Inotropic effects of ET<sub>B</sub> receptor stimulation and their modulation by endocardial endothelium, NO, and prostaglandins

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Leite-Moreira, Adelino F., and Carmen Brás-Silva. Inotropic effects of ET<sub>B</sub> receptor stimulation and their modulation by endocardial endothelium, NO, and prostaglandins. *Am J Physiol Heart Circ Physiol* 287: H1194–H1199, 2004. First published May 6, 2004; 10.1152/ajpheart.00563.2003.—Endothelin (ET)-1 acts on ET<sub>A</sub> and ET<sub>B</sub> receptors. The latter include ET<sub>B1</sub> (endothelial) and ET<sub>B2</sub> (muscular) subtypes, which mediate opposite effects on vascular tone. This study investigated, in rabbit papillary muscles (n = 84), the myocardial effects of ET<sub>B</sub> stimulation. ET-1 (10<sup>-9</sup> M) was given in the absence or presence of BQ-123 (ET<sub>A</sub> antagonist). The effects of IRL-1620 (ET<sub>B1</sub> agonist, 10<sup>-10</sup>–10<sup>-6</sup> M) or sarafotoxin S6c (ET<sub>B2</sub> agonist, 10<sup>-10</sup>–10<sup>-6</sup> M) were evaluated in muscles with intact or damaged endocardial endothelium (EE); intact EE, in the presence of N<sup>3</sup>-nitro-L-arginine (l-NNa); and intact EE, in the presence of indomethacin (Indo). Sarafotoxin S6c effects were also studied in the presence of BQ-788 (ET<sub>B2</sub> antagonist). ET-1 alone increased 64 ± 18% active tension (AT) but decreased it by 4 ± 2% in the presence of BQ-123. In muscles with intact EE, sarafotoxin S6c alone did not significantly alter myocardial performance. Sarafotoxin S6c (10<sup>-6</sup> M) increased, however, AT by 120 ± 27% when EE was damaged and by 39 ± 8% or 23 ± 6% in the presence of l-NNa or Indo, respectively. In the presence of BQ-788, sarafotoxin S6c decreased AT (21 ± 3% at 10<sup>-6</sup> M) in muscles with intact EE, an effect that was abolished when EE was damaged. IRL-1620 also decreased AT (22 ± 3% at 10<sup>-6</sup> M) in muscles with intact EE, an effect that was abolished when EE was damaged or in the presence of l-NNa or Indo. In conclusion, the ET<sub>B</sub>-mediated negative inotropic effect is presumably due to ET<sub>B1</sub> stimulation, requires an intact EE, and is mediated by NO and prostaglandins, whereas the ET<sub>B</sub>-mediated positive inotropic effect, observed when EE was damaged or NO and prostaglandins synthesis inhibited, is presumably due to ET<sub>B2</sub> stimulation.

ENDOTHELIN (ET)-1 was originally detected in vascular endothelial cells (62), but there is now evidence that this peptide can be synthesized in endocardial endothelial cells and myocardial cells as well (33).

Plasma and salivary levels of ET-1 are elevated, correlate with the New York Heart Association functional classes, and are of prognostic value in congestive heart failure (30).

The predominantly abluminal release and short half-life of ET-1 in blood restricts its activity primarily to paracrine and autocrine actions (33). In mammalian tissues, two G protein-coupled receptor types, ET<sub>A</sub> and ET<sub>B</sub>, mediate these actions differentially (2, 48, 56). ET<sub>A</sub> receptor stimulation elicits vasoconstriction (35) and mitogenesis (41) and increases inotropism (22, 28). ET<sub>B</sub> receptor activation promotes vasodilatation mediated by nitric oxide (NO) (10, 20) and prostacyclin (10, 14) release and has growth-inhibitory effects (34, 42) associated with apoptosis (42; see Ref. 12 for a review). These receptors also mediate the pulmonary clearance of circulating ET-1 (17) and the reuptake of ET-1 by endothelial cells (43).

In the vasculature, ET<sub>A</sub> receptors are found in smooth muscle cells, whereas ET<sub>B</sub> receptors are localized on endothelial cells (ET<sub>B1</sub>) and in smooth muscle cells (ET<sub>B2</sub>). ET<sub>B1</sub> and ET<sub>B2</sub> receptor stimulation promote opposite effects on vascular tone: ET<sub>B1</sub> receptors induce vasodilatation through the release of NO, whereas ET<sub>B2</sub> receptors induce direct vasoconstriction (13, 56).

Even though ET<sub>B</sub> receptors have been shown to be present in atrial and ventricular myocardium of various animal species, including humans (1, 9), the myocardial effects of their selective stimulation are still poorly understood. We recently found that, in the presence of selective ET<sub>A</sub> receptor antagonism, ET-1 induced a small, although significant, negative inotropic effect (32). The investigation of these effects and their underlying mechanisms was, therefore, the main goal of the present study.

METHODS

This investigation conforms to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996).

**Experimental preparation.** The study was performed in isolated right papillary muscles (n = 84) from male New Zealand White rabbits (*Oryctolagus cuniculus*; 2.0–3.0 kg). Rabbits were anesthetized with intravenous pentobarbital sodium salt (25 mg/kg). A left thoracotomy was performed, and beating hearts were quickly excised and immersed in modified Krebs-Ringer (KR) solution [composition (in mmol/l) 98 NaCl, 4.7 KCl, 2.4 MgSO<sub>4</sub>-7H<sub>2</sub>O, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 4.5 glucose, 1.8 CaCl<sub>2</sub>·2H<sub>2</sub>O, 17 NaHCO<sub>3</sub>, 15 C<sub>6</sub>H<sub>12</sub>Na<sub>7</sub>O<sub>6</sub>, 5 C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>O<sub>4</sub>, and 0.02 atenolol] at 35°C with cardioplegic 2,3-butanedione monoxime (BDM; 3%) (40) and 5% newborn calf serum and gassed with 95% O<sub>2</sub>-5% CO<sub>2</sub> to obtain a pH between 7.38 and 7.42.

After dissection, papillary muscles (length: 3.8 ± 0.1 mm, weight: 2.9 ± 0.2 mg, preload: 4.4 ± 0.2 mN) were mounted vertically in a 10-ml Plexiglas organ bath containing the above-described KR solution and attached to an electromagnetic length-tension transducer (University of Antwerp; Antwerp, Belgium). Preload was estimated according to muscle dimensions, and the electrical stimulus (0.6 Hz) was set at 10% above threshold. Twenty minutes later, bathing solutions were replaced by corresponding KR solutions without BDM. During the next 2 h, muscles were stabilized. Bathing solutions were then replaced by corresponding KR solutions without calf serum, and maximum physiological length (*L*<sub>max</sub>) was calculated. Protocols were initiated after two similar isotonic and isometric control twitches separated by a 10-min interval were obtained.

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Experimental protocols. The myocardial effects of ET-1, sarafotoxin S6c (SRTXc), and IRL-1620 were studied. ET-1 (10^{-9} M) was given in the absence (n = 9) and presence (n = 9) of BQ-123 (10^{-5} M; selective ET_{A} receptor antagonist). Concentration-response curves to the selective ET_{B} receptor agonist SRTXc (10^{-10}–5 \times 10^{-6} M) were performed in muscles with I intact endocardial endothelium (EE; n = 6); 2 damaged EE (n = 7); 3 intact EE in the presence of the NO synthase inhibitor L-NNA (10^{-5} M, n = 8); 4 intact EE in the presence of the NO synthase inhibitor indo- methacin (Indo; 10^{-5} M, n = 7); 5 intact EE in the presence of the ET_{B2} antagonist BQ-788 (10^{-5} M, n = 6); and 6 damaged EE in the presence BQ-788 (10^{-5} M, n = 6). Concentration-response curves to another selective ET_{B} receptor agonist, IRL-1620 (10^{-10}–6 \times 10^{-6} M), which predominantly activates endothelial ET_{B1} receptors (63), were evaluated in muscles with I intact EE (n = 7); 2 damaged EE (n = 8); 3 intact EE in the presence of the NO synthase inhibitor L-NNA (10^{-5} M, n = 5); and 4 intact EE in the presence of the cyclooxygenase inhibitor Indo (10^{-5} M, n = 6).

The EE was damaged by briefly (1 s) exposing the isolated papillary muscle to a weak solution (0.5%) of the detergent Triton X-100 (5).

Chemicals were obtained from Sigma Chemical (St. Louis, MO). Bovine aprotinin, Isotonic and isotonic twitches were recorded and analyzed. The selected parameters included active tension (AT; in mN/mm^{2}), maximum velocity of tension rise (dT/dt_{max}; in mN/mm^{2}-s^{-1}); maximum velocity of tension decline (dT/dt_{min}; in mN/mm^{2}-s^{-1}); peak isotonic shortening (PS; %L_{max}); maximum velocity of shortening (dL/dL_{max}; in mm/s); maximum velocity of lengthening (dL/dL_{min}; in mm/s); and time to half relaxation (tHR; in ms).

Notwithstanding, only data obtained from isometric twitches are described, as the analysis of isotonic twitches yielded globally similar results. In the various protocols, results are given as the percent change from baseline. For the parameters that are expressed as negative values (e.g., dT/dt_{min}), such percent change refers to the absolute values.

When a pharmacological inhibitor (BQ-123; BQ-788, Indo or L-NNA) was used, the term baseline refers to the condition in the presence of those inhibitors before the addition of ET-1, SRTXc, or IRL-1620. Analysis of the effects of each of those inhibitors per se on myocardial performance revealed that only BQ-123, in the presence of an intact EE, significantly altered the contractile parameters, decreasing AT by 6.5 \pm 1.2\%, dT/dt_{max} by 8.5 \pm 1.4\%, and dT/dt_{min} by 7.2 \pm 1.5\%.

Statistical methods. Values are means \pm SE. The effects of a single concentration of an individual drug on the various contractile parameters were analyzed by a paired t-test, whereas the effects of increasing concentrations were analyzed by one-way repeated-measures ANOVA. When significant differences were detected with the latter, the Student-Newman-Keuls test was selected to perform multiple comparisons. P < 0.05 was accepted as significant.

RESULTS

Mean values of the contractile parameters in papillary muscles with an intact EE (n = 63) were similar in all experimental protocols. Removal of the EE (n = 21) resulted in a negative inotropic effect (Table 1).

Table 1. Mean values of contractile parameters in papillary muscles with an intact or damaged EE.

<table>
<thead>
<tr>
<th>Contractile Parameter</th>
<th>Intact EE</th>
<th>Damaged EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>63</td>
<td>21</td>
</tr>
<tr>
<td>AT, mN/mm^{2}</td>
<td>25.2 \pm 2.0</td>
<td>18.0 \pm 2.3</td>
</tr>
<tr>
<td>dT/dt_{max}, mN/mm^{2}-s^{-1}</td>
<td>414.4 \pm 9.7</td>
<td>433.2 \pm 14.1</td>
</tr>
<tr>
<td>dT/dt_{min}, mN/mm^{2}-s^{-1}</td>
<td>1663.6 \pm 13.1</td>
<td>1097.9 \pm 11.7</td>
</tr>
<tr>
<td>PS, %L_{max}</td>
<td>13.2 \pm 0.01</td>
<td>10.0 \pm 0.01</td>
</tr>
<tr>
<td>dL/dL_{max}, L_{max}/s</td>
<td>0.8 \pm 0.04</td>
<td>0.7 \pm 0.07</td>
</tr>
<tr>
<td>dL/dL_{min}, L_{min}/s</td>
<td>-2.9 \pm 0.2</td>
<td>-2.3 \pm 0.3</td>
</tr>
<tr>
<td>tHR, ms</td>
<td>-127.9 \pm 9.1</td>
<td>85.7 \pm 9.8</td>
</tr>
</tbody>
</table>

Values are means \pm SE; n = no. of muscles. EE, endocardial endothelium; AT, active tension; dT/dt_{max}, maximum velocity of tension rise; dT/dt_{min}, maximum velocity of tension decline; PS, peak isotonic shortening; L_{max}, maximum length; dL/dL_{max}, maximum velocity of shortening; dL/dL_{min}, maximum velocity of lengthening; tHR, time to half relaxation.

When given alone to muscles with an intact EE, SRTXc did not significantly alter myocardial performance, although there was a trend for negative inotropic and lusitropic effects at the two higher concentrations. In these conditions, 10^{-6} M SRTXc decreased AT by 7.8 \pm 4.8\%, dT/dt_{max} by 6.8 \pm 7\%, and dT/dt_{min} by 7.1 \pm 3.8\%. In contrast, when the EE was damaged, or in the presence of L-NNA or Indo, SRTXc induced instead positive inotropic and lusitropic effects (Fig. 1), which were highly significant at 10^{-6} M. At this concentration, when the EE was damaged, SRTXc increased AT by 119.2 \pm 27.2\%, dT/dt_{max} by 85.9 \pm 31.9\%, and dT/dt_{min} by 95.0 \pm 21.0\%. At the same concentration, in the presence of L-NNA, SRTXc increased AT by 38.8 \pm 8.0\%, dT/dt_{max} by 51.4 \pm 11.2\%, and dT/dt_{min} by 35.2 \pm 11.5\%, whereas in the presence of Indo, SRTXc increased AT by 23.4 \pm 6.0\%, dT/dt_{max} by 27.8 \pm 7.0\%, and dT/dt_{min} by 16.0 \pm 8.2\%. The positive inotropic effect elicited by SRTXc, both in muscles with damaged EE and muscles with intact EE in the presence of L-NNA or Indo, started immediately after the addition of the drug and reached a stable maximum 16–19 min later. The effect could be completely, albeit slowly, washed out, taking 90–120 min to return to baseline.

When given in the presence of BQ-788 to muscles with an intact EE, SRTXc induced concentration-dependent negative inotropic and lusitropic effects, which were significant over the entire range of concentrations, decreasing at 10^{-6} M: AT, 20.7 \pm 2.5\%; dT/dt_{max}, 19.1 \pm 2.5\%; and dT/dt_{min}, 17.1 \pm 3.0\%. On the other side, when the EE was damaged, the positive inotropic and lusitropic effects of SRTXc observed in the absence of BQ-788 were abolished in the presence of this blocker (Fig. 2).

Figure 3 illustrates concentration-response curves to the other ET_{B} agonist, IRL-1620. When the EE was intact, this agent promoted concentration-dependent negative inotropic and lusitropic effects. At 10^{-6} M, it decreased AT by 22.1 \pm 2.6\%, dT/dt_{max} by 14.4 \pm 2.0\%, and dT/dt_{min} by 11.1 \pm 6.1\%. This effect was abolished when the EE was damaged or in the
The negative inotropic effect elicited by IRL-1620 in muscles with intact EE started immediately after the addition of the drug and reached a stable maximum 20–25 min later. The effect could be completely washed out, taking 30–35 min to return to baseline.

**DISCUSSION**

The present study showed that in the presence of selective ET<sub>A</sub> antagonism, ET-1 induces negative inotropic and lusitropic effects and an earlier onset of relaxation, indicating that they are presumably due to ET<sub>B</sub> stimulation.

Previous reports also described a negative inotropic effect of ET-1. The relative contribution of ET<sub>A</sub> and ET<sub>B</sub> receptors to this effect remains, however, unclear. Most of these studies showed an initial transient negative, followed by a sustained positive inotropic effect (7, 11, 37, 46, 57, 58). This pattern was mainly reported in atrial tissue (11, 37, 46), being observed in ventricular tissue only with higher concentrations of ET-1 (57, 58). In our study, 10<sup>−9</sup> M of ET-1 produced a sustained positive inotropic effect that began immediately after its administration, whereas the sustained negative inotropic effect was observed only when ET-1 was given in the presence of an ET<sub>A</sub> receptor antagonist. In mouse ventricular tissue, ET-1 also induced a long-lasting negative inotropic effect, which was, however, attributed to ET<sub>A</sub> stimulation (24) and

Fig. 1. Concentration-response curves for the effect of sarafotoxin S6c (SRTXc) on contractile parameters in the various experimental conditions: intact (n = 6) or damaged (n = 7) endocardial endothelium (EE) or intact EE in the presence of N<sup>ω</sup>-nitro-l-arginine (l-NNA, n = 8) or indomethacin (Indo, n = 7). A: active tension (AT). B: peak rate of tension rise (dT/dt<sub>max</sub>). C: peak rate of tension decline (dT/dt<sub>min</sub>). Values are means ± SE (as %baseline). *P < 0.05 vs. baseline.

Fig. 2. Concentration-response curves for the effect of SRTXc in the presence of BQ-788 on contractile parameters in muscles with intact (n = 6) or damaged (n = 6) EE. A: AT. B: dT/dt<sub>max</sub>. C: dT/dt<sub>min</sub>. Values are means ± SE (as %baseline). *P < 0.05 vs. baseline.
To investigate whether a similar distribution of ETB receptor subtypes occurs in the heart (ETB₁ in EE cells and ETB₂ in cardiomyocytes), we analyzed in muscles with intact and damaged EE the myocardial effects of two distinct ETB agonists: 1) SRTXc, which, activating both ETB₁ and ETB₂ subtypes, acts predominantly on muscular ETB₂ receptors (61, 63); and 2) IRL-1620, which selectively activates endothelial ETB₁ receptors (9, 63). The results of these studies support our hypothesis. In fact, in muscles with an intact EE, both agents induced negative inotropic and lusitropic effects, although they were of larger magnitude with IRL-1620 and did not even reach statistical significance with SRTXc. Therefore, in the heart, IRL-1620 apparently also selectively activates endothelial ETB₁ receptors because its effects disappeared when the EE was damaged. On the other side, the nonsignificant negative inotropic and lusitropic effects elicited by SRTXc observed were reversed when the EE was damaged or when the synthesis of NO or prostaglandins was inhibited. Cardiac endothelium, both vascular and endocardial, regulates performance of underlying cardiac muscle, through the release of several substances, namely, ET-1, NO, and prostaglandins (see Ref. 4 for a review).

The inotropic and lusitropic effects of SRTXc may be explained by the balance between its effects on endothelial (ETB₁) and muscular (ETB₂) receptor stimulation. In the presence of an intact EE, ETB₁ effects equilibrate, or slightly exceed, those of ETB₂ stimulation, and therefore SRTXc promotes small and nonsignificant negative inotropic and lusitropic actions. In the presence of BQ-788, which preferentially antagonizes ETB₂ receptors (25, 63), the balance favors ETB₁ effects, and therefore increased and highly significant negative inotropic and lusitropic actions are observed over the entire range of SRTXc concentrations. When the EE is damaged, SRTXc acts preferentially on ETB₂ receptors and elicits positive inotropic and lusitropic actions, which were observed with concentrations of $10^{-8}$ M or higher, although it only reached statistical significance at the concentration of $10^{-6}$ M. Such effects are blocked when BQ-788 is concomitantly administered. Finally, when the synthesis of NO or prostaglandins is inhibited, it is conceivable that the balance might be also shifted toward the effects of ETB₂ stimulation.

NO released either from cardiac endothelial cells or generated within cardiac myocytes can directly influence cardiac contractile function (52). Both endothelium-derived NO and exogenous NO donors induce an earlier onset of myocardial relaxation and/or reduce diastolic tone (18, 44). These effects have been observed in a variety of preparations and species, including rat cardiac myocytes (31, 54, 60); ferret (55), cat (38), and human (16) papillary muscles; isolated ejecting guinea pig hearts (18); and normal human subjects undergoing diagnostic cardiac catheterization (44, 45). Although NO is generally considered to have negative inotropic properties, some studies have suggested that it might increase or decrease inotropism depending on the concentration of NO (29, 38, 50), the integrity of endocardial endothelium (38), the rate of NO release (3), and the presence of cholinergic (38) or β-adrenergic stimulation (36, 38, 49). The negative inotropic and relaxant effects of NO have largely been attributed to a cGMP-mediated reduction in myofilament Ca$^{2+}$ responsiveness, via activation of PKG (60) and possibly subsequent phosphorylation of troponin I (31).

Fig. 3. Concentration-response curves for the effect of IRL-1620 on contractile parameters in the various experimental conditions: intact (n = 7) or damaged (n = 8) EE or intact EE in the presence of l-NNa (n = 5) or Indo (n = 6). A: AT. B: dT/d$\max$. C: dT/d$\min$. Values are means ± SE (as % baseline). *P < 0.05 vs. baseline.

seemed to be specific to this animal species (24, 47). Some authors attributed the negative inotropic of ET-1 to concomitant β-adrenoceptor stimulation (6, 11, 64), a mechanism that presumably did not contribute to the effects we observed because our experiments were performed in the presence of the β-blocking agent atenolol.

In the vasculature, ETB receptors are localized on endothelial cells (ETB₁) and in smooth muscle cells (ETB₂), which promote opposite effects on vascular tone: ETB₁ receptors induce vasodilatation through the release of NO (10, 20) and prostaglandins (10, 14), whereas ETB₂ receptors induce direct vasoconstriction through mechanisms that are not yet clear (13, 56).
ET-1 also stimulates the release of prostaglandins, namely, prostacyclin, as shown in bovine and human vascular endothelial cells (14, 27) and in isolated (26) and in vivo rabbit hearts (59). Prostaglandins act through their specific receptors that are coupled via G proteins to second messenger systems, mainly cAMP and possibly inositol 1,4,5-triphosphate (51). Direct myocardial actions of prostaglandins are still not clear, because both negative (51) and positive (39) inotropic effects were shown in isolated papillary muscles. The positive inotropic effect of EE-released prostaglandins was, however, observed only when NO synthesis was inhibited (39). This interaction between EE-released NO and prostaglandins might help to explain why, in our study, inhibition of the synthesis of NO or prostaglandins resulted in positive inotropic and lusitropic effects of ET<sub>B</sub> stimulation and why such effects were of larger magnitude when NO rather than prostaglandin synthesis was inhibited.

In conclusion, the present study, therefore, adds to our knowledge about the myocardial effects of ET<sub>B</sub> receptor stimulation. It shows that ET<sub>B</sub> stimulation induces a negative inotropic effect, which requires an intact EE and is mediated by NO and prostaglandins. When the EE was damaged, ET<sub>B</sub> stimulation induced a positive inotropic effect. The differential effects of ET<sub>B</sub> stimulation, in the presence and absence of an intact EE, indicate that the analysis of such effects might be used as an experimental tool, unavailable to date, to test the functional integrity of the EE.

These findings might be potentially relevant for the pathophysiology of cardiovascular diseases characterized by increased ET-1 levels and endothelial dysfunction (e.g., heart failure). They might also help to better interpret eventual differences between the therapeutic effects of selective and nonselective ET<sub>A</sub> receptor antagonists, which are being currently tested.

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