Beneficial Effects of $\alpha_2$-Adrenoceptor Activity on Ischemic Myocardium During Coronary Hypoperfusion in Dogs

Masafumi Kitakaze, Masatsugu Hori, Koichi Gotoh, Hiroshi Sato, Katsuomi Iwakura, Akira Kitabatake, Michitoshi Inoue, and Takenobu Kamada

We have previously reported that $\alpha_2$-adrenoceptor stimulation enhances adenosine-induced coronary vasodilation. In the present study, we tested the hypothesis that $\alpha_2$-adrenoceptor activity exerts beneficial effects on myocardial ischemia through augmentation of vasodilatory effects of released adenosine. In open-chest dogs, the left anterior descending coronary artery was perfused through an extracorporeal bypass tube from the carotid artery. Propranolol was infused into the bypass tube to exclude the metabolic effects of norepinephrine. When clonidine (0.24 $\mu$g/kg/min i.e.) was infused for 10 minutes after reduction of coronary blood flow by partial occlusion of the bypass tube, coronary blood flow was increased by 43% from 27±1 ml/100 g/min despite no changes in coronary perfusion pressure (38±5 mm Hg) and a slight decrease in adenosine release. Both fractional shortening and lactate extraction ratio of the perfused area were significantly improved (fractional shortening, 1.8±1.0 to 10.9±1.5%, p<0.001; lactate extraction ratio, -57.8±6.5 to -31.9±2.4%, p<0.005). Identical results were observed in the denervated hearts, indicating that the beneficial effect of clonidine is not attributed to the prevention of norepinephrine release from the sympathetic nerve terminals. The beneficial effects of clonidine were prevented by yohimbine, an $\alpha_2$-adrenoceptor blocking agent. An adenosine receptor antagonist, 8-phenyltheophylline, also prevented the beneficial effects of clonidine, indicating that these beneficial effects are mediated by effects of adenosine. Furthermore, the extent of augmentation of coronary flow in the ischemic heart was coincided with that of augmentation of exogenous adenosine-induced hyperemic flow (40%) by clonidine. Production of cyclic AMP in the coronary artery during myocardial ischemia was augmented by clonidine. In 12 other dogs, myocardial ischemia was produced by intracoronary embolization of microspheres (15 $\mu$m in diameter). Clonidine enhanced (39%) the hyperemic coronary flow and improved both fractional shortening and lactate extraction ratio. Thus, we conclude that $\alpha_2$-adrenoceptor stimulation can ameliorate myocardial ischemia mainly due to enhancement of vasodilatory effects of adenosine released from the ischemic myocardium. (Circulation Research 1989;65:1632–1645)
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heart. The latter observation seems corresponding to reports of Heusch and Deussen and Seitelberger et al. However, if the former mechanism (i.e., enhancement of adenosine-induced coronary vasodilation) overcomes its direct vasoconstriction, \( \alpha_2 \)-adrenoceptor stimulation can increase coronary blood flow during ischemia, because a massive amount of adenosine is released from ischemic myocardium.

Thus, in the present study, to test whether \( \alpha_2 \)-adrenoceptor stimulation has an ability to enhance endogenous adenosine-induced coronary vasodilation and ameliorate myocardial ischemia clonidine, an \( \alpha_2 \)-adrenoceptor agonist, was infused into the coronary artery during hypoperfusion and measured regional contractile and metabolic functions. Furthermore, to exclude the possibility that this beneficial effect of clonidine does not involve the effect of withdrawal of norepinephrine release from the presynaptic vesicles, we measured adenosine and norepinephrine concentrations in the arterial and coronary venous blood and performed identical experiments in the denervated hearts. Finally, to observe the subcellular mechanism of this phenomenon, we measured cyclic AMP of coronary smooth muscles with and without \( \alpha_2 \)-adrenoceptor stimulation during myocardial ischemia.

Materials and Methods

Instrumentation

Fifty-nine mongrel dogs weighing 15–20 kg were anesthetized with pentobarbital sodium (30 mg/kg i.v.). In 10 dogs, systemic chemical sympathectomy was performed by intravenous injection of 50 mg/kg 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) and phentolamine (1 mg/kg), and three fractional doses of 6-hydroxydopamine (10, 20, and 20 mg/kg) were administered over a period of 24 hours.

The trachea was intubated, and the animal was ventilated with room air mixed with oxygen. The chest was opened through the left fifth intercostal space, and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was cannulated and perfused with blood via the left carotid artery through an extracorporeal bypass tube. Coronary perfusion pressure (CPP) was monitored at the tip of the coronary arterial cannula, and coronary blood flow of the perfused area (CBF) was measured with an electromagnetic flow probe attached at the bypass tube. A small, short collecting tube (1 mm in diameter and 7 cm in length) was inserted into a small coronary vein near the center of the perfused area to sample coronary venous blood. The drained venous blood was collected in the reservoir placed at the level of the left atrium and was returned to the jugular vein. High fidelity left ventricular (LV) pressure was measured by a micromanometer (model P-7, Konigsberg, Pasadena, California) placed in the LV cavity through the apex. A pair of ultrasonic crystals were placed in the inner one third of the myocardium about 1 cm apart to measure myocardial segment length with an ultrasonic dimension gauge (Schuessler, 5 MHz). Heart rate averaged 129 beats/min in the intact heart and 96 beats/min in the denervated heart. Heart rate was not changed during each study.

Experimental Protocols

Protocol I: Effects of clonidine on myocardial ischemia produced by coronary hypoperfusion.

Forty-two dogs were used in this protocol. Propranolol was injected (0.3 mg/kg) and continuously infused (10 \( \mu \)g/kg/min) into the perfused area to inhibit metabolic effects of norepinephrine. We confirmed that this treatment of propranolol prevents the effects of isoproterenol (0.1 \( \mu \)g/kg i.c.) on the fractional shortening and coronary blood flow at least for 40 minutes. After hemodynamic stabilization, coronary arterial and venous blood were sampled for blood gas analysis and determination of lactate, adenosine, and norepinephrine concentrations. Hemodynamic functions (i.e., LV pressure [LVP], \( dP/dt \), and segment length of the perfused area) were measured. End-diastolic length (EDL) was determined at the R wave of the electrocardiogram, and end-systolic length (ESL) was determined at the minimal \( dP/dt \). Fractional shortening (FS) was calculated by \( \text{EDL} - \text{ESL} \)/EDL as an index of myocardial contractility of the perfused area. With an occluder attached at the extracorporeal bypass tube, CPP was reduced so that CBF decreased to one third of the control CBF. After a low CPP was determined, the occluder was adjusted exactly to keep CPP constant at the low level (n = 32). All hemodynamic parameters were measured 3, 5, 7, and 10 minutes after the onset of hypoperfusion, and both coronary arterial and venous blood for the metabolic parameters were sampled at 10 minutes. After these measurements, clonidine (0.24 \( \mu \)g/kg/min) was infused into LAD and all hemodynamic and metabolic parameters were measured again. The dose of clonidine was determined so that it maximally enhanced exogenous adenosine-induced coronary vasodilation but did not cause direct coronary vasoconstriction. Although clonidine is reported to be a selective \( \alpha_2 \)-adrenoceptor agonist, we tested that the effects of clonidine were blunted by an \( \alpha_2 \)-adrenoceptor antagonist. Instead of withdrawal of clonidine infusion, yohimbine (9 \( \mu \)g/kg/min) was additionally infused into LAD during coronary hypoperfusion (n = 5). Furthermore, to test that the effect of clonidine during coronary hypoperfusion is related to adenosine’s effect, clonidine was infused into LAD during hypoperfusion under intracoronary infl
sion of 8-phenyltheophylline (30 µg/kg/min i.c.), a potent surface membrane adenosine receptor antagonists (n=5). The treatment of 8-phenyltheophylline was initiated 10 minutes before the coronary hyperperfusion. In the preliminary study, this dose of 8-phenyltheophylline abolished the coronary vasodilatory effect of exogenous adenosine (5 µg/kg/min i.c.).

In five other denervated dogs, identical procedures and measurements of all variables were performed. We confirmed that the norepinephrine content in the myocardium in systemically denervated (n=5) and innervated (untreated) dogs (n=5) was 12±3 and 366±28 pg/mg (p<0.001), respectively. These 10 dogs were killed immediately after anesthesia, and the myocardial tissue was sampled from the LAD area for measurement of norepinephrine content without any intervention.

To examine the subcellular molecular mechanisms of enhancement of coronary vasodilation by α2-adrenoceptor stimulation, cyclic AMP in the LAD coronary artery was measured with (n=5) and without (n=5) clonidine treatment during hyperperfusion. Coronary hyperperfusion was continued for 20 minutes, and clonidine was infused 10 minutes after the onset of hyperperfusion. As a control, the content of cyclic AMP in the left circumflex (LCX) coronary artery was measured. In these 10 dogs, to exclude the effect of β-adrenoceptor activity on cyclic AMP production of both LAD and LCX coronary arteries, propranolol (0.2 mg/kg/min) was infused intravenously. We confirmed that this dose of propranolol blocked effects of isoproterenol (0.1 µg/kg/min).

Protocol II: Effects of α2-adrenoceptor activity on myocardial ischemia induced by coronary microembolization. If α2-adrenoceptor stimulation enhances adenosine-induced coronary vasodilation, myocardial ischemia due to microembolization can be improved by α2-adrenoceptor stimulation because coronary hyperemic flow induced by the embolization of microspheres is attributed to adenosine released from the ischemic foci. To test this idea, microspheres (diameter: 15±1 µm, 5.0±106/CBF (ml/min)); 3M, St. Paul, Minnesota) were repeatedly injected until CBF reduced less than 8 ml/min with (n=5) and without (n=7) intracoronary infusion of clonidine (0.24 µg/kg/min). When hemodynamic parameters were stabilized 5 minutes after embolization and measured, coronary arterial and venous blood was sampled.

Protocol III: Effects of α2-adrenoceptor activity on exogenous adenosine-induced coronary vasodilation. To examine the extent of the enhancement of adenosine-induced coronary vasodilatation by clonidine (0.24 µg/kg/min i.c.) in the normoxic condition, changes in CBF during infusion of adenosine (0.5, 1, and 2 µg/kg/min i.c.) were observed in five dogs with and without intracoronary infusion of clonidine before microsphere embolization.

Since the vasodilatory effect of adenosine is reported to be modified when myocardium is acidic,16 the potency of interaction between adenosine and α2-adrenoceptor activity may be changed. To mimic the condition of myocardial acidosis during ischemia, five dogs were ventilated with room air mixed with 5% CO₂-95% O₂. Five to 10 minutes after this ventilation, pH in arterial blood was reduced from 7.39±0.01 to 7.27±0.02 and reached a stable state, keeping O₂ in the arterial blood at a control level (105±5 mm Hg). Vasodilatory potency of three doses of adenosine were tested with and without clonidine under this acidic condition.

Chemical Analysis

Coronary arteriovenous blood oxygen difference (AVO₂D) was assessed by the difference between coronary arterial and venous oxygen contents. MVO₂ (ml/100 g/min) was calculated by CBF (ml/100 g/min)×AVO₂D(ml/dl). Lactate was assessed by the enzymatic assay, and lactate extraction ratio was obtained by coronary arteriovenous difference in lactate concentration multiplied by 100 and divided by arterial lactate concentration.

Adenosine measurement. The method of adenosine measurement has been reported previously.6,9,17 One milliliter of blood was drawn into a syringe containing 0.5 ml dipyridamole (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 the adenosine content were employed. Briefly, 100 µl dioxane containing succinic acid anhydride and triethyamine succinylated adenosine in the plasma (100 µl). After a 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 anti-adenosine 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 sampling procedure and degradation rate of adenosine were reported negligibly small.6,17

Norepinephrine measurement. The method of norepinephrine measurement has been described previously.19 Five milliliters of coronary arterial and venous blood taken into a tube containing EDTA was immediately placed in iced water and centrifugated for 20 minutes. The plasma was kept at -80°C. Within 2 weeks, plasma norepinephrine
was adsorbed on alumina and separated by high-performance liquid chromatography (pump, LC-3A; column, Zpax-SCX; Shimazu Seisakusho, Kyoto, Japan). Plasma norepinephrine was determined spectrofluorometrically by the trihydroxyindole method (Shimazu spectrofluorophotometer RF-500LCA). In this system, sensitivity of the assay is 10 pg/ml plasma and the intra-assay coefficient of variation is 6.8%.18

To determine norepinephrine concentration in myocardial tissue, myocardial tissue from the LAD 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 the EDTA (0.1 M), NaHSO3 (1 M), and HClO3 (0.05 M) solution. After centrifugation, the norepinephrine concentration in the supernatant was determined by the method described above.

Cyclic AMP measurement. The method of cyclic AMP measurements in tissues has been previously described.19–21 During hypoperfusion of the LAD coronary artery with and without treatment of clonidine, the small segments of LAD and LCX coronary arteries were removed and frozen with precooled stainless steel scissors and tongs in liquid nitrogen and immediately stored at −80°C in liquid nitrogen. After removal of the adventitial connective tissues in the coronary arteries (20–40 mg), the frozen tissue was powdered, homogenized at 4°C in 1 ml of ice-cold 6% trichloroacetic acid, and centrifuged at 2,500g for 20 minutes. The supernatant fluid was removed and extracted three times with 3 ml diethyl ether saturated with water and stored in the freezer (−80°C). The cyclic AMP concentration in the supernatant fluid was measured by the radioimmunoassay method19–21 within 7 days. Briefly, 100 µl dioxanetricyclamidine mixture containing succinic acid anhydride succinylated cyclic AMP in the supernatant (100 µl). After a 10-minute incubation, the reaction mixture was added to 800 µl of 0.3 M imidazole buffer (pH 6.5). One hundred microliters of succinyl cyclic AMP tyrosine methyl ester iodinated with 125I (15,000–20,000 counts/min in an amount less than 10−4 M) was added to the assay mixture containing 100 µl of the supernatant and 100 µl of diluted anti-sera in the presence of chloramine T2; the mixture was kept at 4°C for 24 hours. A cold solution of dextran-coated charcoal (500 µl) was added to the mixture in an ice-cold water bath. The charcoal was spun down, and 0.5 ml of the supernatant was counted for radioactivity in a gamma spectrometer.

Statistical Analysis

Statistical analysis was performed with paired (Figures 2–7 and 11, Tables 1–3) and unpaired t tests (Figures 8–10). Multiple analysis of variance was also used in Figures 9–11 to test the tendency of changes in hemodynamic and metabolic variables versus extent of embolization or dose of adenosine. All values were expressed as mean±SEM, and p<0.05 was considered significant.

Results

Effects of α2-Adrenoceptor Stimulation on Myocardial Ischemia

Intracoronary infusion of propranolol slightly but significantly decreased both coronary arteriovenous oxygen difference (A VO2D) and myocardial oxygen consumption (MVO2) (Table 1). However, intracoronary infusion of clonidine changed neither coronary hemodynamic nor other metabolic parameters (Figures 9–11).

Table 1. Coronary Hemodynamic and Metabolic Parameters Before and After β-Adrenoceptor Blockade in Intact (Innervated) Hearts

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Propranolol (baseline)</th>
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<tbody>
<tr>
<td>CPP (mm Hg)</td>
<td>110±5</td>
<td>107±5</td>
</tr>
<tr>
<td>CBF (ml/100</td>
<td>92±2</td>
<td>86±2</td>
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<tr>
<td>LER (%)</td>
<td>30.8±1.6</td>
<td>28.2±3.1</td>
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<td>AVO2D (ml/dl)</td>
<td>8.58±0.50</td>
<td>8.08±0.57*</td>
</tr>
<tr>
<td>MVO2 (ml/100</td>
<td>7.90±0.54</td>
<td>6.93±0.47*</td>
</tr>
<tr>
<td>g/min</td>
<td>2.0±0.4</td>
<td>1.9±0.5</td>
</tr>
<tr>
<td>AdR (nmol/100</td>
<td>26±3</td>
<td>23±3</td>
</tr>
<tr>
<td>g/min</td>
<td>386±63</td>
<td>376±52</td>
</tr>
<tr>
<td>FS (%)</td>
<td>499±57</td>
<td>397±64</td>
</tr>
<tr>
<td>NE(A) (pg/ml)</td>
<td>2.0±0.4</td>
<td>1.9±0.5</td>
</tr>
<tr>
<td>NE(V) (pg/ml)</td>
<td>10±2</td>
<td>6.93±0.47*</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=7). CPP, coronary perfusion pressure; CBF, coronary blood flow; LER, lactate extraction ratio; AVO2D, coronary arteriovenous oxygen difference; MVO2, myocardial oxygen consumption; AdR, adenosine release; FS, fractional shortening; NE(A) and NE(V), norepinephrine concentrations in coronary arterial and venous blood, respectively.

*p<0.05 vs. the untreated control condition.
FIGURE 1. Representative records of coronary perfusion pressure (CPP), left ventricular pressure (LVP), first derivatives of LVP (dP/dt), coronary blood flow of perfused area (CBF), and segment length (SL) before and during coronary hypoperfusion. The heart was pretreated with propranolol. After initiation of infusion of clonidine, CBF gradually increased from 12 to 17 ml/min and fractional shortening was recovered. Withdrawal of clonidine infusion returned both CBF and fractional shortening to the preclonidine levels. Reduced CPP and systemic hemodynamic parameters were stable throughout this protocol.

increased, indicating that myocardial anaerobic metabolism was improved by clonidine infusion, and thus MVO$_2$ increased ($p<0.001$). Myocardial ischemia produced a significantly ($p<0.01$) greater AVO$_2$D; however, clonidine did not change AVO$_2$D significantly (ischemia before clonidine, 10.9±0.9 ml/dl; ischemia with clonidine, 10.2±0.6 ml/dl; ischemia during withdrawal of clonidine, 10.5±0.8 ml/dl). Coronary venous pH at the baseline condition was 7.39±0.01 and decreased to 7.25±0.01 during coronary hypoperfusion. However, clonidine infusion significantly ($p<0.05$) improved the pH in coronary venous blood (7.32±0.02). The pH of coronary arterial blood did not change throughout this study (7.40±0.01). Intracoronary infusion of clonidine decreased adenosine release and did not change norepinephrine concentrations in coronary arterial and venous blood. Figure 4 shows that these beneficial effects of clonidine are antagonized by intracoronary infusion of yohimbine (9 µg/kg/min). Intracoronary infusion of yohimbine decreased the increased coronary blood flow, fractional shortening, and lactate extraction ratio although intracoronary infusion of clonidine was continued. Thus, the beneficial effect of clonidine during ischemia is not due to its nonspecific effect but attributed to the specific $\alpha_2$-adrenoceptor stimulation. Figure 5 shows that these beneficial effects of clonidine are related to coronary vasodilatory effect of adenosine. The intracoronary infusion of 8-phenyltheophylline did not change coronary hemodynamic and metabolic parameters (Table 2). After reduction of coronary perfusion pressure to decrease coronary blood flow to one third, fractional shortening, myocardial oxygen consumption, and lactate extraction ratio were significantly ($p<0.001$) reduced. Ten minutes after initiation of coronary hypoperfusion, clonidine was initiated. However, coronary blood flow did not increase, and improvement neither in fractional shortening, myocardial oxygen consumption nor lactate extraction ratio was observed. Although adenosine release was enhanced during ischemia compared with the condition of ischemia without 8-phenyltheophylline (Figure 3C), amounts of adenosine release before and after clonidine infusion were not changed (Figure 5F). These results indicate that the beneficial effect of clonidine is related to the coronary vasodilatory action of adenosine.

$\alpha_2$-Adrenoceptor stimulation may decrease norepinephrine release from the presynaptic vesicles and adversely attenuate sympathetic vasoconstriction. We tested this possibility by performing a comparable protocol in the denervated hearts (Table 3). In the denervated hearts (Figure 6), intracoronary infusion of clonidine increased CBF by 40±4% and recovered fractional shortening despite no changes in reduced CPP. Furthermore, clonidine infusion improved lactate metabolism and increased MVO$_2$ (Figure 7). Clonidine infusion in the denervated hearts decreased adenosine release from the ischemic myocardium and did not alter norepinephrine concentrations in coronary arterial and venous blood. These results indicate that clonidine increases reduced CBF during hypoperfusion and improves myocardial ischemia in the denervated dogs. Thus, withdrawal of sympathetic activities by activation
FIGURE 2. Changes in coronary perfusion pressure (panel A), coronary blood flow (panel B), and fractional shortening (panel C) during intracoronary infusion and withdrawal of clonidine infusion in coronary hypoperfusion. Three to 5 minutes after clonidine infusion, coronary blood flow was gradually increased (panel B) and fractional shortening recovered (panel C), indicating that intracoronary infusion of clonidine improves mechanical myocardial function during ischemia. During this protocol, reduced coronary perfusion pressure was kept constant (panel A).

FIGURE 3. Changes in myocardial oxygen consumption (MVO₂, panel A), lactate extraction ratio (panel B), adenosine release (panel C), and norepinephrine concentrations in the coronary arterial and venous blood (panel D) before and after intracoronary infusions of clonidine (0.24 μg/kg/min), and after withdrawal of clonidine during myocardial ischemia. Intracoronary infusions of clonidine significantly improved lactate extraction ratio (panel B), indicating that clonidine infusion improves metabolic function of ischemic myocardium. With this improvement of metabolic function, myocardial oxygen consumption was also increased (panel A).
FIGURE 4. Effects of intracoronary infusion of yohimbine (9 μg/kg/min) on coronary blood flow (panel A), fractional shortening (panel B), and lactate extraction ratio (panel C) during coronary hypoperfusion with intracoronary infusion of clonidine. Clonidine increased coronary blood flow by 32%. Yohimbine infusion significantly (p<0.001) decreased improved coronary blood flow, fractional shortening, and lactate extraction ratio to the pre-clonidine level, although clonidine infusion was continued. Coronary perfusion pressure was kept constant during this protocol.

FIGURE 5. Changes in coronary perfusion pressure (panel A), coronary blood flow (panel B), fractional shortening (panel C), myocardial oxygen consumption (panel D), lactate extraction ratio (panel E), and adenosine release (panel F) by intracoronary infusion of clonidine during coronary hypoperfusion. Adenosine receptors were antagonized by intracoronary infusion of 8-phenyltheophylline.
Figure 6. Changes in coronary perfusion pressure (panel A), coronary blood flow (panel B), and fractional shortening (panel C) during intracoronary infusion and withdrawal of clonidine infusion in coronary hypoperfusion in the denervated hearts. Three to 5 minutes after clonidine infusion, coronary blood flow gradually increased (panel B) and associated with this, fractional shortening increased (panel C). The extents of increases in coronary blood flow and fractional shortening are comparable with those in the innervated hearts, indicating that withdrawal of sympathetic activity by presynaptic α₁-adrenoceptor activation is not involved in the beneficial effect of clonidine in ischemic myocardium. During this protocol, reduced coronary perfusion pressure was kept constant (panel A).

Figure 7. Changes in myocardial oxygen consumption (panel A), lactate extraction ratio (panel B), adenosine release (panel C), and norepinephrine concentrations in the coronary arterial and venous blood (panel D), before and after intracoronary infusion of clonidine (0.24 μg/kg/min), and after withdrawal of clonidine during myocardial ischemia. The hearts were chemically denervated. In the denervated dogs, systemic norepinephrine concentration was higher (panel D) than that in the innervated myocardium (Figure 3D). This may be because of compensatory mechanisms of depletion of norepinephrine in the heart. Intracoronary infusion of clonidine significantly improved lactate extraction ratio (panel B), indicating that clonidine infusion improves metabolic function of ischemic myocardium. This improvement is related with neither the withdrawal of presynaptic sympathetic activity nor the increase in adenosine release from the ischemic myocardium (panel C). According to this improvement of metabolic function, myocardial oxygen consumption (MVO₂) was also increased (panel A).
of presynaptic $\alpha_2$-adrenoceptors is not involved in the mechanism of this beneficial effect of clonidine infusion.

Figure 8 represents the subcellular molecular basis of the interaction between $\alpha_2$-adrenoceptor stimulation and adenosine. Without treatment of clonidine, myocardial ischemia (coronary perfusion pressure, 94±6 to 32±5 mm Hg; coronary blood flow, 82±3 to 27±2 ml/100 g/min) increased cyclic AMP content of the coronary artery to 290±40 pmol/g ($p<0.001$). However, treatment of clonidine during myocardial ischemia further increased ($p<0.05$) cyclic AMP content of the involved coronary artery to 480±70 pmol/g.

Effects of $\alpha_2$-Adrenoceptor Stimulation on Microsphere-Induced Myocardial Ischemia

The doses of microspheres at the maximal embolization with and without clonidine infusion were 5.2±0.2×10$^5$ and 5.4±0.3×10$^5$/g myocardium, respectively (NS). Figure 9 demonstrates the changes in CBF and adenosine release during repetitive embolizations of microspheres. Intracoronary infusion of clonidine significantly ($p<0.05$) enhanced the peak hyperemic CBF by 39% despite no significant changes in adenosine release. Although coronary microembolization produces almost linear decreases in fractional shortening and lactate extraction ratio (Figure 10), these two parameters with clonidine were significantly ($p<0.05$) improved compared with those without clonidine, indicating that clonidine improves the microsphere embolization-induced myocardial ischemia due to the enhancement of adenosine's vasodilatory effect by clonidine.

Effects of $\alpha_2$-Adrenoceptor Stimulation on Exogenous Adenosine-Induced Coronary Vasodilation

Figure 11 shows the responses of CBF to exogenous adenosine with and without clonidine under the condition that pH in the coronary venous blood is 7.38±0.02. Adenosine-induced coronary vasodilation (1 $\mu$g/kg/min i.e.) was enhanced by 28±5% with infusion of clonidine (panel A). Figure 11B shows the responses of CBF to adenosine with and without clonidine under the condition that pH in the coronary venous blood is 7.27±0.03. Treatment of clonidine (1 $\mu$g/kg/min i.e.) enhanced adenosine-induced coronary vasodilation by 40±3%.

Discussion

Although there is consensus that potent $\alpha_2$-adrenoceptor stimulation causes coronary vasoconstriction, we have previously reported that adenosine-induced coronary vasodilation is enhanced by moderate $\alpha_2$-adrenoceptor stimulation. The present study provides evidence that $\alpha_2$-adrenoceptor stimulation can increase coronary blood flow of ischemic myocardium and improve both mechanical and metabolic functions. This increased flow is nutritional to ischemic myocardium.

We showed in the present study that $\alpha_2$-adrenoceptor stimulation by clonidine (0.24 $\mu$g/kg/min i.e.) increases coronary blood flow in the ischemic heart by 32–43% (Figures 2B, 4A, 6B, and 9A). Our previous studies showed that moderate $\alpha_2$-adrenoceptor stimulation enhances exogenous adenosine-induced vasodilation, and the present study indicated that acidic condition enhances the sensitivity of $\alpha_2$-adrenoceptor stimulation on aden-

### Table 2. Coronary Hemodynamic and Metabolic Parameters Before and After Treatments With Propranolol and 8-Phenyltheophylline

<table>
<thead>
<tr>
<th></th>
<th>CPP (mm Hg)</th>
<th>CBF (ml/100 g/min)</th>
<th>LER (%)</th>
<th>AVO$_2$D (ml/dl)</th>
<th>MVO$_2$ (ml/100 g/min)</th>
<th>AdR (nmol/100 g/min)</th>
<th>FS (%)</th>
<th>FS (%)</th>
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<tbody>
<tr>
<td>Untreated</td>
<td>105±7</td>
<td>93±3</td>
<td>28.9±2.5</td>
<td>8.69±0.41</td>
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<td>89±5</td>
<td>30.2±3.3</td>
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<td>7.01±0.55</td>
<td>1.1±0.6*</td>
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<td>8-Phenyltheophylline</td>
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<td>89±5</td>
<td>23.4±4.4</td>
<td>7.93±0.44</td>
<td>7.07±0.71</td>
<td>2.6±0.8</td>
<td>20±1</td>
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Values are mean±SEM (n=5). CPP, coronary perfusion pressure; CBF, coronary blood flow; LER, lactate extraction ratio; AVO$_2$D, coronary arteriovenous oxygen difference; MVO$_2$, myocardial oxygen consumption; AdR, adenosine release; FS, fractional shortening.

*p<0.05 vs. the untreated control condition.

### Table 3. Coronary Hemodynamic and Metabolic Parameters Before and After $\beta$-Adrenoceptor Blockade in Denervated Hearts

<table>
<thead>
<tr>
<th></th>
<th>CPP (mm Hg)</th>
<th>CBF (ml/100 g/min)</th>
<th>LER (%)</th>
<th>AVO$_2$D (ml/dl)</th>
<th>MVO$_2$ (ml/100 g/min)</th>
<th>AdR (nmol/100 g/min)</th>
<th>FS (%)</th>
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<td>Propranolol (baseline)</td>
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<td>22.8±1.6</td>
<td>8.10±1.12</td>
<td>5.97±0.74</td>
<td>1.8±0.7</td>
<td>29±3</td>
<td>500±48</td>
<td>488±56</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=5). CPP, coronary perfusion pressure; CBF, coronary blood flow; LER, lactate extraction ratio; AVO$_2$D, coronary arteriovenous oxygen difference; MVO$_2$, myocardial oxygen consumption; AdR, adenosine release; FS, fractional shortening; NE(A) and NE(V), norepinephrine concentrations in coronary arterial and venous blood, respectively.

*p<0.05 vs. the untreated control condition.
osine's effect by 40% (Figure 11B). This value corresponds well to the increment of coronary blood flow by α2-adrenoceptor stimulation in the ischemic myocardi um. Furthermore, a potent antagonist of adenosine receptors, 8-phenyltheophylline, completely abolished the effects of α2-adrenoceptor stimulation on coronary blood flow, indicating that improvement of myocardial ischemia with increased coronary blood flow is due to enhancement of vasodilatory effects of adenosine released from the ischemic myocardium by α2-adrenoceptor stimulation.

Nevertheless, we should consider other possibilities. α2-Adrenoceptor stimulation may increase adenosine release from the ischemic myocardium and increase coronary blood flow. However, this is not the case because the present study showed no increases in adenosine release (Figures 3C, 7C, and 9B), and our previous study showed that adenosine release is not attributed to the α2-adrenoceptor activity. α2-Adrenoceptor activation is reported to increase in the endothelium-derived relaxing factor and may cause coronary vasodilation. However, intracoronary infusion of clonidine in the normoxic condition did not change baseline CBF (Figure 9A), indicating that this dose of clonidine does not facilitate release of endothelium-derived relaxing factor. In the skeletal muscle, α2-adrenoceptor stimulation produces vasodilation resulting from histamine release. Histamine is reported to increase coronary blood flow through its inotropic and chronotropic effects. However, this mechanism is not likely because 1) intracoronary infusion of clonidine changed neither MVO2 nor baseline CBF and 2) positive inotropic effect of histamine may result in improvement of contractile function but may not improve metabolic function during myocardial ischemia (Figures 3B and 7B). In addition to this, several lines of evidence suggest that α2-adrenoceptor activity itself has a positive inotropic effect, which may enhance the contractility of ischemic region and increase coronary blood flow. However, this mechanism is unlikely because increased contractility neither improves the metabolic function (lactate extraction ratio and pH in coronary venous blood) nor decreases adenosine release. α2-Adrenoceptor stimulation can cause withdrawal of tonic sympathetic activity and exert coronary vasodilation, since activation of presynaptic α2-adrenoceptors may decrease the release of norepinephrine from the presynaptic vesicles. However, in our experiment there is no evidence that clonidine during coronary hypoperfusion decreases norepinephrine concentration in the coronary venous blood (see Figures 3D and 7D), and furthermore, clonidine increases coronary blood flow even in the denervated hearts (Figure 6B). There is also the possibility that intracoronary infusion of clonidine opens functional collateral vessels to the ischemic area, which may reduce the severity of ischemia. However, there is evidence that collateral flow is not regulated by α-adrenoceptor activities. Nathan and Feigl reported that α2-adrenoceptor stimulation during coronary hypoperfusion favorably affects intramyocardial flow distribution by causing subepicardial vasoconstriction. During hypoperfusion, a decrease in subendocardial flow is more prominent than in subepicardial flow, although oxygen demand in the subendocardial region is higher than in the subepicardial region. If subepicardial flow dominantly bypasses the subendocardial region, subendocardial ischemia may be improved. Since coronary resistance vessels in the nonischemic condition are predominantly regulated by α2-adrenoceptor activity, this mechanism may be possible. However, Buffetton and Feigl showed that transmural inhomogeneous flow distribution mediated by α-adrenoceptor coronary vasoconstriction is evident under moderate coronary hypoperfusion (coronary perfusion pressure ≥ 70 mm Hg). This beneficial flow distribution is diminished by the potent metabolic vasodilatory effect when the heart is progressively underperfused (coronary perfusion pressure, 38 mm Hg; lactate extraction ratio, −49%). In our experiment, because coronary perfusion pressure during hypoperfusion was 38±5 mm Hg (Figure 2A), the beneficial effect observed by Nathan and Feigl is not related to our present observation.

Heusch and Deussen demonstrated that postsynaptic α2-adrenoceptor stimulation mediates coronary vasoconstriction produced by sympathetic nerve stimulation during critical reduction of coronary perfusion pressure and concluded that α2-adrenoceptor stimulation is deleterious in myocardial ischemia. Seitelberger et al also demonstrated that α2-adrenoceptor blockade attenuates the severity of exercise-induced myocardial ischemia.
FIGURE 9. Serial changes in coronary blood flow (panel A) and adenosine release (panel B) under repetitive embolization of microspheres into coronary artery with and without intracoronary infusion of clonidine (0.24 \( \mu \)g/kg/min). Extent of embolization indicates cumulative amount of injected microspheres normalized by amount of maximal embolization. The doses of microspheres at maximal embolization with and without clonidine infusion were 5.2±0.2x10\(^6\) and 5.4±0.3x10\(^6\)g myocardium, respectively (NS). \( \alpha_2 \)-Adrenoceptor stimulation with clonidine further enhanced microembolization-induced coronary hyperemia (panel A) despite no significant changes in adenosine release from ischemic foci (panel B).

FIGURE 10. Serial changes in fractional shortening (panel A) and lactate extraction ratio (panel B) under repetitive embolization of microspheres into coronary artery with and without intracoronary infusion of clonidine. Without clonidine, fractional shortening and lactate extraction ratio were significantly (p<0.05) decreased compared with those of the clonidine-treated group, indicating that intracoronary infusion of clonidine significantly improves mechanical and metabolic functions during microsphere-induced ischemia.
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A normal condition

B acidotic condition

FIGURE 11. Effects of intracoronary infusion of clonidine on adenosine-induced coronary vasodilation under arterial blood pH of 7.39±0.01 (panel A) and 7.27±0.01 (panel B). The vasodilatory effect of adenosine is augmented (p<0.01) by clonidine infusion by 28±5% at 1 µg/kg/min of adenosine infusion (panel A); however, in the acidotic condition (panel B), intracoronary infusion of clonidine further enhanced (p<0.05) adenosine-induced coronary vasodilation (40±3%).

two reports seem contradictory to ours. Both exercise and sympathetic nerve stimulation elevate the systemic norepinephrine level to more than 1,000 pg/ml,18,35-37 which is two to three times higher concentrations than that of our results (Figure 3D).

If α₂-adrenoceptor stimulation is potent, direct coronary vasoconstriction is exerted and may overcome the beneficial effect of α₂-adrenoceptor stimulation on adenosine-induced coronary vasodilation. Our previous studies10,12 showed that intracoronary infusion of norepinephrine of 0.24 µg/kg/min enhances adenosine-induced maximal coronary vasodilation under α₁- and β-adrenoceptor blockades; however, 0.60 µg/kg/min of norepinephrine adversely decreases maximal coronary blood flow, indicating that two to three times more potent stimulation of α₂-adrenoceptor activity can reverse its effect on coronary blood flow. Thus α₂-adrenoceptor stimulation increases coronary blood flow and improves myocardial ischemia under low norepinephrine levels in systemic arterial blood, but further activation of α₂-adrenoceptors under high concentration of norepinephrine may decrease coronary blood flow and produce severe ischemia during coronary hypoperfusion. It is noteworthy that α₂-adrenoceptor stimulation by norepinephrine plays a protective role in ischemic myocardium, because several lines of evidence suggest only the deleterious effects of catecholamine,4,7 and the physiological role of α-adrenoceptor activity during ischemia has not been clarified.

Activations of the adenosine receptor (A₂) and β-adrenoceptor in coronary arteries increased cyclic AMP through the activation of adenylyl cyclase activity. However, interference of β-adrenoceptor stimulation during ischemia was minimal because the experiments were done under propranolol infusions, and thus the increase in cyclic AMP level by ischemia was mainly attributed to the stimulation of adenosine receptor in the coronary arteries. Our data (Figure 8) also showed that myocardial ischemia increases cyclic AMP content of the coronary artery. α₂-Adrenoceptor stimulation during myocardial ischemia enhanced this production of cyclic AMP in the coronary artery; however, stimulation of α₂-adrenoceptors alone is not responsible for increases in intracellular cyclic AMP concentrations.31 This result indicates that our observation (i.e., the interaction between α₂-adrenoceptors and adenosine receptors) is tightly coupled to the changes in cyclic AMP levels in the involved coronary arteries. Indeed, several investigators reported that an increase in cyclic AMP level by stimulation of adenosine receptors is amplified by the α₂-adrenoceptor stimulation in the cerebellum38 and islet cells.39

Although we did not elucidate the regulatory mechanisms of amplification of cyclic AMP level by α₂-adrenoceptor stimulation, we provided the novel
evidence that $\alpha_2$-adrenoceptor stimulation can be beneficial in acute myocardial ischemia through the enhancements of adenosine-induced cyclic AMP production and coronary vasodilation.

Acknowledgments

The authors gratefully acknowledge the helpful discussion and generous assistance of Drs. T. Matsumaya, H. Ozaki, and K. Iwai in the catecholamine measurements.

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**KEY WORDS** • alpha-adrenoceptor activity • clonidine • myocardial ischemia • adenosine • catecholamine
Beneficial effects of alpha 2-adrenoceptor activity on ischemic myocardium during coronary hypoperfusion in dogs.

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Circ Res. 1989;65:1632-1645
doi: 10.1161/01.RES.65.6.1632

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