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Characterization of ET_B receptors mediating contractions induced by endothelin-1 or IRL 1620 in guinea-pig isolated airways: effects of BQ-123, FR139317 or PD 145065

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1 We have characterized the receptors mediating contractions to endothelin-1 (ET-1) or IRL 1620, an ET_B receptor selective agonist, in isolated strips of tissue prepared from different parts of the guinea-pig airways. We used as antagonists BQ-123 and FR139317 (ET_A receptor-selective) and PD 145065 (ET_A/ET_B receptor non-selective).

2 ET-1 and IRL 1620 (10^{-10} M to 10^{-6} M) caused similar concentration-dependent contractions of strips of guinea-pig trachea and upper bronchus. In the guinea-pig trachea without epithelium or lung parenchyma, IRL 1620 was less potent than ET-1.

3 In the trachea, contraction to ET-1 ($<10^{-8}$ M) was preceded by a transient relaxation which was inhibited by BQ-123 (10^{-5} M) or FR 139317 (10^{-5} M) or by the removal of the epithelium. The concentration-response curve to ET-1 in the trachea was shifted to the right by PD 145065 (10^{-5} M to 10^{-4} M). PD 145065 (10^{-4} M) also inhibited the response to ET-1 (3×10^{-7} M) by 55%. Contractions induced by IRL 1620 were not affected by BQ-123 (10^{-6} M) or FR139317 (10^{-6} M) but were significantly attenuated by 10^{-5} M of either antagonist. PD 145065 at 10^{-6} M strongly attenuated and at 10^{-5} M abolished contractions induced by IRL 1620.

4 In the trachea, removal of the epithelium potentiated the effects of both agonists. BQ-123 (10^{-5} M) had no effect on contractions of the trachea without epithelium induced by ET-1, but FR139317 (10^{-5} M) caused a significant inhibition. PD 145065 (10^{-5} M to 10^{-4} M) caused a shift to the right of the ET-1 concentration-response curve without affecting the contractile effect at 3×10^{-7} M. All three antagonists inhibited contractions induced by IRL 1620.

5 In the upper bronchus, BQ-123 (10^{-5} M) did not affect contractions induced by ET-1, while FR139317 (10^{-5} M) attenuated (20–26%) only contractions induced by $1-3 \times 10^{-7}$ M ET-1. PD 145065 (10^{-5} M to 10^{-4} M) caused a shift to the right of the ET-1 concentration-response curve. The contractions induced by IRL 1620 were inhibited by BQ-123 or FR139317 (10^{-5} M to 10^{-4} M). PD 145065 (10^{-6} M) strongly inhibited contractions induced by IRL 1620 and PD 145065 (10^{-5} M) totally abolished them.

6 The contractile action of ET-1 in the lung parenchyma was significantly and similarly attenuated by BQ-123 (10^{-5} M) or indomethacin (10^{-5} M), while FR139317 (10^{-5} M) was less effective. PD 145065 (10^{-6} to 10^{-5} M) inhibited contractions to ET-1. IRL 1620, which is less potent than ET-1 in this preparation, was antagonized by PD 145065 (10^{-5} to 10^{-6} M) but unaffected by BQ-123 (10^{-6} M to 10^{-5} M) or FR139317 (10^{-6} M).

7 Thus, ET_B receptors mediate contractions to ET-1 in all four guinea-pig airway preparations. In addition, contractions to ET-1 in the trachea and lung parenchyma are mediated in part by ET_A receptors. In the latter tissue, these ET_A receptors mediate contraction through the release of cyclo-oxygenase metabolites. Similarly, ET_A receptors located on the epithelial cells also mediate the release of prostanoids in the trachea with epithelium but they are responsible for transient relaxations. Interestingly, contractions induced by IRL 1620 were more susceptible to inhibition by the different antagonists, most probably because it binds to the endothelin receptors in a reversible manner. High concentrations (10^{-5} M) of ET_A-selective antagonists also inhibit responses to IRL 1620, most probably by an effect at ET_B receptors in both the trachea and the upper bronchus.

Keywords: Endothelin-1; ET_A/ET_B non-selective agonist; IRL 1620; ET_B selective agonist; BQ-123; FR139317; ET_A-selective receptor antagonist; PD 145065; ET_A/ET_B non-selective receptor antagonist; endothelin receptor subtypes; ET_A receptors; ET_B receptors

Introduction

Endothelin-1 (ET-1) is a 21 amino acid peptide, first isolated from the culture medium of aortic endothelial cells (Yanagisawa *et al.*, 1988). ET-1, the related peptides (ET-2 and ET-3) and the sarafotoxins (SX6a, SX6b, SX6c, SX6d) cause vasoconstriction, vasodilatation, mitogenesis and various endocrine effects (see Rubanyi, 1992). These effects are mediated by at least two distinct endothelin receptors. The ET_A receptor is ET-1-selective (Arai *et al.*, 1990) whereas the ET_B receptor is equisensitive to all ET isopeptides (Sakurai *et al.*, 1990). ET_A and ET_B receptors have both been shown to mediate contractions of various vascular preparations induced by ET/SX peptides (Williams *et al.*, 1991; Bigaud & Pelton, 1992; Cristol *et al.*, 1993).

The endothelins are also potent constrictors of airways *in vitro* and *in vivo* (see Battistini *et al.*, 1992; Hay *et al.*, 1993). In guinea-pigs, these effects appear to be mediated by non-ET_A, presumably ET_B receptors. For instance, in anaesthetized guinea-pigs, ET-1, ET-2 or ET-3, given intravenously, cause similar bronchoconstrictions (Pons *et al.*, 1991) and in

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guinea-pig isolated airways, all three isopeptides are equipotent (Maggi et al., 1989a; Cardell et al., 1993). Similarly, SX6c and IRL 1620, two ET_B-selective agonists (Williams et al., 1991; Takai et al., 1992; Watakabe et al., 1992), cause comparable contractions of guinea-pig bronchi (SX6c; Hay, 1992) and trachea (IRL 1620; Takai et al., 1992). These data suggest mediation by predominantly ET_B receptors. However, others have suggested that ET-1 is more potent than ET-3 in the guinea-pig trachea (Ninomiya et al., 1992) and in the same preparation that SX6c is a much less effective agonist than ET-1 (Hay & Luttman, 1992). Thus, it appears that both ET_A and ET_B receptors may mediate these contractions. Data from experiments employing endothelin receptor antagonists are similarly contradictory. BQ-123 (Ihara et al., 1992b) or FR 139317 (Sogabe et al., 1993), two selective ET_A receptor antagonists, do not affect contractions induced by ET-1 in the isolated bronchus (BQ-123; Hay, 1992) or trachea (FR 139317; Cardell et al., 1993) or bronchoconstriction induced by ET-1 in anaesethetized guinea-pigs (BQ-123; Noguchi et al., 1993), suggesting mediation by ET_B receptors. Conversely, BQ-123 partially inhibits the contractions induced by ET-1 in the guinea-pig trachea (Hay, 1992), suggesting an involvement of ET_A receptors. Similarly, BQ-123 also inhibits 40% of the contraction induced by ET-1 in the rat trachea (Henry, 1993). These conflicting reports are indicative of the fact that the receptors mediating the effects of the ET/SX peptides in the guinea-pig airways have not been systematically characterized. Here, using as agonists ET-1 and IRL 1620 and as antagonists, BQ-123 or FR139317 (ET_A receptor selective) or PD 145065 (ET_A/ET_B receptor non-selective; Cody et al., 1993), we have characterized further the receptors mediating the contractile effects of the endothelins in the guinea-pig trachea (with or without epithelium), upper bronchus and lung parenchyma.

Methods

Tissue preparation

Male Hartley guinea-pigs weighing 350–450 g were killed by cervical dislocation and rapidly bled. The trachea, upper bronchus and lung parenchyma were excised, placed in Krebs-Henseleit buffer (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.18, $NaHCO_3$ 25, $MgSO_4.7H_2O$ 1.18 and glucose 5.6, pH 7.4) and trimmed of connective tissue and fat. The trachea and upper bronchus were cut into spirals. Some tracheal preparations were denuded of their epithelium by gentle rubbing with a wet cotton swab. Strips of peripheral parenchyma were prepared from the anterior surface of the lower lobe. The tissues were mounted in 10 ml organ baths and connected to auxotonic force-displacement transducers (Harvard Bioscience Apparatus) under tensions of 1.5 g. Tissues were allowed to stabilize for 1 h in warmed (37°C) and gassed (95% O_2:5% CO_2) Krebs-Henseleit solution with washing every 15 min. Changes in length were recorded continuously on a Linearcorder (Graphtec, model WR 3300, Japan).

Experimental protocol

Following an equilibration period (60 min) tissues were contracted three times with histamine (5×10^{-6} M) to induce 80% of the maximal contraction, with a 30 min washout period between contractions. Tissues were then incubated (20–30 min) with or without indomethacin (10^{-5} M), BQ-123 (10^{-6}, 10^{-5} M), FR139317 (10^{-6}, 10^{-5} M), PD 145065 (10^{-6}, 10^{-5}, 10^{-4} M) or vehicle before addition of cumulative concentrations of ET-1 (10^{-9} to 3×10^{-7} M) or IRL 1620 (10^{-10} to 10^{-6} M). Each preparation was used for only one concentration-response curve for ET-1 or IRL 1620. Responses are expressed as a percentage of the contraction evoked by 5×10^{-6} M histamine.

Drugs

Endothelin-1 (ET-1) and IRL 1620 (Suc-[Glu^9, $Ala^{11,15}$] ET-1 (8–21)) were synthesized at the INRS-Santé (Montreal, P.Q., Canada). IRL 1620 was also obtained as a gift from the International Research Laboratories, CIBA-GEIGY (Takarazuka, Japan). ET-1 and IRL 1620 were dissolved in distilled water and stored in aliquots of 5×10^{-5} M at − 20°C. Indomethacin (Sigma Chemicals Co., Poole, Dorset) was freshly dissolved each day in 5% w/v $NaHCO_3$. BQ-123 (cyclo (-D-Trp-D-Asp-L-Pro-D-Val-L-Leu-)), FR139317 ((R)2-[(R)-2-[(S)-2-[[1-(hexahydro-1H-azepinyl)] carbonyl] amino-4-methylpentanoyl] amino-3-[3-(1-methyl-1H-indoyl)] proprionyl] amino-3-(2-pyridyl) proprionic acid) and PD 145065 (Ac-D-Bhg-L-Leu-L-Asp-L-Ile-L-Ile-L-Trp; Bhg = 5H-dibenzyl [a,d]cycloheptene-10,11-dihydroglycine) were dissolved in distilled water and stored in aliquots of 10^{-3} M at − 20°C. All drugs were further diluted with Krebs-Henseleit solution when necessary. Histamine dihydrochloride was obtained from Sigma Chemical Co. (Poole, Dorset).

Statistical analysis

All values are expressed as mean ± s.e.mean. Statistical analysis of the data was performed by an unpaired, two-tailed, Mann-Whitney U test and $P < 0.05$ was considered significant.

Results

Endothelin-1 verus IRL 1620

Endothelin-1 or IRL 1620 caused strong concentration-dependent contractions of the four guinea-pig airway preparations (Table 1; Figure 1a-b). In the trachea with epithelium, ET-1 or IRL 1620 induced contractions with similar potencies, and produced similar responses at 3×10^{-7} M (Table 1; Figure 1a-b). At concentrations up to 10^{-8} M, ET-1, but not IRL 1620, induced transient relaxations prior to the contractile response. In the trachea without epithelium, the sensitivities to ET-1 and IRL 1620 were increased by 16 and 5 fold, respectively as compared to trachea whereas the contractions induced by 3×10^{-7} M of either peptide were not significantly affected (Table 1; Figure 1a-b). In the guinea-pig upper bronchus, ET-1 and IRL 1620 were equipotent (Table 1; Figure 1a-b). In the lung parenchyma, ET-1 was more potent than IRL 1620 (Table 1; Figure 1a-b).

Effect of antagonists on responses of the guinea-pig trachea to ET-1

In the trachea, BQ-123 (10^{-5} M) or FR139317 (10^{-5} M) completely inhibited the transient relaxations induced by ET-1. Contractions at 3×10^{-7} M were not affected by either antagonist although the sensitivities to ET-1 were slightly increased (Table 1; Figure 2a). PD 145065 (10^{-5} M and 10^{-4} M) also abolished the transient relaxations and shifted the concentration-response curve to ET-1 to the right by 1.5 and 5 fold, respectively. PD 145065 (10^{-5} M and 10^{-4} M) also significantly attenuated (− 35% and − 55%, respectively) contractions induced by 3×10^{-7} M ET-1 (Table 1; Figure 2b).

In the trachea with epithelium, PD 145065 antagonized contractions to ET-1 causing a shift to the right in the concentration-response curve of 7 fold (10^{-5} M) and 60 fold (10^{-4} M) (Table 1; Figure 2d) without causing any reduction in the responses at 3×10^{-7} M. BQ-123 (10^{-5} M) had no effect, whereas FR139317 (10^{-5} M) caused a significant inhibition (31%) of the contraction induced by 3×10^{-7} M ET-1, and shifted the ET-1 concentration-response curve to the right by 8 fold (Figure 2c).

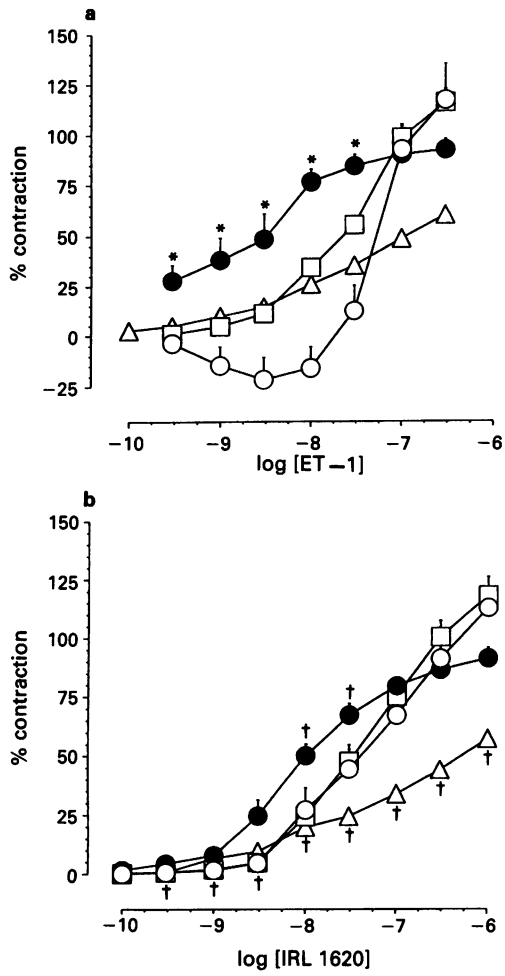

Figure 1 Effect of endothelin-1 (ET-1) (a) and IRL 1620 (b) on guinea-pig isolated trachea (○), trachea without epithelium (●), upper bronchus (□) and lung parenchyma (△); Results are expressed as a percentage of the response to histamine (5×10^{-6} M), and are means of 5–19 observations with s.e.mean. $*P<0.05$ between tracheal strips ± epithelium. $\dagger P<0.05$ between ET-1 and IRL 1620 (compared by an unpaired, two-tailed, Mann-Whitney U test).

Effect of antagonists on responses of the guinea-pig upper bronchus to ET-1

In the guinea-pig upper bronchus, PD 145065 (10^{-5} M) shifted the ET-1 concentration-response curve to the right by 2 fold while 10^{-4} M PD 145065 caused a further parallel shift of 32 fold (Figure 2f). The response to 3×10^{-7} M ET-1 in the presence of 10^{-4} M PD 145065 was also attenuated by 74% (Table 1; Figure 2f). In contrast, BQ-123 (10^{-5} M) or FR139317 (10^{-5} M) did not affect the ET-1 induced concentration-response curves to ET-1 (Figure 2e). The contraction to higher concentrations of ET-1 (10^{-7} to 3×10^{-7} M) was slightly but significantly attenuated (− 20%) by FR139317 (10^{-5} M; Table 1; Figure 2e).

Effect of antagonists on responses of the guinea-pig lung parenchyma to ET-1

In the guinea-pig lung parenchyma, BQ-123 (10^{-5} M) shifted the concentration-curve for ET-1 by 5 fold to the right (Table 1; Figure 2g) as did indomethacin (10^{-5} M; $n=9$; EC_{50}: 54.9 ± 14.0 nM (5 fold to the right); response at 3×10^{-7} M: 49.2 ± 3.2%; Figure 2g). Combination of BQ-123 (10^{-5} M) and indomethacin (10^{-5} M) did not further antagonize contractions induced by ET-1 ($n=4$; data not shown). FR139317 (10^{-5} M) produced a smaller inhibition (2 fold), especially at higher concentrations of ET-1 ($>3 \times 10^{-8}$ M). PD 145065 (10^{-6} M and 10^{-5} M) caused shifts of 3 and >50 fold in the concentration-response curve to ET-1. PD 145065 (10^{-6} M) did not affect the contractions induced by 3×10^{-7} M ET-1, while PD 145065 (10^{-5} M) caused a 62% inhibition (Table 1; Figure 2h). Combination of both indomethacin (10^{-5} M) and PD 145065 (10^{-5} M), completely abolished contractions induced by ET-1 ($n=4$; data not shown).

Effect of antagonists on responses of the guinea-pig trachea to IRL 1620

IRL 1620 caused only contractions of the trachea which were significantly inhibited by BQ-123 (10^{-5} M) or FR139317 (10^{-5} M) (Table 1; Figure 3a). For instance, the two antagonists attenuated contractions to 3×10^{-7} M IRL 1620 by 48% and 59%, respectively. PD 145065 (10^{-6} M) and (10^{-5} M) shifted the concentration-response curve for IRL 1620 to the right by 7 and >22 fold, respectively, and reduced the response to 3×10^{-7} M IRL 1620 by 52% and 79%, respectively (Table 1; Figure 3b).

In the trachea without epithelium, the concentration-response curve to IRL 1620, which was potentiated by the removal of the epithelium, was shifted to the right by more than 100 fold by PD 145065 (10^{-6} and 10^{-5} M) (Table 1; Figure 3d). The responses to 3×10^{-7} M IRL 1620 were attenuated by 73% and 77%, respectively, by the same concentrations of PD 145065 (Table 1; Figure 3d). BQ-123 (10^{-6} M) or FR139317 (10^{-6} M) had no effect. Either antagonist at 10^{-5} M caused a significant inhibition with FR139317 being more effective (Table 1; Figure 3c).

Effect of antagonists on responses of the guinea-pig upper bronchus to IRL 1620

In the upper bronchus, PD 145065 (10^{-6} M) shifted the concentration-response curve to IRL 1620 24 fold to the right, while PD 145065 (10^{-5} M) totally abolished contractions induced by IRL 1620 (Figure 3f). BQ-123 (10^{-6} M) or FR139317 (10^{-6} M) also significantly inhibited contractions to IRL 1620, causing shifts to the right of 7 and 8 fold in the concentration-response curve (Table 1; Figure 3e). Higher concentrations (10^{-5} M) of either antagonist further inhibited the responses to IRL 1620 causing 9 fold (BQ-123) and 25 fold (FR139317) shifts in the concentration-response curve (Table 1; Figure 3e).

Effects of antagonists on responses of the guinea-pig lung parenchyma to IRL 1620

IRL 1620 was significantly less potent than ET-1 in contracting the lung parenchyma. PD 145065 (10^{-6} M) attenuated IRL 1620-induced contractions, causing a 16 fold shift to the right in the concentration-response curve and an inhibition of 54%. PD 145065 (10^{-5} M) totally abolished contractions induced by IRL 1620 (Table 1; Figure 3h). BQ-123 (10^{-6} M to 10^{-5} M) or FR139317 (10^{-6} M) did not affect responses to concentrations of IRL 1620 greater than 10^{-8} M (Table 1; Figure 3g). For instance, FR139317 (10^{-5} M) caused a 7 fold shift to the right of the IRL 1620 concentration-response curve, but no inhibition of the response at 3×10^{-7} M (Table 1; Figure 3g).

BQ-123, FR139317 or PD 145065 (up to 10^{-4} M) had no effect on the basal tension of the tissues or on the responses of any of the four preparations to histamine.

Discussion

The present study demonstrates that contractile responses to ET-1 and IRL 1620 in guinea-pig isolated airways are mediated almost exclusively by ET_B receptors. PD 145065, an ET_A/

Table I Effects of PD 145065, BQ-123 or FR139317 on the contractions at 3×10^{-7} M and apparent EC_{50} values for ET-1 and IRL 1620 in the guinea-pig trachea (± epithelium), upper bronchus and lung parenchyma

	Control			+ PD145065 (10^{-6} M)			+ PD145065 (10^{-5} M)			+ PD145065 (10^{-4} M)		
	(n)	% C_{eff}	EC_{50}	(n)	% C_{eff}	EC_{50}	(n)	% C_{eff}	EC_{50}	(n)	% C_{eff}	EC_{50}
Trachea												
Endothelin-1	(9)	117 ± 18	45 ± 5		ND	ND	(6)	76 ± 16	64 ± 11	(3)	53 ± 12*	238 ± 115
IRL 1620	(5)	92 ± 8	45 ± 18	(3)	44 ± 14	316 ± 102	(4)	19 ± 3*	> 1000		ND	ND
Trachea without epithelium												
Endothelin-1	(7)	93 ± 5	3 ± 3		ND	ND	(8)	117 ± 12	19 ± 6	(4)	87 ± 28	166 ± 78
IRL 1620	(6)	87 ± 4	9 ± 2†	(6)	24 ± 4*	> 1000	(8)	20 ± 3*	> 1000		ND	ND
Upper bronchus												
Endothelin-1	(19)	116 ± 7	32 ± 3		ND	ND	(4)	104 ± 20	77 ± 22	(3)	30 ± 13*	> 1000
IRL 1620	(12)	101 ± 7	35 ± 4	(11)	18 ± 4*	817 ± 77	(6)	0.6 ± 0.3*	> 1000		ND	ND
Lung parenchyma												
Endothelin-1	(19)	60 ± 4	11 ± 2	(4)	61 ± 2	37 ± 11	(7)	23 ± 6	567 ± 145		ND	ND
IRL 1620	(8)	44 ± 3†	26 ± 7†	(8)	21 ± 4*	399 ± 14	(10)	1.8 ± 0.7*	> 1000		ND	ND

	+ BQ123 (10^{-6} M			+ BQ123 (10^{-5} M)			+ FR139317 (10^{-6} M)			+ FR139317 (10^{-5} M)		
	(n)	% C_{eff}	EC_{50}	(n)	% C_{eff}	EC_{50}	(n)	% C_{eff}	EC_{50}	(n)	% C_{eff}	EC_{50}
Trachea												
Endothelin-1		ND	ND	(4)	88 ± 10	18 ± 7		ND	ND	(8)	102 ± 15	13 ± 6
IRL 1620	(5)	108 ± 26	34 ± 9	(5)	47 ± 8*	435 ± 146	(5)	95 ± 17	92 ± 22	(4)	37 ± 10*	710 ± 191
Trachea without epithelium												
Endothelin-1		ND	ND	(4)	98 ± 22	6 ± 4		ND	ND	(4)	64 ± 6*	21 ± 12
IRL 1620	(4)	110 ± 16	9 ± 3	(5)	73 ± 10	93 ± 30	(4)	95 ± 16	8 ± 2	(4)	57 ± 4*	147 ± 59
Upper bronchus												
Endothelin-1		ND	ND	(11)	105 5	17 ± 8		ND	ND	(8)	93 ± 9	34 ± 15
IRL 1620	(5)	66 ± 9*	231 ± 109	(4)	56 ± 14*	316 ± 125	(4)	62 ± 10*	288 ± 148	(4)	29 ± 2*	855 ± 117
Lung parenchyma												
Endothelin-1		ND	ND	(5)	42 ± 6	54 ± 10		ND	ND	(13)	51 ± 2	26 ± 7
IRL 1620	(4)	56 ± 2	14 ± 3	(7)	51 ± 3	39 ± 6	(6)	55 ± 5	36 ± 11	(7)	40 ± 7	172 ± 69

Contractile effects (% C_{eff}) at 3×10^{-7} M of agonist are expressed as the percentage of the contraction to 5×10^{-6} M histamine. Effective concentrations (EC_{50}) values are the concentration ($\times 10^{-9}$ M) eliciting half of the response to 3×10^{-7} M of agonists in the paired control values. Values are mean ± s.e.mean. *Shows significance ($P < 0.05$) for the effects of antagonists and †shows significant difference ($P < 0.05$) between ET-1 and IRL 1630. ND: not determined.

ET_B non-selective endothelin receptor antagonist, shifted all the concentration-response curves in a concentration-related manner, whereas BQ-123 or FR139317 had little if any effect. This amplifies and agrees with data from experiments using both bronchial (Hay, 1992) and tracheal (Cardell *et al.*, 1993) preparations *in vitro*, and from studies examining the responses of guinea-pig airways *in vivo* (Noguchi *et al.*, 1993).

In our study, all three antagonists (at 10^{-5} M) abolished the transient relaxations of the trachea induced by low concentrations ($< 10^{-8}$ M) of ET-1. At the same time, our results show that BQ-123 or FR139317 (10^{-5} M) slightly potentiated contractions to low concentrations of ET-1 ($\leqslant 3 \times 10^{-7}$ M) without affecting those to higher concentrations of ET-1. These transient relaxations (as reported by Turner *et al.*, 1989; White *et al.*, 1991) are mediated via ET_A receptors, and counteract the direct constrictor effect of the endothelins. The receptors mediating this effect are most probably situated on the epithelial cells, for transient relaxations were not seen in preparations without epithelium. Furthermore, removal of the epithelium of the trachea potentiated the contractile effects of ET-1. The relaxation may well be secondary to an increase in production of prostaglandin E_2 (PGE_2), the predominant airway product of arachidonic acid in epithelial cells, which relaxes airway smooth muscle (Goodwin, 1989) and is released from feline tracheal epithelial cells by ET-1 (Wu *et al.*, 1993).

In intact tracheal strips, IRL 1620 induced similar contractions to ET-1 suggesting ET_B receptors mediate this response. Contractions induced by IRL 1620 were strongly inhibited or abolished by PD 145065. Similarly, the higher concentration of PD 145065 (10^{-5} M) slightly reduced the contractions to ET-1 at $\geqslant 10^{-7}$ M. The greater effectiveness of PD 145065 against IRL 1620 is not explained by the lower binding affinity of this selective agonist compared to ET-1, for competitive binding assays revealed almost identical displacement curves (Watakabe *et al.*, 1992). However, IRL 1620 binds to endothelin receptors in a reversible manner, whereas ET-1 only dissociates very slowly from the binding sites (Watakabe *et al.*, 1992). This different sensitivity of agonists to antagonists would agree with our previous observations that PD 142893, PD 145065 and Ro 46-2005 (Clozel *et al.*, 1993), which are all ET_A/ET_B non-selective receptor antagonists, more actively antagonize responses to SX6c or IRL 1620 than ET-1 at ET_B receptors (Warner *et al.*, 1993a,b; unpublished observations). This is of great interest for it suggests that the relative effectiveness of endothelin receptor antagonists varies with the agonist used. It also implies that great care should be taken in assessing the potency of endothelin receptor antagonists by competition experiments employing synthetic ligands.

Neither FR139317 (10^{-6} M) nor BQ-123 (10^{-6} M) had any significant effect on the contractions induced by IRL 1620. However, a concentration of 10^{-5} M of either ET_A-antagonist caused a significant inhibition of contractions induced by IRL 1620, shifting the IRL 1620 concentration-response curve to a similar extent to PD 145065 (10^{-6} M). Similarly, high concentrations of BQ-123 inhibit ET-1-induced bronchoconstriction *in vivo* (Noguchi *et al.*, 1993). Possibly this is because at these high concentrations (10^{-5} M), BQ-123 or FR139317 can displace ET-1 binding to ET_B receptors. Indeed, BQ-123 (IC_{50}: 18 μM) or FR139317 (IC_{50}: 4.6 μM) will displace the binding of ET-1 to ET_B receptors in brain tissue

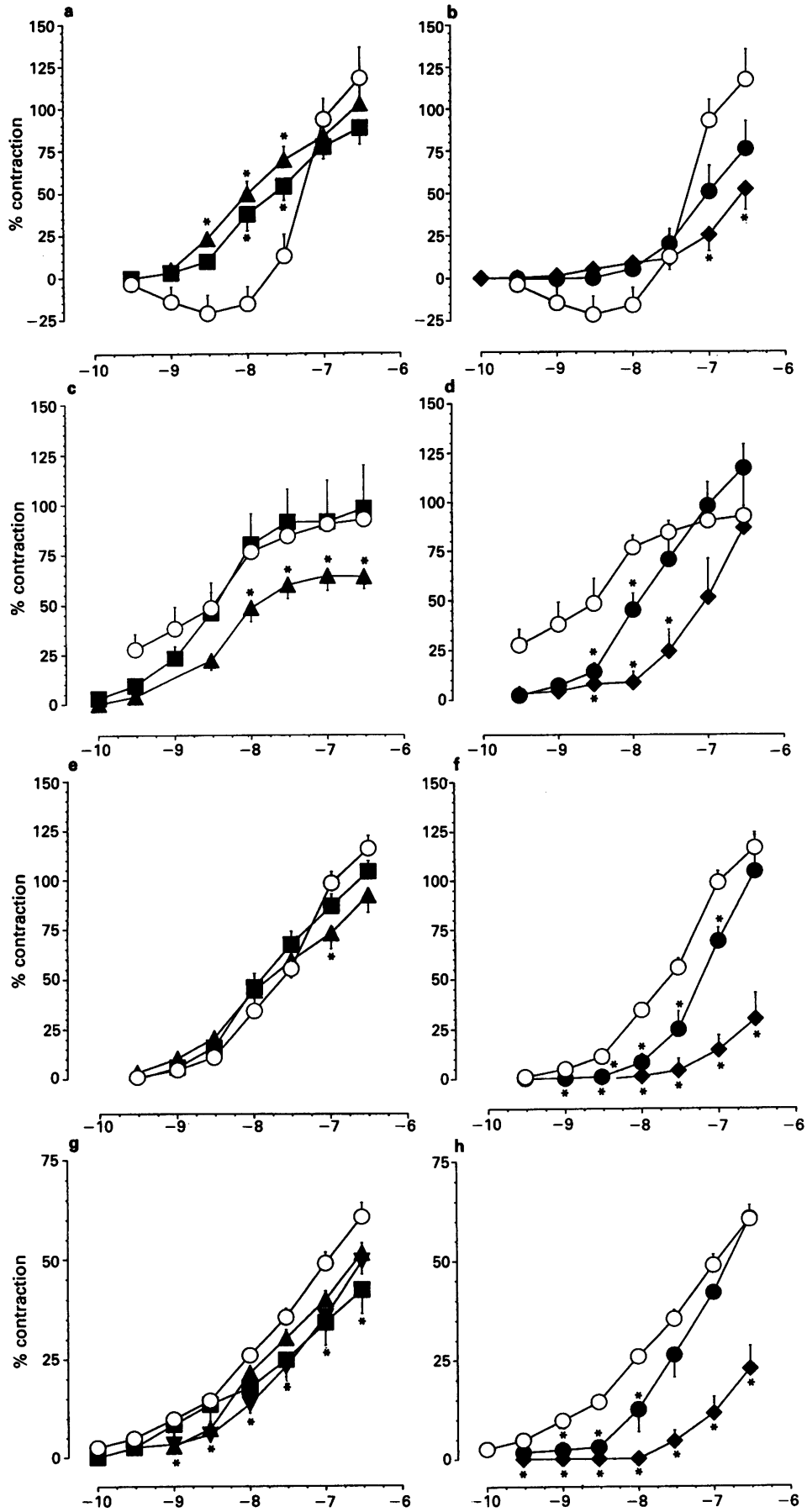

Figure 2 Effects of BQ-123 (10^{-5} M; ■), FR139317 (10^{-5} M, ▲) or PD 145065 (10^{-5} M, ● and 10^{-4} M, ◆) on contractions of guinea-pig isolated trachea (a-b), trachea without epithelium (c-d), upper bronchus (e-f) and lung parenchyma (g-h) induced by endothelin-1 (ET-1) (○). The concentrations of PD 145065 used in the guinea-pig lung parenchyma were 10 times less (10^{-6} M, ● and 10^{-5} M, ◆) than those used in other airway preparations. The effect of indomethacin (10^{-5} M, ▼) is also shown on contractions of lung parenchyma induced by ET-1 (g). Results are expressed as a percentage of the response to histamine (5×10^{-6} M), and are mean of 3–19 observations with s.e.mean. $^*P<0.05$ (compared to ET-1 by a Mann-Whitney U test).

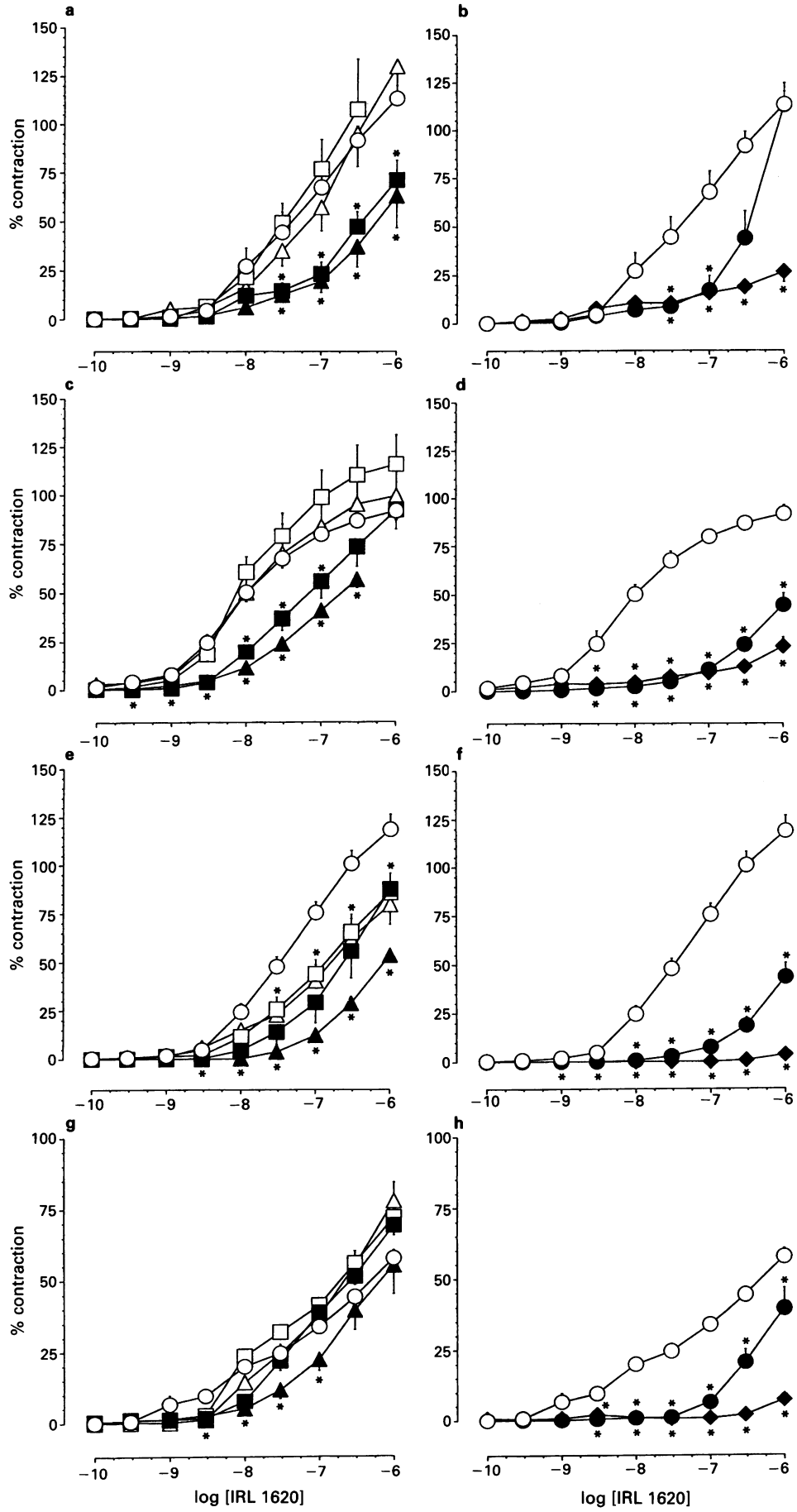

Figure 3 Effects of BQ-123 (10^{-6} M, □, 10^{-5} M ■), FR139317 (10^{-6} M △, 10^{-5} M ▲) or PD 145065 (10^{-6} M ●, 10^{-5} M ◆) on contractions of guinea-pig isolated trachea (a-b), trachea without epithelium (c-d), upper bronchus (e-f) and lung parenchyma (g-h) induced by IRL1620 (○). Results are expressed as a percentage of the response to histamine (5×10^{-6} M) and are mean of 3–12 observations with s.e.mean. $^*P < 0.05$ (compared to ET-1 by a Mann-Whitney U test).

(Ihara *et al.*, 1992; Sogabe *et al.*, 1993; Aramori *et al.*, 1993).

Removal of the epithelium in the trachea potentiated the contractile effects of both ET-1 (as shown by Maggi *et al.*, 1989b; Hay, 1989; 1990) and IRL 1620 (this study), as has been shown for other bronchoconstrictor agents such as histamine, acetylcholine, 5-hydroxytryptamine and leukotriene C_4 and D_4 (Barnes *et al.*, 1985; Holroyde, 1986; Goldie *et al.*, 1986; Hay *et al.*, 1987). Interestingly, while ET-1 and IRL 1620 were equipotent in contracting the intact trachea, ET-1 was three times more potent than IRL 1620 in contracting tracheal preparations without epithelium. This may be explained by the additional loss of the ET-1-induced relaxant response, which is epithelium-dependent (see above). In the trachea without epithelium, BQ-123 (10^{-5} M) had no effect on contractions induced by ET-1 while FR139317 (10^{-5} M) significantly inhibited them. This suggests that the trachea contains a mixed population of endothelin receptors. This would agree with previous reports comparing both agonist potencies and the effects of the endothelin receptor antagonists (Maggi *et al.*, 1989b; Ninomiya *et al.*, 1992; Hay & Luttman, 1992; Henry, 1993).

In the upper bronchus, ET-1 and IRL 1620 produced superimposable concentration-response curves suggesting mediation by a homogeneous ET_B receptor population. BQ-123 or FR139317 were ineffective against ET-1 but PD 145065 antagonized the effects of ET-1 or IRL 1620. As before, high concentrations of BQ-123 or FR139317 inhibited contractions induced by IRL 1620.

The present study supports the suggestion (Filep *et al.*, 1991) that the contractions induced by ET-1 in the lung parenchyma are partly mediated through the release of eicosanoids, particularly thromboxane A_2. BQ-123 may block the release of TxA_2 for it significantly inhibited part of the response curve induced by ET-1 and this inhibition was not additive to that of indomethacin. This agrees with experiments using rat perfused lungs where BQ-123 blocks the release of prostacyclin and/or thromboxane A_2 induced by ET-1 (D'Orleans-Juste *et al.*, 1992). It also provides a role for ET_A receptors, which account for 15% of ET-1 binding in the guinea-pig lung (Ihara *et al.*, 1992a).

Thus, our data indicate that ET-1 activates ET_A receptors on tracheal airway epithelial cells and cells in the lung parenchyma of the guinea-pig to induce the release of prostanoids. In the trachea, this causes a transient relaxation and in the lung parenchyma, contraction through the release of thromboxane A_2 (TxA_2). More importantly $\geqslant 85\%$ of the contractions induced by ET-1 in guinea-pig airways are mediated via ET_B receptors most probably present on the airway smooth muscle. As ET-1 may be an endogenous regulator of airway tone and is secreted from tracheal and bronchial epithelial cells (Black *et al.*, 1989; Mattoli *et al.*, 1990), endothelins may play an active role in diseases such as asthma, bronchitis and adult respiratory distress syndrome. These airway diseases are associated with vascular remodelling, reduction of airway calibre, increased microvascular permeability, ciliary function, ion transport and mucin release which are all affected by the endothelins (Wu *et al.*, 1990; Tamaoki *et al.*, 1991; Plews *et al.*, 1991; Peacock *et al.*, 1992; Helset *et al.*, 1993). The ability of PD 145065 to antagonize ET-1 in airway tissues therefore suggests it may be beneficial in disease states.

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