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Differential effects of BQ-123 against endothelin-1 and endothelin-3 on the rat vas deferens: evidence for an atypical endothelin receptor

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1 Endothelin-1 and endothelin-3 enhanced concentration-dependently the rat vas deferens twitch response to electrical stimulation, endothelin-1 being three times more potent. Sarafotoxin S6c was at least 200 times less active than endothelin-1.

2 The response to endothelin was antagonized in a competitive manner by the supposedly selective ET_A receptor antagonist, BQ-123 (pA_2: 7.0 ± 0.1). In contrast, the endothelin-1 concentration-response curve was only shifted two fold in the presence of 10 μM BQ-123, while no effect was observed at 1 μM.

3 This evidence suggests the rat vas deferens contains an endothelin receptor not conforming to the ET_A/ET_B receptor subtype classification so far proposed.

Keywords: Endothelin-1; endothelin-3; receptor subtypes; BQ-123; sarafotoxin S6c; rat vas deferens

Introduction

The endothelins have a wide spectrum of biological actions, mediated through the interaction with at least two distinct receptor subtypes: ET_A (Arai *et al.*, 1990) and ET_B (Sakurai *et al.*, 1990). These receptor subtypes have been classified by the rank order of potency of the endogenous endothelins, endothelin-1 being at least ten times more active than endothelin-3 on ET_A receptors, while being equally active on the non-selective ET_B receptor. The existence of an additional subtype (ET_C) preferentially binding endothelin-3, has been proposed on bovine endothelial cells (Emori *et al.*, 1990). Recently, new selective ligands have become available for endothelin receptor characterization. Sarafotoxin S6c was described as a selective ET_B receptor agonist (Williams *et al.*, 1991), and BQ-123, as a selective ET_A receptor antagonist with very low affinity for ET_B receptors (Ihara *et al.*, 1992; D'Orléans-Juste *et al.*, 1992). The pharmacological profile of the ET_C receptor obtained with these new tools has not yet been reported.

On the basis of the rank order of agonist potency, facilitation of the rat vas deferens twitch response to endothelins has been attributed to activation of ET_B receptors (Maggi *et al.*, 1989; Telemaque & D'Orléans-Juste, 1991). Because of recent data suggesting further heterogeneity of endothelin receptor subtypes (Harrison *et al.*, 1992), we have used both a selective agonist and antagonist in an attempt to characterize further the endothelin receptor mediating facilitation of the rat vas deferens twitch response.

Methods

Male Sprague Dawley rats (Charles-River, 250–300 g) were killed by cervical dislocation and the vasa deferentia (pars prostatica) rapidly removed, cleaned and placed in tissue baths containing warm (37°C) oxygenated (95% O_2, 5% CO_2) Krebs solution. Activity was recorded isotonically (Ugo Basile, Italy) along the longitudinal axis (1.5 cm), under a resting tension of 0.5 g. The tissues were field stimulated sub-maximally (10 V, 0.25 ms pulse width, pulse interval of 200 ms, trains of 5 s every 60 s) by platinium electrodes connected to a Digit 3T stimulator. Under these conditions the twitch response could be abolished by 1 μM tetrodotoxin ($n = 4$; data not shown).

Following a 60–90 min equilibration period, cumulative concentration-response curves to endothelins were constructed (only one concentration-response curve was established per tissue). In studies with BQ-123, tissues were incubated with the antagonist for 30 min prior to initiation of agonist concentration-response curves. At concentrations up to 10 μM, BQ-123 had no effects, *per se*, on the twitch response.

Endothelin-1 and endothelin-3 were purchased from NovaBiochem (Switzerland), sarafotoxin S6c from Peninsula Laboratories (U.K.), and BQ-123, cyclo(D-Trp-D-Asp-Pro-D-Val-Leu) was synthesized by solid-phase methodology.

Results are presented as mean ± s.e.mean of at least three separate experiments. EC_{50} values were calculated by the Macintosh Allfit programme, and pA_2 values were calculated by Schild plot analysis.

Results

Endothelin-1 and endothelin-3 enhanced in a concentration-dependent manner the rat vas deferens twitch response, with a characteristic long lasting effect which was resistant to washing out. Threshold concentrations for both peptides were between 0.1–0.5 nM.

Maximal stimulatory effects (296 and 262% above basal) were obtained with a concentration of 100 nM of either peptide, and the EC_{50} values were 9.0 and 25.0 nM respectively (Figure 1). Sarafotoxin S6c exhibited very weak agonist activity, with a threshold stimulatory effect at 500 nM (Figure 1), and an increase of 60% above basal at the maximum concentration tested (1 μM).

BQ-123 (1 μM), had no significant effects on the endothelin-1-induced stimulation of the vas deferens twitch response (Figure 2a) whereas at 10 μM, the threshold response was shifted from 0.1 to 0.5 nM, the EC_{50} from 8.0 to 18.0 nM and the maximal response appeared to be somewhat decreased.

BQ-123 antagonized in a competitive manner the

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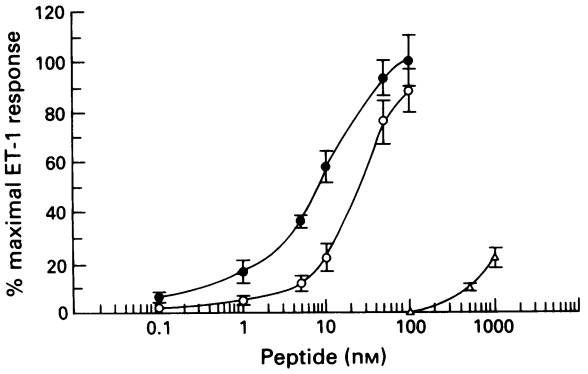

Figure 1 Concentration-response curves to endothelin-1 (●), endothelin-3 (○) and sarafotoxin S6c (△) for facilitation of the twitch response of rat vas deferens to electrical stimulation. Results are presented as % of the maximal response to endothelin-1. Each point represents the mean ± s.e.mean of at least three determinations.

endothelin-3-mediated twitch enhancement of the rat vas deferens as assessed by Schild plot analysis (pA_2: 7.0 ± 0.12; slope = 1.09; Figure 2b). Further, the maximal response to endothelin-3 was not affected at any BQ-123 concentrations.

Discussion

Sarafotoxin S6c, a selective ET_B receptor agonist (Williams *et al.*, 1991) and BQ-123, selective ET_A receptor antagonist (Ihara *et al.*, 1992) were used in an attempt to characterize further the endothelin receptor population present on the rat vas deferens pars prostatica. Endothelin-1 and endothelin-3 induced similar maximal facilitatory effects on the twitch response to field stimulation, with endothelin-1 being almost three times more potent. Under the currently accepted classification scheme (Arai *et al.*, 1990; Sakurai *et al.*, 1990), this would be consistent with the receptor being of the isopeptide-non-selective ET_B type. However, we have found that in the rat vas deferens sarafotoxin S6c induced only weak agonist activity, which would not be consistent with an action on the classical, sarafotoxin S6c-sensitive ET_B receptor (Williams *et al.*, 1991).

In the absence of a selective ET_B receptor antagonist, BQ-123, introduced as a selective antagonist at the ET_A receptor (Ihara *et al.*, 1992), was used. We have shown that BQ-123 antagonizes competitively the endothelin-1-mediated vasoconstriction of the endothelium-denuded rat thoracic aorta to yield a pA_2 value of 7.0 ± 0.1, consistent with that described for the porcine aorta (pA_2: 7.4; Ihara *et al.*, 1992). Remarkably, on the rat vas deferens, BQ-123 was inactive at 1 μM as an antagonist of endothelin-1 and at the higher concentration (10 μM) only a weak antagonism was observed. This antagonism appeared to be non-competitive, as previously observed in neuroblastoma cells (Hiley *et al.*, 1992), since the maximal effect was significantly reduced. Conversely, BQ-123 was a reasonably potent (pA_2 = 7.0) competitive antagonist of the endothelin-3 response. Previous studies have shown BQ-123 to be a selective ET_A receptor

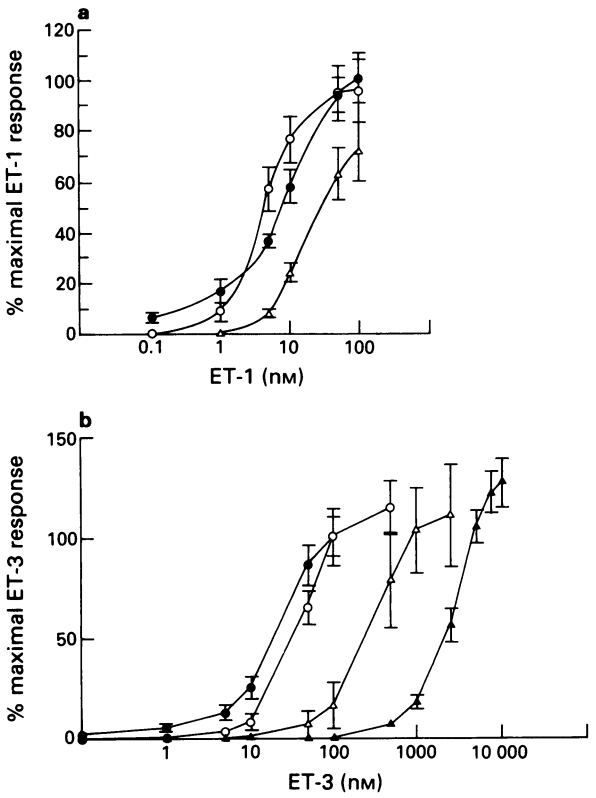

Figure 2 Concentration-response curves to: (a), endothelin-3 in the absence (●) and presence of BQ-123, 0.1 μM (○) and 10 μM (△) and to: (b) endothelin-3 in the absence (●) and presence of BQ-123, 0.1 μM (○), 1 μM (△) and 10 μM (▲) for facilitation of the twitch response of the rat vas deferens to electrical stimulation. Results are presented as % of the maximal response to endothelin-1 or endothelin-3 in the absence of BQ-123. Each point represents the mean ± s.e.mean of at least three determinations.

antagonist with low affinity for ET_B receptors (D'Orléans-Juste *et al.*, 1992; Ihara *et al.*, 1992). The fact that in the rat vas deferens BQ-123 had a preferential antagonist effect on the endothelin-3 response, suggests that the endothelin-1 and endothelin-3-mediated enhancement of the twitch response to electrical stimulation could be mediated by a receptor population distinct from either ET_A or ET_B. Taken together with the weak agonist activity of sarafotoxin S6c, our findings indicate that the pharmacology of endothelin receptor(s) in the rat vas deferens cannot easily be accommodated within the ET_A/ET_B classification. The possibility that the ET_C, non ET_A/ET_B receptor, tentatively described in cultured bovine endothelial cells (Emori *et al.*, 1990) or the site mediating contraction of porcine coronary arteries (Harrison *et al.*, 1992), may be similar to that found in the rat vas deferens deserves further consideration.

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