Endogenous Adenosine Blunts
β-Adrenoceptor–Mediated Inotropic Response in Hypoperfused Canine Myocardium

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Background. Adenosine attenuates β-adrenoceptor–mediated inotropic responses through GTP-binding protein in vitro. The goal of the present study was to test the hypothesis that endogenous adenosine released from the ischemic myocardium blunts the inotropic response to β-adrenergic stimulation.

Methods and Results. In 45 open-chest dogs, the left anterior descending coronary artery was perfused through an extracorporeal bypass tube from the carotid artery. Coronary perfusion pressure was reduced so that coronary blood flow was decreased to 60% of the basal level by partial occlusion of the bypass tube, and the reduced coronary perfusion pressure was kept constant thereafter. Inotropic responses to isoproterenol were assessed by fractional shortening of the myocardium in the perfused area. After the onset of hypoperfusion, lactate extraction ratio (18.8 ± 1.2%) and fractional shortening (20.7 ± 1.1%) were significantly decreased to −8.4 ± 8.0% and 5.9 ± 1.5%, respectively, and coronary arteriovenous differences of adenosine were increased from 4.6 ± 3.6 to 89.4 ± 10.5 pmol/ml. In the untreated condition, an intravenous infusion of isoproterenol (150 ng/kg/min) augmented fractional shortening from 5.9 ± 1.5% to 13.6 ± 0.8%. When adenosine release was attenuated by administration of prazosin (4 μg/kg/min i.c.) during hypoperfusion, the response of fractional shortening to isoproterenol (from 5.3 ± 1.2% to 20.5 ± 1.4%) was much greater (p < 0.05) than that in the untreated control condition. Exogenous administration of adenosine significantly attenuated the inotropic response to isoproterenol in the prazosin-treated hearts. In contrast, an adenosine receptor antagonist, 8-phenyltheophylline, also enhanced the inotropic response to isoproterenol. The attenuation of β-adrenoceptor–mediated inotropic response by adenosine could not be attributed to the inhibition of norepinephrine release from the sympathetic nerve endings, because identical results were observed in the chemically denervated hearts.

Conclusions. Endogenous adenosine released from the ischemic myocardium attenuates β-adrenoceptor–mediated inotropic response in the ischemic heart. (Circulation 1992;85:1594–1603)

KEY WORDS • catecholamine • myocardial ischemia • prazosin • isoproterenol • 8-phenyltheophylline

Adenosine is known to act on coronary vessels as a potent and endogenous vasoactive agent during ischemia.1–6 Adenosine is also reported to attenuate the inotropic response of the myocardium to β-adrenergic stimulation.7–12 Because sympathetic activities are enhanced in severe myocardial ischemia,13–15 adenosine released from the ischemic myocardium may attenuate the enhanced contractile function of the ischemic myocardium. This possible interaction between adenosine and β-adrenergic stimulation may also be observed in the clinical setting. Although previous observations in perfused myocardium and heart dem-
mg/kg). Each dog was intubated with auffed endotracheal tube and ventilated with room air mixed with oxygen (2–5 L/min) by a respirator. A left thoracotomy was performed through the fifth intercostal space, and the heart was suspended in a pericardial cradle. A proximal portion of the left anterior descending coronary artery was cannulated and perfused with blood through an extracorporeal tube from the left carotid artery. An electromagnetic flow probe (Nihon Kohden, FF-050T, Tokyo) was attached to the bypass tube for measurement of coronary blood flow (CBF). Coronary perfusion pressure (CPP) was measured at the proximal portion of the cannula. A small, short tube (1 mm in diameter and 7 cm long) for blood sampling was inserted into the epicardial vein at the center of the perfused area, and drained coronary venous blood was returned to the jugular vein. A miniaturized pressure transducer (model P-7, Konigsberg, Pasadena, Calif.) was inserted into the left ventricular cavity through a stab incision of the left ventricular apex. A pair of ultrasonic crystals (5 MHz, 2 mm in diameter; Schuessler) were implanted into the endocardial one third of the left ventricular anterior wall in the center of the perfused area for measurement of segment length. These hemodynamic parameters were recorded on a multichannel recorder (Nihon Kohden, RM-6000). We calculated fractional shortening (FS) using the equation 
\[
\text{FS} = \frac{\text{EDL} - \text{ESL}}{\text{EDL} \times 100} \%
\]
where EDL and ESL are end-diastolic and end-systolic segment lengths, respectively. Heart rate averaged 148 beats per minute in the intact heart and 111 beats per minute in the denervated heart.

**Experimental Protocols**

**Protocol 1: Effects of 8-phenyltheophylline and prazosin on the inotropic response to β-adrenergic stimulation in the ischemic myocardium.** Twenty-five dogs were used in this protocol. After hemodynamic stabilization, left ventricular pressure, segment length in the perfused area, CBF, and regional CBF were measured. Coronary arterial and venous blood were sampled by glass syringes for blood gas analysis and determination of lactate and adenosine concentrations. With an occluder attached at the extracorporeal bypass tube, CPP was reduced so that CBF decreased to 60% of the control CBF. Once low CPP was determined, the occluder was manually adjusted exactly to maintain CPP constant at the set level. In seven dogs (group 1a), these procedures were done without treatment with 8-phenyltheophylline or prazosin. In six other dogs (group 1b), to eliminate the effect of adenosine on the ischemic myocardium, the same procedure was performed under continuous intracoronary administration of a potent surface membrane adenosine receptor antagonist, 8-phenyltheophylline (30 µg/kg body wt/min). In the preliminary study, this dose of 8-phenyltheophylline abolished the coronary vasodilatory effect of exogenous adenosine (5 µg/kg body wt/min i.c.). Because prazosin inhibits adenosine release from the ischemic myocardium, prazosin (4 µg/kg body wt/min) was also administered into the extracorporeal bypass tube in the seven other dogs (group 1c) before the procedure of coronary hypoperfusion. Furthermore, in five other dogs (group 1d), adenosine (5 µg/kg body wt/min) was infused into the bypass tube in addition to the treatment with prazosin (4 µg/kg body wt/min) to test whether the exogenous adenosine could restore the effects of endogenous adenosine. In group 1d, an infusion of adenosine began 5 minutes after the onset of hypoperfusion. Administrations of prazosin and 8-phenyltheophylline were initiated at least 10 minutes before the onset of coronary hypoperfusion and continued throughout the study. The hemodynamic and metabolic parameters were also measured immediately before the onset of the hypoperfusion.

Under each intervention during coronary hypoperfusion, three doses of isoproterenol (16.7, 50, and 150 ng/kg body wt/min) were infused intravenously in random order at least 5 minutes after the onset of coronary hypoperfusion. Measurements of hemodynamic and metabolic parameters were performed before and 5 minutes after the infusion of isoproterenol. In the preliminary study, we confirmed that hemodynamic and metabolic parameters become stable within 4 minutes after the onset of infusion of isoproterenol.

**Protocol 2: Effects of endogenous adenosine on β-selective adrenoceptor-mediated inotropic response of the ischemic myocardium.** To test whether β-selective adrenoceptor–mediated inotropic response is enhanced by the attenuation of adenosine release, denopamine (TA-064, Tanabe Pharmacol; 1 µg/kg body wt/min) was infused intravenously during coronary hypoperfusion (n=5, group II). All of the parameters were measured at the steady state with and without an intracoronary infusion of prazosin (4 µg/kg body wt/min) in each dog.

**Protocol 3: Effects of prazosin on β-adrenoceptor-mediated inotropic response of the ischemic myocardium in chemically denervated hearts.** Because several lines of evidence suggest that adenosine reduces the release of norepinephrine from nerve terminals through presynaptic A1-adenosine receptors,18–21 attenuated adenosine release by prazosin may increase myocardial contractility of the ischemic heart through enhancement of norepinephrine release from nerve endings. To exclude this possibility, identical procedures and measurements of all variables as in protocol 2 were performed in chemically denervated hearts (n=5, group III). CBF was reduced so that FS decreased to a comparable level as in protocol 1. Systemic chemical sympathectomy was performed by an intravenous injection of 50 mg/kg body wt of 6-hydroxydopamine 5 days before the experiment. Prevention of deleterious side effects of 6-hydroxydopamine was provided by previous injections of propranolol (1 mg/kg body wt) and phentolamine (1 mg/kg body wt), and three fractional doses of 6-hydroxydopamine (10, 20, and 20 mg/kg body wt) were administered over a period of 24 hours.22 In this protocol, a single dose of isoproterenol (50 ng/kg body wt/min) was administered in each dog. We confirmed that norepinephrine contents in the myocardium in systemically denervated and innervated dogs (group 1a; n=7) were 11±2.3 and 366±28 pg/mg (p<0.05), respectively. These dogs were killed immediately after the experimental procedure, and the myocardial tissue was sampled from the perfused area for the measurement of norepinephrine content.

**Protocol 4: Effects of prazosin on β-adrenoceptor-mediated inotropic response.** To exclude the possibility that α-adrenergic blockade directly contributes to the
TABLE 1. Coronary Hemodynamic and Metabolic Parameters Before and During Hypoperfusion in Intact (Innervated) Hearts

<table>
<thead>
<tr>
<th></th>
<th>CPP (mm Hg)</th>
<th>CBF (ml/100 g/min)</th>
<th>HR (bpm)</th>
<th>FS (%)</th>
<th>LER (%)</th>
<th>MVO₂ (ml/100 g/min)</th>
<th>AdoD (pmol/ml)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>108±15</td>
<td>92.0±1.4</td>
<td>159±11</td>
<td>23.1±1.4</td>
<td>26.6±8.6</td>
<td>6.57±0.59</td>
<td>4.6±2.0</td>
<td>7.40±0.04</td>
</tr>
<tr>
<td>Hypoperfusion</td>
<td>49±4*</td>
<td>48.0±2.1*</td>
<td>158±11</td>
<td>6.8±0.9*</td>
<td>-2.9±2.0*</td>
<td>4.26±0.25*</td>
<td>68.3±8.6*</td>
<td>7.30±0.06*</td>
</tr>
<tr>
<td>Denopamine</td>
<td>49±4</td>
<td>53.3±3.3</td>
<td>182±10</td>
<td>12.3±1.9</td>
<td>-33.6±8.5</td>
<td>5.28±0.58</td>
<td>104.3±14.9</td>
<td>7.25±0.04</td>
</tr>
<tr>
<td>± prazosin</td>
<td>50±3</td>
<td>50.7±4.3</td>
<td>181±10</td>
<td>18.9±2.8†</td>
<td>-52.8±10.6</td>
<td>5.83±0.55</td>
<td>72.4±8.2†</td>
<td>7.20±0.04†</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=5).

CPP, coronary perfusion pressure; CBF, coronary blood flow; HR, heart rate; bpm, beats per minute; FS, fractional shortening; MVO₂, myocardial oxygen consumption; AdoD, coronary arteriovenous difference of adenosine concentration; pH, pH in coronary venous blood.

*p<0.05 vs. untreated control condition; †p<0.05 vs. denopamine treatment.

enhancement of β-adrenoceptor-mediated inotropic response,

of prazosin on isoproterenol-induced inotropic response were studied in nonischemic hearts (group IVa) and ischemic hearts in which adenosine receptors were blocked by 8-phenyltheophylline (group IVb). In five dogs (group IVa), inotropic response to isoproterenol was examined in the nonischemic hearts treated with prazosin, which does not affect the release of adenosine in the normoxic heart. Without the constriction of the bypass tube, two doses of isoproterenol were infused intravenously (50 and 150 ng/kg body wt/min), and hemodynamic and metabolic parameters were measured with and without the prazosin treatment (4 μg/kg body wt/min). In five other dogs (group IVb), inotropic response to isoproterenol was also studied in the ischemic hearts treated with 8-phenyltheophylline. In this group, to eliminate the secondary effect of prazosin, i.e., attenuation of adenosine release, 8-phenyltheophylline was administered to block the adenosine receptors. During intracoronary administration of 8-phenyltheophylline (30 μg/kg body wt/min), the coronary bypass tube was constricted, and the hemodynamic response to intravenous infusion of isoproterenol (50 ng/kg body wt/min) was studied. After this experiment, prazosin (4 μg/kg body wt/min) was administered and the identical study was performed.

At the conclusion of each protocol, after the perfusion area was stained by infusion of Evans blue dye, the heart was excised, and weight of the perfused area was determined for the normalization of CBF (ml/100 g myocardium/min) and myocardial oxygen consumption (ml/100 g myocardium/min). The mean tissue weight of the perfusion area in protocols I, II, III, and IV were 32±6, 34±7, 30±5, and 33±6 g, respectively.

Chemical Analysis

Blood gases, including hemoglobin and pH, were measured with an ABL Radiometer (ABL-2, Copenhagen Apparatus, Denmark Radiometer Institute). Coronary arteriovenous blood oxygen difference (AVo₂D) was assessed by the differences between coronary arterial and venous oxygen contents. Myocardial oxygen consumption (MVO₂, ml/100 g/min) was calculated by CBF (ml/100 g/min)×AVo₂D (ml/min). Blood (1 ml) for lactate measurement was rapidly centrifuged, and 0.02 ml of supernatant was analyzed. Lactate was measured by the Determina LA assay kit (Kyowa Medics Corp.) and the automatic Analyzer 705 (Hitachi Corp.). Hydrogen peroxide generated from lactate peroxidase was measured by the peroxidase assay with 4-aminofanipyprine as a substrate. Lactate extraction ratio (LER) was obtained by the coronary arteriovenous difference in the lactate concentration divided by arterial lactate concentration and multiplied by 100.

Adenosine measurement. The method of adenosine measurement has been reported previously from our laboratory. Blood (1 ml) was drawn into a syringe containing 0.5 ml dipyriradime (0.01%) and 0.1 ml MnCl₂ (10 mM) to block the uptake of adenosine by red blood cells and degradation of adenosine. After centrifugation, the supernatant was mixed with an equal volume of 10% trichloroacetic acid to remove the coagulated protein. Residual trichloroacetic acid was removed by water-saturated ether from the extraction of the supernatant, and radioimmunoassay methods for analyzing adenosine content were used. Briefly, adenosine in the plasma (100 μl) was succinylated by 100 μl dioxane containing succinic anhydride and triethylamine. After 10-minute incubation, the mixture was diluted with 800 μl of 0.3 M imidazole buffer (pH 6.5). The assay mixture contained 100 μl of the sample, 100 μl succinyl [3H]adenosine (25,000 counts/min in an amount of 1 pmol), and 100 μl of diluted amidinosine serum. After the mixture was kept in an ice-cold water bath for 24 hours, a cool suspension of dextran-coated charcoal (500 μl) was added. The charcoal was spun down, and 0.5 ml of the supernatant was counted for radioactivity in a liquid scintillation counter. The amount of adenosine degradation during the blood sampling procedure and the degradation rate of adenosine were reported as negligible.

Norepinephrine measurement. The method of norepinephrine measurement has been described previously. To determine norepinephrine concentration in myocardial tissue, myocardial tissue from the left anterior descending coronary artery area was sampled within 5 seconds and was frozen in liquid nitrogen. The frozen tissue was stored at -80°C. Within 1 week, myocardial tissue was homogenized with EDTA (0.1 M), NaHSO₃ (1 M), and HClO₄ (0.05 M) solution. After centrifugation, the norepinephrine in the supernatant was adsorbed on alumina and separated by high-performance liquid chromatography (pump, LC-3A; column, Zpax-SCX; Shimazu Seisakusho, Kyoto, Japan). Norepinephrine content was determined spectrofluorometrically by the trihydroxindole method (Shimazu spectrofluorophotometer RF-500LCA). In this system, sensitivity of the assay is 10 pg/ml plasma, and intra-assay coefficient of variation is 6.8%.26
Table 2. Coronary Hemodynamic and Metabolic Parameters Before and During Hypoperfusion in Denervated Hearts

<table>
<thead>
<tr>
<th>Condition</th>
<th>CPP (mm Hg)</th>
<th>CBF (ml/100 g/min)</th>
<th>HR (bpm)</th>
<th>FS (%)</th>
<th>LER (%)</th>
<th>MVO₂ (ml/100 g/min)</th>
<th>AdoD (pmol/ml)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>99±9</td>
<td>91.7±1.1</td>
<td>111±8</td>
<td>26.3±2.5</td>
<td>19.8±6.2</td>
<td>5.04±0.37</td>
<td>10.2±5.3</td>
<td>7.43±0.03</td>
</tr>
<tr>
<td>Hypoperfusion</td>
<td>49±4*</td>
<td>31.9±1.7*</td>
<td>107±6</td>
<td>7.5±1.5*</td>
<td>-8.8±5.8*</td>
<td>2.92±0.42*</td>
<td>74.2±12.6*</td>
<td>7.31±0.04</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>49±5</td>
<td>34.8±1.5</td>
<td>131±16</td>
<td>14.9±3.9</td>
<td>-20.5±10.8</td>
<td>3.08±0.57</td>
<td>115.4±23.8</td>
<td>7.23±0.04</td>
</tr>
<tr>
<td>Isoproterenol + prazosin</td>
<td>48±5</td>
<td>34.3±1.4</td>
<td>130±16</td>
<td>19.4±5.2†</td>
<td>-34.2±11.9</td>
<td>3.32±0.47</td>
<td>78.8±21.3†</td>
<td>7.19±0.04</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=5).
CPP, coronary perfusion pressure; CBF, coronary blood flow; HR, heart rate; bpm, beats per minute; FS, fractional shortening; MVO₂, myocardial oxygen consumption; AdoD, coronary arteriovenous difference of adenosine concentration; pH, pH in coronary venous blood.
†p<0.05 vs. untreated control condition; †p<0.05 vs. isoproterenol treatment.

Statistical Analysis

Statistical analysis was performed with paired (Tables 1-4) and unpaired t tests (Figures 1-3). Multiple ANOVA was also used in Figures 1–3 to test the differences of the responses of hemodynamic and metabolic variables to the doses of isoproterenol. All values were expressed as mean±SEM, and p<0.05 was considered significant.

Results

Effects of 8-Phenyltheophylline and Prazosin on Myocardial Ischemia

Neither an infusion of 8-phenyltheophylline nor prazosin significantly changed coronary hemodynamic and metabolic parameters in the baseline conditions in each group, indicating that the effects of these agents on the coronary vascular tone were minimal. Myocardial oxygen consumption and arteriovenous differences of adenosine were altered by neither prazosin nor 8-phenyltheophylline in the nonischemic condition (Figures 1–3).

After the onset of hypoperfusion, both FS and LER were decreased, but the extents of decreases in these parameters were not different among the groups. After the onset of hypoperfusion, arteriovenous differences of adenosine were significantly attenuated in the prazosin-treated group (13.5±5.7 pmol/ml) compared with the control condition (89.4±10.5 pmol/ml).

Averaged heart rates were increased by isoproterenol infusion (161±11 beats per minute at isoproterenol of 16.7 ng/kg body wt/min, 172±12 beats per minute at 50 ng/kg body wt/min, and 195±13 beats per minute at 150 ng/kg body wt/min) but were not different among the groups. In all the groups, FS was increased during intravenous infusion of isoproterenol in the dose-dependent manner, but the extents of increments of FS were significantly different among the groups (Figure 1).

In the control group (group Ia), FS was increased from 5.9±1.5% to 13.6±0.8% when the doses of isoproterenol increased from 16.7 to 150 ng/kg body wt/min. In the prazosin-treated group (group Ic), however, FS was more increased, from 5.3±1.2% to 20.5±1.4%, indicating that the inotropic response in the prazosin-treated condition was higher than that in the control group. The effects of prazosin on ß-adrenergceptor-mediated inotropic responses were completely inhibited by an additional intracoronary infusion of adenosine (group Id).

In the 8-phenyltheophylline-treated group (group Ib), increases of FS in response to isoproterenol (from 6.1±2.1% to 19.8±2.2%) were comparable to those in the prazosin-treated condition and were significantly larger than those in the untreated condition. Figure 1B shows that with prazosin treatment (group Ic), arteriovenous differences of adenosine after the onset of hypoperfusion were markedly attenuated, whereas treatment with exogenous adenosine and prazosin significantly increased arteriovenous differences of adenosine. Because CBF and CPP were not different among the groups (Figure 2), these results of arteriovenous

Table 3. Coronary Hemodynamic and Metabolic Parameters Before and During ß-Adrenoceptor Blockade in Intact (Inervated) Nonischemic Hearts

<table>
<thead>
<tr>
<th>Condition</th>
<th>CPP (mm Hg)</th>
<th>CBF (ml/100 g/min)</th>
<th>HR (bpm)</th>
<th>FS (%)</th>
<th>LER (%)</th>
<th>MVO₂ (ml/100 g/min)</th>
<th>AdoD (pmol/ml)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>101±4</td>
<td>90.4±2.7</td>
<td>148±6</td>
<td>22.8±2.2</td>
<td>23.2±3.4</td>
<td>6.81±1.63</td>
<td>5.58±4.92</td>
<td>7.42±0.03</td>
</tr>
<tr>
<td>Isoproterenol (50 ng/kg/min)</td>
<td>98±5</td>
<td>149.5±16.7*</td>
<td>163±4*</td>
<td>29.6±3.1</td>
<td>25.4±8.4</td>
<td>8.72±1.71</td>
<td>35.1±13.2</td>
<td>7.37±0.02*</td>
</tr>
<tr>
<td>Isoproterenol (150 ng/kg/min)</td>
<td>93±8</td>
<td>178.8±25.8*</td>
<td>175±6*</td>
<td>33.7±3.6</td>
<td>28.1±6.3</td>
<td>12.50±1.41*</td>
<td>51.2±12.5*</td>
<td>7.33±0.04*</td>
</tr>
<tr>
<td>Prazosin</td>
<td>101±4</td>
<td>94.7±5.5</td>
<td>150±3</td>
<td>24.0±2.0</td>
<td>23.5±4.0</td>
<td>6.80±1.17</td>
<td>7.70±4.07</td>
<td>7.39±0.03</td>
</tr>
<tr>
<td>Prazosin + isoproterenol (50 ng/kg/min)</td>
<td>94±3</td>
<td>148.2±19.2†</td>
<td>164±4†</td>
<td>29.5±2.8†</td>
<td>23.7±6.2</td>
<td>9.82±1.66†</td>
<td>31.5±7.2†</td>
<td>7.38±0.03</td>
</tr>
<tr>
<td>Prazosin + isoproterenol (150 ng/kg/min)</td>
<td>90±5</td>
<td>180.3±20.1†</td>
<td>178±10†</td>
<td>31.3±2.9†</td>
<td>27.1±6.7</td>
<td>13.58±1.84†</td>
<td>46.5±6.0†</td>
<td>7.31±0.03</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=5).
CPP, coronary perfusion pressure; CBF, coronary blood flow; HR, heart rate; bpm, beats per minute; FS, fractional shortening; MVO₂, myocardial oxygen consumption; AdoD, coronary arteriovenous difference of adenosine concentration; pH, pH in coronary venous blood.
†p<0.05 vs. untreated control condition; †p<0.05 vs. prazosin treatment.
differences of adenosine indicate that endogenous adenosine released from the ischemic myocardium attenuates inotropic responses to isoproterenol. The augmentation of β-adrenoceptor-mediated inotropic response by both prazosin and 8-phenyltheophylline could not be attributed to the attenuation of ischemia, because pH and oxygen content in the coronary venous blood both in the prazosin-treated group and the 8-phenyltheophylline-treated group were lower than those in the untreated group (Figure 3).

β1-Selective Adrenoceptor-Mediated Inotropic Responses With and Without a Massive Adenosine Release

In group II, intracoronary infusions of denopamine (without prazosin) increased FS from 6.8±0.9% to 12.3±1.9%, and this increase was further enhanced by the prazosin treatment (from 12.3±1.9% to 18.9±2.8%, p<0.05 versus denopamine without prazosin treatment, Table 1). Arteriovenous difference of adenosine was significantly increased by denopamine, from 68.3±8.6 to 104.3±14.9 pmol/ml, but this increase was attenuated by the treatment with prazosin. CPP was not altered, as was designed, and CBF was not different between the groups with and without the treatment with prazosin. The treatment with prazosin further increased MVo2 and further decreased both LER and pH during infusions of denopamine compared with the untreated condition (Table 1).

Effects of Prazosin on β-Adrenoceptor-Mediated Inotropic Response in the Denervated Hearts During Hypoperfusion

Table 2 shows coronary hemodynamic and metabolic parameters before and during hypoperfusion in the denervated hearts (group III). Isoproterenol without prazosin treatment increased FS from 7.5±1.5% to 14.9±3.9%, and this increase was further enhanced by the treatment with prazosin (14.9±3.9% to 19.4±5.2%, p<0.05 versus without the treatment with prazosin). Arteriovenous difference of adenosine was increased by isoproterenol from 74.2±12.6 to 115.4±23.8 pmol/ml, and this increase was attenuated by the prazosin treatment (78.8±21.3 pmol/ml; p<0.05 versus without prazosin). CPP was kept constant, and CBF was not different between the groups with and without prazosin treatment. LER and coronary venous pH were decreased by an isoproterenol infusion in the prazosin-treated condition. These results were comparable to those in the innervated hearts, indicating that presynaptic modulation with endogenous adenosine is minimally involved in the inotropic response to isoproterenol.

Effects of Prazosin on β-Adrenoceptor-Mediated Inotropic Response

In the intact nonischemic hearts (group IVa), an intracoronary infusion of prazosin did not affect both hemodynamic and metabolic parameters (Table 3). Arteriovenous differences of adenosine were increased by isoproterenol infusion but were not different between the groups with and without the prazosin treatment. Responses of FS to isoproterenol infusion were unchanged by the prazosin treatment.

In the ischemic hearts (group IVb), FS (21.9±2.1%) was not altered by the treatment with 8-phenyltheophylline (21.8±2.0%) but decreased to 6.0±1.4% during coronary hypoperfusion (Table 4). Although administration of isoproterenol increased FS to 19.0±1.6%, intracoronary administration of prazosin did not further enhance the inotropic response (18.3±1.1%). These results suggest that subcellular mechanisms associated with α-blockade are not directly related to the enhancement of β-adrenoceptor-mediated inotropic response by prazosin in both normoxic and ischemic hearts.

Discussion

There are several lines of evidence that exogenously administered adenosine inhibits full mechanical responsiveness to β-adrenergic stimulation in the intact heart11-12. However, the role of endogenous adenosine in attenuation of contractile response to β-adrenergic stimulation in the ischemic heart is not fully clarified. To elucidate the role of endogenous adenosine, we used two different protocols to test the effects of adenosine: antagonization of adenosine receptors with 8-phenyltheophylline (group Ib) and attenuation of adenosine release from the ischemic myocardium by treatment with prazosin (group Ic). The present study indicates that endogenous adenosine released from the ischemic myocardium blunts the β-adrenoceptor-mediated inotropic response (Figure 1), because 1) an

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**Table 4.** Coronary Hemodynamic and Metabolic Parameters Before and During α1-Adrenoceptor Blockade in Ischemic Hearts

<table>
<thead>
<tr>
<th>Acceptor</th>
<th>CPP (mm Hg)</th>
<th>CBF (ml/100 g/min)</th>
<th>HR (bpm)</th>
<th>FS (%)</th>
<th>LER (%)</th>
<th>MVo2 (ml/100 g/min)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>102±6</td>
<td>90.6±1.9</td>
<td>146±4</td>
<td>21.9±2.1</td>
<td>24.4±7.9</td>
<td>6.34±0.92</td>
<td>7.40±0.02</td>
</tr>
<tr>
<td>8-Phenyltheophylline (30 μg/kg/min)</td>
<td>100±5</td>
<td>90.2±2.0</td>
<td>143±6</td>
<td>21.8±2.0</td>
<td>23.0±6.0</td>
<td>6.15±0.85</td>
<td>7.39±0.02</td>
</tr>
<tr>
<td>Hypoperfusion</td>
<td>60±7*</td>
<td>53.0±2.1*</td>
<td>145±5</td>
<td>6.0±1.4*</td>
<td>-19.5±20*</td>
<td>4.65±0.69*</td>
<td>7.31±0.03*</td>
</tr>
<tr>
<td>Hypoperfusion + isoproterenol (50 ng/kg/min)</td>
<td>59±7</td>
<td>66.6±5.2†</td>
<td>171±4†</td>
<td>19.0±1.6†</td>
<td>-68.4±25.4†</td>
<td>6.20±0.88†</td>
<td>7.23±0.05†</td>
</tr>
<tr>
<td>Hypoperfusion + prazosin + isoproterenol (50 ng/kg/min)</td>
<td>57±7</td>
<td>63.5±6.7</td>
<td>176±3†</td>
<td>18.3±1.1†</td>
<td>-71.4±27.3†</td>
<td>6.23±0.93†</td>
<td>7.23±0.04†</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=5).

CPP, coronary perfusion pressure; CBF, coronary blood flow; HR, heart rate; bpm, beats per minute; FS, fractional shortening; MVo2, myocardial oxygen consumption; AdoD, coronary arteriovenous difference of adenosine concentration; pH, pH in coronary venous blood. *p<0.05 vs. untreated control condition; †p<0.05 vs. hypoperfusion.
adenosine receptor antagonist, 8-phenyltheophylline (30 μg/kg body wt/min), enhanced positive inotropic responses to isoproterenol; 2) inotropic responses to β-adrenergic stimulation by isoproterenol and denopamine were enhanced by the prazosin treatment (Figure 1, Tables 1 and 2); and 3) the upward shift of the inotropic response curve by the treatment with prazosin was completely abolished by an intracoronary infusion of adenosine (Figure 1).

Other possibilities should be also considered, however. Prazosin, which attenuates the release of adenosine, may directly affect coronary vessels and myocardium. Because α₁-adrenergic stimulation exerts coronary vasoconstriction during myocardial ischemia,27–29 prazosin may increase CBF during ischemia and thus improve contractile responses to isoproterenol. In the present study, however, basal CBF was not significantly altered by prazosin infusion (Figure 2), and the extents of the reduction in CBF and CPP during hypoperfusion were not different from those of the untreated group. Thus, a small dose of prazosin (4 μg/kg/min) does not affect the coronary vascular tone and myocardial contraction. There is another possibility: that prazosin dilates the functional collateral vessels to the ischemic area and improves the ischemia. This possibility may be negated, however, because the collateral flow is not regulated by α₁-adrenoceptor activities.30,31 Indeed, in the present study, FS and LER after the onset of hypoperfusion were not different between untreated and prazosin-treated conditions. Thus, direct actions of α₁-adrenoceptor activity on coronary vessels cannot explain our present results.

The previous report23 that α₁-adrenergic stimulation attenuates β-adrenergic responsiveness through the activation of protein kinase C may suggest that α₁-adrenoceptor blockade may enhance β-adrenoceptor-mediated inotropic response. Subcellular α–β interaction may not be a mechanism of the beneficial effects of...
FIGURE 3. Graphs showing changes in myocardial oxygen consumption (panel A), lactate extraction ratio (panel B), pH (panel C), and oxygen saturation (panel D) in coronary venous blood during the control condition and an infusion of isoproterenol in the ischemic heart. During the control condition, all these metabolic parameters were not significantly different among the groups. During hypoperfusion, lactate extraction ratio, pH, and oxygen saturation were decreased in response to isoproterenol infusion. Lactate extraction ratio and pH in the 8-phenyltheophylline-treated group and pH in the prazosin-treated groups were significantly decreased compared with those in the untreated group (multiple ANOVA), indicating that augmentation of β-adrenoceptor-mediated inotropic response by prazosin or 8-phenyltheophylline treatment (see Figure 1) could not be attributed to the attenuation of ischemia. Bars are mean±SEM; C1, no drug; C2, pretreatment; *p<0.05 vs. untreated group.
prazosin, however, because the response of the FS to isoproterenol was not affected by the prazosin treatment in the normoxic hearts (Table 3) and in the ischemic hearts treated with 8-phenyltheophylline (Table 4). Taken together, we consider that the treatment with prazosin enhances the inotropic response to $\beta$-adrenergic stimulation through inhibition of endogenous adenosine release from the ischemic myocardium. This possibility is further supported by the fact that exogenous adenosine completely abolished the effects of prazosin (Figure 1).

In the present study, we also observed that contractile response to isoproterenol is enhanced by adenosine receptor blockade with 8-phenyltheophylline (Figure 1). Although 8-phenyltheophylline has an inhibitory effect of phosphodiesterase and has a potency of inotropic effect, the dose of 8-phenyltheophylline used in this study did not affect myocardial contractility in the control condition (Figure 1), consistent with our previous study. These results indicate that its effect on phosphodiesterase was minimal. On the other hand, several reports suggest that theophylline derivatives improve the transmural redistribution of coronary blood flow from the endocardium to the epicardium, which attenuates myocardial ischemia. In the present study, however, 8-phenyltheophylline did not improve hemodynamic and metabolic parameters during hypoperfusion (Figure 3), indicating that transmural redistribution of the coronary blood flow is minimally influenced by 8-phenyltheophylline in our ischemic model.

There are two subtypes of $\beta$-adrenoceptors, i.e., $\beta_1$ and $\beta_2$-adrenoceptors, and isoproterenol activates both subtypes. In this study, $\beta_2$-selective adrenoceptor-mediated inotropic response is also enhanced by the attenuation of adenosine release (Table 1), suggesting that the enhancement of inotropic responses during attenuation of adenosine release is attributed to activations of $\beta_2$-adrenoceptors of myocardium.

It is well known that adenosine inhibits norepinephrine release from the sympathetic nerve endings. Thus, prazosin and 8-phenyltheophylline may enhance the norepinephrine release and increase contractile function. In the previous study, we observed that restriction of CBF to one third of the control flow did not induce norepinephrine release from nerve endings. Furthermore, in the present study, the augmented inotropic responses to isoproterenol were also observed, even in chemically denervated hearts (Table 2). Thus, contribution of norepinephrine from the sympathetic nerve endings may be minimal in our model.

**Physiological and Clinical Relevance**

Previous biochemical and pharmacological studies in ventricular myocytes demonstrated that adenosine has a negative inotropic action mediated by $A_1$-adenosine receptor coupled to the inhibitory guanine regulatory proteins (Gi). Several mechanisms of inhibition of adenylate cyclase by Gi have been reported: 1) the $\beta\gamma$-subunits of Gi interact with free $\alpha_1$-subunit to inactivate Gi, 2) $\alpha_2$-subunit competes with $\alpha_1$ on the adenylate catalyst, and 3) inhibition of calmodulin-stimulated adenylate cyclase by $\beta\gamma$-subunits. It is also reported that the Gi is linked with K$^+$ and Ca$^{2+}$ channels in cardiac and other cells. Thus, Gi stimulation may affect the cardiac function through the modification of intracellular Ca$^{2+}$ concentration. Although the precise subcellular mechanism was not clarified in this study, inhibition of adenylate cyclase may largely contribute to the negative inotropic response of adenosine, because negative inotropic actions were observed only when cAMP was increased above the basal level by administration of isoproterenol. In physiological conditions, adenosine production is increased during $\beta$-adrenoceptor stimulation but is quickly degraded. Dobson et al. showed that endogenous adenosine attenuates catecholamine-induced contractile response in normoxic hearts treated with adenosine deaminase. In the ischemic heart, adenosine release is sustained and may largely modify the contractile response to catecholamine. In this study, we demonstrated that endogenous adenosine released from the ischemic myocardium blunts the inotropic response to $\beta$-adrenergic stimulation and that inhibition of adenosine release by the $\alpha_1$-blocker prazosin can enhance $\beta$-adenoreceptor-mediated inotropic responses.

A question may be raised as to whether the augmented responsiveness to $\beta$-stimulation during the prazosin treatment is beneficial to the ischemic heart. Our study suggests that prazosin treatment may be deleterious because we observed that pH and oxygen content in the coronary venous blood during infusions of isoproterenol decreased in the prazosin-treated hearts. Indeed, ischemic myocardium seems to be forced to contract at the expense of further progression of myocardial anaerobic metabolism. Thus, endogenous adenosine can be cardioprotective in the ischemic heart by attenuating the contractile response to $\beta$-adrenergic stimulation. This is compatible with other lines of evidence that adenosine is beneficial to prevent ischemic and reperfusion injuries. Because stimulations of $A_1$-adenosine receptors as well as $A_2$-adenosine receptors attenuate myocardial stunning, the negative inotropic action of adenosine during reperfusion may be one of the mechanisms of its beneficial effect. It is also reported that attenuation of $\beta$-adrenoceptor-mediated inotropic action by adenosine contributes to limit the infarct size. This beneficial action of adenosine may be attributed to the preservation of the energy potentials.

Therefore, the negative inotropic actions of adenosine may be clinically important in the treatment of heart failure due to acute myocardial ischemia. We often use the combined therapy: catecholamine as an inotropic agent and $\alpha_1$-adrenoceptor antagonist as a vasodilator. When prazosin is administered together with the treatment with catecholamine, contractility in the ischemic region may be augmented more than expected. This augmentation of contractility, however, is not necessarily beneficial for the ischemic myocardium. The treatment with prazosin may enhance the myocardial anaerobic metabolism and worsen ischemia.

**Limitations of the Study**

There may be several limitations in this study. We used anesthetized animals in the present study. The effects of adenosine on $\beta$-adrenoceptor-mediated inotropic responses may be different from those in the
conscious condition, because the autonomic nervous tone is altered during anesthesia. Therefore, we should be careful to extend our results to conscious animals. Identical results were obtained in denervated hearts, however, and thus, the changes in autonomic nervous tone may not be critical in our experimental model. We should also consider the possibility that reflex autonomic nerve activation caused by a decrease of blood pressure during infusion of isoproterenol may modulate the net myocardial sympathetic input. The results in the denervated hearts could exclude this possibility, however, because we obtained identical results in denervated hearts in which sympathetic nerve activities were reduced.

The myocardial ischemic changes in our hypoperfused hearts (reduction of CBF to 60% of control level) may be much less severe than that of the widely used ischemic model (occlusion of left anterior descending or left circumflex coronary artery). Indeed, reduction of CBF to 60% of the control level induces relatively mild ischemia compared with total coronary occlusion. Hemodynamic and metabolic parameters were significantly altered, however, i.e., lactate production, a decrease of pH in the coronary venous blood, and depressed ventricular wall motion (Figures 1–3). In severe ischemia, the ischemic myocardium may not respond to β-adrenergic stimulation, and release of endogenous norepinephrine may occur; thus, the effect of adenosine on contractile response to catecholamine may be masked by the severe ischemic changes. Therefore, we applied a mild ischemic model in the present study, but we should be careful to extend our results to severely injured ischemic hearts.

In conclusion, this in vivo canine study demonstrated that endogenous adenosine from the hypoperfused myocardium blunts β-adrenoceptor–mediated effects. Although further studies are necessary to clarify the roles of endogenous adenosine released from ischemic myocardium and to elucidate the subcellular mechanism of adenosine, our results suggest that endogenous adenosine attenuates contractile response to β-adrenergic stimulation and acts on the ischemic myocardium as cardioprotection.

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