Beneficial Effects of $\alpha_1$-Adrenoceptor Activity on Myocardial Stunning in Dogs

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

This study was undertaken to elucidate whether $\alpha_1$-adrenoceptor activity is beneficial to contractile dysfunction during reperfusion after a brief period of ischemia (stunned myocardium) in 54 open-chest dogs. Contractile dysfunction assessed by fractional shortening (FS) was observed 3 hours after the onset of reperfusion following 15 minutes of complete occlusion of the left anterior descending coronary artery. Pretreatment with prazosin (4 $\mu$g/kg/min i.c.) further deteriorated contractile dysfunction compared with the untreated condition (12.7±0.6% versus 6.9±0.4% with prazosin treatment, p<0.001). Conversely, $\alpha_1$-adrenoceptor agonists, methoxamine (1.0 $\mu$g/kg/min i.c.) and norepinephrine (0.24 $\mu$g/kg/min i.c.) with rauwolscine and propranolol, significantly attenuated contractile dysfunction (FS in the methoxamine-treated group, 17.3±0.3%, p<0.001 versus the untreated group; FS in the norepinephrine-treated group, 18.0±0.9%, p<0.05 versus 13.6±1.1% in the propranolol group). Both adenosine release and hyperemic coronary flow response during the early reperfusion period were significantly attenuated in the prazosin-treated group, and both were enhanced in the $\alpha_1$-adrenoceptor stimulation groups. These results suggest that beneficial effects of $\alpha_1$-adrenoceptor activity may be due to the enhanced release of adenosine. To test the cause–effect relation between the extent of adenosine release and contractile dysfunction during reperfusion, 8-phenyltheophylline was infused to block adenosine receptors in the methoxamine-treated group. The treatment with 8-phenyltheophylline completely abolished (FS, 7.4±0.3%) the beneficial effect of the enhanced adenosine release by $\alpha_1$-adrenoceptor stimulation. Furthermore, in the prazosin-treated group, adenosine (9 $\mu$g/kg/min) was additionally infused into the left anterior descending coronary artery 5 minutes before and 2 hours after the onset of reperfusion. Both hyperemic coronary flow and contractile dysfunction (FS, 17.3±0.3%) recovered to the levels of the $\alpha_1$-adrenoceptor stimulation groups. However, treatment with papaverine could not prevent deleterious effects of prazosin despite the fact that comparable hyperemic flow was obtained. Instead, lactate production up to 10 minutes after the onset of reperfusion was significantly larger (p<0.01) despite augmented contractile function in the prazosin-treated and the 8-phenyltheophylline with methoxamine–treated groups compared with the untreated group. The electron microscopic examination revealed no irreversible myocardial injury with and without pharmacological interventions. Thus, we conclude that $\alpha_1$-adrenoceptor activity can reduce the magnitude of myocardial stunning and that its cellular mechanism is due to enhanced adenosine release by $\alpha_1$-adrenoceptor activity. The metabolic effects of adenosine, in part, may mediate protective mechanisms for myocardial stunning rather than the coronary hyperemic flow response. (Circulation Research 1991;68:1322–1339)

Even if ischemic heart muscle is reperfused before irreversible injury can occur, contractile function remains impaired for a long period, a phenomenon known as myocardial stunning.1,2 Several lines of evidence suggest that myocardial ischemia facilitates release of catecholamines,3–5 raising the possibility that this release of catecholamines may modulate the severity of myocardial ischemia and reperfusion injury.6–8 To inves-

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tigate this possibility, it is helpful to break down adrenergic stimulation into its individual components. α1-Adrenergic stimulation is particularly worthy of examination, given the multiplicity of its effects. α1-Adrenoceptor stimulation causes coronary vasoconstriction,10-14 and worsens myocardial ischemia.15,16; indeed, it has been reported that prazosin improves the metabolism of hypoxic hearts by improving coronary perfusion.17 Furthermore, α1-adrenoceptor activity has direct effects on myocardium.18-22 Sharma et al23 reported that α1-adrenoceptor stimulation promotes the accumulation of calcium in reperfused myocardium. Because calcium overload during ischemia and reperfusion23-27 figures prominently in the pathogenesis of myocardial stunning,27-29 α1-adrenoceptor activity during ischemia and reperfusion might be expected to augment the magnitude of myocardial stunning. On the other hand, we have previously reported that α1-adrenergic activity regulates the release of adenosine from ischemic myocardium.30 Adenosine is reported to prevent reperfusion injury.31,32 Prolonged hyperemic flow during reperfusion by released adenosine underlies the prompt recovery of the coronary circulation33-35 and may reduce the magnitude of myocardial stunning insofar as microcirculatory disturbances contribute to this process.36 Adenosine is also reported to depress the positive inotropy by β-adrenoceptor stimulation and calcium influx, and this may exert myocardial metabolic effects and prevent calcium overload during reperfusion.37,38 Furthermore, adenosine attenuates activities of leukocytes during ischemia and reperfusion39,40 and thus may attenuate the magnitude of myocardial stunning.41-43 If these processes occur during ischemia and reperfusion periods, α1-adrenoceptor activity may be beneficial for myocardial stunning.44 However, no clear consensus has been reported whether α1-adrenoceptor stimulation is beneficial for reperfusion injury: α1-adrenoceptor stimulation has a potency to worsen the ischemia via coronary vasoconstriction10-17 and increases in myocardial contractility19-22 as well as to improve ischemia via enhanced release of adenosine.30

To determine whether α1-adrenoceptor activity augments or reduces the magnitude of myocardial stunning, in the present study we reperfused a region of the heart for 3 hours after 15 minutes of ischemia with and without either prazosin or methoxamine treatment. We measured adenosine release from the reperfused myocardium as well as the regional ATP content, contractile function, and lactate metabolism. Furthermore, to determine the cause–effect relation between adenosine release and contractile dysfunction, we tested whether 8-phenyltheophylline completely abolishes the beneficial effects of α1-adrenoceptor stimulation and whether exogenous adenosine reverses the contractile dysfunction under the treatment with prazosin to the level of α1-adrenoceptor stimulation.

Materials and Methods

Instrumentation

Fifty-four mongrel dogs weighing 15–20 kg were anesthetized with pentobarbital sodium (30 mg/kg i.v.). Additional doses of pentobarbital sodium (0.5–1.5 mg/kg i.v.) were administered as necessary. 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 was monitored at the tip of the coronary arterial cannula, and coronary blood flow (CBF) of the perfused area was measured with an electromagnetic flow probe attached to the bypass tube. A 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 pressure was measured by a micromanometer (Königsberg P-7, Pasadena, Calif.) placed in the left ventricular 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 (5 MHz, Schuessler, Cardiff by the Sea, Calif.). Heart rate averaged 138±2 beats/min at control conditions, 140±3 beats/min at 15 minutes of ischemia, 139±2 beats/min at 10 minutes of reperfusion, and 138±3 beats/min at 3 hours of reperfusion. There were no significant changes in heart rate during each study.

Experimental Protocols

Protocol 1: Effects of α1-adrenoceptor activities on myocardial stunning. Thirty-nine dogs were used in this protocol. After hemodynamic stabilization, coronary arterial and venous blood were sampled for blood gas analysis and determination of lactate, adenosine, and norepinephrine concentrations. Hemodynamic functions, that is, left ventricular pressure, 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.45 Fractional shortening (FS) was calculated by (EDL−ESL)/EDL as an index of myocardial contractility of the perfused area. In six dogs (the prazosin-treated group), prazosin (4.0 μg/kg/min) was continuously infused into the LAD 5 minutes before coronary occlusion, and hemodynamic parameters were measured. After this procedure, the bypass tube was occluded for 15 minutes and the same hemodynamic parameters were observed 1, 3, 5, 7, 10, and 15 minutes during myocardial ischemia. During the ischemia, prazosin infusion was discontinued because we completely stopped the flow to the LAD from the perfusion line. After an abrupt release of the bypass tube, intracoronary infusion of prazosin was initiated again, and hemodynamic parameters were measured 15, 30, 45, and 60 seconds, and 3, 5,
10, 30, 60, 90, 120, 150, and 180 minutes after the onset of reperfusion. Coronary arterial and venous blood were sampled 30 and 60 seconds and 3, 5, 10, 30, 60, 120, and 180 minutes after ischemia. Three hours after reperfusion, the myocardium perfused by LAD and the left circumflex (LCX) coronary arteries were quickly sampled into liquid nitrogen to measure myocardial tissue ATP content as described below. Myocardium in each area was also sampled for light and electron microscopic examination. In seven dogs, the same procedures were done in the untreated condition (the untreated group).

To elucidate the effect of α₁-adrenoceptor stimulation on myocardial stunning, methoxamine (1.0 μg/kg/min i.c.) was administered in six dogs (the methoxamine-treated group). In five other dogs, norepinephrine (0.24 μg/kg/min) was infused into the LAD after pretreatment with rauwolscine (6.0 μg/kg i.c.) and propranolol (the norepinephrine-treated group). Because α₁-adrenoceptors are reported to be divided into two subtypes and norepinephrine is reported to stimulate both subtypes of α₁-adrenoceptors,40 we examined the effects of norepinephrine on myocardial stunning. Propranolol was injected (0.30 mg/kg) and continuously infused (10 μg/kg/min) into the perfused area to inhibit metabolic effects of norepinephrine except when CBF was interrupted. In the preliminary study, intracoronary infusion of isoproterenol (0.10 μg/kg/min) did not increase FS, CBF, and myocardial oxygen consumption (MV̇ O₂) under these doses of β-adrenoceptor antagonists and this dose of norepinephrine did not cause direct vasoconstriction by α₁-adrenoceptor stimulation of the coronary arteries.40 Ten minutes after the initiation of norepinephrine and rauwolscine infusion under the treatment with propranolol, coronary perfusion to the LAD was interrupted for 15 minutes, and then the LAD was reperfused for 3 hours. During the experiments, the same measurements of hemodynamic and metabolic parameters were performed. Myocardial tissue was sampled for measurements of tissue ATP content and microscopic examinations. As a control, the same procedures of ischemia and reperfusion were done only under β-adrenoceptor blockade by propranolol (n=5, the propranolol group).

These doses of prazosin, norepinephrine, and methoxamine were chosen as the maximal doses that do not alter coronary hemodynamics in the control nonischemic conditions. To test the more potent effects of prazosin and methoxamine treatments on myocardial stunning, either 12 μg/kg/min prazosin (the higher dose of the prazosin-treated group, n=5) or 10 μg/kg/min methoxamine (the higher dose of the methoxamine-treated group) were administered before and after 15 minutes of ischemia.

In protocol 1, all pharmacological interventions, that is, infusions of prazosin, methoxamine, propranolol, rauwolscine, and norepinephrine, were discontinued after 2 hours of reperfusion to obtain the steady state without any pharmacological interventions.

Protocol 2: Roles of released adenosine by α₁-adrenoceptor activity in myocardial stunning. Treatment with prazosin is known to attenuate adenosine release from ischemic myocardium.30 If the amount of released adenosine is the primary determinant of the extent of myocardial stunning in the α₁-adrenoceptor–stimulated or –blocked conditions, exogenous adenosine in the prazosin-treated group augments the magnitude of the stunning to the level of the methoxamine-treated group, and treatment with 8-phenyltheophylline in the methoxamine-treated group increases the magnitude of the stunning to the level of the prazosin-treated group. These pieces of evidence are necessary to prove the cause–effect relation between adenosine release mediated by α₁-adrenoceptor activity and the extent of contractile dysfunction during reperfusion.

To test these ideas, exogenous adenosine (9.0 μg/kg/min i.c., n=5) was infused in the prazosin-treated (4.0 μg/kg/min i.c.) group, and 8-phenyltheophylline (30 μg/kg/min i.c., n=5) was infused in the methoxamine-treated (1.0 μg/kg/min i.c.) group. Five minutes after the initiation of infusion of each drug, hemodynamic and metabolic parameters were observed during a 15-minute LAD occlusion and 3-hour reperfusion. The pharmacological interventions were discontinued 2 hours after the onset of reperfusion. During each run, the same measurements of hemodynamic and metabolic parameters were made as in protocol 1.

To test the possibility that hyperemic flow induced by exogenous adenosine during reperfusion augments the magnitude of the stunning, we infused papaverine (90 μg/kg/min i.c.) to produce hyperemic flow comparable to the exogenous adenosine in the prazosin-treated (4.0 μg/kg/min i.c.) group. Administration of papaverine was done 5 minutes before coronary occlusion and 120 minutes after the onset of reperfusion.

In protocol 2, all pharmacological interventions, that is, infusions of prazosin, methoxamine, 8-phenyltheophylline, adenosine, and papaverine, were discontinued after 2 hours of reperfusion to obtain the steady state without any pharmacological interventions.

Chemical Analysis

Coronary arterial and venous blood oxygen difference (AVO₂D) was assessed by the difference between coronary arterial and venous oxygen contents. MV̇ O₂ (ml/100 g/min) was calculated by CBF (ml/100 g/min)×AVO₂D (ml/dl). Lactate was assessed by enzymatic assay,47 and the lactate extraction ratio (LER) was obtained by AVO₂D in lactate concentration multiplied by 100 and divided by arterial lactate concentration.30,35,48

Adenosine measurement. The method of adenosine measurement has been previously reported.30,48,49 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 the degradation of adenosine. Sampled
blood was quickly cooled to 4°C to prevent release of adenosine from red blood cells. 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 were used for analyzing the adenosine content.

Briefly, adenosine in the plasma (100 μl) was succinylated by 100 μl dioxane containing succinic acid anhydride and triethylamine. After a 10-minute incubation, the mixture was diluted with 800 μl 0.3 M imidazole buffer (pH 6.5). The assay mixture contained 100 μl sample, 100 μl succinyl [3H]adenosine (25,000 cpm in an amount of 1 pmol), and 100 μl 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 (50 μl) was added. The charcoal was spun down, and 0.5 ml supernatant was counted for radioactivity in a liquid scintillation counter. The amount of adenosine degradation during the sampling procedure and degradation rate of adenosine were negligible.

Norepinephrine measurement. The method of norepinephrine measurement has been described previously. Five milliliters of coronary arterial and venous blood taken into a tube containing EDTA was immediately placed in iced water and centrifuged 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 Co., Kyoto, Japan). Plasma norepinephrine was determined spectrofluorometrically by the trihydroxyindole method (Shimazu spectrofluorophotometer, RF-500LCA). In this system, the sensitivity of the assay is 10 pg/ml plasma and the intra-assay coefficient of variation is 6.8%.

ATP measurement. The method of ATP measurement has been previously described. After the last hemodynamic measurements 3 hours after reperfusion, small samples of LAD and LCX coronary arterial areas were removed (40–100 mg), frozen with precooled stainless steel scissors and tongs in liquid nitrogen, and immediately stored at −80°C in liquid nitrogen. The frozen tissue was powdered and homogenized at 4°C in 1 ml ice-cold 6% trichloroacetic acid and then centrifuged at 2,500 g 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). A luciferin-luciferase solution was prepared by dissolving firefly lantern extract (FLE-50, Sigma Chemical Co., St. Louis) in 10 ml distilled water, followed by centrifugation at 8,000 g in ice-cold temperature (0°C) for 1 hour, and the supernatant was used as an enzyme solution. A 0.2-ml sample of the enzyme solution was added to 5 ml ATP standard solution or ATP extract of myocardium, and the intensity of bioluminescence generated from the ATP luciferine-luciferase mixture was measured with a bioluminescence reader. ATP standard solution was prepared by diluting Na2ATP salts with distilled water. The ATP concentration was computed from the intensity of chemiluminescence.

Morphological Studies

After completion of the physiological measurements, heart tissues were prepared for light and electron microscopic analysis to test whether the pharmacological interventions in the present study produce irreversible myocardial injury. Immersion fixation was used. A transmural wedge of the LAD (stunned) and LCX (control) areas were cut rapidly with a sharp razor blade into multiple 1-mm cubes while immersed in a solution of 4% formaldehyde–1% glutaraldehyde in 0.186 M phosphate buffer (pH 7.2). The samples from each region were divided into subepicardium and subendocardium. Subepicardial and subendocardial samples were taken no more than 1 mm in depth from the epicardial and endocardial surfaces, respectively. The samples were then placed in vials containing 5–10 ml of the same fixative. After fixation, the blocks were washed with several changes of 0.1 M phosphate buffer, postfixed for 1 hour with 1% osmium tetroxide in phosphate buffer, dehydrated in a graded series of alcohols and propylene oxide, embedded in epoxy resin, and cut into semithin (1-μm) sections with an ultramicrotome. These sections were stained with toluidine blue and examined by light microscopy. Ultrathin sections (75 nm) were stained with lead citrate and uranyl acetate and were examined with an electron microscope (100CX, Hitachi, Tokyo).

Electron micrographs were evaluated according to the presence and severity of mitochondrial injury (swelling, disruption, and presence of amorphous densities), the extent of contraction and disorganization of sarcomeres, and the clumping of nuclear chromatin as signs of cell injury. All slides and micrographs were read blindly.

Statistical Analysis

Statistical analysis was performed with paired and unpaired t tests. Multiple analysis of variance was also used to assess the differences of time–response curves between each group. All values were expressed as mean±SEM, and p<0.05 was considered significant.

Results

Intracorony infusion of propranolol slightly but significantly decreased both FS and MVo2 (Figures 1, 3, and 4; Table 1); however, intracorony infusions of prazosin (4.0 μg/kg/min) and methoxamine (1.0 μg/kg/min) changed neither systemic and coronary hemodynamic nor any other metabolic parameters (Table 1). Intracorony infusions of norepinephrine and rauwolscine did not exert direct effects on myocardium or coronary circulation in
TABLE 1. Coronary Hemodynamic and Metabolic Parameters Before and After Pharmacological Interventions Prior to Ischemia

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<th>CBF (ml/100 g/min)</th>
<th>LER (%)</th>
<th>MVo2 (ml/100 g/min)</th>
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<th>HR (beats/min)</th>
<th>AoP syst. (mm Hg)</th>
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Values are mean±SEM. n, Number of data; CPP, coronary perfusion pressure; CBF, coronary blood flow; LER, lactate extraction ratio; MVo2, myocardial oxygen consumption; AdC, plasma adenosine concentrations (coronary arteriovenous differences of adenosine); HR, heart rate; AoP, aortic pressure; syst. and diast., systolic and diastolic, respectively; PRZ, prazosin; MTX, methoxamine; 8PT, 8-phenyltheophylline; PROP, propranolol; RWL+NE, rauwolscine with norepinephrine; ADO, adenosine; PAP, papaverine.  
* p<0.05, †p<0.001 vs. control condition.

the nonischemic condition under β-adrenoceptor blockade (Table 1). A higher dose of prazosin significantly increased CBF, and a higher dose of methoxamine decreased CBF (Table 1). Pharmacological interventions in this study did not alter coronary perfusion pressure, systolic and diastolic pressures, or heart rate.

During ischemia, FS in each group decreased to an identical extent and no significant differences were observed in FS at 15 minutes of ischemia or in time courses in the FS decline (Figures 1 and 4). Systemic pressure, coronary perfusion pressure, and heart rate in each group did not change during ischemia and reperfusion.

Effects of α1-Adrenoceptor Activity on Myocardial Stunning

Figure 1 shows time courses of FS before, during, and after 15 minutes of ischemia in the untreated, prazosin-treated, and methoxamine-treated groups. These pharmacological interventions did not significantly change FS before and during ischemia. This observation indicates that these drugs did not change the severity of ischemia. During early reperfusion (up to 10 minutes), FS in the prazosin-treated condition was higher (p<0.05) and in the methoxamine-treated condition was lower (p<0.001) than that of the untreated condition. However, the functional recovery between 10 and 180 minutes after the onset of reper-
fusion in the prazosin-treated condition was significantly worse (p<0.05) than that in the untreated condition, and the functional recovery in the methoxamine-treated condition was better (p<0.001) than that in the untreated condition. Furthermore, 180 minutes after the onset of reperfusion, FS in the prazosin-treated group was significantly lower than that in the untreated condition (12.7±0.6% versus 6.9±0.4% in the prazosin-treated group, p<0.001), and FS in the methoxamine-treated condition was significantly higher (17.3±0.3%, p<0.001) than that in the untreated condition. Figure 2 demonstrates the changes in CBF (panel A) and adenosine concentrations (panel B) during reperfusion. Reactive hyperemic flow was continued up to 10 minutes after reperfusion in these three groups. Hyperemic flow in the prazosin-treated group was significantly (p<0.05) attenuated, and hyperemic flow in the methoxamine-treated group was significantly (p<0.001) augmented compared with the untreated condition (panel A). In accordance with these changes in coronary hyperemic flow, adenosine release in the prazosin-treated group (panel B) was attenuated (p<0.005), and adenosine release in the methoxamine-treated group was augmented (p<0.001) compared with the untreated condition, as was expected from our previous report.30 The changes in LER and MVo2 in these three groups are depicted in Figure 3. At steady state, 180 minutes after the reperfusion, there were no significant differences in LER (panel A) and MVo2 (panel B). However, during early reperfusion (up to 10 minutes after reperfusion) in the prazosin-treated condition, MVo2 was significantly (p<0.001) higher and LER was significantly (p<0.001) lower compared with the untreated condition. During early reperfusion in the methoxamine-treated group, MVo2 was significantly (p<0.001) lower and LER was significantly (p<0.001) higher compared with the untreated condition. These results indicate that a1-adrenoceptor attenuation worsens contractile dysfunction during reperfusion and a1-adrenoceptor stimulation improves it.

Figure 4 strengthens these observations. Figure 4 shows the changes in FS before and during ischemia and reperfusion with and without a1-adrenoceptor stimulation. The recovery of FS with norepinephrine treatment was greater (p<0.001) than that of the group treated with propranolol alone (control). Figures 1 and 4 indicate that a1-adrenoceptor stimulation can improve the decreased FS. In a1-adrenoceptor stimulation caused by norepinephrine, both reactive hyper-
emic flow and adenosine release were significantly enhanced (p<0.05 in both reactive hyperemic flow and adenosine release) during early reperfusion (up to 10 minutes after reperfusion) compared with the propranolol-alone condition (Figure 5). At steady state, 180 minutes after the onset of reperfusion, there were no differences of CBF and adenosine release between these two groups. Figure 6 shows the serial changes in LER (panel A) and MVo2 (panel B). MVo2 with α1-adrenoceptor stimulation caused by norepinephrine was significantly (p<0.05) depressed (panel B), and the recovery of lactate metabolism was significantly (p<0.05) quicker (panel A) than in the group treated with propranolol alone. By comparing Figures 1 and 4, at 180 minutes after reperfusion, FS in the untreated and propranolol groups was 12.7±0.6% versus 13.6±1.1%, respectively. There was no significance in the time course of the functional recovery between these two groups, indicating that β-adrenergic blockade does not attenuate contractile dysfunction during reperfusion.

With the higher dose of prazosin administration (12 μg/kg/min i.c.), although peak CBF was significantly (p<0.01) increased (267±10 ml/100 g/min) compared with the prazosin (4.0 μg/kg/min)-treated group, FS at 3 hours of reperfusion (7.0±0.4%) was comparable to that in the prazosin-treated group. The peak values of adenosine release (100±12 pmol/ml) were attenuated to the comparable levels in the prazosin-treated group. With the higher dose of methoxamine administration (10 μg/kg/min i.c.), FS at 3 hours of reperfusion (13.1±0.7%) was smaller (p<0.001) than that in the methoxamine (1.0 μg/kg/min i.c.)-treated group. The peak values of adenosine release and CBF were 806±17 pmol/ml and 373±6 ml/100 g/min, respectively. FS at 15 minutes of ischemia for the prazosin (4.0 μg/kg/min i.c.)-treated group and the group receiving the higher dose of prazosin (12 μg/kg/min i.c.) was −6.9±2.2% and −6.3±1.2%, respectively, and these values were not significantly different from the values in the untreated condition.

These results indicate that moderate α1-adrenoceptor activation does not deteriorate myocardial function during reperfusion; instead, it can reduce the magnitude of myocardial stunning. Adenosine release controlled by α1-adrenoceptor activity may play a critical role in the reduction of the magnitude of the stunning.
Unique Role of Released Adenosine by 
$\alpha_1$-Adrenoceptor Activity in Myocardial Stunning

Although we showed that $\alpha_1$-adrenoceptor activity can be beneficial for myocardial stunning, possibly because of the enhanced release of adenosine, there is no direct cause–effect relation between adenosine release and attenuation of the magnitude of the stunning because $\alpha_1$-adrenoceptor activity has both myocardial and coronary effects other than the enhancement of adenosine release from the ischemic myocardium. To determine this unique cause–effect relation, we tested whether 8-phenyltheophylline, a specific adenosine receptor antagonist, completely or partially diminishes the beneficial effects of $\alpha_1$-adrenoceptor stimulation. We also tested whether exogenous adenosine administration can improve the stunning comparable to the levels of $\alpha_1$-adrenoceptor stimulation groups. If 8-phenyltheophylline completely inhibits the beneficial effects of $\alpha_1$-adrenoceptor stimulation (methoxamine, 1.0 $\mu$g/kg/min i.c.) to the level of the $\alpha_1$-adrenoceptor attenuated group (prazosin, 4.0 $\mu$g/kg/min i.c.) and exogenous adenosine reduces the magnitude of stunning to the level of the $\alpha_1$-adrenoceptor stimulated condition, we can strongly argue that beneficial effects of $\alpha_1$-adrenoceptor activity on myocardial stunning are uniquely attributed to the enhanced release of adenosine. Figure 7 shows the effect of 8-phenyltheophylline on myocardial stunning in the methoxamine-treated condition and also the effect of exogenous adenosine on myocardial stunning in the prazosin-treated condition. The data of the methoxamine- and prazosin-treated groups are the same data as those in Figure 1. The beneficial effects of methoxamine on myocardial stunning were completely abolished by 8-phenyltheophylline, and FS decreased to the level of the prazosin-treated condition when 8-phenyltheophylline was added in the methoxamine-treated group. The deleterious effects of prazosin were eliminated by exogenous adenosine, and FS increased to the level of the methoxamine-treated condition when exogenous adenosine was added in the prazosin-treated group. This result strongly indicates that the amount of released adenosine mediated by $\alpha_1$-adrenergic activity uniquely determines the extent of myocardial stunning and that the effect of $\alpha_1$-adrenocep-
ator activity on myocardial stunning is attributed to the amount of released adenosine.

The treatment with 8-phenyltheophylline in the methoxamine-treated condition attenuated the peak coronary hyperemic flow (216±4 versus 400±18 ml/100 g/min in the methoxamine-treated group at 15 seconds after the onset of reperfusion, p<0.001), and exogenous adenosine in the prazosin-treated condition augmented the peak hyperemic flow (380±14 ml/100 g/min, p<0.001). The attenuated and augmented levels of hyperemic flow by 8-phenyltheophylline and exogenous adenosine are comparable to the peak coronary hyperemic flow levels of the prazosin- and methoxamine-treated conditions, respectively. The treatment with 8-phenyltheophylline in the methoxamine-treated condition further increased adenosine efflux (794±28 versus 697±27 pmol/ml in the methoxamine-treated group at 1 minute after the onset of reperfusion, p<0.05; 624±51 versus 408±37 pmol/ml in the methoxamine-treated group at 3 minutes after reperfusion, p<0.01). This phenomenon is comparable to the findings of McKenzie et al.\textsuperscript{53} Exogenous adenosine also increased adenosine efflux (1,180±77 pmol/ml at 1 minute after reperfusion; 1,020±43 pmol/ml at 3 minutes after reperfusion). Three hours after the onset of reperfusion, because all pharmacological interventions were discontinued at 2 hours of reperfusion, both CBF and adenosine efflux returned to the control levels (methoxamine and 8-phenyltheophylline--treated group: CBF, 93±6 ml/100 g/min; adenosine efflux, 17±2 pmol/ml; prazosin and adenosine--treated group: CBF, 92±4 ml/100 g/min; adenosine efflux, 20±4 pmol/ml). Figure 8 shows the effects of 8-phenyltheophylline and exogenous adenosine on the LER (panel A) and M\textsubscript{VO\textsubscript{2}} (panel B) in the methoxamine- and prazosin-treated groups, respectively. The treatment with 8-phenyltheophylline in the methoxamine-treated group depressed (p<0.001) the LER and enhanced (p<0.001) M\textsubscript{VO\textsubscript{2}} during early reperfusion compared with the group treated with methoxamine alone. Conversely, exogenous adenosine in the prazosin-treated group increased (p<0.001) LER and decreased (p<0.001) M\textsubscript{VO\textsubscript{2}} to the level of the methoxamine-treated group.

**FIGURE 4.** Serial changes in fractional shortening of the left anterior descending coronary artery area before, during, and after 15 minutes of myocardial ischemia with and without \(\alpha_1\)-adrenoceptor stimulation. Neither propranolol (C2) nor norepinephrine with rauwolscine (C3) for \(\alpha_1\)-adrenoceptor stimulation (closed triangles) changes fractional shortening. As a control (open triangles), propranolol alone is infused into the left anterior descending coronary artery. These pharmacological interventions do not alter decreases in fractional shortening during ischemia. During the early reperfusion period (up to 10 minutes), fractional shortening in the \(\alpha_1\)-adrenoceptor stimulation group is significantly (p<0.05) reduced compared with that in the control condition; however, it becomes larger afterward (p<0.001). At 180 minutes after the onset of reperfusion, fractional shortening in the \(\alpha_1\)-adrenoceptor stimulation group is significantly improved compared with the propranolol group.
Mechanisms Whereby α₁-Adrenoceptor Stimulation Reduces the Magnitude of the Stunning

Although we elucidated that adenosine release mediated by α₁-adrenoceptor activity primarily determines the extent of myocardial stunning, it remains unresolved whether the beneficial effects of adenosine are attributed to the coronary hyperemic response or mechanical depressed effects, because adenosine is known to cause coronary hyperemic response through the adenosine A₂-receptor and to inhibit enhanced contractility through the adenosine A₁-receptor.\textsuperscript{54} To further investigate the role of coronary hyperemic flow on eliminating stunned myocardium, instead of exogenous adenosine, papaverine in the prazosin-treated group was infused into the coronary artery up to 2 hours after the onset of reperfusion. Papaverine in the prazosin-treated condition caused coronary hyperemic flow comparable to that of exogenous adenosine (Table 1). However, papaverine with prazosin treatment could not restore the contractile dysfunction (6.9 ± 0.2% versus 17.3 ± 0.3% in the exogenous adenosine with prazosin–treated group, \(p < 0.001\)). The peak hyperemic flow up to 2 hours of reperfusion was comparable both in the adenosine and papaverine groups (385 ± 12 versus 380 ± 16 ml/100 g/min at 1 minute of reperfusion, \(p = \text{NS}\); 383 ± 18 versus 394 ± 16 ml/100 g/min at 60 minutes of reperfusion, \(p = \text{NS}\); 385 ± 11 versus 389 ± 13 ml/100 g/min at 120 minutes, \(p = \text{NS}\)); however, adenosine efflux in the papaverine with prazosin–treated group was significantly (\(p < 0.001\)) depressed for 2 hours (96 ± 40 ml/100 g/min at 1 minute of reperfusion, 21 ± 8 ml/100 g/min at 60 minutes of reperfusion, 15 ± 4 ml/100 g/min at 120 minutes of reperfusion). After 2 hours of reperfusion, cessation of exogenous adenosine and papaverine infusions returned both CBF and adenosine efflux to the control level. These results indicate that hyperemic flow during reperfusion is not the major cause for attenuation of the magnitude of the stunning because papaverine-induced coronary hyperemia did not mimic this effect.

Because adenosine antagonizes the catecholamine-induced augmentation of contractility, the results in Figures 1, 3, 4, and 6–8 suggest that adenosine release mediated through α₁-adrenergic activity...
inhibits catecholamine-induced hyperfunction during early reperfusion and facilitates recovery from the anaerobic metabolism. In fact, in the untreated condition, the norepinephrine concentration in coronary venous blood was increased to 552±12 pg/ml at 3 minutes after reperfusion (control, 450±34 pg/ml; p<0.05) and returned to the control level at 10 minutes after reperfusion (471±35 pg/ml).

The ATP content of reperfused myocardium with and without pharmacological interventions was significantly decreased compared with that of the non-ischemic myocardium, corresponding well to the features of stunned myocardium. The ATP content of the subepicardium in the prazosin-treated condition was slightly lower and that of the methoxamine-treated condition was higher than that in the untreated condition (Table 2). There were no differences of ATP content in the epicardium of stunned myocardium in each group (Table 2).

Histological Examinations

Four hearts each from the prazosin-treated group and the untreated group were selected randomly for histological examination. With light microscopy, myocardium from both groups appeared normal. In particular, there were no contraction band injuries as are seen in irreversibly injured reperfused myocardium. In the electron microscopic examination, most of the tissues exhibited completely normal ultrastructure (Figure 9). In the rare cells, mild mitochondrial swelling was observed. In these cells, there was no evidence of more extensive or more severe histological injury in the subendocardium, corresponding to the features of stunned myocardium.

Discussion

In the present study, we concluded that α1-adrenoceptor activity protects against myocardial stunning and that the cellular mechanism of stunning is due to enhanced adenosine release by α1-adrenoceptor activity. The metabolic effects may partially mediate the protective mechanisms of myocardial stunning rather than the coronary hyperemic flow response. However, before reaching these conclusions, we need to consider the other effects of α1-adrenoceptor activity leading us to the present results.
Role of $\alpha_1$-Adrenoceptor Activity in Myocardial Stunning

$\alpha_1$-Adrenoceptor activity is reported to cause coronary vasoconstriction and to limit oxygen supply to ischemic and nonischemic myocardium, leading to the idea that the magnitude of myocardial stunning may be augmented because severe ischemia and prolonged anaerobic metabolism during reperfusion is thought to cause severe myocardial stunning. Liang and Jones reported that prazosin increases oxygen supply and exerts beneficial effects in hypoxic hearts. In the present study, although the higher dose of prazosin (12 $\mu$g/kg/min i.c.) can increase CBF in the nonischemic condition, this dose of prazosin did not attenuate the severity of myocardial ischemia because the extent of decreases in FS at 15 minutes of ischemia was not different between the untreated and prazosin-treated conditions (Figure 1 and “Results”). These differences may be attributable to differences of the experimental models and doses of prazosin. In the hypoxic hearts of Liang and Jones, considerably higher doses of prazosin increased CBF and improved the oxygen supply. In our study, however, the extent of decreases in FS during myocardial ischemia was comparable between the untreated and prazosin-treated conditions because we completely clamped the bypass tube to the LAD. Thus, coronary effects of prazosin during ischemia were not exerted in our experimental model. The smaller dose of prazosin (4.0 $\mu$g/kg/min i.c.) used in our study did not change either coronary circulation or myocardial metabolism at the baseline; however, it inhibited adenosine release during reperfusion, indicating that $\alpha_1$-adrenoceptor stimulation by released norepinephrine contributes to adenosine release during reperfusion. Nathan and Feigl reported that $\alpha_1$-adrenoceptor stimulation during coronary hyperperfusion favorably affects intramyocardial flow distribution by causing subepicardial vasoconstriction, suggesting that $\alpha_1$-adrenoceptor stimulation may be beneficial to ischemia and reperfusion injury. However, Buffington and Feigl showed that transmural inhomogeneous flow distribution mediated by $\alpha$-adrenoceptor coronary vaso-
constriction is evident under moderate coronary hyperperfusion (coronary perfusion pressure >70 mm Hg), and this beneficial flow distribution is over-ridden and diminished by potent metabolic vasodilatory effects when the heart is progressively underperfused (coronary perfusion pressure =38 mm Hg).

**TABLE 2.** ATP Content (μmol/g wet wt) of Stunned and Nonstunned Myocardium

<table>
<thead>
<tr>
<th></th>
<th>LAD area</th>
<th>LCX area</th>
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<tr>
<td></td>
<td>END</td>
<td>EPI</td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRZ (4.0 μg/kg/min i.c.)</td>
<td>2.8±0.1</td>
<td>3.0±0.1</td>
</tr>
<tr>
<td>PRZ (12 μg/kg/min i.c.)</td>
<td>2.1±0.1*</td>
<td>2.8±0.1</td>
</tr>
<tr>
<td>MTX (1.0 μg/kg/min i.c.)</td>
<td>3.3±0.1</td>
<td>3.1±0.1</td>
</tr>
<tr>
<td>MTX (10 μg/kg/min i.c.)</td>
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<td>3.1±0.2</td>
</tr>
<tr>
<td>MTX (1.0 μg/kg/min i.c.)+8PT</td>
<td>2.0±0.1*</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td>PRO5</td>
<td>2.9±0.2</td>
<td>3.2±0.1</td>
</tr>
<tr>
<td>NE+RWL+PROP</td>
<td>3.2±0.1†</td>
<td>3.2±0.1</td>
</tr>
<tr>
<td>PRZ+ADO</td>
<td>2.9±0.1</td>
<td>3.0±0.1</td>
</tr>
<tr>
<td>PRZ+PAP</td>
<td>1.9±0.1*</td>
<td>2.8±0.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n, Number of data; LAD, left anterior descending coronary artery; LCX, left circumflex artery; END, endocardium; EPI, epicardium; PRZ, prazosin-treated group; MTX, methoxamine-treated group; MTX+8PT, methoxamine with 8-phenyltheophylline–treated group; PROP, propranolol-treated group; NE+RWL+PROP, norepinephrine with rauwolscine and propranolol–treated group; PRZ+ADO, prazosin with adenosine–treated group; PRZ+PAP, prazosin with papaverine–treated group.

*p<0.001, †p<0.05 vs. untreated condition.
Because our experimental model of ischemia is one of a complete coronary occlusion, the beneficial effect observed by Nathan and Feigl cannot be directly extrapolated to our present observation. Alternatively, prazosin, methoxamine, or norepinephrine may affect collateral flow. If this were the case, prazosin would be expected to worsen the extent of myocardial ischemia because of interruption of collateral flow and thus to further depress the contractile dysfunction during reperfusion. However, in the present study, prazosin, methoxamine, or norepinephrine did not affect the severity of ischemia assessed by FS during ischemia (Figure 1), suggesting that collateral flow is not modulated by these interventions, consistent with a previous report that collateral flow is not regulated by \( \alpha \)-adrenoceptor activity.  

Thus, direct actions of \( \alpha_1 \)-adrenoceptor activity on coronary vessels cannot explain our present results.

On the other hand, \( \alpha_1 \)-adrenoceptor activity exerts several actions on the myocardium that merit careful consideration. \( \alpha_1 \)-Adrenoceptor activity is known to increase the sensitivity of the contractile proteins to \( \text{Ca}^{2+} \) and thus to exert positive inotropism. This result may explain our observation because it seems as if prazosin depressed the regional contractility of stunned myocardium and both norepinephrine and methoxamine increased contractility, although all pharmacological interventions were discontinued 2 hours after reperfusion and FS remained depressed at 3 hours. The doses of norepinephrine (0.24 \( \mu \text{g/kg/min i.c.} \)) and methoxamine (1.0 \( \mu \text{g/kg/min i.c.} \)) in the present study did not increase FS, CBF, or adenosine release in the nonischemic conditions (Figures 1 and 4, Table 1), indicating that direct effects of \( \alpha_1 \)-adrenoceptor activity on myocardial contractility are not related to the beneficial roles of \( \alpha_1 \)-adrenoceptor stimulation in myocardial stunning. Sharma et al.
reported that \( \alpha_1 \)-adrenoceptor blockade prevents calcium overload during reperfusion and thus prevents reperfusion arrhythmia. This effect of \( \alpha_2 \)-adrenoceptor blockade is reported to be linked not to CBF, but directly to myocytes.\(^{59} \) Several investigators\(^{28,29,60–62} \) reported that calcium overload during ischemia and reperfusion is a potential cause of stunning. If so, \( \alpha_1 \)-adrenoceptor blockade would prevent reperfusion injury,\(^{21} \) and our results seem contradictory. In the reports of Sharma et al.,\(^{20} \) the duration of ischemia is 35 minutes, which may start to involve irreversible cell injury.\(^{63} \) In this case, \( \alpha_2 \)-adrenoceptor–mediated cell injury would be responsible for irreversible injury because increases in the number of \( \alpha_1 \)-adrenoceptors\(^{64} \) and the amount of released norepinephrine become prominent 20–30 minutes after the onset of myocardial ischemia.\(^{4,5} \) This \( \alpha_1 \)-adrenoceptor–mediated cell injury that is inhibited by prazosin\(^{20} \) may cause massive calcium influx, presumably through the damaged cell membrane as well as leakage from the intracellular calcium sites. Adenosine release may also be enhanced during 35 minutes of ischemia although this released adenosine may no longer predominate over the increased deleterious effects of \( \alpha_1 \)-adrenoceptor stimulation. On the other hand, in the brief period of ischemia and reperfusion, the situation is quite different. During 15 minutes of ischemia and subsequent reperfusion, \( \alpha_2 \)-adrenoceptors are moderately stimulated only by circulating catecholamines, and the beneficial effects of moderate \( \alpha_1 \)-adrenoceptor stimulation caused by adenosine release can overcome the deleterious effects. Indeed, our present results suggest that deleterious effects of moderate \( \alpha_1 \)-adrenoceptor stimulation did not occur during 15 minutes of ischemia and subsequent reperfusion. The rise in free \( \text{Ca}^{2+} \) during 15–20 minutes of ischemia and subsequent reperfusion, one of the causes of myocardial stunning, is thought to be attributed to \( \text{Na}^{+}–\text{Ca}^{2+} \) exchanges\(^{24,60–62} \) rather than \( \alpha_1 \)-adrenoceptor activity.\(^{20} \) Thus the effect of \( \alpha_1 \)-adrenoceptor stimulation that disturbs cellular homeostasis and promotes calcium accumulation observed during the longer period of ischemia\(^{20} \) is more likely to be relevant to mechanisms of irreversible cell injury rather than to stunning.

It is reported that \( \alpha_1 \)-adrenoceptors have two subtypes, that is, \( \alpha_{1\text{A}} \) and \( \alpha_{1\text{B}} \) subtypes.\(^{46} \) The former activates contractile response by promoting \( \text{Ca}^{2+} \) influx through nifedipine-sensitive channels, and the latter activates inositol phosphate. The present study could not differentiate the subtype of \( \alpha_1 \)-adrenoceptors that is responsible for the improvement in the magnitude of myocardial stunning, because the chemicals that were used for modulation of \( \alpha_1 \)-adrenoceptor activity activated both subtypes.

**Role of Released Adenosine Caused by \( \alpha_1 \)-Adrenoceptor Activity in Stunned Myocardium**

Although Figures 1 and 2 show a correlation between changes in adenosine release and myocardial stunning after modifications of \( \alpha_1 \)-adrenergic activity, it is not necessarily true that adenosine leads directly to changes in myocardial mechanical function. It is possible that adenosine release is simply a concomitant phenomenon related to the state of \( \alpha_1 \)-adrenoceptor stimulation but not directly related to myocardial stunning. However, we showed that 1) treatment with 8-phenyltheophylline, which is not thought to affect \( \alpha_1 \)-adrenergic activity, completely abolishes the beneficial effects of \( \alpha_1 \)-adrenergic activity and augments the magnitude of the stunning to the level of the \( \alpha_1 \)-adrenoceptor–blocked condition and 2) exogenous adenosine, which does not affect \( \alpha_1 \)-adrenergic activity, completely eliminates the deleterious effects of prazosin and improves the contractile dysfunction to the level of the \( \alpha_1 \)-adrenoceptor–stimulated condition. These results strongly indicate the direct cause–effect relations between adenosine release mediated by \( \alpha_1 \)-adrenoceptor activity and myocardial stunning.

Our study further demonstrates that the contractility of the reperfused myocardium with lower adenosine concentrations is transiently augmented during early reperfusion despite the production of large amounts of lactate during early reperfusion (Figures 1 and 3). This enhanced contractility may be due to calcium overload during early reperfusion because several lines of evidence indicate the existence of calcium overload during early reperfusion\(^{20,23–28} \) and transient augmentation of contractility during calcium overload.\(^{29} \) On the other hand, the present study (Figures 1, 3, and 7) shows that massive release of adenosine by \( \alpha_1 \)-adrenoceptor stimulation can inhibit reperfusion-induced myocardial hyperfunction through adenosine \( \alpha_1 \)-receptors. This result supports the view that this enhanced release of adenosine may attenuate calcium overload through adenosine \( \alpha_1 \)-receptors during ischemia and the very early reperfusion period because calcium influx into myocytes is reported to be attenuated by adenosine.\(^{37,38,65,66} \) Since transient calcium overload during the early reperfusion period is responsible for myocardial stunning,\(^{25–29,60–62} \) adenosine may attenuate this calcium overload and, thus, the magnitude of myocardial stunning.

Another possibility for the attenuation of the magnitude of the stunning caused by adenosine may be the preservation of ATP content of reperfused myocardium.\(^{67} \) The preservation of ATP content by adenosine may be attributed to its capacity to modulate energy demand. This adenosine-induced myocardial energy-sparing effect potentially abbreviates the period of anaerobic myocardial metabolism during early reperfusion and attenuates myocardial stunning (Figures 1, 3, 4, and 6–8). Although the extent of ATP depletion and contractile dysfunction seems to be related during reperfusion injury in previous studies\(^{68,69} \) and the present study, the extent of recovery from ATP depletion does not necessarily determine the contractile dysfunction of stunned myocardium\(^{28,29,70,71} \) because repletion of ATP does not restore normal contractile function.\(^{72} \) Although
ATP depletion of stunned myocardium was between 40% and 60% (Table 2) and compatible with previous results,68,69 such an extent of decreases in ATP content of stunned myocardium is not likely to cause contractile dysfunction observed in the present study, because these decreases in ATP are not sufficient to compromise the ionic pump activities of the cellular membrane or the sarcoplasmic reticulum.73

The other possibility is that adenosine A2-receptor–mediated coronary vasodilation may participate in attenuating mechanisms of myocardial stunning. However, this is not the case because of the exogenous papaverine and adenosine protocols. Papaverine preferentially inhibits phosphodiesterase of coronary smooth muscle independent of adenosine A2-receptors68 and dilates coronary arterioles in the subendocardial region as well as in the subepicardial region.74 Nevertheless, papaverine could not attenuate myocardial stunning in the prazosin-treated condition, indicating that hyperemic flow per se is not responsible for preventive effects of adenosine on myocardial stunning. However, there may be the possibility that adenosine attenuates microcirculatory disturbances because these disturbances are reported to cause stunned myocardium.75 There are several possibilities that adenosine prevents microcirculatory disturbances. Activated leukocytes are reported to plug coronary capillaries and release free radicals,39 which may cause stunned myocardium. Adenosine potentially inhibits the deleterious effects of activated leukocytes.40 Engler et al41,42 and Kortuis et al43 observed involvement of leukocytes in myocardial stunning. Platelet aggregation during ischemia and reperfusion is also potentially inhibited by adenosine.76 These beneficial effects of adenosine may contribute to the reduction of the magnitude of the stunning, although our data neither support nor negate this possibility.

**Physiological and Clinical Relevance**

Although we concluded that moderate $\alpha_1$-adrenoceptor stimulation is beneficial for myocardial stunning through enhancement of release of adenosine, the deleterious effects of potent $\alpha_1$-adrenoceptor stimulation on myocardial stunning should be considered. $\alpha_1$-Adrenoceptor stimulation may worsen ischemic and reperfusion damages via coronary vasoconstriction,10–16 increased myocardial contractility,18–22 and $\text{Ca}^{2+}$ overload,63,64 any of which augments the magnitude of myocardial stunning. Indeed, when a higher dose of methoxamine (10 $\mu$g/kg/min i.c.) was administered in the present study, the beneficial effects of $\alpha_1$-adrenoceptor stimulation (methoxamine, 1.0 $\mu$g/kg/min i.c.) were blunted (see "Results"). Although peak reactive hyperemic flow was attenuated by coronary vasoconstriction for the higher dose of methoxamine administration, this attenuation may not worsen the severity of myocardial stunning, because our results demonstrate that coronary hyperemic flow during reperfusion is not directly related to the magnitude of myocardial stunning. On the other hand, $\alpha_1$-adrenoceptor stimulation increases myocardial contractility caused by the enhanced sensitivity of myofilaments,18–22 which may lead to the hyperfunction of myocardium during early reperfusion and augmentation of the magnitude of myocardial stunning. The higher dose of methoxamine may also facilitate calcium overload during ischemia and reperfusion.4,5,63,64 Although our results indicate that moderate $\alpha_1$-adrenoceptor stimulation (methoxamine, 1.0 $\mu$g/kg/min i.c.) attenuates both myocardial contractile dysfunction and $\text{MVO}_2$ during reperfusion through the marked release of adenosine and subsequent stimulation of adenosine A1 receptors, if $\alpha_1$-adrenoceptor stimulation is more prominent (methoxamine, 10 $\mu$g/kg/min i.c.) in this period, myocardial contractility, $\text{MVO}_2$, and $\text{Ca}^{2+}$ accumulation would be more enhanced and augment the magnitude of myocardial stunning despite the beneficial effects of $\alpha_1$-adrenoceptor stimulation. These lines of evidence support the idea that $\alpha_1$-adrenoceptor stimulation has two contradictory actions in the situation of myocardial stunning. Circulating and released catecholamines in the in vivo hearts may not be deleterious during a brief period of ischemia and subsequent reperfusion but may act as an improving factor for myocardial stunning through enhancement of adenosine release. However, we should note that the potent $\alpha_1$-adrenoceptor stimulation increases the severity of myocardial stunning.

During the longer period of ischemia and subsequent reperfusion, the situation would be different and deleterious effects of $\alpha_1$-adrenoceptor stimulation derived from increases in the number of $\alpha_1$-adrenoceptors and massively released catecholamine would overcome its beneficial effects although more adenosine release is expected. Thus we need to consider the balance between the beneficial and deleterious effects of $\alpha_1$-adrenoceptor stimulation during ischemia and reperfusion, as discussed previously, and need to test which effect of $\alpha_1$-adrenoceptor stimulation is prominent.

Another intriguing aspect in the present study is that the linkage between $\alpha_1$-adrenoceptor activity and adenosine release as well as adenosine per se may contribute to the important mechanisms for attenuating ischemic and reperfusion injury. This is because released adenosine is reported to inhibit further release of norepinephrine from neurotransmitters and thus to prevent catecholamine-mediated injury.77 This negative feedback circuit is essential for the prevention of progressive, unchecked deterioration of myocardial contractile function.

Several investigators, including the present authors, have observed beneficial effects of adenosine on reperfusion injury.35,48,67,68 These results merit further evaluation in realistic clinical settings. Percutaneous transluminal coronary recanalization and thrombolysis are often used in acute myocardial infarction; although the beneficial effects of early reperfusion are unquestionable, myocardial stunning can persist for days to weeks. Adenosine infusion or
potentiation of adenosine release may be promising therapeutic adjustments to reperfusion therapy.

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