Comparison of the effects of intra-arterial and aerosol administration of endothelin-1 (ET-1) in the guinea-pig isolated lung

Françoise Pons, Caroline Touvay, Vincent Lagente, ¹Jean Michel Mencia-Huerta & Pierre Braquet

Institut Henri Beaufour, 1, ave des Tropiques, 91952 Les Ulis, France

1 Intra-arterial injection of endothelin-1 (ET-1, 400 pmol; 1 µg) in guinea-pig isolated perfused lungs, induced increases in pulmonary inflation pressure (PIP) and perfusion pressure (PPP), associated with oedema formation and thromboxane B₂ (TxB₂) release but not with the generation of sulphidopeptide leukotrienes or release of histamine. In contrast, aerosol administration of ET-1 (3, 6, 10 µg ml⁻¹, for 2 min) evoked a dose-dependent increase in PIP, without significant changes in PPP, oedema formation or TxB₂ release.

2 Addition of indomethacin (5 µM) or BW 755C (10 or 100 µM), but not nordihydroguaiaretic acid (NDGA, 50 µM) or FPL 55712 (10 µM), to the perfusion medium led to a significant inhibition of the increases in PIP and PPP, TxB₂ release and oedema formation evoked by intra-arterial injection of 400 pmol ET-1. In contrast, indomethacin (5 µM), BW 755C (100 µM) or FPL 55712 (10 µM), added to the perfusion medium 10 min prior to challenge, did not affect the increase in PIP induced by a 2-min aerosol of a solution of ET-1 10 µg ml⁻¹.

3 *In vivo* aerosol administration of indomethacin (100 mg ml⁻¹, for 20 min) to non-anaesthetized guinea-pigs, 15 min before lung removal, did not modify the bronchopulmonary response evoked in isolated perfused lungs by an aerosol of ET-1 10 µg ml⁻¹. However, under the same experimental conditions, indomethacin significantly inhibited TxB₂ release evoked by aerosolized arachidonic acid (2 mg ml⁻¹).

4 In conclusion, the present study shows that when injected by the intra-arterial route, ET-1 effects are mediated primarily via the generation of cyclo-oxygenase metabolites of arachidonic acid, whereas when the aerosol route is used, the peptide appears to act on airway smooth muscle cells, through an indomethacin-insensitive process which may involve some other, as yet unidentified, mediator(s).

Keywords: Endothelin-1; bronchoconstriction; indomethacin; BW 755C; FPL 55712; NDGA; guinea-pig lung; aerosol

Introduction

The spasmogenic effect of endothelin-1 (ET-1), a 21-amino acid peptide isolated from cultured porcine endothelial cells (Yanagisawa *et al.*, 1988), is not restricted to the vascular system. Indeed, this peptide induces the *in vitro* contraction of pulmonary tissue from various species (Uchida *et al.*, 1988; Maggi *et al.*, 1989; Turner *et al.*, 1989; Hay, 1989; Advenier *et al.*, 1990). Intravenous injection of ET-1 in the guinea-pig elicits a dose-dependent bronchoconstriction which is accompanied by a rapid and marked increase in mean arterial blood pressure (Payne & Whittle, 1988; Braquet *et al.*, 1989a; Macquin-Mavier *et al.*, 1989; Touvay *et al.*, 1990). However, it has been suggested that the *in vivo* effects of ET-1 on blood pressure and bronchopulmonary alterations in the guinea-pig are, at least in part, dissociated (Touvay *et al.*, 1990). This was strengthened by the finding that in the guinea-pig, aerosol administration of ET-1 also induces a potent bronchoconstriction without significant changes in blood pressure (Lagente *et al.*, 1989).

Pretreatment of guinea-pigs with the cyclo-oxygenase inhibitor, indomethacin, produced a marked but incomplete inhibition of the bronchoconstriction induced by both intravenous and aerosol administrations of ET-1 (Payne & Whittle, 1988; Braquet *et al.*, 1989b; Macquin-Mavier *et al.*, 1989; Lagente *et al.*, 1989). Furthermore, a marked release of cyclo-oxygenase metabolites, especially thromboxane (Tx) A₂, following injection of ET-1 into guinea-pig lung is observed (De Nucci *et al.*, 1988; Antunes *et al.*, 1989; Braquet *et al.*, 1989a; Touvay *et al.*, 1990). These results suggested that ET-1 exerts its bronchopulmonary activity mostly via the generation of cyclo-oxygenase metabolites such as TxA₂. However, the possible contribution of other mediators such as lipoxygenase-derived

metabolites of arachidonic acid in the ET-1-induced bronchopulmonary effects have yet to be determined.

In the present study, we compared the effects of intra-arterial and aerosol administration of ET-1 in isolated and perfused guinea-pig lungs, in order to analyse the respective contribution of the direct and the indirect bronchopulmonary alterations of the peptide. Furthermore, the pharmacological modulation of the ET-1-induced bronchopulmonary effects, as well as the possible release of histamine, TxA₂ and of the bronchoconstrictor lipoxygenase metabolites of arachidonic acid upon challenge with the peptide were also investigated.

Methods

Lung perfusion experiments

Male Hartley guinea-pigs (400–500 g, Charles River, Saint-Aubin-les-Elbeuf, France) were anaesthetized with sodium pentobarbitone (60 mg kg⁻¹, i.p.) and the lungs were prepared as described by Pons *et al.* (1989). Briefly, after mid-thoracotomy the lungs were washed *in situ* through the pulmonary artery with 50 ml of Krebs solution containing 2% bovine serum albumin (Krebs-BSA) and heparin 20 units ml⁻¹. Then, the trachea was cannulated and the lungs rapidly excised and suspended in a warmed (37°C) glass chamber. The trachea was connected to a respiratory pump (Ugo Basile pump, Varese, Italy, 60 strokes min⁻¹, 1 ml 100 g⁻¹ body weight) and the pulmonary inflation pressure (PIP) was monitored with a Gould transducer (Cleveland, Ohio, USA) connected to the ventilation system and linked to a multichannel recorder (Gould). The lungs were perfused at a flow rate of 5 ml min⁻¹ through the pulmonary artery with thermostated (37°C) and oxygenated (95% O₂:5% CO₂) Krebs-BSA. The pulmonary perfusion pressure (PPP) was

¹ Author for correspondence.

monitored via a transducer (Gould) connected to the perfusion system. The lungs were allowed to equilibrate for 10 min before intra-arterial or aerosol administration of ET-1 or the various agents tested. For the aerosol administrations, a Devilbiss 'Pulmosonic' ultrasonic Nebuliser (Somerset, Pennsylvania, USA) was inserted in series with the afferent limb of the ventilator circuit (as modified from Payne & De Nucci, 1987). The various drugs tested, indomethacin (5 μM), BW 755C (10 and 100 μM), FPL 55712 (10 μM), nordihydroguaiaretic acid (NDGA; 50 μM), were added to the perfusion medium 10 min before the stimulation with ET-1 or the various agents. The lung effluent was collected for 1 min before and 5 min following intra-arterial or aerosol challenge for the measurement of histamine, inactive metabolite of TxA_2 : thromboxane B_2 (TxB_2), and immunoreactive sulphidopeptide leukotrienes. In order to evaluate oedema formation, the wet weights of the lungs were measured prior to and at the end of the experiments. The increment of the lung weight is expressed as a percentage calculated over the value measured before the experiment.

Pretreatment of guinea-pigs by aerosol administration of indomethacin

In some experiments, non-anaesthetized guinea-pigs were placed in a plexiglass chamber (12 $\times$ 30 $\times$ 12 cm) and exposed to an aerosol of a solution of indomethacin 100 mg ml^{-1} for 20 min. The aerosol was generated by a Devilbiss 'Pulmosonic' ultrasonic nebuliser, directly connected to the plexiglass chamber. The guinea-pig lungs were prepared as described above, 15 min after the end of the aerosol of indomethacin.

Mediator assays

TxB_2 and sulphidopeptide leukotrienes were assayed according to the method described by Pradelles *et al.* (1985) and with commercial kits (Stallergenes, Fresnes, France). The minimal detectable concentration of TxB_2 was 10 pg ml^{-1} and within the range of 10–400 pg ml^{-1} the intra-assay variation was less than 10%. Cross-reactivities with TxB_1 and dinor TxB_2 were 17% and 11%, respectively. Cross-reactivity with other prostanoids was less than 1%. Background values of TxB_2 in the effluent, prior to ET-1 challenge never exceeded 1 ng ml^{-1} . The minimal detectable concentration of sulphidopeptide leukotriene in lung effluent was equivalent to 10 pg ml^{-1} leukotriene C_4 (LTC_4) and cross-reactivities with LTD_4 , LTE_4 and LTB_4 were 56%, 2% and 0.1%, respectively.

For the assessment of histamine, 2 ml aliquots of the lung perfusate were mixed with 0.2 ml of a 4.8 N perchloric acid solution in water. After centrifugation for 20 min at 3500 r.p.m. and at 4°C, the supernatants were collected and stored at 4°C. The spectrofluorimetric assay for histamine was performed according to the method described by Shore (1959).

Drugs

The composition of the Krebs solution was (in mM): NaCl 118, KCl 4.7, CaCl_2 2.5, MgSO_4 7H $_2\text{O}$ 1.2, KH_2PO_4 1.2, NaHCO_3 25 and glucose 5.6. The following drugs were used: sodium pentobarbitone (Nembutal, Clin-Midy, Saint-Jean-de-la-Ruelle, France), pancuronium bromide (Pavulon, Organon Technika, Fresnes, France), heparin (Roche, Neuilly-sur-Seine, France), BSA, TxB_2 , histamine hydrochloride, NDGA (Sigma, Saint Louis, MO, U.S.A.). NDGA was solubilized in a 50% ethanol solution in water and then added to the Krebs-BSA. The inhibitor of cyclo-oxygenase and lipoxygenase, BW 755C (3-amino-1-[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline, a gift from Drs M.D. Cooke and S. Moncada, Wellcome Research Laboratories, Beckenham, U.K.), and the antagonist of peptidoleukotrienes, FPL 55712 (7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylic acid, a gift from Dr Shipman, Fisons

Pharmaceuticals, Loughborough, U.K.), were dissolved in distilled water. The cyclo-oxygenase inhibitor, indomethacin, was solubilized either in 0.5 N NaOH in 0.15 M NaCl and the solution was then adjusted to pH 7.4–7.6 for aerosol administration, or dissolved in absolute ethanol and further diluted in Krebs-BSA. ET-1 (mol. wt. = 2492, Peptide Institut Inc., Osaka, Japan) was dissolved in distilled water and further diluted in a 0.15 M NaCl solution (saline).

Statistical analysis

The results are expressed as mean $\pm$ s.e.mean. Differences between means were analysed by Student's *t* test for unpaired data or by use of two-way analysis of variance for the time-course studies.

Results

Effect of intra-arterial and aerosol administration of endothelin-1 in isolated guinea-pig lungs

Aerosol administration of ET-1 (1, 3, 6 or 10 $\mu\text{g ml}^{-1}$ solutions) for 2 min provoked a dose-dependent increase in PIP, developing slowly and reaching a maximal value by 2–3 min following the beginning of the aerosol (Figure 1). Aerosol administration of a solution containing 1 $\mu\text{g ml}^{-1}$ ET-1 evoked no significant change in PIP, as compared to the effect of the solvent alone. When a solution containing ET-1, 3 $\mu\text{g ml}^{-1}$, was administered by aerosol an important increase in PIP was observed. This bronchoconstrictor response was not significantly different from the one induced by 6 $\mu\text{g ml}^{-1}$ ET-1, but significantly lower than that evoked by aerosol administration of 10 $\mu\text{g ml}^{-1}$ ET-1 (two way analysis of variance). These results are in keeping with the ones previously obtained when investigating the *in vivo* aerosol administration of the peptide in guinea-pigs (Lagente *et al.*, 1989).

The increase in PIP induced by aerosol administration of 10 $\mu\text{g ml}^{-1}$ ET-1 was of lower intensity than the one induced by intra-arterial injection of 400 pmol (approx. 1 μg) of the peptide (Figure 2a). When administered by the intra-arterial route, the ET-1-induced bronchoconstriction was associated with an increase in PPP (Figure 2b), which lasted for at least 5 min following injection of the peptide. In contrast, the bronchoconstrictor effect induced by aerosolized ET-1 (10 $\mu\text{g ml}^{-1}$) was not associated with such a significant change in PPP (Figure 2b).

As presented in Figure 3, intra-arterial injection of 400 pmol ET-1 provoked a 10% oedema formation and generation of

Figure 1 Time-course of the increases in pulmonary inflation pressure (PIP) induced by aerosol administration of 1 (○, $n = 7$), 3 (●, $n = 6$), 6 (□, $n = 7$) or 10 $\mu\text{g ml}^{-1}$ (■, $n = 15$) for 2 min in isolated and perfused guinea-pig lungs. PIP is expressed in mmHg as means, with s.e.mean shown by vertical bars.

Figure 2 Time-course of the increases in pulmonary inflation pressure (PIP) (a) and pulmonary perfusion pressure (PPP) (b) induced by intra-arterial (400 pmol, $\circ$, $n = 25$) or aerosol ($10 \mu\text{g ml}^{-1}$ for 2 min, $\bullet$, $n = 15$) administration of endothelin-1 (ET-1) in isolated and perfused guinea-pig lungs. PIP and PPP are expressed in mmHg as means, with s.e.mean shown by vertical bars.

TxB₂ in the lung effluent; however, there was no detectable release of histamine or sulphidopeptide leukotrienes (data not shown). In contrast, no generation of TxB₂ and little, if any, oedema formation was observed after aerosol administration of $10 \mu\text{g ml}^{-1}$ ET-1 (Figure 3).

Pharmacological modulation of the bronchopulmonary effects induced by intra-arterial injection of endothelin-1

The effects of indomethacin, BW 755C, FPL 55712 and NDGA on the bronchopulmonary alterations induced by intra-arterial injection of 400 pmol of ET-1 were investigated. Addition of indomethacin ($5 \mu\text{M}$) or BW 755C (10 or $100 \mu\text{M}$) to the perfusion medium of isolated and perfused guinea-pig lungs led to a significant inhibition of the increase in PIP, TxB₂ release and oedema formation induced by ET-1 (Figure 4a, c and d). In the presence of indomethacin or BW 755C, the increase in PPP was also reduced, but this inhibition did not reach a significant level (Figure 4b). The effect of BW 755C was concentration-related and that of $100 \mu\text{M}$ abrogated the

Figure 3 Comparison of the effects of intra-arterial (400 pmol, open columns, $n = 25$) or aerosol ($10 \mu\text{g ml}^{-1}$ for 2 min, stippled columns, $n = 15$) administration of endothelin 1 (ET-1) on pulmonary inflation pressure (PIP), pulmonary perfusion pressure (PPP), oedema formation, thromboxane B₂ (TxB₂) release in isolated and perfused guinea-pig lungs. Increases in PIP (Δ PIP) and PPP (Δ PPP) are expressed in mmHg. Lung oedema (Δ L.W.) is expressed as percentage increase in the wet weight of the organ, as compared to the pre-experiment value. TxB₂ release is expressed in ng ml^{-1} . Results are presented as means with s.e.mean shown by vertical bars. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (t test).

increase in PIP and in lung weight, as well as the TxB₂ release, induced by ET-1 (Figure 4a, c and d). In contrast, FPL 55712 ($10 \mu\text{M}$) or NDGA ($50 \mu\text{M}$), added to the perfusion medium, did not significantly affect the bronchopulmonary alterations evoked by ET-1 (Figure 4a, b, c and d). On the contrary, in the presence of NDGA ($50 \mu\text{M}$), the increases in PIP and PPP, TxB₂ release and oedema formation evoked by ET-1 were slightly enhanced (Figure 4a, b, c and d).

Pharmacological modulation of the bronchopulmonary effects induced by aerosol administration of endothelin-1

The effects of addition of indomethacin, BW 755C or FPL 55712 into the perfusion medium were investigated only on the increase in PIP, since no changes in PPP, TxB₂ release and lung wet weight were observed following ET-1 aerosol administration in guinea-pig isolated lungs (see above). The presence of indomethacin ($5 \mu\text{M}$) in the perfusion medium did not significantly modify the increase in PIP after aerosol administration of ET-1 (3, 6 or $10 \mu\text{g ml}^{-1}$, for 2 min) (Table 1). In addition, *in vivo* aerosol administration of indomethacin (100 mg ml^{-1} , for 20 min) to non-anaesthetized guinea-pigs, 15 min before lung removal, did not modify the bronchopulmonary response induced by an aerosol of $10 \mu\text{g ml}^{-1}$ ET-1 to isolated perfused lungs (data not shown). Furthermore, BW 755C ($100 \mu\text{M}$) and FPL 55712 ($10 \mu\text{M}$), added to the perfusion medium, also failed to affect the increase in PIP induced by an aerosol of $10 \mu\text{g ml}^{-1}$ ET-1. Indeed, the ET-1-induced increases in PIP were 17.7 ± 1.5 mmHg, 18.2 ± 4.5 mmHg and 17.0 ± 2.9 mmHg in the absence and in the presence of BW 755C and FPL 55712, respectively.

Table 1 Effects of indomethacin on the increase in pulmonary inflation pressure (PIP) induced by aerosol administration of endothelin-1 (ET-1)

ET-1 ($\mu\text{g ml}^{-1}$)	n	Increase in PIP induced by aerosolized ET-1 (mmHg)			
		Control		Indomethacin	
3	6	13.3 $\pm$ 2.3	6	9.2 $\pm$ 2.6	
6	7	15.4 $\pm$ 2.6	6	19.0 $\pm$ 3.9	
10	15	17.7 $\pm$ 1.5	4	18.0 $\pm$ 1.9	

Indomethacin ($5 \mu\text{M}$) was added to the perfusion medium 10 min before administration of ET-1. Increases in PIP following aerosol administration of ET-1 (3, 6 or $10 \mu\text{g ml}^{-1}$, for 2 min) are expressed in mmHg as mean $\pm$ s.e.mean of the indicated number (n) of experiments. No significant differences were observed between control and indomethacin-treated lungs, regardless of the dose of ET-1 used.

Figure 4 Effects of indomethacin (Indo, 5 µM), BW 755C (10 or 100 µM), FPL 55712 (10 µM) and nordihydroguaiaretic acid (NDGA, 50 µM) on the increases in pulmonary inflation pressure (PIP) (a) and pulmonary perfusion pressure (PPP) (b), thromboxane B₂ (TxB₂) release (c) and oedema formation (d) induced by intra-arterial injection of 400 pmol endothelin-1 (ET-1). The various drugs were added separately to the perfusion medium 10 min before the injection of ET-1. Increases in PIP and PPP are expressed in mmHg. TxB₂ release is expressed in ng ml⁻¹. Lung oedema is expressed as percentage increase in the wet weight of the organ, as compared to the pre-experimental value. Results are presented as mean with s.e.mean shown by vertical bars of the number of experiments indicated inside the bars of (a). * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$ (*t* test).

Bronchopulmonary effects induced by arachidonic acid administered by aerosol

The effect of indomethacin on the bronchopulmonary actions of aerosolized arachidonic acid was also studied, in order to ascertain that the dose of indomethacin chosen in this series of experiments was adequate. In isolated perfused guinea-pig lungs, aerosol administration of arachidonic acid (2 mg ml⁻¹) for 3 min provoked a slowly developing increase in PIP with a maximal value of 12.9 ± 1.5 mmHg by 3–4 min (Table 2). This peak increase in PIP was associated with no significant change in PPP and with only a minimal alteration (less than 3.9%) in the lung weight (data not shown). However, aerosol administration of arachidonic acid induced a marked generation of TxB₂ in the lung effluent (Table 2). Addition of indomethacin (5 µM) to the perfusion medium did not significantly affect the increase in PIP induced by arachidonic acid,

although it reduced by 95% the generation of TxB₂ (Table 2). Also, *in vivo* aerosol administration of indomethacin to non-anaesthetized guinea-pigs, 15 min before lung removal, led to a 60% reduction ($P < 0.05$) of the arachidonic acid-induced generation of TxB₂ by isolated lung, without significant alteration of the bronchoconstrictor response (Table 2).

Discussion

Intra-arterial injection of ET-1 in isolated perfused lungs of the guinea-pig has been shown to result in a dose-dependent increase in PIP, associated with TxB₂ release (Antunes *et al.*, 1988; De Nucci *et al.*, 1988; Braquet *et al.*, 1989a; Touvay *et al.*, 1990). The present results indicate that ET-1 also induces an *in vitro* increase in PIP in isolated and perfused guinea-pig lungs when administered by aerosol. The threshold concentra-

Table 2 Effects of indomethacin on the increase in pulmonary inflation pressure (PIP) and thromboxane B₂ (TxB₂) release induced by aerosol administration of arachidonic acid

Treatment	n	Increase in PIP (mmHg)	TxB ₂ release (ng ml ⁻¹)
Control	8	12.9 ± 1.5	15.3 ± 3.1
Indo (5 µM)	6	9.2 ± 1.7	$0.8 \pm 0.3^{***}$
Indo (100 mg ml ⁻¹)	6	20.0 ± 4.1	$6.3 \pm 1.7^*$

Indomethacin was added to the perfusion medium (5 µM) or administered by aerosol to non-anaesthetized guinea-pigs (100 mg ml⁻¹, for 20 min). Isolated and perfused guinea-pig lungs were challenged with arachidonic acid (2 mg ml⁻¹) for 3 min. Increases in PIP are expressed in mmHg and TxB₂ in ng ml⁻¹. Results are presented as mean $\pm$ s.e.mean of the indicated number (*n*) of experiments. * $P < 0.05$; *** $P < 0.001$ (*t* test).

tion of the peptide was $3\mu\text{g ml}^{-1}$, and in the range of $3\text{--}10\mu\text{g ml}^{-1}$, the ET-1-induced effects were dose-dependent. These results are in agreement with those reported by Lagente *et al.* (1989), who initially described the *in vivo* bronchoconstrictor effects of aerosolized ET-1 in the guinea-pig. Administered by the aerosol route, however, ET-1 had no significant effect on PPP, oedema formation or generation of TxB_2 in the lung effluent. This contrasts with intra-arterial injection of the peptide.

The inhibition of the bronchopulmonary effects of intra-arterially administered ET-1 by indomethacin or BW 755C indicates that when this route is used, ET-1-induced effects are primarily mediated by cyclo-oxygenase metabolites of arachidonic acid, a result in agreement with previous reports (De Nucci *et al.*, 1988; Antunes *et al.*, 1988). In addition, no release of immunoreactive sulphidopeptide leukotrienes and no inhibitory effect of NDGA and FPL 55712 were observed, suggesting that lipoxygenase metabolites of arachidonic acid are not involved in the bronchopulmonary alterations induced by intra-arterial injection of ET-1.

The present results also indicate that, when administered by aerosol, ET-1 effects appear not to be mediated through the generation of cyclo-oxygenase metabolites. Indeed, no generation of TxB_2 was detected following aerosol administration of ET-1, under experimental conditions where aerosolized arachidonic acid evoked a marked release of this metabolite in the lung effluent. Furthermore, the bronchopulmonary response induced by aerosolized ET-1 was unaltered when indomethacin, BW 755C or NDGA were added to the perfusion medium. Previous work by Lagente *et al.* (1989) demonstrated that indomethacin, given intravenously to anaesthetized guinea-pigs, reduced by 60% the *in vivo* bronchopulmonary response evoked by aerosolized ET-1, suggesting that when the aerosol route is used, ET-1-induced effects are only partially mediated by the release of cyclo-oxygenase metabolites. Such a discrepancy between *in vivo* and *in vitro* results indicates that other components or cell types (like circulating blood cells) could be recruited during *in vivo* aerosol challenge with ET-1 and contribute to the indomethacin-sensitive part of the bronchopulmonary activity of the peptide. On the other hand, it has been recently reported (Battistini *et al.*, 1990) that the TxA_2 antagonist, BM 13177, markedly reduced the *in vitro* contraction of guinea-pig trachea induced by ET-1. However, in this study, the effect of indomethacin was not evaluated. In this regard, Maggi *et al.* (1989) and Advenier *et al.* (1990) have reported only a slight inhibition of

the contraction of guinea-pig trachea and human bronchus induced by low concentrations of ET-1 in the presence of indomethacin. The lack of effect of this drug added to the perfusion medium under our experimental conditions is in keeping with these last results, but can also indicate that, in isolated and perfused lungs, large and small bronchi rather than trachea are the primary targets of ET-1.

In the present study, no inhibitory effect of indomethacin was observed when non-anaesthetized guinea-pigs were pretreated with this drug by aerosol. This pretreatment, however, significantly reduced the release of TxB_2 induced by aerosolized arachidonic acid, and has been reported to suppress the bronchoconstriction induced by aerosolized FMLP in the guinea-pig. Indeed, Boukili *et al.* (1989) have demonstrated that indomethacin administered by aerosol to anaesthetized guinea-pigs significantly reduced the bronchoconstriction induced by both intravenous and aerosol administration of FMLP and abrogated that evoked by intravenous injection of arachidonic acid, suggesting a predominant cyclo-oxygenase-dependent component in these experiments. Under our experimental conditions, aerosol administration of indomethacin (100 mg ml^{-1} , for 20 min) suppressed the *in vivo* bronchoconstriction evoked by intravenous injection of arachidonic acid (0.5 mg kg^{-1} ; Pons *et al.*, unpublished results). Interestingly, although pretreatment of the animals or addition of indomethacin to the perfusion medium significantly decreased TxB_2 formation induced *in vitro* by arachidonic acid by more than 65%, minimal effect of this drug on the increase in PIP was noted. Nevertheless, Manzini *et al.* (1989) suggested that arachidonic acid-induced bronchoconstrictor activity is not exclusively mediated by the generation of cyclo-oxygenase metabolites but also through a local release of neuropeptides. The possibility that the bronchoconstriction evoked by aerosolized ET-1 may involve mediators other than the indomethacin-sensitive ones, for example platelet-activating factor or neuropeptides, deserves investigation.

In summary, these results demonstrate that in isolated and perfused guinea-pig lungs, ET-1 administered by the intra-arterial or aerosol route induces bronchopulmonary effects. When ET-1 is injected by the intra-arterial route, its effects are primarily mediated via the generation of cyclo-oxygenase metabolites of arachidonic acid, whereas when the aerosol route is used, the peptide appears to act on airway smooth muscle cells through an indomethacin-insensitive process which may involve some other, as yet unidentified, mediator(s).

References

- ADVENIER, C., SARRIA, B., NALINE, E., PUYBASSET, L. & LAGENTE, V. (1990). Contractile activity of three endothelins on the human isolated bronchus. *Br. J. Pharmacol.*, **100**, 168–172.
- ANTUNES, E., DE NUCCI, G. & VANE, J.R. (1988). Endothelin releases eicosanoids from and its removed by isolated perfused lungs of the guinea-pig. *J. Physiol.*, **407**, 40P.
- BATTISTINI, B., FILEP, J. & SIROIS, P. (1990). Potent thromboxane-mediated *in vitro* bronchoconstrictor effect of endothelin in the guinea-pig. *Eur. J. Pharmacol.*, **178**, 141–142.
- BOUKILI, M.A., BUREAU, M.F., LELLOUCH-TUBIANA, A., LEFORT, J., SIMON, M.T. & VARGAFTIG, B.B. (1989). Alveolar macrophages and eicosanoids but not neutrophils, mediate bronchoconstriction induced by FMLP in the guinea-pig. *Br. J. Pharmacol.*, **98**, 61–70.
- BRAQUET, P., TOUVAY, C., LAGENTE, V., VILAIN, B., PONS, F., HOSFORD, D., CHABRIER, P.E. & MENCIA-HUERTA, J.M. (1989a). Effect of endothelin-1 on blood pressure and bronchopulmonary system of the guinea-pig. *J. Cardiovasc. Pharmacol.*, **13**, suppl. 5, S143–S146.
- BRAQUET, P., TOUVAY, C., LAGENTE, V., VILAIN, B., PONS, F., LEJEUNE, V. & MENCIA-HUERTA, J.M. (1989b). Indomethacin inhibits *in vitro* and *in vivo* endothelin induced bronchoconstriction in the guinea-pig. *Eur. Resp. J.*, **2** (suppl. 5), 274S.
- DE NUCCI, G., THOMAS, R., D'ORLEANS-JUSTE, P., ANTUNES, E., WALDER, C., WARMER, T.D. & VANE, J.R. (1988). Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 9797–9800.
- HAY, D.W.P. (1989). Guinea-pig tracheal epithelium and endothelin. *Eur. J. Pharmacol.*, **171**, 241–245.
- LAGENTE, V., CHABRIER, P.E., MENCIA-HUERTA, J.M. & BRAQUET, P. (1989). Pharmacological modulation of the bronchopulmonary action of the vasoactive peptide, endothelin, administered by aerosol in the guinea-pig. *Biochem. Biophys. Res. Commun.*, **158**, 625–632.
- MAGGI, C.A., PATACCHINI, R., GUILLIANI, S. & MELI, A. (1989). Potent contractile effect of endothelin in isolated guinea-pig airways. *Eur. J. Pharmacol.*, **160**, 179–182.
- MACQUIN-MAVIER, I., LEVANE, M., ISTIN, N. & HARF, A. (1989). Mechanisms of endothelin-mediated bronchoconstriction in the guinea-pig. *J. Pharmacol. Exp. Ther.*, **250**, 740–745.
- MANZINI, S., BALLATI, L., GEPPETTI, P., RUBINI, I., MEINI, S. & PERRETTI, F. (1989). Arachidonic acid-induced bronchomotor responses are partially mediated by release of sensory neuropeptides from capsaicin-sensitive structures. *Br. J. Pharmacol.*, **98**, 1077–1079.
- PAYNE, A.N. & DE NUCCI, G. (1987). Anaphylaxis in guinea-pigs induced by ovalbumin aerosol. *In vivo* and *in vitro* methods. *J. Pharmacol. Methods*, **17**, 83–90.
- PAYNE, A.N. & WHITTLE, B.J.R. (1988). Potent cyclooxygenase-mediated bronchoconstrictor effects of endothelin in the guinea-pig *in vivo*. *Eur. J. Pharmacol.*, **158**, 303–304.

- PONS, F., TOUVAY, C., LEJEUNE, V., CARRE, C., VILAIN, B., BROQUET, C., MENCIA-HUERTA, J.M. & BRAQUET, P. (1989). Action of platelet-activating factor (PAF) antagonists on the bronchopulmonary effects of PAF in the guinea-pig. *J. Lipids Mediators*, **1**, 329–340.
- PRADELLES, P., GRASSI, J. & MACLOUF, J. (1985). Enzyme immunoassays of eicosanoids using acetylcholinesterase as label: an alternative to radioimmunoassay. *Anal. Chem.*, **57**, 1170–74.
- SHORE, P.A. (1959). A method for the fluorometric assay of histamine in tissues. *J. Pharmacol. Exp. Ther.*, **127**, 182–187.
- TOUVAY, C., VILAIN, B., PONS, F., CHABRIER, P.E., MENCIA-HUERTA, J.M. & BRAQUET, P. (1990). Bronchopulmonary and vascular effect of endothelin in the guinea-pig. *Eur. J. Pharmacol.*, **176**, 23–33.
- TURNER, N.C., POWER, R.F., POLAK, J.M., BLOOM, S.R. & DOLLERY, C.T. (1989). Contraction of rat tracheal smooth muscle by endothelin. *Br. J. Pharmacol.*, **96**, 103P.
- UCHIDA, Y., NINOMIYA, H., SAOTOME, M., NOMURA, A., OHTSUKA, M., YANAGISAWA, M., GOTO, K., MASAKI, T. & HASEGAWA, S. (1988). Endothelin, a novel vasoconstrictor peptide, as potent bronchoconstrictor. *Eur. J. Pharmacol.*, **154**, 227–228.
- YANAGISAWA, M., KURIHARA, H., KIMURA, S., TOMOBE, Y., KOBAYASHI, M., MITSUI, Y., GOTO, K. & MASAKI, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, **332**, 411–415.

(Received March 23, 1990

Revised November 6, 1990

Accepted November 13, 1990)