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Endothelin ET_A and ET_B receptors mediate vascular smooth muscle contraction

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1 We have investigated the receptors mediating endothelin-induced contraction of rabbit isolated jugular vein (RJV) and rat isolated thoracic aorta (RTA).

2 Endothelin-1 (ET-1) and endothelin-3 (ET-3) contracted RJV preparations with similar potency (EC_{50} values $\sim$1 nM), whereas, ET-1 (EC_{50}:4.5 nM) was $\sim$80 fold more potent than ET-3 in contracting RTA. In addition, the ET_B receptor-selective agonist [$Ala^{1,3,11,15}$]ET-1 contracted RJV (EC_{50}:2.1 nM) but not RTA.

3 The ET_A receptor antagonist, BQ123, competitively antagonized (pA_2 6.93) the contraction of RTA produced by ET-1, but had no effect (at 10 μM) on the contractile effects of either ET-1, ET-3 or [$Ala^{1,3,11,15}$]ET-1 in RJV.

4 These data suggest that both ET_A and ET_B receptors can mediate vascular smooth muscle contraction.

Keywords: Endothelin; receptor subtypes; vascular smooth muscle contraction; ET_A antagonist; BQ123

Introduction

The discovery of endothelin-1 (ET-1; Yanagisawa *et al.*, 1988) and its isopeptides, endothelin-2 (ET-2) and endothelin-3 (ET-3; Inoue *et al.*, 1989) has stimulated considerable interest. The differential potencies of ET-1, ET-3 and [$Ala^{1,3,11,15}$]ET-1 ([Ala_4]ET-1) suggests that subtypes of the endothelin receptor occur (Masaki *et al.*, 1991; Saeki *et al.*, 1991). Indeed, two subtypes have been cloned and sequenced (Arai *et al.*, 1990; Sakurai *et al.*, 1990) and denoted ET_A, which shows selectivity for ET-1 over ET-3 and mediates contraction, and ET_B at which ET-1 and ET-3 are equipotent, and mediate vasorelaxation, possibly via the release of endothelium-derived relaxant substances (Masaki *et al.*, 1991). Recently, a cyclic pentapeptide antagonist (BQ123; D-Val, Leu, D-Trp, D-Asp, Pro) has been described with a marked selectivity for the ET_A receptor, both *in vitro* and *in vivo* (Ihara *et al.*, 1991; 1992). In this study, we have used BQ123 to characterize the endothelin receptors mediating contraction of rabbit isolated jugular vein (RJV) and rat isolated thoracic aorta (RTA) preparations.

Methods

Ring preparations (2–3 mm) from RJV and RTA were mounted in organ baths for isometric tension measurement. Tissues were equilibrated at 37°C in a gassed (95% O_2, 5% CO_2) Krebs solution (composition, mM: NaCl 118, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 0.6, D-glucose 11, $CaCl_2$ 1.3) at a resting tension of 0.5 g (RJV) or 2 g (RTA). Following 50 mM KCl (RTA only), preparations were contracted with either histamine (100 μM, RJV) or with the stable thromboxane A_2-mimetic, U-46619 (10 nM, RTA). The endothelium of RTA preparations was mechanically removed. This could not be undertaken in the more fragile RJV, therefore these preparations were treated with L-N^G-nitroarginine methyl ester (L-NAME, 100 μM) and indomethacin (3 μM) to inhibit, endothelium-derived, nitric oxide and prostanoid formation, respectively. In tissues with induced tone, carbachol (1 μM, RJV) or acetylcholine (1 μM, RTA) were used to assess endothelial cell function. Tissues were then washed prior to a cumulative concentration-effect curve to an endothelin peptide. In experiments with BQ123 tissues were pretreated for 30 min.

Values are the arithmetic mean ($\pm$ s.e.mean) or geometric mean (with 95% confidence intervals; for agonist EC_{50} and concentration-ratio (CR) values) from n animals. The EC_{50} value is defined as the concentration of agonist producing 50% of its own maximum response. Agonist CR values were calculated by dividing the EC_{50} obtained in the presence of the antagonist by that obtained in its absence, and subjected to Schild analysis (Arunlakshana & Schild, 1959). $P < 0.05$ was taken to reflect a significant difference (unpaired Student's t test).

Drugs

Endothelins were obtained from Peninsula and carbachol from BDH. Acetylcholine chloride, L-N^G-nitroarginine methyl ester (L-NAME), bacitracin, leupeptin, phosphoramidon and histamine dihydrochloride were obtained from Sigma. All the above were prepared in Krebs solution. U-46619 (11,9-epoxymethano-prostaglandin H_2), [Ala_4]ET-1 and BQ123 (D-Val, Leu, D-Trp, D-Asp, Pro) (Glaxo Group Research), were dissolved in 1% (w/v) $NaHCO_3$, 0.1% (w/v) ammonium acetate and distilled water, respectively.

Results

Agonist potencies

ET-1 and ET-3 produced well-sustained, concentration-dependent contractions of the RJV and RTA preparations. ET-1 and ET-3 (0.1–100 nM) showed equivalent potencies (EC_{50} values $\sim$1 nM) on the RJV. In contrast, ET-3 was some $\sim$80 times less potent than ET-1 on the RTA preparation, although it behaved as a full agonist (Table 1). Interestingly, [Ala_4]ET-1 contracted the RJV (Table 1), but was neither an agonist nor an antagonist (versus ET-1) at concentrations up to 1 μM in the RTA. Protease inhibitors (50 μg ml^{-1} bacitracin, 5 μg ml^{-1} leupeptin and 100 μM phosphoramidon) did not affect the potency of ET-1 or ET-3 on

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Table 1 Potencies of endothelin-1 (ET-1), ET-3 and [Ala$_4$]ET-1 as contractile agonists in preparations of rabbit isolated jugular vein (RJV) and rat isolated thoracic aorta (RTA)

	RJV		*RTA*	
Isopeptide	EC_{50} (nM)	*Max response* (% histamine)	EC_{50} (nM)	*Max response* (% U-46619)
ET-1	0.7 (0.4–1.1)	131 (± 5)	4.5 (3.6–5.6)	131 (± 5)
ET-3	0.9 (0.5–1.8)	111* (± 4)	370 (230–580)	121 (± 6)
[Ala$_4$]ET-1	2.1 (0.6–8.0)	106* (± 8)	--NSE-- at 1 μM	

Cumulative concentration-effect curves to endothelin-1 (ET-1), endothelin-3 (ET-3) and [Ala^{1,3,11,15}]ET-1 ([Ala$_4$]ET-1) were produced for contraction of RJV and RTA. Values are arithmetic means (± s.e.mean) or geometric means (95% confidence limits) from 6–32 experiments. Maximum responses are expressed relative to the contraction produced by either histamine (100 μM, RJV, mean 0.92 ± 0.1 g) or U-46619 (10 nM, RTA, mean 1.9 ± 0.1 g).
*Significantly different ($P < 0.05$) from ET-1. NSE: no significant effect.

either preparation. It is unlikely that nitric oxide or prostanoid formation in the RJV influenced the contractile activity to the endothelins, as L-NAME and indomethacin, did not affect either the EC_{50} or the maximum contraction to ET-1 or ET-3 (data not shown).

Antagonist potencies

BQ123 (0.3–3 μM) produced a concentration-dependent, rightward parallel displacement of the ET-1 concentration-effect curve in RTA, with no suppression of the maximum response (Figure 1a). Schild analysis yielded a mean pA_2 value for BQ123 of 6.93 ± 0.06 and slope of 1.00 ± 0.06 ($n = 5$). BQ123 also antagonized the contractile effects of ET-3 on this preparation. The weak agonist potency of ET-3 precluded the determination of a full pA_2 value but using a single concentration (30 nM) of BQ123, a mean pK_B value of 8.3 ± 0.1 ($n = 4$) was estimated. In addition, BQ123 (3 μM) also antagonized the contractile effects of ET-1 (CR = 26.6 [8.76–81.8], $n = 4$) in endothelium-intact RTA preparations exposed to L-NAME (100 μM) and indomethacin (3 μM). In contrast, BQ123 even at 10 μM, had no effect on the contraction of RJV to ET-1 (CR = 1.0 [0.3–3.5], $n = 5$; Figure 1b), ET-3 (CR = 0.7 [0.2–1.7], $n = 5$) or [Ala$_4$]ET-1 (CR = 1.3 [0.7–2.3], $n = 3$). Consistent with its ET receptor specificity, BQ123 (10 μM) did not change either the resting tension or the contractile response to either histamine (100 μM, RJV) or to U-46619 (10 nM, RTA).

Discussion

This study demonstrates that at least two receptor subtypes mediate endothelin-induced vascular smooth muscle contraction and can be distinguished on the basis of both agonist and antagonist selectivities. One type of receptor, as found in the RTA, can be classified as ET_A in that (1) ET-1 was more potent than ET-3, whilst [Ala$_4$]ET-1 was inactive, and (2) the actions of ET-1 were competitively antagonized by BQ123, the pA_2 value of 6.93 being comparable to that reported elsewhere (Ihara *et al.*, 1992). In contrast, a second endothelin receptor, as found in the RJV, resembles the ET_B receptor, since the endothelins ET-1, ET-3 and [Ala$_4$]ET-1 showed comparable agonist potencies which were importantly not antagonized by BQ123.

On the RTA, ET-3-induced contractions were also antagonized by BQ123 but with an estimated potency ($pK_B = 8.3$) greater than the pA_2 value of 6.9 obtained against ET-1 on this preparation. The reason(s) for this is not clear. However, the weak agonist potency of ET-3 precluded its use at the very high concentrations required to produce full concentration-effect curves in the presence of BQ123. Consequently, the potency estimates for BQ123 against ET-3-induced contractions may be of limited value. It is possible, however, that ET-3 may act through a different receptor from ET-1 which is more sensitive to BQ123.

The data from our study suggest that endothelin-induced vascular smooth muscle contraction can be mediated via both ET_A and ET_B reeptors. Alternatively, there may be

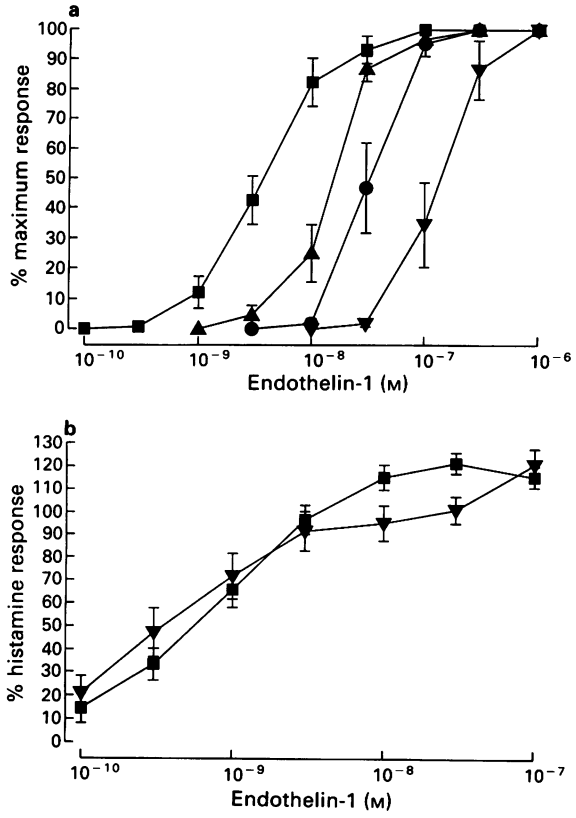

Figure 1 Effects of BQ123 on endothelin-1 (ET-1)-induced contraction of rat isolated thoracic aorta (RTA) and rabbit isolated jugular vein (RJV) ring preparations. (a) RTA rings were pretreated for 30 min with either vehicle (controls, ■) or BQ123 at 0.3 μM (▲), 1 μM (●), or 3 μM (▼), before exposure to endothelin-1. Cumulative concentration-effect curves were constructed and EC_{50} values determined. Agonist concentration-ratios were calculated and used to obtain pA_2 and slope values from Schild plots. The data are means (with s.e.mean shown by the vertical bars) from 5 determinations, yielding mean pA_2 and slope values of 6.93 (± 0.06) and 1.00 (± 0.06) respectively. (b) RJV rings were pretreated for 30 min with either vehicle (control, ■) or BQ123 (10 μM, ▼) before exposure to ET-1. Data are means (with s.e.mean shown by the vertical bars) from 5 determinations, yielding a mean agonist concentration-ratio for ET-1 of 1.0 [0.3–3.5].

subtypes of the ET_A contractile receptor in which one type exhibits an agonist profile (the potency of ET-1 = ET-3) comparable to that observed at ET_B receptors. Thus, the concept of a smooth muscle ET_A receptor mediating vasoconstriction and an endothelial cell ET_B receptor mediating vasodilatation (Masaki *et al.*, 1991) needs reconsideration. Consistent with our proposal are the very recent data from Moreland *et al.* (1992), using a variety of isolated arterial and venous smooth muscle preparations from several species, which indicate that ET_A-like contractile receptors predominate on arterial preparations and ET_B-like contractile receptors on venous smooth muscle. Similarly, Fukuroda and colleagues (1992) noted that in porcine isolated coronary and pulmonary blood vessels part of the ET-1-induced contractile responses was resistant to antagonism with the cyclic pentapeptide ET_A antagonist, BQ153.

It remains to be firmly established which type of ET receptor mediates the vasorelaxant effects of the endothelins. For example, an ET_C receptor, (potency of ET-3 $\gg$ ET-1), has been described in bovine (Emori *et al.*, 1990), but not in human (White *et al.*, 1992) cultured endothelial cells. In addition, ET-1 and ET-3 have been reported to produce relaxation of endothelium-intact porcine pulmonary artery vessels which is unaffected by BQ123, in keeping with an ET_B-mediated response (Fukuroda *et al.*, 1992). However, the ET_B-selective agonist sarafotoxin 6c does not elicit a relaxation response in rabbit carotid arteries with an intact endothelium (Moreland *et al.*, 1992).

In conclusion, the present study has demonstrated that vascular smooth muscle contraction evoked by the endothelins can be mediated by at least two receptor subtypes, which can be differentiated on the basis of the relative potencies of ET-1, ET-3 and [Ala_4]ET-1 and by susceptibility to antagonism by the ET_A receptor antagonist, BQ123.

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