Positive inotropic responses mediated by endothelin $\text{ET}_A$ and $\text{ET}_B$ receptors in human myocardial trabeculae

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ABSTRACT

The aim of the present study was to determine possible inotropic effects mediated by endothelin $\text{ET}_A$ and $\text{ET}_B$ receptors in human myocardial trabeculae from the right atrium and the left ventricle. Isolated trabeculae from human hearts were paced at 1.0 Hz in tissue baths, and changes in isometric contractile force upon exposure to agonist were studied. Endothelin-1 (ET-1) and ET-3 had a strong positive inotropic effect in all trabeculae. ET-1 was significantly more potent than ET-3 in both atrial ($P < 0.001$) and ventricular ($P < 0.05$) trabeculae. Preincubation with the ET A receptor antagonist FR139317 (1 $\mu$M) decreased significantly ($P < 0.005$) the potency of ET-1 in both atrial and ventricular trabeculae, without any significant changes in $E_{\text{max}}$ (maximum effect obtained with an agonist). The ETB receptor agonist IRL 1620 had a positive inotropic effect only in some trabeculae, and the ETB receptor antagonist BQ 788 (1 $\mu$M) almost completely blocked this effect. These results suggest that both $\text{ET}_A$ and $\text{ET}_B$ receptors mediate positive inotropic responses at both the atrial and ventricular level in the human heart.

INTRODUCTION

Endothelin (ET) was originally detected in vascular endothelial cells [1], but there is now evidence that this peptide can be synthesized in endocardial endothelial cells and in myocardial cells as well [2–6]. Direct positive inotropic effects of ET have been demonstrated in isolated cardiac tissue from both laboratory animals [3,7–10] and humans [11,12]. Interestingly, though, ET did not exert any positive inotropic effects in isolated hearts from rat [13] and rabbit [14], and even negative inotropic effects of ET in isolated rat hearts have been reported [15]. Also, in in vivo studies on laboratory animals, a negative inotropic effect of ET was observed, probably secondary to its potent vasoconstrictor effect on the coronary arteries and in the peripheral circulation [16–18]. Increased plasma levels of ET-1 have been measured in heart failure patients [19,20]. Furthermore, experiments on laboratory animals have demonstrated an increase in ventricular ET levels as well as in its ventricular binding sites in congestive heart failure and pressure overload, suggesting that the ET-receptor-mediated signal transduction system in the heart is stimulated and that endogenous ET may be involved in

Key words: atrial function, endothelins, inotropic agents, ventricular function.

Abbreviations: ET, endothelin; $E_{\text{max}}$, maximum effect obtained with an agonist; $pEC_{50}$, negative logarithm of the EC$_{50}$.

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the maintenance of cardiac function under these conditions, possibly via a direct positive inotropic effect [21,22].

There are three known isopeptides of ET: ET-1, ET-2 and ET-3 [23]. Two distinct ET receptors have been cloned, expressed and characterized: ET$_A$ [24] and ET$_B$ [25]. The ET$_A$ receptor is selective for ET-1/ET-2 relative to ET-3, whereas the ET$_B$ receptor is non-selective with respect to the three isopeptides [26,27]. Both ET$_A$ and ET$_B$ receptors seem to be present in human atrial and ventricular myocardium [28–30], but there is controversy as to which receptor subtype(s) mediates a positive inotropic effect. From some studies on cardiac tissue from laboratory animals it has been suggested that ET$_B$ receptors mediate positive inotropic responses [31,32], whereas ET$_A$ receptors have been implicated in positive inotropic responses in the human heart [12,33]. It has further been proposed that ET may have a different physiological role in the atria from that in the ventricles [34,35].

The aim of the present study was to determine possible inotropic effects of ET-1 and ET-3 on isolated trabeculae from the right atrium and the left ventricle of human hearts, as well as the functional role of ET$_A$ and ET$_B$ receptors in the mediation of these responses. For this purpose we used the specific ET$_A$ receptor agonist IRL 1620, the ET$_A$ receptor antagonist FR139317 and the ET$_B$ receptor antagonist BQ 788 [26,27,36,37].

**METHODS**

The investigation conformed with the principles outlined in the Declaration of Helsinki (1989) of the World Medical Association. The collection of human tissues was in accordance with institutional guidelines, and the local ethics committee approved the project.

**Experimental setting**

The human hearts were kindly provided by the Rotterdam Heart Valve Bank (Bio Implant Services Foundation/Eurotransplant Foundation) after removal of the aortic and pulmonary valves for homograft valve implantation. Myocardial trabeculae were excised from the inner surface of the right atrium and the left ventricle. The hearts came from nine males and ten females in the age range 15–63 years (mean ± S.E.M. 46 ± 2.5 years). They were all previously healthy individuals that had died from cerebrovascular accidents or head trauma. The hearts were stored in a chilled, sterile organ protection solution [UW (Eurocollins) or HTK (Brett schneider)] [38] until the physiological studies were performed 12–24 h after explantation.

Trabeculae were excised with the heart placed in chilled Krebs buffer of the following composition (mM): NaCl 118, KCl 4.7, CaCl$_2$ 2.5, MgSO$_4$ 1.2, NaHCO$_3$ 25, KHPO$_4$ 1.2 and glucose 8.3. Only trabeculae that were free from the wall of the heart and with a diameter of approx. 0.5–0.7 mm for atrial trabeculae and 0.8–1.0 mm for ventricular trabeculae, corresponding to a cross-sectional area of approx. 0.2–0.4 mm$^2$ and 0.5–0.8 mm$^2$ for atrial and ventricular trabeculae respectively, were used. Care was taken not to damage the endothelial surface of the tissue. The trabeculae were mounted in organ baths (15 ml) containing the above-described Krebs buffer, which was kept at 37°C and gassed continuously with a mixture of 95% O$_2$ and 5% CO$_2$, giving a pH of approx. 7.4. The ends of the trabeculae were tied with silk sutures and connected to a Harvard transducer for measurement of isometric tension. The trabeculae were paced at 1.0 Hz using field stimulation (5 ms; voltage 20% above threshold for initiation of contractile response), through electrodes placed in the organ baths. Depending on the size of the trabeculae, resting tension was set to approx. 750 mg for atrial trabeculae and 1950 mg for ventricular trabeculae, a pretension established from previous experiments with human trabeculae in our laboratory [39]. During continuous pacing, the tissues were allowed to stabilize for approx. 90 min before the baseline contractile amplitude was measured.

Concentration–response curves for noradrenaline were obtained for some trabeculae, showing that a concentration of 10 µM gave a near-maximum response to noradrenaline. This concentration of noradrenaline was used to test the responsiveness of each trabecula and for comparison with other positive inotropic agents. Trabeculae with an increase in contractile force of less than 25 mg upon exposure to 10 µM noradrenaline were excluded from the study. After several wash-outs with normal Krebs buffer and stabilization at baseline contractile force, cumulative concentrations of agonist were added and changes in contractile force were measured. At the end of the experiments, the reactivity of the trabeculae was again tested by exposure to noradrenaline (10 µM). When testing the effect of specific ET antagonists, the antagonist at a concentration of 1 µM was added to the tissue bath 15 min prior to adding cumulative concentrations of agonist. The antagonist concentration was chosen from knowledge of their binding affinities, so that this concentration of antagonist could be expected to cause an almost maximal inhibitory effect [26,36,37,40].

**Analysis of data**

The maximum effect obtained with an agonist ($E_{max}$) and the negative logarithm of the EC$_{50}$ (pEC$_{50}$) were derived from concentration–response curves for each trabecula. Values are given as means ± S.E.M. for all trabeculae.
tested. One-factor ANOVA followed by Fisher’s Protected Least Significant Difference test was used to determine statistical significance with respect to differences in $E_{\text{max}}$ and $pEC_{50}$ values. The significance level, $\alpha$, was set at 0.05.

**Drugs**

Noradrenaline was obtained from Sigma (St. Louis, MO, U.S.A.). Human ET-1 and human ET-3 were from Bachem (Bubendorf, Switzerland). IRL 1620 ($N$-Suc-$[\text{Glu}^9,\text{Ala}^{11,13}]$ET-1-(8–21)) was from Research Biochemicals International (Natick, MA, U.S.A.). BQ 788 ($N$-cis-2,6-dimethylpiperidino-carbonyl-L-$\gamma$-methyl-leucyl-D-1-methoxy carbonyltryptophanyl-D-norleucine) and FR139317 ($[R]2-((R)-2-[[S]-2-[[1-(hexahydro-1H-azepinyl)]-carbonyl]amino-4-methylpentanoyl]amino-3-[3-(1-methyl-1H-indolyl)]propionyl]amino-3-(2-pyridyl)propionic acid) were from Neosystem S.A. (Strasbourg, France). The drugs were dissolved in distilled water.

**RESULTS**

**Concentration–response curves for noradrenaline**

Cumulative concentrations of noradrenaline up to 100 $\mu$M were applied to 11 atrial and eight ventricular trabeculae (one or two trabeculae from each atrium and ventricle) with baseline contractile amplitudes of $34 \pm 5$ mg and $154 \pm 32$ mg (means $\pm$ S.E.M.) respectively. The $E_{\text{max}}$ values for noradrenaline were $1181 \pm 168$% in atrial trabeculae and $348 \pm 90$% in ventricular trabeculae, these values representing the mean $\pm$ S.E.M. of the increase in contractile force (contractile amplitude after adding noradrenaline minus baseline contractile amplitude), measured as percentage of baseline contractile amplitude, in each individual trabecula (Figure 1). The $E_{\text{max}}$ values were significantly ($P < 0.005$) higher in atrial compared with ventricular trabeculae, measured as a percentage of baseline contractile amplitude. The same tendencies to greater increases in contractile amplitude in atrial compared with ventricular trabeculae were also seen for ET-3 and IRL 1620, but these differences were not statistically significant. ET-1, but not ET-3 or IRL 1620, was also significantly ($P < 0.05$) more potent in atrial than in ventricular trabeculae. In atrial trabeculae the $E_{\text{max}}$ value for ET-1 was significantly ($P < 0.05$) higher than those for ET-3 and IRL 1620. ET-1 was significantly more potent than ET-3 in both atrial ($P < 0.001$) and ventricular ($P < 0.05$) trabeculae, and in atrial trabeculae ET-1 was also significantly ($P < 0.001$) more potent than IRL 1620. There were no significant differences in $E_{\text{max}}$ or $pEC_{50}$ values between ET-3 and IRL 1620 in either atrial or ventricular trabeculae.

**ET receptor agonists**

ET-1 and ET-3 induced strong and potent increases in contractile force in all trabeculae, and IRL 1620 increased contractile force in more than half of the atrial and ventricular trabeculae tested (Table 1; Figures 2 and 3). The increase in contractile amplitude developed slowly, and it was also slow to reverse when washing out with normal buffer solution after finishing the agonist dose–response curves. The $E_{\text{max}}$ value for ET-1 was significantly ($P < 0.005$) higher in atrial than in ventricular trabeculae, measured as a percentage of baseline contractile amplitude. The same tendencies to greater increases in contractile amplitude in atrial compared with ventricular trabeculae were also seen for ET-3 and IRL 1620, but these differences were not statistically significant. ET-1, but not ET-3 or IRL 1620, was also significantly ($P < 0.05$) more potent in atrial than in ventricular trabeculae. In atrial, but not ventricular, trabeculae, the $E_{\text{max}}$ value for ET-1 was significantly ($P < 0.05$) higher than those for ET-3 and IRL 1620. ET-1 was significantly more potent than ET-3 in both atrial ($P < 0.001$) and ventricular ($P < 0.05$) trabeculae, and in atrial trabeculae ET-1 was also significantly ($P < 0.001$) more potent than IRL 1620. There were no significant differences in $E_{\text{max}}$ or $pEC_{50}$ values between ET-3 and IRL 1620 in either atrial or ventricular trabeculae.

**ET receptor antagonists**

The effects of ET receptor antagonists are shown in Figure 3. Preincubation with the $\text{ET}_A$ receptor antagonist

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Table 1  Positive inotropic effects of ET-1, ET-3 and various ET receptor agonists and antagonists

The effects of the various agents were tested on isolated trabeculae from human right atria and left ventricles. \( n \), number of trabecula tested; \( n_2 \), number of trabecula responding to agonist (one or two trabeculae from each atrium and ventricle). Calculations were carried out for each individual trabecula, and values are given as means ± S.E.M. for all trabeculae tested, including those trabeculae not responding to agonist. Baseline (mg) is the contractile amplitude before exposure to agonist, measured in mg. Increase by NA (%) is the increase in contractile amplitude after exposure to noradrenaline (10 \( \mu \)M), measured as a percentage of the baseline contractile amplitude. \( E_{\text{max}} \) is expressed as a percentage of baseline contractile amplitude. Significance of differences: (1) * in both atrial and ventricular trabeculae, preincubation with FR139317 (1 \( \mu \)M) resulted in significantly (\( P < 0.005 \)) lower pEC50 values for ET-1, without any significant changes in \( E_{\text{max}} \) values; (2) †preincubation with BQ 788 (1 \( \mu \)M) almost completely abolished positive inotropic responses to IRL 1620 in both atrial and ventricular trabeculae; (3) ‡ET-3 had a significantly lower pEC50 value than ET-1 in both atrial (\( P < 0.001 \)) and ventricular (\( P < 0.05 \)) trabeculae; (4) §IRL 1620 had a significantly (\( P < 0.001 \)) lower pEC50 value than ET-1 in atrial trabeculae; (5) in atrial trabeculae, the \( E_{\text{max}} \) value for ET-1 was significantly (\( P < 0.05 \)) higher than that of ET-3; and ¶IRL 1620.

<table>
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<tr>
<th>Agonist</th>
<th>( n_1 )</th>
<th>( n_2 )</th>
<th>Baseline (mg)</th>
<th>Increase by NA (%)</th>
<th>( E_{\text{max}} ) (%)</th>
<th>pEC50</th>
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<td>(a) Atria</td>
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<tr>
<td>ET-1</td>
<td>9</td>
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<td>39 ± 9</td>
<td>737 ± 125</td>
<td>507 ± 173</td>
<td>8.56 ± 0.13</td>
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<tr>
<td>FR139317 + ET-1</td>
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<td>9</td>
<td>59 ± 23</td>
<td>969 ± 347</td>
<td>560 ± 196</td>
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<tr>
<td>ET-3</td>
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<td>6</td>
<td>30 ± 14</td>
<td>1200 ± 246</td>
<td>113 ± 40†</td>
<td>7.68 ± 0.22‡</td>
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<td>11</td>
<td>7</td>
<td>47 ± 9</td>
<td>932 ± 228</td>
<td>150 ± 84¶</td>
<td>7.64 ± 0.08§</td>
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<td>1183 ± 387</td>
<td>4 ± 4†</td>
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<tr>
<td>ET-1</td>
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<td>10</td>
<td>105 ± 23</td>
<td>416 ± 84</td>
<td>52 ± 8</td>
<td>8.04 ± 0.22</td>
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<td>174 ± 41</td>
<td>481 ± 101</td>
<td>35 ± 9</td>
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<tr>
<td>ET-3</td>
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<td>128 ± 56</td>
<td>559 ± 110</td>
<td>40 ± 14</td>
<td>7.46 ± 0.25‡</td>
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<tr>
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<td>4</td>
<td>220 ± 58</td>
<td>323 ± 174</td>
<td>17 ± 9</td>
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<tr>
<td>BQ 788 + IRL 1620</td>
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<td>1</td>
<td>109 ± 43</td>
<td>541 ± 112</td>
<td>2 ± 2†</td>
<td>8.50</td>
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</table>

Figure 2  Representative tracings from atrial and ventricular trabeculae showing changes in isometric contractile amplitude upon exposure to cumulative concentrations of ET-1 and the ET\(_6\) receptor agonist IRL 1620.
DISCUSSION

Inotropic responses in atrial compared with ventricular trabeculae

Noradrenaline caused an enormous increase in contractile force – approx. 10-fold in atrial trabeculae and more than 3-fold in ventricular trabeculae. The responses to noradrenaline were significantly stronger in atrial compared with ventricular trabeculae, measured as a percentage of baseline contractile amplitude. Noradrenaline is known to mediate its positive inotropic effect via $\beta_1$-adrenoceptors to evoke maximum positive inotropic effects in both atria and ventricles [41]. If the maximal responses to noradrenaline are taken as an approximation of the maximal positive inotropic responses in human tissues, then our results would suggest a higher potential for modulation of contractile force in atrial than in ventricular tissue. Whereas the contribution of atrial contraction to ventricular filling in healthy individuals under resting conditions has been estimated to be only 29% and 23% for the left and right atrium respectively [42,43], this raises interesting questions as to the role of the atria during conditions of diminished cardiac reserve. Both exercise and pathological conditions such as heart failure are accompanied by an increase in sympathetic tone [44,45], and atrial contraction seems to be particularly important to maintain cardiac output and exercise performance under critical conditions [46].

In the present study, ET-1 and ET-3 induced strong and potent increases in contractile force in both atrial and ventricular human trabeculae. ET-1 induced significantly greater increases in contractile amplitude in atrial compared with ventricular trabeculae, measured as a percentage of baseline contractile amplitude. It thus seems that ET-1, similar to noradrenaline, has a more important role in the atria than in the ventricles as a modulator of contractile force. In rats, radioligand studies revealed a higher density of myocardial ET$_A$ than ET$_B$ receptors, and competition analysis revealed that left ventricular tissue had lower receptor densities and higher receptor affinities than the atria, suggesting a different physiological role for ET in the atria from that in the ventricles [34]. Dissimilarities in receptor coupling between atria and ventricles have also been suggested [35]. With regard to our results comparing functional responses between right atria and left ventricles, it ought to be kept in mind that right and left chambers may exhibit different proportional responses that may contribute to eventual differences seen between right atria and left ventricles. Furthermore, the approach of comparing responses measured as a percentage of baseline contractile force may have some limitations due to the fact that baseline contractile force may differ between atrial and ventricular tissue.
The increased responsiveness to ET-1 as well as to noradrenaline in the atria compared with the ventricles found in our study could be physiologically useful in helping to maintain an adequate cardiac output during conditions of diminished cardiac reserve, when the contribution of atrial contraction to ventricular filling becomes more important.

**ET receptors**

As the ET<sub>A</sub> receptor has a higher selectivity for ET-1 than for ET-3 [26,27], the significantly greater potency of ET-1 compared with ET-3 in both atrial and ventricular trabeculae found in the present study suggests the involvement of ET<sub>A</sub> receptors in mediating positive inotropic responses. This is in agreement with previous studies showing that ET-3 was much less potent than ET-1 in increasing inositol phosphate accumulation in human right atrial slices [35,47]. The effect of the ET<sub>A</sub> receptor antagonist FR139317 in the present study further supports the view that ET<sub>A</sub> receptors mediate positive inotropic effects. Preincubation with FR139317 significantly lowered the pEC<sub>50</sub> values for ET-1 in both atrial and ventricular trabeculae, without any significant changes in E<sub>max</sub> values, which would suggest a competitive antagonism at the ET<sub>A</sub> receptor. In a previous radioligand binding study using human hearts, the ET<sub>A</sub> receptor antagonist BQ-123 competitively inhibited ET-1 binding [28]. It has further been shown that BQ-123 antagonizes the positive inotropic effect of ET-1 in human atrial [12] and ventricular [33] muscle strip preparations. In the latter study, BQ-123 shifted the concentration–response curve for ET-1 to the right, whereas the ET<sub>B</sub> receptor agonist sarafotoxin S6c had no functional effects [33]. In the present study, however, the ET<sub>B</sub> receptor agonist IRL 1620 elicited a positive inotropic effect in more than half of the atrial and ventricular trabeculae, and the effect of IRL 1620 was almost completely blocked by the ET<sub>B</sub> receptor antagonist BQ 788. We have no ready explanation as to why ET<sub>B</sub> receptor antagonism by BQ 788 could not be overcome by increasing the concentration of IRL 1620, except that this might suggest a non-competitive antagonism. Our results do, however, suggest that ET<sub>B</sub> receptors also mediate positive inotropic responses in human atrial and ventricular myocardium. The fact that only some of the trabeculae responded to IRL 1620, whereas others did not, is rather intriguing, but possible explanations could involve a different density or functional state of specific receptors or second messenger mechanisms. Interestingly, a previous study with vascular smooth muscle cells demonstrated plasticity in the smooth muscle cell expression of contractile ET<sub>B</sub> receptors [48]. Although radioligand binding studies have demonstrated the presence of ET<sub>B</sub> receptors in human atrial and ventricular muscle [28,30,35], previous studies have not been able to clearly assess the role of ET<sub>B</sub> receptors in the mediation of positive inotropic responses. In a study using isolated rabbit papillary muscles, both the ET<sub>A</sub> receptor agonist [Thr<sup>5</sup>]sarafotoxin S6b and the ET<sub>B</sub> receptor agonist [Glu<sup>5</sup>]sarafotoxin S6b had a positive inotropic effect, whereas the ET<sub>B</sub> receptor agonist IRL 1620 had no effect. It was concluded that the positive inotropic response might be mediated by a novel ET receptor subtype or an atypical ET<sub>A</sub> or ET<sub>B</sub> receptor [49]. The presence of a novel or atypical ET receptor does not offer any explanation for our present findings, but the possibility cannot be excluded that the functional effects seen after stimulation with ET agonists or antagonists could be obscured by an effect mediated via a novel/atypical ET receptor. It has been suggested from studies on rabbit papillary muscle that ET<sub>B</sub> receptors, as well as different subtypes of ET<sub>B</sub> receptors, may mediate positive inotropic responses [50,51]. Furthermore, in a study on open-chest rats, in contrast with the non-selective activation of ET<sub>A</sub> and ET<sub>B</sub> receptors by ET-1, IRL 1620 had a significant positive inotropic effect, and this discrepancy was explained by a less pronounced vasoconstriction with the absence of myocardial ischaemia after ET<sub>B</sub> receptor activation by IRL 1620 [32].

Our present results suggest that both ET<sub>A</sub> and ET<sub>B</sub> receptors are likely candidates in the regulation of cardiac contractility. As ET appears to be produced locally in the heart [2,52] and this production seems to increase in heart failure [21,22] and myocardial ischaemia [53], ET may play an important role in the regulation of cardiac contractility, especially under certain pathophysiological conditions.

**Conclusions**

Noradrenaline, ET-1 and ET-3 had strong positive inotropic effects in all trabeculae from both the right atrium and the left ventricle of human hearts. Higher E<sub>max</sub> values for noradrenaline and ET-1 in atrial compared with ventricular trabeculae, measured as a percentage of baseline contractile amplitude, suggest a higher potential for modulation of contractile force in atrial than in ventricular tissue. Different responses to ET-1 and ET-3 and an antagonistic effect of the ET<sub>A</sub> receptor antagonist FR139317 on responses to ET-1 suggest the involvement of ET<sub>A</sub> receptors in the mediation of positive inotropic responses. Furthermore, the positive inotropic effect of the ET<sub>B</sub> receptor agonist IRL 1620, which was blocked by the ET<sub>B</sub> receptor antagonist BQ 788, suggests that ET<sub>B</sub> receptors also mediate positive inotropic responses in atrial and ventricular tissue.

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REFERENCES


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