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Endothelin-induced contraction and mediator release in human bronchus

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1 To elucidate the role of acetylcholine and various autacoids in endothelin-1 (ET-1)-induced contraction in human bronchus, the effects of various receptor antagonists were examined. In addition, the ability of ET-1 to stimulate the release of histamine, peptidoleukotrienes and prostanoids was determined.

2 ET-1 was a potent and effective contractile agonist in human bronchus, possessing similar potency and efficacy to leukotriene D_4 (LTD_4); EC_{50} ($-\log M$): ET-1 = 7.76 ± 0.09, $n = 7$; LTD_4 = 8.46 ± 0.53, $n = 7$; $P > 0.2$; maximum response (% 10 μM pre-carbachol): ET-1 = 103.8 ± 17.4, $n = 7$; LTD_4 = 95.5 ± 9.3, $n = 7$; $P > 0.6$.

3 The cyclo-oxygenase inhibitor, sodium meclofenamate (1 μM) or the potent and selective thromboxane receptor antagonist, SQ 29,548 (1 μM) were without significant effect on ET-1 concentration-response curves.

4 In the presence of sodium meclofenamate (1 μM), the muscarinic receptor antagonist, atropine (1 μM), the platelet activating factor (PAF) receptor antagonist, WEB 2086 (1 μM) or the combination of the H_1-histamine receptor antagonist, mepyramine (10 μM) and the leukotriene receptor antagonist, SK&F 104353 (10 μM), were without marked effect on ET-1 concentration-response curves. In addition, the combination of all four receptor antagonists did not antagonize ET-1-induced contraction.

5 ET-1 (0.3 μM) did not stimulate the release of histamine or immunoreactive leukotrienes from human bronchus.

6 ET-1 (0.3 μM) significantly stimulated the release of prostaglandin D_2 (PGD_2), 9α, 11β PGF_2 (PGD_2 metabolite), PGE_2, 6-keto $PGF_{1\alpha}$ (PGI_2 metabolite), $PGF_{2\alpha}$ and thromboxane B_2 (TxB_2) a lower concentration, 10 nM, was without effect on prostanoid release. The production of PGD_2 was increased 7.5 fold, whereas the release of the other prostanoids was stimulated only about 1.6 to 2.7 fold.

7 These data provide evidence that ET-1 elicits contraction of human isolated bronchus predominantly via a direct mechanism with no significant involvement of the release of acetylcholine, leukotrienes, histamine or PAF. Although ET-1 increased the release of several prostanoids they did not have a significant modulatory effect on the smooth muscle contraction.

Keywords: Endothelin-1; human bronchus; SK&F 104353; mepyramine; WEB 2086; SQ 29,548; peptidoleukotriene release; histamine release; sodium meclofenamate; prostanoid release

Introduction

Yanagisawa and co-workers described the isolation, purification, cloning and pharmacological characterization of a potent vasoconstrictor peptide, designated endothelin, which was released from porcine aortic endothelial cells (Yanagisawa *et al.*, 1988). Endothelin is a 21-amino acid peptide, with two sets of intrachain disulphide bridges, which bears a close structural homology with the sarafotoxins, a group of snake venom toxins (Lee & Chiappinelli, 1988; Kloog *et al.*, 1988). Subsequent research indicated that endothelin, named endothelin-1 (ET-1), is only one member of a family of mammalian endothelins: Thus, Inoue and co-workers cloned three distinct ET-related genes by screening a human genomic DNA library (Inoue *et al.*, 1989). These three 21-amino acid peptides, which have only minor differences in amino acid sequence, were designated ET-1 (the orginal porcine/human ET), ET-2 (two amino acid substitution from ET-1) and ET-3 (six amino acid substitution from ET-1) (Yanagisawa & Masaki, 1989a,b).

Although the focus of the research to date on the endothelins has been on their effects and potential pathophysiological relevance in the cardiovascular system, they produce an array of activities in a variety of other systems (Yanagisawa & Masaki, 1989a,b). For example, shortly after its discovery, ET-1 was reported to be a potent contractile agonist of guinea-pig trachea (Uchida *et al.*, 1988). This observation has been confirmed (Hay, 1989; Maggi *et al.*, 1989; Henry *et al.*, 1990) and extended to isolated airway tissues from a variety of species including rat (Turner *et al.*, 1989), ferret (Lee *et al.*, 1990), rabbit (Grunstein *et al.*, 1991) and man (Henry *et al.*, 1990; Hemsén *et al.*, 1990; Advenier *et al.*, 1990; Brink *et al.*, 1991; McKay *et al.*, 1991). In fact, it has been proposed that the endothelins play a role in the pathophysiology of pulmonary disorders including asthma and pulmonary hypertension (Cernacek & Stewart, 1989; Mattoli *et al.*, 1991a; Springall *et al.*, 1991; Hay *et al.*, 1993).

By use of [^{125}I]-ET-1, specific binding sites of high density were detected in smooth muscle of human isolated trachea (Power *et al.*, 1989) and bronchus (Henry *et al.*, 1990; McKay *et al.*, 1991), in human cultured bronchial smooth muscle cells (Mattoli *et al.*, 1990) and in human lung membranes (Brink *et al.*, 1991). The ET-1 EC_{50} in human bronchus is generally in the 10–30 nM range (Henry *et al.*, 1990; Hemsén *et al.*, 1990; Advenier *et al.*, 1990; Brink *et al.*, 1991; McKay *et al.*, 1991).

The role of indirect mechanisms in endothelin-induced airway smooth muscle contraction is controversial. *In vivo* studies have indicated that endothelin-induced bronchocon-

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striction in guinea-pigs can be substantially inhibited by cyclo-oxygenase or thromboxane synthase inhibitors (Payne & Whittle, 1988; Nambu *et al.*, 1990). However, there is conflicting information on the effects of cyclo-oxygenase inhibitors (Maggi *et al.*, 1989; Sarriá *et al.*, 1990; Henry *et al.*, 1990; Hay, 1990) or thromboxane receptor antagonists (Filep *et al.*, 1990; Hay, 1990) on endothelin-induced contraction of guinea-pig isolated trachea. ET-1 elicits histamine release from guinea-pig isolated lung parenchymal mast cells (Uchida *et al.*, 1992), and in preliminary reports it was proposed that the mast-cell derived products, histamine and the peptidoleukotrienes, played a role in endothelin-induced responses in guinea-pig isolated trachea (Ninomiya *et al.*, 1989; Nomura *et al.*, 1990). However, a later study, which included direct measurement of the release of these mediators from the trachea, failed to corrobate this hypothesis (Hay & Undem, 1993). There is little information about potential indirect mechanisms in endothelin-induced contraction of human airways. Several investigations, however, have demonstrated that indomethacin is without effect on contraction elicited by ET-1 in human bronchus (Henry *et al.*, 1990; Advenier *et al.*, 1990; McKay *et al.*, 1991). The purpose of this study was to examine further the potential role of secondary mediators in ET-1-induced contraction in human bronchus. This was conducted by evaluating the effects of various potent and selective receptor antagonists for inflammatory mediators on the contractile response to ET-1 and also by measuring directly the effect of ET-1 on the release of histamine, the leukotrienes and various prostanoids. A preliminary account of the results has been presented by Hay *et al.* (1992).

Methods

Tissue preparation for contraction studies

Human lung tissue from organ donors was obtained from the International Institute for the Advancement of Medicine (IIAM, Exton, PA, U.S.A.) and the National Disease Research Interchange (NDRI, Philadelphia, PA, U.S.A.). Lungs were received within 24 h of removal. The donors had no known respiratory disorders. The bronchi were removed from the lung by carefully placing a glass probe within individual segments and dissecting away lung parenchymal and vascular tissue. First- and fifth-generation bronchial strips (4–15 mm diameter) were prepared and each one was placed in a 10 ml water-jacketed tissue bath containing modified Krebs-Henseleit solution which was maintained at 37°C and continuously aerated with 95% O_2/5% CO_2; according to this classification, the main bronchus is regarded as the first generation airway. The composition of the Krebs-Henseleit solution was (mM): NaCl 113, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 5.5. One end of each preparation was attached with a silk suture to a glass tissue holder and the other end was tied to a Grass model FT03C force-displacement transducer (Grass Instrument Co., Quincy, MA, U.S.A.) for the recording of isometric tension on multichannel Grass polygraphs. The tissues were then placed under about 2 g of passive tension and equilibrated for 60 min, during which they were washed every 15 min with fresh physiological solution, before the start of each experiment.

Concentration-response curves

After the equilibration period, and before construction of ET-1 concentration-response curves, tissues were exposed to 10 μM carbachol. After this reference contraction had reached a plateau, which in preliminary experiments was shown to represent 89.0 ± 4.6% ($n = 4$) of the maximum contractile response to carbachol (100 μM), tissues were washed several times over 15–30 min until the tension returned to baseline level. The preparations were then left for at least 30 min before the start of the experiment. Agonist concentration-response curves were obtained by their cumulative addition to the organ bath in 3 fold 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 most experiments examining the effects of drugs, tissues were exposed to these agents for 30 min before addition of ET-1. ET-1 concentration-response curves were generally conducted in the presence of 1 μM sodium meclofenamate, the cyclo-oxygenase inhibitor, which was added 45 min before initiation of the curves. Only one agonist concentration-response curve was generated per tissue. The receptor antagonists, and their concentrations, employed in this study were 10 μM SK&F 104353 (a peptidoleukotriene receptor antagonist; Hay *et al.*, 1987), 10 μM mepyramine (an H_1-histamine receptor antagonist), 1 μM atropine (a muscarinic receptor antagonist), 1 μM SQ 29,548 (a thromboxane receptor antagonist; Ogletree *et al.*, 1985) and 10 μM WEB 2086 (a PAF receptor antagonist; Casals-Stenzel, 1987a,b). The concentration of SK&F 104353 used has been observed previously to produce marked, competitive antagonism of leukotriene-induced contraction in human bronchus ($pK_B > 8$), and, in combination with 10 μM mepyramine, to abolish antigen-induced contraction in this tissue (Hay *et al.*, 1987). SQ 29,548 has been shown to be a potent antagonist of contractions in guinea-pig trachea elicited by U-46619, the thromboxane-mimetic, or PGD_2 ($pA_2 = 8.2$ and 8.3, respectively; Ogletree *et al.*, 1985). WEB 2086 (1 μM) was reported to inhibit substantially contractions elicited by PAF in human bronchus (Johnson *et al.*, 1990). Furthermore, in the present series of experiments, in four human bronchi, mepyramine (10 μM) and atropine (1 μM) produced marked inhibition of contractions produced by histamine or carbachol, respectively (data not shown); the estimated pK_Bs were 8.5 for mepyramine and 9.3 for atropine.

Tissue preparation for mediator release studies

Human lung tissue was obtained from organ donors as described above. In addition, tissues were obtained from lung resections of anonymous lung cancer patients. The lungs were immediately placed in RPMI 1640 (4°C) solution (Gibco Co., Grand Isle, NY, U.S.A.) and transported to the laboratory. Within 24 h the bronchi (2–12 mm inner diameter) were dissected free of parenchymal tissue with the aid of a dissecting microscope. The bronchi were cut into small pieces and divided into aliquots each containing approximately 175 mg (wet weight). The bronchial tissues were incubated in 2 ml of Krebs-Henseleit solution, which was gassed with 95% O_2/5% CO_2 and maintained at 37°C. The physiological buffer was replaced at 15 min intervals for 90 min. Following this equilibration period, 2 ml of Krebs-Henseleit, containing or lacking ET-1 (10 nM or 0.3 μM), was added for 15 min. After this time the supernatant was taken to assay histamine and eicosanoid release. In addition, to determine the total tissue content of histamine, 2 ml of 0.4 N perchloric acid was added to the tissue, which was then placed in a hot water bath for 15 min. The supernatant fluid was assayed to measure the total histamine content.

Measurement of mediator release

Histamine was assayed by the automated fluorometric technique described by Siraganian (1974). Histamine release is expressed as a percentage of the total histamine content.

Leukotrienes released from bronchi were assayed by the radioimmunoassay previously described by Undem *et al.* (1987). Aliquots (100 μl) were stored at 4°C and assayed without prior purification within 48 h of each experiment. The limit of sensitivity of this assay was approximately

0.03 pmol, as defined by that amount required to inhibit [^{3}H]-LTC_4 binding by 10%. The anti-peptidoleukotriene antibody is highly selective with little affinity (cross-reactivity $<1\%$) for a variety of heterologous eicosanoids. The antibody does not, however, distinguish markedly between LTC_4, LTD_4 and LTE_4; accordingly the data indicate the levels of immunoreactive LTs (i-LTs). Standard curves with authentic LTC_4, LTD_4 and LTE_4 were parallel; the amounts of LTC_4, LTD_4 and LTE_4 required to inhibit [^{3}H]-LTC_4 binding by 50% were found to be approximately 0.4, 0.5 and 0.6 pmol/0.1 ml, respectively.

Prostanoid release into the supernatant fluid was assayed by combined gas chromatography (negative ion chemical ionization) mass spectrophotometry (GC/MS) as previously described (Hubbard *et al.*, 1986). Briefly, a 100 µl aliquot of the supernatant was added to 250 µl of acetone in a silanized vial. A mixture containing a known quantity (about 1 ng) of 3,3,4,4-tetradeuterated PGE_2, PGD_2, $PGF_{2\alpha}$, TxB_2 and 6-keto $PGF_{1\alpha}$ was added to provide internal standards for the identification and quantification of these prostanoids. In addition, the identification of 9α, 11β-PGF_2, was based on its retention to the tetradeuterated $PGF_{2\alpha}$. Samples were then dried down under a stream of nitrogen and the residue was treated with 2% methoxymine hydrochloride dissolved in pyridine. Excess pyridine was evaporated under nitrogen and the residue was subjected to sequential procedures for the synthesis of pentafluorobenzyl ester and trimethylsilyl ether derivatives as previously described (Hubbard *et al.*, 1986). CG/MS analysis of the derivatized samples (1 µl volume) was performed with a Finnigan Model 9611 gas chromatograph interfaced with a Finnigan MAT 4610B EI/CI mass spectrophotometer (Finnigan MAT Corp., San Jose, CA, U.S.A.) supplied with a Superincos data system. The sensitivity of this technique is <0.1 fmol/injection for each of the six prostanoids assayed.

Analysis of data

Agonist-induced responses for each tissue were expressed as a percentage of the reference carbachol-induced contraction added at the beginning of the experiment ('pre-carbachol'). Geometric mean EC_{50} values were calculated from linear regression analyses of data. In some tissues, due to insufficient supply of ET-1 because of cost constraints, a true maximum response could not be obtained. In these instances the contractile response to the maximum concentration of ET-1 used, 0.3 µM, was regarded as the maximum response for data analyses. Results for control- and treated-tissues were analysed for differences in both the EC_{50}s and also the maximum contractile response produced by ET-1. Mediator release is expressed as a function of the wet weight of tissues. In addition, histamine release is expressed as a percentage of total content. All data are given as the mean ± s.e.mean. Statistical analysis was conducted by ANOVA or two-tailed Student's *t* test for paired or unpaired samples where appropriate; a probability value less than 0.05 was regarded as significant.

Drugs

The following drugs were used: endothelin-1 (human, porcine) was purchased from Peninsula Laboratories (Belmont, CA, U.S.A.) or Sigma Chemical Co. (St. Louis, MO, U.S.A.). SK&F 104353 (2(S)-hydroxy-3(R)-(2-carboxyethylthio)-3-[2-(8-phenyloctyl)phenyl]-propanoic acid) was synthesized at SmithKline Beecham Pharmaceuticals (King of Prussia, PA, U.S.A.). Carbachol and mepyramine were obtained from Sigma Chemical Co. and WEB 2086 (3-(4-(2-chlorophenyl)-9-methyl-6H-thieno-(3,2-f) (1,2,4)-triazolo-(4,3-a) (1,4)-diazepine-2-yl)-1-(4-morpholinyl)-1-propanone), SQ 29,548 ([1S-[1α,2β(5Z),3β,4α]]-7-[3-[[2-[(phenylamino) carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid), sodium meclofenamate and zileuton were generous gifts from Boehringer Ingelheim (Richfield, CT, U.S.A.), Squibb Institute of Medical Research (Princeton, NJ, U.S.A.), Warner Lambert (Ann Arbor, MI, U.S.A.) and Abbott Laboratories (Chicago, IL, U.S.A.), respectively.

Results

Contractile studies

As shown in Figure 1, ET-1 was a potent and effective contractile agonist in human bronchus with an EC_{50} = 17.4 nM. In some tissues, due to insufficient supply of ET-1, a true maximum contractile response could not be obtained. Notwithstanding this caveat, and based on the contraction to 0.3 µM ET-1, the highest concentration used, representing the maximum response for data analyses, ET-1 possessed a similar potency and efficacy as LTD_4; EC_{50} (− log M): ET-1 = 7.76 ± 0.09, $n = 7$; LTD_4 = 8.46 ± 0.53, $n = 7$; $P > 0.05$; maximum response (% 10 µM pre-carbachol): ET-1 = 10.38 ± 17.4, $n = 7$; LTD_4 = 95.5 ± 9.3, $n = 7$; $P > 0.05$. Both ET-1 and LTD_4 were less efficacious agonists than carbachol: carbachol contraction at the end of the experiment ('post-carbachol') = 166 ± 8.4% of pre-carbachol ($P < 0.05$, compared to ET-1 or LTD_4).

Sodium meclofenamate (1 µM), the cyclo-oxygenase inhibitor, although it increased the contractile response to 10 nM and 30 nM ET-1, was without significant effect on the ET-1 EC_{50} or maximum contractile response; EC_{50} (− log M): control = 7.35 ± 0.09, $n = 7$; + sodium meclofenamate = 7.59 ± 0.05, $n = 7$; $P > 0.05$; maximum contractile response (% 10 µM pre-carbachol): control = 99.2 ± 25.8, $n = 7$; + sodium meclofenamate = 98.7 ± 12.4, $n = 7$; $P > 0.05$ (Figure 2a). Furthermore, in the absence of sodium meclofenamate, the potent and selective thromboxane receptor antagonist, SQ 29,548 (1 µM), had no effect on ET-1 concentration-responses curves (Figure 2b).

A series of studies, conducted in the presence of sodium meclofenamate, was performed to examine the effects of various receptor antagonists on ET-1-induced contraction. Previous studies in our laboratory have indicated that SK&F 104353 or mepyramine generally had minor effects on antigen-induced contraction of guinea-pig trachea or human bronchus, whereas the combination of the antagonists essentially abolished the response (Hay *et al.*, 1987). In this experi-

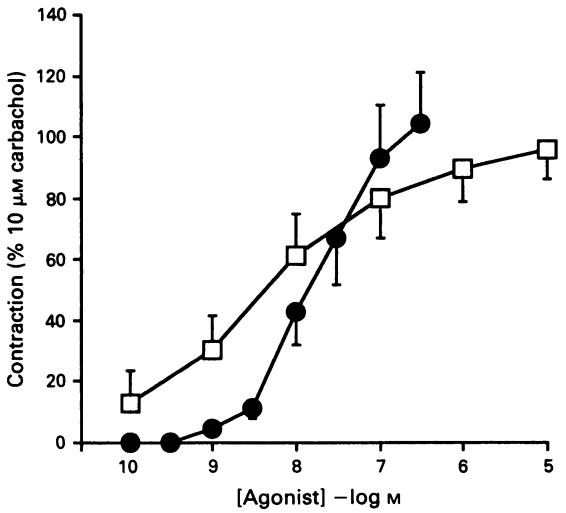

Figure 1 Comparison of endothelin-1 (ET-1) and leukotriene D_4 (LTD_4) concentration-response curves in human isolated bronchus. Results are expressed as a percentage of the response to 10 µM pre-carbachol and are the mean ± s.e.mean of 4 experiments. (●) ET-1; (□) LTD_4. Studies were conducted in the presence of 1 µM sodium meclofenamate.

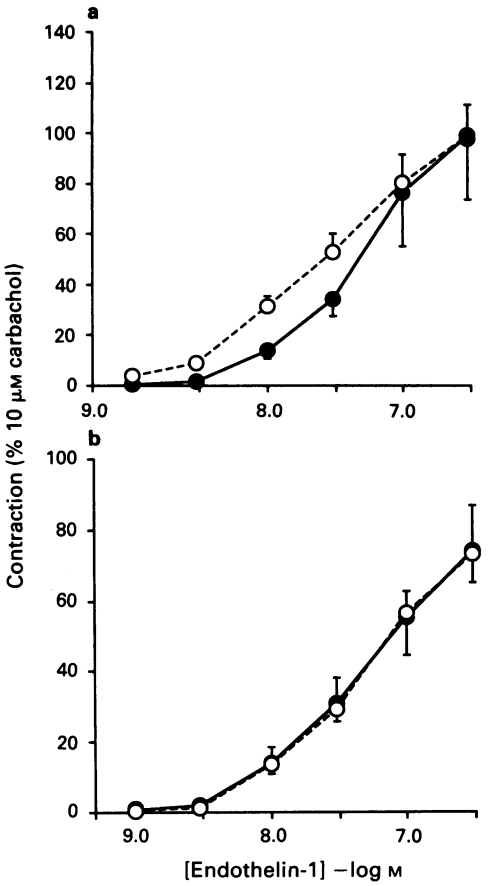

Figure 2 Effects of (a) the cyclo-oxygenase inhibitor, sodium meclofenamate (1 μM), or (b) the thromboxane receptor antagonist, SQ 29,548 (1 μM) on endothelin-1 (ET-1) concentration-response curves in human isolated bronchus. Results are expressed as a percentage of the response to 10 μM pre-carbachol and are the mean ± s.e.mean of (a) 7 or (b) 6 experiments; (a) (●) control; (○) + 1 μM sodium meclofenamate; (b) (●) control; (○) + 1 μM SQ 29,548.

ment the combination of SK&F 104353 (10 μM) and mepyramine (10 μM) had no effect on contraction elicited by ET-1 (Figure 3a). In addition, WEB 2086 (10 μM) or atropine (1 μM) had no significant effect on ET-1 concentration-response curves (Figures 3b and c). Furthermore, the combination of SK&F 104353 (10 μM), mepyramine (10 μM), WEB 2086 (1 μM) and atropine (1 μM) also was without effect on ET-1-induced contractions (Figure 4).

Mediator release

The ability of 0.3 μM ET-1, the highest concentration of ET-1 used in contractile studies, to elicit the release of leukotrienes (measured by RIA), histamine (measured fluorometrically) and six prostanoids (measured by GC/MS methods) from human bronchus was examined. All the mediators were found to be released under basal conditions (Table 1). The two major prostanoids released under basal conditions were PGE_2 and the prostacyclin metabolite, 6-keto $PGF_{1\alpha}$. The spontaneous release of immunoreactive LTs (i-LTs) was relatively high, averaging 14 ng g^{-1} tissue. The immunoreactivity was not further evaluated regarding the authenticity of the reactant; however, in a separate series of 9 experiments the spontaneous release of i-LT was only marginally inhibited by 10 μM zileuton, the 5-lipoxygenase inhibitor (Carter *et al.*, 1989); control = 8.6 ± 2.0 ng g^{-1} tissue; + zileuton = 6.5 ± 1.8 ng g^{-1} tissue ($P = 0.053$). The spontaneous release of histamine was less than 1% of total histamine content.

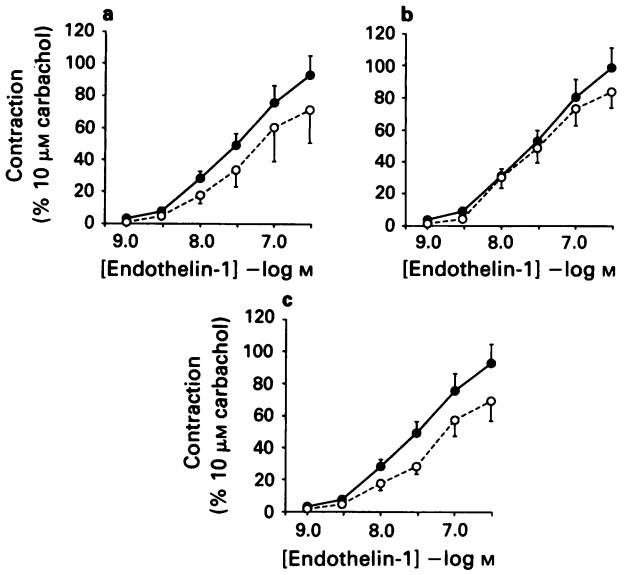

Figure 3 Effects of (a) the combination of the leukotriene receptor antagonist, SK&F 104353 (10 μM) and the H_1-histamine receptor antagonist, mepyramine (10 μM); (b) the PAF receptor antagonist, WEB 2086 (1 μM), or (c) the muscarinic receptor antagonist, atropine (1 μM) on endothelin-1 (ET-1) concentration-response curves in human isolated bronchus. Results are expressed as a percentage of the response to 10 μM pre-carbachol and are the mean ± s.e.mean of (a) 8, (b) 7 and (c) 8 experiments; (a) (●) control; (○) + 10 μM SK&F 104353 and 10 μM mepyramine; (b) (●) control; (○) + 1 μM WEB 2086; (c) (●) control; (○) + 1 μM atropine. Studies were conducted in the presence of 1 μM sodium meclofenamate.

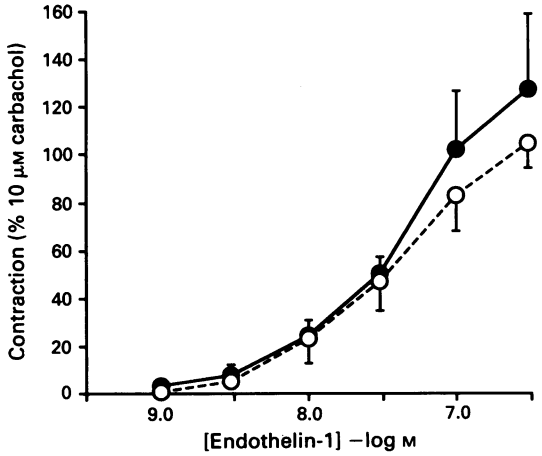

Figure 4 Effects of the combination of the leukotriene receptor antagonist, SK&F 104353 (10 μM), the H_1-histamine receptor antagonist, mepyramine (10 μM), the PAF receptor antagonist, WEB 2086 (1 μM) and the muscarinic receptor antagonist, atropine (1 μM) on endothelin-1 (ET-1) concentration-response curves in human isolated bronchus. Results are expressed as a percentage of the response to 10 μM pre-carbachol and are the mean ± s.e.mean of 5 experiments. (●) control; (○) + receptor antagonists. Studies were conducted in the presence of 1 μM sodium meclofenamate.

ET-1 (0.3 μM; 15 min exposure) had no significant effect on the release of histamine or i-LT from the bronchi. In contrast, ET-1 produced a marked increase in the production of each of the six prostanoids analysed. The most robust effect was on the production of PGD_2 where a 7.5 fold increase was observed. Approximately 10% of the PGD_2 was released from the tissue as its metabolite 9α, 11β PGF_2. The increase in the other prostanoids averaged about 1.6 to 2.7 fold over spontaneous release levels. These data are summarized in Table 1. Note, a lower concentration of ET-1, 10 nM, did not elicit a significant increase in the release of any of the prosta-

Table 1 Endothelin-1 (ET-1)-induced autacoid release from human isolated bronchus[a]

Autacoid	Spontaneous	Release ET-1 (0.3 μM)	n[c]
Eicosanoids (ng g^{-1})			
PGD_2	0.73 ± 0.31	5.51 ± 2.11**[b]	12
9α, 11β PGF_2	0.09 ± 0.03	0.56 ± 0.19**	12
TxB_2	0.17 ± 0.07	0.45 ± 0.17**	12
PGE_2	3.11 ± 0.87	6.07 ± 1.20**	12
6-keto $PGF_{1\alpha}$	2.26 ± 0.81	3.77 ± 0.90**	12
$PGF_{2\alpha}$	0.60 ± 0.17	0.94 ± 0.22*	12
i-LT	14.1 ± 9.3	10.4 ± 3.7 NS	6
Histamine (%)	0.76 ± 0.12	1.32 ± 0.21 NS	7

[a]The release of various autacoids during a 15 min period, from human isolated bronchial tissue in the absence or presence of ET-1 (0.3 μM) was quantified as outlined in Methods. Briefly, human bronchi were cut into small fragments and equilibrated at 37°C in 2 ml of buffer solution, which was replaced at 15 min intervals. After 60 min vehicle was added for a 15 min period and the solution was analysed for 'spontaneous' mediator release. The buffer was again replaced and ET-1 or vehicle was added for 15 min and the supernatant fluids analysed for ET-1-induced mediator release values or time-control values, respectively. There was no significant difference between two consecutive 15 min spontaneous release values for any mediator except prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$. With respect to these mediators the increase in release over time averaged 1.4, 1.2 and 1.3 fold, respectively. The release of the prostanoids and i-LT are expressed as ng g^{-1} tissue wet weight. The release of histamine is expressed as a percentage of total histamine content which averaged 4.1 μg g^{-1} ($n = 7$).

[b]Denotes a statistically significant difference (*$P < 0.05$; **$P < 0.01$) between the amount of autacoid released in the absence (spontaneous release) and presence of ET-1, based on Student's t test for paired observations. For PGE_2, $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$ (prostanoids whose release was found to increase with the 15 min incubation time used, see above), the asterisk denotes that the mediator release after ET-1 was significantly greater than that observed with time alone (Student's t test for unpaired data). NS denotes no significant increase.

[c]n indicates the number of experiments (each lung provided tissue for a single experiment).

noids ($n = 4$, data not shown). In two separate experiments 1 μM sodium meclofenamate abolished ET-1-induced release of all the prostanoids (data not shown).

Discussion

The results confirmed that ET-1 is a potent contractile agonist of human bronchus with an EC_{50} of approximately 17 nM, which is in agreement with values reported previously (Advenier *et al.*, 1990; Hemsén *et al.*, 1990; Henry *et al.*, 1990; Brink *et al.*, 1991; McKay *et al.*, 1991). The potency of ET-1 observed in this study is similar to that which has been reported generally for guinea-pig isolated trachea (Hay, 1989; 1990; Maggi *et al.*, 1989; Henry *et al.*, 1990).

The functional data from the present study also demonstrate that the contractile response to ET-1 in human bronchus does not appear to involve the release of significant amounts of acetylcholine, histamine, leukotrienes, PAF or thromboxane. Thus, atropine, the classical muscarinic receptor antagonist, WEB 2086, the PAF receptor antagonist (Casals-Stenzel, 1987a,b), SQ 29,548, the thromboxane receptor antagonist (Ogletree *et al.*, 1985), or the combination of mepyramine, the H_1-histamine receptor antagonist, and SK&F 104353, the leukotriene receptor antagonist (Hay *et al.*, 1987), were without marked effect on ET-1 concentration-response curves. The concentrations of these receptor antagonists employed in this study were those which had been observed to inhibit markedly contractions produced by the natural ligands in human bronchus and/or guinea-pig trachea (see above). It has been demonstrated previously that the combination of mepyramine and SK&F 104353 was much more effective than either agent alone at inhibiting antigen-induced, mast cell-dependent contraction of human bronchus or guinea-pig trachea, suggesting that in the presence of the antagonist of one mediator, there is a sufficient quantity of the other mediator released to elicit the maximum, or close to the maximum, contractile response (Hay *et al.*, 1987). It is possible that a similar, or even more complex, scenario may occur with ET-1-induced contraction of human bronchus, such that ET-1 may stimulate the release of multiple mediators which contribute to the contractile response. However, this does not appear to be the case, as the combination of atropine, mepyramine, SK&F 104353 and WEB 2086, in the presence of sodium meclofenamate, the cyclo-oxygenase inhibitor, was without marked effect on ET-1 concentration-response curves. In agreement with functional studies, 0.3 μM ET-1, which is close to the maximally effective concentration, did not stimulate the release of histamine or i-LTs from human bronchus. In contrast to these findings it has been reported recently that ET-1 potently stimulates histamine release from guinea-pig lung parenchymal, but not peritoneal mast cells (Uchida *et al.*, 1992).

We have previously reported that ET-1 did not stimulate the release of histamine and various potent and selective receptor antagonists were without effect on contraction elicited by ET-1 in guinea-pig trachea (Hay & Undem, 1983). These and the present data suggest that ET-1 contracts human isolated bronchus and guinea-pig trachea by a similar, direct mechanism(s) which does not involve the release of secondary mediators. However, other studies have provided evidence that ET-1-induced contraction of guinea-pig trachea is in part mediated via the release of histamine, PAF and/or thromboxane (Ninomiya *et al.*, 1989; Nomura *et al.*, 1990; Battistini *et al.*, 1990; Filep *et al.*, 1990; Uchida *et al.*, 1992).

Contractile responses to ET-1 in human bronchus and guinea-pig trachea have a similar resistance to inhibition by voltage-dependent calcium channel inhibitors and appear to involve comparable calcium translocation mechanisms, i.e. predominantly the release of intracellular calcium (Maggi *et al.*, 1989; Hay, 1990; Sarriá *et al.*, 1990; Advenier *et al.*, 1990; McKay *et al.*, 1991). Thus, in both tissues ET-1-induced contraction appears to be mediated via an interaction with specific ET receptors and subsequent stimulation of the phosphatidylinositol pathway (Hay, 1990; Mattoli *et al.*, 1991b). Collectively, the above data indicate that the guinea-pig trachea is a good *in vitro* model tissue for human isolated airways to examine the bronchoconstrictor effects of ET-1.

Sodium meclofenamate, a cyclo-oxygenase inhibitor was without marked effect on ET-1 concentration-response curves. This finding is similar to those of previous studies which indicated that indomethacin had no effect on ET-1-induced contraction of human bronchi (Henry *et al.*, 1990; Advenier *et al.*, 1990; McKay *et al.*, 1991) and suggests that cyclo-oxygenase products do not significantly modulate ET-1-induced contraction in human bronchus.

Despite cyclo-oxygenase inhibitors exerting no effect on the ET-1-induced contractile responses. ET-1, albeit in a high concentration of 0.3 μM, was an effective stimulant of prostaglandin production in the human bronchus, enhancing the release of all six prostanoids measured. ET-1 caused a 7.5 fold increase in the production of PGD_2 in human isolated bronchus. The cellular source of the PGD_2 or the other prostanoids cannot be discerned from our results. Mast cells and macrophages are two types of cell capable of producing PGD_2 in the human airways (Lewis *et al.*, 1981; Balter *et al.*, 1988; Yoss *et al.*, 1990). The fact that ET-1 failed to enhance significantly histamine or i-LT production suggests either mast cells were not stimulated, or they were stimulated in a

unique manner such that only cyclo-oxygenase metabolites were formed. ET-1-induced PGD_2 production, in the absence of histamine release, is consistent with its effect on guinea-pig isolated trachea (Hay, Hubbard & Undem, unpublished observations), and canine bronchoalveolar lavage preparations (Ninomiya *et al.*, 1992). Although the human lung macrophages produce PGD_2, the major prostanoid produced upon stimulation of this cell is thromboxane A_2 (Balter *et al.*, 1988; Yoss *et al.*, 1990). In contrast, ET-1 in the present study stimulated the release of about 17 times more PGD_2 than thromboxane. The microvasculature may be a source for some of the ET-1 induced prostanoid production in the airway. Thus, ET-1 has been found to stimulate the production of prostacyclin from bovine cultured aortic endothelial cells (Filep *et al.*, 1991). In guinea-pig trachea experiments using epithelium-containing and epithelium-denuded tissues provided evidence to suggest that the epithelium is not the source of the prostanoids released by ET-1 (Hay, Hubbard & Undem, unpublished observations).

In summary, the present data provide evidence that ET-1 produces potent contraction of human bronchus predominantly via a direct action, which does not involve a significant contribution of the release of acetylcholine, histamine, leukotrienes or PAF. ET-1 does not evoke the release of histamine or i-LT from the human bronchus, but is an effective stimulus for prostanoid production. However, the released prostanoids exert no significant modulatory influence on ET-1-induced contraction in human bronchus, although the possibility remains that these autacoids may play a significant role in mediating or modulating other effects of ET-1 in the respiratory system.

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