Activation of Adenosine Receptors Before Ischemia Enhances Tolerance Against Myocardial Stunning in the Rabbit Heart

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Objectives. This study examined whether stimulation of adenosine receptors before ischemia enhances myocardial resistance to stunning in vivo.

Background. We previously demonstrated the attenuation of myocardial stunning by ischemic preconditioning through adenosine receptor activation in rabbits.

Methods. 1) To confirm the efficacy of an intravenous infusion of adenosine to stimulate adenosine receptors in the heart, we assessed the effect of an adenosine infusion on the inotropic response to an isoproterenol challenge. 2) Myocardial stunning was induced by 10 min of coronary occlusion and reperfusion. The regional thickening fraction was monitored by an epicardial Doppler sensor. Rabbits were pretreated with either no drug (control group), adenosine, 8-phenyltheophylline or a combination of 8-phenyltheophylline plus adenosine.

Results. An intravenous infusion of adenosine at 0.15 mg/kg body weight per min attenuated by 50% the elevation of left ventricular dP/dt max by isoproterenol (0.075 μg/kg per min). The same dose of adenosine infused for 15 min before ischemia significantly improved the postischemic recovery of the thickening fraction, and the thickening fraction at 30 min reperfusion was 76.8 ± 3.3% (mean ± SE) of the baseline value, which was significantly higher than the control value (42.9 ± 4.5%). The relation between thickening fraction and systolic left ventricular pressure after reperfusion was shifted toward higher thickening fraction by adenosine. This beneficial effect of adenosine was not detected in rabbits given 8-phenyltheophylline before adenosine, and 8-phenyltheophylline-treated rabbits showed a time course of thickening fraction similar to that in the control group.

Conclusions. An intravenous infusion of adenosine is capable of protecting rabbit hearts against stunning through adenosine receptor activation.

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Recently, the cardioprotective effects of ischemic preconditioning against myocardial infarction have been given much attention by investigators (13). In a recent study (14), we found that myocardial stunning is also alleviated by preconditioning with 1 min of ischemia in rabbit hearts. Furthermore, this effect of preconditioning was abolished by an adenosine receptor blocker, 8-phenyltheophylline (14), suggesting the involvement of adenosine receptor activation. If this is indeed the case, it is possible that adenosine infusion mimics preconditioning and could be a therapeutic approach to myocardial stunning. The present study aimed to test this possibility. We used the same rabbit preparation as in our previous studies (7,10,12,14) because of two advantages of this model of stunning: 1) The level of xanthine oxidase in the heart, which contributes to myocardial stunning by generating oxygen-free radicals (15), is low in rabbits (16,17), as it is in humans (17-19), in contrast to that in rats and dogs (16-19). 2) The variation of myocardial stunning by the coronary collateral blood flow (20) does not need to be considered in this collateral artery-deficient species (21).

The dose of adenosine was selected so as not to reduce the systemic blood too greatly, thus minimizing the alteration of ventricular afterload. To confirm the activation of cardiac adenosine receptors by a relatively low dose of adenosine, we...
assessed whether adenosine infusion could suppress inotropic response to beta-adrenergic stimulation.

**Methods**

This study conformed to the Guidelines of Sapporo Medical University on Animal Usage.

**Experiment 1: effect of intravenous adenosine infusion on inotropic response to isoproterenol.** This series of experiments was performed to test whether the dose of adenosine that we selected (0.15 mg/kg body weight per min) was sufficient to stimulate adenosine receptors in rabbit hearts in situ.

**Surgical preparation.** Male albino rabbits (Japanese white, n = 5) weighing 2.0 to 2.5 kg were anesthetized with pentobarbital (30 mg/kg intravenously) and ventilated with a Harvard rodent respirator (model 684) using room air and an oxygen supplement. Ventilation rate and oxygen flow were adjusted to maintain arterial blood gas within the physiologic range. The chest was opened by left thoracotomy, and the heart was exposed. A catheter-tipped manometer (Nihon-Kohden CTD-096N) was placed in the left ventricle through the left atrium, and fluid-filled catheters were inserted into the right carotid artery for blood pressure monitoring and into the jugular and femoral veins for drug infusion. Preordial electrodes were placed across the chest wall for electrocardiographic (ECG) monitoring. Left ventricular pressure and its first time derivative (left ventricular dP/dt), left ventricular end-diastolic pressure, systemic blood pressure and the ECG were continuously recorded on an eight-channel direct-writing recorder (Nihon-Kohden WT-687G).

**Experimental protocol.** After a 10-min stabilization period, baseline hemodynamic variables were measured, and isoproterenol was infused intravenously at 0.075 µg/kg per min for 5 min, at which time the hemodynamic data were collected. The isoproterenol infusion was then discontinued, and the rabbit's heart was allowed to recover for ~15 min. After heart rate, blood pressure and left ventricular dP/dt returned to baseline levels, adenosine was administered intravenously at 0.15 mg/kg per min for 20 min, during which time the isoproterenol challenge was repeated. Finally, 5 min after discontinuation of adenosine and isoproterenol infusions, 10 mg/kg of 8-phenyltheophylline was injected intravenously. The response to isoproterenol with an adenosine infusion was reassessed 10 min later.

**Experiment 2: effect of adenosine infusion before ischemia on myocardial stunning.** Surgical preparation. Male albino rabbits (n = 31) were prepared under pentobarbital anesthesia; a catheter-tipped manometer was placed in the left ventricle, and fluid-filled catheters were placed in the carotid artery and jugular veins, as in Experiment 1. Then, 4-0 silk thread was passed around the marginal branch of the left coronary artery using a taper needle to make a coronary snare. In addition, a single epicardial Doppler sensor (22) was secured with 6-0 stitches to the epicardium in the territories of the coronary branch. The Doppler sensor was connected to a Pulsed Doppler Dimension System VF-1 (Crystal Biotech) to measure the regional thickening fraction. The systolic thickening fraction was calculated as the ratio of the difference between end-systolic and end-diastolic wall thickness to end-diastolic thickness, multiplied by 100 (22). The onset of systole was determined as the initial increase in left ventricular dP/dt, and the end of systole was considered to be coincident with the peak negative left ventricular dP/dt. For each sample period, hemodynamic variables and the thickening fraction were measured for at least 5 beats and then averaged.

**Experimental protocol.** As shown in Figure 1, rabbits were assigned to four experimental groups, and myocardial stunning was induced by 10 min of coronary artery occlusion and reperfusion. The group that received only vehicle (saline) served as the control group. The adenosine group received adenosine at 0.15 mg/kg per min intravenously for 15 min before ischemia, and the adenosine infusion was discontinued immediately before coronary occlusion. In the 8-phenyltheophylline/adenosine group, 10 mg/kg of 8-phenyltheophylline was injected intravenously as a bolus 25 min before the coronary occlusion, and adenosine was administered in the same way as in the adenosine group. In the 8-phenyltheophylline group, only 8-phenyltheophylline was administered.
coronary artery occlusion. One group of rabbits received a saline infusion, and the other was given the same dose of adenosine intravenously as in Experiment 2 (0.15 mg/kg per min) for 15 min of the preischemic period. After 10 min of the ischemic period, Monastral blue dye was injected into the left atrium, and the heart was quickly excised and immediately immersed in an ice-cold isotonic potassium chloride solution. Transmural myocardial tissues were transected from the nonischemic region (the region stained with Monastral blue dye) and from the ischemic region (the region not stained with the blue dye) and stored in liquid nitrogen until assay. The time interval between the excision of the heart and freezing of the heart tissue in liquid nitrogen was <1 min.

Tissue metabolite assay. Myocardial tissue samples (100 to 500 mg) were lyophilized and homogenized in ice-cold 0.6 N perchloric acid. The homogenate was centrifuged at 3,000 rpm, -4°C, to obtain the supernatant, which was neutralized with 30% potassium hydroxide and then centrifuged again. The recentrifuged supernatant was used for an enzymatic assay of adenosine nucleotides and lactate (23).

Statistics. Differences in heart weight and tissue metabolite levels were tested by one-way analysis of variance (ANOVA). The difference between the variables in each group for the various time periods of measurement was tested by a repeated-measures ANOVA. The time course of the thickening fraction and hemodynamic variables between the experimental groups were compared using two-way ANOVA with the Greenhouse-Geisser correction (24). The difference in linear regression lines was tested by analysis of covariance. The ANOVA was conducted by using STATISTICA (Statsoft, Inc.). Results are reported as mean value ± SE.

Results

Experiment 1. Alterations in hemodynamic variables during isoproterenol and adenosine infusion are summarized in Table 1. Isoproterenol infusion increased heart rate and left ventricular dP/dt_{max} by ~30 beats/min and 2,200 mm Hg/s, respectively, whereas mean blood pressure was slightly reduced. An adenosine infusion blunted the response to isoproterenol, and elevation of left ventricular dP/dt_{max} was attenuated by ~50% during adenosine infusion. This inhibitory effect of adenosine was not detected after an 8-phenyltheophylline injection. These results suggest that an intravenous infusion of adenosine at 0.15 mg/kg per min is sufficient to activate adenosine receptors in rabbit hearts in situ, which suppressed adenylyl cyclase activity stimulated by the beta-receptor agonist.

Experiment 2. Hemodynamic data during ischemia/reperfusion are summarized in Table 2. Under baseline conditions, heart rate, blood pressure and left ventricular end-diastolic pressure were comparable in all experimental groups. Mean blood pressure immediately before coronary occlusion was slightly lower in the adenosine group than in the 8-phenyltheophylline group, but there was no significant difference between the adenosine and control groups. Elevation of left ventricular end-diastolic pressure during coronary occlusion was also similar in all groups.

Under baseline conditions, the regional systolic thickening fraction was comparable in all study groups: 14.2 ± 1.2% in the control group, 11.3 ± 0.6% in the adenosine group, 12.3 ± 0.5% in the 8-phenyltheophylline/adenosine group and 13.8 ± 2.0% in the 8-phenyltheophylline group. For a clear presentation of the effect of drugs on postischemic recovery of the thickening fraction, Figure 2 illustrates the time course of the thickening fraction, which was normalized by the baseline values. In the control group, the thickening fraction was reduced to approximately ~40% during ischemia. After reperfusion, the thickening fraction gradually improved but remained depressed at 42.9 ± 4.5% of the baseline value after 30 min of reperfusion. In the adenosine group, the thickening fraction during the reperfusion period was consistently higher than that in the control group at corresponding time points and recovered to 76.8 ± 3.3% of baseline at 30 min after reperfusion (Fig. 2A). These time courses of postischemic recovery of the thickening fraction in the adenosine and control groups were significantly different by two-way repeated-measures ANOVA. Conversely, the time course and extent of postischemic recovery of the thickening fraction in the 8-phenyltheophylline/adenosine and 8-phenyltheophylline groups were not different from those in the control group (Fig. 2, B and C).

Because alteration in left ventricular afterload and myocardial oxygen consumption (12) could have influenced postischemic recovery of the thickening fraction, we plotted the thickening fraction against the left ventricular systolic pressure at 30 min of reperfusion (Fig. 3) and against the rate-pressure products immediately before the coronary occlusion (Fig. 4).
### Table 2. Summary of Hemodynamic Variables in Experiment 2

<table>
<thead>
<tr>
<th>Variable and Study Group</th>
<th>Baseline</th>
<th>Pre-Occ</th>
<th>Occlusion</th>
<th>Rep 1 min</th>
<th>Rep 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>256 ± 11</td>
<td>254 ± 11</td>
<td>252 ± 12</td>
<td>247 ± 11</td>
<td>246 ± 13</td>
</tr>
<tr>
<td>ADO</td>
<td>253 ± 10</td>
<td>258 ± 11</td>
<td>259 ± 10</td>
<td>251 ± 10</td>
<td>250 ± 12</td>
</tr>
<tr>
<td>8-PT</td>
<td>248 ± 16</td>
<td>269 ± 22</td>
<td>265 ± 21</td>
<td>248 ± 20</td>
<td>249 ± 21</td>
</tr>
<tr>
<td>8-PT/ADO</td>
<td>249 ± 8</td>
<td>259 ± 11</td>
<td>260 ± 12</td>
<td>256 ± 9</td>
<td>253 ± 6</td>
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<tr>
<td>SBP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>88 ± 6</td>
<td>87 ± 6</td>
<td>78 ± 6</td>
<td>82 ± 7</td>
<td>79 ± 6</td>
</tr>
<tr>
<td>ADO</td>
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<td>81 ± 5</td>
<td>79 ± 4</td>
<td>85 ± 5</td>
<td>84 ± 5</td>
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<tr>
<td>8-PT</td>
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<td>98 ± 8</td>
<td>94 ± 8</td>
<td>90 ± 7</td>
<td>91 ± 9</td>
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<tr>
<td>8-PT/ADO</td>
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<td>98 ± 6</td>
<td>90 ± 4</td>
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<td>89 ± 4</td>
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<td>DBP (mm Hg)</td>
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<tr>
<td>Control</td>
<td>69 ± 6</td>
<td>69 ± 6</td>
<td>60 ± 6</td>
<td>65 ± 7</td>
<td>63 ± 6</td>
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<td>ADO</td>
<td>68 ± 4</td>
<td>57 ± 5</td>
<td>60 ± 4</td>
<td>64 ± 4</td>
<td>66 ± 4</td>
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<tr>
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<td>76 ± 7</td>
<td>69 ± 6</td>
<td>70 ± 9</td>
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<td>8-PT/ADO</td>
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<td>76 ± 5</td>
<td>71 ± 4</td>
<td>70 ± 7</td>
<td>67 ± 4</td>
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<tr>
<td>RPP (×100)</td>
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</tr>
<tr>
<td>Control</td>
<td>225 ± 20</td>
<td>225 ± 21</td>
<td>199 ± 22</td>
<td>205 ± 24</td>
<td>200 ± 24</td>
</tr>
<tr>
<td>ADO</td>
<td>218 ± 17</td>
<td>213 ± 19</td>
<td>186 ± 19</td>
<td>212 ± 18</td>
<td>212 ± 19</td>
</tr>
<tr>
<td>8-PT</td>
<td>248 ± 28</td>
<td>265 ± 33</td>
<td>251 ± 33</td>
<td>229 ± 32</td>
<td>232 ± 37</td>
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<tr>
<td>8-PT/ADO</td>
<td>238 ± 24</td>
<td>257 ± 25</td>
<td>237 ± 20</td>
<td>241 ± 25</td>
<td>227 ± 14</td>
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<td>LVEDP (mm Hg)</td>
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<tr>
<td>Control</td>
<td>2.8 ± 0.6</td>
<td>2.7 ± 0.5</td>
<td>5.5 ± 0.8</td>
<td>3.6 ± 0.9</td>
<td>3.3 ± 0.7</td>
</tr>
<tr>
<td>ADO</td>
<td>2.9 ± 0.4</td>
<td>3.8 ± 0.4</td>
<td>5.3 ± 0.5</td>
<td>3.9 ± 0.4</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td>8-PT</td>
<td>5.2 ± 0.5</td>
<td>5.0 ± 0.6</td>
<td>7.0 ± 0.6</td>
<td>4.4 ± 0.7</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td>8-PT/ADO</td>
<td>3.7 ± 0.3</td>
<td>3.9 ± 0.3</td>
<td>6.4 ± 0.9</td>
<td>4.4 ± 0.5</td>
<td>4.3 ± 0.4</td>
</tr>
</tbody>
</table>

Data presented are mean value ± SE. Occlusion = 1 min after coronary occlusion; Pre-Occ = 1 min before coronary occlusion; Rep 1 min = 1 min after reperfusion; Rep 30 min = 30 min after reperfusion; RPP = rate-pressure product; other abbreviations as in Table 1.

Correlation between the thickening fraction and the concurrent left ventricular systolic pressure did not reach statistical significance in the control group (y = 42.39 - 0.01x, r = -0.01, p = NS), but a correlation was observed in the adenosine group (y = 110.76 - 0.40x, r = -0.59, p < 0.05) (Fig. 3). These two regression lines differ in intercepts, but slopes were not significantly different by analysis of covariance, suggesting an upward shift of the relation by adenosine. Similarly, the relation between rate-pressure products before ischemia and the thickening fraction 30 min after reperfusion was significant only in the adenosine group, and the regression line for this group had larger y-intercepts than those for the control group, although its slope was not significantly different from that in the control group (y = 101.0 - 1.14 × 10⁻³x, r = -0.66, p < 0.05, vs. y = 45.5 - 0.17 × 10⁻³x, r = -0.31, p = NS).

**Experiment 3.** Table 3 summarizes the data of adenine nucleotide metabolites and lactate in the myocardium. The levels of myocardial adenosine triphosphate (ATP), adenosine diphosphate, adenosine monophosphate, energy charge potential and lactate in the nonischemic region were comparable between the control and adenosine-treated rabbits. The levels of ATP and the other adenine nucleotides and energy charge potential were lowered to similar levels in ischemic myocardium in both the control and the adenosine-treated rabbits. However, tissue lactate accumulation in the ischemic myocardium was significantly less in the adenosine-treated group than in the control group (108.1 ± 19.8 vs. 165.3 ± 7.5 µmol/g dry weight, p < 0.05).

**Discussion**

In the present study, regional contractile dysfunction after 10 min of ischemia was improved by an adenosine infusion before the ischemia, and this improvement of contractile function was not accompanied by significant alterations of left ventricular end-diastolic pressure or systolic left ventricular pressure. Cardioprotection by an adenosine infusion was completely abolished by pretreatment with 8-phenyltheophylline. These findings suggest that activation of adenosine receptors before ischemia may enhance myocardial resistance against stunning in rabbit hearts in situ.

**Efficacy of adenosine infusion.** An intravenous infusion is an easily accessible route in the clinical setting. However, it is not an ideal method for delivering adenosine to the myocardium because a large part of the adenosine is taken up by red blood cells (25) and vascular endothelial cells (26,27) before it reaches the cardiomyocytes. Thus, we first tried to confirm the efficacy of an intravenous infusion of adenosine for activating myocardial adenosine receptors. It is well known that adenosine A₁ receptor activation suppresses adenylate cyclase activity stimulated by beta-adrenoceptor activation (28). As shown in Table 1, 0.15 mg/kg per min of adenosine significantly
attenuated the inotropic response to an isoproterenol challenge without concurrent changes in left ventricular end-diastolic pressure or heart rate. Systolic blood pressure was slightly reduced during adenosine infusion; however, this would not cause overestimation, but rather underestimation of the negative inotropic effect of adenosine on left ventricular dP/dt_{max}. It is unlikely that beta-adrenoceptors were desensitized by the first transient 5-min infusion of isoproterenol, causing the blunted response to the second isoproterenol challenge. Furthermore, the effect of adenosine on the inotropic response to the beta-adrenoceptor agonist was blocked by 8-phenyltheophylline, a nonselective adenosine receptor blocker. These results indicate that the present dose of adenosine was sufficient for activating adenosine receptors in the ventricular myocytes in the open chest rabbit preparation.

**Figure 2.** Effect of adenosine (ADO) and 8-phenyltheophylline (8-PT) on the regional systolic thickening fraction during 10 min of ischemia and 30 min of reperfusion. The thickening fraction was normalized by the baseline values. A, Control group versus adenosine group. B, Control group versus 8-phenyltheophylline group. C, Control group versus 8-phenyltheophylline/adenosine group. The adenosine group received adenosine intravenously at 0.15 mg/kg per min for 15 min before coronary artery occlusion. The 8-phenyltheophylline group was given 10 mg/kg of 8-phenyltheophylline 25 min before the 10 min of ischemia. The 8-phenyltheophylline/adenosine group received both an injection of 8-phenyltheophylline and an adenosine infusion (see Fig. 1 for details). There was significant difference in the time course of the thickening fraction between the control and adenosine groups (A) by two-way repeated-measures analysis of variance with the Greenhouse-Geisser correction. Oc = 1 min after coronary occlusion; Pre-Oc = point just before coronary artery occlusion (end point of adenosine infusion).

**Effect of adenosine infusion on regional contractile function.** One limitation of the present study is that we assessed myocardial stunning using the systolic thickening fraction. This index of the systolic thickening fraction reflects contractility but is also influenced by heart rate and ventricular load. However, heart rate was the same for all study groups, and left ventricular end-diastolic pressure was similar during both the ischemic and reperfusion periods, suggesting that ventricular preload was comparable in all the groups. However, there was

**Figure 3.** Scatterplot of thickening fraction versus systolic left ventricular pressure at 30 min after reperfusion. There was a weak inverse correlation between the two variables in the adenosine group (solid circles) \((y = 110.76 - 0.40x, r = -0.592, p < 0.05)\) but not in the control group (open circles) \((y = 42.39 - 0.01x, r = -0.008, p = NS)\). The regression line for the adenosine group has a larger y-intercept than that for the control group, but the slopes were not statistically different.


**Table 3. Summary of Myocardial Metabolites**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>TAN</th>
<th>ECP</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonischemic region</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>19.4 ± 1.7</td>
<td>4.3 ± 0.6</td>
<td>2.4 ± 0.8</td>
<td>15.6 ± 1.6</td>
<td>0.9 ± 0.0</td>
<td>32.8 ± 14.9</td>
</tr>
<tr>
<td>Adenosine</td>
<td>5</td>
<td>18.1 ± 1.8</td>
<td>6.3 ± 0.9</td>
<td>2.8 ± 0.4</td>
<td>27.2 ± 2.4</td>
<td>0.8 ± 0.0</td>
<td>30.7 ± 6.1</td>
</tr>
<tr>
<td>Ischemic region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>8.4 ± 0.7</td>
<td>4.4 ± 1.1</td>
<td>3.2 ± 0.3</td>
<td>16.0 ± 1.4</td>
<td>0.7 ± 0.0</td>
<td>165.3 ± 7.5</td>
</tr>
<tr>
<td>Adenosine</td>
<td>5</td>
<td>8.6 ± 0.8</td>
<td>6.4 ± 0.4</td>
<td>2.9 ± 0.5</td>
<td>17.9 ± 1.2</td>
<td>0.7 ± 0.0</td>
<td>108.1 ± 19.8*</td>
</tr>
</tbody>
</table>

*p < 0.05 versus control. Data are expressed as μmol/g dry weight, mean value ± SE. ATP = adenosine triphosphate; AMP = adenosine monophosphate; ADP = adenosine diphosphate; Control = vehicle (saline); ECP = energy charge potential; n = number of samples; TAN = total adenine nucleotide.

Figure 4. Scatterplot showing relation between the thickening fraction at 30 min after reperfusion and rate-pressure product (RPP) immediately before coronary artery occlusion. In the adenosine group (solid circles), the thickening fraction at 30 min after reperfusion correlated with the rate-pressure product before ischemia (y = 101.0 - 1.14 × 10^-3x, r = -0.66, p < 0.05), but there was no significant correlation in the control group (open circles) (y = 45.5 - 0.17 × 10^-3x, r = -0.31, p = NS). The regression line for the adenosine group has a larger y-intercept than that for the control group, but slopes were not statistically different.

A weak correlation between the thickening fraction and systolic left ventricular pressure 30 min after reperfusion (Fig. 3). This correlation suggests that a slight reduction in ventricular afterload could partially contribute to the improved regional contractile function by adenosine. However, Figure 3 shows that the thickening fraction 30 min after reperfusion was higher in the adenosine-treated group than in the control group, regardless of the level of systolic pressure; that is, the regression line in the adenosine group was shifted upward to the control data with essentially no overlap. This finding suggests that an afterload-independent effect of adenosine also improved the thickening fraction during reperfusion. Taken together, the present dose of adenosine had a beneficial effect on contractile dysfunction after ischemia by two mechanisms: reduction in ventricular afterload and attenuation of myocardial stunning. As seen in Figure 3, the latter mechanism appears predominant over the former in the present preparation.

One possible explanation for the attenuation of myocardial stunning by adenosine may be an improvement in myocardial oxygen supply/demand balance as a result of the reduction of myocardial oxygen demand by this agent. A recent study (12) from our laboratory showed that an ~20% reduction in rate-pressure products before ischemia by verapamil is capable of attenuating myocardial stunning. Furthermore, there was strong correlation between the postischemic thickening fraction and preischemic rate-pressure products in verapamil-pretreated rabbits (12). In the present study, the thickening fraction after reperfusion correlated with rate-pressure products before coronary occlusion in adenosine-treated rabbits, but there was no significant correlation in the control group (Fig. 4). However, the regression line in the adenosine group was shifted upward to the control data, indicating that a reduction in myocardial oxygen demand by adenosine alone cannot explain the improved postischemic recovery of contraction.

Although it was not statistically significant, the baseline thickening fraction in the adenosine group tended to be somewhat lower than the values in the other groups. To test whether this slight difference in the baseline thickening fraction contributed to a higher recovery of the normalized thickening fraction in the adenosine group (Fig. 2A), we plotted the normalized thickening fraction at 30 min after reperfusion against the absolute value of the thickening fraction under baseline conditions in each rabbit. As shown in Figure 5, recovery of the normalized thickening fraction was inversely correlated with the absolute values of the baseline thickening fraction in the untreated control rabbits (r = -0.70, p < 0.05). However, improvement in recovery of the thickening fraction in the adenosine group was independent of the baseline thickening fraction; the regression line for the adenosine group had a significantly larger y-intercept than that in the control group, and the slopes of those two regression lines were similar. The relations in the 8-phenyltheophylline/adenosine and 8-phenyltheophylline groups were similar to those of the untreated controls (Fig. 5). These results indicate that the higher recovery of the normalized thickening fraction after reperfusion in the adenosine groups cannot be attributed to their slightly lower absolute thickening fraction before the onset of myocardial ischemia.

**Effect of adenosine infusion on myocardial metabolism.** In adenosine-treated rabbits, lactate accumulation in the myocardium during 10 min of ischemia was 35% less than that in the untreated control group (Table 3). This result suggests that glycolysis was suppressed in adenosine-treated hearts, because washout of lactate from the ischemic region should be negli-
levels in the nonischemic region may have been somewhat
maintained by consumption of creatine phosphate and anaerobic
ATP and ADP levels were not changed within 1 to 3 rain of
data suggest that the adenine nucleotide levels were main-
ischemia, whereas creatine phosphate was depleted by 70%,
the tissues sampled as in the present study, they showed that
samples quickly frozen by a precooled rongeur and those in
canine hearts. By comparisons with tissue metabolite levels in
Jennings et al. (33) previously evaluated this possibility in
mulation might have occurred during the cooling process,
versely, ATP after 10 rain of ischemia was not different
was not statistically different in the two groups. The relation between
percent recovery of the thickening fraction and the baseline thickening
fraction at 30 min after reperfusion and the absolute value of
8-phenyltheophylline/adenosine and 8-phenyltheophylline
groups was similar to that in the control group.

Figure 5. Scatterplot showing the relation between the thickening
fraction at 30 min after reperfusion and the absolute value of
thickening fraction before the onset of myocardial ischemia. Open
triangles = 8-phenyltheophylline/adenosine group; open squares =
8-phenyltheophylline group. In the control group (open circles), there
was significant inverse correlation between the two variables ($y =
81.4 - 2.7x$, $r = -0.70$, $p < 0.05$), and the regression line is shown. In
the adenosine group (solid circles), the thickening fraction at 30 min
after reperfusion was clearly higher than that in the control group
regardless of the thickening fraction at baseline, and the regression
line for the adenosine group ($y = 97.6 - 1.9x$, $r = -0.33$, $p = 0.35$) has
a larger y-intercept than the control regression line, although the slope
was not statistically different in the two groups. The relation between
percent recovery of the thickening fraction and the baseline thickening
fraction in the 8-phenyltheophylline/adenosine and 8-phenyltheophylline
groups was similar to that in the control group.

gable in collateral-channel deficient rabbit hearts (21). Con- 
versely, ATP after 10 min of ischemia was not different
between adenosine-treated and untreated rabbits, although
earlier studies (29–32) have reported a deceleration in ATP
depletion during ischemia by adenosine receptor activation.
However, if glycolysis (and thus anaerobic ATP synthesis) was
indeed suppressed by the adenosine infusion, a similar level of
ATP in the adenosine-treated and untreated rabbits might
reflect suppressed ATP consumption during ischemia in
adenosine-pretreated hearts.

A limitation of the tissue metabolite analysis in the present
experiments was a delay in the freezing of the myocardial
tissues after the sampling. As stated in Methods, it took 30 s to
1 min to excise the heart and separate the nonischemic and
ischemic tissues in ice-cold potassium chloride solution before
dipping the tissue samples in liquid nitrogen. This delay would
have caused ischemic alterations in the metabolism in both
nonischemic and ischemic samples. Although we do not have
direct data for how much ATP degradation and lactate accu-
mulation might have occurred during the cooling process,
Jennings et al. (33) previously evaluated this possibility in
canine hearts. By comparisons with tissue metabolite levels in
the samples quickly frozen by a precooled rongeur and those in
the tissues sampled as in the present study, they showed that
ATP and ADP levels were not changed within 1 to 3 min of
ischemia, whereas creatine phosphate was depleted by 70%,
and lactate levels increased approximately threefold. These
data suggest that the adenine nucleotide levels were main-
tained by consumption of creatine phosphate and anaerobic
glycolysis during that time period. Thus, the tissue lactate
levels in the nonischemic region may have been somewhat
overestimated in the present study, but data for adenine
nucleotide levels are considered to be reasonable estimates of
the values in vivo, and we believe that interpretation of the
effect of adenosine infusion on tissue ATP and lactate during
 coronary occlusion was not seriously compromised by the
technical limitation.

Intracellular mechanism of action. In the present study,
the intracellular mechanism through which adenosine receptor
activation enhances myocardial resistance against stunning was
not clarified. However, a few possible explanations can be
suggested.

1. Suppression of glycolysis during ischemia by adenosine
(34) could result in less accumulation of proton and of sodium
through sodium–proton exchange during ischemia (35). This
would lead to less calcium overload in the myocyte through a
sodium–calcium exchange (36). This explanation is supported
by a recent study by Fralix et al. (30), who showed that
pretreatment with adenosine delayed the accumulation of
proton and calcium during global ischemia in isolated rat
hearts.

2. An infusion of adenosine may activate the $A_1$-receptor-
linked ATP-sensitive potassium channel ($K_{ATP}$), thus enhanc-
ing myocardial tolerance against stunning. This explanation is
supported by a recent study in our laboratory (10) using the
same rabbit preparation. That study showed that an infusion of
nicorandil, a $K_{ATP}$ opener, before ischemia was capable of
attenuating stunning (10), and this cardioprotection was abol-
ished by glibenclamide, a blocker of $K_{ATP}$. Similar results were
also obtained in canine models of myocardial stunning (9).

3. Eckert et al. (37) found that in isolated myocytes,
adenosine receptor activation decreases the calcium current
but increases the intracellular calcium level. This response was
attenuated by ryanodine and thapsigargin and mimicked by
inositol 1,4,5-trisphosphate, which suggests that calcium re-
lease from the intracellular store may cause an elevation of
intracellular calcium after adenosine receptor activation. If this
is the case, adenosine receptor activation might deplete cal-
cium in its intracellular store sites and thus suppress the
calcium overload from this source during ischemia/reperfusion
(37). However, this explanation is not supported by a study by
Fralix et al. (30), who did not detect any significant elevation of
the intracellular calcium level during an adenosine infusion.

This discrepancy in the observation of the effect of adenosine
on intracellular calcium may be caused by a difference in the
dose of adenosine or the conditions of the myocytes (isolated
myocytes vs. crystalloid-perfused whole heart).

Possible species difference. The present findings are con-
sistent with earlier studies using isolated rat hearts (30,38), in
which preischemic treatment with adenosine significantly
improved the recovery of the global contractile function from 10
to 30 min of ischemia. Although “stunning” of the isolated
hearts may not be strictly comparable with stunned myocard-
dium in vivo (39), those studies support the hypothesis that
adenosine receptor activation before ischemia enhances myocar-
dial tolerance against stunning. In contrast, two studies
using canine in vivo models (40,41) failed to observe that a
large dose of adenosine or 2-chloro-N\textsuperscript{6}-cyclopentyladenosine, an A\textsubscript{1}-receptor agonist, could precondition the myocardium against myocardial stunning. The failure of the adenosine infusion to attenuate the stunning cannot be explained by the dose of adenosine (4 mg/min), because one-tenth of that dose infused for 10 min was shown to precondition the canine heart successfully against infarction (42). A recent study using a swine preparation (43) also could not detect any significant attenuation of myocardial stunning by infusion of adenosine into the left atrium during the preischemic period. However, these different outcomes of preischemic administration of adenosine among the canine (40,41), swine (43), rabbit (Fig. 2) and rat (30,38) hearts appear comparable to the different results of ischemic preconditioning against stunning in these species (14,44–46). Myocardial stunning was attenuated by preconditioning, which was abolished by 8-phenyltheophylline, in the rabbit heart (14). Similarly, Steenbergen et al. (44) found that preconditioning the isolated rat heart with four cycles of 5 min of ischemia and 5 min of reperfusion significantly improved recovery of left ventricular pressure from 30 min of ischemia. Furthermore, the protective effect of preconditioning on contractile function was prevented by 8-sulfophenyltheophylline. Because infarct size limitation by preconditioning in rat hearts was not blocked by 8-sulfophenyltheophylline (47), the improved contractile function by preconditioning in the Steenbergen et al. (44) study is most likely attributable to attenuation of stunning. In contrast, various protocols of preconditioning reportedly failed to protect the canine and swine hearts against stunning (42,46). It is unclear why both adenosine and preconditioning protect the heart against stunning in the rabbit (14) and rat (30,44) but not in the dog and pig (40,41,43,45,46). A possible explanation is that a modest improvement in contractile function by adenosine and preconditioning might have been masked by the variation in stunning because of various collateral flow levels in the dog (19), and the beneficial effect was detectable in collateral flow-independent models of stunning (i.e., collateral-channel deficient rabbit hearts and isolated rat hearts). However, this does not explain the negative results in the swine heart, in which the native coronary collaterals are poorly developed, as in the rabbit and the rat. Possible differences in the extent of K\textsubscript{ATP} activation after adenosine receptor stimulation and in the level of free radical production on reperfusion (48) may be responsible for the contradictory findings. However, there is no clear evidence supporting these speculations.

Conclusions. Adenosine receptor stimulation before ischemia significantly enhances myocardial resistance against stunning in the rabbit heart in situ. This adenosine-mediated protection may play a role in attenuation of myocardial stunning by ischemic preconditioning in rabbits.

References

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