Catecholamines Can Induce Adenosine Receptor–Mediated Protection of the Myocardium but Do Not Participate in Ischemic Preconditioning in the Rabbit

Jon D. Thornton, J.F. Daly, Michael V. Cohen, Xi-Ming Yang, James M. Downey

The role of catecholamines in ischemic preconditioning is unclear. Accordingly, the effects of tyramine-induced norepinephrine release and α₁-receptor blockade were examined. Ischemic preconditioning with a 5-minute coronary occlusion and 10 minutes before a 30-minute ischemic interval resulted in only 7.7±3.1% infarction of the risk area, significantly less than that in control rabbits with isolated 30-minute coronary occlusions (34.4±3.2% P<.01). Intravenous infusion of tyramine 10 minutes before 30 minutes of ischemia also protected the heart from infarction to an extent similar to that seen with ischemic preconditioning (6.9±2.4% infarction). This protection observed with tyramine infusion was eliminated by α₁-receptor blockade with BE 2254 (36.8±2.6% infarction) but was unaffected by β-blockade with propranolol (10.5±2.4% infarction). Furthermore, the protection was unaffected when the tyramine-induced hypertension was attenuated by allowing blood to flow into a volume reservoir (3.9±0.8% infarction, P<.01 vs control value). The nonselective adenosine-receptor blocker PD 115,199 also eliminated tyramine-induced protection (40.2±5.6% infarction), indicating that adenosine is involved in adrenergic-mediated protection. BE 2254 could not block ischemic preconditioning (3.9±1.1% infarction, P<.01 vs control value). Therefore, catecholamine release before prolonged ischemia can protect the heart from infarction via the α₁-receptor, but adenosine receptor stimulation is also involved. α₁-Adrenergic stimulation does not appear to be critical to the protection observed after ischemic preconditioning. (Circ Res. 1993;73:649–655.)

KEY WORDS • adenosine • adrenergic agonists • myocardial infarction • preconditioning • α₁-receptors • tyramine

Preconditioning the heart with a brief period of ischemia renders the myocardium resistant to infarction from a subsequent ischemic insult. This phenomenon has been demonstrated by various investigators in dogs,1–3 pigs,4 rabbits,5,6 and rats.7 Attenuation of ischemia following successive coronary occlusions during coronary angioplasty in humans may also be related to preconditioning, although infarction was not the end point in the human studies, and other explanations of the observations could not be excluded.8 Recent work in this laboratory has indicated that adenosine production and α₁-receptor occupancy trigger this effect. Whereas adenosine receptor blocking agents eliminate the protective effect of ischemic preconditioning, substitution of infusions of adenosine or α₁-selective adenosine analogues for the brief ischemia confer equal protection to the heart.9,10 Furthermore, a pertussis toxin–sensitive G protein is a critical intracellular messenger for mediation of this protection,5 and the muscarinic agonist carbachol, whose receptor also couples to G proteins, including G₁, additionally protects the ischemic heart.5

Numerous investigators have reported that myocardial ischemia also increases catecholamine levels.11–15 Catecholamine release during ischemia can be either local nonexocytotic release11,12,15 or exocytotic release following activation of cardiac sympathetic nerves.12,16 Catecholamines have previously been considered to exacerbate the damage of ischemic myocardium14,17 and facilitate serious arrhythmias18 by increasing oxygen requirements and worsening the supply-demand imbalance.19,20 Catecholamines may also have a deleterious effect on the myocardium by serving as a source of free radicals.21 However, recent experiments from our laboratory indicate that the involvement of catecholamines in infarction may be minor, since whole-body pharmacological sympathectomy failed to protect ischemic rabbit hearts22 and other investigators have demonstrated that β-blockers have failed to alter infarct size.23–25 Catecholamines may also be protective against ischemia. Norepinephrine released after the induction of ischemia in the isolated rat heart has been reported to be the mediator that preconditions the heart and protects it from ischemia.26 Furthermore, it was proposed that the protection was the result of an α₁-adrenergic receptor mechanism.26 Kitakaze et al.27 also reported better preservation of myocardial function after isch-
emia in animals pretreated with an α-adrenergic agonist. This study was undertaken to determine whether the release of endogenous catecholamines could mimic ischemic preconditioning and cause the heart to be protected against ischemia. Catecholamine stimulation was induced by tyramine, which triggers the release of endogenous catecholamines.28 Furthermore, the relative importance of both adrenergic and adenosine receptor stimulation to the possible protection induced by ischemic preconditioning as well as by catecholamines was also determined. The studies outlined below suggest that catecholamines can pharmacologically precondition the rabbit heart, but those studies fail to indicate a role for them in ischemic preconditioning.

**Materials and Methods**

**Surgical Preparation of the Animals**

New Zealand White rabbits of either sex weighing approximately 2 kg were used for this study. Selection of this animal simplified the protocol. Because rabbits lack preformed collateral vessels,29 measurement of collateral blood flow during ischemia was not necessary.

The rabbits were anesthetized with an intravenous injection of sodium pentobarbital (30 mg/kg). The rabbits were intubated through a tracheotomy and mechanically ventilated with 100% oxygen with a positive-pressure respirator (MD Industries, Mobile, Ala). Ventilation rate was 30 to 35 breaths per minute, and tidal volume was approximately 15 mL. The respiratory rate was adjusted to keep the blood pH in the physiological range. A PE-90 catheter was placed in the right carotid artery for blood gas and blood pressure monitoring. Another catheter was placed in the jugular vein for drug injection. All catheters were flushed with heparinized saline (10 U/mL) to prevent clotting during the experiment. A left thoracotomy was performed in the fourth intercostal space, and the pericardium opened to expose the heart. A 2-0 silk thread was passed beneath a prominent anterior branch of the left coronary artery with a curved needle, and the ends of the ligature were passed through a short segment of pliable vinyl tubing to form a snare. The coronary branch was occluded by pulling the silk through the tubing. Myocardial ischemia was confirmed by the appearance of regional cyanosis of the myocardial surface. Release of the snare resulted in reperfusion, with visible hyperemia of the previously cyanotic area. In all animals, the coronary artery was occluded for 30 minutes, followed by 3 hours of reperfusion.

**Measurement of Risk Area and Infarct Size**

At the end of the reperfusion period, the hearts were removed and mounted on a modified Langendorff apparatus and perfused with saline at room temperature. After 1 minute of perfusion to flush blood from the heart, the coronary artery branch that had been occluded previously was reoccluded by ligating the silk thread that had been placed around it. Two milliliters of a 1% solution of 1 to 10 μm zinc/cadmium sulfide fluorescent particles (Duke Scientific Corp, Palo Alto, Calif) was infused into the perfusate to identify the ischemic region. The fluorescent particles filled the nonischemic region of the heart, and the coronary field that was supplied by the occluded artery, ie, the ischemic region, could be visualized as a nonfluorescent perfusion defect. The hearts were frozen and then sliced into 2- to 3-mm sections, thawed, and incubated in 1% triphenyltetrazolium chloride in sodium phosphate buffer at 37°C for 20 minutes. Tetrozolium salts react with dehydrogenase enzymes and cofactors, such as NADH, present in viable tissue to produce an intensely red-colored formazan pigment,30 whereas infarcted tissues that have lost these cofactors do not stain and appear tan or brown. All slices were then compressed between two plastic plates separated by exactly 2 mm. The outline of each ventricular slice and areas of triphenyltetrazolium chloride-positive or infarcted regions identified under white light and nonfluorescent or risk zones identified under UV light were traced onto superimposed plastic overlays. Then these areas of interest were measured by planimetry with the aid of a digitizing tablet (SAC, Norwalk, Conn) interfaced to a computer. Volumes of ischemic or risk zone tissue and infarct were calculated for the entire heart, and infarct size was expressed as a percentage of the ischemic zone infarcted.

**Protocol**

Eight groups of animals with at least one 30-minute ischemic period were studied. Groups 1 and 2 were untreated control and preconditioned animals, respectively. The former experienced only a single 30-minute coronary occlusion and reperfusion. The latter received a single 5-minute coronary occlusion and then a 10-minute reperfusion period before the 30-minute coronary occlusion. To determine whether catecholamines could also induce protection, a bolus of intravenous tyramine (1.4 mg/kg, Sigma Chemical Co, St Louis, Mo), dissolved in saline was administered to animals in group 3 as a substitute for ischemic preconditioning. A 10-minute recovery period followed the injection to duplicate the timing of the ischemic preconditioning protocol. To assess whether the hemodynamic changes mediated by tyramine might have caused the antiinfarct effect of this agent, its hypertensive effect was blunted. Cannulas inserted into the carotid arteries of rabbits of group 4 were connected to a reservoir that was permitted to fill with blood after tyramine administration and empty as the hypertensive effect faded. To study the possible mechanisms of the effect of tyramine on ischemic myocardium, three receptor-blocking agents were used. Group 5 animals also received a tyramine injection but were pretreated with the specific α1-adrenergic antagonist BE 2254 (2 mg/kg) dissolved in saline 5 minutes before tyramine treatment. This dose was selected because of its demonstrated effectiveness at blocking the hypertensive effects of an intravenous injection of 2 mg methoxamine. Propranolol (5 mg/kg, Sigma) dissolved in saline was administered to the animals in group 6 before tyramine treatment. Group 7 rabbits were treated with the nontoxic adenosine-receptor antagonist PD 115,199 (3 mg/kg, Parke-Davis Pharmaceuticals, Ann Arbor, Mich), 5 minutes before receiving tyramine. This agent was solubilized in a vehicle composed of 1 part 95% ethanol, 4 parts 0.1N hydrochloric acid, and 11 parts saline, after which the pH was adjusted to 6.0 with 0.1N sodium hydroxide. Finally, the animals in group 8 were treated with BE 2254 5 minutes before ischemic preconditioning to
determined whether catecholamines might be involved in the protection afforded by ischemic preconditioning.

In these experiments, animals of groups 3, 5, and 7 were studied concurrently and were assigned to groups according to the day of the week. Rabbits of groups 1 and 2 were studied throughout the duration of the protocols. Rabbits in groups 4, 6, and 8 were randomly assigned and also were studied concurrently, but after the completion of studies in the other groups.

Myocardial Blood Flow

An additional four animals were used exclusively to measure the effect of tyramine on myocardial perfusion. In these rabbits, catheters were introduced into the jugular vein for drug administration, femoral artery for timed withdrawal of an arterial reference sample, and left atrium for injection of radioactive microspheres. Both carotid arteries were also cannulated and connected to a reservoir to permit unloading of the arterial system as indicated above. Radioactive microspheres labeled with either 46Sc, 85Sr, or 142Ce were injected in random order for the three flow determinations in each animal. Microspheres were ultrasonicated and mechanically agitated for at least 10 minutes before injection. Approximately 0.5 to 1.0 x 10^6 spheres suspended in 1 mL saline were injected into the left atrium over 10 to 15 seconds and then flushed with 1 mL saline while an arterial sample was simultaneously collected at the rate of 1.56 mL/min. The collection was terminated after 90 seconds. Blood flow measurements were made under control conditions, at the peak hypertensive response after intravenous administration of tyramine (1.4 mg/kg), and again after tyramine injection and unloading of the arterial system. In the latter situation, the radioactive microspheres were injected at the same interval required after tyramine administration for attainment of the observed maximal blood pressure response seen when the arterial system was not unloaded.

After the third blood flow determination, the heart was excised and sliced into rings from apex to base. The left ventricular myocardium was divided into free wall and septum, and each of these pieces was in turn subdivided into inner and outer halves. Each myocardial piece was weighed, and all left ventricular and blood samples were placed in tubes for counting of radioactivity in a gamma well counter (1282 Compugamma, LKB-Wallac, Turku, Finland).

Raw radioactive counts per minute (CPM) were corrected for energy overlap by standard computer programs. CPM for all left ventricular pieces as well as for inner and outer pieces separately were summed and divided by the respective collective tissue mass. Flow (milliliters per minute per gram) was calculated as (CPM/g x f)/CPM, where CPM, and f, are CPM and pump withdrawal rate, respectively, of the reference arterial blood sample.

Myocardial Lactate Concentration

Eight rabbits were used to measure left ventricular lactate concentration under baseline conditions (n=4) and during the peak hypertensive response to intravenous administration of tyramine (1.4 mg/kg, n=4). In all animals, catheters were inserted into a jugular vein for drug or saline injections and a carotid artery for measurement of arterial pressure. The heart was exposed through a left thoracotomy, and a 4-0 silk suture was passed through the left ventricular myocardium at the apex. In treated rabbits, a bolus of tyramine was administered, and further manipulation of the heart was suspended for approximately 30 seconds until the maximal hypertensive response to the α-agonist was well established. In all hearts, the suture at the apex was pulled to raise the heart out of the chest, and simultaneously the heart was quick-frozen in Wollenberger tongs precooled in liquid nitrogen. The great vessels were quickly transected immediately above the edge of the tongs, and the heart was dropped into liquid nitrogen. Hearts were stored at −72°C until all eight specimens had been collected. Then the hearts were placed in ice and allowed to thaw. The left ventricle was separated from the other chambers, and two 0.5-g pieces were cut away for duplicate determinations of tissue lactate concentrations. Commercially available kits (Sigma) and a spectrophotometer (Lambda 3A, Perkin-Elmer, Oak Brook, Ill) were used to measure lactate. Duplicate measurements for each heart were averaged.

Statistics

All parameters are presented as mean±SEM. Results were tested for statistical significance by Student's unpaired t test, analysis of variance, and Scheffe’s post hoc test. A value of P<.05 was accepted as indicating statistical significance.

Results

Each of the eight groups contained five to eight animals (see Table 1), and a total of 52 rabbits were used. Table 1 presents the hemodynamic data. Tyramine infusion resulted in significant hypertension and tachycardia. These effects persisted for approximately 2 minutes; afterwards, there were no significant differences in heart rate and mean pressure between rabbits treated with tyramine and control animals for the remainder of the protocol.

Table 2 indicates that the average animal weights, heart weights, and the risk areas of the various groups were not significantly different. Infarct size in the control group averaged 34.4±3.2% of the ischemic region (Table 2 and Fig 1). As expected, ischemic preconditioning in group 2 significantly protected the heart from infarction, which measured only 7.7±3.1% of the risk zone (P<.01 vs control value). Tyramine pretreatment resulted in protection that was equivalent to that of ischemic preconditioning and produced an infarct percentage significantly smaller than the control value, 6.9±2.4% (P<.01 vs control value). Because of the possibility that the marked hypertensive response following tyramine injection rather than a direct effect of the drug itself was causing ischemic myocardium to be protected, the hemodynamic changes were blunted by effectively withdrawing blood from the arterial system in group 4 rabbits as the blood pressure rose and reinfusing it as pressure normalized. As noted in Table 1, this manipulation did minimize the rise in blood pressure. Table 2 and Fig 1 demonstrate that tyramine, even when divested of its hypertensive action, continued to protect the heart.

In view of these observations that tyramine was able to salvage ischemic myocardium, various receptor block-
ers were used to help define a mechanism of action. BE 2254, a selective α₁-adrenergic receptor blocker, was administered to group 5 rabbits. It effectively prevented the hypertensive effect of tyramine (Table 1). This agent also abolished the protective action of tyramine, resulting in 36.8±2.6% infarction of the risk zone (P=NS vs control value) (Table 2 and Fig 2). On the other hand, β-blockade with propranolol was unable to abolish the protective effect of tyramine (10.5±2.4% infarction, P<.01 vs control value) (Fig 2). Propranolol did, however, block both the hypertension and the tachycardia (Table 1). The nonselective adenosine antagonist PD 115,199 also prevented tyramine from protecting the heart, resulting in 40.2±5.6% infarction (Fig 2).

To determine whether the ability of BE 2254 to block the salutary effect of tyramine on ischemic myocardium could be extended to other methods of protection, group 8 animals were pretreated with this adrenergic blocking drug before ischemic preconditioning. In these animals, the protective effect of ischemic preconditioning was unaffected (3.9±1.1% infarction, P<.01 vs control value) (Table 2 and Fig 2).

To establish whether tyramine injection might be causing any myocardial ischemia, both left ventricular blood flow and lactate concentration were measured. Under baseline conditions, myocardial blood flow averaged 2.37±0.12 mL·min⁻¹·g⁻¹ and the inner/outer (I/O) left ventricular wall blood flow ratio was 1.11±0.02. Lactate concentration was 1.48±0.13 mmol/L. After tyramine administration, blood pressure rose as noted above, and tissue blood flow doubled to 4.88±0.32 mL·min⁻¹·g⁻¹ (P<.01). However, neither the I/O flow ratio (1.10±0.04) nor lactate level (1.74±0.24 mmol/L) changed significantly. Finally, when tyramine was injected and blood pressure was prevented from rising, the flow was unchanged from the baseline level (2.51±0.14 mL·min⁻¹·g⁻¹), and the I/O flow ratio was unaltered (1.16±0.08).

**Discussion**

The results of this study indicate that catecholamines can protect the heart from infarction to an extent similar to that seen after ischemic preconditioning. Tyramine was used in the study because it causes the endogenous release of catecholamines within the heart. Tyramine is β-hydroxylated to octopamine in

### TABLE 1. Serial Hemodynamic Changes in Rabbits Treated With Tyramine and Adrenergic and Adenosine Receptor Blockers

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>n</th>
<th>HR (bpm)</th>
<th>BP (mm Hg)</th>
<th>HR (bpm)</th>
<th>BP (mm Hg)</th>
<th>HR (bpm)</th>
<th>BP (mm Hg)</th>
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<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>8</td>
<td>268±9</td>
<td>62±4</td>
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<td>...</td>
<td>261±4</td>
<td>56±2</td>
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<tr>
<td>2</td>
<td>PC</td>
<td>6</td>
<td>249±18</td>
<td>74±6</td>
<td>...</td>
<td>...</td>
<td>241±18</td>
<td>69±5</td>
</tr>
<tr>
<td>3</td>
<td>TYR</td>
<td>7</td>
<td>225±13</td>
<td>62±6</td>
<td>281±16*</td>
<td>118±11†</td>
<td>232±8</td>
<td>69±5</td>
</tr>
<tr>
<td>4</td>
<td>TYR+UNL</td>
<td>5</td>
<td>226±24</td>
<td>51±5</td>
<td>238±20</td>
<td>81±10†</td>
<td>216±12</td>
<td>49±6</td>
</tr>
<tr>
<td>5</td>
<td>BE+TYR</td>
<td>7</td>
<td>259±15</td>
<td>68±5</td>
<td>297±9†</td>
<td>67±4‡</td>
<td>248±13</td>
<td>56±4</td>
</tr>
<tr>
<td>6</td>
<td>PR+TYR</td>
<td>7</td>
<td>255±4</td>
<td>76±4</td>
<td>215±11‡</td>
<td>79±8‡</td>
<td>212±9</td>
<td>64±3</td>
</tr>
<tr>
<td>7</td>
<td>PD+TYR</td>
<td>6</td>
<td>259±21</td>
<td>61±5</td>
<td>345±24*</td>
<td>127±4*</td>
<td>252±15</td>
<td>59±3</td>
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<tr>
<td>8</td>
<td>BE+PC</td>
<td>6</td>
<td>241±12</td>
<td>65±6</td>
<td>...</td>
<td>...</td>
<td>229±9</td>
<td>52±4</td>
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</tbody>
</table>

n, Number of rabbits; HR, heart rate; BP, blood pressure; TYR, tyramine; REP, reperfusion; PC, preconditioning ischemia; UNL, unloaded state; BE, BE 2254; PR, propranolol; and PD, PD 115,199. Values are mean±SEM.

### TABLE 2. Risk Areas and Infarct Sizes in Rabbits Treated With Tyramine and Adrenergic and Adenosine Receptor Blockers

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>n</th>
<th>Body weight (kg)</th>
<th>Body weight (g)</th>
<th>Risk area (cm²)</th>
<th>Infarct area (cm²)</th>
<th>I/R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>8</td>
<td>2.2±0.1</td>
<td>8.0±0.2</td>
<td>0.61±0.08</td>
<td>0.22±0.05</td>
<td>34.4±3.2*</td>
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<tr>
<td>2</td>
<td>PC</td>
<td>6</td>
<td>2.0±0.1</td>
<td>7.8±0.4</td>
<td>0.57±0.08</td>
<td>0.03±0.01†</td>
<td>7.7±3.1‡</td>
</tr>
<tr>
<td>3</td>
<td>TYR</td>
<td>7</td>
<td>2.6±0.1</td>
<td>6.7±1.4</td>
<td>0.63±0.09</td>
<td>0.05±0.02†</td>
<td>6.9±2.4‡</td>
</tr>
<tr>
<td>4</td>
<td>TYR+UNL</td>
<td>5</td>
<td>2.3±0.1</td>
<td>7.1±0.5</td>
<td>0.66±0.04</td>
<td>0.03±0.01†</td>
<td>3.9±0.8‡</td>
</tr>
<tr>
<td>5</td>
<td>BE+TYR</td>
<td>7</td>
<td>2.4±0.1</td>
<td>7.6±0.6</td>
<td>0.67±0.05</td>
<td>0.25±0.03</td>
<td>36.8±2.6*</td>
</tr>
<tr>
<td>6</td>
<td>PR+TYR</td>
<td>7</td>
<td>2.3±0.1</td>
<td>6.2±0.4</td>
<td>0.83±0.06</td>
<td>0.08±0.02†</td>
<td>10.5±2.4‡</td>
</tr>
<tr>
<td>7</td>
<td>PD+TYR</td>
<td>6</td>
<td>2.3±0.2</td>
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<td>40.2±5.6*</td>
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<tr>
<td>8</td>
<td>BE+PC</td>
<td>6</td>
<td>2.0±0.3</td>
<td>7.0±0.9</td>
<td>0.74±0.14</td>
<td>0.03±0.01†</td>
<td>3.9±1.1‡</td>
</tr>
</tbody>
</table>

n, Number of rabbits; I/R, percentage of risk zone infarcted; PC, preconditioning ischemia; TYR, tyramine; UNL, unloaded state; BE, BE 2254; PR, propranolol; PD, PD 115,199. Values are mean±SEM.

*P<.01 vs group 3; †P<.05 and ‡P<.01 vs group 1.
the adrenergic nerve terminals and stored in vesicles in this form. This causes displacement of the norepinephrine present in the vesicle and subsequent release.\textsuperscript{31}

The catecholamine-induced protection appears to be mediated by $\alpha$-adrenergic receptors since the protective effect of tyramine could be blocked by administration of the $\alpha$-antagonist BE 2254. $\beta$-Blockade with propranolol was not able to inhibit the tyramine-induced protection, although the cardiac effects of $\beta$-receptor occupancy were clearly blocked. These results are in partial agreement with those of a previous study by Kitakaze et al.,\textsuperscript{27} who also reported better preservation of myocardial function after ischemia in animals pretreated with an $\alpha$-adrenergic agonist.

The present results are not in agreement with the study concluding that norepinephrine released during ischemic preconditioning in rat hearts was responsible for the protection.\textsuperscript{26} That study also reported that norepinephrine acting via an $\alpha_1$-receptor mechanism could be protective, as was seen here, but that BE 2254 was able to block protection from ischemic preconditioning. In the present study, the protective effect of ischemic preconditioning could not be blocked with BE 2254. In the rabbit heart at least, the mechanism of catecholamine-induced protection clearly differs from that of ischemic preconditioning.

The above differences noted in rabbits and rats may be related to observed differences in the mechanism of induction of ischemic preconditioning in the two species. Although ischemic preconditioning attenuates ischemic injury in both rat\textsuperscript{7,26} and rabbit\textsuperscript{32,33} hearts, the protection in the rabbit depends on endogenously produced adenosine.\textsuperscript{34} Adenosine-receptor blockers are not effective in blocking the protective effect of ischemic preconditioning on either myocardial stunning\textsuperscript{35} or infarction\textsuperscript{7} in rat hearts. Although exogenous adenosine or $A_1$-selective adenosine receptor agonists can protect the rat heart from ischemic dysfunction\textsuperscript{36,37} and infarction,\textsuperscript{7} adenosine does not appear to be the endogenous stimulus for ischemic preconditioning in that species.

Adenosine is clearly involved in tyramine-induced protection in the rabbit heart. Fig 2 demonstrates that tyramine-induced protection can be eliminated by blocking adenosine receptors with PD 115,199. Therefore, catecholamine-induced protection and the protection afforded by ischemic preconditioning are both critically dependent on endogenous adenosine production. Because $\alpha_1$-receptors can couple to pertussis toxin–sensitive $G$ proteins, it was first thought that catecholamines might be able to precondition the heart by a direct $G$ protein mechanism, much as was seen with carbachol.\textsuperscript{5} The observation that adenosine receptor blockade prevents the protection argues against a direct $G$ protein mechanism. A more likely mechanism is that the increased myocardial oxygen demand caused by catecholamine release in turn induces adenosine release, which then precedes the heart by populating adenosine receptors. Adrenergic receptor stimulation

**FIG 1.** Infarct size plotted as a percentage of risk zone for control rabbits and those treated with ischemic preconditioning (PRECON) and tyramine with and without unloading (UNL). Open circles represent individual data points; filled-in circles represent means. Vertical bars depict SEM. All interventions significantly reduced infarct size compared with that in control rabbits ($P<.01$).

**FIG 2.** Infarct size plotted as a percentage of risk zone for the same control animals and rabbits treated with tyramine (TYR) as depicted in Fig 1. Also demonstrated are effects of TYR on infarct size after pretreatment with BE 2254 (BE), propranolol (PR), and PD 115,199 (PD) and the effect of BE on the protection of ischemic preconditioning (PRECON). Both BE and PD successfully blocked the protective effect of TYR. Propranolol did not affect the successful salvage by TYR of ischemic tissue, and infarct size was significantly reduced compared with that in control rabbits ($P<.01$). Finally, the ability of preconditioning ischemia to significantly diminish infarct size vs control group infarct size was not dampened by BE ($P<.01$).
increases adenosine production by the myocardium in guinea pigs and dogs. Although adenosine production by the heart was not measured in our rabbit model, it is also likely to be increased by catecholamines and probably is central to the mechanism of the protective effect of tyramine.

It would have been useful to have measured the effect of tyramine on adenosine production in our in situ rabbit hearts. Previously, evaluation of adenosine production by in situ myocardium has been done in large animals, in which it was possible either to put catheters into the coronary sinus to collect effluent blood or to place epicardial wells to collect interstitial fluid. Neither of these techniques is feasible for the small rabbit heart. In vitro studies of adenosine production have been done in small animals, but it is unclear whether tyramine stimulation of isolated hearts without the typical hemodynamic effects would be comparable to that seen in vivo. The recently described microdialysis technique for the monitoring of interstitial adenosine production is promising, although there are concerns that even insertion of the tiny fiber into the myocardium results in local myocardial damage and resultant adenosine production. Therefore, at present, the methodology for adenosine measurement in our rabbits remains problematic.

It is interesting that increased adenosine production appears to be a function of α-receptor stimulation. Blunting the hypertensive effect of tyramine with an α-blocker is not critical to the attenuation of the protective effect of tyramine, since mechanical control of blood pressure failed to block protection. Therefore, the protective effect requires stimulation of cardiac and not peripheral vascular α-receptors. The heart rate and inotropic effects of tyramine were, of course, unaffected by BE 2254. Propranolol, which did block both the chronotropic and inotropic effects but not the hypertensive effects of tyramine, was also unable to block the protective effect of tyramine. Kitakaze et al have proposed that α-receptor stimulation causes increased adenosine release by ischemic myocardium through upregulation of 5'-nucleotidase activity. The ability of α-receptor stimulation to augment adenosine production would be expected to increase adenosine release whenever the oxygen demand of the heart from either increased afterload or inotropic stimulation is elevated.

Adenosine appears to be the endogenous mediator of ischemic preconditioning in the rabbit. Since adenosine plays a key role in the preconditioning effect of tyramine, it was wondered whether α-mediated coronary vasoconstriction following tyramine administration might, in fact, be causing coronary hyperperfusion resulting in myocardial ischemia. If this were the case, then tyramine would be preconditioning myocardium simply by causing ischemically induced adenosine production. That seems unlikely, since Biffing and Feig were unable to demonstrate any abnormalities of transmural myocardial flow distribution or myocardial lactate extraction during norepinephrine infusion in dogs. We investigated that possibility by measuring left ventricular blood flow, transmural flow distribution, and tissue lactate levels during tyramine injection. Although tyramine significantly raised myocardial flow when blood pressure was permitted to rise normally, there was no change in the I/O blood flow ratio. Furthermore, tissue lactate levels were not significantly elevated after tyramine injection. Therefore, tyramine does not appear to precondition the heart by simply creating myocardial ischemia.

Infarct size in this study was determined by staining with triphenyltetrazolium chloride. Early determination of the extent of infarction by this method has generally been found to be an accurate measure of ultimate infarct size in untreated animals when reperfusion is permitted before death. It has been demonstrated that tetrazolium staining several hours after reperfusion yields virtually the same infarct size as histological determination several days after reperfusion in pigs and dogs. We must consider whether tetrazolium is revealing a true salvage in our protected animals or simply a delay in necrosis. Iwamoto et al measured infarct sizes by histology in rabbits subjected to a 30-minute occlusion period and preconditioning by the same protocol used in the present study and then 3 days of reperfusion. In control rabbits, 45% of the risk zone was infarcted, whereas only 13% was infarcted in preconditioned rabbits. Those infarct sizes compare very favorably to the data obtained in the present study. There are no available data as to whether tetrazolium is an accurate indicator of salvage induced by tyramine, but at this point, there is little reason to doubt its validity.

The results of the present study indicate that tyramine-induced catecholamine release can protect the myocardium from infarction during a subsequent ischemic insult in a manner very similar to that seen with ischemic preconditioning. The protection results from α-adrenergic receptor stimulation and probable subsequent adenosine production. However, it was not possible to demonstrate any role of α-receptors in ischemic preconditioning.

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