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# Contractile activity of three endothelins (ET–1, ET–2 and ET–3) on the human isolated bronchus

[1]C. Advenier, B. Sarria, E. Naline, L. Puybasset & *V. Lagente

Department of Pharmacology, Faculté de Médecine Paris-Ouest, 15, rue de l'Ecole de Médecine, F-75270 Paris Cedex 06 and * Institut Henri Beaufour, 1 Avenue des Tropiques, F-91952 Les Ulis, France

**1** The effects of three endothelins: (i) the classical or human/porcine endothelin (ET–1); (ii) [Trp$^6$, Leu$^7$] endothelin (ET–2) and (iii) [Thr$^2$, Phe$^4$, Thr$^5$, Tyr$^6$, Lys$^7$, Tyr$^{14}$] endothelin or rat endothelin (ET–3) were tested on the human isolated bronchus.

**2** ET–1 produced a concentration-dependent contraction of the human isolated bronchus that proceeded in two different steps. The first step was observed at very low concentrations ($pD_2 = 11.01 \pm 0.17$, $n = 10$) but corresponded to a low intrinsic activity ($E_{max} = 15.6 \pm 1.8\%$ of $E_{max}$ induced by acetylcholine (ACh) $3 \times 10^{-3}$ M, $n = 10$). This effect was potentiated by Bay K 8644 $10^{-7}$ M ($E_{max} = 26.1 \pm 2.9\%$ of ACh $3 \times 10^{-3}$ M, $n = 5$, $P < 0.05$), reduced by nicardipine $10^{-6}$ M ($E_{max} = 6.0 \pm 2.6\%$ of ACh $3 \times 10^{-3}$ M, $n = 5$, $P < 0.05$) and strongly inhibited in calcium-free medium. The second step of the action of ET–1 corresponded to a lesser potency ($pD_2 = 7.90 \pm 0.17$, $n = 9$) but a higher intrinsic activity ($E_{max} = 82.5 \pm 4.7\%$ of ACh $3 \times 10^{-3}$ M). This effect was not significantly modified by nicardipine $10^{-6}$ M or by Bay K 8644 $10^{-7}$ M. Neither of the two effects was modified by indomethacin $3 \times 10^{-6}$ M.

**3** The effects of ET–2 and ET–3 were qualitatively similar to those of ET–1 but quantitatively different; for these two steps of contracting activity and for potency and efficacy the ranking was: ET–1 > ET–2 = ET–3.

**4** Thus, ET-1 appears to be the most potent of these three substances in its effect on the human isolated bronchus. Its activity seems to involve the action of voltage-dependent calcium channels at low concentrations ($10^{-12}$ to $10^{-9}$ M), whereas other mechanisms are involved at higher concentrations ($10^{-8}$ to $3 \times 10^{-7}$ M).

## Introduction

Endothelin (ET–1) is an endothelium-derived 21-residue peptide recently described by Yanagisawa *et al.* (1988). It possesses a very potent vasoconstrictor activity on several isolated blood vessels where its action seems to be critically dependent upon the influx of calcium ions from the extracellular space through dihydropyridine-sensitive calcium channels (Yanagisawa *et al.*, 1988). ET–1 ($10^{-12}$–$3 \times 10^{-7}$ M) also exerts a potent contractile effect on guinea-pig and human isolated bronchial tissue (Uchida *et al.*, 1988; Maggi *et al.*, 1989a; Borges *et al.*, 1989) which is partially inhibited by the dihydropyridine calcium blockers nicardipine ($10^{-8}$ M) or nifedipine ($10^{-6}$ M) (Uchida *et al.*, 1988; Maggi *et al.*, 1989a). On the rat trachea, Turner *et al.* (1989) observed that contractile responses to ET–1 ($10^{-8}$–$10^{-5}$ M) were attenuated following incubation for 1 h in calcium-free solution and almost completely abolished following incubation in the presence of EGTA. In contrast, these responses were unaffected by treatment with nicardipine ($10^{-7}$ M) (Turner *et al.*, 1989).

ET–1 administered *in vivo* either intravenously or by inhalation induces in the guinea-pig a marked and sustained bronchoconstriction which is suppressed by pretreatment with the cyclo-oxygenase inhibitors indomethacin or meclofenamate (Payne & Whittle, 1988; Braquet *et al.*, 1989; Lagente *et al.*, 1989; Macquin-Mavier *et al.*, 1989).

Recently, three distinct endothelin-isopeptides, produced by three separate human genes have been described by Inoue *et al.* (1989). They have been called (i) ET–1, the 'classical' human and porcine endothelin, first described by Yanagisawa *et al.* (1988), (ii) ET–2, [Trp$^6$, Leu$^7$] endothelin and (iii) ET–3, [Thr$^2$, Phe$^4$, Thr$^5$, Tyr$^6$, Lys$^7$, Tyr$^{14}$] endothelin or rat endothelin (Table 1). The three peptides induce potent vasoconstrictor activity *in vitro* and a transient depressor response followed by a sustained pressor response *in vivo*, but the quantitative profiles of their pharmacological activities are different (Inoue *et al.*, 1989; Minkes *et al.*, 1989; Rodman *et al.*, 1989).

The effects of ET–2 and ET–3 on the bronchi have not yet been tested. In the course of an extensive study of the action of endothelins on airway smooth muscle, we studied the contractile effect of these substances on the human isolated bronchus and its modifications by calcium channel blockers or openers or by reduction of calcium in the medium.

## Methods

### *Human bronchial tissue preparation*

Bronchial tissues were removed from 13 patients with lung cancer at the time of the surgical operation (12 men and 1 women; mean age, 61.3 ± 1.6 yr; 8 squamous cell carcinomas, 3 oat cell carcinomas and 2 adenocarcinomas). Just after resection, segments of human bronchi with inner diameter of 3 to 5 mm were taken as far away as possible from the malignancy. They were placed in oxygenated Krebs-Henseleit solution (composition, mM: NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $KH_2PO_4$ 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 11.7) at 4°C and stored overnight at 4°C. After removal of adhering fat and connective tissue, two to eight adjacent rings from the same bronchus were prepared and suspended under an initial tension of 2.5 g in Krebs-Henseleit solution, bubbled with 95%$O_2$, 5%$CO_2$, and maintained at 37°C. Changes of tension were measured isometrically with strain gauge amplifiers and I.O.S.-Moise 2 recorder system (Celaster, Celle l'Evescault, 86600 Lusignan, France).

### *Protocol*

Each experiment began by contraction of the bronchial strips to maximal tension with acetylcholine (ACh $3 \times 10^{-3}$ M), then maximal relaxation was induced with theophylline ($3 \times 10^{-3}$ M). During the next 120 min, the tissues were

[1] Author for correspondence.

**Table 1** Primary structure of the endothelins used in the present study

| | |
|---|---|
| **ET-1** | Cys[1]-Ser-Cys-Ser-Ser-Leu-Met-Asp-Lys-Glu[10]-Cys-Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile[20]-Trp |
| **ET-2** | Cys[1]-Ser-Cys-Ser-Ser-**Trp[6]**-**Leu[7]**-Asp-Lys-Glu-Cys-Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile[20]-Trp |
| **ET-3** | Cys-**Thr[2]**-Cys-**Phe[4]**-**Thr[5]**-**Tyr[6]**-**Lys[7]**-Asp-Lys-Glu-Cys-Val-Tyr-**Tyr[14]**-Cys-His-Leu-Asp-Ile-Ile-Trp |

**ET-1:** Endothelin-1 (human-porcine ET); **ET-2:** Endothelin-2; **ET-3:** Endothelin-3 (rat ET).

washed every 15 min, and the resting tension was adjusted to 2 to 2.5 g, which has been found to be optimal for recording contractions in such tissues (Advenier *et al.*, 1986; Naline *et al.*, 1989). Thereafter, cumulative concentration-response curves to endothelins were constructed by applying increasing concentrations at 15 to 20 min intervals in logarithmic increments. Acetylcholine ($3 \times 10^{-3}$ M) was added at the end of the concentration-response curve to determine the maximal response of the preparation. Concentrations of endothelins higher than $3 \times 10^{-7}$ M were not tested because of the limited availability of the peptides. In some experiments, indomethacin, Bay K 8644 or nicardipine were added to the bath 30 min before the contractile agents. In all experiments, only one concentration-response curve to endothelins was recorded in each strip.

Concentration-response curves to histamine, acetylcholine, neurokinin A and leukotriene $D_4$ ($LTD_4$) were obtained under similar conditions.

In experiments performed in $Ca^{2+}$-free medium, after control contractions elicited by endothelin ($10^{-10}$ or $3 \times 10^{-8}$ M), ACh ($3 \times 10^{-3}$ M) or KCl ($3 \times 10^{-2}$ M) in normal Krebs solution, the human bronchi were incubated for 45 min in $Ca^{2+}$-free Krebs solution, and immediately thereafter for 15 min in the same solution to which ethylenediaminetetraacetic acid (EDTA) $10^{-3}$ M had been added. Then the preparation was immersed again in $Ca^{2+}$-free Krebs solution without EDTA, and after equilibration the contractile agents were added to the bath (Godfraind *et al.*, 1968; Advenier *et al.*, 1984). The effects of the contractile agents in $Ca^{2+}$-free medium are expressed as a percentage of control contractions obtained in the presence of $Ca^{2+}$.

The data are expressed in terms of $pD_2$ and in mg of tension or as a percentage of the maximum tension induced by ACh ($3 \times 10^{-3}$ M). $pD_2$ values were derived from the log concentration-effect curves and defined as the negative log of the drug concentration that caused 50% of maximal effect. The maximal effect ($E_{max}$) was calculated as the maximal increase in tone for each endothelin or contractile agent.

### *Statistical evaluation of data*

Statistical analysis of the results was performed with Student's *t* test for paired or unpaired data. All values in the text and tables are expressed as mean ± s.e.mean.

### *Drugs*

Drugs used were: endothelin ET-1, ET-2 and ET-3 (Peptide Institute Inc, Osaka, Japan), acetylcholine dihydrochloride (PCH, Paris, France), histamine HCl (Sigma, St. Louis, U.S.A.), $LTD_4$ (Sigma, St. Louis, U.S.A.), neurokinin A (NKA, Novabiochem, 4448 Läufelfingen, Switzerland), (±)-Bay K 8644, methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (Bayer, Wuppertal, West Germany), nicardipine HCl (Sandoz, Basel, Switzerland), indomethacin (Sigma, St. Louis, U.S.A.), phosphoramidon [N-(alpha-L-rhamnopyranosyloxyhydroxy- phosphinyl)-L-leucyl-L-trytophan] (Sigma, St. Louis, U.S.A.). All agents were dissolved in water, except nicardipine, indomethacin and (±)-Bay K 8644 which were dissolved in ethanol. As with the other drugs, these solutions were then diluted with Krebs solution.

## Results

### *Contractile activity of ET-1*

ET-1 ($10^{-12}$–$3 \times 10^{-7}$ M) induced a concentration-dependent contraction of the human isolated bronchus (Figure 1). The contraction reached a plateau 12 to 15 min after addition of the peptide to the bath, whereas under similar conditions a plateau was reached in 3–5 min with acetylcholine (ACh). Two different levels of contraction could be observed in the ET-1-induced contraction. The first one was observed for low concentrations of ET-1 ($10^{-12}$ to $10^{-9}$ M) with a maximal response of 15.6 ± 1.8% of the maximal contractile activity

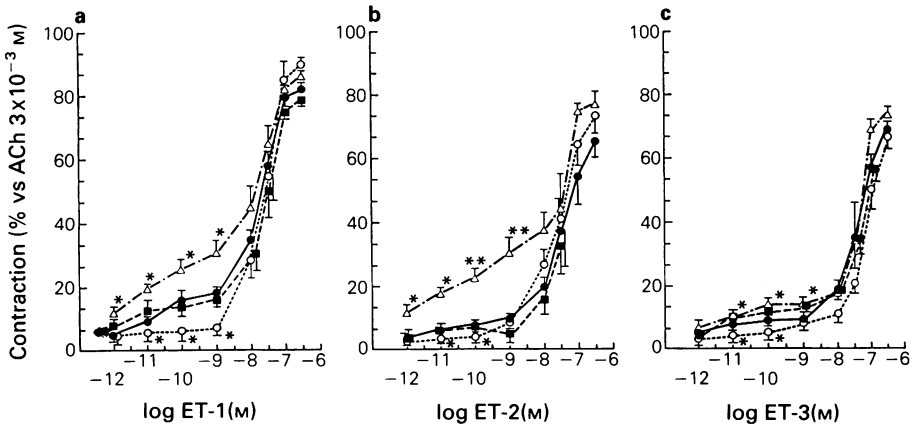


**Figure 1** Response of human isolated bronchi to endothelin-1 (ET-1) (a), endothelin-2 (ET-2) (b) or endothelin-3 (ET-3) (c) in the absence (●) and in the presence of nicardipine $10^{-6}$ M (○), Bay K 8644 $10^{-7}$ M (△) or indomethacin $3 \times 10^{-6}$ M (■). Points represent means; vertical bars represent s.e.mean. Statistical differences are (*) $P < 0.05$ and (**) $P < 0.01$ as compared to control tissues by use of Student's *t* test for unpaired data. Number of experiments are: 7 to 10 for control concentration-response curves, 5 (ET-1) and 4 (ET-2 and ET-3) for concentration-response curves in the presence of nicardipine, Bay K 8644 or indomethacin.

**Table 2** Apparent affinities ($pD_2$) and maximum effects ($E_{max}$) expressed in milligrams of tension (mg) or as percentage of the maximum effect of acetylcholine (% of ACh) measured with endothelin-1 (ET-1), histamine, acetylcholine, leukotriene $D_4$ ($LTD_4$) and neurokinin A (NKA) on human isolated bronchus

| | n | $pD_2$ | $E_{max}$ (mg) | $E_{max}$ (% of ACh) |
|---|---|---|---|---|
| ET-1 first step | 10 | 11.01 ± 0.17* | 279 ± 46* | 15.6 ± 1.8* |
| ET-1 second step | 9 | 7.90 ± 0.17 | 1276 ± 264 | 82.5 ± 4.7 |
| Histamine | 8 | 6.02 ± 0.11 | 1602 ± 293 | 92.4 ± 2.5 |
| Acetylcholine | 8 | 4.96 ± 0.12 | 1947 ± 372 | 100 |
| $LTD_4$ | 4 | 7.67 ± 0.19 | 1755 ± 570 | 80.5 ± 6.8 |
| NKA | 6 | 8.48 ± 0.24 | 1749 ± 284 | 77.6 ± 6.1 |

Values are means ± s.e.mean. Experiments with NKA were performed in the presence of phosphoramidon $10^{-5}$ M.
* Assimilated to the effect observed for $10^{-10}$ M.

induced by ACh ($3 \times 10^{-3}$ M) and a $pD_2$ of 11.01 ± 0.17 (Table 2).

The second step of contraction was observed for higher concentrations ($10^{-9}$ to $3 \times 10^{-7}$ M) with a maximal effect of 82.5 ± 4.7% of the maximal contraction obtained with ACh $3 \times 10^{-3}$ M; $pD_2$ value for this second step was 7.90 ± 0.17 (Table 2).

When compared with other potent constrictor agents, ET-1 appeared to be about 1,122,000 (first step) and 870 fold (second step) more potent than ACh; however, the comparison is questionable, at least as regards the first step, since the contraction observed was 7.8% with ET-1 and 50% with ACh. Under similar experimental conditions, histamine, $LTD_4$ and NKA were 11.5, 512 and 3311 fold respectively more potent than ACh.

When the $E_{max}$ of the second step was examined, ET-1 appeared to have a slightly but significantly lower efficacy than ACh ($P < 0.05$), but a similar efficacy to histamine, $LTD_4$ and NKA (Table 2).

ET-1 responses were not modified by indomethacin ($3 \times 10^{-6}$ M), whatever the concentration (Table 3 and Figure 1). In contrast, the contractions induced by low concentrations of ET-1 ($10^{-12}$ to $10^{-10}$ M) were suppressed by nicardipine ($10^{-6}$ M, $P < 0.05$) or potentiated by Bay K 8644 ($10^{-7}$ M, $P < 0.05$). But responses to ET-1 observed with high concentrations ($10^{-9}$ to $3 \times 10^{-7}$ M) were not altered by nicardipine or by Bay K 8644 (Table 3).

In calcium-free medium, the ET-1 ($10^{-10}$ M)-induced response was markedly reduced, since the contractile response reached only 11.3 ± 7.5% of the control response in normal calcium medium (Table 4). A strong reduction was also observed in the case of $3 \times 10^{-2}$ M KCl. In contrast, the ET-1 $3 \times 10^{-8}$ M-induced contraction was slightly reduced, reaching 56.3 ± 8.1% of the control contraction. This reduction of response was not significantly different from that observed with ACh $3 \times 10^{-3}$ M (Table 4).

### *Contractile activity of ET-2 and ET-3*

ET-2 and ET-3 elicited potent contractile activities in the human isolated bronchus (Figure 1). However, compared with ET-1, the contractile responses induced by low concentrations of ET-2 and ET-3 were lower (Figure 1). For example, at $10^{-10}$ M, ET-2 and ET-3 induced contractions that were 7.1 ± 0.9% and 8.0 ± 1.3% respectively of the maximal contraction induced by ACh $3 \times 10^{-3}$ M, compared with the 15.6 ± 1.8% with ET-1. In concentrations higher than $10^{-9}$ M, the effects induced by ET-2 and ET-3 were qualitatively similar to those of ET-1. However, ET-1 was significantly ($P < 0.01$) more potent than ET-2 and ET-3 since the −log molar concentrations required to obtain 50% of the maximal contraction induced by ACh were respectively 7.77 ± 0.11 ($n = 9$), 7.23 ± 0.14 ($n = 8$) and 7.21 ± 0.08 ($n = 7$).

As was the case for ET-1, the contractile activities recorded for ET-2 and ET-3 were not altered by addition of indomethacin ($3 \times 10^{-6}$ M) (Figure 1 and Table 5). In contrast, in the presence of Bay K 8644 ($10^{-7}$ M), the responses ($E_{max}$, %ACh) induced by small concentrations of both peptides

**Table 3** Effect of indomethacin $3 \times 10^{-6}$ M, nicardipine $10^{-6}$ M and Bay K 8644 $10^{-7}$ M on the potency ($pD_2$) and maximal effect ($E_{max}$) of endothelin-1 (ET-1), on human isolated bronchus

| | *First step* | | | | | | *Second step* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Pretreatment* | n | $pD_2$ | P | $E_{max}$ (mg) | $E_{max}$ (% of ACh) | P | n | $pD_2$ | P | $E_{max}$ (mg) | $E_{max}$ (% of ACh) | P |
| Control | 10 | 11.01 ± 0.17 | | 279 ± 46 | 15.6 ± 1.8 | | 9 | 7.90 ± 0.17 | | 1276 ± 264 | 82.5 ± 4.7 | |
| Indomethacin $3 \times 10^{-6}$ M | 5 | 10.93 ± 0.21 | NS | 270 ± 90 | 15.2 ± 3.5 | NS | 5 | 7.85 ± 0.10 | NS | 1182 ± 310 | 81.3 ± 4.5 | NS |
| Nicardipine $10^{-6}$ M | 5 | — | | 64 ± 25 | 6.0 ± 2.6 | <0.05 | 5 | 7.77 ± 0.08 | NS | 935 ± 160 | 90.3 ± 1.5 | NS |
| Bay K 8644 $10^{-7}$ M | 5 | 11.35 ± 0.38 | NS | 445 ± 131 | 26.1 ± 2.9 | <0.05 | 5 | 8.15 ± 0.28 | NS | 1035 ± 165 | 87.2 ± 2.8 | NS |

$E_{max}$ is assimilated to the effects of ET-1 $10^{-10}$ M (first step) and $3 \times 10^{-7}$ M (second step).
Values are means ± s.e.mean.
$n$ = number of experiments.
$P$ = statistical difference between pretreated and control preparations (Student's $t$ test for unpaired data).
NS = not significant.

**Table 4** Residual contraction to acetylcholine (ACh), KCl, endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3) after pre-incubation of isolated human bronchi in calcium-free Krebs solution

| | | n | *Control contraction in normal Krebs solution* (mg) | *Contraction in calcium-free Krebs solution* (mg) | *% of control* |
|---|---|---|---|---|---|
| ACh | $3 \times 10^{-3}$ M | 7 | 2224 ± 235 | 1500 ± 230 | 66.1 ± 6.4 |
| KCl | $3 \times 10^{-3}$ M | 4 | 890 ± 264 | 88 ± 18 | 13.6 ± 4.3 |
| ET-1 | $10^{-10}$ M | 4 | 163 ± 35 | 25 ± 18 | 11.3 ± 7.5 |
| ET-1 | $3 \times 10^{-8}$ M | 6 | 1278 ± 176 | 691 ± 115 | 56.3 ± 8.1 |
| ET-2 | $10^{-10}$ M | 3 | 138 ± 33 | 6.0 ± 2.6 | 2.5 ± 2.5 |
| ET-2 | $3 \times 10^{-8}$ M | 3 | 683 ± 298 | 550 ± 278 | 76.8 ± 13.6 |
| ET-3 | $10^{-10}$ M | 3 | 145 ± 15 | 3 ± 2 | 2.5 ± 2.5 |
| ET-3 | $3 \times 10^{-8}$ M | 3 | 655 ± 260 | 525 ± 225 | 81.2 ± 12.5 |

$n$ = number of experiments.
Values are mean ± s.e.mean.

**Table 5** Effect of indomethacin $3 \times 10^{-6}$ M, nicardipine $10^{-6}$ M and Bay K 8644 $10^{-7}$ M on the maximal effect ($E_{max}$) of endothelin-2 (ET-2) ($10^{-10}$ M) and endothelin-3 (ET-3) ($10^{-10}$ M), on human isolated bronchus

| *Pretreatment* | n | $E_{max}$ (mg) | P | $E_{max}$ (% of ACh) | P |
|---|---|---|---|---|---|
| *ET-2 $10^{-10}$ M* | | | | | |
| Control | 10 | 108 ± 17 | | 7.1 ± 0.9 | |
| Indomethacin $3 \times 10^{-6}$ M | 4 | 72.5 ± 49.0 | NS | 5.7 ± 3.8 | NS |
| Nicardipine $10^{-6}$ M | 4 | 55 ± 19 | NS | 4.5 ± 0.6 | <0.05 |
| Bay K 8644 $10^{-7}$ M | 4 | 365 ± 122 | NS | 22.8 ± 2.6 | <0.01 |
| *ET-3 $10^{-10}$ M* | | | | | |
| Control | 10 | 170 ± 33 | | 8.0 ± 1.3 | |
| Indomethacin $3 \times 10^{-6}$ M | 4 | 146 ± 32 | NS | 10.6 ± 1.5 | NS |
| Nicardipine $10^{-6}$ M | 4 | 75 ± 43 | NS | 4.7 ± 1.6 | <0.05 |
| Bay K 8644 $10^{-7}$ M | 4 | 285 ± 105 | NS | 14.3 ± 1.5 | <0.05 |

Values are means ± s.e.mean.
$n$ = number of experiments.
$P$ = statistical difference between pretreated and control preparations (Student's test for unpaired data). NS = not significant.

($10^{-10}$ M) were markedly enhanced (Figure 1 and Table 5), so much so that the contractions observed with $10^{-10}$ M of either ET-1 or ET-2 were not significantly different (respectively 26.1 ± 2.9% and 22.8 ± 2.6%). In the presence of nicardipine ($10^{-6}$ M), the responses ($E_{max}$, %ACh $3 \times 10^{-3}$ M) induced by small concentrations ($10^{-10}$ M) of both ET-2 and ET-3 were significantly ($P < 0.05$) inhibited (Table 5). In calcium-free medium responses to low concentration ($10^{-10}$ M) of ET-2 and ET-3 were abolished, whereas the responses obtained with $3 \times 10^{-8}$ M were 76.8 ± 13.6 and 81.2 ± 12.5% respectively of the control contraction (Table 4).

## Discussion

It has recently been shown that endothelin (ET-1) elicits contractile effects on airway smooth muscle *in vitro* with concentrations ranging from $10^{-12}$ to $3 \times 10^{-7}$ M in the guinea-pig trachea (Uchida *et al.*, 1988; Maggi *et al.*, 1989a; Borges *et al.*, 1989) or from $10^{-8}$ to $10^{-5}$ M in the rat trachea (Turner *et al.*, 1989). On human airways, Uchida *et al.* (1988) also described a contractile activity for ET-1, without reporting quantitative data concerning the concentration-effect relationship. ET-1 was first considered, principally in blood vessels, to be an endogenous modulator of voltage-dependent calcium channels (Yanigasawa *et al.*, 1988), so that particular attention has been paid to the role of calcium flux in the contractile activity of ET-1. It has been demonstrated that the *in vivo* airway response to ET-1 was partially sensitive to the dihydropyridine calcium channel blockers, such as nicardipine ($10^{-8}$ M) (Uchida *et al.*, 1988) or nifedipine ($10^{-6}$ M) (Maggi *et al.*, 1989a). In contrast, a decrease of the contractile response was observed in calcium-free medium on the rat trachea, while no inhibitory effect of nicardipine ($10^{-7}$ M) was observed (Turner *et al.*, 1989). Thus a relationship between transmembrane calcium flux and airway contractile activity has been established for the guinea-pig, rat and human airways.

The present results clearly show that in the human isolated bronchus the contractile activities of the three endothelins proceed in a stepwise manner. The first step is observed for concentrations of endothelin below $10^{-9}$ M. The maximum response observed with ET-1 is recorded for $10^{-10}$ M, corresponding to 15.6 ± 1.8% of the maximal tissue response obtained by addition of ACh $3 \times 10^{-3}$ M at the end of the experiment, indicating a high potency but a low efficacy of the peptide. This step involves calcium flux and suggests an effect dependent upon dihydropyridine-sensitive calcium channels ($L_m$ channels, Nayler, 1988), since the contractile effect of ET-1 is enhanced by Bay K 8644 and inhibited by nicardipine, drugs which respectively increase and decrease the voltage-dependent calcium transfer in the guinea-pig or human airways (Foster *et al.*, 1983a,b; Advenier *et al.*, 1984; 1986; Allen *et al.*, 1985). This is emphasized by the results obtained in calcium-free medium where the contractile effect of ET-1 ($10^{-10}$ M) is markedly reduced, and to an extent similar to that of KCl (Advenier *et al.*, 1986).

The second step of the peptide airway response recorded for concentrations higher than $10^{-9}$ M is characterized by a higher efficacy but a lower potency than those observed for the first step. However, at these concentrations, endothelin appears to be more potent than other bronchoconstrictor agents such as histamine, ACh, NKA and $LTD_4$. This second step is not altered by Bay K 8644 or by nicardipine, and it is modified by the same amount as ACh in calcium-free medium, suggesting a mechanism unrelated to the dihydropyridine-dependent calcium channels. On the rat uterus, Kozuka *et al.* (1989) similarly showed dissociation between rhythmic contractions involving voltage-dependent calcium channels and slowly developing monophasic contractions insensitive to calcium channel blockers. Therefore, a direct effect of endothelin on intracellular calcium via the activation of phosphatidyl inositol hydrolysis, may be considered, as has recently been demonstrated with high concentrations ($10^{-8}$ to $10^{-5}$ M) on rat or rabbit aorta (Marsden *et al.*, 1989; Huang *et al.*, 1989; Ohlstein *et al.*, 1989), in cultured A10 cells (with $10^{-7}$ M ET-1) (Xuan *et al.*, 1989) or in isolated canine coronary arteries (Pang *et al.*, 1989).

The cyclo-oxygenase inhibitor indomethacin ($3 \times 10^{-6}$ M) does not significantly modify the response to ET-1 on the isolated human bronchus. This is not concordant with the data obtained *in vivo* in the guinea-pig, where indomethacin (10 mg kg$^{-1}$) or meclofenamate (0.5 to 2 mg kg$^{-1}$) suppressed the bronchoconstriction induced by intravenous or inhaled ET-1 (Payne & Whittle, 1988; Lagente *et al.*, 1989; Macquin-Mavier *et al.*, 1989). The discrepancy may be explained by recent reports showing that the human bronchial smooth muscle may generate important quantities of prostanoids which do not modulate the contractile responses *in vitro* (Douglas & Brink, 1987; De Jongste *et al.*, 1987; Naline *et al.*, 1989).

The present data also demonstrate that ET-2 and ET-3 exert similar qualitative effects, but are quantitatively different, since they appear to be slightly less potent and/or less efficient than ET-1, if we consider the two steps of the contractile response. From a qualitative point of view, the ET-2 and ET-3 contractile activities may be divided, as with ET-1, into two steps. One is characterized by high potency and low efficacy, implicating voltage-dependent calcium channels, and the other by lower potency but higher efficacy, independent of transmembrane calcium channels.

The qualitative differences between ET-1 on the one hand and ET-2 and ET-3 on the other are similar to data obtained on the contractile responses of porcine and rat artery strips *in vitro* and on the pressor responses of anaesthetized rats or cats

*in vivo* (Inoue *et al.*, 1989; Rodman *et al.*, 1989; Minkes *et al.*, 1989). Indeed, ET-1 is as potent or more potent than ET-2 and more potent than ET-3. Nevertheless, in the rat, the initial transient depressor response *in vivo* was most profound with ET-3 (Inoue *et al.*, 1989); but the discrepancy between the results obtained by these authors and ours might be due to differences in animal species (rat versus guinea-pig) or in systems (cardiovascular versus respiratory). The differences we observed in the activities of the endothelins might result from differences in efficacy and/or tissue factors such as receptor density, efficiency of receptor/effector coupling and selective inactivation. They may also suggest the existence of different subtypes of endothelin receptors. The last suggestion might be supported by recent data demonstrating different high-affinity binding sites in rat lungs (Kanse *et al.*, 1989). Furthermore, it is possible to discriminate between different endothelin receptors on the guinea-pig bronchus, as demonstrated with an ET-1 analogue (Maggi *et al.*, 1989b). However, whether these endothelin receptors exist in human bronchial tissue is unknown.

To conclude, our results present evidence that the three endothelins have potent contractile activities on human isolated bronchus and that at least two mechanisms are involved in this effect. One of these, which acts when low concentrations are used, is probably dihydropyridine-sensitive calcium channel activation. Other mechanisms operate at higher endothelin concentrations, and in particular a direct effect on intracellular calcium via phosphatidyl inositol hydrolysis activation may be suggested for concentrations higher than $10^{-9}$ M (Marsden *et al.*, 1989; Huang *et al.*, 1989; Ohlstein *et al.*, 1989; Xuan *et al.*, 1989; Pang *et al.*, 1989).

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