Effects of ischemic preconditioning on contractile and metabolic function during hypoperfusion in dogs

TETSUO MINAMINO, MASAFUMI KITAKAZE, HIROSHI SATO, HIROHARU FUNAYA, YASUNORI UEDA, HIROSHI ASANUMA, TSUNEHIKO KUZUYA, AND MASATSUGU HORI
First Department of Medicine, Osaka University School of Medicine, Osaka 565, Japan

Minamino, Tetsuo, Masafumi Kitakaze, Hiroshi Sato, Hiroharu Funaya, Yasunori Ueda, Hiroshi Asanuma, Tsunehiko Kuzuya, and Masatsugu Hori. Effects of ischemic preconditioning on contractile and metabolic function during hypoperfusion in dogs. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H684–H693, 1998.—We examined the effects of ischemic preconditioning (IP) on metabolic and contractile function during coronary hypoperfusion in dogs. After the left anterior descending coronary artery (LAD) was occluded for 5 min (IP) and reperfused for 10 min, coronary blood flow (CBF) of the LAD was decreased to 33% of the control. IP increased (P < 0.05) lactate extraction ratio and the pH of coronary venous blood and decreased (P < 0.05) myocardial oxygen consumption and fractional shortening during hypoperfusion compared with those in the control group, although IP did not change the endocardial-to-epicardial blood flow ratio of the regional myocardium during hypoperfusion. IP increased (P < 0.05) the adenosine levels in coronary venous blood during hypoperfusion. IP increased (P < 0.05) myocardial ecto-5'-nucleotidase activity. Administration of 8-sulfophenyltheophylline or α,β-methyleneadensosine 5'-diphosphate blunted the IP-induced changes in metabolic and contractile parameters during hypoperfusion. These results suggest that IP reduced the severity of anaerobic myocardial metabolism of ischemic hearts by increasing the adenosine levels via an extracellular pathway.

adensosine; 8-sulfophenyltheophylline; α,β-methyleneadensosine diphosphate.

BRIEF PERIODS OF ISCHEMIA preceding sustained ischemia markedly delays the progression of myocardial infarction (22, 27) and reduces the frequency of reperfusion arrhythmia (30), which is known as “ischemic preconditioning” (IP). Adenosine plays an important role in the infarct size-limiting effects of IP (15, 28). We have previously found that IP increases adenosine levels and ecto-5'-nucleotidase (ecto-5'-N) activity (13), and that the inhibition of ecto-5'-N blunts the infarct size-limiting effect of IP (12). However, it has not been determined whether IP improves myocardial contractile and metabolic dysfunction during coronary hypoperfusion, which are pathophysiological conditions quite different from those of myocardial infarction. The most common medical approaches used to reduce myocardial ischemia are reduction of myocardial oxygen consumption and dilatation of the coronary arteries (3, 32). After a brief period of exercise-induced ischemia, the myocardium becomes more resistant to subsequent episodes of myocardial ischemia in patients with effort angina, which is known as the “warmup” phenomenon (10). Warmup phenomenon superficially resembles the phenomenon of IP (17). We have recently demonstrated in the clinical setting that the warmup phenomenon is not due to increased coronary flow but instead to attenuation of myocardial oxygen consumption, which may be mediated by activation of adenosine A1 receptors (25). However, we did not identify a direct causal relation between the increased levels of adenosine and the attenuation of the extent of metabolic and contractile dysfunction or the precise mechanisms by which adenosine levels were increased.

We hypothesized that IP increases ecto-5'-N activity in myocardium, resulting in increased levels of adenosine and the improvement of contractile and metabolic dysfunction during coronary hypoperfusion. To test this idea, first, we examined the effects of IP on myocardial contractile and metabolic function during coronary hypoperfusion. Second, we examined the effects of IP on the adenosine levels during coronary hypoperfusion and assessed myocardial 5'-N activity with and without IP. Third, we examined the effects of IP on myocardial contractile and metabolic function with and without an adenosine receptor antagonist or an ecto-5'-N inhibitor.

MATERIALS AND METHODS

Instrumentation

We anesthetized 69 mongrel dogs weighing 15–21 kg with pentobarbital sodium (30 mg/kg iv). The tracheas were intubated, and the animals were ventilated with room air mixed with oxygen (100% O2, 1 to 2 l/min). The chest was opened through the left fifth intercostal space, and the heart was suspended in a pericardial cradle. After an intravenous administration of heparin (500 U/kg), the left anterior descending coronary artery (LAD) was ligated, cannulated, and perfused with blood from the left carotid artery through an extracorporeal bypass tube. Coronary blood flow (CBF) in the perfused area was measured with an electromagnetic flow probe attached to the bypass tube, and the coronary perfusion pressure (CPP) was monitored at the tip of the coronary artery cannula. A small coronary vein near the center of the perfused area was cannulated with a small, short collecting tube (1 mm in diameter and 7 cm in length) for sampling of coronary venous blood. The drained venous blood was collected in a reservoir placed at the level of the left atrium and returned to the jugular vein. A miniature pressure transducer (model P-5, Konigsberg Instruments, Pasadena, CA) was inserted into the left ventricular (LV) cavity through the LV apex, and the first derivative of LV pressure (LV dP/dt) was determined. A pair of ultrasound crystals dimension gauge (5 MHz; 2 mm in diameter; Schuessler, Cardiff-by-the-Sea, CA) was implanted in the endomyocardial segment of the LV anterior wall in the center of the perfused area to measure segmental length. The lengths of the end-diastolic (EDL) and end-systolic (ESL) myocardial segments were determined at the peak of the R wave on electrocardiogram and at the minimum point of the first derivative of LV pressure, respectively. Fractional shortening [FS = (EDL – ESL)/EDL] was calculated as an index of myocardial performance in the
perfused area. All hemodynamic parameters were recorded on a multichannel recorder (model RM-6000; Nihon-Kohden, Tokyo, Japan).

Measurement of Regional Myocardial Blood Flow

Regional myocardial blood flow was determined by the microsphere technique using nonradioactive microspheres (Sekisui Plastic, Tokyo) made of inert plastic labeled with different types of stable heavy elements as previously described (20). The endocardial-to-epicardial blood flow ratio of each myocardial region (Endo-to-Epi flow ratio) was determined by calculating the microspheres in the inner half of LV wall to that in the outer half.

Experimental Protocols

Experimental protocols are depicted in Fig. 1.

Protocol 1. Effects of IP on myocardial contractile and metabolic function and adenosine levels during coronary hypoperfusion. After hemodynamic stabilization, hemodynamic parameters (LV pressure, LV dP/dt, segment length in the perfused area, CPP, and CBF) were measured. Coronary arterial and venous blood was sampled for blood gas analysis and determinations of lactate and the adenosine levels. After these baseline measurements were obtained, microspheres [1.0 × 10⁴ microspheres/ml of baseline CBF (ml/min)] were injected through the bypass tube to determine the Endo-to-Epi flow ratio. The LAD was completely occluded for 5 min by clamping the bypass tube followed by reperfusion for 10 min (IP). After we confirmed that coronary hyperemic flow was returned to the baseline, CPP was decreased with an occluder attached to the bypass tube until CBF was decreased to 33% of the control flow. When the decrease in CPP was confirmed, the occluder was adjusted manually to maintain CBF at a constant level for 10 min. All hemodynamic and metabolic parameters were measured and the Endo-to-Epi flow ratio was determined just before and 10 min after the ischemia.

Protocol 2 (8-SPT) or 3 (AMP-CP)

Protocol 2a (8-SPT) or 3a (AMP-CP)

Protocol 2b (8-SPT) or 3b (AMP-CP)

Protocol 4

Fig. 1. Schematic diagram of experimental protocols. 8-SPT, 8-sulfophenyltheophylline; AMP-CP, α,β-methyleneadenosine 5'-diphosphate; IP, ischemic preconditioning.
onset of coronary hypoperfusion (protocol 1a, n = 8). In protocol 1b, 15 min after the baseline measurements, CBF was decreased to 33% of the control and maintained at a constant level for 10 min as in protocol 1a. All hemodynamic and metabolic parameters were measured and the Endo-to-Epi flow ratio was determined at the same time points in protocol 1a (protocol 1b, n = 8). To examine the effects of IP on myocardial contractile and metabolic function on different conditions, 5 min after the onset of administration of AMP-CP, the LAD was completely occluded for 5 min followed by reperfusion for 10 min (protocol 2a). We must notice that the extent of myocardial ischemia in the present study is more severe than the pacing-induced and exercise-induced ischemia in patients with effort angina (25, 26, 33).

Protocol 2. Effects of IP on myocardial contractile and metabolic function during coronary hypoperfusion with an adenosine receptor antagonist. The role of adenosine in the effects of IP on myocardial contractile and metabolic function during coronary hypoperfusion was evaluated using 8-sulfophenylthiophene (8-SPT), a specific adenosine receptor antagonist. After contractile and metabolic parameters were measured and the Endo-to-Epi flow ratio was determined under baseline conditions, the administration of 8-SPT (25 mg·kg⁻¹·min⁻¹) into the coronary artery via the bypass tube was started and continued throughout the experimental protocol except during coronary complete occlusion time. An intracoronary dose of 25 mg·kg⁻¹·min⁻¹ of 8-SPT is approximately equal to a concentration of 5–10 × 10⁻⁵ mol/l. This dose of 8-SPT completely abolishes the coronary vasodilatory effect of an intracoronary infusion of exogenous adenosine (100 mg/kg) (19). In protocol 2a, 5 min after the onset of administration of 8-SPT, the LAD was completely occluded for 5 min followed by reperfusion for 10 min (n = 8). After we confirmed that coronary hyperemic flow was returned to the baseline, CBF was decreased to 33% of the control and maintained at constant level for 10 min as in protocol 1a. Contractile and metabolic parameters were measured and the Endo-to-Epi ratio was determined under baseline conditions, 5 min after the onset of administration of 8-SPT, and just before and 10 min after the onset of coronary hypoperfusion. In protocol 2b, 20 min after the onset of administration of 8-SPT, CBF was decreased to 33% of the control and maintained at constant level for 10 min as in protocol 1b (protocol 2b, n = 8). Contractile and metabolic parameters were measured and the Endo-to-Epi ratio was determined at the same time points in protocol 2a.

Protocol 3. Effects of IP on myocardial contractile and metabolic function during coronary hypoperfusion with the inhibition of ecto-5'-N. Adenosine can be produced intracellularly by cytosolic 5'-N and S-adenosylhomocysteine and extracellularly by ecto-5'-N (9). To determine by which pathway IP increases adenosine levels during coronary hypoperfusion, we administered α,β-methyladenosine 5'-diphosphate (AMP-CP), an inhibitor of ecto-5'-N, via the bypass tube throughout the experimental protocol at a rate of 80 mg·kg⁻¹·min⁻¹. In protocol 3a, 5 min after the onset of administration of AMP-CP, the LAD was completely occluded for 5 min followed by reperfusion for 10 min (protocol 3a, n = 8). After we confirmed that coronary hyperemic flow had returned to the baseline, CBF was decreased to 33% of the control and maintained at constant level for 10 min as in protocol 1a. Contractile and metabolic parameters were measured and the Endo-to-Epi ratio was determined under baseline conditions, 5 min after the onset of administration of AMP-CP, and just before and 10 min after the onset of coronary hypoperfusion. In protocol 3b, 20 min after the onset of administration of AMP-CP, CBF was decreased to 33% of the control and maintained at constant level for 10 min as in protocol 1b (n = 8). Contractile and metabolic parameters were measured and the Endo-to-Epi ratio was determined at the same time points in protocol 3a.

Chemical Analysis

The plasma concentration of lactate was determined enzymatically (2), and lactate extraction ratio (LER) was calculated using the following formula: (arterial lactate concentration – coronary venous lactate concentration)/arterial lactate concentration × 100 (19). The coronary arterial and venous blood oxygen difference (a-V O₂) was assessed by the difference between the coronary arterial and venous oxygen content. Myocardial O₂ consumption (MVO₂) was calculated as follows: CBF (in ml·100 g⁻¹·min⁻¹) × a-V O₂ (in mmHg). The adenosine levels were measured according to a previously described method (19). Since practically all the adenosine in coronary venous blood is produced in the heart (14), we measured the adenosine levels in coronary venous blood. The activity of 5'-N was assessed by an enzymatic assay using Sigma 5'-ND assay kit which contains AMP (3.2 mmol/l), NADH (0.2 mmol/l), 2-oxoglutarate (3.7 mmol/l), glutamic dehydrogenase (11,000 UI/l), adenosine deaminase (400 UI/l), β-glycerophosphate buffers, and stabilizers (6, 18). Myocardial tissue samples were homogenized and were divided into membrane and cytosolic fractions according to a previously described method (13, 18). We defined 5'-N activity in membrane and cytosolic fraction as ecto-and cytosolic 5'-N activity, respectively. Results are expressed as units of moles per milligram protein per minute. The protein concentration was determined using the method of Lowry et al. (16) using bovine serum albumin as the standard.

Statistical Analysis

Values are means ± SE. Contractile and hemodynamic parameters, the Endo-to-Epi flow ratio, and the adenosine levels 10 min after the onset of coronary hypoperfusion with and without IP were compared using two-way repeated measures analysis of variance and the Bonferroni multiple comparison test. Ecto-5'-N activity with and without IP was compared with paired t-test. P < 0.05 was considered statistically significant.

RESULTS

Effects of IP on Myocardial Contractile and Metabolic Function During Ischemia

Systolic (144 ± 4 mmHg) and diastolic (84 ± 4 mmHg) blood pressures, heart rate (143 ± 2 beats/min), CBF (90 ± 2 ml·100 g⁻¹·min⁻¹), CPP (104 ± 3 mmHg), FS (24.0 ± 0.9%), LER (23.6 ± 1.1%), MVO₂ (7.1 ± 0.2 ml/dl), the pH of coronary venous blood (7.41 ± 0.04), the Endo-to-Epi flow ratio (1.13 ± 0.04), and the adenosine levels in coronary arterial (19 ± 4 pmol/ml)
and venous (21 ± 4 pmol/ml) blood under the baseline conditions in protocol 1a did not differ from those in other protocols (protocols 1b, 1c, 1d, 2a, 2b, 3a, and 3b) performed in the present study. The hyperemic flow due to IP returned to baseline in 297 ± 7, 292 ± 9, 227 ± 5, and 210 ± 4 s in protocols 1a, 1c, 2a, and 3a, respectively.

CBF (30 ± 1 ml·100 g⁻¹·min⁻¹) and CPP (46 ± 2 mmHg) 10 min after the onset of coronary hypoperfusion in protocol 1a did not differ from those in protocol 1b. LER and the pH in coronary venous blood 10 min after the onset of coronary hypoperfusion in protocol 1a were higher (P < 0.05), and MV̇O₂ and FS at the same time points in protocol 1c were higher (P < 0.05) than those in protocol 1d (Fig. 3). The Endo-to-Epi flow ratio 10 min after the onset of coronary hypoperfusion in protocol 1c did not differ from that in protocol 1d (0.55 ± 0.05 vs. 0.54 ± 0.06). The adenosine levels in coronary venous blood 10 min after the onset of coronary hypoperfusion in protocol 1c were higher (P < 0.05) than those in protocol 1d (812 ± 74 vs. 484 ± 25 pmol/ml).

Effects of IP on Myocardial Contractile and Metabolic Function During Ischemia With an Adenosine Receptor Antagonist

8-SPT did not change hemodynamic and metabolic parameters or the Endo-to-Epi ratio under baseline conditions in protocols 2a and 2b. In protocol 2a, when CBF was decreased to 33% of the control (32 ± 2 ml·100 g⁻¹·min⁻¹), CPP was reduced to 51 ± 2 mmHg,
which was higher (P < 0.05) than that in protocols 1a and 1b. LER (−64 ± 4%), the pH of coronary venous blood (7.13 ± 0.02), MVO$_2$ (2.4 ± 0.2 ml·100 g$^{-1}$·min$^{-1}$), and FS (−1.6 ± 1.4%) in protocol 2a 10 min after the onset of coronary hypoperfusion were lower (P < 0.05) than those in protocol 1a. These parameters 10 min after the onset of coronary hypoperfusion in protocol 2a were comparable to those in protocol 1c. LER, pH of coronary venous blood, MVO$_2$, and FS 10 min after the onset of coronary hypoperfusion in protocol 2a did not differ from those in protocol 1b. These parameters 10 min after the onset of coronary hypoperfusion in protocol 2a were comparable to those in protocol 1c. LER, pH of coronary venous blood, MVO$_2$, and FS 10 min after the onset of coronary hypoperfusion in protocol 2a did not differ from those in protocol 2b (Fig. 4). The Endo-to-Epi ratio 10 min after the onset of coronary hypoperfusion in protocol 2a did not differ from that in protocol 2b (0.57 ± 0.04 vs. 0.58 ± 0.04). 8-SPT increased (P < 0.05) the adenosine levels in coronary venous blood before the onset of coronary hypoperfusion compared with that under baseline condition in protocol 2a (20 ± 5 vs. 25 ± 6 pmol/ml) and 2b (22 ± 4 vs. 28 ± 5 pmol/ml), respectively. The adenosine levels in coronary venous blood 10 min after the onset of coronary hypoperfusion in protocols 2a (763 ± 66 pmol/ml) and 2b (390 ± 23 pmol/ml) were higher than those in protocols 1a and 1b, respectively.

Contractile and Metabolic Function During Ischemia With Inhibition of Ecto-5′-N

AMP-CP did not change hemodynamic and metabolic parameters, the Endo-to-Epi ratio, and the adenosine levels under baseline conditions in protocols 3a and 3b. In protocol 3a, when CBF was decreased to 33% of the control (31 ± 2 ml·100 g$^{-1}$·min$^{-1}$), CPP was reduced to 48 ± 2 mmHg, which was slightly higher than those in protocols 1a and 1b, but it did not reach statistically significance. LER (−61 ± 4%), the pH of coronary venous blood (7.12 ± 0.03), MVO$_2$ (2.5 ± 0.1 ml·100 g$^{-1}$·min$^{-1}$), and FS (0.6 ± 1.1%) 10 min after the onset of coronary hypoperfusion in protocol 3a were lower (P < 0.05) than those values in protocol 1a. These parameters 10 min after the onset of coronary hypoperfusion in protocols 3a were almost comparable to those in protocol 1c. AMP-CP inhibited (P < 0.05) the increases in the adenosine levels in coronary venous blood (94 ± 12 pmol/ml) 10 min after the onset of coronary hypoperfusion. LER, pH of coronary venous blood, MVO$_2$, and FS 10 min after the onset of coronary hypoperfusion in protocol 3a did not differ from those
values in protocol 3b (Fig. 5). The Endo-to-Epi ratio (0.58 ± 0.06 vs. 0.59 ± 0.05) and the adenosine levels in coronary venous blood (128 ± 16 vs. 117 ± 9 pmol/ml) 10 min after the onset of coronary hypoperfusion in protocol 3a did not differ those in protocol 3b.

Effects of IP on Ecto-5'-N Activity

Five-minute occlusion of the LAD increased ecto- and cytosolic 5'-N activity in the LAD-perfused myocardium compared with the LCX-perfused myocardium (Fig. 6).

DISCUSSION

Effects of Endogenous Adenosine on Myocardial Ischemia

We demonstrated that the adenosine levels were much increased when CBF was decreased to 15% or 33% of the control. Blocking adenosine receptors by 8-SPT 10 min after the onset of coronary hypoperfusion decreased LER, pH of coronary vein blood, MV\textsubscript{O}2, and FS, suggesting that blockade of adenosine receptors by itself was deleterious to cardiac function. This finding implies that increased adenosine 10 min after the onset of coronary hypoperfusion improves metabolic and contractile function independently or that it improves metabolic function, resulting in contractile function. The latter is likely, because adenosine inhibits glycolysis (5) and increases glucose oxidation (6), both of which may reduce intracellular acidosis during ischemia (21), and it has negative inotropic effects (9). Adenosine and dipyridamole, an inhibitor of an adenosine transporter, have been believed to induce myocardial ischemia in patients with coronary artery disease (24, 31) by the “coronary steal” mechanism. However, the doses of adenosine and dipyridamole used for the intravenous administration in the clinical setting often reduce systemic blood pressure and subsequently CPP, which may be a major cause for worsening myocardial ischemia in patients with coronary artery diseases. Locally increased adenosine in the ischemic myocardium may play a cardioprotective role without causing significant coronary steal phenomenon.
Mechanisms of IP-Induced Improvement of Anaerobic Myocardial Metabolism

IP increased both LER and the pH of coronary venous blood and decreased both \( \dot{MVO}_2 \) and FS when CBF was reduced to 33% and 15% of the control. This finding suggests that IP increases metabolic parameters as a result of the suppression of contractile function 10 min after the onset of coronary hypoperfusion or that IP increases metabolic parameters and decreases contractile parameters 10 min after the onset of coronary hypoperfusion independently. Since IP increased adenine production 10 min after the onset of coronary hypoperfusion and blockade of adenosine receptor by 8-SPT blunted the changes of metabolic and contractile parameters induced by IP, the IP-induced changes were mediated by adenosine. However, we must consider the possibility that blockade of adenosine receptors 10 min after the onset of coronary hypoperfusion induced a hypometabolic state that could not permit myocardium to be improved by IP. When CBF was reduced to 15% of the control, LER, the pH of coronary venous blood, \( \dot{MVO}_2 \), and FS were reduced to values comparable with those obtained when CBF was reduced to 33% of the control in the presence of 8-SPT. Notably, in this ischemic condition of reduction of CBF to 15% of the control, IP increased LER and the pH of coronary venous blood and decreased \( \dot{MVO}_2 \) and FS. These findings suggest that the effects of IP were apparent even when CBF was reduced to 15% of the control, which was a hypometabolic state comparable with reduction of CBF to 33% of the control in the presence of 8-SPT. Therefore, 8-SPT blunted the effects of IP by the blockade of adenosine receptors but not by inducing the hypometabolic state that cannot be further improved by IP.

If the effects of IP were mediated by increased adenosine, then the finding that IP increased LER and pH of coronary venous blood and decreased \( \dot{MVO}_2 \) and FS seems inconsistent with the results obtained 10 min after the onset of coronary hypoperfusion in the presence of 8-SPT. 8-SPT decreased LER and pH of coronary venous blood, suggested that...
adenosine 10 min after the onset of coronary hypoperfusion may increase MV\textsubscript{O2} and FS as well as LER and pH of coronary venous blood. One possibility to explain this discrepancy is the difference of amount of adenosine produced during coronary hypoperfusion. Since adenosine is reported to inhibit glycolysis resulting in the improvement of anaerobic myocardial metabolism, the improved anaerobic metabolism may improve myocardial contractile function. However, in the case of IP, adenosine appears to decrease contractile function. Adenosine production during coronary hypoperfusion after IP increased by 140% compared with that in the absence of IP. Since exogenous and endogenous adenosine attenuated catecholamine-induced positive inotropic effect, which further improves myocardial metabolic function. Therefore, we assume that the two different directions of contractile function caused elevated adenosine levels, which may depend on the multiplicity of the effects of adenosine and increased levels of adenosine. Although the precise mechanism cannot be clarified in the present study, 8-SPT or AMP-CP blunted the IP-induced changes, suggesting that adenosine produced via the extracellular pathway plays a major role in the IP-induced changes of metabolic and contractile function. We demonstrated that AMP-CP, an inhibitor of ecto\textsuperscript{5}\textsuperscript{8}N, attenuated the increases in adenosine production during coronary hypoperfusion, suggesting that increased adenosine is produced mainly extracellularly by ecto\textsuperscript{5}\textsuperscript{8}N. Furthermore, AMP-CP blunted the IP-induced changes in metabolic and contractile function, suggesting that adenosine produced via the extracellular pathway plays a major role in the IP-induced changes of metabolic and contractile function. One of the possible mechanisms that increase adenosine production via the extracellular pathway during coronary hypoperfusion is the increased ecto\textsuperscript{5}\textsuperscript{8}N activity. We and others have shown that both ischemia and hypoxia increase ecto\textsuperscript{5}\textsuperscript{8}N activity in the canine myocardium (12, 13) and in the isolated rat heart (8). The mechanism of activation of ecto\textsuperscript{5}\textsuperscript{8}N in the myocardium is not well understood. As shown in the present study, one cycle of 5-min ischemia and 10-min reperfusion activates ecto\textsuperscript{5}\textsuperscript{8}N, suggesting that de novo protein synthesis was unlikely to be responsible for the increase in ecto\textsuperscript{5}\textsuperscript{8}N activity. We found that protein kinase C activated by ischemia per se and by norepinephrine released during brief periods of ischemia activates ecto\textsuperscript{5}\textsuperscript{8}N (11). Further investigations are needed to clarify the cellular mechanisms by which ecto\textsuperscript{5}\textsuperscript{8}N is activated. We emphasize that we only show that a correlation between the increased ecto\textsuperscript{5}\textsuperscript{8}N activity and the increased adenosine production. Since hypoxia increases 5'-AMP release from vessels (1), another possible mechanism for the increase in the adenosine levels via the extracellular pathway is an increase in the concentration of 5'-AMP, the substrate for adenosine.
The weakness of our hypothesis is that there are reports that the adenosine levels in the interstitial space are not augmented during sustained ischemia following IP (7, 29), although the adenosine levels in coronary venous blood during coronary hypoperfusion are augmented in the present study. When ecto-5'-N is activated due to IP, the adenosine levels surrounding ecto-5'-N are thought to increase. One possibility to explain this difference between the results of Van Wylen and co-workers (7, 29) and our results is that ecto-5'-N may be activated in endothelial cells more than cardiomyocytes. If this is the case, then, since the adenosine levels in the coronary venous blood are largely affected by endothelial ecto-5'-N, the differences between our study and the other study can be explained. Second, it is possible that even if the adenosine levels in the microenvironment surrounding ecto-5'-N on the cellular membrane are increased by the activation of 5'-N, the alteration of interstitial volume determined by myocardial cellular swelling and the rate of washout due to lymphatic stream may change the interstitial the adenosine levels. In any of these possible situations, the temporal and topical increases in the adenosine levels surrounding ecto-5'-N may be responsible for direct activation of adenosine receptors located at the same cellular membrane, which may not contradict the results of Van Wylen and co-workers (7, 29). This dose juxtaposition may explain how 5'-N activates the adenosine receptors. Further investigation is absolutely necessary to determine this hypothesis between activation of ecto-5'-N activity and adenosine production in IP.

In conclusion, the present study demonstrated that increased adenosine production via an extracellular pathway in the ischemic myocardium reduced the severity of anaerobic myocardial metabolism of ischemic hearts. These findings suggest that a local increase in adenosine production in the ischemic myocardium or the activation of ecto-5'-N may represent new strategies for treating patients with coronary artery diseases.

We thank Makoto Hasegawa for preparing instrumentation of dogs and Kayoko Yoshida and Sachiyu Nomura for measuring 5'-nucleotidase activity.

Tetsuo Minamino is a Research Fellow of Japan Society for the Promotion of Science (JSPS) for Young Scientists. This study was supported by Grant-in-Aid for JSPS Fellows from the Japanese Ministry of Education, Science, and Culture, and by a Japan Heart Foundation/Pfizer Pharmaceuticals Grant for Research on Coronary Artery Disease. Address for reprint requests: M. Kitakaze, First Dept. of Medicine, Osaka Univ. School of Medicine, 2-2, Yamadaoka, Suita, Osaka 565, J. p.


