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Endothelin$_B$ receptor-mediated contraction in human pulmonary resistance arteries

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1 Using wire myography, we have examined the endothelin (ET) receptor subtypes mediating vasoconstriction to ET peptides in human pulmonary resistance arteries (150-200 μm, i.d.).

2 Cumulative concentration-response curves to ET-1, sarafotoxin 6c (SX6c) and ET-3 were constructed in the presence and absence of the selective antagonists FR 139317 (ET_A-selective), BMS 182874 (ET_A-selective) and BQ-788 (ET_B-selective).

3 All agonists induced concentration-dependent contractions. However, the response curves to ET-1 were biphasic in nature. The first component demonstrated a shallow slope up to 1 nM ET-1. Above 1 nM ET-1 the response curve was markedly steeper. Maximum responses to ET-3 and SX6c were the same as those to 1 nM ET-1 and 30% of those to 0.1 μM ET-1. The order of potency, taking 0.3 μM as a maximum concentration was SX6c>>ET-3>ET-1 (pEC_{50} values of: 10.75 ± 0.27, 9.05 ± 0.19, 8.32 ± 0.08 respectively). Taking 1 nM ET-1 as a maximum, the EC_{50} for ET-1 was 10.08 ± 0.13 and therefore ET-1 was equipotent to ET-3 and SX6c over the first component of the response curve.

4 Responses to ET-1 up to 1 nM were resistant to the effects of the ET_A receptor antagonists, FR 139317 and BMS 182874 but were inhibited by the ET_B receptor antagonist, BQ-788. Conversely, responses to ET-1 over 1 nM were inhibited by the ET_A receptor antagonists, FR 139317 and BMS 182874 but unaffected by the ET_B receptor antagonist, BQ-788.

5 The results suggest that at concentrations up to 1 nM, responses to ET-1 are mediated via the ET_B receptor, whilst the responses to higher concentrations are mediated by ET_A receptors.

Keywords: Endothelin receptors; human pulmonary arteries; vasoconstriction

Introduction

There is a growing evidence that endothelins (ETs) may be involved in the pathogenesis of human forms of pulmonary hypertension. Increased plasma endothelin-1 (ET-1) levels have been observed in patients with primary pulmonary hypertension (Stewart *et al.*, 1991) and secondary pulmonary hypertension, due to chronic congestive heart failure and congenital heart defects (Cody *et al.*, 1992; Yoshibayashi *et al.*, 1991). Cody *et al.* (1992) also demonstrated that the plasma levels of ET-1 were well correlated with the degree of pulmonary hypertension observed and with the prognosis of the patient. High levels of ET-1 and ET-1 mRNA are present in the pulmonary vascular endothelial cells of patients with primary and secondary pulmonary hypertension (Giaid *et al.*, 1993).

ET-1 may also be implicated in pulmonary hypertensive vascular remodelling due to its proliferative effect on pulmonary vascular smooth muscle cells and fibroblasts (Janakidevi *et al.*, 1992; Hassoun *et al.*, 1992; Peacock *et al.*, 1992).

Targeting the actions of ET-1 in the pulmonary circulation using ET receptor antagonists has therefore been suggested as a possible therapeutic approach for the treatment of pulmonary hypertension. It is therefore of importance to characterize the receptors mediating the actions of ETs in the pulmonary vasculature.

We have previously demonstrated that the receptor subtype mediating ET-1-induced vasoconstriction in isolated pulmonary arteries of the rat varies, depending on the size and/or location of the artery under study (MacLean *et al.*, 1994). The majority of studies in human vessels have been carried out on large diameter pulmonary arteries (average 3-5 mm, i.d.) with the smallest diameter pulmonary artery used in functional studies being approximately 1 mm, i.d. and these vessels demonstrate ET_A-receptor-mediated vasoconstriction (Fukuroda *et al.*, 1994a). It is thought that the pulmonary resistance arteries are functionally important in resistance changes observed in pulmonary hypertensive states and we wished therefore, to examine responses in small intrapulmonary resistance arteries from the human lung.

Preliminary data from these studies have been presented previously (McCulloch *et al.*, 1994a, b; McCulloch & MacLean, 1995).

Methods

Human pulmonary arteries (150-200 μm, i.d.) were dissected from grossly normal sections of human lung removed from postoperative bronchial carcinoma tissue. Samples were refrigerated in fresh Krebs solution and were collected and studied no longer than 12 h postoperative. The vessels were mounted on a Mulvany Halpern small-vessel myograph (Mulvany & Halpern, 1977). Tension was raised to give an equivalent transmural pressure of approximately 16 mmHg. All vessels were bubbled with 16% O_2, 5% CO_2, balance N_2. This gave a final bath O_2 concentration (measured with an oxygen electrode and blood gas analyser) of approximately 120 mmHg and CO_2 tensions of approximately 35 mmHg to yield values equivalent to those found in pulmonary arteriolar blood. As these vessels have walls that are typically only 20 μm thick, O_2 diffusion problems are not encountered with active bubbling (Pittman & Duling, 1973; Fishman, 1976). After 1 h equilibration period the vessels were contracted with 50 mM KCl. Following this, vessels were washed out three times with fresh Krebs solution and allowed to return to baseline tension. The vessels were then again pre-contracted with a second challenge 50 mM KCl. Following washout and return to baseline tension the vessels were subjected to the following protocols: (A) 45 min equilibration period followed by a cumulative concentration-response curve (CCRC: 0.01 pM to 0.3 μM) to either endothelin-1 (ET-1), endothelin-3 (ET-3) or

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sarafotoxin 6c (SX6c); (B) 45 min incubation with one concentration of the antagonist under study followed by CCRC to selected agonist.

Drugs and solutions

The composition of the Krebs–bicarbonate saline was as follows (in mM): NaCl 118.4, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 0.6, $CaCl_2$ 2.5, glucose 11. The following drugs were used: endothelin-1 (Thistle, Glasgow, UK), Endothelin-3 (Peninsula Laboratories), sarafotoxin S6c (Sigma), BQ 788 (*N-cis*-2,6-dimethylpiperidinocarbonyl L-γ-MeLeu-D-Trp ($COOCH_3$)-D-Nle; Peptide International), FR139317 (*N*-CO-L-Leu-D-1-Me-Trp-D-3(2-Pyridyl) Ala-OH; Neosystems) and BMS 182874 (5-dimethylnamino)-*N*-(3,4-dimethyl-5-isoxzolyl)-1-naphthalenesulphonamide). Stock solutions of sarafotoxin S6c were prepared in 0.1% acetic acid and those to BQ 788 in 0.1% DMSO. All other drugs and dilutions were prepared in distilled H_2O.

Data analysis

Results are expressed graphically as percentage of the reference contraction to the second application of 50 mM KCl or as a percentage of the maximum response to ET-1. The pEC_{50} values were calculated by computer interpolation from individual CCRCs. Statistical comparisons of the means of groups of data were made by Student's *t* test for paired or unpaired data; $P<0.05$ was considered statistically significant. An estimated pK_B value for BQ-788 against ET-3 was calculated from the following equation: $pK_B = -\log([B]/DR-1)$ where B represents the concentration of antagonist studied and DR is the dose-ratio produced by the antagonist. Due to the nature of antagonism displayed by BMS 182874 and BQ-788 vs. ET-1, it was not possible to calculate pK_B values for these compounds. n/n in text refers to the number of preparations in the number of lungs studied.

Results

The average internal diameter of pulmonary resistance arteries mounted on the myograph was 188 ± 9 μm at an average transmural pressure of 16.3 ± 0.7 mmHg ($n=12$). KCl (50 mM) induced contractions of 113 ± 10 mg wt. tension ($n=12/10$). Figure 1 illustrates the tissue responses to ET-1, ET-3 and SX6c and data for pEC_{50} values are summarized in Table 1.

The response to ET-1 comprised two components, one being a gradual slope up to 1 nM and the second a steeper component at higher concentrations. Taking the CCRC to ET-1 as a whole, ET-3 was approximately five times more potent that ET-1 in this preparation (see Table 1) but produced a maximum contraction of approximately 30% of the contraction to 1 μM ET-1 ($P<0.001$). The selective ET_B agonist, SX6c, was more potent than ET-1 and ET-3 in producing contractions in human pulmonary resistance arteries. These contractions were completely resistant to the actions of the ET_A receptor antagonist, FR 139317 (Table 1). However, in a similar fashion to ET-3, SX6c produced a maximum contraction of approximately 30% of the contraction to 1 μM ET-1 ($P<0.001$).

Incubation with antagonists at all concentrations studied had no effect on resting vascular tone. FR 139317 did not antagonize responses to ET-1 when present at 1 μM (Figure 2; Table 1). However, when present at 10 μM, the antagonist caused a significant decrease in the response to ET-1 at 30 nM and 0.1 μM ($P<0.001$). BMS 182874 (a selective ET_A receptor antagonist, 10 μM) did cause a significant decrease in the absolute contractile response to ET-1, but only in the range of 10 nM to 0.1 μM (Figure 3; Table 2). The first, shallow component of the ET-1 CCRC was unaffected by BMS 182874.

BQ-788 (a selective ET_B receptor antagonist) inhibited responses to ET-1 up to 1 nM but not to higher con-

Table 1 pEC_{50} values for endothelin-1(ET-1), endothelin-3 (ET-3) and sarafotoxin S6c (SX6c) in human pulmonary resistance arteries

Agonist ± antagonist	*pEC_{50}*	n/n
ET-1	8.32 ± 0.08	12/10
ET-3	9.05 ± 0.19**	10/10
SX6c	10.65 ± 0.27***†††	10/6
ET-1 + 1 μM FR 139317	7.98 ± 0.30	8/6
ET-1 + 10 μM FR 139317	8.80 ± 0.21	4/4
ET-1 + 10 μM BMS 182874	8.20 ± 0.26	8/5
ET-3 + 1 μM BQ 788	7.43 ± 0.13†††	7/5
SX6c + 1 μM FR 139317	10.55 ± 0.25	3/3

Data are expressed as mean ± s.e.mean. *n/n*: number of preparations from number of lungs. Statistical comparisons were made by Students unpaired *t* test. **$P<0.01$, ***$P<0.001$ vs. ET-1. †††$P<0.001$ vs. ET-3.

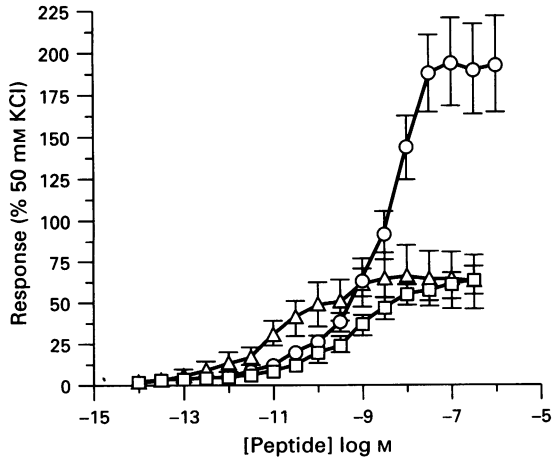

Figure 1 Cumulative concentration-response curves to endothelin-1 (○, $n=12/10$), endothelin-3 (□, $n=10/10$) and sarafotoxin S6c (△, $n=10/6$) in human pulmonary resistance arteries. Data are expressed as percentage reference contraction to 50 mM KCl in each vessel preparation. Each point represents the mean ± s.e.mean.

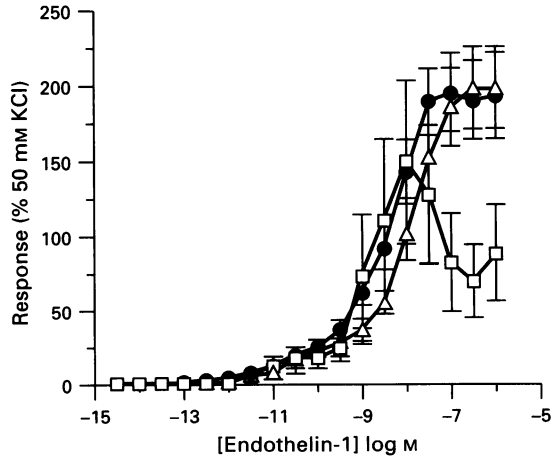

Figure 2 Endothelin-1 (ET-1) induced vasoconstriction in human pulmonary resistance arteries: effect of the ET_A receptor antagonist, FR 139317. Cumulative concentration-response curves to ET-1 (●, $n=12/10$), in the presence of 1 μM FR 139317 (△, $n=8/6$) and in the presence of 10 μM FR 139317 (□, $n=4/4$). Data are expressed as percentage reference contraction to 50 mM KCl in each vessel preparation. Each point represents the mean ± s.e.mean.

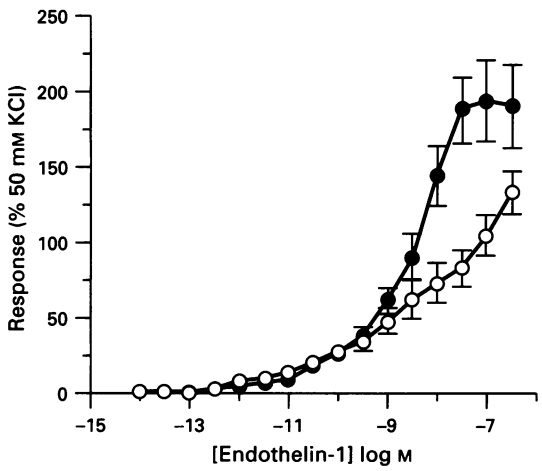

Figure 3 Endothelin-1 (ET-1)-induced vasoconstriction in human pulmonary resistance arteries: effect of the ET_A receptor antagonist, BMS 182874. Cumulative concentration-response curves to ET-1 (●, $n=12/10$) and in the presence of 10 μM BMS 182874 (○, $n=8/5$). Data are expressed as percentage reference contraction to 50 mM KCl in each vessel preparation. Each point represents the mean ± s.e.mean.

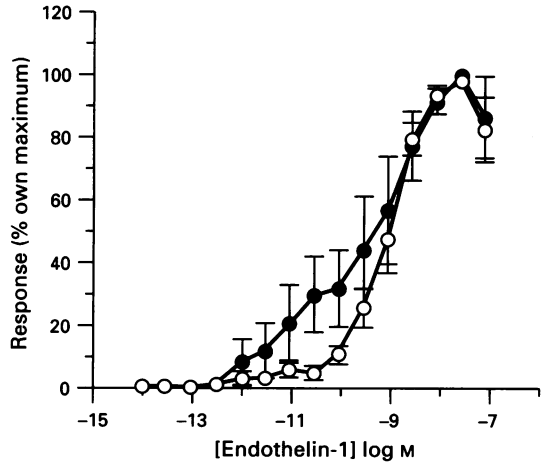

Figure 4 Endothelin-1 (ET-1) induced vasoconstriction in human pulmonary resistance arteries: effect of the ET_B receptor antagonist, BQ-788. Cumulative concentration-response curves to ET-1 (●, $n=4/4$) and in the presence of 1 μM BQ-788 (○, $n=4/4$). Data are expressed as percentage own maximum contraction in each vessel preparation. Each point represents the mean ± s.e.mean.

centrations (Figure 4). The maximum contraction to ET-1 was not altered in the presence of BQ-788 (control maximum contraction = 373 ± 112% of 50 mM KCl response, and in the presence of 1 μM BQ-788 = 211 ± 38% of 50 mM KCl response).

The presence of the antagonists further suggested that there were two components to the ET-1 CCRC, an ET_B-mediated component up to 1 nM and an ET_A-mediated component at higher concentrations. Assuming that 1 nM ET-1 gave the maximum response for the ET_B component, the EC_{50} for this component was 10.08 ± 0.13. ET-1 was, therefore, equipotent to ET-3 and SX6c over the 'ET_B' component of the response curve.

BQ-788 (1 μM) caused a rightward shift in the response curves to ET-3 in human pulmonary resistance arteries, without affecting the maximal contractile response to the peptide (Figure 5, Table I). The estimated pK_B value for BQ-788 against ET-3 was 7.72 ± 0.22 ($n=7$ preparations from 5 lungs).

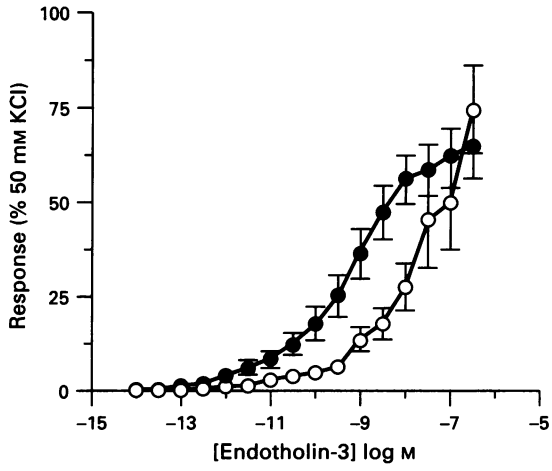

Figure 5 Endothelin-3 (ET-3)-induced vasoconstriction in human pulmonary resistance arteries: effect of the ET_B receptor antagonist, BQ-788. Cumulative concentration-response curves to ET-3 (●, $n=10/10$) and in the presence of 1 μM BQ-788 (○, $n=7/5$). Data are expressed as percentage reference contraction to 50 mM KCl in each vessel preparation. Each point represents the mean ± s.e.mean.

Discussion

The response curve to ET-1 in human pulmonary resistance arteries is biphasic in nature, which suggests a heterogeneous population of ET-receptors. That ET-1-induced vasoconstriction is not mediated entirely by a typical ET_A-receptor in human pulmonary resistance arteries is suggested by its resistance to both of the ET_A-receptor antagonists studied. However, although the antagonists did not competitively shift the ET-1-induced response, both FR 139317 (10 μM) and BMS 182874 (10 μM) significantly decreased the contractile response to high concentrations of ET-1. This would suggest that ET-1 may be acting at ET_A-receptors at high concentrations but at another receptor at low concentrations. A similar effect of the ET_A receptor antagonist BQ 123 has been observed in rabbit pulmonary artery where ET_A and ET_B receptors co-exist (La Douceur *et al.*, 1993).

The selective ET_B receptor agonist, SX6c, was extremely potent at producing contractile responses, being over 200 fold more potent that ET-1. These contractions were resistant to the actions of the ET_A receptor antagonist, FR 139317. The CCRC to SX6c followed a similar course to that of the first component of the CCRC to ET-1. The maximum contraction induced by SX6c was however only 30% of that caused by high concentrations of ET-1. Concentration-dependent contractile responses were also observed to ET-3. This peptide proved to be less potent than SX6c but 5 fold more potent than ET-1. In a similar fashion to SX6c, the maximum contraction induced by ET-3 was only some 30% of the maximum response induced by ET-1 in this preparation. The results also show that the CCRC to ET-3 follows a similar course to that of SX6c and the first component of the CCRC to ET-1. ET-1 and ET-3 would be expected to be equipotent at a classical ET_B receptor subtype. However, in our results it can be seen that ET-3 is more potent that ET-1 when 0.3 μM ET-1 is considered as the concentration causing maximum contraction. A receptor selective for ET-3 over ET-1 (denoted ET_C) has been cloned in *Xenopus laevis* dermal melanophores (Karne *et al.*, 1993). The relative potency of ET-3 over ET-1 in human pulmonary resistance arteries may suggest the presence of such an ET_C receptor; however, a mammalian vascular counterpart of this receptor has yet to be identified.

The ET_B receptor antagonist, BQ-788, antagonized the contractile responses to concentrations of ET-1 up to 1 nM, suggesting that ET-1 is activating vascular ET_B receptors within this concentration-range. The inability of BQ-788 to

inhibit responses to ET-1 at concentrations greater than 1 nM confirms the heterogeneity of the receptor population and combined with the ability of the selective ET_A receptor antagonists to inhibit the responses to ET-1 above 1 nM, confirms that ET-1 activates ET_A receptors at these high concentrations. The ability of BQ-788 to antagonize responses to ET-3 confirms that ET-3 is mediating its response via ET_B receptors in this preparation. Given that the results suggested an ET_B-receptor-mediated response to ET-1 at concentrations up to 1 nM, we re-analyzed the sensitivity of the tissue to ET-1 over this component. The results show ET-1 was equipotent to both SX6c and ET-3, taking 1 nM as a maximum concentration. This further supports the concept that ET_B-receptors are involved in the response to ET-1 up to 1 nM.

What are the physiological and pathophysiological concentrations of ET-1 to which the pulmonary vascular smooth muscle may be exposed? Under normal physiological conditions, ET-1 circulates within the plasma in the low picomolar range, with values often in the range of 0.5 to 5 pg ml^{-1} of plasma (Miyauchi *et al.*, 1991; Lam *et al.*, 1991; Goerre *et al.*, 1995; Ferri *et al.*, 1995). There is considerable variation between groups as to the precise levels under normal conditions and this is probably due to sample populations, the assay techniques used for measurement and the site at which the samples are taken. However, all relevant studies are consistent in showing that the ET-1 levels are significantly increased in cases of pulmonary hypertension. The most dramatic increases in circulating ET-1 levels and ET-1 expression, appear to be associated with primary pulmonary hypertension (Stewart *et al.*, 1991; Cacoub *et al.*, 1993; Giaid *et al.*, 1993; Nootens *et al.*, 1995); although ET-1 levels are also significantly increased in pulmonary hypertension secondary to hypoxia (Stewart *et al.*, 1991; Ferri *et al.*, 1995), congenital heart defects (Yoshibayashi *et al.*, 1991; Cacoub *et al.*, 1993; Vincent *et al.*, 1993), valvular heart disease (Stewart *et al.*, 1991; Chang *et al.*, 1993; Yamamoto *et al.*, 1994; Zhu *et al.*, 1994), chronic heart failure (Cody *et al.*, 1992; Kiowski *et al.*, 1995) and the adult respiratory distress syndrome (Langleben *et al.*, 1993). Plasma ET-1 levels can be increased four fold in some of these studies. Although plasma levels of ET-1 give an indication of production, the actions of ET-1 may be more paracrine than endocrine in nature. It is thought that approximately 75% of ET-1 synthesized is secreted towards the vascular smooth muscle cells (Yoshimoto *et al.*, 1991; Wagner *et al.*, 1992), and given the small volumes of interstitial fluid, ET-1 concentrations may be significantly greater at the smooth muscle cell level compared to plasma levels. The results from our study would suggest that *in vivo*, ET-1 will cause contraction of the pulmonary resistance artery via the ET_B receptor when present at concentrations below 1 nM. In the context of our *in vitro* studies, plasma ET-1 concentrations would be at the threshold level (pEC_{10}) required for contraction. Threshold concentrations of ET-1 have also been shown to facilitate contractions to other vasoactive compounds such as 5-hydroxytryptamine, angiotensin II and α_2-adrenoceptor agonists (MacLean & McGrath, 1990; Takeshita *et al.*, 1991; Itoh *et al.*, 1992). Indeed, it has been demonstrated in the forearm circulation of healthy human volunteers, under control conditions, that infusion of the ET_A receptor antagonist, BQ-123, mediates prolonged vasodilatation suggesting that ET-1 plays a role in the regulation of human vascular tone under normal physiological conditions (Haynes *et al.*, 1995).

Interaction of ETs with the vascular endothelium in human pulmonary vessels is not well documented. Endothelial ET_B receptors have been shown to mediate pulmonary vasodilatation in rats (Eddahibi *et al.*, 1991) and lambs (Wong *et al.*, 1995), but whether this occurs in the human pulmonary circulation is not yet clear. It has also been reported that responses to ET-1 in human large diameter intrapulmonary arteries were not affected by cyclo-oxygenase inhibition (indomethacin) or NO synthase inhibition (L-NOARG), suggesting that local endogenous release of endothelium-derived relaxing factors may not be important in regulating the contractile responses to ET-1 in human pulmonary arteries *in vitro* (Pussard *et al.*, 1995).

Unfortunately little is known about the actions of ETs in human lungs *in vivo*. From studies of isolated systemic vessels *in vitro*, it was generally thought that ET-1 contracted the majority of human arterial and venous preparations via the activation of ET_A receptors (Davenport *et al.*, 1994; Maguire *et al.*, 1995). However, comparative studies *in vivo* would indicate that both ET_A and ET_B receptors mediate vasoconstriction in human resistance and capacitance vessels (Haynes *et al.*, 1995). This discrepancy may be due to the size and or type of preparation studied, as there is growing evidence *in vitro* to suggest the presence of vascular ET_B receptors in human arteries and veins (Seo *et al.*, 1994; White *et al.*, 1994; Dashwood *et al.*, 1995). We therefore may expect to see contributions of both ET_A and ET_B receptors in mediating ET-1-induced vasoconstriction in the human pulmonary circulation *in vivo*. ET_A and ET_B receptors mediate contraction in the human bronchi and in rabbit pulmonary artery. In these tissues, dual blockade of both receptors is required to antagonize responses to ET-1 fully (LaDouceur *et al.*, 1993; Fukuroda *et al.*, 1994b; 1996). Non-selective ET antagonists may, therefore, prove to be more effective antagonists in the human pulmonary arterial bed.

ET-induced responses and ET receptor subtypes in the pulmonary vasculature are altered in animal models of pulmonary hypertension (Eddahibi *et al.*, 1991; Yorikane *et al.*, 1993; Li *et al.*, 1994; MacLean *et al.*, 1995). Whether pulmonary vascular responses to ET-1 are altered in human pulmonary hypertension is not yet known and will be an important factor in determining the possible use of ET receptor antagonists as a therapy for pulmonary hypertension.

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