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The mechanism of action of endothelin in human lung

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1 The peptides endothelin-1 (ET-1) and endothelin-2 (ET-2) elicited potent and sustained contractions of human isolated bronchus and pulmonary artery.

2 ET-1 is one of the most potent contractile agonists investigated in these tissues with an EC_{50} value of 18.3 nM (95% confidence interval: 12.9, 25.9 nM; $n = 26$) in bronchus and 3.2 nM (95% confidence interval: 0.4, 23.9 nM; $n = 5$) in the arterial preparation.

3 ET-1 is 2.5 times more potent than ET-2 in both the airway and vascular tissues, and both forms of the peptide have geometric mean EC_{50} values 5 times greater in the isolated bronchial tissue than in the pulmonary artery.

4 Neither pretreatment with the voltage-dependent calcium (VDC) channel antagonist verapamil (10 μM) nor with indomethacin (25 μM) significantly altered the response curve to ET-1 in human isolated bronchus. Removal of calcium from the Krebs-Henseleit solution did not affect ET-1-induced responses.

5 Specific binding on the smooth muscle of human airway and pulmonary arterial tissue to both ET-1 and ET-2 was detected in autoradiographic studies. There appeared to be no difference between the peptides in the location nor the density of binding sites.

6 We conclude that contraction of human bronchial tissue by ET-1 is not dependent upon influence of extracellular calcium nor release of prostaglandins or thromboxane A_2. It is likely that the action of ET-1 in this tissue is due to binding of this peptide to specific receptors located on the smooth muscle.

Introduction

Endothelin is a 21 amino acid peptide which has extremely potent vasoconstrictor properties and is produced by vascular endothelial cells (Yanagisawa *et al.*, 1988). It is now apparent that there are three forms of endothelin (endothelin-1 (ET-1), ET-2 and ET-3), hence there is the possibility of more than one endothelin receptor subtype (Inoue *et al.*, 1989). It may be that endothelin, the most potent vasoconstrictor substance yet isolated, has properties which are relevant to respiratory diseases such as asthma. In a recent report, markedly elevated levels of endothelin were detected in the lavage fluid of a patient in 'status asthmaticus' (Nomura *et al.*, 1989). Little is known of the conditions under which endothelin is released but hypoxia (Hiley, 1989) or a decrease in temperature (Fyhrquist *et al.*, 1990) may be provoking stimuli, both of these factors being associated with asthma. Events occurring in the vasculature (where endothelin is probably produced), may affect not only the calibre of the airways themselves, but also local concentrations of bronchoactive mediators.

The effects and mechanism of action of ET-1 on the vasculature of many species have been investigated in some depth. Ohtsuka *et al.* (1989) have detailed the contractile action of ET-1 on human pulmonary artery strips and have also reported binding sites for this peptide on the smooth muscle of these vessels. Intravenous or aerosol administration of endothelin to guinea-pigs results in bronchoconstriction (Braquet *et al.*, 1989). *In vitro* studies have shown that both ET-1 and ET-3 induce contraction of guinea-pig isolated trachea and bronchus (Maggi *et al.*, 1989; 1990; Battistini *et al.*, 1990), but the mechanism by which this contraction is generated remains unclear. There are as yet no quantitative data available on the functional effects of endothelin in human isolated airways, although there is an isolated report of the contractile action on human bronchus (Uchida *et al.*, 1988).

The mode of action of endothelin has attracted considerable interest. Results of initial studies carried out in vascular tissue indicate that endothelin may act as an endogenous ligand for voltage-dependent calcium (VDC) channels, while others have found that it promotes calcium entry into the cell via activation of non voltage-dependent rather than voltage-dependent calcium channels. The results of a recent study suggest that contraction of guinea-pig trachea and bronchus may be due to release of thromboxane A_2 (Battistini *et al.*, 1990).

In the present study, we compared the contractile effects of ET-1 and ET-2 on human isolated pulmonary arterial and bronchial segments and used *in vitro* autoradiography to identify possible binding sites for both of these forms of endothelin in these tissues. In addition, we examined the contractile activity of ET-1 in human bronchus under conditions of calcium exclusion and in the presence of the VDC antagonist, verapamil, to investigate whether ET-1 acts as a modulator of the VDC channel in these tissue preparations. We also studied contractions generated in the presence of the cyclo-oxygenase inhibitor indomethacin, to determine if the action of ET-1 is due to the liberation of prostaglandins or thromboxane A_2.

Methods

Functional studies

Tissue was obtained after removal from patients undergoing lung resection for carcinoma as previously described (Armour *et al.*, 1986; Black *et al.*, 1986). Consent had been obtained prior to surgery and the protocol had been approved by the Medical Ethical Review Committee of The University of Sydney. Segments of bronchus and pulmonary artery 3–5 mm in internal diameter were dissected free of surrounding tissue and cut into rings measuring approximately 3–4 mm in length.

Subsequently, paired tissue preparations were suspended on stainless steel hooks under an optimal load of 1 g (bronchus) or 1–1.5 g (artery) (Armour *et al.*, 1988) in jacketed organ baths containing Krebs-Henseleit solution (composition in mM: NaCl 118.4, KCl 4.7, $CaCl_2 \cdot 2H_2O$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and D-glucose 11.1) maintained at 37°C and continuously aerated with 5% CO_2 in 95% O_2. During a 1 to 3 h equilibration period, the tension was readjusted to the load originally applied and the bath fluid was changed at 15–20 min intervals.

Changes in tension in response to the addition of an agonist to the bath were measured isometrically with Grass FT03

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transducers and were recorded on Grass polygraphs. Whenever possible the tissue pairs were studied in duplicate.

Bronchial tissue

Studies of the comparative potency and efficacy of endothelin-1 and endothelin-2 Initially, comparisons of two of the forms of endothelin were made by obtaining cumulative concentration-response curves (10 pM to 0.3 μM) in parallel in four tissues from the same patient. When baseline tone was stable, a bolus dose of acetylcholine (1 mM) was added to the bronchial preparations. When the contractile response to acetylcholine had reached a maximum, the tissues were washed repeatedly until a stable baseline tone had been re-established. Cumulative concentration-response curves were then obtained to either ET-1 or ET-2 in each tissue pair.

The effect of verapamil Following establishment of baseline tone, one of each of the pair of tissues received 10 μM verapamil and 30 min later the ET-1 cumulative concentration-response curve was started. After completion of the concentration-response curve, and with no intervening washing of the tissues, a bolus dose of carbachol (1 mM) was administered to each tissue.

The effect of calcium-free Krebs-Henseleit solution In this series of experiments, the contractile responses to both ET-1 and histamine were elicited in the presence and absence of extracellular calcium. The maximal contractile response generated as a result of the addition of 1 mM acetylcholine was determined as above. When the maximal response had been attained, half of the tissues were washed twice (at 15 min intervals) with Krebs-Henseleit solution from which the calcium had been omitted and to which 1 mM EGTA had been added. Subsequently, the bathing solution was changed to one with no calcium and no EGTA and the tissues were washed three times with this solution. The remaining tissues, which acted as controls, were washed as previously with Krebs-Henseleit solution of the usual composition. When baseline tone was stable in all tissues, cumulative concentration-response curves to either ET-1 or histamine were started.

The effect of indomethacin In these experiments, following addition of 1 mM acetylcholine and washout as above, indomethacin (25 μM) was added to half of the tissues, the other half being left as controls. After 30 min and when tone was stable, cumulative concentration-response curves to ET-1 were started in all tissues.

Vascular tissue

In this series of experiments, a comparison was made of the relative potency and efficacy of ET-1 and ET-2 in pulmonary arterial tissue. Following addition of 1 mM noradrenaline the tissues were washed and when baseline tone had been reestablished, cumulative concentration-response curves were obtained (10 pM to 0.3 μM) for ET-1 and ET-2 in paired tissues.

Autoradiographic studies

To investigate the location of binding sites for ET-1 and ET-2, autoradiographic studies were carried out on human bronchus ($n = 6$) and pulmonary artery ($n = 10$). The source of tissue for these experiments was generally the same as that for the functional studies. In some cases ($n = 2$) however, bronchial tissue was obtained at autopsy. Specimens were frozen in Tissue-Tek embedding medium over liquid nitrogen and stored at -70°C until required. Twelve serial sections of 10 μM thickness from each specimen were cut on a cryostat and thaw mounted onto glass slides with a gelatin coating. Slides of adjacent serial sections were formed into six pairs, one of each pair being used to designate 'total binding', while the other was used to estimate 'non-specific binding'. After three 5 min washes in Tris-HCl buffer (pH 7.4) containing NaCl 100 mM, $MgCl_2$ 5 mM, bacitracin 40 mg ml^{-1} and bovine serum albumin 0.3%, six slides from each patient (i.e. those labelled 'total binding') were incubated for 30 min at 21°C in buffer containing 25 pM [^{125}I]-ET-1 (specific activity: 1500 Ci mmol^{-1}).

'Non-specific binding' was assessed in the presence of 1 μM unlabelled ET-1, this concentration being in excess to ensure that all ET-1 binding sites were occupied by the unlabelled agonist. The six remaining slides from each patient were placed in this solution for 30 min.

At the end of the incubation period, the slides were washed in ice-cold Tris buffer, then in water and dried under a stream of cold air. The slides were placed in a dessicator and kept in the refrigerator overnight. Glass coverslips coated with Amersham LM-1 liquid emulsion (diluted 1 : 1 with water) were then placed in apposition to the slides the following day. The slide assemblies were stored in light-proof X-Ray boxes at 4°C for 14–16 days.

At the conclusion of this time, the slide/coverslip assemblies were developed with Ilford Phenisol developer and the sections stained with methyl-green pyronin. After dehydration of the sections in increasing concentrations of alcohol, the coverslips were attached to the slides with mounting medium.

The various histological structures were first identified under bright-field microscopy at which time the light condenser was changed to a dark-field one and the endothelin binding sites were visualised as illuminated silver grains over the tissue.

The above procedure was then repeated, this time with 25 pM [^{125}I]-ET-2 (specific activity: 2000 Ci mmol^{-1}) and 1 μM unlabelled ET-1.

Analysis of results

In each tissue preparation, contractile responses to each concentration of agonist were expressed as a percentage of the maximal response to acetylcholine, carbachol, or in the case of vascular tissue, to noradrenaline. A graph was then constructed relating cumulative concentration of agonist to response. From this an EC_{50} value was derived. Where preparations were studied in duplicate, a mean response curve was constructed for each experiment and a geometric mean EC_{50} value calculated. An overall mean curve was then constructed for all experiments for both control and treated tissues and a geometric EC_{50} value with 95% confidence limits obtained from individual experimental values. Responses to all concentrations of agonist obtained in the presence and absence of calcium and in the presence and absence of verapamil or indomethacin were then compared by a two-tailed Student's t test for paired data. Differences were considered significant when $P < 0.05$.

Drugs used

Both ^{125}I-radiolabelled and unlabelled ET-1 were obtained from Auspep (Australia); ET-2 from The Peptide Institute (Japan); [^{125}I]-ET-2 from Amersham (U.K.); acetylcholine, carbamylcholine chloride (carbachol), ethylene glycol-*bis*(β-aminoethyl ether)N,N,N′,N′-tetraacetic acid (EGTA), histamine acid phosphate, indomethacin and noradrenaline ((−)-arterenol) from Sigma (U.S.A.) and verapamil from Knoll (U.S.A.). Both forms of endothelin were dissolved in 0.1 M acetic acid and noradrenaline in 10 mM HCl. Stock solutions of other drugs were prepared in distilled water, except for indomethacin which was prepared in 5% $NaHCO_3$. Aliquots were kept frozen between experiments and diluted in Krebs-Henseleit solution on the day of the experiment. Where applicable, separate series of dilutions of ET-1 and histamine were made in calcium-free Krebs-Henseleit solution.

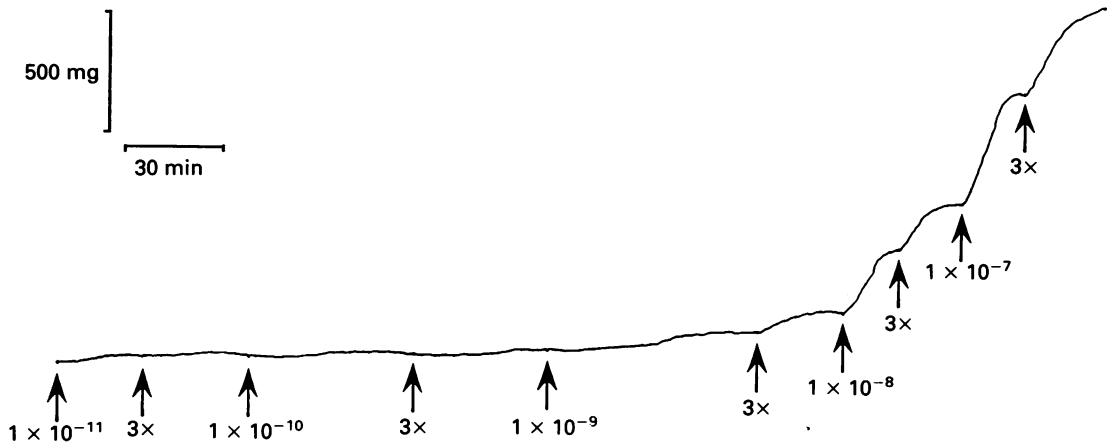

Figure 1 An example of the contractile effect of endothelin-1 on a human bronchial segment. Endothelin-1 was cumulatively added to the organ bath to give the final concentration shown (M).

Results

Functional studies

Endothelin produced protracted contractions of both the bronchial and arterial preparations. The contractile response to each concentration took up to 40 min to reach a plateau and thus a cumulative concentration-response curve took approximately 6 h to complete (Figure 1). Therefore, only one response curve was carried out in each tissue. In some experiments we attempted to reverse the effects of endothelin by repeated washing of the tissues. Washout was extremely slow and was incomplete up to 90 min.

Bronchial tissue

Studies of the comparative potency and efficacy of endothelin-1 and endothelin-2 Both forms of endothelin induced potent contractions of human isolated bronchial tissue (Figure 2). ET-1 and ET-2 proved to be equally efficacious in airway tissue, producing average contractions in response to 0.3 μM ET-1 or ET-2 that were equivalent to 106 ± 12% and 95 ± 11% ($n = 6$) of those produced by 1 mM acetylcholine. There did however, appear to be a difference in the threshold concentrations required for contraction and the EC_{50} values. The initial contraction resulting from addition of ET-1 to the organ bath occurred at 0.1 nM, whereas in the case of ET-2 the threshold contraction occurred at 1 nM. Parallel studies of ET-1 and ET-2 in bronchial tissue resulted in an EC_{50} value of 17.9 nM [95% confidence interval: 8.0, 40.3 nM] for ET-1 while that for ET-2 was 44.8 nM [95% CI: 20.5, 97.7 nM] and this difference was statistically significant ($P = 0.04$, $n = 6$).

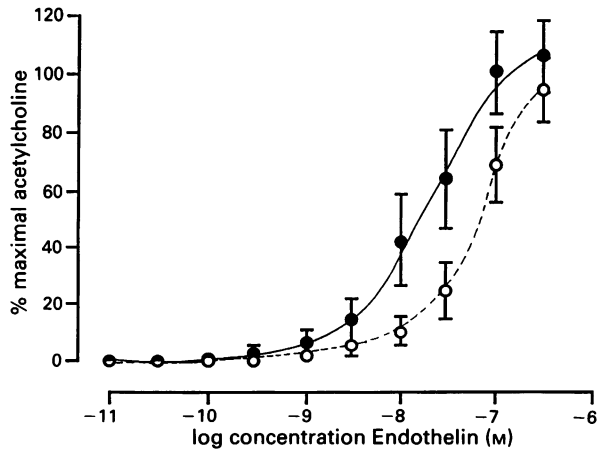

Figure 2 Mean cumulative concentration-response curves for endothelin-1 (●) and endothelin-2 (○) on human isolated bronchus. Mean responses from 6 experiments are expressed as a percentage of the maximal response to 1 mM acetylcholine. S.e.mean values are shown as vertical bars.

The effect of verapamil Blockade of the voltage-dependent calcium channels with 10 μM verapamil did not alter the potency or the efficacy of ET-1 (Table 1). Addition of verapamil to the bath did however, decrease the baseline tension by an average of 281 ± 72 mg ($n = 10$ tissues).

The effect of calcium-free Krebs-Henseleit solution The ET-1 cumulative concentration-response curve was also unaffected by removal of extracellular calcium (Figure 3a), the geometric mean EC_{50} in this instance being 10.8 nM [95% CI: 2.9, 40.0 nM ($n = 4$)] and the maximal contraction generated was 92 ± 25% of that induced by 1 mM acetylcholine (not significantly different from the values obtained in the presence of calcium). Contractile responses to histamine were however, markedly attenuated in a calcium-free environment obtained by the same method of calcium exclusion (Figure 3b). The mean maximal contraction to histamine in the presence of calcium was 127 ± 5% of the acetylcholine maximum whereas in the absence of calcium it was 41 ± 20% ($n = 4$).

The effect of indomethacin Neither the potency nor the efficacy of the contraction generated by ET-1 was affected by 25 μM indomethacin (Table 1). Addition of indomethacin to the bath induced a 306 ± 80 mg ($n = 3$) decrease in the baseline tone.

Vascular tissue

Contraction in response to addition of ET-1 to pulmonary artery rings occurred over the concentration-range 30 pM to 0.3 μM. For ET-2 however, the initial contraction was elicited at a bath concentration of 1 nM. At 0.3 μM, the highest concentration used, the cumulative concentration-response curves appeared to have reached plateaus, suggesting that the full contractile response of the tissue had been elicited (Figure 4). Neither the potency nor the efficacy of ET-1 was significantly different from those of ET-2. The geometric mean EC_{50} for ET-1 was 3.24 nM [95% CI: 0.44, 23.93 nM ($n = 5$)] while that for ET-2 was 8.04 nM [95% CI: 3.74, 17.22 nM]. The corresponding maximal contractions generated were 256 ± 71% and 226 ± 52% of the response to 1 mM noradrenaline in these tissues.

Autoradiographic studies

Binding sites appeared as uniformly sized white spots when dark field illumination was used. Dense, specific binding sites for [^{125}I]-ET-1 and [^{125}I]-ET-2 were localized on the smooth muscle of both the airway and pulmonary arterial tissue

Table 1 Mean values for maximum tension and sensitivity to endothelin in human bronchus in the presence and absence of verapamil or indomethacin

	Control	*Verapamil*	*Control*	*Indomethacin*
Mean T_{max} mg (±s.e.mean)	1260 (443)	1260 (415)	663 (100)	898 (204)
Mean $\%T_{max}$ (±s.e.mean)	67.1 (6.8)	70.3 (7.5)	129.1 (25.4)	139.0 (4.6)
Mean EC_{50} M (95% CI)	2.9×10^{-8} (1.3, 6.1)	2.3×10^{-8} (1.4, 3.8)	0.7×10^{-8} (0.1, 7.4)	1.4×10^{-8} (0.1, 19.0)
n	7	7	3	3

Maximum tension (T_{max}) is expressed as mg or as a % of the response to 1 mM carbachol/acetylcholine. Standard error of the mean (s.e.mean) values are shown in parentheses. Geometric mean EC_{50} values and the 95% confidence intervals (CI) are also shown; *n* refers to the number of experiments performed.

(Figure 5), in addition to binding over airway ganglia and alveoli. There appeared to be no binding associated with the epithelium or the endothelium. There was no obvious difference between either form of endothelin in the density or the location of binding. 'Non-specific binding' was uniformly low and was unassociated with histological structures for both peptides. The location and density of binding sites over the section appeared to be independent of the source of the tissue (i.e. whether it was from surgical or post mortem specimens).

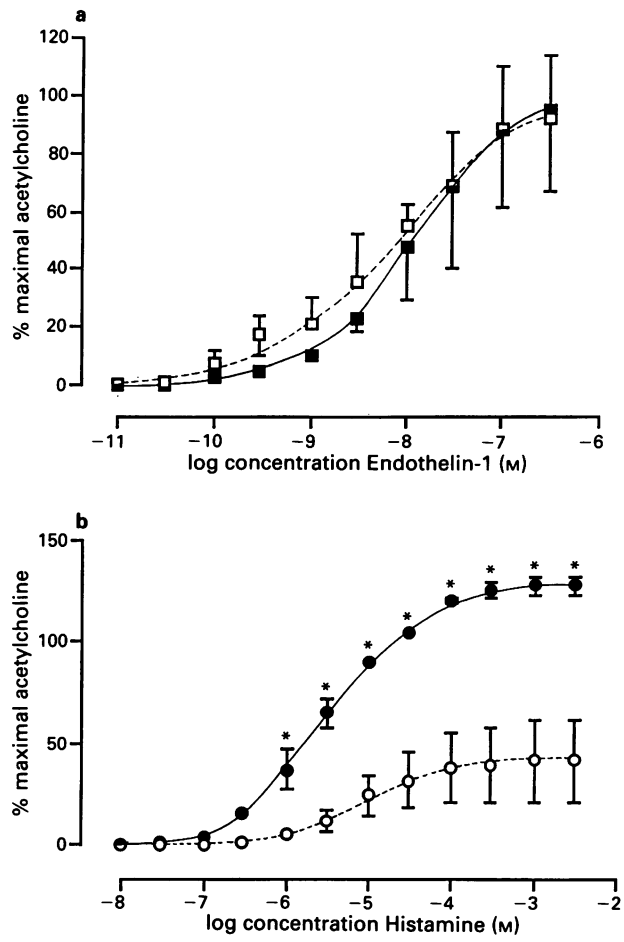

Figure 3 (a) Mean cumulative concentration-response curves for endothelin-1 in the presence (■) and absence (□) of extracellular calcium. Mean responses from 4 experiments are expressed as a percentage of the maximal response to 1 mM acetylcholine. S.e.mean values are shown as vertical bars. (b) Mean cumulative concentration-response curves for histamine in the presence (●) and absence (○) of extracellular calcium. Mean responses from 3 experiments are expressed as a percentage of the maximal response to 1 mM acetylcholine. S.e.mean values are shown as vertical bars. *indicates significant ($P < 0.05$) differences in the maximal responses.

Discussion

This study has shown that endothelin has an extremely potent contractile action on human airway and vascular tissue. Its action in isolated airways cannot be attributed to activation of voltage-dependent calcium channels or the liberation of cyclo-oxygenase products, and is independent of extracellular calcium. In addition, binding sites for two of the forms of endothelin (ET-1 and ET-2) have been visualized on sections of human bronchus and pulmonary artery suggesting that there are distinct receptors for endothelin on the smooth muscle of the vessels and bronchus of human lung.

The potency of ET-1 as a contractile agent of bronchial tissue in this study represented by a geometric EC_{50} of 18.3 nM was similar to that of leukotriene D_4 (Hanna *et al.*, 1981) and the thromboxane A_2-mimetic U46619 (Armour *et al.*, 1989), but far greater than that of carbachol and histamine (Black *et al.*, 1986). Its efficacy was however, less than that of carbachol and therefore of U46619 which produces a greater contractile response than carbachol in human airway tissue. Due to limited availability of the peptide, the maximal bath concentration attained in these experiments was 0.3 μM. At this point it was not possible to ascertain whether a maximal response had been reached. Thus, it is possible that the values representative of the efficacy of endothelin, namely T_{max} and $\%T_{max}$

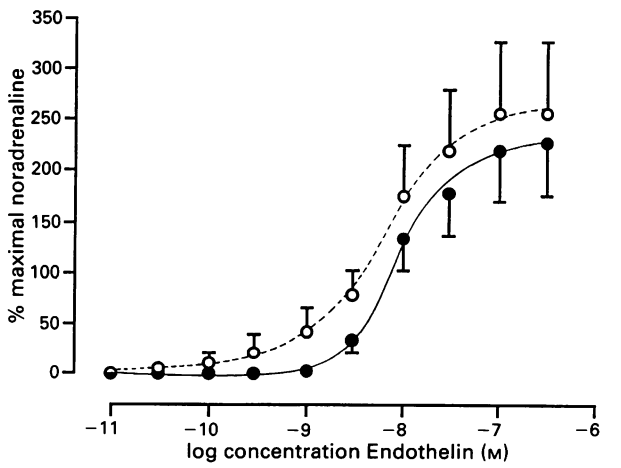

Figure 4 Mean cumulative concentration-response curves for endothelin-1 (○) and endothelin-2 (●) on human isolated pulmonary artery. Mean responses from 5 experiments are expressed as a percentage of the maximal response to 1 mM noradrenaline. S.e.mean values are shown as vertical bars.

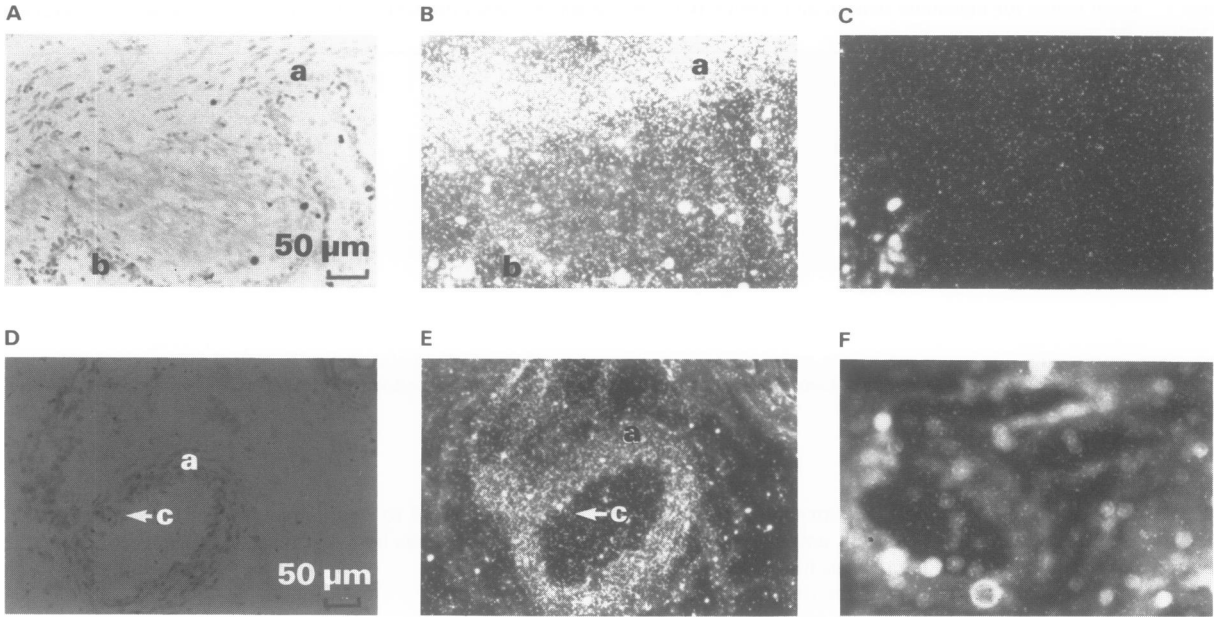

Figure 5 Photomicrographs of sections of human bronchus (A, B and C) and pulmonary artery (D, E and F). (A) and (D) are light field photomicrographs; central and right hand panels are dark field photomicrographs of adjacent sections incubated in [^{125}I]-endothelin-1 (B and C) and [^{125}I]-endothelin-2 (E and F) alone and in the presence of unlabelled endothelin-1 (C and F). (a) Smooth muscle, (b) epithelium and (c) endothelium.

may be underestimated in this preparation. If this were the case, then the mean values for EC_{50} may in fact be higher.

There is only one previous description of the contractile activity of ET-1 in human isolated airway tissue. Uchida *et al.* (1988), have briefly reported that endothelin contracts human bronchus. They commented that 'the dose response curve for endothelin was 2 orders of magnitude smaller than those of histamine and neurokinin A'. Whether this refers to the potency or the efficacy is unclear and as no data are presented, a comparison of experimental results in the two studies is not possible.

ET-1 and ET-2 have been shown to differ both in the potency and efficacy of their contractile action on porcine coronary artery strips (Inoue *et al.*, 1989). ET-1 was significantly more potent than ET-2, while ET-2 produced a far greater maximal tension in this tissue. From the results of the present study it would seem that these differences in activity are not as apparent in human isolated airways. Both forms of endothelin were equally efficacious in this tissue. There was however, a difference in the potency of the peptides, the geometric mean EC_{50} value for ET-2 being 2.5 times greater than that for ET-1 in human isolated bronchial tissue.

It has been suggested that the contractile action of endothelin on vascular smooth muscle results from an action of endothelin as an endogenous ligand for voltage-dependent calcium channels (Yanagisawa *et al.*, 1988). The results of recent studies have not conclusively supported or rejected this proposed mechanism. Maggi *et al.* (1989) demonstrated that in guinea-pig airways, partial inhibition of the endothelin-induced contraction can be achieved by pre-incubation with nifedipine, while in rat trachea the voltage-dependent calcium channel antagonist nicardipine was without effect (Turner *et al.*, 1989) although nifedipine slightly attenuated the response in this tissue (Borges *et al.*, 1989). The present study has shown that, in human isolated bronchial tissue, the presence of verapamil has no effect on the contractile activity of ET-1. Thus, it would seem unlikely that ET-1 acts as an endogenous ligand for the voltage-dependent calcium channel in human airways.

The finding that the action of ET-1 on human airway tissue was independent of extracellular calcium was unexpected. Yanagisawa *et al.* (1988) concluded that contraction of porcine coronary arteries in response to the application of endothelin was due, at least in part, to the influx of extracellular calcium into the cytoplasm of the smooth muscle cell. Removal of extracellular calcium from an *in vitro* preparation of rat trachea (Turner *et al.*, 1989) attenuated the contraction generated by endothelin. By contrast, in human omental vessels (Hughes *et al.*, 1989) as well as in the human bronchial tissue studied in this investigation, the removal of calcium from the extracellular environment was without effect on the resultant ET-1-induced contraction. It is difficult to compare results from these studies and others, as calcium-free conditions have been achieved by use of a number of different protocols. These include omission of calcium from the Krebs-Henseleit solution and in some cases, the addition of various concentrations of EDTA or EGTA. Moreover, these varying 'calcium-free solutions' remained in contact with the tissues for a range of time periods. Incubation of tissue in a calcium-free medium without chelating agents may not be sufficient to remove all the extracellular calcium (Keatinge, 1972). Conversely, prolonged exposure to EGTA may not only affect the residual extracellular calcium ions, it may also remove calcium from the intracellular stores. In the present study, the protocol described by Creese & Denborough (1981) was employed. Incubation of the tissue with EGTA for only 30 min and then the replacement of the EGTA solution with calcium-free Krebs-Henseleit solution, results in removal of the extracellular but not intracellular calcium, as evidenced by the markedly decreased contractile action of histamine in this extracellular calcium-free environment. It is thus apparent that this protocol is a suitable one for distinguishing those agonists with a contractile action dependent upon extracellular calcium from those able to elicit contractions in an extracellular calcium-free environment.

The contraction induced by the addition of endothelin to the organ baths was extremely slow in onset. Its prolonged nature was similar to the contraction which results from the application of leukotrienes to isolated bronchial tissue (Hanna *et al.*, 1981). Endothelin may therefore elicit contraction via endogenous leukotriene release. De Nucci *et al.* (1988), have demonstrated thromboxane A_2 and prostaglandin release from guinea-pig lung perfused with a solution containing ET-1. More recently, a decreased response to ET-1 in guinea-pig trachea and bronchus was observed when the contractions were generated in the presence of a thromboxane A_2 receptor

antagonist (Battistini *et al.*, 1990). There was therefore, a possibility that the endothelin contraction in human bronchus in the present series of experiments was mediated by the release of products of the arachidonic acid pathway. Incubation of the human bronchial rings with the cyclo-oxygenase inhibitor indomethacin, resulted in no change in the contraction induced by ET-1. On the basis of these results, the action of ET-1 in human bronchial tissue cannot therefore, be explained by the release of endogenous prostaglandins or thromboxane A_2. Indomethacin does not however, prevent the formation of products of the lipoxygenase pathway, so it is still possible that leukotrienes may be released as a result of addition of endothelin to the organ bath. Further studies using either leukotriene receptor antagonists or inhibitors of leukotriene release are indicated to address this issue.

The potent and efficacious nature of endothelin as a vasoconstrictor agent on human arterial vascular tissue has been confirmed by this study. Application of endothelin to rings of pulmonary artery resulted in a contraction that was both slow in onset and sustained over time. The geometric EC_{50} values of 3.24 nM (ET-1) and 8.04 nM (ET-2) establish that endothelin is as potent if not more potent a vasoconstrictor as a bronchoconstrictor agent. ET-1 and ET-2 were approximately 5 times as potent in the arterial preparations as in the bronchial tissues. This would seem to suggest that these forms of endothelin are acting at the same receptor subtype in both tissues. In contrast to the results obtained in bronchial tissue, the responses obtained at 0.3 μM ET-1 and ET-2 appeared to have reached a plateau in the arterial preparations and thus represent the true maximal responses in this tissue type.

In this study, the existence of binding sites for ET-1 on the smooth muscle of human airway tissue as reported by Power *et al.* (1989), was confirmed. Binding sites for ET-2 were also visualized on the smooth muscle as well as the parasympathetic ganglia associated with the bronchial tissue. This is the first reported localization of binding sites for ET-2 in any tissue from any species. In addition to the localization of ET-1 and ET-2 binding sites in human airway tissue, dense binding of both forms of endothelin was observed on sections of pulmonary arteries.

The results of autoradiographic studies carried out on a wide variety of tissue from many different species provide evidence for the suggestion of a distinct endothelin receptor. Studies in human tissue have localized ET-1 binding sites to coronary artery (Dashwood *et al.*, 1989), adrenal gland (Davenport *et al.*, 1989), and intrapulmonary vessels in addition to bronchus (Power *et al.*, 1989). This binding was not displaced by co-incubation with vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP), atrial natriuretic peptide (ANP) or gastrin and was unaffected by nicardipine (Power *et al.*, 1989), verapamil or the presence of EDTA or EGTA (Kohzuki *et al.*, 1989). The evidence for the existence of a discrete receptor for endothelin is thus supported by the present results.

Future investigation into the mechanism of action of endothelin on human airway and vascular smooth muscle promises to be very exciting in the light of the finding of raised levels of endothelin in human lung during an acute asthma episode (Nomura *et al.*, 1989). Our results have demonstrated that it is one of the most potent contractile agents studied on human airway and vascular smooth muscle, its action is independent of the release of cyclo-oxygenase products and the influx of extracellular calcium into the cytoplasm of the smooth muscle cell, and there are specific receptors for endothelin on this muscle as well as on neural tissue. These findings suggest that in human airways, endothelin acts by binding at these receptors located on the smooth muscle and may subsequently alter the mobilization of intracellular calcium resulting in smooth muscle contraction. Since a number of events associated with an acute attack of asthma seem to be stimuli for endothelin release, further studies will clarify if and how endothelin has a role in the pathophysiology of asthma.

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