to that provided in other rabbit ischemia-reperfusion studies where phenylephrine is not used. This suggests that phenylephrine use during the reperfusion period for hemodynamic support is benign with respect to infarct size. Furthermore, it has also been shown that inotropic doses of dobutamine administered during reperfusion have no effect on infarct size. Our use of phenylephrine during the reperfusion period is to ensure that coronary perfusion pressure is sufficient for the washout of cardiac enzymes, which is necessary for macrohistochemical staining with TTC.

**Catecholamines, Preconditioning, and Myoprotection**

We expected to find an interrelation between norepinephrine and adenosine in the preconditioning model. As adenosine is known to inhibit the release of catecholamines under both normoxic and ischemic conditions, the attenuated catecholamine release could be considered a viable mechanism for the myoprotection attainable with ischemic preconditioning. Alternately, it may be the release of catecholamines during preconditioning ischemia that may augment the production of adenosine during the prolonged ischemic period.

Murry has shown that ischemic preconditioning slows the depletion of ATP during a subsequent ischemic event in comparison to nonpreconditioned myocardium, and it was the preservation of myocardial ATP that was thought to sustain vital cell functions and delay irreversible ischemic injury. In a subsequent study from the same laboratory, Vander Heide has recently demonstrated a similar preservation of ATP under ischemic conditions in myocardium that had been pretreated with adenosine, indicating that exogenous adenosine can modulate the expenditure of ATP in ischemic myocardium. In contrast, adenosine failed to modulate ATP degradation when reserpine was used to deplete the myocardial norepinephrine stores before ischemia, suggesting that adenosine-mediated protection was dependent on the presence of norepinephrine.

Preischemic administration of tyramine has been shown by Thornton25 to produce infarct size reduction in the heart analogous to preconditioning. As tyramine is known to stimulate the release of norepinephrine from sympathetic nerve terminals, it is possible that norepinephrine released locally in the myocardium initiates or mediates preconditioning. Przyklenk has introduced the concept of “remote” preconditioning by showing that a brief coronary occlusion of the left circumflex coronary artery can induce tolerance to ischemia in myocardium perfused by the left anterior

**TABLE 1. Data for Heart Rate**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Preconditioning</th>
<th>Reserpine</th>
<th>Reserpine+Preconditioning</th>
</tr>
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<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td>171±24</td>
<td>147±10</td>
<td>150±10</td>
<td>140±7</td>
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<tr>
<td>5' Preconditioning</td>
<td>...</td>
<td>171±8</td>
<td>...</td>
<td>159±5</td>
</tr>
<tr>
<td>10' After preconditioning</td>
<td>...</td>
<td>145±11</td>
<td>...</td>
<td>143±9</td>
</tr>
<tr>
<td>5' Ischemia</td>
<td>197±28</td>
<td>164±8</td>
<td>171±13</td>
<td>156±7</td>
</tr>
<tr>
<td>25' Ischemia</td>
<td>188±25</td>
<td>161±7</td>
<td>173±16</td>
<td>153±9</td>
</tr>
<tr>
<td>15' Reperfusion</td>
<td>174±26</td>
<td>135±8</td>
<td>164±13</td>
<td>151±11</td>
</tr>
<tr>
<td>60' Reperfusion</td>
<td>165±28</td>
<td>148±9</td>
<td>162±13</td>
<td>160±11</td>
</tr>
<tr>
<td>120' Reperfusion</td>
<td>176±28</td>
<td>135±10*</td>
<td>164±16</td>
<td>166±16</td>
</tr>
</tbody>
</table>

Values are mean±SEM (min⁻¹).

*P<.05 vs control group.

**TABLE 2. Data for Mean Arterial Pressure**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Preconditioning</th>
<th>Reserpine</th>
<th>Reserpine+Preconditioning</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td>79.9±4.1</td>
<td>81.8±2.7</td>
<td>81.7±7.8</td>
<td>82.7±5.1</td>
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<td>5' Preconditioning</td>
<td>...</td>
<td>71.1±2.1</td>
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<td>73.7±5.5</td>
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<tr>
<td>10' After preconditioning</td>
<td>...</td>
<td>74.7±2.4</td>
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<td>79.3±6.2</td>
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<tr>
<td>5' Ischemia</td>
<td>72.5±4.3</td>
<td>71.4±2.8</td>
<td>67.7±7.3</td>
<td>74.7±5.9</td>
</tr>
<tr>
<td>25' Ischemia</td>
<td>71.8±5.2</td>
<td>69.4±3.4</td>
<td>65.5±7.0</td>
<td>73.6±5.7</td>
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<tr>
<td>15' Reperfusion</td>
<td>70.7±5.8</td>
<td>74.7±4.2</td>
<td>70.7±4.4</td>
<td>71.7±5.6</td>
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<tr>
<td>60' Reperfusion</td>
<td>67.7±3.9</td>
<td>74.2±6.0</td>
<td>68.8±4.4</td>
<td>70.4±5.6</td>
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<tr>
<td>120' Reperfusion</td>
<td>56.2±3.7</td>
<td>74.1±3.6*</td>
<td>65.7±5.1</td>
<td>61.7±4.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM (mm Hg).

*P<.05 vs control group.
TABLE 3. Pressure-Rate Product

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Preconditioning</th>
<th>Reserpine</th>
<th>Reserpine+Preconditioning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>16 208±2406</td>
<td>12 612±2910</td>
<td>13 447±1155</td>
<td>13 839±1426</td>
</tr>
<tr>
<td>5' Preconditioning</td>
<td>. . .</td>
<td>13 458±592</td>
<td>. . .</td>
<td>14 196±1419</td>
</tr>
<tr>
<td>10' After preconditioning</td>
<td>. . .</td>
<td>11 822±741</td>
<td>. . .</td>
<td>13 503±1821</td>
</tr>
<tr>
<td>5' Ischemia</td>
<td>16 732±2640</td>
<td>12 669±362*</td>
<td>13 221±1005</td>
<td>14 009±1517</td>
</tr>
<tr>
<td>25' Ischemia</td>
<td>14 794±2236</td>
<td>12 260±489</td>
<td>12 992±1187</td>
<td>13 332±1634</td>
</tr>
<tr>
<td>15' Reperfusion</td>
<td>13 742±2341</td>
<td>10 931±984</td>
<td>13 747±1208</td>
<td>13 074±1711</td>
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<tr>
<td>60' Reperfusion</td>
<td>13 263±2188</td>
<td>12 657±804</td>
<td>13 213±1253</td>
<td>14 072±1976</td>
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<tr>
<td>120' Reperfusion</td>
<td>11 184±1802</td>
<td>11 017±1058</td>
<td>12 754±1279</td>
<td>12 380±1862</td>
</tr>
</tbody>
</table>

Values are mean±SEM (mm Hg · min⁻¹).

*P<.05 vs control group.

descending coronary artery. McClanahan27 has demonstrated that ischemic tolerance in the heart can be produced using a 5-minute "preconditioning" occlusion of the renal artery. Perhaps the experiments of Thornton,21 Przyklenk,22 and McClanahan23 have a common feature in that all of these interventions might be expected to have produced a substantial release of catecholamines in the time interval immediately preceding the prolonged ischemic insult and that this norepinephrine release initiates the protective effect.

Potential Mechanism

There is already evidence that supports the role of norepinephrine in preconditioning. Kitakaze28 has shown that 5′-nucleotidase activity (conversion of AMP to adenosine) is increased after ischemic preconditioning, an occurrence that may be promoted by protein kinase C in response to α₁-adrenoceptor stimulation. The authors suggested that the α₁-receptor is stimulated during the preconditioning ischemia and that the resultant increase in 5′-nucleotidase activity augments the production of adenosine during the more prolonged ischemia. In a recent preliminary report,29 Kitakaze has also demonstrated that α₁-receptor antagonists are able to block the effects of ischemic preconditioning on infarct size, whereas agonists at the α₁-receptor can reduce the extent of myocardial necrosis. Our data appear supportive of the findings of Kitakaze28,29 in that we determined that the presence of norepinephrine is necessary for preconditioning to be protective in our model. However, we have not ruled out the possibility that norepinephrine may be acting through an entirely different mechanism that is independent of adenosine or that reserpine itself may have had an effect on adenosine production or potassium channel activity.

One question that remains on the potential role of norepinephrine is whether or not norepinephrine is released into myocardium during preconditioning. Previous work by Richardt30 and Schomig31 have demonstrated in the isolated perfused rat heart that there is not a buildup of norepinephrine during periods of ischemia less than 10 minutes. This was demonstrated to be the result of reuptake mechanisms in nerve terminals and the presence of adenosine, which inhibits neurotransmitter release. These findings would suggest that a 5-minute preconditioning ischemia is insufficient to cause a significant buildup of norepinephrine in the myocardium. However, Richardt30 and Schomig31 also demonstrated that when the reuptake mechanism was blocked by desipramine, the accumulation of norepinephrine increased dramatically with periods of ischemia as short as 2 minutes. In summary, these studies suggest that there is a large release of norepinephrine early in ischemia (2 minutes) but that buildup does not occur until 10 minutes of ischemia because of functioning reuptake mechanisms. Would the amount of norepinephrine that is released and reuptaken during a 5-minute preconditioning ischemia be sufficient to trigger the mechanism suggested by Kitakaze28,29? This point remains an open question.

Implications

Although the heart can apparently create a milieu where the formation of adenosine is promoted, rendering the heart resistant to ischemic injury, the generation of such an environment seems to require a brief nonlethal stimulus immediately before ischemia. Although the myocardial preservation afforded by preconditioning is highly significant, it is a distinct disadvantage that preconditioning protects the heart only transiently.

TABLE 4. Myocardial Tissue Weights: Left Ventricle, Risk Region, and Necrotic Region

<table>
<thead>
<tr>
<th>Group</th>
<th>LV (grams)</th>
<th>Risk (grams)</th>
<th>Necrotic (grams)</th>
<th>Risk:LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.02±0.18</td>
<td>1.78±0.31</td>
<td>0.70±0.13</td>
<td>41.7±5.1</td>
</tr>
<tr>
<td>Preconditioning</td>
<td>3.35±0.27</td>
<td>1.07±0.12</td>
<td>0.16±0.04*</td>
<td>31.6±3.7</td>
</tr>
<tr>
<td>Reserpine</td>
<td>3.61±0.44</td>
<td>1.26±0.12</td>
<td>0.48±0.08</td>
<td>36.3±3.0</td>
</tr>
<tr>
<td>Reserpine+Preconditioning</td>
<td>3.86±0.34</td>
<td>1.25±0.15</td>
<td>0.53±0.09</td>
<td>32.1±2.6</td>
</tr>
</tbody>
</table>

LV indicates mass of entire left ventricle (grams); Risk, mass of myocardium in risk region (grams); Necrotic, mass of necrotic myocardium (grams); and Risk:LV, mass of myocardium in risk region expressed as a percentage of left ventricular mass.

*P<.05 vs all other groups.
The development of agents that can pharmacologically amplify the endogenous cardioprotective mechanism of the heart may be of clinical importance. Liu et al. used R-PIA (an adenosine-A<sub>1</sub> agonist) to reduce infarct size, analogous to ischemic preconditioning, in a setting that most likely did not involve the preischemic release of norepinephrine because stimulation of A<sub>1</sub>-receptors is known to inhibit norepinephrine release. However, whereas the present study demonstrates that norepinephrine is necessary for preconditioning to be protective, the study of Liu et al. suggests that this element of the preconditioning mechanism (norepinephrine release) can be side-stepped by stimulating adenosine-A<sub>1</sub> receptors directly. However, the Liu study noted a profound hypotensive effect of R-PIA that may, in certain circumstances, promote coronary steal thus making the systemic use of such an agent impractical.

With regard to adenosine, it may be more reasonable to promote its actions locally rather than systemically. Alternative therapeutic approaches are emerging that increase local concentration of adenosine under ischemic conditions. The novel adenosine promotor acadesine (AICA-riboside, AICAr) has been found to augment adenosine production during ischemia. This finding suggests that acadesine might successfully emulate the protection attainable with ischemic preconditioning in clinically relevant situations such as elective angioplasty or coronary bypass surgery. In fact, the presence of acadesine has recently been shown to lower the time threshold necessary for ischemic preconditioning in the rabbit, suggesting that acadesine significantly potentiates adenosine production. Alternately, preservation of local buildup of adenosine by inhibiting its degradation (ie, adenosine deaminase inhibitors) has also been shown to be cardioprotective under ischemic conditions and thus may also have important clinical implications.

Summary

While adenosine is largely regarded as the mediator of preconditioning and adenosine may exert its effects through a potassium channel mechanism, we have revealed another essential element of this intriguing phenomenon: the presence of norepinephrine. Considering the data at hand and that present in the literature, it appears that infarct size reduction via ischemic preconditioning involves three distinct elements: norepinephrine release, adenosine receptor stimulation, and potassium channel activity, all three of which may represent an opportunity for therapeutic intervention in salvaging myocardium.

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