Actions of endothelins and sarafotoxin 6c in the rat isolated perfused lung

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- 1 Endothelin (ET) receptors within the vasculature and airways were studied in a rat perfused lung model in which pulmonary perfusion pressure (PPP), pulmonary inflation pressure (PIP) and lung weight were continuously monitored.
- 2 The vascular potencies of ETs (ET-1 > ET-2 > ET-3) suggest an action via ET_A receptors. This was confirmed by use of the antagonist, BQ123 (2 µM). The vasoconstrictor effects of sarafotoxin 6c (SX6C) also indicated the presence of ET_B receptors.
- 3 Lung weight increases induced by ETs appeared to be a consequence of their vasoconstrictor potencies. The mixed ET receptor antagonist, bosentan (5 µM), markedly attenuated the responses of ET-1 and SX6C on PPP and lung weight, further implicating activation of both ET_A and ET_B receptors in these responses.
- 4 Endothelin-1 (ET-1) induced an accumulation of albumin-bound Evans blue dye in orthogradely perfused lungs. Retrograde perfusion attenuated the extravasation and increase in lung weight due to ET-1 but significantly augmented those induced by SX6C.
- 5 The bronchoconstrictor actions of ETs (ET-1 = ET-2 = ET-3) and SX6C suggest this is an ET_B-mediated response. However SX6C was more potent than ETs and the dose-response curve was significantly steeper and achieved a higher maximum.
- 6 Indomethacin did not affect the vascular or bronchial responses to ET-1 or SX6C.
- 7 These findings indicate that rat pulmonary vasculature contains both ET_A and ET_B receptors. Retrograde perfusion suggests that ET_B receptors are located arterially whereas ET_A receptors are predominantly venous in distribution. Differences in the bronchoconstrictor potency of SX6C (compared to ETs) and the antagonism by bosentan may indicate ET_B receptor heterogeneity in the airways.

Keywords: Endothelins; sarafotoxin 6c; BQ123; bosentan; rat lung; pulmonary vasoconstriction; bronchoconstriction; lung weight

Introduction

Endothelin (ET) was discovered as a potent vasoactive peptide produced by cultured endothelial cells (Yanagisawa *et al.*, 1988). It is now apparent that there are four forms of endothelin [endothelin-1 (ET-1), ET-2 and ET-3] (Inoue *et al.*, 1989) including vasoactive intestinal contractor (VIC) (Saida *et al.*, 1989). Sarafotoxins (SX6A, -B, -C and -D) also possess structural features and pharmacological activities similar to those of ETs (Kloog *et al.*, 1988). Based on differential affinities for ETs and related peptides, four types of ET receptor have also been described namely ET_A, ET_B, ET_C and ET_{AX}. The ET_A receptor is characterized by the rank order of binding affinities ET-1 ≥ ET-2 > ET-3 (Arai *et al.*, 1990), while the ET_B receptor is non-isopeptide-selective (ET-1 = ET-2 = ET-3) (Sakurai *et al.*, 1990) and at the ET_C receptor ET-3 is more potent than ET-1 (Karne *et al.*, 1993). The ET_{AX} receptor cloned recently shares 74%, 60% and 51% identities with human ET_A, human ET_B and *Xenopus* ET_C receptors (Kumar *et al.*, 1994). SX6B has a higher affinity for ET_A receptors whereas SX6C selectively binds to ET_B receptors (Williams *et al.*, 1991).

The actions of ETs in the lung have attracted considerable interest. ETs are potent constrictors of pulmonary blood vessels (McKay *et al.*, 1991; White *et al.*, 1993; Cardell *et al.*, 1993; MacLean *et al.*, 1994). In a recent report it has been shown that the constrictor action of ETs in segments of rat large pulmonary artery are mediated by ET_A receptors whereas constriction in the small pulmonary arteries is mediated by the activation of ET_B receptors (MacLean *et al.*, 1994). ETs also constrict tracheal rings (Black *et al.*, 1989; Cardell *et al.*, 1993;

Battistini *et al.*, 1994) and lung parenchymal strips (Battistini *et al.*, 1994), an action primarily mediated via ET_B receptors (Battistini *et al.*, 1994).

In the present study we have utilised an isolated perfused lung model (Lal *et al.*, 1994a) to investigate the effects of ETs and SX6C with a view to identifying the subtypes of ET receptor present. As an adjunct we have also studied the effects of an ET_A receptor antagonist, BQ123 (Ihara *et al.*, 1992) and bosentan, the mixed ET_A/ET_B receptor antagonist (Clozel *et al.*, 1994), on the responses to ETs and SX6C. In some experiments retrograde perfusion of the lungs via the pulmonary vein was used to localize further the sites of action of ET-1 and SX6C. Preliminary findings from these studies have been presented to the British Pharmacological Society (Lal *et al.*, 1994b,c).

Methods

Isolated ventilated perfused lung preparation

Lungs were isolated and perfused as described previously (Lal *et al.*, 1994a). Male Wistar rats (300–350 g) were anaesthetized with Sagatal (60 mg kg⁻¹ body weight, i.p.). Heparin (500 iu) was injected i.v. via the tail vein; 5 min later the chest was opened and the pulmonary artery cannulated with a stainless steel cannula (external diameter = 1.5 mm, internal diameter = 1 mm) via the right ventricle. The left atrium was cut and the major part of the ventricles removed to allow free efflux of the perfusate. The trachea was cannulated, the lungs were isolated and immediately transferred into a warming jacket maintained at 37°C. Lungs were perfused via the pulmonary artery at 5 ml min⁻¹ with Krebs solution of the fol-

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lowing composition (mM): potassium chloride 4.7, potassium dihydrogen phosphate 1.2, calcium chloride 1.25, magnesium sulphate 1.2, sodium chloride 118, sodium bicarbonate 25, glucose 11.1, (gassed with 95% O₂ and 5% CO₂). Pulmonary perfusion pressure (PPP) was recorded via a pressure transducer connected to the pulmonary artery cannula. The tracheal cannula was attached to a ventilator and the lungs were ventilated with room air, stroke volume (1 ml) using 28 strokes min⁻¹ (no positive end expiratory pressure). A pressure transducer was attached to the tracheal cannula for recording the pulmonary inflation pressure (PIP). Lungs were suspended from an isometric transducer for recording changes in lung weight. All parameters were recorded on a multichannel recorder (Grass model 7D polygraph). Lungs were allowed to stabilize for 30 min before drug administration. After the initial stabilization, basal values for recorded parameters were as follows: PPP, 6.7 ± 0.5 mmHg; PIP, 5.0 ± 0.01 mmHg and lung weight, 2.8 ± 0.06 g, $n = 28$. Drugs were injected as bolus doses (10–100 μ l) via the pulmonary artery.

Effects of indomethacin and endothelin antagonists

In a series of experiments, BQ123 (2 μ M) or bosentan (5 μ M) was added to the perfusate 20 min or 30 min, respectively, before giving the bolus injections of drugs via the pulmonary artery cannula. These concentration ranges have previously been shown to produce inhibition of ET-1-induced effects (Hay et al., 1993; Clozel et al., 1994). Experiments were also performed with indomethacin (10 μ M) to study the involvement of cyclo-oxygenase products. Indomethacin (10 μ M) was added to the perfusate 30 min before agonist injection to inhibit prostanoïd production (Palmer et al., 1973).

Retrograde perfusion

In some experiments a cannula was introduced into the left atrium via the left ventricle and lungs were perfused via the pulmonary vein and pulmonary venous pressure monitored together with LW and PIP. Drugs were injected as bolus doses (10–100 μ l) via the pulmonary vein cannula.

Determination of albumin-bound dye accumulation

Evans blue dye was added to a 10% (w/v) solution of bovine serum albumin (BSA) in saline to give a final concentration of 1 mg ml⁻¹. The mixture was dialysed overnight in Visking tubing (22/23) against an excess of 0.9% saline. Aliquots of the solution were infused into the pulmonary artery (0.1 ml min⁻¹) for 5 min, 1 min after ET-1 or SX6C injection. Lungs were perfused for a further 10 min to remove unbound dye from the vasculature, lungs were removed and after removal of cardiac tissue and trachea, the lungs were oven dried (50–60°C) overnight and dry weight noted. Lungs were then digested with formamide (2 ml) at 40°C in a water bath for 20 h and the contents were centrifuged (Eppendorf centrifuge 5414) at 9880 g for 30 min. The absorbance of standard concentrations of Evans blue (0.25–20 μ g ml⁻¹) or lung extracts were read at 622 nm in a spectrophotometer (Pye Unicam). The concentration of dye in lung extracts was calculated by reference to the standard curve. From this value, fluid accumulation in the lung could be calculated by reference to the concentration of dye in the fluid perfusing the lung.

Drugs used

BQ123 cyclo[D-Asp-L-Pro-D-Val-L-Leu-D-Trp]) was supplied by Dr. K. Clark, Glaxo Group Research (Ware, Herts) and bosentan (Ro47-0203; 4-tert-butyl-N-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2'-bipyrimidin-4-yl]-benzenesulphonamide sodium salt) was provided by Hoffmann-La Roche Ltd (Basel, Switzerland). Endothelin-1, endothelin-2 and endothelin-3 were from Novabiochem (Nottingham), indomethacin was from Sigma (Poole, Dorset) and sarafotoxin 6c from Peninsula

Laboratories (Merseyside). Stock solutions of drugs were prepared in 0.9% normal saline and stored at –20°C.

Data analyses

Increases in PPP, PIP and lung weight are expressed as relative to the basal values prior to the addition of each dose. When lung weight measurement was needed for comparative purposes, weight gain was calculated 7 min after the maximum dose of agonist. Data are expressed as mean $\pm$ standard error of the mean (s.e.mean). ANOVA, Dunnett's and Student's *t* test were used to test the levels of significance. Probability values of $P < 0.05$ were considered significant.

Results

Pulmonary vascular and bronchial actions of ETs and SX6C

Effects on pulmonary perfusion pressure (PPP) The accumulated results for ETs (ET-1, ET-2 and ET-3) and SX6C on PPP are shown in Figure 1. From Figure 1a, it can be seen that over the dose-range used the order of potency of the ETs was ET-1 > ET-2 > ET-3. SX6C was of similar potency to ET-1 with ED₅₀ values of 165 ± 19 pmol, $n = 6$ and 130 ± 17 pmol respectively ($n = 10$).

Effects on lung weight and albumin accumulation Figure 1b shows the accumulated results for the effects of ETs and SX6C on lung weight. The order of potency of the ETs was ET-1 > ET-2 > ET-3. In contrast, SX6C which was equipotent with ET-1 on PPP caused no significant change in lung weight over the dose-range used.

In a separate set of experiments the increase in lung weight caused by ET-1 (800 pmol) was shown to be associated with a large accumulation of albumin bound dye (97.5 ± 22 ng mg⁻¹ dry weight of tissue compared to 1.32 ± 0.54 ng mg⁻¹ in control lungs without ET-1; $P < 0.01$, $n = 4$). Back-calculation of fluid retention from retained dye indicated that 800 pmol of ET-1 caused an accumulation of 1.62 ± 0.28 ml of fluid ($n = 4$). Assuming a specific gravity of 1 for this fluid a comparison of this calculated weight gain with weight gain recorded experimentally (4.25 ± 0.25 g, $n = 4$) illustrates that the values are significantly different ($P < 0.01$), i.e. fluid accumulation without albumin.

Effects on pulmonary inflation pressure (PIP) As shown in Figure 1c the three ETs were equipotent on PIP (ED₅₀ values for ET-1, ET-2 and ET-3 on PIP were 216 ± 2 pmol, $n = 10$; 277 ± 7 pmol, $n = 4$ and 268 ± 4 pmol, $n = 8$ respectively). However SX6C was the most potent bronchoconstrictor agent (ED₅₀ 80 ± 6 pmol, $n = 6$). In addition the slope of the PIP response to SX6C was significantly steeper than that for any of the ETs ($P < 0.001$).

Effects of indomethacin and endothelin antagonists

Indomethacin (10 μ M) had no effect on basal PPP, PIP or lung weight ($n = 14$), and did not affect the actions of ET-1 on PPP (ED₅₀ value of 132 ± 14 pmol was not different from control ED₅₀ of 130 ± 17 pmol, $n = 10$), PIP (ED₅₀ of 200 ± 2 pmol compared to control ED₅₀ of 216 ± 2 pmol, $n = 10$) or lung weight (maximum increase in lung weight produced by 400 pmol ET-1 in the presence and absence of indomethacin was 4 ± 0.9 g and 4.5 ± 0.8 g $n = 10$ respectively).

SX6C-mediated responses were also unaffected by indomethacin (10 μ M). PPP ED₅₀ values for SX6C in the presence and absence of indomethacin were 142 ± 11 pmol ($n = 4$) and 165 ± 19 pmol ($n = 6$) respectively, while the corresponding PIP ED₅₀ values in the presence and absence of indomethacin were 81 ± 8 pmol ($n = 4$) and 80 ± 6 pmol ($n = 6$). SX6C had no significant effect on lung weight with or without indomethacin.

BQ123 (2 μ M), a selective ET_A receptor antagonist, had no effect on basal PPP, PIP and lung weight ($n=4$). However, perfusion of BQ123 significantly attenuated the effects of lower doses of ET-1 on PPP (Figure 2a). The ED₅₀ value for ET-1 was increased from 130 ± 17 pmol ($n=10$) to 311 ± 3 pmol ($n=4$; $P<0.01$) in the presence of BQ123. However, BQ123 had no effect on the maximal increase in PPP induced by ET-1. The maximum increase in lung weight produced with 400 pmol ET-1 in control lungs (4.5 ± 0.8 g, $n=10$) was significantly reduced in the presence of BQ123 (0.75 ± 0.1 g, $n=4$, $P<0.01$) as shown in Figure 2b. Interestingly, BQ123 significantly augmented the effects of ET-1 (200 pmol) on PIP ($P<0.05$) but it had no effect on responses to other doses of ET-1 (Figure 2c).

BQ123 (2 μ M) did not affect the PPP response to SX6C; control ED₅₀ was 165 ± 19 pmol ($n=6$) and in the presence of BQ123 was 184 ± 20 pmol ($n=3$). Similarly, SX6C-induced changes in PIP were not altered by BQ123; control ED₅₀ was 80 ± 6 pmol ($n=6$) and in presence of BQ123 was 68 ± 2 pmol ($n=3$).

Figure 1 Actions of endothelin-1 (ET-1, ○), ET-2 (●), ET-3 (△) and sarafotoxin 6c (SX6C, ▲) on (a) pulmonary perfusion pressure (PPP), (b) lung weight and (c) pulmonary inflation pressure (PIP). Each point represents mean $\pm$ s.e.mean, $n=4-10$ experiments.

Bosentan (5 μ M), a mixed endothelin receptor antagonist, had no effect on the basal PPP, PIP and lung weight ($n=4$). However, it significantly ($P<0.001$, $n=4$) reduced the ET-1-induced PPP and lung weight responses as shown in Figure 3a and 3b, respectively. In contrast, bosentan did not affect ET-1-induced PIP responses (Figure 3c). Responses to SX6C on PPP, PIP and lung weight were significantly ($P<0.001$, $n=4$) attenuated by bosentan (Figure 3).

Effects of ET-1 and SX6C in retrogradely perfused lungs

Figure 4 illustrates the actions of ET-1 and SX6C on recorded parameters in orthogradely and retrogradely perfused lungs. ET-1 (50–400 pmol) and SX6C (50–800 pmol) produced dose-dependent increases in PPP, PIP and lung weight. However, a comparison of the upper panels shows that the actions

Figure 2 Endothelin-1 (ET-1)-induced increases in (a) pulmonary perfusion pressure (PPP), (b) lung weight and (c) pulmonary inflation pressure (PIP) in the absence (○, control data as in Figure 1) and presence of BQ123 (2 μ M) (●). Each point represents mean $\pm$ s.e.mean, $n=4-10$ experiments. * $P<0.05$; ** $P<0.01$; *** $P<0.001$: significantly different from control ET-1.

Figure 3 Effects of bosentan (5 μ M) on endothelin-1 (ET-1, ○, ●) and sarafotoxin 6c (SX6C, △, ▲) on (a) pulmonary perfusion pressure (PPP) (b) lung weight and (c) pulmonary inflation pressure (PIP) in absence (open symbols) and presence of bosentan (closed symbols). Each point represents mean $\pm$ s.e. mean, $n=4-13$ experiments. *** $P<0.001$: significantly different from control ET-1 or SX6C.

of ET-1 on lung weight were markedly reduced by retrograde perfusion. Using orthograde perfusion, 400 pmol of ET-1 produced an increase in lung weight of 6.9 ± 0.21 g ($n=4$) whereas 400 pmol of ET-1 in retrograde perfusion produced only a 1.8 ± 0.36 g ($n=4$, $P<0.01$) increase. Furthermore, albumin bound dye accumulation (1.48 ± 0.85 ng mg^{-1} of dry wt, $n=4$) produced by a higher dose of ET-1 (800 pmol) with retrograde perfusion was still significantly lower ($P<0.01$) than the accumulation seen with 400 pmol ET-1 (25 ± 9.5 ng mg^{-1} of dry wt., $n=4$) given via orthograde perfusion.

Comparison of the lower panels in Figure 4 shows that the actions of SX6C on lung weight were markedly increased by retrograde perfusion. Using orthograde perfusion, 800 pmol of SX6C produced a 0.11 ± 0.06 g ($n=4$) increase in lung weight whereas the same dose given via retrograde perfusion produced a 7.5 ± 0.29 g ($n=4$, $P>0.001$) increase in lung weight. Furthermore, in a separate set of experiments accumulation of

albumin bound dye (216 ± 2.6 ng mg^{-1} dry tissue, $n=4$) caused by SX6C (800 pmol) in retrograde perfusion was significantly greater ($P<0.01$) than albumin bound dye accumulation (70 ± 4 ng mg^{-1} dry wt, $n=4$) caused by SX6C in orthograde perfusion. Back calculation of fluid retention from retained dye indicated that 800 pmol of SX6C caused an accumulation of 1.44 ± 0.06 ml and 6.6 ± 0.39 ml fluid in orthograde and retrogradely perfused lungs, respectively ($n=4$). Assuming a specific gravity of 1 for this fluid a comparison of this calculated weight gain with weight gain recorded experimentally (1.46 ± 0.24 g and 7.3 ± 0.15 g, $n=4$, in orthograde and retrogradely perfused lung, respectively) illustrates that the values are in good agreement.

Discussion

We have studied the effects of ETs and SX6C in a rat perfused lung model which allows simultaneous measurement of PPP, PIP and lung weight. The pulmonary vasoconstrictor potencies of ETs (ET-1 > ET-2 > ET-3) suggest an action mediated via ET_A receptors (Webb, 1991). This was supported by the finding that a low concentration of BQ123, a selective ET_A receptor antagonist (Ihara *et al.*, 1992), markedly attenuated the pulmonary vasoconstrictor actions of ET-1. A similar finding has been reported by Bonvallet *et al.* (1993). However SX6C, a highly selective ET_B agonist (Williams *et al.*, 1991) was also found to be a pulmonary vasoconstrictor with a potency similar to ET-1. This shows that stimulation of ET_B receptors can also produce vasoconstriction in the lung; a similar finding has been reported in guinea-pig lung (Noguchi *et al.*, 1993). In addition ET_B receptors have been shown to be present on isolated rings of rat small pulmonary artery (MacLean *et al.*, 1994). In the present experiments, perfusion of bosentan a mixed ET_A/ET_B receptor antagonist (Clozel *et al.*, 1994) blocked the responses of ET-1 and SX6C on PPP confirming the findings that pulmonary vasoconstriction is mediated via both ET_A and ET_B receptors.

Our data indicate that ETs are also very potent in producing increases in lung weight, which supports the findings of Ercan *et al.* (1993). The potency profile of ETs in causing increases in lung weight paralleled their vasoconstrictor potencies which indicates that changes in lung weight are secondary to their vasoconstrictor actions. Interestingly SX6C produced similar increases in PPP to the ETs but caused very little increase in lung weight. We have previously reported that phenylephrine selectively increases PPP whereas bradykinin increases both PPP and lung weight. These different effects were explained by phenylephrine producing arteriolar vasoconstriction whereas bradykinin caused venoconstriction (Lal *et al.*, 1994a). Thus SX6C, like phenylephrine is probably acting on the arteriolar side of the pulmonary circulation whereas ETs, acting like bradykinin, cause venoconstriction, an action ETs are known to exert (Horgan *et al.*, 1991; Rodman *et al.*, 1992) and oedema formation. In order to investigate this possibility retrograde perfusion was used. Under these conditions, arterial vasoconstriction will now cause an increase in hydrostatic pressure within the microcirculation and increase lung weight via hydrostatic means whereas venous constriction would be expected to cause little effect. This is exactly what was found, SX6C now produced significant increases in lung weight, whilst ET-1 had a reduced effect on lung weight, suggesting that ET_B receptors stimulated by SX6C are predominantly located on the arterial side of the pulmonary circulation whereas the majority of ET_A receptors appear to be located in the pulmonary venous vessels. These results agree with findings in other vascular beds (D'Orleans-Juste *et al.*, 1993; White *et al.*, 1994; MacLean *et al.*, 1994).

Increases in lung weight produced by ET-1 were associated with large accumulations of albumin bound dye. We have previously reported that increases in venous outflow pressure in this lung model can cause significant retention of albumin bound dye (Lal *et al.*, 1994a). In the present experiments ET-1

Figure 4 Experimental traces comparing the effects of endothelin-1 (ET-1, upper section) and sarafotoxin 6c (SX6C, lower section) on pulmonary perfusion pressure (PPP), lung weight (LW) and pulmonary inflation pressure (PIP) in rat lung perfused orthogradely (right hand panels) and retrogradely (left hand panels). This figure represents one of 4 similar experiments.

when given in the orthograde mode of perfusion produced marked accumulation of albumin bound dye, whereas when ET-1 was given via the retrograde mode of perfusion significantly less dye retention was seen. These findings further support the suggestion that increases in lung weight and retention of dye produced with ET-1 are due to potent venoconstriction. Furthermore increases in lung weight resulting from fluid accumulation were significantly higher than the expected lung weight increases calculated from the retention of

dye. This suggests that accumulation of fluid in the lung is primarily due to hydrostatic oedema rather than to a direct increase in vascular permeability to albumin.

When SX6C was given via orthograde perfusion it also caused retention of albumin bound dye which indicates the presence of ET_B receptors mediating constriction of pulmonary venous smooth muscle which would contribute to the pulmonary oedema via a hydrostatic mechanism. However when SX6C was given via retrograde mode of perfusion it

produced a significantly higher retention of albumin bound dye compared to orthograde perfusion. This provides further evidence that the majority of ET_B receptors are located at the arteriolar level.

BQ123 significantly attenuated the changes in lung weight produced by ET-1 suggesting the involvement of ET_A receptors in this action. Filep *et al.* (1992, 1993) have also recently concluded that ET-1 increases vascular permeability via an action on ET_A receptors. However, these findings are not in agreement with those of Bonvallet *et al.* (1993), who showed that in the rat isolated perfused lung, ET-1-associated hydrostatic oedema was unaffected by BQ123. Bosentan, a mixed ET_A/ET_B receptor antagonist, was shown to block the increases in PPP and lung weight produced by ET-1 or SX6C. This again indicates that stimulation of both ET_A and ET_B receptors can produce pulmonary oedema.

The bronchoconstrictor profile of ETs (ET-1 ≈ ET-2 ≈ ET-3) and SX6C suggests the presence of ET_B receptors in the bronchial smooth muscle. The fact that BQ123 at a concentration which reduced ET-1 induced increases in PPP and oedema, did not produce any reduction in the bronchoconstrictor effects of ET-1 or SX6C shows that bronchoconstriction was not mediated by ET_A receptors. Similar findings have been reported in guinea-pig (Hay *et al.*, 1993; Battistini *et al.*, 1994) and human bronchus (Hay *et al.*, 1993). The finding that SX6C was more potent than the ETs in causing bronchoconstriction and that the slope of the dose-response curve was significantly steeper and achieved a higher maximum could be due to a number of factors. Firstly ETs are known to be more susceptible to breakdown by neutral endopeptidase present in the lung, this could reduce the effects of ETs compared with SX6C (Sokolovsky *et al.*, 1990). However, we feel this is unlikely as we have shown that the bronchoconstrictor actions of ETs were not influenced by the addition of the neutral endopeptidase inhibitor phosphoramidon (unpublished observation). A recent report by Hisaki *et al.* (1994) has also shown that in rat isolated lung, pressor responses to ET-1 were not influenced by phosphoramidon. Secondly, ETs could be releasing some bronchodilator substance resulting in a physiological antagonism, as reported by Uchida *et al.* (1991). In the present study use of the selective ET_A receptor antagonist,

BQ123, significantly augmented the bronchoconstrictor response of ET-1 at 200 pmol. This could indicate that activation of ET_A receptors may release a bronchodilator substance which antagonizes the bronchoconstrictor responses of ET-1. Interestingly, Battistini *et al.* (1994) also reported that BQ123 potentiated the ET-1-induced contractions of guinea-pig isolated trachea.

Perfusion with the mixed ET_A/ET_B receptor antagonist, bosentan, produced a significant reduction in the bronchoconstrictor responses of SX6C without having any effect on the bronchoconstrictor responses of ET-1. This could indicate that in bronchial smooth muscles, ET_B receptors activated by ET-1 are different from the ET_B receptors activated by SX6C. The existence of different ET_B receptor subtypes in the guinea-pig isolated trachea has also been suggested by Battistini *et al.* (1993). Alternatively the strong binding affinity of ET-1 for ET_B receptors may explain this discrepancy (Takasuka *et al.*, 1992; Wu-Wong *et al.*, 1994). Indomethacin did not inhibit the vascular and bronchial actions of ET-1 or SX6C suggesting that the effects of ETs or SX6C were not mediated by eicosanoids. Similar findings have been reported by several other workers (O'Donnell *et al.*, 1990; Raffestin *et al.*, 1991; Rodman *et al.*, 1992).

In summary, our experiments show that activation of both ET_A and ET_B receptors can lead to constriction in the pulmonary vascular bed. In addition we have provided indirect evidence that ET_A receptors are predominantly present on the venous side of the pulmonary circulation whereas ET_B receptors are located arterially. Changes in lung weight probably reflect hydrostatic oedema in the pulmonary microvasculature resulting from intense venoconstriction mediated by ET_A receptors. Results also suggest the possibility of two ET_B receptor subtypes in mediating bronchial smooth muscle contraction.

BQ123 was a generous gift of Glaxo and bosentan was a generous gift of Hoffmann-La Roche Ltd. H.L. is an Indian Government Scholar.

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(Received November 24, 1994

Revised February 1, 1995

Accepted February 21, 1995)