Endothelin$_A$ Receptor Subtype Mediates Endothelin-Induced Contractility in Left Ventricular Cardiomyocytes Isolated from Rabbit Myocardium

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ABSTRACT
Endothelin (ET)-1 is a potent positive inotropic agent, the effects of which are mediated by increases in cytosolic Ca$^{2+}$ in the myocardium. The object of this study was to examine 1) the influence of ET$_A$ and ET$_B$ receptor subtypes, and 2) the role of the phospholipase C (PLC) pathway in mediating ET-1-induced contraction. Left ventricular cardiomyocytes were isolated from the hearts of New Zealand White rabbits (2–2.5 kg) by the use of Langendorf perfusion with collagenase. Cardiomyocyte function was examined during unloaded, electrically stimulated (0.5 Hz) contractions with a video-edge detection system. ET-1 increased cell shortening with greater potency than ET-3: mean EC$_{50}$ values were 1.1 and 2.6 $\times$ 10$^{-11}$ M, respectively. With the same order of potency, ET-1 and ET-3 increased (P $<$ .05) velocity of cell shortening. The ET$_A$ receptor-selective antagonist ABT-627 shifted the ET-1-induced cell shortening response curve to the right with a pA$_2$ value of 10.3. The ET$_B$ receptor-selective antagonist A-192621 (10$^{-8}$–10$^{-7}$ M) did not alter the concentration-response of ET-1. Moreover, the ET$_B$ receptor-selective agonist sarafotoxin 6c did not have any effect on cell shortening over the concentration range of 10$^{-11}$ to 10$^{-7}$ M. ET-1 in the presence of the PLC inhibitor U-73122 did not alter the contractile amplitude. However, ET-1 in the presence of the protein kinase C inhibitor bisindolylmaleimide increased cell shortening. These findings indicate that 1) the ET$_A$ receptor subtype, and not the ET$_B$ receptor subtype, mediates the positive inotropic effect of ET-1, and 2) the response of ET-1 is mediated by a PLC pathway, but not through protein kinase C, in ventricular cardiomyocytes isolated from rabbit myocardium.

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ABBRévIATIONS: ET, endothelin; ET$_A$, endothelin receptor subtype A; ET$_B$, endothelin receptor subtype B; BQ-123, cyclo[6-Trp-6-Asp-Pro-6-Val-Leu]; Sfx, sarafotoxin 6c; PLC, phospholipase C; ABT-627, 2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[N,N-di(n-butyl)amino carbonyl methyl]-pyrrolidine-3-carboxylic acid; A-192621, 2-(4-propoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[2,5-ethylphenyl]larnino carbonyl methyl]-pyrroldine-3-carboxylic acid; BIM, bisindolylmaleimide I; U-73122, 1-[6-[[17β]-3-methoxyestra-1,3,5(10)-tri-en-17-yl]amino[hexyl]-1H-pyrole-2,5-dione; PKC, protein kinase C; SR, sarcoplasmic reticulum.

ET-1 is more potent than ET-3 as an inotropic agent, whereas both peptides are equipotent chronotropic agents (Moravec et al., 1989; Ishikawa et al., 1991), suggesting that the inotropic response is likely to be mediated, at least in part, by the ET$_A$ receptor subtype, whereas the chronotropic response is more likely to be mediated by the ET$_B$ receptor subtype. However, all three isopeptides from the ET family, namely, ET-1, ET-2, and ET-3, were reported to produce similar positive inotropic effects in rabbit papillary muscle, suggesting that the contractile effect may be mediated by the ET$_A$ receptor subtype (Takanashi and Endoh, 1991; Molenaar et al., 1993). The use of ET receptor-selective antagonists as pharmacological tools to study functional responses has exposed increased complexity of interactions. For example, based on sensitivity to BQ-123, ET-1 has been reported to produce a positive chronotropic effect through the ET$_B$ receptor subtype and a negative chronotropic effect through the ET$_A$ receptor subtype and a negative chronotropic effect through...
the ET<sub>1</sub> receptor subtype (Ono et al., 1994), and exemplifies the modulatory role of the peptide in cardiac regulation.

There is a distinct lack of clarity regarding the influence of ET receptor subtype(s) that mediate the contractile response in the myocardium. The positive inotropic effects of ET isopeptides in mammalian myocardium tend to vary extensively and depend on the preparation used and the species investigated. Although rabbit myocardium has a high density of binding sites for ET, density does not appear to reflect wholly the magnitude of functional response. For example, ET-3 has no cardiotoxic effect in ventricle of rat and a potent effect in ventricle of rabbit, although the density of binding sites for I<sup>125</sup>-ET-3 is much higher in ventricle of rat (Ishikawa et al., 1991) than rabbit (Kasai et al., 1994).

The influence of specific ET receptor subtypes on contractile parameters has not previously been examined with isolated ventricular cardiomyocytes. Isolated ventricular cells are devoid of endogenous neuronal, humoral, or endothelial-derived effects that are likely to influence myocardial contractile function. Using such a single population of cells also may avert any disparity caused by myocardial tissue preparations, which have additional cell types that also potentially contain high densities of ET receptors (Ishikawa et al., 1991). In this study, ventricular cardiomyocytes were isolated from myocardium of rabbit, a species that, in contrast to the rat, responds markedly to interventions that increase intracellular Ca<sup>2+</sup> (Li et al., 1991). Cumulative concentration-response relationships were assessed in single cells with the agonists ET-1 (ET<sub>1</sub>B receptor nonselective), ET-3, and sarafotoxin 6c (Sfx) (ET<sub>3</sub> receptor selective). Receptor-selective antagonists investigated by noncumulative dose-effect relationships that had both high potency and selectivity. Both ABT-627 and A-192621 (Abbott Laboratories, Chicago, IL) are reported to have 1400-fold selectivity for the ET<sub>1</sub> and ET<sub>3</sub> receptor subtypes, respectively (Douglas et al., 1994).

The influence of phospholipase C (PLC) pathway also was investigated with selective signaling transduction inhibitors.

### Experimental Procedures

#### Isolation of Ventricular Cardiomyocytes.
Ventricular cardiomyocytes were obtained after enzymatic dissociation. New Zealand White male rabbits (2.5–3 kg) were anesthetized with sodium pentobarbitone (50 mg/kg i.v.) after heparinization (400 I.U./kg i.v.). The chest was opened and the heart quickly removed and cannulated through the ascending aorta, on a modified Langendorff perfusion apparatus (Keslo et al., 1995). Blood was flushed from the coronary vasculature with a Ca<sup>2+</sup>-free modified Krebs-Ringer buffer containing 110 mM NaCl, 2.6 mM KCl, 25 mM NaHCO<sub>3</sub>, 1.2 mM MgSO<sub>4</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, and 11 mM glucose (pH 7.4; 37°C), which had just previously been aerated with 95% O<sub>2</sub>, 5% CO<sub>2</sub>. The perfusate was subsequently supplemented with 0.12% (w/v) collagenase and recirculated for approximately 15 min while maintained at 37°C and continuously aerated with 95% O<sub>2</sub>, 5% CO<sub>2</sub>. After enzymatic digestion, the hearts were cut at the atrioventricular junction, sliced into segments with a Ca<sup>2+</sup>-free modified Krebs-Ringer solution containing 110 mM NaCl, 2.6 mM KCl, 25 mM NaHCO<sub>3</sub>, 1.2 mM MgSO<sub>4</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, and 11 mM glucose (pH 7.4; 37°C), which had just previously been aerated with 95% O<sub>2</sub>, 5% CO<sub>2</sub>. The perfusate was subsequently supplemented with 0.12% (w/v) collagenase and recirculated for approximately 15 min while maintained at 37°C and continuously aerated with 95% O<sub>2</sub>, 5% CO<sub>2</sub>. After enzymatic digestion, the hearts were cut at the atrioventricular junction, sliced vertically toward the apex, and chopped into pieces of 0.7 mm<sup>3</sup> with a mechanical tissue chopper (McIlwain, Mickle Laboratory Engineering Co. Ltd., Surrey, UK). The minced tissue was placed in the collagenase-containing perfusate that had been supplemented with 0.2% (w/v) BSA, and the mixture triturated with a 10-ml serological pipette for approximately 5 min. The dispersed cells were filtered through a nylon mesh gauze of pore size 200 μm and washed twice. Ca<sup>2+</sup> was restored by means of centrifugation at 25g twice, and the cells were resuspended in modified Krebs-Ringer solutions containing 250 and 500 μM CaCl<sub>2</sub>, respectively. Finally, the cells were layered onto a solution of 4% (w/v) BSA containing 1 mM CaCl<sub>2</sub>, and left to settle by gravity, at 37°C. After approximately 5 min, the supernatant was aspirated and the resulting cell material resuspended at a density of 1 to 2 mg of protein/ml in a storage medium (M199 with Earle's salts, containing 5 mM creatine, 5 mM taurine, 2 mM carnitine, 100 I.U/ml streptomycin, 100 μg/ml penicillin, pH 7.4) at 37°C. Suspension of cardiomyocytes were >70% viable as estimated by their elongated rod-shaped morphology.

#### Contractile Measurement.
An aliquot of the cell suspension was placed in a transparent recording chamber (~150 μl) mounted on a heated microscope stage (37°C; Zeiss, Jena, Germany), and allowed to settle for 10 min before being bathed with an aerated (100% O<sub>2</sub>) Krebs-Henseleit solution of the following composition: 125 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO<sub>4</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 2 mM CaCl<sub>2</sub>, 10 mM HEPES, and 11 mM glucose (pH 7.4). Cells were field stimulated (Grass stimulator) at 0.5 Hz with biphasic pulses of 0.5-ms duration at 60 V via Ag/AgCl wires embedded in the wall of the recording chamber. The cells were visualized at 1280× magnification and cell shortening was monitored with a video-edge detection system (VED 40; Creonetive, Sandy, UT). The resulting signal was digitized (Digidata 1200; Axon Instruments, Foster City, CA) and recorded on computer for subsequent analysis with WCP software (Dr. J. Dempster, Department of Pharmacology, University of Strathclyde, Strathclyde, Scotland). This system has a time resolution of 16.7 ms and a spatial resolution of 1 in 512. Eight consecutive contractions were signal averaged to produce data under each discrete set of conditions. Cardiomyocyte contractile function was assessed by measuring the following: absolute cell shortening (μm); time to peak contraction (time taken for the signal to rise from 10 to 90% of peak amplitude, ms); velocity of cell shortening (maximum rate of rise of the signal measured between 10 and 90% of peak amplitude, μm/s); and relaxation time (time taken for the signal to fall from peak amplitude to 10% of peak amplitude, ms). Viable cells were used for each experiment as defined by 1) a rod-shaped appearance without sarcolemmal blebbing, 2) quiescent when unstimulated, and 3) stable baseline contractions to electrical stimulation in Krebs-Henseleit solution. The solution bathing the cells could be changed within 30 s, by use of a gravity-fed, multichannel solution exchanger consisting of eight reservoirs, a series of solenoid valves, and an eight-channel input manifold attached to the bath.

#### Protocols.
After an equilibration period to allow cardiomyocyte contractions to stabilize, the effects of stimulation were monitored in control cells (without drugs) for 1 h. The temporal effects of ET-1 and ET-3 were assessed to establish the time of maximum response of acute exposure. Concentration-response relationships were established in a cumulative manner for ET-1 (10<sup>−12</sup> to 10<sup>−7</sup> M), ET-3 (10<sup>−11</sup> to 10<sup>−7</sup> M), and Sfx (10<sup>−11</sup> to 10<sup>−7</sup> M). Cells were exposed to each concentration of drug for 8 to 12 min. After stabilization, cells were preincubated with the respective receptor antagonists alone for 8 min, before continuous exposure to the antagonist in the presence of cumulative doses of agonist.

#### Data and Statistical Analysis.
Data are expressed as mean ± S.E. Concentration-response curves for the contractile responses were normalized to their respective baseline values. Data were fitted by nonlinear regression, and the concentrations of agonist producing 50% of maximum contractile amplitude (EC<sub>50</sub>) were established from log-probit plots of the individual concentration-response relationships. Data were analyzed statistically by ANOVA followed by Dunnett's or Student-Neuman-Keuls multiple comparisons test. Values of P < .05 were taken as indicating statistical significance.

#### Materials.
ET-1, ET-3, and Sfx were obtained from American Peptide Company (Santa Clara, CA). ABT-627 [2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(N,N-di(n-butyl)amino carbonyl-methyl)-pyrroolidine-3-carboxylic acid] and A-192621 [2-(4-propoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(2,5-ethyllyphenyl)amino carbonyl-methyl)-pyrroolidine-3-carboxylic acid] were obtained as gifts from Abbott Laboratories (Abbott Park, IL). ET-1 and ET-3 were dissolved in water and stored in aliquots of 10<sup>−4</sup> M at −20°C. The ET receptor-
selective antagonists were dissolved in dimethyl sulfoxide and stored in aliquots of $10^{-4}$ M at $-20^\circ$C; the final concentration of dimethyl sulfoxide was $<0.01\%$. Collagenase (type I) was purchased from Serva Feinbiochemica (Heidelberg, Germany). Medium 199 was obtained from Gibco Ltd. (Middlesex, UK). Bisindolylmaleimide I (BIM) and U-73122 [1-[(1β)-3-methoxyestr-1,3,5(10)-tri-en-17-yl]amino]-hexyl]-1H-pyrrole-2,5-dione] were obtained from Alexis Corporation (Nottingham, UK). All other chemicals were of analytical grade. Double distilled water, which had been deionized through a Millipore Q system (Millipore, Harrow, UK) was used in all experiments.

Results

Response of Cardiomyocytes to Basal Contractile Stimulation. Rabbit cells were selected only if they showed no signs of spontaneous contraction when stimulated at 0.5 Hz during the 10-min equilibration period in 2 mM calcium. Mean cell length was $117 \pm 1.4 \ \mu m$ (190 cells). Cell shortening, time to peak contraction, velocity of cell shortening, and relaxation time did not change, over a period of 60 min, from basal values of $7.85 \pm 0.66 \ \mu m$, $184 \pm 14 \ \mu s$, $125 \pm 18 \ \mu m/s$, and $235 \pm 25 \ \mu s$, respectively (data not shown).

Positive Inotropic Effects of ET-1 and ET-3 but Not Sfx. In electrically paced ventricular cardiomyocytes isolated from rabbit myocardium, the maximum positive contractile effects ($P < .05$) were attained after 8 min of exposure of the cells to either ET-1 or ET-3 (data not shown). Both ET-1 and ET-3 increased cell shortening in a concentration-dependent manner (Fig. 1a). The potency of ET-1 was greater ($P < .05$) than ET-3, whereas the efficacy of ET-1 and ET-3 was similar. EC$_{50}$ values for ET-1 and ET-3 were $1.1 \times 10^{-11}$ and $2.6 \times 10^{-10} \ \text{M}$, respectively, indicating a 23-fold difference in potency. In contrast, Sfx had no effect on cell shortening or indeed any of the contractile parameters examined (Fig. 1). $E_{\text{max}}$ values of changes in cell shortening for ET-1 and ET-3 were approximately 50% less than isoprenaline, which was used as a positive control (data not shown). Moreover, the magnitude of the temporal effects produced by either ET-1 or ET-3 was much less than the response produced by isoprenaline: ET-1 and ET-3 decreased time to peak contraction, maximally at $10^{-10} \ \text{M}$, by $13.7 \pm 3.8$ and $13.8 \pm 6 \ \text{ms}$, respectively, whereas isoprenaline decreased time to peak contraction by $92 \pm 6 \ \text{ms}$.

Inotropic Effect of ET-1 Inhibited by an ET$_{\alpha}$ but Not ET$_{\beta}$ Receptor-Selective Antagonist. The ET receptor-selective antagonists (ABT-627, $10^{-10}$ to $10^{-9} \ \text{M}$; A-192621, $10^{-8}$ to $10^{-7} \ \text{M}$) did not alter basal contractile function per se from basal values of $7.44 \pm 0.50$ and $7.44 \pm 0.47 \ \mu m$, respectively. Maximal inhibition of the effects of ET-1 was observed after 3 min of preincubation with ABT-627. ABT-627 ($10^{-10}$ to $10^{-9} \ \text{M}$) produced rightward shifts in the concentration-response curve of ET-1 on cell shortening (Fig. 2a). The pA$_2$ value for ABT-627, determined from the Schild plot, was 10.3; the slope of the regression line was $1.6 \pm 0.3$ and was not found to be significantly different from unity; and goodness of fit of the regression was 0.961. pD$_2$ values for ET-1 in the presence of ABT-627, at concentrations of $3 \times 10^{-10}$ and $10^{-9} \ \text{M}$ ($9.37 \pm 0.22$ and $8.80 \pm 0.7$, respectively) were less ($P < .05$) than ET-1 alone ($10.84 \pm 0.13$). The increase in velocity of cell shortening was attenuated by ABT-627 and was almost completely blocked by the highest concentration of antagonist used (Fig. 2b). Moreover, the small decrease in time to peak contraction was abolished by all concentrations of the antagonist used. The ET$_{\beta}$ receptor-selective antagonist A-192621 ($10^{-8}$ to $10^{-7} \ \text{M}$) did not alter the concentration-
response relationship of ET-1 on contractile parameters of cell shortening (Fig. 3a), velocity of cell shortening (Fig. 3b), relaxation time, or time to peak contraction (data not shown). ABT-627, at a concentration of $10^{-10}$ M, had no effect on the positive contractile effect of ET-3 ($10^{-8}$ M). However, ABT-627, at a concentration of $10^{-9}$ M, attenuated the positive contractile effects of ET-3 (Fig. 4). When the cells were exposed to ET-3, in the presence of ABT-627 ($10^{-9}$ M), there was an increase in cell shortening that appeared to be arrested at 6 min and subsequently reversed thereafter (Fig. 4a); the increase in cell shortening, from basal, after a 12-min exposure to ET-3 ($10^{-8}$ M), in the presence of ABT-627 ($10^{-9}$ M), was 3.8-fold less than ET-3 alone. ET-3 in combination with ABT-627 ($10^{-9}$ M) produced a similar trend in attenuating the increased velocity of cell shortening (3.6-fold after 12 min; Fig. 4b) and relaxation time (4-fold after 12 min). ET-3 ($10^{-8}$ M) did not affect time to peak contraction.

**Inotropic Effect of ET-1 Inhibited by PLC Inhibitor, but Not Protein Kinase C (PKC) Inhibitor.** The PLC inhibitor U-73122 (1 $\mu$M) per se did not alter cell shortening from a basal value of $7.5 \pm 0.4 \mu$m. Moreover, ET-1, in combination with U-73122, did not alter cell shortening ($7.8 \pm 0.5 \mu$m) from basal (Fig. 5a). The PKC inhibitor BIM (5 $\mu$M) maximally decreased ($P < .05$) cell shortening after 2 min to $5.1 \pm 0.3 \mu$m from a control amplitude of $6.2 \pm 0.3 \mu$m, and was completely reversed ($6.1 \pm 0.4 \mu$m) within 2 min after washout in control buffer (data not shown). ET-1 in the presence of BIM ($6.7 \pm 0.3 \mu$m) was not different from control, but increased ($P < .05$) cell shortening compared with BIM alone. ET-1 alone produced a further increase ($P < .05$) in cell shortening ($8.5 \pm 0.4 \mu$m) compared with BIM in combination with ET-1, indicating the reversible nature of the effects of BIM (Fig. 5b); the difference between control values and ET-1 alone ($1.92 \pm 0.4 \mu$m) were similar to the difference between BIM alone and in combination with ET-1 ($1.63 \pm 0.2 \mu$m).
ET-1 exerts a positive inotropic effect in cardiac muscle isolated from various species, albeit with differing potencies (Takanashi and Endoh, 1991). Both ETA and ETB receptor subtypes coexist in cardiomyocytes, as has been demonstrated with in situ hybridization techniques (Hori et al., 1992). Findings from this study indicate that the contractile response of acute exposure to ET isopeptides, in rabbit ventricular cardiomyocytes, is mediated by the ETA receptor subtype, and not influenced by the ETB receptor subtype. Moreover, this response was not found to be mediated by PKC, although it was coupled to PLC.

ET-1 has a particularly potent action on ventricular cardiomyocytes isolated from rabbit myocardium with an EC50 value of 10 pM. A similar sensitivity has been observed for ET-1 on ventricular cardiomyocytes isolated from rats (Kelly et al., 1990) and pigs (Thomas et al., 1996) with EC50 values of 50 and 64 pM, respectively. Differences observed in studies between cells, which contract without load, and tissues, which undergo isometric contractions, are not uncommon. Neurotransmitters or hormones within the tissue can influence the responses to exogenous inotropic agents (Harding et al., 1991). The potency of ET-1 on contraction with ventricular (Concas et al., 1989; Shah et al., 1989; Ishikawa et al., 1991) or atrial (Hu et al., 1988; Ishikawa et al., 1988; Hattori et al., 1993) tissue preparations, or indeed, whole heart preparations (Firth et al., 1990), is in the nanomolar range. Removal of the endothelial layer of the endocardium has been found to increase the sensitivity of the myocardium to ET (Li et al., 1991; Mebazaa et al., 1993; McCellan et al., 1994). Damage to the endocardial endothelium of ventricular trabeculae, isolated from rat, has been reported to shift the threshold contractile effects from the nanomolar range to the picomolar range (McCellan et al., 1994). Nitric oxide, in addition to other factor(s), has been reported to play a negative modulatory role at subnanomolar concentrations of ET-1 (McCellan et al., 1996).

The kinetics of the acute response of the ET isopeptides is of slow onset (8 min) and sustained duration of action, in contrast to the response of the positive inotropic agent isoprenaline, which is relatively fast (2 min) and quickly reversed. Moreover, ET isopeptides prolong the duration of contraction, predominantly as a result of increased relaxation time (Meyer et al., 1995), in contrast to the abbreviated response of isoprenaline. Although the peptides were found to increase the relaxation times in experiments that were performed with a single maximum concentration of the peptides, this effect was attenuated in concentration-response experiments. It is not clear why such a difference was observed, but it may reflect sensitization of cells to gradual increases in concentration of the peptide, or it may result from opposing actions in the kinetics of the peptide.

In isolated ventricular cardiomyocytes, evidence supporting an ETA-mediated role, and excluding an ETB-mediated role, in the positive inotropic effect of ET isopeptides includes the following: 1) order of potency of the ET agonists to increase contractile activity, 2) absence of contractile effect in the presence of Sfx, 3) inhibition by the ETA receptor-selective antagonist ABT-627, and 4) lack of effect of the ETB receptor-selective antagonist A-192621 on ET-1-induced contractile activity. In ventricular tissue preparations, however, it is suggested the positive inotropic effect of ET-1 is mediated by both ET\textsubscript{A1} (sensitive to BQ-123) and ET\textsubscript{A2} (resistant to BQ-123) receptor subtypes, depending on the concentration used, whereas the effect of ET-3 is mediated predominantly by ET\textsubscript{A1} and partially by ET\textsubscript{B} receptor subtypes (Kasai et al., 1994; Endoh et al., 1996, 1998). It would appear that the endothelium (or indeed noncardiomyocytes) contributes to the ETB receptor-mediated influence in tissue preparations. It is possible that ABT-627 is nonselective for the ETA and ETB receptor subtype. However, the small subnanomolar ETA receptor subtype effect (20% of the maximum response to ET-1) observed by Kasai et al. (1994) would likely be masked with isolated ventricular cardiomyocytes due to the shift in the concentration-response relationship observed between tissue preparations and cell preparations (McCellan et al., 1994). However, although the contractile response of ET-3 appears to be mediated by the ETA receptor subtype, the temporal differences between the responses of ET-1 and ET-3 would suggest that receptor activation might well be more complex.

$B_{\text{max}}$ values of labeled ET-1 and ET-3, in rabbit ventricular myocardium, are reported in the order of 230 and 42 fmol/mg of protein (Kasai et al., 1994); however, ET-1 was observed to occupy a greater proportion of ETA (82%) to ETB (18%) receptor-binding sites than ET-3 (41 and 59%, respectively; Endoh et al., 1996). ET-1 and ET-3 produced maximum contractile responses of similar magnitude in this study, implying that, consistent with previous reports (Kasai et al., 1994; Yang et al., 1997), the density of receptors does not wholly reflect the magnitude of the functional response. Such disparity also has been noted in cardiomyocytes isolated from pigs after the development of pacing-induced heart failure. ET-1 produced a negative contractile effect that was not associated with alterations in the relative density of ETA and ETB receptor subtypes, but was thought to be due to changes in ETA receptor-mediated intracellular transduction (Thomas et al., 1996).

Several signal transduction mechanisms are likely to be involved in ET-1-induced contraction. It is clear from this study and others (Kramer et al., 1991; Hattori et al., 1993; Evans et al., 1994; Meyer et al., 1995) that ET-1 activates PLC, thereby generating inositol trisphosphate and diacylglycerol (Rubanyi and Polokoff, 1994). However, inhibition of PKC did not influence the positive contractile effect of ET-1 in ventricular cardiomyocytes isolated from rabbit myocardium. The PKC inhibitor BIM produced a rapid decrease in basal contractile response that could be reversed; hence, this compound did appear to be penetrating the cell membrane. BIM is a potent and selective inhibitor (Touleec et al., 1991) that was reported to inhibit the PKC-dependent trophic response to phenylephrine in isolated cardiomyocytes (Bell et al., 1995). Coupling subsequent to activation of PKC is complex. An inhibitory action of ET-1 is reported as a result of activation of PKC that phosphorylates troponin I and tropinin T, resulting in a decrease in Ca\textsuperscript{2+}-stimulated Mg\textsuperscript{2+}-ATPase activity and a negative inotropic effect or inhibition of the positive inotropic effect (Meyer et al., 1995). In rat cardiomyocytes, the positive inotropic action of ET-1 was reported to be due to stimulation of sarcoslemmal Na\textsuperscript{+}-H\textsuperscript{+} exchanger by a PKC-mediated pathway, resulting in alkalization and sensitization of cardiac myofilaments to intracellular Ca\textsuperscript{2+} (Kramer et al., 1991). Indeed, we have observed
that the hypertrophic response of ET-1 in rat cardiomyocytes was abolished by the PKC inhibitor BIM (Cullen et al., 1998). Because ET-1 did not shorten the relaxation time, as was the case with isoprenaline, the peptide is unlikely to have an effect on Ca\(^{2+}\) uptake by the sarcoplasmic reticulum (SR). However, ET-1 has been reported to increase contraction by accelerating the Ca\(^{2+}\) transport from the uptake site to the release site within the SR (Vigne et al., 1990). It also has been reported to inhibit Ca\(^{2+}\) leakage from the SR to the cytoplasm during the rest period, which would increase Ca\(^{2+}\) load and contraction. Because the time course effect of the ET-3 positive contractile response was different from that of ET-1, it is possible that the postreceptor mechanisms of action may well be different.

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References


