Role of Ca$^{2+}$-Activated K$^+$ Channels in the Protective Effect of ACE Inhibition Against Ischemic Myocardial Injury

Koichi Node, Masafumi Kitakaze, Hiroaki Kosaka, Tetsuo Minamino, Hidezo Mori, Masatsugu Hori

Abstract—Angiotensin-converting enzyme (ACE) inhibitors increase the production of nitric oxide (NO) and prostacyclin and open Ca$^{2+}$-activated K$^+$ channels. The effects of these actions of ACE inhibitors on infarct size were investigated in open-chest dogs subjected to myocardial ischemia and reperfusion. Infarct size was assessed 6 hours after the onset of reperfusion, subsequent to 90 minutes of occlusion of the left anterior descending coronary artery. The ACE inhibitor cilazaprilat was administered into the coronary artery 10 minutes before coronary occlusion, and infusion was continued until 1 hour after reperfusion. The bradykinin and NO concentrations in coronary venous blood 10 minutes after the onset of reperfusion were significantly higher in dogs treated with cilazaprilat (3 μg · kg$^{-1}$ · min$^{-1}$) than in control animals. Although there were no significant differences in collateral flow during ischemia, infarct size in the cilazaprilat group was smaller than that in the control group (15.1±3.0% versus 46.7±4.2% of the area at risk, $P<0.0001$). The infarct size–limiting effect of cilazaprilat was partially reduced by either $N^6$-nitro-L-arginine methyl ester (an inhibitor of NO synthase) or iberiotoxin (a blocker of Ca$^{2+}$-activated K$^+$ channels) and was abolished by $N^6$-nitro-L-arginine methyl ester plus iberiotoxin. Indomethacin (an inhibitor of cyclooxygenase) had no effect on the beneficial action of cilazaprilat. Inhibition of ACE thus reduced myocardial infarct size, an effect that was mediated by NO and the opening of Ca$^{2+}$-activated K$^+$ channels in canine hearts. (Hypertension. 1998;31:1290-1298.)

Key Words: angiotensin-converting enzyme • nitric oxide • potassium channels • prostacyclin • bradykinin • infarction

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ngiotensin-converting enzyme inhibitors block the degradation of bradykinin by inhibiting kininase II, and the consequent increase in bradykinin concentrations results in the generation of NO through B$^2$ receptor activation in endothelial cells. NO (1) increases CBF during ischemia and reperfusion, (2) inhibits platelet aggregation, (3) reduces catecholamine-induced increases in myocardial contractility, (4) inhibits catecholamine release, (5) reduces Ca$^{2+}$ overload in ischemic and reperfused myocardium, and (6) inhibits neutrophil activation. Thus, enhancement of NO production by ACE inhibitors during ischemia and reperfusion may reduce ischemia and reperfusion injury. Furthermore, bradykinin hyperpolarizes the cell membrane potential by opening K$_{Ca}$ channels. ACE inhibitors potentiate the endothelium-dependent hyperpolarization induced by bradykinin, which may attenuate Ca$^{2+}$ overload during ischemia and reperfusion.

To investigate the effect of an ACE inhibitor on infarct size in canine hearts, we infused cilazaprilat into the coronary artery before and after coronary occlusion. We measured plasma end products of NO metabolism and examined the possibility that any infarct size–limiting effect of the ACE inhibitor was attributable to an increase in NO release or the opening of K$_{Ca}$ channels by L-NAME (an inhibitor of NO synthase) or IBTX (K$_{Ca}$ channel blocker), respectively.

Methods

Instrumentation

Mongrel dogs (body mass, 15 to 23 kg) were anesthetized with intravenous sodium pentobarbital (30 mg/kg of body mass), intubated with a cuffed endotracheal tube, and ventilated with room air mixed with $O_2$ (1.5 L/min) with the use of a respirator. A left thoracotomy was performed through the fifth intercostal space, and the heart was suspended in a pericardial cradle. After the intravenous administration of heparin (500 U/kg), a proximal portion of the LAD was cannulated and perfused with blood through an extracorporeal tube from the left carotid artery. An electromagnetic flow probe (FF-050T, Nihon Kohden) was attached to the bypass tube for measurement of CBF, CPP was measured at the proximal portion of the cannula. The femoral artery was cannulated to obtain blood flow sample for determination of the absolute value of the regional myocardial blood flow. The left atrium was cannulated for microsphere injection. For blood sampling, a small-caliber (1-mm), short (70-mm) tube was inserted into the epicardial vein at the center of the perfused area, and the drained coronary venous blood was returned to the jugular vein. Arterial blood was obtained from the femoral artery.

Heart rate was 141±2 bpm for the intact hearts. Thirty to 40 minutes was required for the setup of the experimental preparation. CPP (101±3 versus 100±2 mm Hg), CBF (88±1 versus 87±2 mL · 100 g$^{-1}$ · min$^{-1}$), LER (24.1±4.5% versus 21.7±2.5%), and pH of the coronary venous blood (7.42±0.02 versus 7.39±0.01) did not differ significantly between 1 and 8 hours after the experimental setup. All studies conformed to the position of the American Heart
in the Effect of ACE Inhibitor on Infarct Size

Protocol 1: Effect of ACE Inhibitor on Infarct Size

After hemodynamic stabilization, coronary arterial and venous blood was sampled for blood gas analysis and determination of the concentrations of bradykinin, end products of NO metabolism (nitrate plus nitrite), lactate, and NE. Hemodynamic parameters, including systolic and diastolic aortic blood pressure, heart rate, CBF, and CBF, were measured. Twenty minutes after the onset of hemodynamic stability, an infusion of either cilazaprilat (0.3, 3, or 30 μg · kg⁻¹ · min⁻¹; Nippon Roche) or vehicle (saline) was initiated into the bypass tube at a rate of 0.1 mL · kg⁻¹ · min⁻¹ and continued until 1 hour after reperfusion with the exception of the occlusion period (number of control dogs, n = 8; ACE inhibitor, 0.3 μg · kg⁻¹ · min⁻¹, n = 7; 3 μg · kg⁻¹ · min⁻¹, n = 7; and 30 μg · kg⁻¹ · min⁻¹, n = 7). Ten minutes after infusion began, the coronary artery was occluded for 90 minutes and then reperfused for 6 hours. The occluder was attached to the bypass tube from a carotid-to-LAD shunt and was clamped at zero CBF. Coronary arterial and venous blood was sampled immediately before coronary occlusion and after 10 minutes of reperfusion; assays were then conducted for bradykinin, lactate, NE, and nitrate plus nitrite. Hemodynamic parameters were measured before sustained ischemia, 80 minutes after the onset of ischemia, and 10 minutes and 3 hours after reperfusion was initiated.

In a preliminary study, we had tested three doses of cilazaprilat (0.3, 3, and 30 μg · kg⁻¹ · min⁻¹) during coronary hyperperfusion (n = 3 at each dose in three dogs). In the groups treated with 0.3 and 3 μg · kg⁻¹ · min⁻¹ cilazaprilat, CBF increased to 35±1 and 42±2 mL · 100 g⁻¹ · min⁻¹, respectively, from 29±2· mL · 100 g⁻¹ · min⁻¹ during coronary hyperperfusion, and the 30 μg · kg⁻¹ · min⁻¹ dose of cilazaprilat increased CBF to 42±2· mL · 100 g⁻¹ · min⁻¹. Therefore, we decided to perform the experiments with doses of 0.3, 3, and 30 μg · kg⁻¹ · min⁻¹ cilazaprilat.

Protocol 2: Roles of NO, Prostacyclin, and Kca Channels in the Effect of ACE Inhibitor on Infarct Size

The infarct size-limiting effect of cilazaprilat was examined in dogs treated with L-NAME, indomethacin (an inhibitor of cyclooxygenase), IBTX, or L-NAME plus IBTX. An infusion of L-NAME (10 μg · kg⁻¹ · min⁻¹, n = 7; Sigma Chemical Co), indomethacin (10 μg · kg⁻¹ · min⁻¹, n = 6; Sigma), IBTX (1 μg · kg⁻¹ · min⁻¹, n = 8; Research Biomedical Institute), or L-NAME plus IBTX (n = 7) into the bypass tube was initiated 10 minutes before infusion of cilazaprilat (3 μg · kg⁻¹ · min⁻¹) or vehicle (20 minutes before the onset of coronary occlusion) and continued (with the exception of the 90-minute occlusion period) until 1 hour after the 6-hour reperfusion period had elapsed. We also determined the effect of L-NAME (n = 7), indomethacin (n = 7), IBTX (n = 7), and L-NAME and IBTX (n = 7) on infarct size. We had previously shown that this dose of L-NAME abolished the release of NO during ischemia. Indomethacin treatment prevented the coronary vasodilatory effect of intracoronary infusion of arachidonic acid (600 μg), demonstrating effective inhibition of cyclooxygenase. This dose of IBTX maximally inhibited coronary vasodilation induced by intracoronary administration of bradykinin (20 ng · kg⁻¹ · min⁻¹), and hemodynamic values and blood samples were taken at the same time as in protocol 1.

In another 20 dogs, we measured the cGMP content of the coronary arteries in the nonischemic myocardium at baseline and ischemic myocardium in control, cilazaprilat (3 μg · kg⁻¹ · min⁻¹), and cilazaprilat + L-NAME (n = 5) each group. Before coronary occlusion or 10 minutes after reperfusion was started, we rapidly removed the epicardial LAD (ischemic region) (n = 6) with precooled stainless steel scissors and tongs and stored the tissue in LN₂.

Protocol 3: Effect of ACE Inhibitor on Infarct Size in Chemically Denervated Hearts

Inhibition of ACE may increase NE uptake into nerve terminals, which may reduce both local NE concentrations and catecholamine-induced injury. To clarify the role of such a decrease in NE concentration in the effects of cilazaprilat, we administered this ACE inhibitor (3 μg · kg⁻¹ · min⁻¹) as in protocol 1 to dogs that had undergone chemical denervation (denervation group, n = 7; ACE inhibitor+denervation group, n = 7). Systemic chemical sympathomimetic was performed by intravenous injection of 6-hydroxydopamine (50 mg/kg body mass) administered in three fractional doses (10, 20, and 20 mg/kg body mass) over 24 hours 5 days before the main experiment. The deleterious side effects of 6-hydroxydopamine were prevented by prior injection of propranolol (1 mg/kg body mass). In a preliminary study, 5 control and 5 denervated dogs were killed after the experimental procedure, and myocardial tissue of the perfused area was sampled for the measurement of NE concentration. The NE content of the denervated and innervated myocardium was 12:53 and 342±22 pg/mg of tissue (mean±SEM; n = 5, P < 0.001), respectively.

Chemical Analysis

MVO₂ (in mL · 100 g⁻¹ · min⁻¹) was calculated as the product of CBF (mL · 100 g⁻¹ · min⁻¹) and the coronary arteriovenous blood oxygen difference (μL/L). Lactic acid concentration was measured by enzymatic assay, and LER was calculated by dividing the coronary arteriovenous difference in lactate concentration by the arterial lactate concentration and multiplying by 100.

Bradykinin Measurement

Bradykinin was measured by radioimmunoassay as described previously. One milliliter of blood from the sample tube was rapidly transferred to a siliconized polyethylene tube containing 4 mL of 96% ethanol; the tube and its contents were then centrifuged at 2500g at 4°C for 15 minutes. The supernatant was decanted into a siliconized 250-mL round-bottomed flask, and the pellet was resuspended in 20 mL of 75% ethanol and recentrifuged. The resulting supernatant was combined with the first supernatant, and after 0.5 mL of octanol was added to prevent frothing, the ethanol was removed and the volume reduced to ≈2 mL by evaporation at 60°C under reduced pressure. The residual solution was acidified with 5 mL of 10 mmol/L HCl and extracted twice with 20 mL of diethyl ether; the ether supernatant was subsequently removed by suction. The aqueous phase remaining in the flask was then evaporated to dryness with a rotary evaporator, and the dry residue was stored at −80°C for 18 hours before assay. The dried samples were redissolved in 2.5 mL of 0.1 mol/L Tris-HCl (pH 7.5) containing 22.5% gelatin, 0.1% neomycin, and 10 mmol/L EDTA. The reaction mixture for the radioimmunoassay consisted of 0.1 mL of 10 mmol/L 1,10-phenanthroline HCl, 0.5 mL of diluent buffer containing the unknown or standard bradykinin, 0.1 mL of antisera diluted 1:600 with diluent buffer, and 0.1 mL of [³⁵S-Tyr]iodobradykinin (∼8000 counts per minute) dissolved in normal saline. The mixture was incubated in a polyethylene tube at 4°C for 24 hours, after which dextran-coated charcoal was added to separate the free labeled antigen from that bound to the antibody. Three replicate tubes containing only buffer, phenan- throline, and [³⁵S-Tyr]iodobradykinin were incubated and treated with.

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**Selected Abbreviations and Acronyms**

ACE = angiotensin-converting enzyme  
CBF = coronary blood flow  
CPP = coronary perfusion pressure  
EDHF = endothelium-derived hyperpolarizing factor  
IBTX =iberotoxin  
Kca = Ca²⁺-activated K⁺ (channels)  
L-NAME = N⁰-nitro-l-arginine methyl ester  
LAD = left anterior descending (coronary artery)  
LER = lactate extraction ratio  
MVO₂ = myocardial oxygen consumption  
NE = norepinephrine  
NO = nitric oxide
dextran-coated charcoal to determine the amount of labeled antigen that remained in the supernatant in the absence of antibody; the resulting mean value was subtracted from the amount of radioactivity in the supernatant of the antibody-containing tubes. The resulting values were used to calculate the proportion of labeled antigen bound to the antibody.

**NE Measurement**

Coronary arterial or venous blood (5 mL) was collected into a tube containing EDTA, immediately placed on ice, and subsequently centrifuged for 20 minutes. The plasma supernatant was stored at −80°C, and within 2 weeks plasma NE was assayed on alumina, segmented by high-performance liquid chromatography (LC-3A system, Zpak-SCX column; Shimadzu Seisakusho), and assayed spectrophotometrically by the trihydroxyindole method (Shimazu spec- trifluorophotometer RF-500LCA). This assay can detect NE at 10 pg · mL⁻¹, and the intra-assay coefficient of variation was 6.8%.

**NO Measurement**

Blood was collected into heparinized tubes and centrifuged within 30 seconds for 5 minutes at 2000g. The plasma fraction was diluted 1:1 with nitrite- and nitrate-free distilled water, and 400 μL of the diluted sample was centrifuged at 2000g in an Ultracentrifuge MC microcentrifuge device (Millipore) to remove substances >10 kDa. The filtrate was analyzed by an automated procedure based on the Griess reaction.¹⁷ In brief, nitrite in the sample was measured by absorbance at 540 nm after being mixed with the Griess reagent, which consisted of 0.1% naphthylethenediamide in 5% (vol/vol) H₂PO₄. Absorbance at 540 nm was also measured after passing the samples through a copper-plated cadmium column to reduce nitrate to nitrite; this value represented the total amount of plasma NO end products (nitrate plus nitrite). The difference in the total concentration of nitrate plus nitrite between coronary venous and arterial blood reflects the amount of NO released from the myocardium.

**Measurement of cGMP Concentration**

After removal of the adventitial connective tissue from the coronary arteries, the frozen material (20 to 40 mg) was ground to a powder and homogenized at 4°C in 1 mL of ice-cold 6% (wt/vol) trichloroacetic acid. The homogenate was centrifuged at 2500g for 20 minutes, and the resulting supernatant was removed, extracted three times with 3 mL of diethyl ether saturated with water, and stored at −80°C. The concentration of cGMP was measured by radioimmunoassay within 7 days. In brief, cGMP in the supernatant (100 μL) was succinylated by incubation for 24 hours at 4°C with 0.1 mL of 125I-labeled succinyl cGMP tyrosine methyl ester (15 000 to 20 000 cpm; concentration<0.01 pmol/mL) and 100 mL of diluted antiserum to cGMP in the presence of chloramine T2¹. The reaction mixture was then added to 800 μL of 0.3 mol/L imidazole buffer (pH 6.5). A portion (100 μL) of the resulting solution was incubated for 24 hours at 4°C with 0.1 mL of 15F-labeled succinyl cGMP (yrosine methyl ester (15 000 to 20 000 cpm; concentration<0.01 pmol/mL) and 100 mL of diluted antiserum to cGMP in the presence of chloramine T2¹. After addition of 0.5 mL of an ice-cold suspension of dextran-coated charcoal the mixture was centrifuged, and 0.5 mL of the supernatant was assayed for radioactivity with a gamma spectrometer. The amount of cGMP was normalized by protein content as assayed by the method of Lowry et al.¹⁶

**Criteria for Exclusion**

To ensure that all of the animals included in the analysis of infarct size data were healthy and exposed to a similar extent of ischemia, the following criteria were adopted to exclude unsatisfactory data: (1) a subendocardial collateral flow >15 mL · 100 g⁻¹ · min⁻¹, (2) a heart rate >170 bpm, or (3) more than two consecutive attempts required to correct ventricular fibrillation with low-energy DC pulses applied directly to the heart.

**Measurement of Infarct Size**

After 6 hours of reperfusion, the LAD was reoccluded and perfused with autologous blood, and Evans blue dye was injected into a systemic vein to determine the anatomic area at risk and the nonischemic area in the heart. The heart was then removed immediately and sliced into serial transverse sections 6 to 7 mm thick. The nonischemic area was identified by blue stain, and the ischemic region was incubated at 37°C for 20 to 30 minutes in sodium phosphate buffer (pH 7.4) containing 1% neotetrazolium chloride (Sigma). Neotetrazolium chloride–stained the noninfarcted myocardium brick red, indicating the presence of a formazan precipitate formed as a result of neotetrazolium chloride reduction by dehydrogenase enzymes in viable tissue. The photographic slides of the entire area at risk area of each heart slice were projected (×10) and traced. The extents of the area at risk and area of necrosis in each slice were then quantified by planimetry, corrected for the weight of the tissue slice, and summed for each heart. Infarct size was expressed as a percentage of the area at risk.

**Measurement of Regional Myocardial Blood Flow**

Regional myocardial blood flow was determined by the microsphere technique.¹⁹ Nonradioactive microspheres (Sekisu Plastic) made of inert plastic and labeled with bromine or zirconium (mean diameter, 15 μm; specific gravity, 1.34 and 1.36, respectively) were suspended in isotonic saline with 0.01% Tween 80 to prevent aggregation. The microspheres were sonicated for 5 minutes and then agitated with a vortex mixer for 5 minutes immediately before injection of ~1 mL of the suspension (2×10⁶ to 4×10⁶ microspheres) into the left atrium, followed by several warm (37°C) saline flushes (5 mL). Microspheres were administered 45 minutes after the onset of coronary occlusion. A reference blood flow sample was obtained from the femoral artery at a constant rate of 8 mL · min⁻¹ for 2 minutes immediately before microsphere injection.

The x-ray fluorescence of the stable heavy elements was measured with a wavelength-dispersive spectrometer (PW 1480 Philips); specifications have been described in detail elsewhere.¹⁹ Myocardial blood flow (mL · 100 g⁻¹ · min⁻¹) was calculated from tissue counts multiplied by reference flow and divided by reference counts. We measured the endocardial blood flow of the inner half of the left ventricular wall.

**Statistical Analysis**

Data are expressed as mean±SEM. Statistical significance was assessed by ANOVA followed by Dunnett’s test with the exception of the effect of collateral blood flow on infarct size; this was analyzed by ANCOVA, with regional collateral flow in the inner half of the left ventricle wall as the covariate. A level of P<0.05 was considered statistically significant.

**Results**

**Mortality and Exclusions**

We excluded nine dogs from data analysis because their subendocardial collateral blood flow was >15 mL · 100 g⁻¹ · min⁻¹. No dogs were excluded because of a heart rate >170 bpm. At least one episode of ventricular fibrillation occurred in 42 dogs; ventricular fibrillation that matched the exclusion criterion was detected in 14 of these animals during ischemia or reperfusion (Table 1).

**Effects of Cilazaprilat on Infarct Size**

No significant differences in either systolic (~142 mm Hg) or diastolic (~86 mm Hg) blood pressure or heart rate (~141 bpm) were detected before sustained ischemia, 80 minutes after the onset of ischemia, or 10 minutes or 3 hours after the onset of reperfusion among the various groups of innervated dogs. Heart rate in the denervated dogs (~121 bpm) was lower than that in the innervated dogs. CPP, CBF, pH of coronary arterial and venous blood, NE concentrations in coronary arterial and venous blood, LER, and MVO₂ did not differ significantly among the innervated dogs immediately
before the onset of 90 minutes of ischemia (Table 2). Relative to baseline values, the arteriovenous difference in bradykinin and NO and cGMP contents of the LAD were significantly increased after 10 minutes of reperfusion in the control group; the increases in these parameters were even greater in dogs treated with cilazaprilat, but the effects of this ACE inhibitor on the arteriovenous difference in NO and cGMP contents were prevented by L-NAME (Figure 1).

The area at risk and collateral blood flow were similar in all groups (Table 3). Cilazaprilat at 0.3 μg · kg⁻¹ · min⁻¹ significantly reduced infarct size, and the effect was more marked at a dose of 3 μg · kg⁻¹ · min⁻¹; the extent of infarct size reduction apparent with cilazaprilat at 30 μg · kg⁻¹ · min⁻¹ was similar to that apparent at 3 μg · kg⁻¹ · min⁻¹ (Figure 2). Furthermore, cilazaprilat (3 μg · kg⁻¹ · min⁻¹) limited infarct size in denervated dogs to an extent comparable to that in innervated animals.

The infarct size–limiting effect of cilazaprilat was reduced by either L-NAME or IBTX and abolished by L-NAME plus IBTX. Indomethacin had no effect on the infarct size–limiting action of the ACE inhibitor (Figure 2). Similar results were obtained by plotting infarct size (normalized by risk area) against the collateral blood flow to the inner half of the LAD-dependent endomyocardium during sustained ischemia (Figure 3).

**Discussion**

We have shown that the ACE inhibitor cilazaprilat reduces myocardial infarct size and that this effect is attenuated by

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**TABLE 2. Baseline Coronary Hemodynamic and Metabolic Parameters Before Sustained Ischemia**

<table>
<thead>
<tr>
<th>Group</th>
<th>CPP, mm Hg</th>
<th>CBF, mL · 100 g⁻¹ · min⁻¹</th>
<th>MVV₀, mL · dL⁻¹</th>
<th>LER, %</th>
<th>pH (A)</th>
<th>pH (V)</th>
<th>NE (A), nmol · L⁻¹</th>
<th>NE (V), nmol · L⁻¹</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>101±3</td>
<td>88.2±0.9</td>
<td>6.45±.2</td>
<td>24.1±4.5</td>
<td>7.42±0.1</td>
<td>7.42±0.2</td>
<td>2.26±0.1</td>
<td>2.30±0.2</td>
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<tr>
<td>ACE-I, 0.3 μg · kg⁻¹ · min⁻¹</td>
<td>102±4</td>
<td>91.5±0.4</td>
<td>6.76±.25</td>
<td>25.2±2.1</td>
<td>7.41±0.2</td>
<td>7.40±0.2</td>
<td>2.22±0.2</td>
<td>2.42±0.1</td>
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<tr>
<td>ACE-I, 3 μg · kg⁻¹ · min⁻¹</td>
<td>101±3</td>
<td>93.2±1.5</td>
<td>6.33±.3</td>
<td>24.9±3.2</td>
<td>7.42±0.3</td>
<td>7.42±0.1</td>
<td>1.88±0.1</td>
<td>2.02±0.1</td>
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<tr>
<td>ACE-I, 30 μg · kg⁻¹ · min⁻¹</td>
<td>102±1</td>
<td>95.6±2.1</td>
<td>6.43±.40</td>
<td>23.7±3.1</td>
<td>7.41±0.2</td>
<td>7.42±0.1</td>
<td>1.92±0.2</td>
<td>2.17±0.1</td>
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<td>ACE-I+L-NAME</td>
<td>99±2</td>
<td>88.2±1.4</td>
<td>5.98±.22</td>
<td>20.2±3.1</td>
<td>7.40±0.2</td>
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<td>2.23±0.1</td>
<td>2.37±0.1</td>
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<td>ACE-I+IBTX</td>
<td>100±3</td>
<td>88.9±2.1</td>
<td>6.19±.43</td>
<td>23.6±2.6</td>
<td>7.41±0.2</td>
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<td>1.78±0.3</td>
<td>1.91±0.2</td>
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<tr>
<td>ACE-I+L-NAME+IBTX</td>
<td>103±4</td>
<td>90.1±1.6</td>
<td>6.89±.18</td>
<td>23.7±2.1</td>
<td>7.43±0.3</td>
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<td>ACE-I+indomethacin</td>
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<td>92.8±1.4</td>
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<td>23.5±2.3</td>
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<td>IBTX</td>
<td>102±5</td>
<td>89.1±2.3</td>
<td>5.56±.21</td>
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<td>6.22±.40</td>
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<td>7.41±0.2</td>
<td>7.42±0.2</td>
<td>1.98±0.2</td>
<td>1.92±0.2</td>
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<tr>
<td>ACE-I (denervation)</td>
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<td>92.2±1.9</td>
<td>6.21±.22</td>
<td>20.0±2.1</td>
<td>7.41±0.1</td>
<td>7.41±0.2</td>
<td>2.63±0.1*</td>
<td>2.77±0.1*</td>
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<tr>
<td>Denervation</td>
<td>100±2</td>
<td>87.2±2.2</td>
<td>6.18±.35</td>
<td>23.7±2.4</td>
<td>7.41±0.2</td>
<td>7.42±0.1</td>
<td>2.55±0.2*</td>
<td>2.68±0.2*</td>
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</table>

(A) indicates arterial; (V), venous; and I, inhibitor. Data are mean±SEM.

*P<0.05 vs control (Bonferroni’s test).
both inhibition of NO synthase and antagonism of KCa channels but not by inhibition of cyclooxygenase in dogs. These results suggest that augmentation of endogenous NO release and the opening of KCa channels induced by ACE inhibitors contribute to alleviation of irreversible ischemia-reperfusion injury.

Endothelium-dependent relaxation in coronary arteries is thought to be attributable to at least three different mechanisms mediated by NO, prostacyclin, and EDHF. Bradykinin is thought to stimulate the release of EDHF as well as that of NO in various endothelium-containing tissues, and EDHF relaxes smooth muscles by opening KCa channels. However, the possible role of the opening of KCa channels in ischemia-reperfusion injury in the heart has not been previously determined. We have now provided in vivo evidence that the opening of KCa channels is an important component in the infarct size-limiting effect of an ACE inhibitor.

This result is predicated on the premise that IBTX is a specific inhibitor of KCa channels, and we have confirmed the specificity of IBTX for the inhibition of KCa channels during myocardial ischemia in the previous study. 13

Validity of the Experimental Model
An important assumption in all of the protocols in the present study is that the intracoronary infusion of vasoactive chemicals, such as cilazaprilat, L-NAME, indomethacin, and IBTX, has no effect on the peripheral vessels, so that the observed changes in the LAD area are due to only local effects on the heart. If pharmacological interventions in the LAD area also affect systemic hemodynamics, then the beneficial effects of the ACE inhibitor may be secondary to systemic hemodynamic effects, such as afterload reduction. However, in the present study, systolic and diastolic blood pressures as well as heart rate were not affected by the intracoronary infusion of pharmacological agents, suggesting that such interventions had minimal if any effects on systemic hemodynamic parameters. Thus, the beneficial effects of cilazaprilat are likely to be attributable to local coronary vascular and myocardial actions rather than to changes in systemic hemodynamic parameters.

ACE inhibitors curtail the accumulation of angiotensin II and accumulate bradykinin in the myocardium. First, since angiotensin II is reported to promote the release of NE from presynaptic vesicles, ACE inhibitors may decrease the release of NE from these vesicles and the subsequent withdrawal of catecholamine injury in the ischemic myocardium. However, in our experiment, there is evidence that beneficial effects of

### Table 3. Risk Area and Collateral Blood Flow During Myocardial Ischemia

<table>
<thead>
<tr>
<th>Group</th>
<th>Risk Area, % of Left Ventricle</th>
<th>Collateral Flow During Ischemia, mL·100 g⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33±2.7</td>
<td>7.6±2.1</td>
</tr>
<tr>
<td>ACE-I, 0.3 µg·kg⁻¹·min⁻¹</td>
<td>33±2.8</td>
<td>7.2±2.3</td>
</tr>
<tr>
<td>ACE-I, 3 µg·kg⁻¹·min⁻¹</td>
<td>36±2.3</td>
<td>7.0±2.2</td>
</tr>
<tr>
<td>ACE-I, 30 µg·kg⁻¹·min⁻¹</td>
<td>41±3.8</td>
<td>7.0±1.8</td>
</tr>
<tr>
<td>ACE-I+L-NAME</td>
<td>31±3.8</td>
<td>7.3±1.2</td>
</tr>
<tr>
<td>ACE-I+IBTX</td>
<td>37±4.4</td>
<td>7.1±1.9</td>
</tr>
<tr>
<td>ACE-I+L-NAME+IBTX</td>
<td>33±2.1</td>
<td>8.0±2.3</td>
</tr>
<tr>
<td>ACE-I+indomethacin</td>
<td>34±3.2</td>
<td>7.0±1.8</td>
</tr>
<tr>
<td>L-NAME</td>
<td>37±2.9</td>
<td>7.0±2.1</td>
</tr>
<tr>
<td>IBTX</td>
<td>39±2.6</td>
<td>6.2±2.0</td>
</tr>
<tr>
<td>L-NAME+IBTX</td>
<td>46±3.2</td>
<td>6.8±2.3</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>35±3.8</td>
<td>8.2±1.5</td>
</tr>
<tr>
<td>ACE-I (denervation)</td>
<td>39±5.4</td>
<td>7.0±1.9</td>
</tr>
<tr>
<td>Denervation</td>
<td>42±4.0</td>
<td>8.7±2.4</td>
</tr>
</tbody>
</table>

I Indicates inhibitor. Data are mean±SEM and did not differ significantly among the groups (Bonferroni's test).

---

**Figure 1.** Effects of cilazapril (3 µg·kg⁻¹·min⁻¹) in the absence or presence of L-NAME on the arteriovenous difference in bradykinin (A) and NO (B) concentrations and cGMP content in the LAD (C) 10 minutes after onset of reperfusion. Data are mean±SEM. *P<0.001 vs baseline value; **P<0.001 vs control group.
the ACE inhibitor were not blunted in the denervated ischemic myocardium. These observations suggest that withdrawal of sympathetic nerve activity is not a likely mechanism of the infarct size–limiting effect. Second, angiotensin II also directly increases myocardial contractility, which may explain the mechanisms for the infarct size–limiting effect of cilazaprilat. However, in a preliminary study, CV11974, an inhibitor of angiotensin II receptors, did not significantly decrease infarct size, suggesting that inhibition of angiotensin II accumulation is not the major factor. Third, our results suggest that bradykinin accumulation due to ACE inhibitor administration is a major factor for the infarct size–limiting effect.

There are several studies showing that the beneficial effects of ACE inhibitors are mediated through prostacyclin.\textsuperscript{21-23} The discrepancy between the previous reports and the present study may be attributable to species differences (rats versus dogs), experimental preparation (isolated hearts versus whole hearts), or factors evaluated (arrhythmia and contractile function versus infarct size).

Mechanisms of ACE Inhibitor–Induced NO Release and $K_{Ca}$ Channel Opening

The present study revealed that cilazaprilat augmented bradykinin release during reperfusion and that L-NAME plus IBTX abolished the infarct size–limiting effect of cilazaprilat, indicating that the cardioprotective effect of the ACE inhibitor is attributable to NO accumulation and the opening of $K_{Ca}$ channels. It is possible that bradykinin activates constitutive NO synthase by increasing both the concentration of inositol trisphosphate and the release of Ca\textsuperscript{2+} in endothelial cells.\textsuperscript{24} It is also possible that the antioxidant properties of ACE inhibitors\textsuperscript{25} prolong the half-life of NO.\textsuperscript{26}

Bradykinin increases the activity of the large conductance $K_{Ca}$ channels in isolated rabbit endothelial cells and
rat Langendruff-perfused hearts. In the present study, IBTX and L-NAME appeared to act in an additive manner in blunting the infarct size–limiting effect of cilazaprilat. The hyperpolarization of vascular smooth muscle cells elicited by EDHF is mediated by an increase in the K<sup>1</sup> conductance of the cell membrane that results from activation of KCa channels. Endothelium-dependent hyperpolarization and subsequent vascular relaxation are inhibited by a blocker of KCa channels in coronary arteries, and the activation of KCa channels appears to play an important role in coronary vasodilation in the ischemic myocardium. Therefore, cilazaprilat may induce the opening of KCa channels in smooth muscle cells via EDHF released from the endothelium.

Mechanisms of Infarct Size–Limiting Effect Mediated by NO and Opening of KCa Channels
Potentiation of NO release may be an effective pharmacological intervention to limit myocardial infarct size, given that administration of an NO donor markedly attenuates ischemia-reperfusion injury in dogs. Several possible mechanisms may underlie the beneficial effects of NO on infarct size. One such mechanism may relate to the observation that NO regulates Ca<sup>2+</sup> current in cardiomyocytes, which may reduce the severity of ischemia by reducing the cytosolic accumulation of Ca<sup>2+</sup>. NO may also reduce MV<sub>O</sub> as a result of a direct negative inotropic effect, inhibition of NE release from sympathetic nerve terminals, and an increase in ATP generation by stimulation of glycolysis. In addition to the energy-sparing effects of NO on the myocardium, cGMP-mediated coronary vasodilation may help reduce myocardial ischemia. NO may also inhibit platelet aggregation in the ischemic heart. On the other hand, the NO synthase inhibitor N<sup>6</sup>-monomethyl-L-arginine preserved myocardial function after ischemia/reperfusion in the isolated rat heart, and L-NAME protected the isolated working rabbit heart from ischemia-reperfusion injury. The reason for these dis-
crepan observations is unclear but may be related to differences in the duration of the ischemic period, the degree of production of $O_2^-$, the route of administration of NO synthase inhibitors, or the change in blood pressure induced by L-NAME. The opening of $K_{ca}$ channels may hyperpolarize the cellular membrane and reduce Ca$^{2+}$ overload during ischemia and reperfusion, and these effects may mediate a protective effect that is similar to the opening of ATP-sensitive $K^+$ channels.37

Clinical Implications

ACE inhibitors are effective in reducing infarct size after myocardial ischemia and reperfusion.38,39 Martorana et al9 showed that via the action of kinins, ACE inhibitors reduce infarct size from 55% to 25% of the area at risk in dog hearts. Furthermore, ACE inhibitors also inhibit ventricular remodeling after acute myocardial infarction, thereby preventing enlargement of the ventricles.40 Our data contribute to a clarification of the roles of NO and $K_{ca}$ channels in the beneficial effects of ACE inhibitors in a canine experimental model of ischemic heart disease. Meanwhile, the number of large clinical studies is growing, and they are showing the efficacy of ACE inhibitors for the treatment of myocardial infarction (eg, SAVE41 and AIR24).

Acknowledgments

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Role of Ca\(^{2+}\)-Activated K\(^{+}\) Channels in the Protective Effect of ACE Inhibition Against Ischemic Myocardial Injury

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