British Journal of Pharmacology (1996) 118, 1209–1217 © 1996 Stockton Press All rights reserved 0007–1188/96 $12.00

Comparison of endothelin $_B$ (ET_B) receptors in rabbit isolated pulmonary artery and bronchus

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1 To explore potential differences between endothelin (ET) receptors in airway versus vascular smooth muscle from the same species, the ET_B receptors mediating contractions produced by ET-1, ET-3 and the selective ET_B ligands, sarafotoxin S6c (S6c) and BQ-3020, in rabbit bronchus and pulmonary artery were investigated by use of peptide and non-peptide ET receptor antagonists.

2 In rabbit pulmonary artery SB 209670 (10 μM), a mixed ET_A/ET_B receptor antagonist, was a more potent antagonist of contractions produced by S6c ($pK_B = 7.7$; $n = 9$; $P < 0.05$), than those elicited by ET-1 ($pK_B = 6.7$; $n = 6$) or ET-3 ($pK_B = 6.7$; $n = 5$). BQ-788 (10 μM), an ET_B receptor antagonist, inhibited responses produced by ET-3 ($pK_B = 5.1$; $n = 8$), BQ-3020 ($pK_B = 5.2$; $n = 4$) or S6c ($pK_B = 6.2$; $n = 9$; $P < 0.05$ compared to potency versus ET-3- or BQ-3020-induced contractions), but was without inhibitory effect on ET-1-induced contractions ($n = 5$). RES-701 (10 μM), another selective ET_B receptor antagonist, was without effect on contractions produced by S6c ($n = 4$) or ET-1 ($n = 4$), and potentiated ET-3- ($n = 5$) or BQ-3020-induced responses ($n = 4$).

3 The combination of BQ-788 (10 μM) and BQ-123 (10 μM), an ET_A-selective receptor antagonist, antagonized contractions produced by lower concentrations of ET-1 (1 and 3 nM) in rabbit pulmonary artery, but was without effect on responses elicited by higher concentrations of ET-1 ($n = 5$). The combination of RES-701 (10 μM) and BQ-123 (10 μM) potentiated responses elicited by ET-1, producing a 3.7 fold shift to the left in the agonist concentration-response curve ($n = 5$).

4 In rabbit bronchus SB 209670 (3 μM) had similar potency for antagonism of contractions produced by ET-1 ($pK_B = 6.3$; $n = 6$), ET-3 ($pK_B = 6.5$; $n = 6$) or S6c ($pK_B = 6.1$; $n = 8$). BQ-788 (3 μM) was without effect on responses elicited by ET-1, ET-3 or S6c ($n = 6$) but antagonized BQ-3020-induced contractions ($pK_B = 6.4$; $n = 4$). RES-701 (3 μM) was without effect on contractions produced by S6c ($n = 6$) or BQ-3020 ($n = 4$), and potentiated rather than antagonized ET-1- or ET-3-induced responses ($n = 6$), reflected by a significant (about 6 fold) shift to the left in ET-1 or ET-3 concentration-response curves. The combination of BQ-788 (3 μM) and BQ-123 (3 μM) was without effect on contractions produced by ET-1 in rabbit bronchus ($n = 6$). The combination of RES-701 (3 μM) and BQ-123 (3 μM) potentiated responses elicited by ET-1, producing a 5.2 fold shift to the left in the agonist concentration-response curve ($n = 5$).

5 BQ-123 (3 or 10 μM), an ET_A-selective receptor antagonist, was without effect on ET-1, ET-3 or S6c concentration-response curves ($n = 3-6$) in rabbit pulmonary artery or rabbit bronchus.

6 These data indicate that contractions induced by ET-1, ET-3, S6c and BQ-3020 in rabbit pulmonary artery or rabbit bronchus appear to be mediated predominantly via stimulation of ET_B receptors. However, the qualitative and quantitative differences in the relative profiles of the various structurally diverse peptide and non-peptide antagonists examined suggests that responses produced by the ET ligands may not be mediated by a homogeneous ET_B receptor population. In addition, the results suggest that differences exist in the ET_B receptors mediating contraction in pulmonary vascular versus airway tissues in the same species. These receptors are not very sensitive to the standard ET_B receptor antagonists, BQ-788 and RES-701. Furthermore, the results also provide further evidence that the potencies of ET receptor antagonists depend upon the ET agonist.

Keywords: ET-1; ET-3; sarafotoxin S6c; rabbit pulmonary artery; rabbit bronchus; ET_B receptor subtypes; SB 209670; BQ-788; RES-701; BQ-3020

Introduction

The endothelins (ETs) are a family of 21-amino acid peptides whose members include ET-1, ET-2 (two amino acid substitution from ET-1) and ET-3 (six amino acid substitution from ET-1) (Yanagisawa *et al.*, 1988; Inoue *et al.*, 1989; Masaki *et al.*, 1992). ET-1 produces diverse effects in the lung and enhanced expression and/or levels of ET-1 have been demonstrated in various lung diseases. These findings have formed the basis of the speculation that ET-1 plays a significant role in the pathophysiology of pulmonary disorders, notably asthma and pulmonary hypertension (Springall *et al.*, 1991; Stewart *et al.*, 1991; Hay *et al.*, 1993a; Hay & Goldie, 1995). An important component of these latter diseases is contraction of airway and pulmonary vascular smooth muscles.

There is unequivocal evidence from pharmacological, biochemical and molecular biological studies that the diverse effects of the ETs are mediated via distinct receptor subtypes (Yanagisawa & Masaki, 1989; Masaki *et al.*, 1992; Sakurai *et al.*, 1992). A few years ago it was proposed that there are two major ET receptor subtypes: an ET_A receptor which has a higher affinity for ET-1 or ET-2 compared with ET-3, and an ET_B receptor which has equal affinity for the three ETs (Arai *et al.*, 1990; Sakurai *et al.*, 1990; Masaki *et al.*, 1992). There is also evidence for another putative ET receptor subtype, designated ET_C, which is selective for ET-3 (Martin *et al.*, 1990; Samson *et al.*, 1990; Masaki *et al.*, 1992; Douglas *et al.*, 1995).

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However, recent research indicates that the above ET receptor subtype classification appears to be incomplete (Sokolovsky *et al.*, 1992; Warner *et al.*, 1993; Bax & Saxena, 1994). Furthermore, controversy exists as to the identity of the ET receptors responsible for the contractile effects of the ETs in vascular and airway smooth muscles. For example, initial evidence suggested that ET_A receptor activation mediates the vasoconstrictor effects of the ETs whereas vasodilatation is elicited by stimulation of ET_B receptors (and release of NO) located on endothelial cells (Sakurai *et al.*, 1992; Masaki *et al.*, 1992; Warner *et al.*, 1993). However, subsequent studies revealed that ET_B receptor activation produces contraction in several vascular smooth muscles (Fukuroda *et al.*, 1992; Sudjarwo *et al.*, 1993; 1994; Douglas *et al.*, 1995) including rabbit pulmonary artery (Ihara *et al.*, 1992b; Panek *et al.*, 1992; LaDouceur *et al.*, 1993; Warner *et al.*, 1993; Fukuroda *et al.*, 1994; Ohlstein *et al.*, 1994a,b). Based on these and other data it has been proposed that there are two subtypes of ET_B receptors: 'ET_{B1}-like', which mediate the vasodilator effects, and 'ET_{B2}-like' which are responsible for the vasoconstrictor activity of the ETs (Sokolovsky *et al.*, 1992; Warner *et al.*, 1993). In large airways there are species differences in the relative contributions of ET_A and ET_B receptors to the contractile responses produced by ET-1 (Hay *et al.*, 1993a,b; Henry, 1993; Goldie *et al.*, 1994; Yoneyama *et al.*, 1995).

Some of the reported differences in the potencies of the various ET receptor antagonists and related evidence for further ET receptor subtypes may be attributed to species differences in the tissue compared. Accordingly, the primary aim of the present study was to explore, with the use of peptide and non-peptide receptor antagonists, whether differences exist in the ET_B receptors mediating contractions elicited by the ET ligands, ET-1, ET-3, S6c (ET_B-selective agonist; Williams *et al.*, 1991) or BQ-3020 (ET_B-selective agonist; Ihara *et al.*, 1992b), in isolated pulmonary tissues from the same species, namely, rabbit bronchus and pulmonary artery. The compounds used were the selective ET_B receptor antagonists BQ-788 (Ishikawa *et al.*, 1994) and RES-701 (Tanaka *et al.*, 1994), the combined ET_A and ET_B receptor antagonist, SB 209670, which has high affinity for the ET_A receptor and lower but significant affinity for the ET_B receptor (Ohlstein *et al.*, 1994a,b), and the selective ET_A receptor antagonist, BQ-123 (Ihara *et al.*, 1992a).

Methods

All experiments were performed in accordance with the guidelines of the Animal Care and Use Committee, SmithKline Beecham Pharmaceuticals.

Tissue preparation

Rabbit bronchus and pulmonary artery Lungs were removed from male New Zealand White rabbits (H.A.R.E. Rabbitry, Hewitt, NJ, U.S.A.; 2–3 kg body weight) following sodium pentobarbitone overdose (100 mg kg^{-1} via the central ear vein) and placed in modified Krebs-Henseleit solution. The composition of the Krebs-Henseleit solution, which was gassed with 95% O_2:5% CO_2 and maintained at 37°C, was (mM): NaCl 112.0; KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2 and dextrose 11.0. Following removal of parenchyma, adherent fat and connective tissue, the rabbit bronchus was cut open along its longitudinal axis, directly opposite the smooth muscle, and strips consisting of two adjacent cartilage rings were prepared. The epithelium was removed mechanically by gently rubbing the luminal surface with a cotton-tipped applicator. The pulmonary arteries were located, cleaned of adherent tissue and cut into rings of 4 mm width and about 3–4 mm length. The endothelium was removed by gently rubbing the intimal surface of the vessel with a stainless steel rod (Ohlstein *et al.*, 1994a).

Tension recording and reference contractions Each preparation was then placed in a 10 ml water-jacketed organ bath containing modified Krebs-Henseleit solution and connected via a silk suture to Grass FT03C force-displacement transducers (Grass Instrument Co., Quincy, MA, U.S.A.). Mechanical responses were recorded isometrically by MP100WS/Acknowledge data acquisition system (BIOPAC Systems, Goleta, CA, U.S.A.) run on Macintosh computers or Beckman R-611 dynographs (Sunnyvale, CA, U.S.A.). Tissues were equilibrated under approximately 1.5 g resting load for bronchus and 1 g for pulmonary artery for 60–90 min, washed every 15 min with fresh Krebs-Henseleit solution, before the start of each experiment. After the equilibration period, and before addition of the agonist to be investigated, bronchial tissues were exposed to 10 μM carbachol and arterial tissues were exposed to 60 or 100 mM KCl. In arterial tissues, to confirm successful removal of the endothelium (i.e., little or no relaxation upon exposure to muscarinic agonists) precontracted preparations were exposed to carbachol (1 μM) or acetylcholine (0.1 μM). Upon completion of the reference contractions, tissues were washed several times over 30–60 min to return tension to baseline level. Subsequent responses in the preparations were expressed as a percentage of this reference response ('% carbachol maximal' or '% KCl maximal'). The preparations were then left for at least 30 min before the start of the experiment.

Concentration-response curves

ET-1, ET-3, S6c or BQ-3020 concentration-response curves were obtained by the cumulative addition of agonists to the organ bath in half-log increments according to the technique of Van Rossum (1963). Each drug concentration was left in contact with the preparation until the response reached a plateau before addition of the subsequent agonist concentration. In experiments examining the effects of antagonists, tissues were exposed to the appropriate compound or solvent for 30 min before the initiation of agonist concentration-response curves. Only one agonist concentration-response curve was generated per tissue. Experiments on the bronchus were conducted in the presence of 1 μM sodium meclofenamate, to inhibit the activity of bronchoactive prostanoids.

Analysis of data

Agonist-induced responses for each tissue were expressed as a percentage of the reference contraction (10 μM carbachol and 60 or 100 mM KCl for bronchial and arterial tissues, respectively) obtained at the beginning of the experiment. Concentration-response curves were analysed by nonlinear least squares regression (Ohlstein *et al.*, 1994a). Geometric mean EC_{50} values (pD_2s) were calculated from linear regression analyses of data. It is recognized that the ETs and related ligands may not interact with their receptors in a classical manner which will result in a reversible competitive interaction between agonist, antagonist and receptor (Marsault *et al.*, 1991; Waggoner *et al.*, 1992; Ohlstein *et al.*, 1995). However, antagonist potencies were calculated assuming a classical competitive interaction, and expressed as pK_B; $pK_B = -\log$ [antagonist]/X − 1, where X is the ratio of agonist concentration required to elicit 50% of the maximal contraction in the presence of the antagonist compared with that in its absence (Arunlakshana & Schild, 1959). Results for control- and treated-tissues were analysed for differences in both the pD_2s ($-\log EC_{50}$s) and also the maximal contractile responses. All data are given as the mean or mean ± s.e.mean and n represents the number of tissues studied in a particular group. Statistical analysis was conducted using ANOVA (Fisher's protected least squares difference) or Student's two-tailed t test for paired samples where appropriate with a probability value less than 0.05 regarded as significant.

Drugs

All drug solutions were made daily (from stock solutions or powder) and stored on ice. The following drugs were used: endothelin-1, endothelin-3, sarafotoxin S6c and *N*-acetyl-[Ala^{11,15}]endothelin-1 (6–21) (BQ-3020) were purchased from Peninsula Laboratories (Belmont, CA, U.S.A.), American Peptide Co. (Sunnyvale, CA, U.S.A.) or Sigma Chemical Co. (St. Louis, MO, U.S.A.). Carbachol, acetylcholine bromide and dimethylsulphoxide (DMSO) were purchased from Sigma Chemical Co. and *cyc*(DTrp-DAsp-Pro-DVal-Leu)(BQ-123) from American Peptide Co. (+)-(1S,2R,3S)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5-(prop-1-yloxy)-indane-2-carboxylic acid (SB 209670) and *N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxy-carbonyltryptophanyl-D-uorleucin (BQ-788) were synthesized by colleagues in the Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals. Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp-Trp-Phe-Phe-Asn-Tyr-Tyr-Trp (RES-701) and sodium meclofenamate were gifts from Warner Lambert (Ann Arbor, MI, U.S.A.) and Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan) respectively.

Results

Contractile effects of ET-1, ET-3, S6c and BQ-3020

ET-1, ET-3, S6c or BQ-3020 potently produced concentration-dependent contractions of rabbit pulmonary artery and rabbit bronchus, with EC_{50}s in the low to high nM range. In both tissues S6c was more potent than ET-1, ET-3 or BQ-3020 ($P<0.0001$). In rabbit pulmonary artery ET-1 was more potent than ET-3 and, in particular, BQ-3020. In bronchus ET-1 and ET-3 were more potent than BQ-3020 (Table 1). The four agonists possessed equivalent efficacies in rabbit bronchus, whereas in rabbit pulmonary artery S6c produced a smaller maximal contraction than ET-1, ET-3 or BQ-3020; BQ-3020 was also more efficacious than ET-1 (Table 1). The influence of SB 209670 (ET_A/ET_B receptor antagonist), RES-701 (ET_B receptor antagonist), BQ-788 (ET_B receptor antagonist) or BQ-123 (ET_A receptor antagonist) against contractions induced by ET-1, ET-3, S6c or BQ-3020 was explored.

Effects of antagonists against ET-1-, ET-3-, S6c- or BQ-3020-induced contractions in rabbit pulmonary artery

SB 209670 (10 μM) was a more potent antagonist (about 10 fold) of contractions produced by S6c ($pK_B=7.7$; $n=9$; $P<0.05$ compared to potency against contractions induced by ET-1 or ET-3), than those elicited by ET-1 ($pK_B=6.7$; $n=6$) or ET-3 ($pK_B=6.7$; $n=5$) in rabbit pulmonary artery (Table 2). In a more extensive analysis, SB 209670 (0.1–10 μM) produced a concentration-dependent antagonism of S6c- or ET-1-induced contractions (Figures 1a and 1c, respectively). SB 209670 also increased the maximal contraction produced by S6c (Figure 1a), but not ET-1 (Figure 1c); the increase, about 30%, was the same with the three concentrations of SB 209670 (Figure 1a).

BQ-788 (10 μM) antagonized contractions elicited by S6c ($pK_B=6.2$; $n=9$) (Figure 2a), ET-3 ($pK_B=5.1$; $n=8$) or BQ-3020 ($pK_B=5.2$; $n=4$; Figure 2c) but was without inhibitory effect on responses produced by ET-1 ($n=5$; Figure 5a) in rabbit pulmonary artery (Table 2). BQ-788 significantly increased, by about 20%, the maximal contraction elicited by S6c (0.3 μM; $P<0.001$; Figure 2a) or ET-1 (0.3 μM; $P<0.001$; Figure 5a).

RES-701 (3 μM) was without effect on contractions produced by S6c ($n=4$) (Figure 3a) or ET-1 ($n=4$) but potentiated ET-3 ($n=5$; Figure 3c)- or BQ-3020-induced contractions ($n=4$) (Table 2), as reflected by a 2.8 fold shift to the left in the BQ-3020 concentration-response curve (data not shown) and an increase in contractions produced by low concentrations of ET-3 (≤3 nM; Figure 3c); there was no effect on the maximal contractions induced by the ET ligands (Figures 3a and c; Table 2).

BQ-123 (10 μM) was without effect on ET-1 ($n=4$; Figure 4a), ET-3 ($n=3$) or S6c ($n=4$; Figure 4c) concentration-response curves in rabbit pulmonary artery (Table 2).

The combination of BQ-788 (10 μM) and BQ-123 (10 μM) antagonized contractions produced by lower concentrations of ET-1 (1 or 3 nM), but was without effect on contractions elicited by higher ET-1 concentrations in rabbit pulmonary artery. Overall there was no effect on the ET-1 pD_2: control = 8.1 ± 0.1; + BQ-788 and BQ-123 = 7.9 ± 0.1 ($n=5$; $P>0.05$) (Figure 5c). The combination of RES-701 (10 μM) and BQ-123 (10 μM) potentiated ET-1-induced contractions

Table 1 Comparison of the potencies and efficacies of ET-1, ET-3, S6c and BQ-3020 in rabbit pulmonary artery (endothelium-free) and rabbit bronchus (epithelium-free)

	Rabbit pulmonary artery		*Rabbit bronchus*	
Agonist	*pD_2*	*Maximal contraction*	*pD_2*	*Maximal contraction*
ET-1	8.6 ± 0.1 (19)[b]	107.8 ± 3.4 (19)[c]	7.5 ± 0.1 (11)[c]	110.0 ± 5.1 (11)
ET-3	8.2 ± 0.1 (8)	120.5 ± 14.9 (8)	7.8 ± 0.1 (9)[c]	98.4 ± 9.6 (9)
S6c	9.9 ± 0.1 (26)[a]	88.7 ± 4.7 (26)[a]	9.1 ± 0.1 (11)[a]	94.7 ± 5.9 (11)
BQ-3020	7.6 ± 0.1 (4)	142.0 ± 9.0 (4)	7.0 ± 0.1 (4)	101.4 ± 4.9 (4)

Results are presented as pD_2 (–log M) and maximal contraction (% reference contraction) and are given as mean ± s.e.mean; the *n* values are indicated in parentheses. [a] Significant versus ET-1, ET-3 and BQ-3020, $P<0.01$; [b] Significant versus ET-3 and BQ-3020, $P<0.05$; [c] Significant versus BQ-3020, $P<0.05$.

Table 2 Effects of SB 209670, BQ-788, RES-701, BQ-123 against contractions produced by ET-1, ET-3, S6c or BQ-3020 in rabbit pulmonary artery (endothelium-free), rabbit bronchus (epithelium-free)

	pK_B							
Compound	*Rabbit pulmonary artery*				*Rabbit bronchus*			
	ET-1	*ET-3*	*S6c*	*BQ-3020*	*ET-1*	*ET-3*	*S6c*	*BQ-3020*
SB 209670	6.7 (6)[a]	6.7 (5)[a]	7.7 (9)	ND	6.3 (6)	6.5 (6)	6.1 (8)	ND
BQ-788	NE (5)	5.1 (8)[a]	6.2 (9)	5.2 (4)[a]	NE (6)	NE (6)	NE (6)	6.4 (4)
RES-701	NE (4)	LS (5)	NE (4)	LS (4)	LS (6)	LS (6)	NE (6)	NE (4)
BQ-123	NE (4)	NE (3)	NE (4)	ND	NE (4)	NE (6)	NE (6)	ND

Results are expressed as pK_B; the *n* values are given in parentheses. NE = no effect; LS = potentiation, reflected by a shift to the left in the concentration-response curve; ND, not determined. [a] Significant compared to potency versus S6c in rabbit pulmonary artery, $P<0.05$.

producing a 3.7 fold shift to the left of the agonist concentration-response curve; pD_2s: control = 7.8 ± 0.1; + RES-701 and BQ-123 = 8.5 ± 0.1; $n = 5$; $P < 0.005$ (data not shown). There was no effect of the combination of RES-701-1 and BQ-123 against the maximal contraction induced by ET-1 (data not shown).

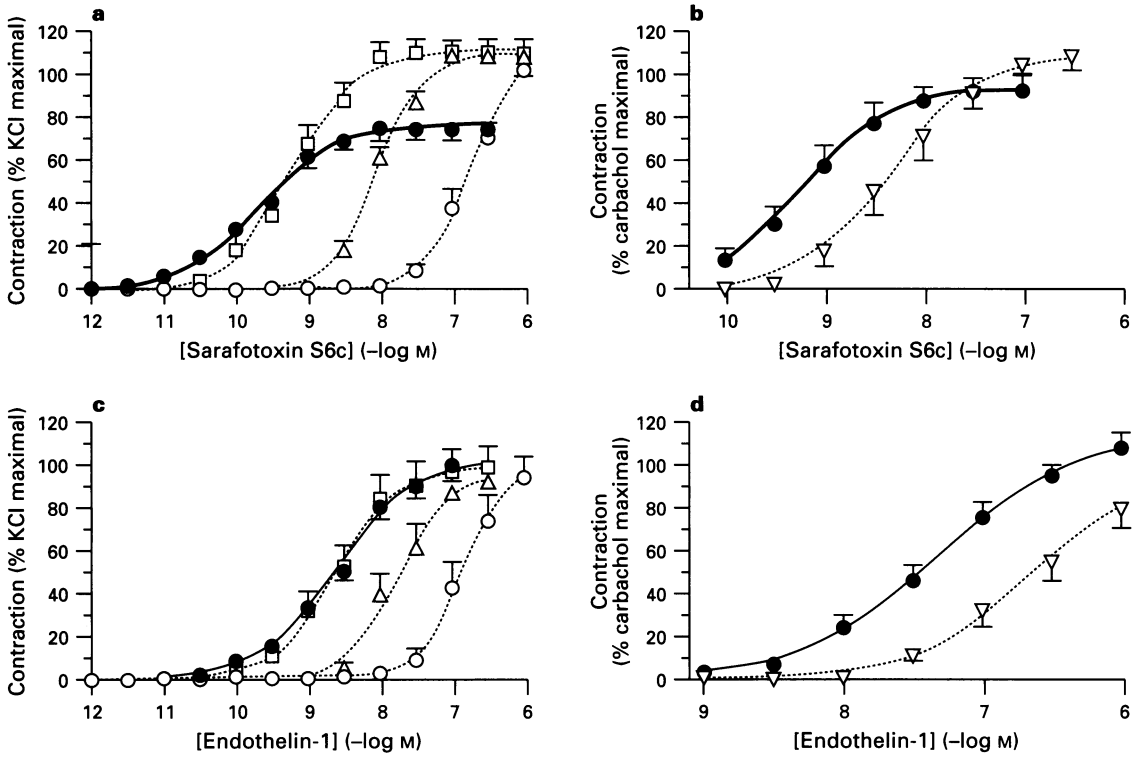

Figure 1 Effect of SB 209670 on (a,b) S6c or (c,d) ET-1 concentration-response curves in (a,c) rabbit pulmonary artery (endothelium-free) or (b,d) rabbit bronchus (epithelium-free). Results are expressed as a percentage of the response to a maximally-effective concentration of KCl (a,c) or carbachol (b,d) and are given as the mean ± s.e.means of (a) 9, (b) 8 or (c,d) 6 experiments. (a,c): (●) Control; (□) + 0.1 μM SB 209670; (△) + 1 μM SB 209670; (○) 10 μM SB 209670; (b,d): (●) control; (▽) + 3 μM SB 209670.

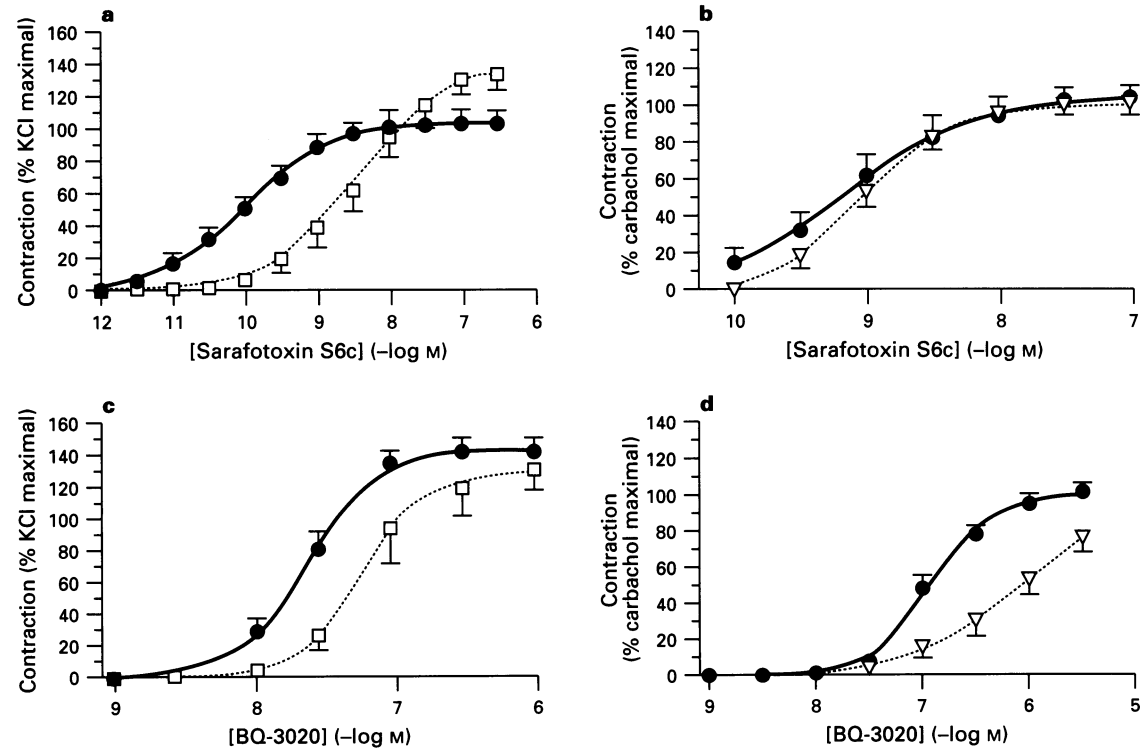

Figure 2 Effect of BQ-788 on (a,b) S6c or (c,d) BQ-3020 concentration-response curves in (a,c) rabbit pulmonary artery (endothelium-free) or (b,d) rabbit bronchus (epithelium-free). Results are expressed as a percentage of the response to a maximally-effective concentration of KCl (a,c) or carbachol (b,d) and are given as the mean ± s.e.mean of (a) 9, (b) 6 or (c,d) 4 experiments. (a,c): (●) Control; (□) + 10 μM BQ-788; (b,d): (●) control; (▽) + 3 μM BQ-788.

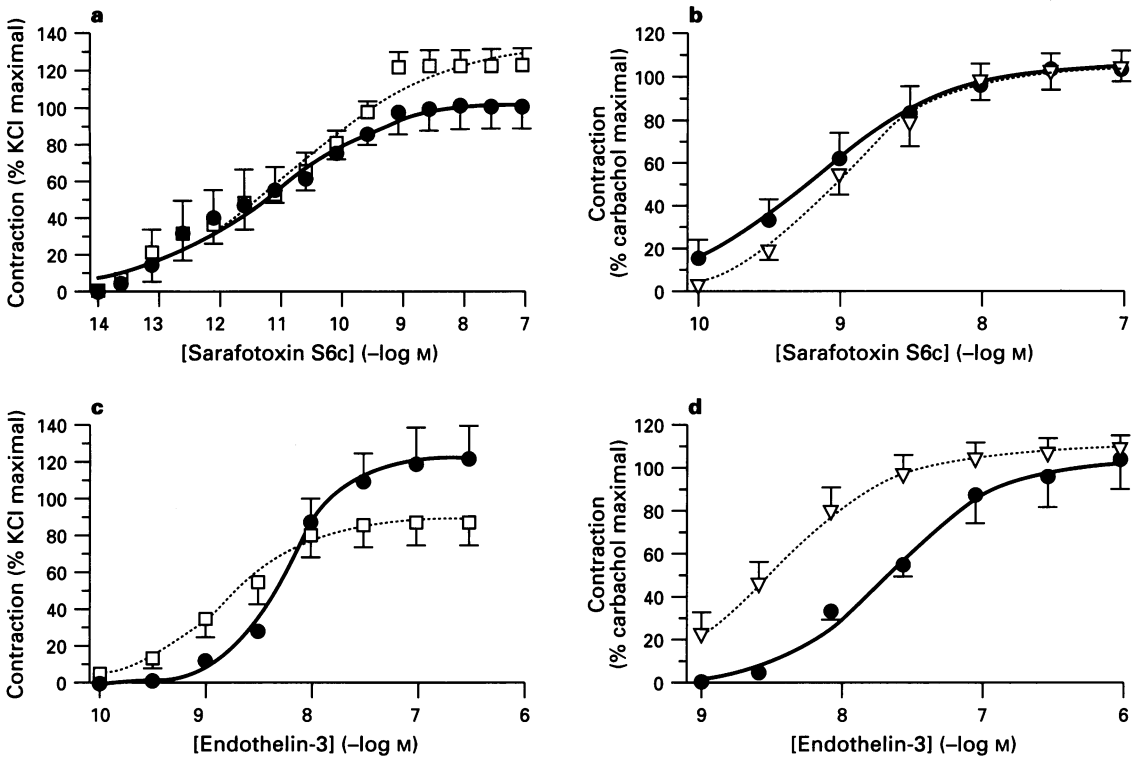

Figure 3 Effect of RES-701 on (a,b) S6c or (c,d) ET-3 concentration-response curves in (a,c) rabbit pulmonary artery (endothelium-free) or (b,d) rabbit bronchus (epithelium-free). Results are expressed as a percentage of the response to a maximally-effective concentration of KCl (a,c) or carbachol (b,d) and are given as the mean ± s.e.mean of (a) 4, (b) 6 or (c,d) 4 experiments. (a,c): (●) Control; (□) + 10 μM RES-701; (b,d): (●) control; (▽) + 3 μM RES-701.

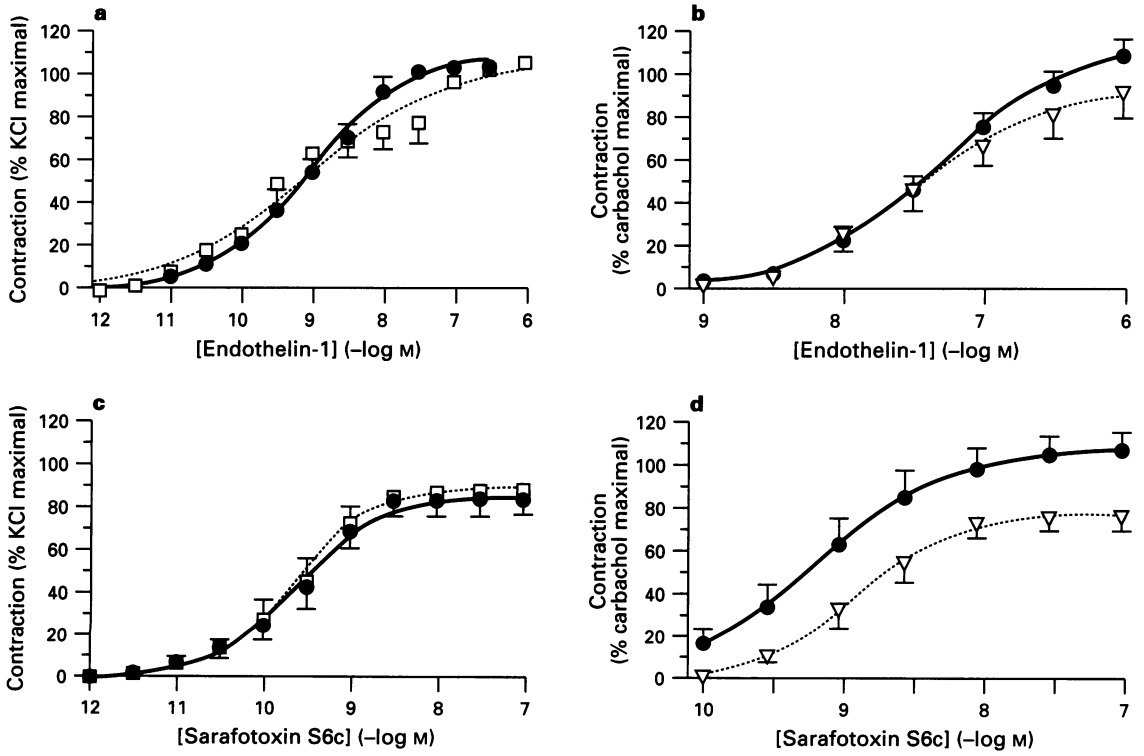

Figure 4 Effect of BQ-123 on (a,b) ET-1 or (c,d) S6c concentration-response curves in (a,c) rabbit pulmonary artery (endothelium-free) or (b,d) rabbit bronchus (epithelium-free). Results are expressed as a percentage of the response to a maximally-effective concentration of KCl (a,c) or carbachol (b,d) and are given as the mean ± s.e.mean of (a,b,c) 4 or (d) 6 experiments. (a,c): (●) Control; (□) + 10 μM BQ-123; (b,d): (●) control; (▽) + 3 μM BQ-123.

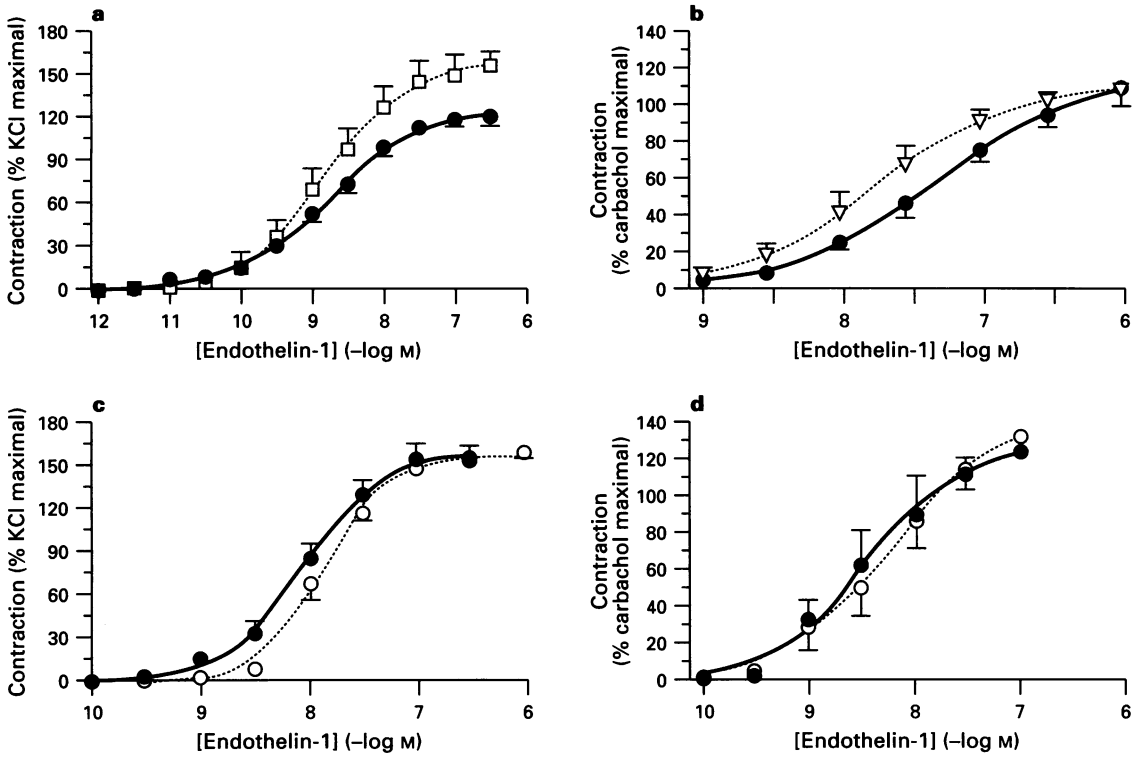

Figure 5 Effect of BQ-788 on ET-1 concentration-response curves in (a,c) rabbit pulmonary artery (endothelium-free) or (b,d) rabbit bronchus (epithelium-free) in the absence (a,b) or presence (c,d) of BQ-123. Results are expressed as a percentage of the response to a maximally-effective concentration of KCl (a,b) or carbachol (c,d) and are given as the mean±s.e.mean of (a,c) 5 or (b,d) 6 experiments. (a): (●) Control; (□)+10 μM BQ-788; (b): (●) control; (▽)+3 μM BQ-788; (c): (●) control; (○)+10 μM BQ-788 and 10 μM BQ-123; (d): (●) control; (○)+3 μM BQ-788 and 3 μM BQ-123.

Effects of antagonists against ET-1-, ET-3-, S6c- or BQ-3020-induced contractions in rabbit bronchus

In rabbit bronchus SB 209670 (3 μM) had similar potency for antagonism of contractions produced by ET-1 ($pK_B = 6.3$; $n = 6$), ET-3 ($pK_B = 6.5$; $n = 6$) or S6c ($pK_B = 6.1$; $n = 8$) (Table 2; Figure 1b,d). Further experiments revealed that SB 209670 (0.3–30 μM) produced a concentration-dependent antagonism of S6c-induced contractions (data not shown). There was no significant effect of SB 209670 (3 μM) on the maximal contraction elicited by S6c (Figure 1b); however, a higher concentration, 30 μM, significantly increased (by about 25%) the maximal contraction to S6c in rabbit bronchus: maximal contraction (% 10 μM carbachol), control = 81.3±3.7; +SB 209670 30 μM = 105.7+9.2, $n = 4$; $P < 0.05$.

BQ-788 (3 μM) was without effect on responses elicited by ET-1, ET-3 or S6c ($n = 6$; Figure 2b), but antagonized BQ-3020-induced contractions with a pK_B of 6.4 ($n = 4$) (Figure 2d) (Table 2).

RES-701 (3 μM) did not antagonize contractions produced by ET-1 ($n = 6$), S6c ($n = 6$; Figure 3b), ET-3 ($n = 6$) (Figure 3d), or BQ-3020 ($n = 4$) in rabbit bronchus (Table 2). In fact, a significant potentiation of ET-1- and ET-3-induced responses was observed, reflected by a 6.3 and 6.5 fold shift to the left in their respective concentration-response curves; there was no effect on the maximal responses induced by ET-1 (data not shown) or ET-3 (Figure 3d); PD_2s: ET-1: control = 7.6±0.1; +RES-701 = 8.7±0.5, $n = 6$, $P < 0.05$; ET-3, control = 7.8±0.1; +RES-701 = 8.7±0.2, $n = 6$, $P < 0.05$).

BQ-123 (3 μM) was without effect on ET-1-, ET-3- or S6c-induced responses in rabbit bronchus ($n = 4–6$; Figure 4d; Table 2).

Like BQ-788 alone, the combination of BQ-788 (3 μM) and BQ-123 (3 μM) was without effect on ET-1 concentration-response curves (Figure 5d); pD_2: control = 8.4±0.3; +BQ-788 and BQ-123 = 8.4±0.2 ($n = 6$; $P > 0.05$); maximal contraction (% 10 μM carbachol), control = 124.7±5.8; +BQ-788 and BQ-123 = 131.5±7.9 ($n = 6$; $P > 0.05$). Similar to the findings with RES-701 alone, the combination of RES-701 (3 μM) and BQ-123 (3 μM) potentiated ET-1-induced contractions in rabbit bronchus, reflected by a 5.2 fold shift to the left in the agonist concentration-response curve; there was no effect on the maximal response (pD_2: control = 7.7±0.1; +RES-701 and BQ-123 = 8.3±0.1; $n = 5$; $P < 0.05$; maximal response (% 10 μM carbachol): control = 115.0±5.6; +RES-701 and BQ-123 = 141.5±12.4; $n = 5$; $P > 0.05$).

Discussion

The major findings of the present study are: (1) contractions induced by ET-1, ET-3, S6c and BQ-3020 in rabbit pulmonary artery or rabbit bronchus appear to be mediated predominantly, if not exclusively, via stimulation of ET_B receptors; (2) qualitative and quantitative differences in the relative profiles of the various structurally diverse peptide and non-peptide antagonists examined suggests that responses produced by the ET ligands in the two tissues may not be mediated by a homogeneous ET_B receptor population; thus, differences may exist in the ET_B receptors mediating contraction in vascular versus airway tissues in the same species; (3) the ET_B receptors mediating contraction in rabbit pulmonary artery and rabbit bronchus are not sensitive to standard ET_B receptor antagonists, BQ-788 and RES-701; (4) the results support previous observations that the potencies of ET receptor antagonists depends upon the ET ligand studied.

Considerable research in the ET field is being directed towards the characterization of receptor subtypes, including their distribution and physiological and pathophysiological significance. It has been proposed that there are several ET receptor subtypes, with the present classification suggesting the existing of ET_A, ET_{B1}, ET_{B2} and ET_C receptors (Masaki *et al.*,

1992; Sokolovsky *et al.*, 1992; Warner *et al.*, 1993; Bax & Saxena, 1994; Douglas *et al.*, 1995) although it has been speculated that others exist (Karaki *et al.*, 1994a; Sudjarwo *et al.*, 1994; Yoneyama *et al.*, 1995). However, there remains considerable uncertainty regarding the number, location and function of the proposed ET receptors, in particular ET_B receptor subtypes. To circumvent potential problems of using tissues from different species, and to explore potential differences in ET receptor subtypes between airway versus pulmonary vascular smooth muscle, we examined the effects of peptide and non-peptide ET receptor antagonists against contractions produced by ET-1, ET-3, S6c or BQ-3020 in rabbit bronchus and rabbit pulmonary artery.

The ability of the selective ET_B agonists, S6c (Williams *et al.*, 1991), and BQ-3020 (Ihara *et al.*, 1992b) to contract rabbit bronchus and rabbit pulmonary artery and lack of effect of BQ-123, a selective ET_A receptor antagonist (Ihara *et al.*, 1992a), against contractions produced these agonists, and also ET-1 and ET-3, as well as the increased potency of S6c relative to ET-1 and ET-3, indicate that responses produced by ET ligands in these preparations are mediated predominantly, if not exclusively, via ET_B receptor activation (Fukuroda *et al.*, 1994). However, LaDouceur and co-workers demonstrated that BQ-123 antagonized ET-1-induced responses in endothelium-denuded rabbit pulmonary arteries, especially in tissues in which ET_B receptors had been desensitized by exposure to S6c (LaDouceur *et al.*, 1993). It was concluded that both ET_A and ET_B receptors contribute to ET-1-induced responses in rabbit pulmonary artery. In the present study BQ-123 either alone or in the presence of the ET_B receptor antagonists BQ-788 (Ishikawa *et al.*, 1994) or RES-701 (Tanaka *et al.*, 1994), was without significant effect on responses induced by ET-1 in rabbit pulmonary artery. In addition, another study reported that BQ-123 (1 μM) was without effect on ET-1 concentration-response curves in rabbit pulmonary artery (Fukuroda *et al.*, 1994). The differences between the results of these studies in rabbit pulmonary artery may be a consequence of the utilization of different segments of this tissue. The lack of effect of BQ-123 against ET-1-induced contraction in rabbit pulmonary artery contrasts with its ability to antagonize potently responses produced by ET-1 in human pulmonary artery (Hay *et al.*, 1993b; Fukuroda *et al.*, 1994). The results of the functional studies providing evidence that ET-1-induced responses in rabbit pulmonary artery are predominantly ET_B-receptor mediated and those in human pulmonary artery are ET_A-receptor mediated are supported by binding studies which indicated that the relative proportions of ET_A : ET_B receptors in membrane preparations of human and rabbit pulmonary artery are 93 : 7 and 23 : 77, respectively (Fukuroda *et al.*, 1994).

The qualitative and quantitative differences in the activity of the various ET receptor antagonists against contractions elicited by the ET ligands suggests that differences exist between the ET_B receptors in rabbit pulmonary artery and bronchus. For example, SB 209670 was 10 fold more potent against S6c-induced responses than those produced by ET-1 or ET-3 in pulmonary artery whereas it was equipotent against contractions induced by all three ligands in bronchus. BQ-788 was about 10 fold more potent against responses produced by S6c than those produced by ET-3 in pulmonary artery and was without significant effect on S6c-, ET-1- or ET-3-induced contractions in bronchus. In addition, BQ-788 was over 10 fold more potent against BQ-3020-induced contractions in bronchus versus pulmonary artery.

Some of the present data in rabbit bronchus are at odds with the recent report by Yoneyama and co-workers who concluded that in rabbit trachea ET ligands induce contraction via four ET receptor subtypes: BQ-123-sensitive ET_{A1}, BQ-123-insensitive ET_{A2}, RES-701-1-sensitive ET_{B1} and RES-701-1-insensitive ET_{B2} subtypes (Yoneyama *et al.*, 1995). Thus, they observed that RES-701 antagonized contractions induced by ET-3 (7 fold shift in concentration-response curve with 3 μM) or IRL 1620 (74 fold shift) but was without effect on contractions produced by S6c, which were antagonized by BQ-788 (about 26 fold shift with 3 μM). In contrast, in our study it was noted that in rabbit bronchus RES-701 did not antagonize but actually potentiated ET-3-induced contractions, and BQ-788 had no effect on S6c-induced contractions. These discrepancies may be regulated again to regional differences in the relative distribution of ET receptor subtypes, a phenomenon which has been noted previously in guinea-pig (Hay *et al.*, 1993b; Battistini *et al.*, 1994) and porcine airways (Nakamichi *et al.*, 1992).

An observation related to the profile of the antagonists was that their functional potencies were significantly less than previously reported and also less than perhaps envisaged from the results of binding experiments (Ishikawa *et al.*, 1994; Tanaka *et al.*, 1994; Ohlstein *et al.*, 1994a,b). For example, in the initial publication on BQ-788, it was reported to have a pK_B of 8.4 against BQ-3020-induced contractions in endothelium-denuded rabbit pulmonary artery (Ishikawa *et al.*, 1994). In the present study, BQ-788 possessed a pK_B of only 5.2 against responses produced by BQ-3020 in this tissue. The reason for this difference in potency of BQ-788 is not known, although we confirmed from binding studies that BQ-788 is a reasonably potent ET_B receptor antagonist, albeit about 40 fold less potent than reported by Ishikawa *et al.* (1994) (data not shown). BQ-788 potently inhibited S6c-induced contractions in rabbit saphenous vein (about 100 fold shift to the right in agonist concentration-response curve with 3 μM) and in guinea-pig ileum, at a concentration of 10 μM, abolished both phases of ET-3-induced relaxation (Karaki *et al.*, 1994a). It was concluded that BQ-788 is an antagonist at both ET_{B1} and ET_{B2} receptors. Binding studies indicate that RES-701 potently inhibits [^{125}I]-ET-1 binding in rabbit lung (IC_{50} = 20 nM) (Tanaka *et al.*, 1995). However, in rabbit bronchus (this study) or trachea (Yonemaya *et al.*, 1995) RES-701 had minor or no effect on contractions induced by ET ligands. Differences between radioligand binding and functional potencies may be related to the pseudoirreversible binding nature of the endothelins (Marsault *et al.*, 1991; Waggoner *et al.*, 1992; Ohlstein *et al.*, 1995). The slow dissociation of endothelin from its receptor may create non-equilibrium conditions which may affect the estimation of receptor dissociation constants. Therefore, the predicted potency from radioligand binding experiments may not agree with potency values obtained in functional studies. The lack of effect or limited potency that was generally observed in this study with BQ-788 and RES-701 suggests that ET_B-induced responses in rabbit pulmonary artery and bronchus are not sensitive to these prototypical ET_B receptor antagonists. Due to the weak potency of the compounds it was not possible to explore the effects of various concentrations, and thus determine pA_2 values, which would have permitted a more comprehensive understanding of the ET_B receptors in rabbit pulmonary artery and bronchus.

RES-701 was demonstrated to antagonize the initial depressor response to ET-1 in rats *in vivo*, and has been classified as an ET_B-receptor antagonist (Tanaka *et al.*, 1994; Karaki *et al.*, 1994b). In this study RES-701 did not antagonize responses produced by any of the ET ligands explored in either rabbit pulmonary artery or bronchus, and in some cases potentiated contractions; this potentiation phenomenon exhibits agonist-dependence. These data would support the proposal that RES-701 does not antagonize the contractile ET_B receptor (ET_{B2}-like?). The potentiation of the responses to some ET agonists may reflect inhibition by RES-701 of an ET receptor whose activation normally inhibits contraction; as these experiments were conducted in endothelium-free and epithelium-free preparations the location of this proposed receptor would appear to be the smooth muscle.

A notable feature of the present results was that the potencies of the antagonists SB 209670 and BQ-788 was dependent upon the ET ligand. This phenomenon has been demonstrated previously (Warner *et al.*, 1993; Kizawa *et al.*, 1994; Yoneyama *et al.*, 1995) and may be related to the presence of heterogeneous populations of ET receptors for which

different ET receptor antagonists have different affinities. Alternatively, ET-1, S6c, ET-3 and BQ-3020 may interact with different binding domains within a single population of ET_B receptors, and receptor antagonists may have differential affinities for these domains (Hiley *et al.*, 1992). The possibility also exists that a single population of ET_B receptors exists in different conformation states as has been previously proposed for the angiotensin II receptor (Robertson *et al.*, 1994). This version of Gero's two-state receptor model suggests that the ET_B receptors exists in two conformational states; an active state coupled to contraction, and an inactive state that is not coupled to contraction. If a receptor antagonist has a higher affinity for the 'inactive' form of the receptor, then the 'active' form may predominate, and at high concentrations a greater maximal contractile response may occur. It was observed that SB 209670 and BQ-788 significantly potentiated the maximal response produced by S6c in rabbit pulmonary artery. In contrast, SB 209670 (except at a high concentration of 30 μM) and BQ-788 did not potentiate contractions produced by S6c in rabbit bronchus. The mechanism underlying this phenomenon, which has been reported previously for SB 209670 and other receptor antagonists in this tissue (Ohlstein *et al.*, 1994a) is unknown although it may be related to the above two-state receptor model.

In conclusion, these data suggest that contractions induced by ET-1, ET-3, S6c or BQ-3020 in rabbit pulmonary artery or rabbit bronchus are mediated predominantly via stimulation of ET_B receptors. However, the qualitative and quantitative differences in the relative profiles of the various structurally diverse peptide and non-peptide antagonists suggest that responses produced by the ET ligands in these preparations may not be mediated by a homogeneous ET_B receptor population. Thus, within the same species there may be differences in the ET_B receptors mediating contraction in vascular versus airway tissues. It remains to be clarified how these receptors fit into the proposed ET_{B1} and ET_{B2} classification. Furthermore, the results also provide further evidence that the potencies of ET receptor antagonists may depend on the ET agonist studied.

The authors thank Dotti Lavan for secretarial assistance, Kyowa Hakko Kogyo and Warner Lambert for their gifts of RES-701 and sodium meclofenamate, respectively, and John D. Elliott, Amparo Lago and Aming Gao for synthesis of SB 209670 and BQ-788.

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(Received January 18, 1996
Revised March 7, 1996
Accepted March 8, 1996)