Role of Endothelin-A Receptors in Ischemic Contracture and Reperfusion Injury

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Background—Circulating endothelin (ET)-1 is elevated in ischemia/reperfusion and may exert proischemic effects. The aim of the present study was to characterize the effects of ET-1 in rat isolated hearts using subtype-selective ET receptor antagonists, agents modulating the cytosolic Ca\(^{2+}\) concentration, or the activity of cGMP-dependent protein kinase.

Methods and Results—Rat hearts perfused at constant pressure were made ischemic by reducing flow to 0.2 mL ⋅ min\(^{-1}\) ⋅ g\(^{-1}\), followed by reperfusion at normal pressure (each phase, 25 minutes). Drugs were infused during the ischemic phase only. Parameters monitored were extent and time-to-onset of contracture in ischemia, left ventricular developed pressure (LVEDP), coronary flow (CF), and diastolic relaxation during reperfusion. The ET\(_A\) receptor-selective antagonist PD 155080 (50 nmol/L) reduced peak ischemic contracture (−49%) and delayed its time to onset (−56%) and improved recovery of reperfusion LVEDP (−12%), CF (−16%), and diastolic relaxation (−50%). Infusion of an ET\(_A\)/ET\(_B\)-nonselective antagonist, PD 142893 (200 nmol/L), had similar effects on all parameters, whereas infusion of BQ-788 (20 nmol/L), an ET\(_B\) receptor-selective antagonist, was without effect. Exogenous ET-1 (100 pmol/L) hastened contracture and increased its extent (−23%) and reduced recovery of both LVEDP (−31%) and CF (−18%), effects that were counteracted by HOE 642 (10 μmol/L), a Na\(^+/H^+\) exchange inhibitor, but not by nicardipine (30 μmol/L), a Ca\(^{2+}\) entry blocker; activation of cGMP-dependent protein kinase by the cell-permeable cGMP analog Sp-8-p-chlorophenylthio-guanosine-3',5'-cyclic monophosphorothioate (10 μmol/L) improved function without preventing the effects of ET-1.

Conclusions—The data indicate that ET-1 exacerbates ischemic contracture and worsens ventricular and coronary reperfusion dysfunction by activating ET\(_A\) receptors via a mechanism likely involving activation of Na\(^+/H^+\) exchange in this model. (Circulation. 1998;97:391-398.)

Key Words: endothelin ■ nitric oxide ■ ischemia ■ reperfusion ■ hemodynamics

Endothelium is synthesized in vascular and endocardial endothelial cells and exerts potent cardiovascular actions, including physiological vasodilation, pathophysiological vasoconstriction, and positive inotropic actions. The effects of ET-1 are mediated by distinct receptors now classified into two subtypes: ET\(_A\) and ET\(_B\). The ET\(_A\) receptor is expressed in vascular smooth muscle cells, whereas the ET\(_B\) receptor has been localized to endothelial cells and vascular smooth muscle cells. The role of these receptor subtypes in maintaining coronary and peripheral vascular tone, in both experimental animals and humans, is gradually being unraveled, but the direct myocardial actions of ET-1 have yet to be clearly defined. These actions include variable effects on contractility, a deleterious effect on diastolic relaxation, and direct arrhythmogenic effects. A general difficulty with a number of studies is the use of very high (nanomolar and higher) concentrations of ET-1, whereas cardiac tissue levels in both health and disease are in the low picomolar range. Therefore, the use of receptor antagonists may yield more physiologically relevant results.

A prominent feature of ischemic dysfunction in rat hearts perfused at physiological temperature is myocardial contracture, defined as a rise in resting force (or tension) during prolonged global ischemia. This phenomenon has attracted much attention since the recognition of the “stone heart” as a complication of cardiopulmonary bypass. Severe contracture heralds the onset of irreversible injury during coronary occlusion. Two primary cellular mechanisms have been proposed: (1) severe depletion of ATP with inability to sequester Ca\(^{2+}\) and (2) a raised diastolic cytosolic Ca\(^{2+}\) concentration. Although Ca\(^{2+}\) entry blockers of different classes may prevent ischemic contracture, the pathophysiological mediators of Ca\(^{2+}\) entry are not known.

In view of the potent Ca\(^{2+}\)-dependent effects of ET-1 and its increased release in ischemia and/or reperfusion, we hypothesized that endogenous ET-1 may mediate or exacerbate ischemic contracture and reperfusion myocardial and vascular dysfunction, possibly via a receptor subtype-selective action. Therefore, we investigated whether ET receptor...
subtyping-selective antagonists affected time to onset and extent of ischemic contracture. The ET receptor antagonists used in the present study were PD 155080 (subtype A), BQ-788 (subtype B), and, for comparison, the subtype-nonselective buffer composed of (in mmol/L): NaCl 118, NaHCO3 25, KH2PO4 1.2, KCl 4.8, MgSO4 1.2, CaCl2 1.25, glucose 11 with the use of the mounting within 2 minutes of thoracotomy for retrograde perfusion. Rats were anesthetized with diethyl ether, and the hearts were rapidly mounted within 2 minutes of thoracotomy for retrograde perfusion. An epicardial ECG was recorded throughout the experimental period as described previously. Ventricular tachycardia was defined as three or more consecutive, morphologically similar ventricular complexes. The test substances PD 155080 (5, 50, and 500 nmol/L), PD 142893 (200 nmol/L), BQ-788 (2, 20, and 200 nmol/L), ET-1 (100 pmol/L), HOE 642 (100 nmol/L), nicardipine (30 µmol/L), thapsigargin (5 µmol/L), Sp-8-pCPT-cGMPS (10 µmol/L), and Rp-8-pCPT-cGMPS (5 µmol/L) were added to the perfusion medium during the ischemic phase only. In some cases, the NO donor SNAP (200 µmol/L) and L-NNA (100 µmol/L) were also used.

Evaluation of Ventricular Arrhythmias
An epicardial ECG was recorded throughout the experimental period as described previously. Ventricular tachycardia was defined as three or more consecutive, morphologically similar ventricular complexes, and ventricular fibrillation was defined as six or more rapid, morphologically irregular ventricular complexes.

Measurement of Ischemic Contracture
Ischemic contracture was taken as the rise in diastolic tension after the onset of ischemia. It was calculated as the ratio of the peak diastolic pressure reached during ischemia over the control LVEDP (peak systolic minus diastolic pressure) and expressed as a percentage. Time to onset was defined as the time when diastolic pressure reached 5% of control LVEDP.

Determination of ET-1
The peptide was concentrated by solid-phase extraction followed by quantitative RIA as described previously. Briefly, coronary effluents were chromatographed on C2 Ethyl Spe-ed cartridges, ET-1 was eluted with acetonitrile (70%), the elute was freeze-dried, and ET-1 contained in the sediment was dissolved in buffer and determined with a sensitive RIA using an antibody specific for ET-1 without cross-reactivity for other ET isomers (RAS 6901; Peninsula Laboratories).

Drugs and Chemicals
PD 155080 (2-benzo[1,3]dioxol-5-yl-3-benzyl-4-(4-methoxy-phenyl)-4-oxobut-2-enoate sodium salt; lot U), BQ-788 (N-α-tosyl-2,6-dimethyl-piperdinocarbonyl-L-γ-methylleucyl-d-1-methoxy carbonyl-γ-thyrtosphanyl-d-norleucine sodium salt; internal code: PD 169000–0015; lot P), and PD 142893 (Ac-o-3,3-diphenylalanine-4-Asp-L-Ile-L-Ile-L-Trp disodium salt; lot 7/V) were gifts from Dr Annette Doherty (Parke-Davis Pharmaceutical Research, Ann Arbor, MI). HOE 642 (4-isopropyl-3-methylsulfonylbenzoyl-guanidine-methanesulfonate) was a gift from Dr Gabriele Wiemer (Hoechst AG, Frankfurt, Germany). Thapsigargin was from Alamone Labs, nicardipine hydrochloride was from Yamanouchi Chemicals, Sp-8-pCPT-cGMPS and Rpe-8-pCPT-cGMPS (sodium salts) were from Biolog Life Science Institute, L-NNA hydrochloride was from Sigma Chemical, and SNAP was from Tocris Cookson. Drugs were freshly dissolved in perfusion buffer at the final concentrations given in the text.

Presentation of Data and Statistical Analysis
Group data are presented as arithmetic mean±SEM values for five hearts in each group (where applicable, data were normalized to 1 g of heart wet wt). Hemodynamic parameters were subjected to a two-way ANOVA for repeated measurements to account for different treatments (control, ischemia, reperfusion) and factors (vehicle and drugs).

Experimental Protocol
Hearts were perfused for 15 minutes to establish stable perfusion conditions (equilibration period), followed by control perfusion for 25 minutes, after which measurements were taken (baseline). Hearts were then perfused at 0.2 mL · min⁻¹ · g⁻¹ of heart wet wt for 25 minutes (ischemic period) and reperfused at normal pressure for another 25 minutes (total duration of experiment, 90 minutes). A model of low-flow ischemia was chosen in accordance with our previous studies showing that low-flow, but not total global, ischemia stimulated ET-1 secretion in rat hearts. The study substances PD 155080 (5, 50, and 500 nmol/L), PD 142893 (200 nmol/L), BQ-788 (2, 20, and 200 nmol/L), ET-1 (100 pmol/L), HOE 642 (100 nmol/L), nicardipine (30 µmol/L), thapsigargin (5 µmol/L), Sp-8-pCPT-cGMPS (10 µmol/L), and Rp-8-pCPT-cGMPS (5 µmol/L) were added to the perfusion medium during the ischemic phase only. In some cases, the NO donor SNAP (200 µmol/L) and L-NNA (100 µmol/L) were also used.

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facilitate comparison between treatments. The χ² test was used to analyze the incidence of ventricular tachycardias or fibrillations. A probability of <5% was considered significant.

**Results**

**Functional Effects of Test Drugs in the Absence of Ischemia**

First, the effects of test drugs were studied in normoxic perfused hearts to document their functional effects independent of ischemia. The compounds were infused over 25 minutes (ie, between the 40th and 65th minute to match infusion during the ischemic period), and functional parameters were monitored up to 90 minutes. None of the compounds significantly affected heart rate or LVDevP. ET-1 reduced coronary flow from 10.1±0.2 mL/min (baseline) to 8.0±0.2 mL/min at 65 minutes (P<.05). This vasoconstrictor effect was still present at the end of 90 minutes’ perfusion (7.2±0.1 mL/min). When ET-1 was infused together with Sp-8-pCPT-cGMPS or HOE 642, coronary flow was restored to vehicle level. Coronary flow was not affected by ET receptor antagonists or HOE 642.

**Role of ET Receptor Subtypes in Exacerbating Ischemic Contracture**

In the absence of drug (vehicle), peak ischemic contracture (attained after ≈15 minutes of ischemia) was 38±2 mm Hg (ie, 48±3% of control LVDevP). The effects of the ET₄ receptor-selective antagonist PD 155080 and the ET₄ receptor antagonist BQ-788 on peak contracture are shown in Fig 1. PD 155080 reduced peak contracture in concentration-dependent fashion, but BQ-788 was without effect (48±2%). In comparison, the mixed ET₄/ET₅ receptor antagonist PD 142893 reduced peak contracture to the same extent (22±1%) as PD 155080. The corresponding concentration-effect data for time to onset of ischemic contracture are given in Fig 2. In the absence of drug (vehicle), time to onset was 8.9±1.1 minutes. PD 155080 delayed the occurrence of contracture, whereas BQ-788 was without effect, and PD 142893 delayed time to onset to a similar extent as PD 155080 (16.2±0.6 minutes; 1.8-fold). In all subsequent experiments, PD 155080 was used at 50 nmol/L and BQ-788 was used at 20 nmol/L to retain the desired subtype selectivity.

**Role of Intracellular Ca²⁺ in ET-1–Mediated Effects on Ischemic Contracture**

We investigated the effect of exogenous ET-1 and several compounds presumably affecting intracellular Ca²⁺ homeostasis on ischemic contracture. Peak contracture (Fig 3) was increased through a pathophysiological concentration of exogenous ET-1 (100 pmol/L) (59±1%), whereas it was reduced by the Na⁺/H⁺ exchange inhibitor HOE 642 (26±2%) or the Ca²⁺ entry blocker nicardipine (28±1%); thapsigargin increased contracture to a similar extent as ET-1 (58±3%). The effect of ET-1 was reduced by HOE 642 to the level observed in the absence of ET-1 (29±3%), but neither nicardipine nor thapsigargin significantly affected ET-1–induced peak contracture.

Similar results were obtained for time to onset of ischemic contracture (Fig 4). Compared with vehicle (8.9±1.1 minutes), HOE 642 and nicardipine delayed time to onset 1.5-
fold, whereas it was shortened by exogenous ET-1 (5.6 ± 0.4 minutes) or thapsigargin (5.6 ± 0.4 minutes). When infused together with ET-1, HOE 642 prevented the deleterious effects of the peptide, so time to onset of contracture was not different from vehicle level, whereas nicardipine did not delay ET-1–induced contracture; the contracture hastening effect of thapsigargin was not increased further in hearts coinfused with ET-1.

cGMP-Dependent Protein Kinase and ET-1–Mediated Effects on Ischemic Contracture

We studied the effects of a stimulator and inhibitor of cGMP-dependent protein kinase in the presence of exogenous ET-1 or ET receptor blockade on ischemic contracture. Compared with vehicle (48 ± 3%), peak contracture (Fig 5) was similarly reduced by stimulating the kinase with Sp-8-pCPT-cGMPS in both the absence (27 ± 2%) and presence of PD

155080 (24 ± 1%). The deleterious effects of exogenous ET-1 were not prevented, only reduced to vehicle level. The protein kinase inhibitor Rp-8-pCPT-cGMPS by itself worsened peak contracture (57 ± 3%), potentiated the deleterious effect of ET-1 (63 ± 2%; P < .05 versus vehicle and versus Sp-diastereoisomer in each case), but also diminished the protective effect of PD 155080 (40 ± 3%; P < .05 versus Sp-diastereoisomer). With respect to time to onset of contracture, a similar protection was observed with Sp-8-pCPT-cGMPS and a similar deterioration was observed with Rp-8-pCPT-cGMPS (Fig 6). The NO donor SNAP (200 μmol/L) was similarly protective as Sp-8-pCPT-cGMPS, whereas the NO synthase inhibitor L-NNA (100 μmol/L) was similarly injurious as exogenous ET-1 vis-à-vis both aspects of contracture (n = 5 in each case; data not shown).

Effects on Reperfusion Myocardial and Vascular Function

For vehicle, baseline heart rate was 298 ± 5 bpm, LVDevP was 80 ± 2 mm Hg, CF was 9.8 ± 0.3 mL/min, and LVEDP was 5.1 ± 1 mm Hg; at end of reperfusion, heart rate had completely recovered, LVDevP was 62 ± 2 mm Hg (78% recovery), CF was 7.1 ± 0.1 mL/min (72% recovery), and LVEDP was 12 ± 1 mm Hg (2.4-fold increase). The effects of test drugs on reperfusion function compared with vehicle are shown in Fig 7. Recovery of LVDevP (Fig 7A) was improved by PD 155080, PD 142893, HOE 642, and Sp-8-pCPT-cGMPS; recovery was unaffected by BQ-788 and reduced by exogenous ET-1, nicardipine, and Rp-8-pCPT-cGMPS. The deleterious effect of exogenous ET-1 on LVDevP recovery was prevented in the presence of HOE 642, reduced to vehicle level by Sp-8-pCPT-cGMPS, but little affected in the presence of Rp-8-pCPT-cGMPS or nicardipine. A similar pattern of protection or deterioration was observed for coronary flow (Fig 7B) and LVEDP, a measure of diastolic relaxation (Fig 7C).

Effect on Reperfusion Cardiac Rhythm

After reintroduction of normal coronary flow, hearts quickly resumed beating and stabilized within several minutes. As
would be expected for a model of global ischemia of medium duration, the incidence of reperfusion ventricular tachyarrhythmias (ventricular tachycardia and/or reversible ventricular fibrillation) was high (80%) in these unpaced hearts (Fig 8). No episodes of irreversible ventricular fibrillation were observed. Treatment of hearts with PD 155080, PD 142893, HOE 642, HOE 642 plus ET-1, Sp-8-pCPT-cGMPS, or Sp-8-pCPT-cGMPS plus ET-1 reduced the incidence of arrhythmias, but the other drugs exerted no significant effect.

**Effect on ET-1 Secretion**

For vehicle, the secretion rate of ET-1 under normoxic conditions was 0.27±0.004 pg/min, reduced during ischemia (~60%), and increased twofold to threefold within the first minutes of reperfusion; secretion was back to baseline level within ~30 minutes. The corresponding ET-1 concentrations were 0.029±0.0006 pg/mL at baseline, 0.54±0.025 pg/mL during ischemia (20-fold increase), and 0.08±0.002 pg/mL on reperfusion (2.7-fold increase) (data not shown). The effects of SNAP, L-NNA, HOE 642, and Sp-8-pCPT-cGMPS on ET-1 secretion into coronary effluent are shown in Fig 9. SNAP and Sp-8-pCPT-cGMPS decreased ET-1 secretion in ischemia (~35% and ~18%) and reperfusion (~28% and ~26%); L-NNA increased it 1.5-fold (mean) in reperfusion. HOE 642 was without effect on ET-1 secretion in ischemia and reperfusion.

**Discussion**

This study supports the view that activation of ET<sub>A</sub> receptors by endogenous ET-1 exacerbates ischemic and reperfusion...
injury. We also showed that stimulating the NO/cGMP pathway or inhibiting Na⁺/H⁺ exchange counterbalanced the deleterious effects of ET₁ receptor stimulation, whereas a Ca²⁺ entry blocker was ineffective. Although drugs were administered during ischemia only, heart function was clearly affected in both ischemia and reperfusion in this rat heart model.

**Selectivity of ET Receptor Antagonists**

PD 155080 and BQ-788 have previously been shown to be selective for different ET receptor subtypes. In binding studies using human ventricular membranes, PD 155080 was a potent competitive inhibitor with an IC₅₀ value of 7.8 nmol/L at ET₁ receptors and 3.5 µmol/L at ET₂ receptors (>400-fold selectivity). A reverse biochemical and pharmacological profile was found for BQ-788. This compound potently inhibited binding of ET-1 to ET₂ receptors on human heart cells (IC₅₀ ≈1 nmol/L) and competitively antagonized the vasoconstriction of rabbit pulmonary artery induced by an ET₂-selective agonist (Kᵣ = 4 nmol/L). The very low antagonist concentrations used in this study ensured that only one subtype was occupied at a time. The efficacy of the two compounds was tested in concentration-response curves that clearly showed the deleterious effects of ET-1 are mediated exclusively by ET₁ receptors (Figs 1 and 2).

**Effects of Test Compounds on Cardiac Function in Absence of Ischemia**

When tested in hearts not subjected to ischemia, neither ET receptor antagonists, Sp-8-pCPT-cGMPS, or HOE 642 affected myocardial and coronary function, indicating that these drugs did not by themselves alter the effects of ischemia; in particular, they had no negative inotropic effect that could have decreased the severity of ischemia. However, exogenous ET-1 reduced coronary flow by ≈25%, which may have contributed to its contracture-hastening actions, but did not affect reperfusion ventricular function, as would be expected in these buffer-perfused hearts known to have a high coronary reserve. Thus, the interpretation of the present results, obtained through infusion of test drugs during the ischemic phase only, appears simpler than that of several previous investigations in which test agents were infused starting before ischemia and infusion was continued throughout the ischemic and reperfusion periods or started within the ischemic period and maintained for part of the reperfusion period.

**Effects of ET-1 on Diastolic Pressure-Volume Relationship (Ischemic and Reperfusion Contracture)**

One of our important findings is that endogenous ET-1 augments myocardial and coronary injury induced by ischemia/reperfusion by consistently activating one subtype, ET₁ receptors. The activation of these receptors potently hastened onset and increased peak ischemic contracture and increased diastolic stiffness throughout reperfusion. The mechanism behind this rise in diastolic tension is largely unknown but may involve an increase in intracellular Ca²⁺ concentration (Ca²⁺ overload), depletion of energy stores due to the positive inotropic effect of ET-1, or increased Ca²⁺ sensitivity of myofibrils. Evidence for these explanations includes the direct Ca²⁺-increasing effects of exogenous ET-1 in cultured myocytes, the reduction in glycogen stores by exogenous ET-1 in reperfused hearts, and the potent positive inotropic effect of picomolar concentrations of ET-1 in isolated myocytes accompanied by changes in intracellular Ca²⁺ concentration. Complementary evidence is provided by the inhibition of ischemic contracture, and attendant cardioprotection on reperfusion, by nisoldipine, an L-type Ca²⁺ channel antagonist. In any event, the increased production/release of ET-1 during ischemia or reperfusion, possibly together with an increased availability of ET-1 binding sites through externalization triggered in these states, clearly exerted proischemic effects in the present model that were attenuated by antagonists with high affinity for ET₁ receptors. It is inferred that the contracture-enhancing effect exerted by 100 pmol/L ET-1 was also mediated by ET₁ receptors. Using another antagonist, BQ-123, others have also concluded that ET₁ receptors mediate the proischemic effects of exogenous ET-1. ET₁ receptors are also expressed by cardiac myocytes, although in comparatively low density (one tenth of ET₂ receptors), but did not mediate any of the proischemic effects. Rather, they appear to be involved in the inactivation of ET-1 by binding and sequestering the peptide.

The Na⁺/H⁺ exchange inhibitor HOE 642 effectively antagonized the ischemic rise in left ventricular diastolic pressure and abolished the contracture-hastening effects of exogenous ET-1. These results are in support of a deleterious role for ET₁-mediated activation of Na⁺/H⁺ exchange in the ischemic heart and agree with a similar previous conclusion reached with methylisobuthyl amiloride, a rather nonspecific inhibitor of the antipporter. ET-1 is known to activate the Na⁺/H⁺ exchanger in the heart, resulting in intracellular alkalization as well as increased myofilament sensitivity to intracellular Ca²⁺. Therefore, it is likely that stimulation of Na⁺/H⁺ exchange consequent to activation of ET₁ receptors of cardiac myocytes contributes to the increase in resting tension induced by ET-1.

The role of Ca²⁺ in ET-1 action was probed with nicardipine, which inhibits Ca²⁺ entry via L-type Ca²⁺ channels, or thapsigargin, which inhibits its reuptake from the cytoplasm into the sarcoplasmic reticulum. Although nicardipine by itself was similarly protective as the ET₁ receptor antagonist (see Figs 1 through 4), the deleterious effect of exogenous ET-1 was hardly affected. On the other hand, the contracture-hastening effect of thapsigargin, presumably due to increased cytosolic Ca²⁺ activity, was not increased further by ET-1 (Fig 4). Thus, although Ca²⁺ influx during ischemia clearly contributed to contracture development, the proischemic action of ET-1 appears not to be directly dependent on Ca²⁺.

**Recovery of Reperfusion Contractile Function**

Despite antagonism of ET receptors during the ischemic phase only, both ischemic and reperfusion functions were improved in this study. In fact, the two receptor antagonists PD 155080 and PD 142893 ameliorated recovery of cardiac function throughout reperfusion, resulting in a higher recovery of LVEDP, higher coronary flow, and better diastolic function (lower LVEDP). The similar protection obtained with HOE
642 and nicardipine and the corresponding deterioration with thapsigargin support the view that improved reperfusion function was likely the result of a better maintained Ca\(^{2+}\) homeostasis during ischemia. However, because the deleterious effects of ET-1 were considerably reduced in the presence of HOE 642 but not of nicardipine, it is doubtful whether Ca\(^{2+}\) mobilization plays a major role as part of ET-1-mediated damage. The amelioration of function with HOE 642 was likely due to attenuation of the deleterious effects of ET-1 during ischemia, rather than a reduced release of the peptide, because the latter was unaffected (Fig 9). Other authors have also studied the effects of ET-1 on reperfusion myocardial function with varying results, probably due to differences in experimental design. In the study by Khandoudi et al,\(^{27}\) ET-1 (0.4, 2, and 4 nmol/L, ie, 4 to 40 times the concentration used here) was applied during the reperfusion phase and resulted in depressed contractile recovery, which was completely reversed by methyisobutyl amiloride, suggesting a causal role for ET-1-mediated activation of Na\(^+\)/H\(^+\) exchange in reperfusion injury independent of ischemia.

Reperfusion Arrhythmias
Recent studies have established that exogenous ET-1 has direct proarrhythmic effects on rat myocardium. With low concentrations of ET-1 likely to be attained in vivo (40 or 50 pmol/L), the peptide has increased the incidence of reperfusion ventricular extrasystoles\(^{30}\) and ventricular fibrillation.\(^{36}\) These arrhythmogenic effects were in all likelihood mediated by ETA receptors without participation of ETB receptors, as evident from the profile of activity of the antagonists used in the present study. These receptors are probably localized on the myocardial cell and may be activated by ET-1 generated locally by cardiomyocytes as a consequence of ischemia.\(^{37}\) Although ET-1–induced coronary vascular injury, possibly leading to the no-reflow phenomenon, may have contributed to the proarrhythmic effect, the increased availability of cytosolic Ca\(^{2+}\) in myocytes was probably the predominant mechanism as evident from the potent inhibitory effects of HOE 642, SNAP, and the cGMP analog (see below).

Myocardial and Coronary Protective Effects of the NO/cGMP System and Mechanism of ET-1 Action
Another important finding of this study is the functional antagonism exerted by the NO/cGMP system vis-à-vis the deleterious effects of exogenous and endogenous ET-1, during both ischemia and reperfusion. This was evident from the positive effect of Sp-8-pCPT-cGMPS (and SNAP) and the deleterious effect of Rp-8-pCPT-cGMPS (and NO synthase inhibition by L-NNA) on ischemic contracture and all aspects of reperfusion function studied. In addition, SNAP and Sp-8-pCPT-cGMPS inhibited ET-1 release during reperfusion (Fig 9), probably as a consequence of raised intracellular Ca\(^{2+}\) levels.\(^{35}\) The mechanism of ET-1 action was probed using the activator and inhibitor of cGMP-dependent protein kinase, in both the absence and presence of ET receptor activation and blockade. Clearly, the proischemic effect of 100 pmol/L ET-1 was reduced but not prevented by Sp-8-pCPT-cGMPS, indicating that ET-1 exerts its effects through a mechanism unrelated to this pathway. On the other hand, in the combined presence of Sp-8-pCPT-cGMPS and PD 155080, protection was different from that observed in the presence of Sp-8-pCPT-cGMPS alone (Fig 5) or PD 155080 alone (Fig 1), which might suggest a role of the NO/cGMP pathway for endogenous ET-1 in mediating ischemic contracture. Also, both peak and time to onset of contracture were not significantly different after infusion of Rp-8-pCPT-cGMPS alone, Rp-8-pCPT-cGMPS together with ET-1, or infusion of ET-1 alone, which supports the involvement of the NO/cGMP pathway in ET-1 action, whereas data for Rp-8-pCPT-cGMPS together with PD 155080 do not (P<.05 versus Rp-8-pCPT-cGMPS alone; Figs 5 and 6). Thus, the precise relationship between NO/cGMP and ET-1 is unclear on the basis of these experiments, and further studies are required.

Antagonism of ETA receptors or substitution of NO was also effective in increasing reperfusion coronary flow. Recently, ET-1 was shown to contribute to abnormal endothelium-dependent relaxation after ischemia/reperfusion in rabbit hearts,\(^{38}\) possibly due to impaired efficacy of endogenous NO,\(^{39}\) which may allow Ca\(^{2+}\)-mediated damage to progress unchecked.

Possible Clinical Application
In human heart, in situ hybridization showed ET\(_A\) and ET\(_B\) receptor mRNA localized to ventricular and atrial myocardium and the atrioventricular and endocardial conducting system,\(^{40}\) and these observations have been verified with receptor autoradiographic studies.\(^{41}\) Moreover, plasma ET-1 levels are elevated in patients with heart disease,\(^{2}\) and cardiac ET-1 production is stimulated in patients undergoing reperfusion procedures. The present study clearly indicates the ability of endogenous ET-1 to impair ventricular and coronary function after ischemia/reperfusion and suggests that ET\(_A\) receptor antagonists might be effective in diminishing the deleterious effects of ET-1 in clinical situations, including cardiopulmonary bypass and acute myocardial infarction.\(^{42,43}\)

Study Reservations
Although the pharmacological compounds used in this study are widely used agents in experimental investigations of this kind and some of them partly or completely reversed the proischemic effects of ET-1, this by itself does not constitute proof that these pathways specifically mediate the ET-1–related enhancement in ischemic injury. Therefore, the conclusions drawn, especially regarding to the mechanism by which ET\(_A\) receptor stimulation results in deleterious mechanical effects, are, of necessity, somewhat inferential. Additional studies are in progress regarding the role of intracellular Ca\(^{2+}\) in the deleterious effects of ET-1 through the use of direct measurements of Ca\(^{2+}\) with aequorin. In addition, the role of the endogenous NO/cGMP system and its modulatory effect on the ET system, possibly involving changes in cytosolic Ca\(^{2+}\) levels, must be addressed.

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Endogenous Endothelin and Ischemia/Reperfusion Damage

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