Inhibition of Inositol(1,4,5)Trisphosphate Generation by Endothelin-1 During Postischemic Reperfusion
A Novel Antiarrhythmic Mechanism
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Background—Reperfusion of ischemic rat hearts in the presence of thrombin or norepinephrine but not endothelin-1 causes the generation of inositol 1,4,5-trisphosphate (Ins 1,4,5P₃) and arrhythmias. The present study investigates the effect of endothelin-1 on these responses.

Methods and Results—Ins 1,4,5P₃ generation was quantified by use of [³H] labeling and high-performance liquid chromatography as well as by mass analysis. Twenty minutes of global ischemia followed by 2 minutes of reperfusion increased [³H]Ins 1,4,5P₃ from 2828±265 to 5033±650 cpm/g tissue in the presence of thrombin 2.5 IU/mL and to 4561±286 cpm/g tissue in response to release of norepinephrine (n=4, P<0.01) in both cases. Reperfusion in the presence of endothelin-1 alone caused no change in Ins 1,4,5P₃ (2762±240 cpm/g tissue), but when added together with thrombin or norepinephrine, endothelin-1 reduced the Ins 1,4,5P₃ responses to 2313±197 and 1764±168 cpm/g tissue, respectively (n=4, P<0.01 in both cases). Similar inhibitory interactions between endothelin-1 10 nmol/L and thrombin 2.5 IU/mL were observed under normoxic conditions in nonperfused ventricle, eliminating the possibility that excessive vasoconstriction was responsible. In parallel studies, endothelin-1 suppressed the development of reperfusion arrhythmias initiated by either thrombin (ventricular fibrillation, 75% to 39%, n=16 to 18) or norepinephrine (83% to 8%, n=12 to 22) (P<0.01 in both cases).

Conclusions—Inhibition of Ins 1,4,5P₃ generation during myocardial reperfusion by endothelin-1 represents a novel antiarrhythmic mechanism. (Circulation. 1999;99:823-828.)

Key Words: inositol trisphosphate • arrhythmias • endothelin • thrombin • receptors, adrenergic, alpha

Under conditions of myocardial ischemia and reperfusion, both norepinephrine and thrombin exert direct proarrhythmic effects.¹⁻³ The arrhythmogenic responses to norepinephrine are mediated by α₁-adrenergic receptors and can be initiated by norepinephrine released from the sympathetic nerves under these conditions.⁴⁻⁵ Both α₁-receptors and thrombin receptors activate phosphatidylinositol (PtdIns) turnover in the myocardium with generation of inositol phosphates (InsPs) and sn-1,2-diacylglycerol.⁶⁻⁸ Our studies have shown that the proarrhythmic actions of both of these effectors are related to the generation of IP₃ within the ventricle.⁹⁻¹⁰ Studies from other laboratories have also pointed to a proarrhythmic activity of IP₃. In isolated cardiomyocytes, direct intracellular application of IP₃ but not inositol 1,3,4-trisphosphate [Ins(1,3,4)P₃] caused electrophysiological perturbations, especially action potential prolongation, which could lead to arrhythmogenesis.¹¹ In addition, IP₃ has been shown to cause Ca²⁺ oscillations in myocardial tissue¹² and to activate Na⁺/Ca²⁺ exchange,¹³ both of these actions being potentially arrhythmogenic.

Under normoxic conditions, activation of cardiac α₁-adrenergic receptors does not cause detectable increases in IP₃ content when experiments are performed using preparations of intact heart tissue, and InsPs accumulated appear to derive primarily from Ins(1,4)P₂.¹⁴ Under conditions of early reperfusion, the InsP response to norepinephrine changes so that rapid generation of IP₃ is observed.¹⁵ The thrombin-induced InsP response to norepinephrine changes so that rapid generation of IP₃ is observed.¹⁶ In marked contrast to findings with norepinephrine or thrombin, reperfusion of catecholamine-depleted hearts in the presence of endothelin-1 (ET-1) does not cause any increase in IP₃ and does not cause arrhythmias.¹⁷ In the course of studies to delineate the
pathways mediating the responses to the different agents, it was found that ET-1 was able to suppress the IP₃ responses both to thrombin and to norepinephrine under reperfusion conditions. Our previous studies would predict that inhibition of IP₃ responses during myocardial reperfusion should be antiarrhythmic. The present studies were undertaken to investigate this possibility further.

**Methods**

Adult male Sprague-Dawley rats (weight, 160 to 200 g) were used. All experiments were approved by the Alfred Hospital and Baker Institute Animal Ethics Committee.

**[³H]InsP Responses in Right Ventricle Strips**

Right ventricles were dissected from rat hearts under ice-cold saline, and thin strips (10 to 20 mg) were mounted in siliconized 3-mL organ baths in HEPES-buffered Krebs medium equilibrated with 5% CO₂/95% O₂ at 37°C. The medium contained the following, in mmol/L: HEPES buffer (pH 7.4) 20, glucose 11, Na⁺ 138, K⁺ 4.5, Mg²⁺ 1.2, HCO₃⁻ 25, PO₄⁻ 1.2, and Ca²⁺ 2. After equilibration, the strips were labeled by incubation in medium containing [³H]inositol 20 μCi/mL for 4 hours. This procedure was repeated after the same medium was removed and was replaced with medium containing LiCl 10 mmol/L and propranolol 1 μmol/L before addition of agonists as indicated. [³H]InsP generation was terminated after 20 minutes by placing the strips into liquid N₂. Tissue was weighed and maintained at −70°C before extraction.

**[³H]InsP Responses in Perfused Rat Hearts**

Where indicated, rats were treated with reserpine 5 mg/kg IP 18 hours before the experiment to deplete endogenous norepinephrine. Catecholamine depletion was confirmed by a reduction in heart norepinephrine content from 582±310 to 25±18 μg/g wet weight (n=6, P<0.01).16 All rats were given heparin (1 IU/g IP) 30 minutes before decapitation. Hearts were removed immediately, chilled in ice-cold saline, and cannulated via the ascending aorta to initiate Langendorff perfusion with HEPES-buffered Krebs medium, pH 7.4, at 37°C, at 7 mL/min. After a 15-minute equilibration period, hearts were weighed, and InsPs were extracted and quantified as described below. The deacylation procedure was checked by counting the organic phase together with any remaining insoluble residue. On this basis, <5% of the [³H]-labeled lipids remained unhydrolyzed. The [³H]-labeled compounds were identified as deacylated PtdIns, PtdIns(4)P, and PtdIns(4,5)P₂ by removing the mobile phase by repeated lyophilization and then performing anion-exchange HPLC as described above.

**Measurement of IP₃ and PtdIns(4,5)P₂ Mass**

Tissue was extracted as described above, except that ATP 5 mmol/L replaced phytic acid in the extraction medium, the proteinase K step was omitted, and urea was not added before lyophilization. Lyophilized samples were resuspended in water and neutralized with NaHCO₃ plus NaOH as required. The IP₃ content of the neutralized samples was measured with a commercial kit (Amersham).

Lipids were extracted from the TCA pellets remaining after IP₃ extraction from unlabeled tissue (see above) by the method described below. The deacylated lipids were subsequently deglycerated with sodium periodate and dimethylhydrazine as described elsewhere.17 Mass assay of the IP₃, resulting from decylation and deglyceration of PtdIns(4,5)P₂, was carried out as above. The identity of the compound measured in the assay as IP₃ and thus the progenitor lipid as PtdIns(4,5)P₂ was validated by treating aliquots of representative samples with pure IP₃, 5'-phosphatase (10 ng) for 30 minutes at 37°C, followed by boiling to inactivate the enzyme. This treatment removed material that displaced [³H]IP₃ in the binding assay, identifying the measured substance as IP₃ or PtdIns(4,5)P₂.

**Arrhythogenic Responses**

Rats were anesthetized with pentobarbital 60 mg/kg IP and given heparin 200 IU IV. Hearts were cannulated in situ via the ascending aorta and perfused at 7 mL/min with Krebs-Henseleit medium constantly gassed with 5% CO₂/95% O₂, pH 7.4, at 37°C. The perfusate included propranolol 1 μmol/L to block β-adrenergic receptors and LiCl 10 mmol/L to replicate the conditions used in studies of InsP release. This preparation is essentially similar to a standard Langendorff preparation, except that the heart remains attached to the open chest of the animal and the perfusion flow rate is controlled by a peristaltic pump. A 10-minute period was allowed to stabilize the preparation before the experiment, then the left with 1 mL water. Urea 0.05 mol/L final was added, and samples were lyophilized before high-performance liquid chromatographic (HPLC) analysis.14 [³H]-labeled InsPs were separated by anion-exchange HPLC and quantified with an on-line β-counter (Radiomatic Instruments, model CR) as described previously.14

**Extraction and Quantification of [³H]Insolitols Phospholipids**

TCA pellets obtained as described above were extracted with 3 mL of chloroform:methanol:HCl 200:100:1 by sonication and vigorous vortexing. One milliliter of 1 mmol/L EDTA was added, and the phases were separated by centrifugation. The interface was reextracted, and the final organic phase was evaporated under N₂. The dried lipids were deacylated and analyzed by HPLC as described above.

**Intracellular IP₃ Generation**

With whole frozen ventricle (≈1 g) in 3.5 mL of 5% trichloroacetic acid (TCA) containing 2.5 mmol/L EDTA and 5 mmol/L phytic acid with a Polytron homogenizer, followed by sonication as described previously.14 After centrifugation at 5000g for 10 minutes at 4°C, supernatants were removed and TCA pellets were reextracted with 1.5 mL TCA/EDTA/aphylic acid. The combined aqueous phases were pooled and extracted with a 1:1 mixture of Freon and tri-N-octylamine 0.75 mL/mL supernatant. The final aqueous phase was collected and treated with proteinase K 50 μg/mL (2 hours, 50°C), and the samples were then passed through a 1-mL Dowex-50 column (4% cross-linked, 4 to 400 mesh size) and eluted with 1 mL water. Urea 0.05 mol/L final was added, and samples were lyophilized before high-performance liquid chromatographic (HPLC) analysis.14 [³H]-labeled InsPs were separated by anion-exchange HPLC and quantified with an on-line β-counter (Radiomatic Instruments, model CR) as described previously.14
coronary artery was ligated to produce regional ischemia, involving 30% to 40% of the heart, judged from the reduction in coronary flow required to maintain coronary perfusion pressure (CPP). After 20 minutes, the ligature was released to initiate reperfusion. The perfusion flow rate was adjusted coincidently with coronary occlusion and reperfusion to maintain a constant CPP. We have previously demonstrated that the incidences of ischemic ventricular tachycardia (VT) and ventricular fibrillation (VF) were significantly reduced by propranolol to 40% and 30%, respectively, compared with 80% and 75% in the control group (both P<0.05). Ischemic ventricular arrhythmias were unaffected by LiCl.7 The incidence of reperfusion arrhythmias was not altered by propranolol or LiCl alone or in combination. During the 20 minutes of ischemia and 5 minutes of reperfusion, the epicardial ECG and the CPP were monitored. Ventricular arrhythmias were quantified according to the Lambeth Convention guidelines.18

Materials
Reserpine, propranolol, norepinephrine, thrombin, and LiCl were obtained from Sigma Chemical Co. ET-1 was obtained from Peninsu lara Laboratories. Thrombin receptor–activating peptide (TRAP, SFLLRN) was from Auspep. myo-[3H]inositol, [3H]IP₃, [14C]Ins(1)P, and IP₃ mass assay kits were from Amersham. [3H]Ins(1,3,4)P₃, [3H]Ins(1,4)P₂, and [3H]Ins(4)P were from New England Nuclear and were supplied by Auspep. All other reagents were analytical reagent grade, and solutions were prepared in Milli-Q water.

Statistics
Group differences for nonparametric data (incidence and duration of VT and VF) were examined with a χ² test with Fisher’s exact calculation or by a Mann-Whitney rank-sum test. Parametric data (InsP responses) were examined by ANOVA followed by Student’s unpaired t test if significant group differences were found. A value of P<0.05 was considered significant.

Results
InsP Responses in Normoxic Ventricle in the Presence of ET-1 and Thrombin
Strips of right ventricle were labeled with [3H]inositol, preincubated in medium containing LiCl 10 mmol/L and BSA 0.1% for 10 minutes, and then stimulated with ET-1 10 mmol/L or thrombin 2.5 IU/mL for 20 minutes. [3H]InsPs were extracted and quantified. ET-1 caused increases in [3H]Ins(1)P₁, [3H]Ins(4)P₂, and [3H]Ins(4)P₃, but there was no increase in [3H]IP₃. Thrombin caused a smaller overall increase in labeled InsPs but, unlike ET-1, caused an increase in [3H]IP₃ in addition to increases in the lower InsPs. When ET-1 was added together with thrombin, the overall [3H]InsP response was higher than the thrombin response but lower than the response to ET-1. Importantly, no increase in [3H]IP₃ was observed (Figure 1, top).

Similar experiments were performed in perfused rat ventricles. [3H]Inositol-labeled hearts were perfused with medium containing LiCl and BSA for 10 minutes, and then ET-1 10 mmol/L, thrombin 2.5 IU/mL, or both agonists were added and perfusion was continued for 20 minutes. Responses to the 2 agonists were similar to those described for right ventricle strips. ET-1 caused increases in [3H]-labeled InsPs without any increase in IP₃. Thrombin caused a smaller stimulation but did produce an increase in [3H]IP₃. Addition of ET-1 together with thrombin prevented the thrombin-induced IP₃ response (Figure 1, bottom).

InsP Responses During Postischemic Reperfusion: Effects of Endothelin and Thrombin
We have previously reported that, under conditions of early reperfusion, the IP₃ response to thrombin is markedly enhanced.19 Experiments were performed to determine whether ET-1 inhibited the IP₃ response to thrombin under reperfusion conditions. Hearts from catecholamine-depleted rats were labeled with [3H]inositol and subsequently subjected to 20 minutes of global zero-flow ischemia followed by 2 minutes of reperfusion with oxygenated medium at 7 mL/min. In the absence of added agonists, the [3H]InsP level after 2 minutes of reperfusion was not different from that after 20 minutes of ischemia, as described previously.14 When thrombin 2.5 IU/mL or TRAP 50 µmol/L was added to the perfusate immediately before perfusion was restarted, generation of [3H]IP₃ was observed. When ET-1 10 nmol/L was added, there was no increase in [3H]IP₃, as described previously.15 Experiments were performed in which both thrombin 2.5 IU/mL and ET-1 10 mmol/L were added to the perfusate immediately before reperfusion. In the presence of both agonists, the rise in [3H]IP₃, seen in the presence of thrombin alone was eliminated (Figure 2, top left). A similar reduction in the IP₃ response to thrombin was observed when ET-1 was added at 3 nmol/L (data not shown).
Similar experiments were performed using unlabeled hearts and quantifying IP3 mass. Two minutes of reperfusion of catecholamine-depleted ischemic hearts in the presence of thrombin 2.5 IU/mL or TRAP 50 μmol/L caused an increase in IP3 content. No such increase was observed in hearts perfused with ET-1 10 nmol/L (Figure 2, top right).

**Phospholipid Changes During Reperfusion in the Presence of ET-1 and Thrombin**

Competition for phospholipid precursors might provide an explanation for the inhibitory effect of adding ET-1 and thrombin in combination. To address this possibility, the levels of 3H-labeled PtdIns, PtdIns(4)P, and PtdIns(4,5)P2 were measured in the hearts subjected to the procedures described above. In catecholamine-depleted ventricles, levels of PtdIns(4,5)P2, both 3H-labeled and mass, were high, and these levels decreased when thrombin or TRAP was added during reperfusion. ET-1 caused no decrease in PtdIns(4,5)P2 and prevented the decrease observed in the presence of thrombin (Figure 2, bottom left). Thus, competition for substrate is an unlikely explanation for the observed mutually inhibitory interactions between thrombin and ET-1.

**Interaction Between ET-1 and Norepinephrine During Reperfusion**

Under reperfusion conditions, InsP responses to norepinephrine and thrombin appear similar, and both agents initiate arrhythmias.10 Experiments similar to those involving ET-1 and thrombin were performed in which the effects of ET-1 on the IP3 response to norepinephrine were investigated. Intact 3H-labeled hearts (without catecholamine depletion) were subjected to the ischemia/reperfusion procedure in the presence or absence of ET-1 10 nmol/L. In the absence of ET-1, reperfusion caused the generation of [3H]IP3, as described previously,9 and ET-1 prevented this response. Similar results were obtained when catecholamine-depleted hearts were perfused with norepinephrine 100 μmol/L (Figure 3). In contrast to the inhibitory action of ET-1, addition of thrombin 2.5 IU/mL during reperfusion did not reduce the IP3 caused by endogenously released norepinephrine (Figure 3).

**Effect of ET-1 on Reperfusion Arrhythmias Initiated by Norepinephrine or Thrombin**

Intact perfused hearts (without reserpine pretreatment) were subjected to 20 minutes of regional ischemia, followed by reperfusion. An ECG was recorded during the first 5 minutes of reperfusion, and all reperfusion VT and VF occurred within the first 2 minutes after reperfusion was commenced. ET-1 at 10 nmol/L was added at reperfusion and maintained for a period of 4 minutes. The addition of ET-1 largely prevented the onset of VT and VF (Table), a finding in keeping with the inhibition by ET-1 of reperfusion-mediated IP3 release (Figure 3). In hearts treated with ET-1 at 10 nmol/L, reperfusion VT and VF occurred within the first 2 minutes after reperfusion was commenced. ET-1 at 10 nmol/L was added at reperfusion and maintained for a period of 4 minutes. The addition of ET-1 largely prevented the onset of VT and VF (Table), a finding in keeping with the inhibition by ET-1 of reperfusion-mediated IP3 release (Figure 3). In hearts treated with ET-1 at 10 nmol/L, reperfusion VT and VF occurred within the first 2 minutes after reperfusion was commenced. ET-1 at 10 nmol/L was added at reperfusion and maintained for a period of 4 minutes. The addition of ET-1 largely prevented the onset of VT and VF (Table), a finding in keeping with the inhibition by ET-1 of reperfusion-mediated IP3 release (Figure 3).
nmol/L, a progressive rise in CPP was observed (from 33±1 mm Hg at 0 minutes to 44±2, 54±3, 63±4, and 67±4 mm Hg at 1, 2, 3, and 4 minutes of reperfusion, respectively, n=8, P<0.01 by ANOVA). Because hearts were perfused at a constant flow rate, the increase in CPP indicated vasoconstriction. Although the flow was delivered by a pump, an uneven distribution of myocardial flow might ensue in the presence of ET-1. To address this question, ischemic hearts were perfused with ET-1 at 3 nmol/L, a concentration that has been shown to effectively activate the release of InsPs during reperfusion. Although ET-1 at this dose had minimal vasoconstrictor effects (CPP increased from 34±1 mm Hg to 38±1, 41±2, 44±2, and 45±3 mm Hg at 1, 2, 3, and 4 minutes of reperfusion, respectively, n=8, P<0.01), reperfusion arrhythmias were essentially eliminated (Table). Thus, this finding argues against coronary vasoconstriction with resultant slower reperfusion as an explanation for the antiarrhythmic action of ET-1 under reperfusion conditions.

Similar experiments were performed in catecholamine-depleted hearts perfused with thrombin during reperfusion. Depletion of catecholamines caused a reduction in reperfusion arrhythmias from 100% (VT incidence) and 83% (VF incidence) in intact hearts to 17% (VT) and 0% (VF) in the reserpine-treated group. The addition of thrombin 2.5 IU/mL increased the incidence and duration of reperfusion arrhythmias relative to the catecholamine-depleted controls (Table). Addition of ET-1 10 nmol/L alone did not increase either the incidence or duration of arrhythmias over the 5-minute reperfusion period. However, when added together with thrombin 2.5 IU/mL, ET-1 10 nmol/L reduced the arrhythmogenic action of thrombin (Table).

**Discussion**

Under conditions of ischemia and reperfusion, both norepinephrine and thrombin are arrhythmogenic, and ET-1 is not. Our previous studies have provided evidence that the onset of arrhythmias is related to the generation of IP₃ under reperfusion conditions in the presence of thrombin or norepinephrine but not in the presence of ET-1. Under reperfusion conditions, ET-1 induces a robust InsP response that does not involve an increase in IP₃; furthermore, our data indicated that the InsPs generated after ET-1 stimulation did not derive from IP₃. The marked difference between the InsP responses initiated by norepinephrine or thrombin on the one hand and those by ET-1 on the other indicates that the changes caused under reperfusion conditions are specific to certain receptor classes. Furthermore, the finding that ET-1 is not arrhythmogenic even though it causes InsP generation points to IP₃ specifically and as being related to arrhythmogenic responses.

In the present study, ET-1 was shown to inhibit the IP₃ responses to both thrombin and norepinephrine during early reperfusion. This inhibitory action of ET-1 did not appear to be due to intense vasoconstriction preventing effective reperfusion, for 2 reasons. First, similar inhibition of IP₃ responses was observed under normoxic conditions and in nonperfused ventricle strips (Figure 1). Second, ET-1 inhibited the reperfusion-induced IP₃ response at concentrations below those that caused observable vasoconstriction. Furthermore, our studies showed that the inhibitory action of ET-1 was not caused by competition for phospholipid precursors, because the content of the immediate precursor of IP₃, PtdIns(4,5)P₂, was high in the presence of both ET-1 and thrombin (Figure 2). Thus, it seems likely that ET-1 receptor activation can inhibit the generation of IP₃ when this is stimulated by either norepinephrine or thrombin. However, whether this inhibitory action occurs as a direct consequence of ET-receptor activation or whether it is mediated by some distal component of the ET-receptor signal transduction pathway remains to be established. Furthermore, the inhibition of IP₃ generation might be directed at the level of the receptors, the G proteins, or the phospholipase C enzymes. A recent study described the phosphorylation and partial inactivation of α₁B-adrenergic receptors after stimulation of ET₄ receptors in Rat-1 fibroblasts, and a similar mechanism might be involved in this instance. However, the finding that responses to norepinephrine...
rine and thrombin were similarly affected argue for an action at a target common to both receptor systems. 

Although norepinephrine and thrombin are proarrhythmic under reperfusion conditions (Table),2,3 there are conflicting reports concerning the arrhythmogenic potential of ET-1. Direct proarrhythmic activity was reported in studies of perfused pig hearts, but these arrhythmias may have been secondary to vasoconstriction.20 Other studies have reported potentially antiarrhythmic actions of ET-1.21 We have found that prolonged infusion with ET-1 (>10 minutes) causes arrhythmias even under normoxic conditions, and these are most likely secondary to severe vasoconstriction. During reperfusion in the absence of other proarrhythmic agents, ET-1 has no effect on arrhythmogenesis,15 but the present studies show that, in the presence of thrombin or norepinephrine, ET-1 is effectively antiarrhythmic (Table). Thus, the apparent discrepancies in the observed effects of ET-1, proarrhythmic, antiarrhythmic, or nonarrhythmic, are related to the different conditions studied. The effects of ET-1 in vivo would comprise a complex mixture of these opposing activities. Although ET-1 is clearly unsuitable for use as an antiarrhythmic agent, the antiarrhythmic action of ET-1 points to a novel antiarrhythmic mechanism that might provide scope for the development of entirely novel agents that inhibit the reperfusion-induced generation of IP3, and thus prevent the initiation of arrhythmias. Although our studies have implicated IP3 in the genesis of arrhythmias under ischemic22 and reperfusion conditions,9,10 recent studies have extended the pathological role of IP3 to inflammatory heart diseases by showing that inhibitors of the IP3 pathway prevent the development of electrophysiological disturbances in cardiomyocytes caused by the presence of activated lymphocytes.11,23 Responses to combinations of thrombin and ET-1 or norepinephrine and ET-1 together are different from the responses to either effector alone, and this applies to InsP3 generation as well as arrhythmogenesis. In the in vivo situation, all of these factors are likely to be present in the ischemic myocardium. In addition, the presence of inflammatory lymphocytes could further complicate such interactions by causing IP3 generation within the myocardium. Thus, IP3 levels in the ischemic and reperfused myocardium in vivo are under multifactorial control, and the overall arrhythmogenic response may well depend critically on combinations of effectors. Despite the complexities, the present findings stress the importance of IP3 in initiating arrhythmias. Agents that prevent IP3 generation may prove useful in preventing the development of intractable arrhythmias.

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References
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