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Evidence for atypical endothelin receptors and for presence of endothelin-converting enzyme activity in the mouse isolated vas deferens

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Abstract

The endothelin receptors controlling sympathetic neurotransmission and the presence of endothelin-converting enzyme were investigated in the mouse vas deferens. Endothelin-1 or endothelin-3 (0.01–100 nM) enhanced contractions evoked by field stimulation, yielding EC_{50} (geometric mean and 95% confidence limits) of 0.7 nM (0.4–1.6) and 13.7 nM (10.2–14.1) and E_{max} (mean ± S.E.M. increase in twitch tension, in mg/10 mg wet tissue) of 473 ± 35 and 520 ± 51, respectively. The selective endothelin ET_B receptor agonists IRL 1620 (Suc-[Glu^9,$Ala^{11,15}$]endothelin-1) and sarafotoxin S6c were inactive up to 100 nM. Responses to endothelin-3 were progressively inhibited by the selective endothelin ET_A receptor antagonist BQ-123 (cyclo[D-Trp-D-Asp-Pro-D-Val-Leu]) (10, 30 and 100 nM). At 100 nM, BQ-123 almost abolished the response to endothelin-3 (100 nM). In contrast, at 100, 300 nM and 1 μM, BQ-123 shifted the curve to endothelin-1 to the right only 2-, 5- and 6-fold, respectively. The selective endothelin ET_B receptor antagonist BQ-788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methyl-leucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine) (100 nM) did not modify responses to endothelin-1 or endothelin-3 (0.01–100 nM). Big-endothelin-1 (0.3–30 nM) was 10-fold less potent than endothelin-1 in increasing neurogenic responses (EC_{50} 6.8 nM, 4.7–9.6; E_{max} 457 ± 37 mg/10 mg wet tissue). Preincubation with phosphoramidon (100 μM) reduced responses to big-endothelin-1, but not endothelin-1. Thus, endothelin-1 and endothelin-3 potently enhance sympathetic neurotransmission in the mouse vas deferens via stimulation of BQ-788-insensitive endothelin receptors, which are variably sensitive to blockade by BQ-123. It remains to be clarified if these findings are due to the presence of an atypical population of endothelin receptors in this tissue, or reflect the binding of endothelin-1 and endothelin-3 to distinct subdomains of a single sub-type of endothelin ET_A receptor. This tissue also displays pronounced phosphoramidon-sensitive endothelin-converting enzyme activity.

Keywords: Endothelin; Sarafotoxin; Vas deferens; BQ-123; IRL 1620; BQ-788; Endothelin-converting enzyme; Phosphoramidon; Neurotransmission; (Mouse)

1. Introduction

Endothelins are produced by many cell types, including peripheral and central neurones (Shinmi et al., 1989; Matsumoto et al., 1989), and can cause variable effects at peripheral cardiovascular and non-vascular sympathetic junctions. Endothelin-1 inhibits sympathetic neurotransmission in the guinea pig femoral artery (Wiklund et al., 1988) and rat heart (Reid et al., 1989), but enhances nerve-mediated vasoconstriction in guinea pig pulmonary artery (Wiklund et al., 1989) and rat perfused arterial (Tabuchi et al., 1989; Han et al., 1990) and venous mesentery (Warner et al., 1990). Endothelin-1 also markedly potentiates neurogenic contractions of the vas deferens of the guinea pig (Wiklund et al., 1990), rat (Maggi et al., 1989; Wiklund et al., 1990; Donoso et al., 1992), and mouse (Rae and Calixto, 1990), in part by increasing sensitivity of smooth muscle to ATP and noradrenaline.

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To date, 3 distinct heptahelical G-protein-coupled endothelin receptors have been cloned. The endothelin ET_A receptor has higher affinity for endothelins-1 and -2, than for endothelin-3 (Arai et al., 1990) and is blocked selectively by several antagonists including BQ-123 (Ihara et al., 1992a; for review see Huggins et al., 1993). The endothelin ET_B receptor does not discriminate between the 3 mammalian endothelins (Sakurai et al., 1990), is preferentially stimulated by sarafotoxin S6c, BQ-3020 and IRL 1620 (Williams et al., 1991; Ihara et al., 1992b; Takai et al., 1992;) and is blocked selectively by BQ-788 (Ishikawa et al., 1994). Finally, the endothelin ET_C receptor, which has been cloned from an amphibian genome (Karne et al., 1993), displays higher affinity for endothelin-3 than endothelin-1 (Emori et al., 1990). Functional evidence also suggests that there are subtypes of endothelin ET_A and ET_B receptors, and atypical receptors (Bertelsen et al., 1992; Harrison et al., 1992; Kumar et al., 1993, 1994; Warner et al., 1993b).

The fact that endothelin-3 is as potent as endothelin-1 in potentiating neurogenic twitch contractions of the rat vas deferens would suggest these effects are mediated via endothelin ET_B receptors (Télémaque and D'Orléans-Juste, 1991). However, this view has been challenged recently by studies showing that the selective endothelin ET_B receptor agonist sarafotoxin S6c is 300-fold less potent than endothelin-1, and that the selective endothelin ET_A receptor antagonist BQ-123 and the endothelin ET_A/ET_B receptor antagonist PD 142893 each block the effects of endothelin-3 to a much larger extent than those of endothelin-1 (Eglezos et al., 1993; Warner et al., 1993a). On the other hand, endothelin-3 is about 30-fold less potent than endothelin-1 in the mouse vas deferens (Rae and Calixto, 1990), which would suggest twitch potentiation in this species may involve endothelin ET_A receptors. The current study attempts to address this issue more adequately, by analysing the effects the selective endothelin ET_B receptor agonists sarafotoxin S6c and IRL 1620 (Williams et al., 1991; Takai et al., 1992), as well as the influences of the selective endothelin ET_A receptor antagonist BQ-123 (Ihara et al., 1992a) and a new selective endothelin ET_B receptor antagonist, BQ-788 (Ishikawa et al., 1994), on twitch potentiations triggered by endothelin-1 and endothelin-3 in the mouse vas deferens.

Big-endothelin-1, the immediate precursor of endothelin-1, is almost as active as endothelin-1 itself in increasing neurogenic contractions of the rat vas deferens, due to presence of a phosphoramidon-sensitive endothelin-converting enzyme (Télémaque and D'Orléans-Juste, 1991; Mattera et al., 1993). In this regard, the present study also assesses if the mouse vas deferens displays significant endothelin-converting enzyme activity.

2. Materials and methods

2.1. Preparations

Male Swiss albino mice (25–35 g), raised in a temperature-controled (22 ± 2°C) ambient under a 12 h light/dark cycle, were lightly anaesthetized with ether and killed by a sharp blow to the head and cervical dislocation. Both vasa deferentia were removed, placed in a Petri dish containing warm physiological salt solution (see composition below) and freed of adhering connective and adipose tissues. Each vas deferens was then transferred to a double-jacketed organ bath containing 5 ml of Krebs' solution at 37°C continuously gassed with 95% of O_2 and 5% of CO_2, connected to a strain gauge transducer coupled to a pen recorder (DMP 4B, Narco Biosystems, USA) and submitted to an initial resting tension of 0.5 g. The Krebs' solution had the following composition (mM): NaCl 118; KCl 4.7; $CaCl_2$ 2.5; $NaHCO_3$ 25; KH_2PO_4 0.9 and glucose 11 (pH 7.2–7.4). An equilibration period of at least 45 min was allowed before any drug additions, during which the bath solution was renewed every 15 min. Field stimulation was induced using trains of 4 rectangular 0.5 ms pulses of supramaximal strength (65–70 V) delivered at 10 Hz, every 20 s, via a pair of platinum electrodes consisting of a hook below and a ring above each preparation.

Once the twitch contractions evoked by field stimulation attained a steady level, a single cumulative concentration-response curve was obtained to the twitch-potentiating effects of endothelin-1, endothelin-3, sarafotoxin S6c or IRL 1620 (0.01–100 nM). Some experiments assessed the influence of the selective endothelin ET_A receptor antagonist BQ-123 (10 nM–1 μM) and of the selective endothelin ET_B receptor antagonist BQ-788 (100 nM) on potentiation of neurogenic contractions by endothelins. For this, each vas deferens was exposed to a single concentration of one of the antagonists or vehicle (phosphate-buffered solution) for 30 min, and a concentration-response curve to either endothelin-1 or endothelin-3 was obtained in its presence. The specificity of BQ-123 action against endothelin-induced responses was confirmed by testing its influence on twitch potentiations induced by equieffective concentrations of endothelin-1 (1 nM), endothelin-3 (30 nM), bradykinin (30 nM, in the presence of captopril 3 μM) or substance P (3 nM). Following a first control challenge with bradykinin or substance P for 2 min, the bathing solution was renewed several times, BQ-123 (300 nM) was added to the bathing medium and another response to the same agonist was obtained in its presence 30 min later. However, due to the very slow reversibility of twitch potentiations induced by endothelins, both vasa deferentia from a given animal were each challenged only once with

either endothelin-1 or endothelin-3, one of them in the presence of BQ-123 (300 nM).

Another set of experiments was carried out to investigate the occurrence of endothelin-converting enzyme, whereby each vas deferens from a given animal was exposed to endothelin-1 or its immediate precursor big-endothelin-1 (0.01–30 nM) either in the absence (vehicle only, i.e. phosphate-buffered saline) or presence of phosphoramidon (100 μM, incubated 45 min prior to agonist additions).

In all experiments only one curve to an endothelin receptor agonist was obtained or one concentration of an endothelin receptor antagonist was tested in each preparation. After each experiment the tissue was blotted on filter paper and weighed.

2.2. Statistical analysis

Agonist-induced changes in twitch response magnitude are expressed as mean ± S.E.M increases in twitch tension (in mg/10 mg wet tissue), relative to the last basal twitch response prior to drug addition. E_{max} indicates maximal increase of twitch tension induced by a given agonist. EC_{50} values in individual experiments (i.e. the concentrations of agonist needed to cause half-maximal potentiation of twitch responses) were obtained by graphical interpolation and are presented as geometric means accompanied by their 95% confidence limits (Fleming et al., 1972). Statistical comparisons were performed by analysis of variance followed by Student's t-test for paired or unpaired samples, where appropriate, and $P < 0.05$ was considered significant.

2.3. Drugs

Drugs used were: sarafotoxin S6c, bradykinin, substance P, captopril, clonidine hydrochloride, tetrodotoxin, guanethidine sulfate, ATP disodium, lithium α,β-methylene ATP, noradrenaline bitartrate and prazosin hydrochloride (all from Sigma Chemical Company, St. Louis, USA), endothelin-1, endothelin-3 and human big-endothelin-1 (Peptide Institute, Osaka, Japan), phosphoramidon (Peninsula Labs., Belmont, USA) and BQ-123 (cyclo[D-Trp-D-Asp-Pro-D-Val-Leu]; Bachem, Torrance, USA). BQ-788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine) was synthetized by Banyu Pharmaceutical Co. (Tsukuba, Japan). IRL 1620 (Suc-[Glu^{9},Ala^{11,15}]endothelin-1) was kindly donated by Ciba-Geigy (Tarazuka, Japan). Most stock solutions were made up in phosphate-buffered saline, but those of prazosin and noradrenaline were made up in ethanol 100% and HCl 0.1 N, respectively. Stock solutions (10 μM–100 mM) were stored at −18°C and diluted to the desired concentrations with phosphate-buffered saline just prior to use. Ethanol did not modify twitch tension or noradrenaline-induced contractions at the concentration of 0.01% (final concentration in the bathing medium when applying prazosin).

3. Results

3.1. Characterization of field stimulation-evoked twitch contractions

Electrical field stimulation of the mouse vas deferens induced twitch contractions of 514 ± 10 mg/10 mg wet tissue (n = 60), which were abolished by tetrodotoxin (100 nM) or guanethidine (5 μM, n = 6 for each; results not shown), thus confirming their neurogenic and sympathetic origin. Twitch contractions were mediated mainly by ATP, as they were fully suppressed by desensitization of P_{2X} purinergic receptors with α,β-methylene ATP (10 μM), but inhibited by only about 25% by the α_1-adrenoceptor antagonist prazosin (100 nM) (n = 6; results not shown).

3.2. Effects of endothelin receptor agonists on neurogenic contractions

Cumulative additions of endothelin-1 (0.01–30 nM) induced concentration-dependent potentiation of contractions evoked by field stimulation (Fig. 1), with an EC_{50} of 0.7 nM (0.4–1.6) and an E_{max} of 473 ± 35 mg/10 mg wet tissue (Table 1). Twitch potentiations usually started 30 s after addition of the peptide and reached a plateau within 5–10 min, which remained stable for at least 1 h. Furthermore, significant twitch

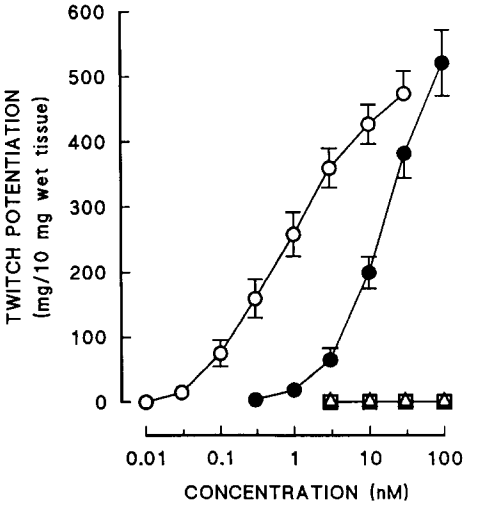

Fig. 1. Mean cumulative concentration-response curves for the effects of endothelin-1 (○), endothelin-3 (●), sarafotoxin S6c (□) and IRL 1620 (△) on contractions of the mouse isolated vas deferens induced by field stimulation. Each value is the mean ± S.E.M. of 4–6 experiments.

Table 1
Effects of endothelin receptor agonists on responses of the mouse isolated vas deferens to electrical field stimulation

Agonist	EC_{50} (nM)	DR	E_{max}	n
Endothelin-1	0.7 (0.4– 1.6)	–	473±35	6
Endothelin-3	13.7 (10.2–14.1)	19.6	520±51	6
Sarafotoxin S6c	Inactive up to 100 nM	–	–	4
IRL 1620	Inactive up to 100 nM	–	–	4
Big-endothelin-1	6.8 (4.7– 9.6)	9.7	457±37	6

EC_{50} are presented as geometric means accompanied by 95% confidence limits. DR (dose ratio) indicates potency relative to that of endothelin-1. E_{max} are presented as the mean±S.E.M. and represent the maximal change in twitch contraction amplitude (expressed in mg/10 mg wet tissue). Number of experiments indicated by 'n'.

potentiation was still evident 2 h after washout of the peptide (30 nM) followed by several renewals of the bathing medium every 15 min ($n = 6$; results not shown). Likewise, endothelin-3 also potentiated neurogenic contractions, but was 20-fold less potent than endothelin-1, yielding an EC_{50} of 13.7 nM (10.2–14.1) and an E_{max} of 520 ± 51 mg/10 mg wet tissue (Fig. 1 and Table 1). In sharp contrast, the selective endothelin ET_B receptor agonists sarafotoxin S6c and IRL 1620 did not alter neurogenic contractions up to 100 nM (Fig. 1 and Table 1). Both of these compounds (0.1–100 nM) caused concentration-dependent contractions of the guinea pig gallbladder strip ($n = 6$; Rae et al., unpublished observations; results not shown).

3.3. Influence of BQ-123 and BQ-788 on responses to endothelin-1 and endothelin-3

Preincubation with the selective endothelin ET_A receptor antagonist BQ-123 (up to 1 μM) failed to modify neurogenic responses of the vas deferens per se, but shifted to the right the concentration-response curve for the twitch-potentiating effect of endothelin-1. However, as shown in Fig. 2, the degree of antagonism produced by BQ-123 against this agonist was modest and not clearly concentration-dependent, as the EC_{50} of endothelin-1 was increased 2-, 5- and 6-fold by 0.1, 0.3 and 1 μM of BQ-123, respectively (Table 2). Furthermore, BQ-123 (0.3 and 1 μM) significantly increased the E_{max} of endothelin-1 (Fig. 2 and Table 2). On the other hand, the curve for potentiation of neu-

Table 2
Influence of the selective endothelin ET_A receptor antagonist BQ-123 and of the selective endothelin ET_B receptor antagonist BQ-788 on the effects of endothelin-1 (ET-1) and endothelin-3 (ET-3) in the mouse isolated field-stimulated vas deferens

Agonist	Condition	EC_{50} (nM)	DR	E_{max}	n
ET-1	Alone [a]	0.8 (0.6– 1.1)	–	479±38	14
	+BQ-123 100 nM	1.9 (1.2– 3.0) [b]	2.4	498±47	6
	+BQ-123 300 nM	4.2 (2.7– 6.6) [b]	5.2	629±57 [b]	6
	+BQ-123 1 μM	5.0 (3.9– 6.5) [b]	6.2	695±40 [b]	6
ET-1	Alone	0.6 (0.3– 1.3)	–	505±51	4
	+BQ-788 100 nM	0.5 (0.2– 1.0)	0.8	552±73	4
ET-3	Alone [a]	14.3 (11.3–18.7)	–	472±28	8
	+BQ-123 10 nM	n.d.	n.d.	236±69 [c]	4
	+BQ-123 30 nM	n.d.	n.d.	94±31 [c]	4
	+BQ-123 100 nM	n.d.	n.d.	10± 4 [c]	4
ET-3	Alone	8.5 (6.2–11.8)	–	640±72	5
	+BQ-788 100 nM	15.8 (11.5–21.9)	1.9	695±51	5

EC_{50} are presented as geometric means accompanied by 95% confidence limits. 'DR' indicates the dose ratio between EC_{50} in the presence and absence of antagonist. E_{max} are presented as mean± S.E.M. and represent the maximal change in twitch contraction amplitude (expressed in mg/10 mg wet tissue). 'n.d.' denotes not determined because E_{max} was not reached. The number of experiments is indicated by 'n'.
[a] As data from different control groups did not differ (ANOVA test) they have been pooled. [b] $P < 0.05$ relative to respective control value (ANOVA followed by unpaired Student t-test). [c] $P < 0.05$ relative to response induced by 100 nM of the agonist in the absence of antagonist (ANOVA followed by unpaired Student t-test).

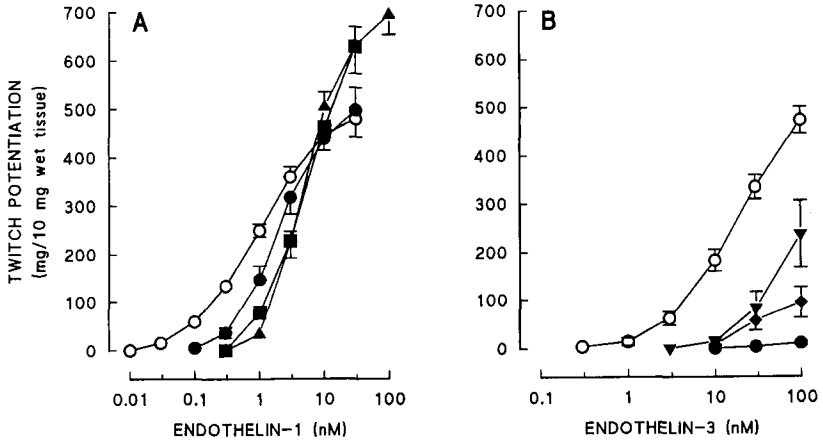

Fig. 2. Influence of the selective endothelin ET_A receptor antagonist BQ-123 on the potentiation of neurogenic contractions of the mouse vas deferens induced by endothelin-1 (panel A) and endothelin-3 (panel B). Effects produced by either agonist alone (○), or in the presence of BQ-123 10 nM (▼), 30 nM (◆), 100 nM (●), 300 nM (■) or 1 μM (▲), added to the bathing medium 30 min beforehand. Each value is the mean ± S.E.M. of 6–14 experiments.

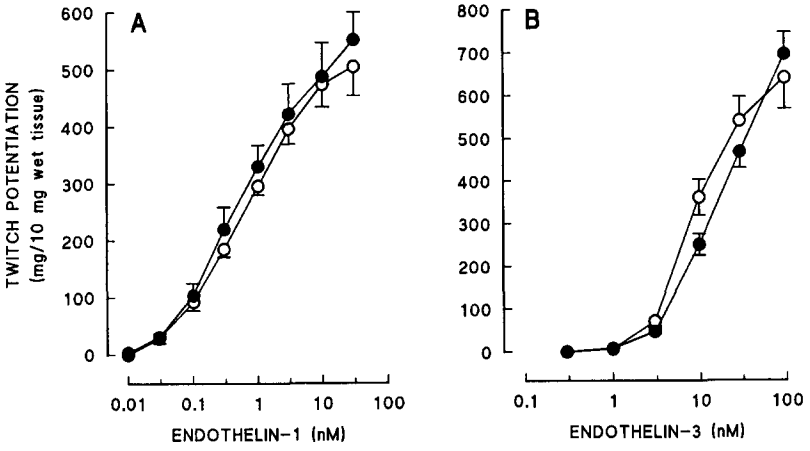

Fig. 3. Influence of the selective endothelin ET_B receptor antagonist BQ-788 on the potentiation of neurogenic contractions of the mouse vas deferens induced by endothelin-1 (panel A) and endothelin-3 (panel B). Effects caused by either agonist alone (○), or in the presence of 100 nM of BQ-788 (●), added to the bathing medium 30 min beforehand. Each value is the mean ± S.E.M. of 4–5 experiments.

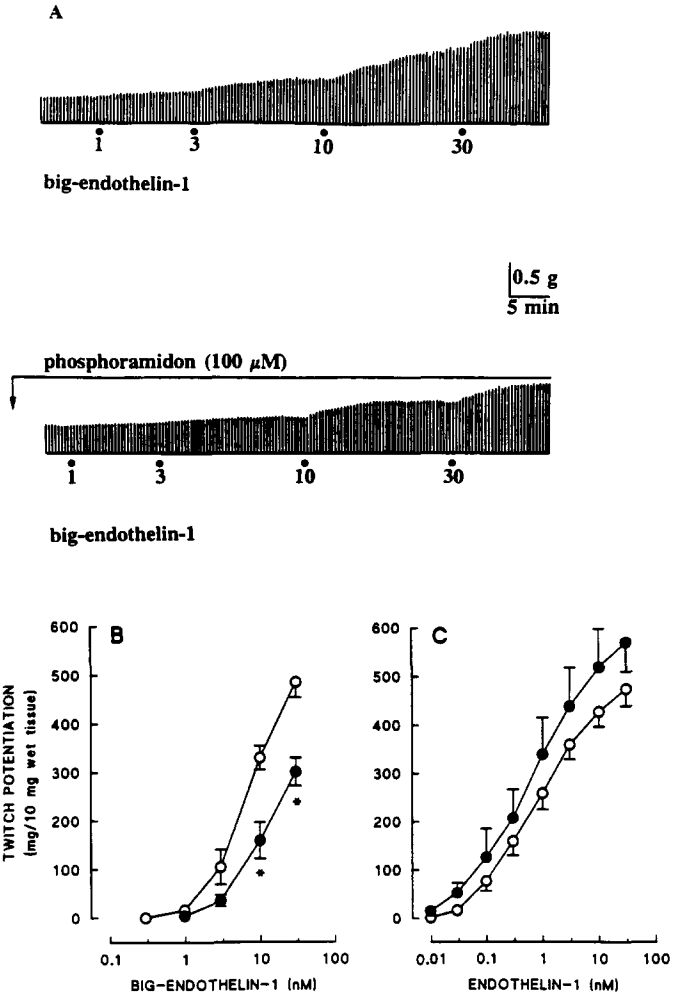

Fig. 4. Influence of phosphoramidon on the potentiation of neurogenic contractions of the mouse vas deferens induced by big-endothelin-1 and endothelin-1. Panel A: Typical tracings of the effects of cumulative additions of big-endothelin-1 (at the nM concentrations indicated) alone or in the presence of phosphoramidon (100 μM) on isometric contractions induced by electrical field stimulation. Mean cumulative concentration-response curves for big-endothelin-1 (panel B) or endothelin-1 (panel C) in the absence (○) or presence (●) of phosphoramidon (100 μM), added to the bathing medium 45 min beforehand. Each value is the mean ± S.E.M. of 5–6 experiments. Asterisks denote $P < 0.05$ when compared to its respective control value (paired Student t-test).

rogenic contractions by endothelin-3 was inhibited in a concentration-dependent fashion by much lower concentrations of BQ-123 (10, 30 and 100 nM) (Fig. 2 and Table 2). The effects of BQ-123 were specific as, at 300 nM, it markedly reduced twitch potentiations (in mg/10 mg of wet tissue) induced by endothelin-1 (1 nM; from 269 ± 28 to 79 ± 26 mg/10 mg wet tissue; $P < 0.05$ paired Student t-test) or endothelin-3 (10 nM; from 198 ± 25 to 0 ± 0 mg/10 mg wet tissue; $P < 0.05$), but did not change similar responses triggered by equieffective concentrations of substance P (3 nM; from 254 ± 35 to 262 ± 39 mg/10 mg wet tissue; $P > 0.05$) or bradykinin (30 nM; from 202 ± 28 to 234 ± 41 mg/10 mg wet tissue; $P > 0.05$; $n = 5$-6).

The selective endothelin ET_B receptor antagonist BQ-788 (100 nM) also did not affect basal twitch tension per se. However, unlike BQ-123, BQ-788 did not inhibit potentiation of neurogenic contractions induced by endothelin-1 and only slightly (albeit significantly) reduced the response to 10 nM endothelin-3 (Fig. 3).

3.4. Effects of big-endothelin-1 on neurogenic contractions

Big-endothelin-1 (0.3–100 nM) induced concentration-dependent potentiations of twitch contractions (Fig. 4). The latency for onset of this effect (ca. 10 min) was somewhat greater than that observed for endothelin-1. In addition, considering the response to 100 nM as the E_{max} of big-endothelin-1 (which is similar to that of endothelin-1), the EC_{50} of the precursor (6.8 nM, 4.7–9.6) was only 10-fold greater than that of endothelin-1 (0.7 nM, 0.4–1.6; Table 1). Preincubation with the endopeptidase inhibitor phosphoramidon (100 μM; 45 min beforehand), which did not affect basal neurogenic contractions or responses to endothelin-1, significantly reduced responses induced by big-endothelin-1 (Fig. 4).

4. Discussion

The present results extend our previous findings (Rae and Calixto, 1990), by demonstrating that endothelins markedly enhance sympathetic neurotransmission in the mouse vas deferens through activation of an unusual population of endothelin receptors. We also present functional evidence for the presence of pronounced endothelin-converting enzyme activity in this tissue.

Endothelin-1 was substantially (20-fold) more potent than endothelin-3 in potentiating neurogenic contractions of the mouse vas deferens, whereas the selective endothelin ET_B receptor agonists sarafotoxin S6c and IRL 1620 were found to be completely inactive up to 100 nM. These results would suggest that the effects of endothelin-1 and endothelin-3 were mediated via endothelin ET_A receptors (Sakamoto et al., 1993; for review see Huggins et al., 1993). This view is further substantiated by the finding that the new potent endothelin ET_B-selective antagonist BQ-788 (Ishikawa et al., 1994), at 100 nM, failed to influence responses to endothelin-1 and only marginally reduced responses to endothelin-3 in this tissue. In sharp contrast, BQ-788 (10 nM) inhibited by 90–100% the BQ-123-insensitive release of thromboxane A_2 induced by endothelin-1 or IRL 1620 in the guinea pig perfused lung (D'Orléans-Juste et al., 1994).

The selective endothelin ET_A receptor antagonist BQ-123, which at 300 nM failed to modify the increases in neurogenic contractions induced by bradykinin or substance P, significantly shifted the curve to endothelin-1 to the right. However, this effect was quite moderate and not clearly concentration-dependent. At 0.6 μM, BQ-123 increases the EC_{50} for endothelin-1-induced contractions of the rat uterus by about 30-fold (Rae et al., 1993), yet at 1 μM it increased the EC_{50} for the agonist in the mouse vas deferens by a mere 6-fold and actually enhanced its E_{max}. More importantly, endothelin-3-induced twitch potentiations were remarkably sensitive to inhibition by BQ-123. The curve to endothelin-3 was displaced to the right by as little as 10 nM of BQ-123, and responses to 100 nM of the agonist were nearly abrogated by 100 nM of the antagonist. Unfortunately, the low potency of endothelin-3, allied to our limited peptide supply, prevented a more accurate characterization of the type of antagonism afforded by BQ-123 against this agonist.

In the rat vas deferens, where endothelin-1 and endothelin-3 are approximately equipotent in increasing neurogenic contractions (Télémaque and D'Orléans-Juste, 1991), BQ-123 also antagonizes twitch potentiations induced by endothelin-3 to a much larger extent than those triggered by endothelin-1 (Eglezos et al., 1993; Warner et al., 1993a). Eglezos et al. (1993) suggested that endothelins activate an atypical endothelin receptor in the rat vas deferens, whereas Warner et al. (1993a) proposed that the effects of endothelin-1 and endothelin-3 (and sarafotoxin S6b) may be mediated via multiple non-ET_B receptors not identical to the endothelin ET_A receptor present in the rat aorta. Likewise, the current results would suggest that receptors for endothelins in the mouse vas deferens do not seem to be typical endothelin ET_A or ET_B receptors.

In the light of classical receptor theory, the fact that a single antagonist, BQ-123, differentially affects similar responses to two closely-related agonists, endothelin-1 and endothelin-3, would constitute functional evidence for receptor heterogeneity in both rat and mouse vasa deferentia. However, it has been shown that ago-

nists and antagonists bind to distinct receptor domains of the tachykinin NK_1 receptor (Fong et al., 1993; Gether et al., 1993b). Somewhat surprisingly, different tachykinin agonists can also bind to varying degrees with several domains of the tachykinin NK_1 receptor (Gether et al., 1993a). Sakamoto et al. (1993) demonstrated that certain structural modifications of the endothelin ET_A receptor can markedly decrease the affinity of BQ-123, without affecting that of endothelin-1 or endothelin-3, and proposed that endothelin-3 lacks certain structural requirements to interact with high affinity with the endothelin ET_A receptor. Therefore, the possibility that, in the mouse and rat vas deferens, the different susceptibilities of responses triggered by endothelin-1 and endothelin-3 to blockade by BQ-123 actually reflect differential binding of these agonists to a single type of receptor cannot be dismissed. Whichever hypothesis is correct, the greater potency of BQ-123 against responses triggered by endothelin-3 in the mouse vas deferens (present study), as compared to that seen in the rat vas deferens (Eglezos et al., 1993), as well as the greater difference in potencies of both endothelins in the former species, may well reflect species variation in endothelin receptor structure.

Big-endothelin-1 is 50- to 150-fold less potent than endothelin-1 to constrict blood vessels without endothelium (Kimura et al., 1989; Nakajima et al., 1989) and 100-fold less potent to displace radiolabelled endothelin-1 from vascular smooth muscle membranes (Hirata et al., 1990). In contrast, we have found big-endothelin-1 to be only 10-fold less potent than endothelin-1 at potentiating neurogenic contractions of the mouse vas deferens, which suggests that this tissue displays pronounced endothelin-converting enzyme activity. The fact that the endopeptidase inhibitor phosphoramidon partially prevented responses to the immediate precursor, leaving those to endothelin-1 unaffected, further strengthens this view.

Marked phosphoramidon-sensitive endothelin-converting enzyme activity is also present in the rat vas deferens, where big-endothelin-1 and big-endothelin-2 are only 2-fold less potent than their active metabolites (Télémaque and D'Orléans-Juste, 1991; Mattera et al., 1993). However, in this species, big-endothelin-3 is either inactive (Télémaque and D'Orléans-Juste, 1991), or is 20-fold less potent than endothelin-3 in potentiating neurogenic contractions and, unlike big-endothelin-1, is inhibited either by phosphoramidon or thiorphan (Mattera et al., 1993). Perhaps the discrepancy between both studies regarding the activity of big-endothelin-3 can be ascribed to the use of isotonic force transducers by Mattera et al. (1993), which rendered the preparations about 10-fold more sensitive to endothelins than that found by Télémaque and D'Orléans-Juste (1991) using isometric force transducers. A phosphoramidon-sensitive, but thiorphan-insensitive, endothelin-converting enzyme has recently been cloned from rat endothelial cell cDNA, which cleaves big-endothelin-1, but not big-endothelin-2 or big-endothelin-3 (Shimada et al., 1994). Further studies are required to determine if this is the endothelin-converting enzyme responsible for conversion of big-endothelin-1 in the mouse vas deferens, and if this tissue can also process big-endothelin-3.

Although to our knowledge no studies have yet demonstrated presence of endogenous endothelins in the vas deferens, high levels of immunoreactive endothelin-1 have been detected throughout the male genital tract, particularly in the testicles (Matsumoto et al., 1989), seminal fluid (Casey et al., 1992; Hammami et al., 1994) and prostate (Langenströer et al., 1993). The results of the current study show that the mouse vas deferens expresses marked endothelin-converting enzyme activity, and that exogenous endothelins produce long-lasting potentiation of contractions elicited by stimulation of sympathetic nerves, via activation of endothelin receptors which do not conform to the typical endothelin ET_A or ET_B types. It is tempting to speculate that, by such an action, endogenous endothelins may play significant roles in controlling the motility of the vas deferens and, hence, the transfer of spermatic fluid to the prostate.

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