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The contractile effects of endothelins on the smooth muscle of the rat prostate gland

Angela Salamoussa, Winnie A.K. Lau [1], Jocelyn N. Pennefather, Sabatino Ventura [*]

Department of Pharmacology, Monash University, PO Box 13E, Victoria 3800, Australia

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Abstract

Endothelin-1 elicited tonic contractions of rat prostatic smooth muscle that were unaffected by prazosin (0.5 μM), tetrodotoxin (1 μM) or guanethidine (10 μM). The rank order of potency of the endothelin isopeptides was endothelin-1 > endothelin-2 ≥ endothelin-3. Sarafotoxin S6B was approximately equipotent with endothelin-1 in eliciting tonic contractions, but neither of the selective endothelin ET_B receptor-agonists, sarafotoxin S6C (0.1 nM–0.3 μM) and BQ3020 (Ac-[Ala ^{11,15}]endothelin-1(6–21); 0.1 nM–0.3 μM), affected prostatic smooth muscle tone. The selective endothelin ET_A receptor antagonist, BQ123 (cyclo(D-Asp-L-Pro-D-Val-L-Leu-D-Trp; 1 μM), attenuated responses to endothelin-1, -2 and -3, while the non-selective endothelin receptor antagonist bosentan (1 μM) and the selective endothelin ET_B receptor antagonist BQ788, (Dmpc-γ-MeLeu^9-D-Trp(l-CO_2CH_3)-D-Nle-OH; 1 μM) attenuated responses to endothelin-3 only. Contractions induced by exogenous administration of noradrenaline were unaffected by preincubation of tissues in BQ123 (1 μM) indicating the selectivity of this antagonist. These data suggest that endothelins mediate contractions of the rat prostate by action at endothelin ET_A receptors.

Keywords: Endothelin; Prostate; (Rat); Neuromuscular transmission; Endothelin ET_A receptor

1. Introduction

Endothelins-1, -2 and -3 are cyclic 21 amino acid peptides that share potent contractile, mitogenic and neuromodulatory properties (Yanagisawa et al., 1988; Battistini et al., 1993; Takimoto et al., 1993; Davenport and Maguire, 1994; Rubanyi and Polokoff, 1996; Hay, 1995; Sokolovsky, 1995). There are two major types of endothelin receptor: ET_A and ET_B. Endothelin-1 and endothelin-2 are more potent than endothelin-3 at endothelin ET_A receptors, whereas all three have similar potencies at endothelin ET_B receptors (for reviews see Gray and Webb, 1996; Rubanyi and Polokoff, 1996).

Endothelin precursors and the endothelin-converting enzyme have been reported to be expressed in the human prostate gland (Rossi et al., 1995; Prayer-Galetti et al., 1997; Walden et al., 1998). The two major endothelin receptor subtypes (Masaki et al., 1994), designated endothelin ET_A and endothelin ET_B receptors have been detected in human cultured prostatic smooth muscle cells (Saita et al., 1998). Endothelin ET_A receptors predominate in human prostatic membrane homogenates (Le Brun et al., 1996) and slide-mounted prostatic sections (Kobayashi et al., 1994a; Imajo et al., 1997). There is some controversy in the literature about the subtype expressed in the prostate stroma and epithelium, but receptor autoradiographic studies by Kobayashi et al. (1994b) indicate a preferential localisation of endothelin ET_A and ET_B receptors in stroma and epithelium, respectively.

Endothelins cause contraction of human prostate stroma (Langenstroer et al., 1993; Moriyama et al., 1996; Imajo et al., 1997). There is some controversy about the receptor activated, with some workers reporting the involvement of an endothelin ET_B receptor (Webb et al., 1995; Raschack et al., 1998) as well as an endothelin ET_A receptor (Kobayashi et al., 1994a). While homogenate binding studies with the guinea-pig prostate have indicated the presence of both endothelin ET_A and ET_B receptors in this

* Corresponding author. Tel.: +61-3-99054762; fax: +61-3-99055851.
E-mail address: sab.ventura@med.monash.edu.au (S. Ventura).
[1] Present address: Department of Medical Laboratory Science, RMIT University, Victoria 3001, Australia.

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species, endothelins have no effect on resting prostatic smooth muscle tone but enhance neuromuscular transmission to the smooth muscle through activation of endothelin ET_A receptors (Lau et al., 1999).

Homogenate receptor binding studies on the rat prostate have shown a predominance of endothelin ET_A receptors (Saito et al., 1996; Auger-Pourmarin et al., 1998), although mRNA for both endothelin ET_A and ET_B receptor subtypes has been detected (Auger-Pourmarin et al., 1998). The effects of endothelins on the tone of prostate smooth muscle from this species have not, however, been investigated in detail. The aim of this study was to determine whether endothelin-1 caused prostatic smooth muscle contraction and/or influenced neurotransmission to the prostate in this species. Classification of the receptor-mediating effects of the endothelins has been described using the endothelin isopeptides, the selective endothelin ET_B receptor agonists sarafotoxin S6C (Williams et al., 1991) and BQ-3020 (Ihara et al., 1992a), and the selective endothelin ET_A and ET_B receptor antagonists, BQ-123 (Ihara et al., 1992b) and BQ-788 (Ishikawa et al., 1994), respectively.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (250–400 g) were housed at 22°C and exposed to a photoperiod of 12-h light/12-h dark. Rats were allowed access to food and water ad libitum. Ethical approval was obtained from the Monash University Standing Committee of Animal Ethics in Animal Experimentation (Ethics number 97/008).

2.2. Experimental procedure

Rats were killed by cervical dislocation. An abdominal incision was made, exposing the male urogenital tract. The left and right lobes of the prostate were removed, providing two prostate preparations from each rat. The prostate lobes were placed in a Petri dish containing Krebs–Henseleit solution (in mM: NaCl, 118.1; KCl , 4.69; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.7; $MgSO_4$, 0.5; $CaCl_2$, 2.5) and the prostatic capsule was removed along with excess fat and connective tissue. The isolated prostates were mounted on tissue holders which incorporated parallel platinum electrodes and were placed in 5 ml siliconised organ baths. The organ baths contained Krebs–Henseleit solution, bubbled with 5% CO_2 in O_2 and maintained at 37°C. One end of the prostate was attached to an isometric Grass FT03 force-displacement transducer, which was connected to a MacLab data acquisition system run on a Macintosh LC630 computer. Tissues were equilibrated for 1 h, under a resting force of 0.5 g. During the 1 h equilibration period, the bath medium was changed every 10 min due to the frothing that occurred in the organ bath as a result of spontaneous prostatic secretions (Lau et al., 1998).

Cumulative log concentration–response curves to endothelin-1, endothelin-2, endothelin-3, sarafotoxin S6B and the selective endothelin ET_B receptor agonists sarafotoxin S6C and BQ3020 (0.1 nM–0.3 μM) were constructed using a concentration progression ratio of half a log unit, to examine their effects and relative potencies on prostatic smooth muscle tone. Each concentration was left in contact with the tissue for 2–3 min before the addition of the next concentration. If further drug addition was delayed beyond this contact time, smooth muscle tone began to fade rapidly.

Cumulative log concentration–response curves to endothelin-1, endothelin-2 and endothelin-3 were constructed in the presence of: BQ123 1 μM (selective endothelin ET_A receptor antagonist), BQ788 (1 μM; selective endothelin ET_B receptor antagonist) and bosentan (1 μM; non-selective endothelin receptor antagonist). To avoid tachyphylaxis to endothelins in the rat prostate which we had observed in pilot studies, only one concentration–response curve was constructed on each tissue and time controls and/or vehicle (0.01% dimethyl sulphoxide (DMSO)) experiments were carried out on parallel preparations from the contralateral prostate. Following the 1 h initial equilibration period, the antagonist or vehicle was added to the organ bath and left in contact with the tissue for a further 30 min before a log concentration–response curve was constructed. Pilot experiments with these endothelin receptor antagonists revealed that equilibrium was reached after 10–15 min and remained stable for at least 1 h. Antagonists were washed out and replaced two to three times throughout the further incubation period.

Cumulative log concentration–response curves to endothelin-1 were also conducted in the presence of prazosin (0.5 μM), tetrodotoxin (1 μM) and guanethidine (10 μM) to determine whether the response to this peptide was indirectly mediated by release of neuronal constituents. These drugs were present in the bathing medium throughout the equilibration period and throughout the duration of the experiment.

Cumulative log concentration–response curves were constructed to noradrenaline (0.1–300 μM) in the absence and presence of BQ123 (1 μM) to determine whether a component of the response to noradrenaline is mediated indirectly by released endothelins.

In a separate set of experiments, tissues were allowed to equilibrate for 30 min, then nerve terminals within the tissues were field-stimulated using electrodes connected to a Grass S88 stimulator to deliver trains of 0.5 ms pulse duration, 80 V, at 10 Hz for 2 s every 60 s. This stimulation of the rat prostate produces twitch-like contractions that are tetrodotoxin sensitive and attenuated by guanethidine (Lau et al., 1999), indicating that these con-

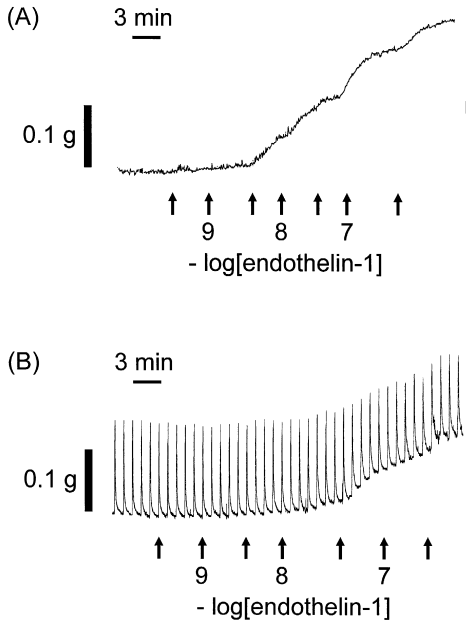

Fig. 1. Representative traces showing the effects of cumulative addition of endothelin-1 on unstimulated preparations (A) and electrical field stimulation induced contractions (B) of the isolated rat prostate.

tractions are mediated by nerves which are predominantly sympathetic in nature. Cumulative log concentration–response curves to endothelin-1, endothelin-2 and endothelin-3 were constructed on these stimulated preparations to examine the effects of endothelins on neuromuscular transmission to the rat prostate.

2.3. Measurement and analysis of data

Graphs showing mean log concentration–response curves were constructed, using Graph Pad Prism (version 3.0). Contractile responses to endothelin agonists in the absence and presence of endothelin antagonists, tetrodotoxin, prazosin and guanethidine were determined by measuring the peak force developed (in grams) to each concentration of agonist.

At the concentrations of agonists used, maximal contractile responses were not reached. To compensate, a two-way repeated measures analysis of variance (ANOVA) was carried out to compare differences between the two treatment groups at all concentration points on the concentration–response curve. A post hoc Bonferroni's test was conducted when multiple comparisons were made between treatment groups. These tests were carried out using Sigmastat® (version 1.0). The P values used to evaluate statistical significance were the probabilities of a significant interaction between dose and treatment and in all cases, $P < 0.05$ was considered significant. Estimates of the differences in agonist potency and shifts caused by antagonists were made by determining the mean concentration of agonist that produced a contractile response of 0.05 g. This was determined by linear regression using Graph Pad Prism® (version 3.0).

2.4. Drugs and solutions

Endothelin-1, endothelin-2, endothelin-3, BQ3020 (Ac-[Ala^{11,15}]-endothelin-1(6–21)), sarafotoxin S6C, sarafotoxin S6B, BQ123 (cyclo-D-Asp-L-Pro-D-Val-Leu-D-Trp) and BQ788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-α-methyl-Leu-D-Trp [1-CO_2CH_3-D-Nle-OH] were obtained from the American Peptide Company. Prazosin and tetrodotoxin were obtained from Sigma. Guanethidine was obtained from CIBA-GEIGY and bosentan was a gift to J.N.P. from F. Hoffmann-La Roche.

Stock concentrations of endothelins (0.1 mM), BQ788 (0.1 mM) and bosentan (1 mM) were made up in 5% DMSO. All other compounds were made up in distilled water. Subsequent dilutions of drugs were made daily in Krebs–Henseleit solution. Drug aliquots of 50 μl were stored at −20°C and thawed on demand.

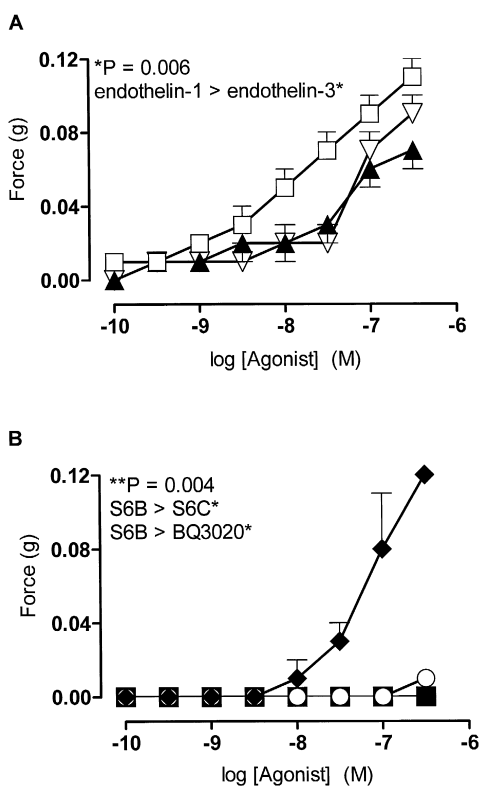

Fig. 2. Mean log concentration–response curves for the naturally occurring endothelins (A): endothelin-1(□), endothelin-2(▲) and endothelin-3(▽) and the more stable endothelin receptor analogues (B): sarofotoxin S6B (♦), sarofotoxin S6C (○) and BQ3020 (■). Each point represents the mean ± S.E.M. of 4–29 experiments. P values represent the difference in the concentration–response curves according to the agonist used. Asterisks indicate a significant difference ($^{*}P < 0.05$; $^{**}P < 0.005$; two-way repeated measures ANOVA, followed by post hoc Bonferroni correction).

3. Results

3.1. Agonist responses

Cumulative addition of endothelins and sarafotoxin S6B produced concentration-dependent tonic contractions of the rat prostate (Figs. 1 and 2). Electrical field stimulation (10 Hz, 0.5 ms, 80 V applied for 2 s every 60 s) of nerve terminals evoked contractions of the rat prostate, but endothelin-1 (Fig. 1), endothelin-2 and endothelin-3 (0.3 nM–0.3 μM) were all without effect on these field stimulation-induced contractions ($P \geq 0.753$; $n = 6$, for all).

Endothelin-1 ($n = 29$) was approximately seven-fold more potent than endothelin-3 ($n = 23$) (potency ratio = 7.1 ± 0.5) in producing contractions of the rat prostate (Fig. 2). Although, endothelin-2 appeared to be approximately six-fold less potent than endothelin-1 (potency ratio = 6.0 ± 0.6), the mean log concentration–response curve to endothelin-2 was not significantly different from endothelin-1 or endothelin-3. The rank order of potency was therefore endothelin-1 ≥ endothelin-2 ≥ endothelin-3. The non-selective endothelin receptor agonist sarofotoxin S6B produced tonic contractions (Fig. 2; $n = 6$). Its potency was approximately five-fold less than that of endothelin-1 (potency ratio = 4.8 ± 0.4). The selective endothelin ET_B receptor agonists, sarofotoxin S6C ($n = 4$) and BQ3020 ($n = 4$) were without effect on the prostatic smooth muscle tone.

3.2. Effects of endothelin receptor antagonists on agonist responses

The selective endothelin ET_A receptor antagonist, BQ123 (1 μM), consistently caused approximately three-fold shifts to the right in the mean log concentration–response curves to endothelin-1 and endothelin-2 (Fig. 3; $P \leq 0.002$; $n = 6$). Endothelin-3 (up to 0.3 μM) was without contractile effect in the presence of BQ123 (1 μM) (Fig. 3; $P < 0.001$; $n = 6$).

BQ788 (1 μM), a selective endothelin ET_B receptor antagonist, slightly attenuated the response to endothelin-3

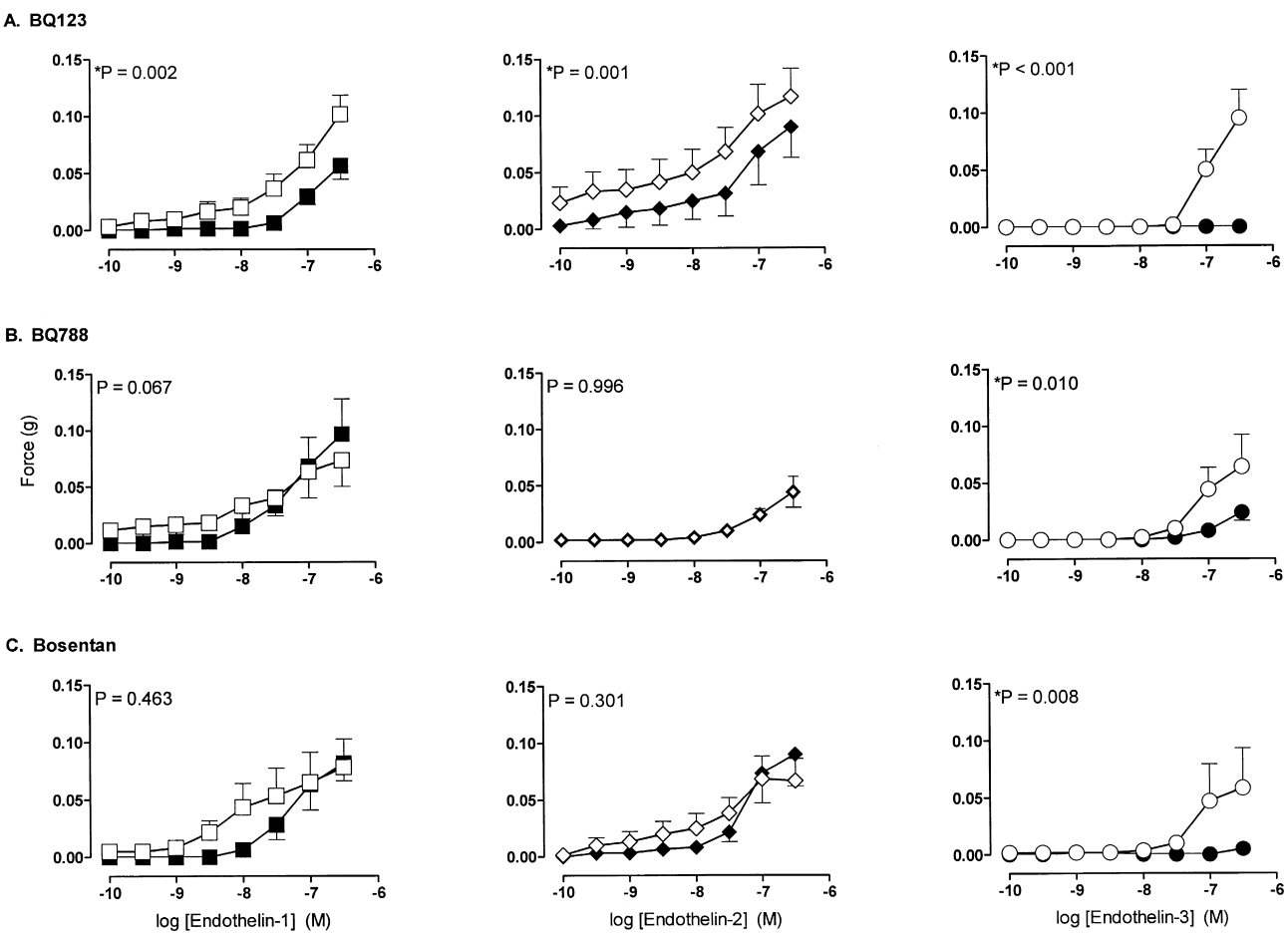

Fig. 3. Mean log concentration–response curves to endothelin-1 (left column), endothelin-2 (centre column) and endothelin-3 (right column) were constructed in the absence (open symbols) and presence (closed symbols) of (A) BQ123 (1 μM), (B) BQ788 (1 μM) and (C) bosentan (1 μM). Each point represents the mean ± S.E.M. of six experiments. P values represent the difference in the concentration–response curves in the absence and presence of antagonist. Asterisks indicate a significant difference ($^{*}P < 0.05$; $^{**}P < 0.005$; two-way repeated measures ANOVA).

(Fig. 3; $P = 0.010$; $n = 6$) but did not affect responses to either endothelin-1 or endothelin-2 (Fig. 3; $P \geq 0.067$; $n = 6$ for each). Similarly, there was no significant inhibition on the contractile responses to endothelin-1 or endothelin-2, by the non-selective endothelin receptor antagonist, bosentan (1 μM) (Fig. 3; $P \geq 0.301$; $n = 6$ for each), but bosentan (1 μM) significantly attenuated the response to endothelin-3 (Fig. 3; $P = 0.008$; $n = 6$).

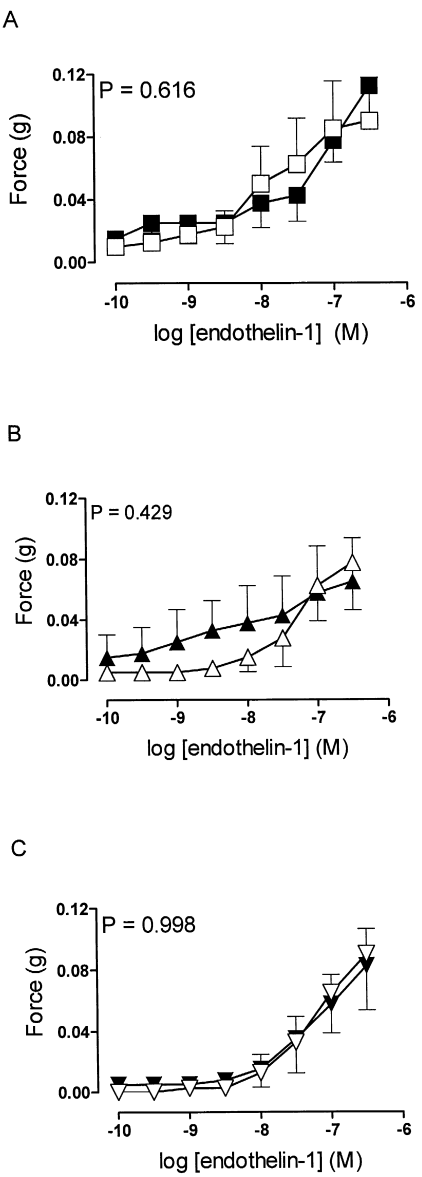

Fig. 4. Mean log concentration–response curves to endothelin-1 were constructed in the absence (open symbols) and presence (closed symbols) of (A) prazosin (0.5 μM), (B) guanethidine (10 μM) and (C) tetrodotoxin (1 μM). Each point represents the mean ± S.E.M. of six experiments. P values represent the difference in the concentration–response curves in the absence and presence of prazosin, guanethidine or tetrodotoxin. Asterisks indicate a significant difference ($^{*}P < 0.05$; $^{**}P < 0.005$; two-way repeated measures ANOVA).

3.3. Mechanisms of action

The α_1-adrenoceptor antagonist prazosin (0.5 μM), had no effect on the contractile response of endothelin-1 on the rat prostate (Fig. 4; $P = 0.616$; $n = 6$). Similarly, the neurotoxin, tetrodotoxin (1 μM), did not affect the contractile responses to endothelin-1 (Fig. 4; $P = 0.429$; $n = 6$). The noradrenergic neuron-blocking drug guanethidine (10μM) was also without effect on the contractile response to endothelin-1 (Fig. 4; $P = 0.998$; $n = 6$).

Preincubation with BQ123 (1 μM) had no effect on the contractile response to noradrenaline (0.1 μM–0.3 mM) of the rat prostate ($P = 0.438$; $n = 5$).

4. Discussion

The present study showed that endothelins-1, -2, -3 and sarafotoxin S6B cause concentration-dependent tonic contractions of the rat prostatic smooth muscle. Thus, endothelins have a similar effect on contractile function of prostatic smooth muscle cells in both rats and humans (Langenstroer et al., 1993; Moriyama et al., 1996; Imajo et al., 1997; Raschack et al., 1998).

Although the endothelins increased the tone of the rat prostatic smooth muscle, they had no effect on electrically evoked neuromuscular transmission to the rat prostate. Thus, the effects of endothelins on rat prostate are in contrast to those observed with guinea-pig prostate, in which they potentiated electrically evoked nerve-mediated contractile responses to the smooth muscle of the prostate via activation of endothelin ET_A receptors, but lacked effect on the tone of the smooth muscle (Lau et al., 1999).

Neither tetrodotoxin, which blocks voltage-sensitive sodium channels in nerve fibres, nor the noradrenergic neuron-blocking drug, guanethidine, affected the responses to endothelin-1 on the prostate, indicating that the response to endothelin-1 is not mediated by stimulation of the excitatory nerve supply to the prostate. Similarly, prazosin, an α_1-adrenoceptor antagonist, did not affect the contractile response to endothelin-1 on the rat prostatic smooth muscle tone. This suggests that the response to endothelin-1 is not mediated by release of noradrenaline, acting on post-junctional α_1-adrenoceptors. This observation is consistent with findings in the human prostate, where terazosin, also an α_1-adrenoceptor antagonist had no effect on the response of endothelin-1 on prostatic smooth muscle tone (Langenstroer et al., 1993).

Endothelin-converting enzyme has been shown to be located on actin filaments in smooth muscle cells (Barnes and Turner, 1999). This prompted a set of experiments to determine whether endothelins might participate in part in mediating the contractile action of noradrenaline on the prostate. This was not the case, since contractile responses to noradrenaline were not affected by the endothelin ET_A receptor antagonist BQ123.

The rank order of potency in causing contraction of rat prostatic smooth muscle was: endothelin-1 ≥ endothelin-2 ≥ endothelin-3, with endothelin-1 being significantly more potent than endothelin-3. As in the canine prostate (Normandin and Lodge, 1996), this rank order of agonist potency indicates that an endothelin ET_A receptor subtype mediates endothelin-induced contractions in the rat prostate.

Two sarofotoxins, the non-selective endothelin receptor agonist, sarofotoxin S6B and the selective endothelin ET_B receptor agonist, sarofotoxin S6C (Williams et al., 1991) as well as the selective endothelin ET_B receptor agonist BQ3020 (Ihara et al., 1992b), were also employed in this study. The finding that sarofotoxin S6B, but neither of the selective endothelin ET_B receptor agonists, contracted the prostate further indicates the function of endothelin ET_A rather than ET_B receptors in prostatic smooth muscle in the rat.

BQ123 has been used as a pharmacological tool in binding and functional experiments to determine the nature of endothelin receptor subtypes. It is a very selective endothelin ET_A receptor antagonist, with a $K_i = 7.3$ nM at endothelin ET_A receptors and 18 μM at endothelin ET_B receptors, respectively (Gray and Webb, 1996). This antagonist has previously been shown to be a potent inhibitor of radiolabelled ^{125}I[endothelin-1] binding in the human prostate (Le Brun et al., 1996). In the present study, BQ123 (1 μM) attenuated the contractile responses of the rat prostate to endothelin-1, endothelin-2 and endothelin-3. This further reinforces the probability that endothelin ET_A receptors mediate the endothelin-induced contractions of the rat prostate. Shifts in the presence of BQ123 (1 μM) of mean log concentration–response curves constructed to endothelin-1 and endothelin-2 were, however, less than in our previous study on guinea-pig prostate where shifts to the right of approximately 100-fold were seen (Lau et al., 1999).

The selective endothelin ET_B receptor antagonist BQ788 1 μM, ($K_i = 1.2$ nM and 1.3 μM at endothelin ET_B and ET_A receptors, respectively; Opgenorth, 1995; Gray and Webb, 1996;), had no effect on the responses to endothelin-1 or endothelin-2, but significantly attenuated the response to endothelin-3. BQ788 (1 μM) produced a smaller shift in the mean log concentration–response curve to endothelin-3 than did BQ123 (1 μM; Fig. 3). Thus, while endothelin ET_B receptors may be present, the concentration of BQ788 used was close to the K_i value for endothelin ET_A receptors.

The reason underlying the relatively high selectivity of both of these antagonists versus endothelin-3 remains unexplained, as does that of bosentan (1 μM), which was without effect on the contractile responses to endothelin-1 or endothelin-2, but attenuated the response to endothelin-3. Since bosentan is a relatively non-selective antagonist ($K_i = 4.7$ nM at endothelin ET_A receptors and $K_i = 95$ nM at endothelin ET_B receptors; Gray and Webb, 1996), it was anticipated that it would attenuate the responses to all three endothelin peptides, as did BQ123. The reason for its inactivity in the present experiments cannot readily be explained but species differences maybe a potentially confounding factor. These previously reported K_i values were determined in human tissues and these antagonists may have correspondingly lower affinities at rat endothelin receptors.

Previous competition radioligand binding studies on the homogenates of rat prostate indicated a predominance of endothelin ET_A receptors (Saito et al., 1996; Auger-Pourmarin et al., 1998). These results are supported by the present study, where clearly, endothelin ET_A receptors play some role in mediating prostate contractions in the rat. Nevertheless, since the endothelin ET_A receptor antagonist BQ123 and the non-selective antagonist bosentan were relatively ineffective in antagonising the effects of endothelin, a number of questions about the precise mechanisms of action remain.

Acknowledgements

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