

Pharmacological Properties of Endothelin-1 in the Rabbit Corpus Cavernosum

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Abstract. *Background:* Endothelin (ET-1) may play a role in the regulation of erection but this has not been conclusively demonstrated. Augmented cavernosal smooth muscle (CSM) contraction in the rat occurs following exposure to both ET-1 and phenylephrine (PE; alpha-1 agonist). The aim of this study was to assess the effect of ET-1 and its possible role in the α_1 -adrenergic pathway during the erectile process. *Materials and Methods:* Organ bath studies were performed on CSM strips of penises obtained from 12 age-matched New Zealand White rabbits. The effect of ET-1 and PE alone on CSM tone in the absence and presence of ET_A (BQ123) and ET_B (BQ788) antagonists was assessed. Tissue responses were measured as tension (newton, N). EC₅₀ values are expressed as mean \pm S.E.M. *Results:* PE (10^{-8} - 10^{-4} M) and ET-1 (10^{-10} - 10^{-6} M) produced a concentration-dependent contraction in rabbit CSM strips. The EC₅₀ values were 1.7×10^{-7} M \pm 1.1 and 3.4×10^{-9} M \pm 1.5, respectively. BQ123 10^{-5} M significantly inhibited ET-1-mediated CSM contractions more than BQ788 10^{-5} M (both ANOVA p < 0.01). The EC₅₀ were 1.3×10^{-6} M \pm 2.6 and 2.0×10^{-7} M \pm 2.1, respectively. Neither the ET_A or ET_B receptor antagonist had a significant influence on α_1 -adrenergic receptor-mediated CSM contraction. *Conclusion:* ET_A receptors may play a greater role than ET_B receptors in ET-1-induced rabbit CSM contraction and the detumescence process. The α_1 -adrenergic-dependent pathway does not involve the ET_A or ET_B receptors.

Normal erectile function involves a balance between the effects of several mediators on cavernosal smooth muscle (CSM) tone (1). In the flaccid state the penile smooth muscle is in a state of tonic contraction, whereas during erection it relaxes. These two opposing effects involve the integration of complex actions of various neurotransmitters and neuropeptides; principal among them are nitric oxide (NO) and noradrenaline (2-5).

Evidence exists for the synthesis and release of endothelin (ET) in the corpus cavernosum (6). Therefore, ET may play a role in regulating erectile function. Two receptor subtypes (ET_A and ET_B) have been identified and cloned (7). The functional properties of these receptors have not been fully characterized in the CSM. It has also been reported that ET-1 enhances α_1 -adrenergic receptor-mediated CSM contraction (8, 9). Wingard *et al.* (9) demonstrated that a combination of ET-1 and phenylephrine (PE) augmented constrictor responses in rat CSM by a mechanism involving the RhoA-Rho kinase pathway.

Overall, there is a lack of conclusive evidence for ET involvement in the physiological regulation of erectile function. Therefore, we evaluated the possible role of ET-1 in the erectile process. We also investigated the interactions between ET-1 and the α_1 -adrenergic pathways in the CSM. As normal human penile tissues are difficult to obtain, we used penile tissues from normal rabbits (New Zealand White).

Materials and Methods

Materials. Phenylephrine was obtained from Sigma Chemical Co. (Poole, UK). The following chemicals were obtained from Tocris Cookson Ltd. (Bristol, UK): ET-1, BQ123 and BQ788.

Methods. Age-matched New Zealand White rabbits (n=12) were purchased from Harlan Ltd. (Oxford, UK). The rabbits were killed by the Schedule 1 method following approval from the local ethics committee. The penises were excised and CSM strips were

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prepared and mounted vertically in organ baths. The strips were perfused with Krebs solution and bubbled with a mixture of 95% O₂ and 5% CO₂, maintained at 37°C with a pH of 7.4. An initial tension of 2 g was applied to the suspended tissue strips. The tension was recorded with a force displacement transducer (FT-03, Grass Instruments, Quincy, MA, USA) on a Grass Polygraph (model 7D). All the strips were equilibrated for at least 60 min. At the end of the equilibration period, as well as at the end of each experiment, the strips were challenged with KCl (124 mM). Tissues were accepted for our study only if two reproducible contractions varying in magnitude by less than 10% were obtained by the KCl challenge. The tissue responses were measured as tension (newton; N).

Characterisation of ET receptors mediating CSM contractile responses. Concentration responses curves (CRCs) to ET-1 (10⁻¹⁰–10⁻⁶M; n=16) were performed in the absence and presence of either ET_A (BQ123 10⁻⁵M; n=8) or ET_B (BQ788 10⁻⁵M; n=8) receptor antagonist. The concentration of 10⁻⁵ M was used for both ET antagonists, as this was the optimal blocking dose in preliminary experiments. Cumulative addition of increasing concentrations of ET-1 was performed once a response had reached a plateau.

Effect of ET receptor antagonists on α₁-receptor-mediated CSM contraction. CRCs to PE (10⁻⁸–10⁻⁴M; n=16; cumulative addition once plateau had been reached) were first performed. After washing the CSM strips for 30 min with Krebs solution, the strips were incubated with either ET_A or ET_B receptor antagonist (both 10⁻⁵M; n=8) for 20 min. CRCs to PE were then repeated to evaluate the effect of each ET receptor subtype blockade on α₁-adrenergic receptor-mediated CSM contraction.

Statistical analysis. ANOVA and the Student's *t*-test were performed to compare curves and tensions, with significance accepted at *p*<0.05. EC₅₀ values are expressed as mean±S.E.M.

Results

Contractile responses to PE and ET-1. PE (10⁻⁸–10⁻⁴M) and ET-1 (10⁻¹⁰–10⁻⁶M) produced a concentration-dependent contraction in rabbit CSM strips. The maximal contractile response to ET-1 was 52±3% of that obtained with PE. The EC₅₀ values for PE and ET-1 were 1.7x10⁻⁷M±1.1 and 3.4x10⁻⁹M ±1.5, respectively.

Effects of ET_A and ET_B receptor antagonists on CSM contraction to ET-1. ET-1-mediated CSM contractile responses were inhibited by ET_A (BQ123) and ET_B (BQ788) receptor antagonists (both 10⁻⁵M), respectively, (ANOVA *p*<0.01) (Figure 1). The EC₅₀ value for ET-1 (3.4x10⁻⁹M ±1.5) was decreased (*p*<0.05) in the presence of BQ123 10⁻⁵M (1.3x10⁻⁶M±2.6). Although, ET-1-mediated CSM contractile responses were inhibited (ANOVA *p*<0.01) by BQ788 (10⁻⁵M; EC₅₀ value was 2.0 x 10⁻⁷M±2.1), the inhibition was greater (*p*<0.01) with BQ123 at 10⁻⁵ M (Figure 1).

Effects of ET_A and ET_B receptor antagonists on CSM contractile responses to PE. PE (10⁻⁸–10⁻⁴M) caused concentration-dependent CSM contractions. Neither ET receptor antagonist (BQ123 10⁻⁵M; BQ788 10⁻⁵M) had an influence on the α₁-adrenergic receptor-mediated CSM contraction (Figure 2). The EC₅₀ values for PE were 1.7x10⁻⁷ M ±1.1 with and without pre-exposure to either of the ET receptor antagonists.

Discussion

ET-1 contracted isolated rabbit CSM strips in a concentration-dependent manner, as previously described (4). The ET_A and ET_B receptor antagonists inhibited these ET-1-mediated CSM contractile responses. The shift of the CRCs to the right had no significant effect on the maximal contractile responses achieved by ET-1, indicating that they are competitive ET receptor antagonists. The inhibition of CSM contractions by the ET_B receptor antagonist was less than that with the ET_A receptor antagonist. This implies that ET_A receptor-mediated contractions play a greater role than that of ET_B receptors in ET-1-induced rabbit cavernosal contraction.

The ET receptor antagonists had no effect on α-1 adrenergic receptor-mediated CSM contraction. Therefore, it would seem that the α-1 adrenergic pathway does not involve the ET_A and ET_B receptors in CSM contraction. Thus, the previously described enhancement of α-1 adrenergic receptor-mediated CSM contraction by ET-1 may represent an additive effect (8, 9).

Further work using human tissue is needed to establish whether ET-1 plays a role in the human erectile process, since Kim *et al.* (10) found disparity between laboratory (rabbits) and clinical studies (men with mild-to-moderate erectile dysfunction, ED) on assessing the effects of ET-1 on erection. The insignificant effect of BMS-193884 on erection in clinical studies in contrast to the ET_A antagonist-inhibited ET-1-mediated CSM contractions in rabbits might be attributed to species variation.

ET might affect human erection more significantly in pathological conditions such as diabetes than in normal men, since the basal plasma ET-1 concentrations in peripheral veins were increased in diabetic patients with ED compared to the controls (11). Increased ET-1 bioactivity might contribute to diabetes-related ED when coupled with diminished NO-mediated CSM relaxation (12) by causing an overall increase in CSM tone. Future studies should address the question of whether ET-1 might contribute to the pathogenesis of ED associated with other conditions (13, 14).

In conclusion, ET-1 elicited rabbit CSM contraction via the ET_A more than the ET_B receptor. ET-1 may play a role in the detumescence process. The α₁-adrenergic-dependent pathway does not involve the ET receptors.

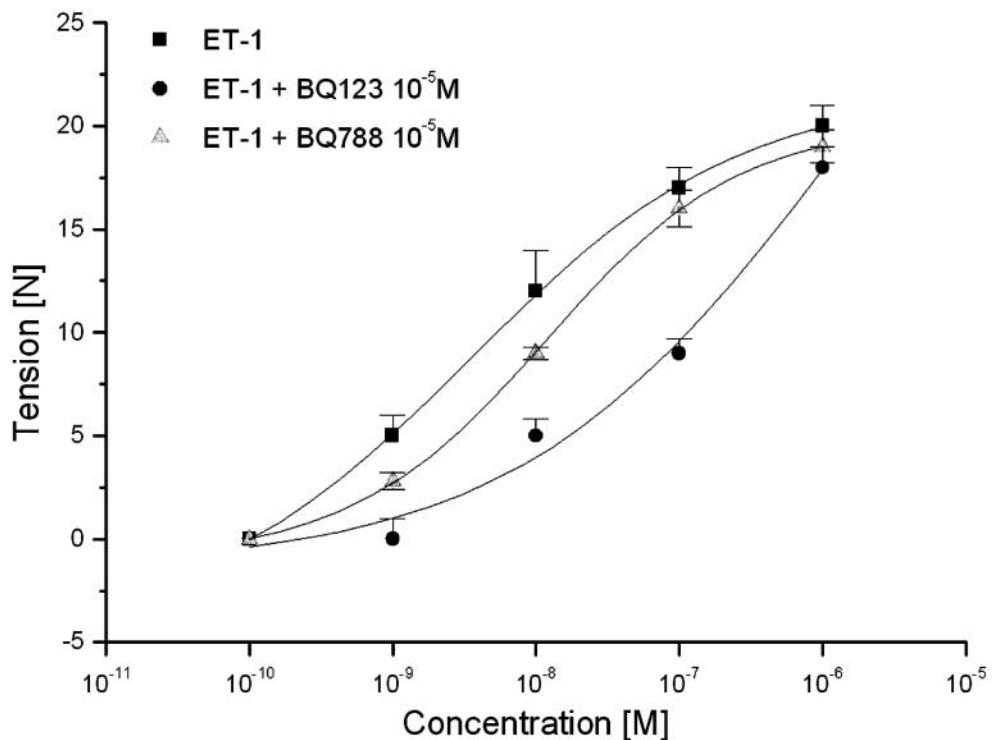


Figure 1. Effects of endothelin-1 (ET-1; 10^{-10} M to 10^{-6} M; n=16) on cavernosal smooth muscle alone and in the presence of either the ET_A receptor antagonist (BQ123; 10^{-5} M; n=8) or the ET_B receptor antagonist (BQ788; 10^{-5} M; n=8). The results are presented as tension (newton; N). Bars on symbols denote S.E.M.

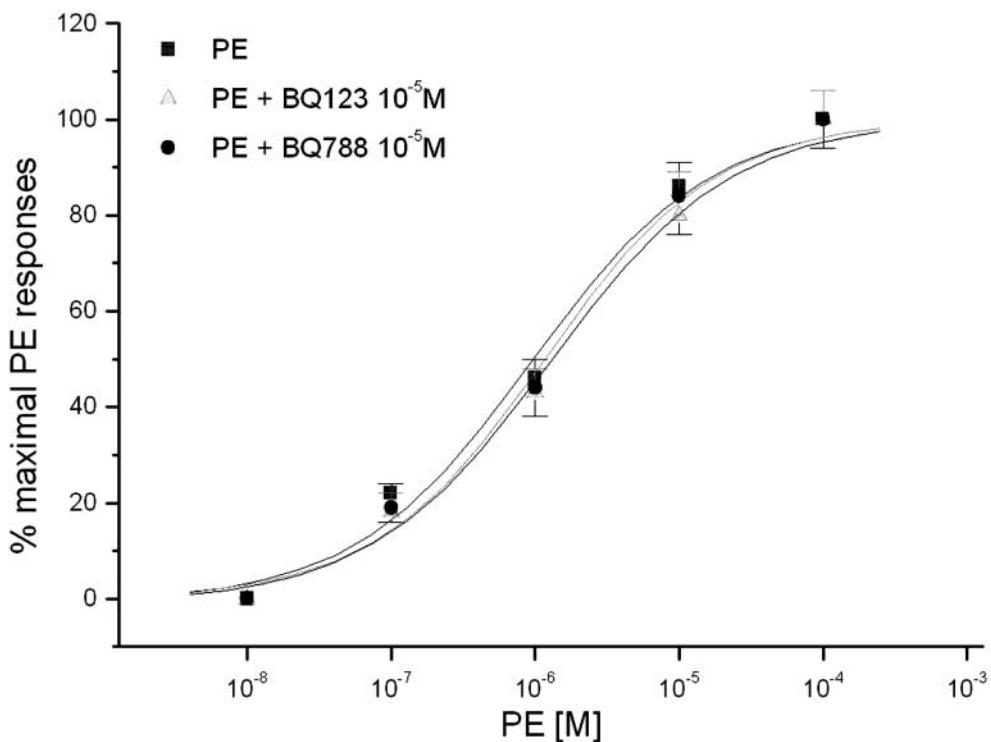


Figure 2. Effects of phenylephrine (PE; 10^{-8} M to 10^{-4} M; n=16) on cavernosal smooth muscle alone and in the presence of either the ET_A receptor antagonist (BQ123; 10^{-5} M; n=8) or the ET_B receptor antagonist (BQ788; 10^{-5} M; n=8). The contractions are expressed as a percentage of the maximal PE responses. Bars on symbols denote S.E.M.

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