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Pharmacological evaluation of a guinea-pig tracheal epithelium-derived inhibitory factor (EpDIF)

Lynette B. Fernandes & [1]Roy G. Goldie

Department of Pharmacology, University of Western Australia, Perth, Nedlands, Australia, 6009

1 An epithelium-derived inhibitory factor (EpDIF) released by guinea-pig tracheal epithelium was evaluated in a co-axial bioassay system consisting of an epithelium-intact guinea-pig tracheal tube surrounding endothelium-denuded rat aortic strip.

2 Histamine and several muscarinic agonists induced concentration-dependent relaxation of phenylephrine-contracted rat aorta via the release of EpDIF. However, several other agonists did not induce the release of EpDIF from guinea-pig trachea. These included the nicotinic cholinoceptor agonists nicotine (25 μM), 1,1-dimethyl-4-phenylpiperazinium (DMPP) (25 μM), calcium ionophore A23187 (0.5 μM), bradykinin (0.05–0.5 μM), substance P (5 μM), platelet activating factor (PAF, 1–100 nM), the leukotrienes (LT) LTC_4, LTD_4 and LTE_4 (0.1–10 nM) as well as hyperosmotic stimuli.

3 Prostaglandin E_2 (PGE_2) induced concentration-dependent contraction of endothelium-denuded rat aortic preparations, indicating that this prostanoid could not be EpDIF. Furthermore, relaxation to histamine and methacholine, mediated via EpDIF, was not significantly altered in the presence of phenidone (50 μM) the cyclo-oxygenase/lipoxygenase inhibitor with radical scavenging properties or the cytochrome P-450 inhibitors metyrapone (1 mM) and SKF 525A (25 μM). This suggests that EpDIF is neither a prostanoid nor a cytochrome P-450 metabolite of arachidonic acid.

4 The soluble guanylate cyclase inhibitor, methylene blue (50 μM), caused small but significant increases in the potencies of both histamine and methacholine in co-axial assemblies, indicating that EpDIF did not activate this enzyme and therefore was not NO or a related substance. The β-adrenoceptor antagonist, (−)-propranolol (1 μM), and the PAF-receptor antagonist, WEB 2086 (50 μM), also failed to alter significantly EpDIF-modulated relaxations. These data suggest that EpDIF is neither a stimulant of β-adrenoceptors nor of PAF receptors.

5 The present study provides some evidence that this vascular smooth muscle-sensitive EpDIF may not be related to the putative EpDIF previously hypothesized to modulate directly spasmogen-induced airway smooth muscle tone.

Introduction

Asthma is often associated with non-specific bronchial hyperreactivity to spasmogens (Boushey *et al.*, 1980) as well as with damage to the airway epithelium (Laitinen *et al.*, 1985; Jeffery *et al.*, 1989). It is now well established *in vitro* that the epithelium has a significant role to play in modulating airway smooth muscle responsiveness to spasmogenic and relaxant agonists in several species including man (Barnes *et al.*, 1985; Flavahan *et al.*, 1985; Goldie *et al.*, 1986; Raeburn *et al.*, 1986; Aizawa *et al.*, 1988; Vanhoutte, 1988). It has been suggested that the airway epithelium may secrete an inhibitory factor which can modulate airway smooth muscle responsiveness to various agonists (Flavahan *et al.*, 1985). Thus substantial damage to or loss of airway epithelium, as commonly occurs in asthma (Laitinen *et al.*, 1985; Dunnill, 1987), with consequently reduced production of this inhibitory factor, may lead to increased airway sensitivity to spasmogens.

Direct evidence for the release of an inhibitory factor by airway epithelium has been demonstrated in co-axial bioassay systems consisting of guinea-pig trachea surrounding rabbit aorta (Ilhan & Sahin, 1986), rat anococcygeus muscle (Guc *et al.*, 1988) or rat aorta (Fernandes *et al.*, 1989). In the present study, we have used this co-axial bioassay technique to evaluate some of the pharmacological properties of epithelium-derived inhibitory factor (EpDIF) released by guinea-pig tracheal epithelium.

Methods

Tissue preparation for organ bath experiments

Male Wistar rats (220–250 g) were killed by stunning and exsanguination and the thoracic aorta removed and opened; cross-cut preparations (7–8 mm in length) were obtained as previously described (Fernandes *et al.*, 1989). The endothelium was removed from some vascular preparations with a cotton wool-coated probe. All preparations were suspended under 500 mg tension and equilibrated for 1 h in Krebs solution maintained at 37°C and aerated with 5% CO_2 in O_2. In all experiments, the Krebs solution also contained the cyclo-oxygenase inhibitor indomethacin (5 μM).

Endothelium-denuded rat aortic preparations were challenged with successively lower concentrations of phenylephrine (Phe) between 0.2 and 0.05 μM. The lowest concentration of Phe producing sustained stable, near maximal contractions was 0.05 μM and was used routinely to induce tone in vascular preparations. Preparations were then exposed to histamine (100 μM) or methacholine (25 μM). The presence of significant numbers of endothelial cells was indicated by relaxant responses to histamine and/or methacholine, while successful removal of vascular endothelium was confirmed by the absence of such relaxation. In some experiments, endothelium-intact and endothelium-denuded vascular preparations were precontracted with Phe (0.05 μM) and re-challenged with either histamine (100 μM) or methacholine (25 μM) in the presence or absence of the soluble guanylate cyclase inhibitor methylene blue (50 μM), the cyclo-oxygenase/lipoxygenase inhibitor BW 755C (150 μM), the cyclo-oxygenase/lipoxygenase inhibitor, radical scavenger and antioxidant phenidone (50 μM), the lipoxygenase inhibitor, radical scavenger and antioxidant nordihydroguaiaretic acid (NDGA) (10 μM), the lipoxygenase inhibitor and antioxidant gossypol (30 μM), the leukotriene receptor antagonist FPL 55712 (10 μM), the β-adrenoceptor antagonist (−)-propranolol (1 μM), the platelet activating factor (PAF) receptor antagonist WEB 2086 (50 μM), the cytochrome P-450 inhibitors metyrapone (1 mM) and SKF 525A (25 μM) or the free radical scavenger superoxide dismutase (50 u ml^{-1}). In some experiments, endothelium-denuded

[1] Author for correspondence.

vessel strips alone, were challenged with prostaglandin E_2 (PGE_2) (0.05–200 μM).

Co-axial bioassay assemblies

Male guinea-pigs (SR/C Tricolour) weighing 450–500 g were killed by cervical dislocation and the trachea removed, dissected free of surrounding tissue and cut into tubular segments approximately 7 mm in length. The epithelium was deliberately removed from some tracheal segments with a cotton wool-coated probe as previously described for tracheal ring preparations (Goldie *et al.*, 1986). Complete removal of the airway epithelium from such preparations was confirmed by histological examination.

Following initial pharmacological verification of the presence or absence of endothelium, aortic preparations were set-up in co-axial assemblies within guinea-pig tracheal tube segments as previously described (Fernandes *et al.*, 1989). After equilibration for 45 min, the vascular preparation was precontracted with Phe (0.05 μM) and re-challenged with histamine (5–200 μM), methacholine (1–200 μM), acetylcholine (1–200 μM), carbachol (1–200 μM), bethanechol (1–200 μM), arecoline (5–500 μM), oxotremorine (5–500 μM), nicotine (25 μM), 1,1-dimethyl-4-phenylpiperazinium (DMPP) (25 μM), calcium ionophore A23187 (0.5 μM), bradykinin (0.05–0.5 μM), substance P (5 μM), PAF (1–100 nM), leukotriene C_4 (LTC_4), LTD_4, or LTE_4 (0.1–10 nM), urea (160 mM) or mannitol (160 mM).

The effects of single selective concentrations of methylene blue (50 μM; Martin *et al.*, 1985), BW 755C (150 μM), phenidone (50 μM), NDGA (10 μM) gossypol (30 μM), metyrapone (1 mM), SKF 525A (25 μM) (Forstermann *et al.*, 1988), FPL 55712 (10 μM; Jancar *et al.*, 1987), propranolol (1 μM), WEB 2086 (50 μM), or superoxide dismutase (50 u ml^{-1}) on responses to histamine (100 μM) and methacholine (25 μM) in co-axial bioassay systems were examined as previously described. In addition, the effects of methylene blue (50 μM) or phenidone (50 μM) on responses to histamine (5–200 μM) or methacholine (1–200 μM) were determined. The effects of the neutral endopeptidase inhibitor phosphoramidon (1 μM; Fine *et al.*, 1989) on responses to histamine (5–200 μM) were also examined.

Analysis of results

Unless otherwise stated, numerical data are presented as mean ± standard error of the mean (± s.e.mean). The potencies of agonists causing concentration-dependent responses of rat aortic preparations are expressed as pD_2 values or as molar EC_{50} values, i.e. concentrations producing 50% of the maximum response, derived from mean estimates of pD_2 where $pD_2 = -\log_{10}(EC_{50})$. Relaxation response data are expressed either as % reductions in the level of vascular tone induced by phenylephrine (0.05 μM), or as % E_{max} values, where E_{max} = maximal reduction in the level of tone induced by Phe (0.05 μM). The probability of differences between mean results was determined with Student's two-tailed t test (paired or non-paired) and was considered significantly different if $P < 0.05$. The effects of enzyme inhibitors on the potency of agonists were assessed as mean shifts (with 95% confidence limits) derived from paired data i.e. from pD_2 values for the agonists obtained in the presence and absence of the antagonist.

Drugs

Drugs used were: A23187, acetylcholine chloride, arecoline hydrobromide, bethanechol chloride, carbachol chloride, methacholine chloride, oxotremorine sesquifumarate, bradykinin acetate, gossypol-acetic acid, histamine diphosphate, indomethacin, metyrapone, nordihydroguaiaretic acid, PAF (L-α-phosphatidylcholine β-acetyl-γ-O-alkyl), phenidone, phenylephrine hydrochloride, phosphoramidon, prostaglandin E_2, substance P (Sigma); BW 755C (3-amino-1[*m*-(trifluoromethyl)-phenyl]-2 pyrazoline, Wellcome); DMPP (1,1-dimethyl-4-phenylpiperazinium, ICN-K&K Lab.); 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, Boehringer Ingelheim); FPL 55712 (7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylic acid, Fisons); mannitol, urea (analytical grade, Ajax Chem.); methylene blue (Gurr); nicotine (B.D.H. Lab. Chem.); (−)-propranolol hydrochloride (I.C.I.); LTC_4, LTD_4, LTE_4, Proadifen (SKF 525A, Smith Kline, Beecham Pharmaceuticals); superoxide dismutase (Boehringer Mannheim).

All drugs tested in organ bath studies were prepared daily in 0.9% w/v NaCl solution (saline). BW 755C and FPL 55712 were prepared in distilled water. Prostaglandin E_2 was prepared in absolute ethanol. Gossypol was prepared in dimethylsulphoxide (DMSO). Indomethacin was prepared in 5 mg ml^{-1} sodium carbonate solution. PAF was obtained dissolved in chloroform. More dilute solutions of PAF were prepared in 0.25% bovine serum albumin (BSA; Sigma) in polypropylene containers. The Krebs solution used throughout the study had the following composition (mM): NaCl 117.6, KCl 5.4, $NaHCO_3$ 25, KH_2PO_4 1.03, $MgSO_4$ 0.57, D-glucose 11.1 and $CaCl_2$ 2.5.

Results

Neither endothelium-intact nor endothelium-denuded rat aorta preparations relaxed significantly in response to the β-adrenoceptor agonist (−)-isoprenaline (5 μM), indicating the absence of natural tone. Phe (0.05 μM) increased baseline tone (233 ± 8 mg; $n = 42$) which could be reduced in a concentration-dependent manner by (−)-isoprenaline (pD_2 = 7.77 ± 0.05; E_{max} = 113 ± 6%; $n = 8$ preparations). In endothelium-intact aortic strips, histamine (100 μM) and methacholine (25 μM) reduced Phe-induced tone by 81 ± 3% ($n = 19$ preparations from 11 animals) and 79 ± 4% ($n = 13$ preparations from 8 animals), respectively. These responses were abolished in the presence of the soluble guanylate cyclase inhibitor, methylene blue (50 μM), indicating that relaxation had been caused by the release of endothelium-derived relaxant factor (EDRF). Relaxation responses caused by this factor were enhanced by superoxide dismutase. In sharp contrast, endothelium-denuded rat aorta failed to respond to histamine or methacholine, thereby confirming the absence of endothelial cells. PGE_2 caused concentration-dependent contraction in endothelium-denuded rat aorta preparations and was therefore unlikely to be EpDIF. The mean pD_2 value obtained to this spasmogen was 6.01 ± 0.09 and the mean E_{max} value was 234 ± 23 mg ($n = 8$ preparations).

Effect of agonists in co-axial bioassay assemblies

When epithelium-intact guinea-pig tracheal tube segments were used as EpDIF donor preparations in a co-axial bioassay system, endothelium-denuded rat aorta, precontracted with Phe (0.05 μM) relaxed in a concentration-dependent manner in response to histamine and the muscarinic cholinoceptor agonists methacholine, acetylcholine, carbachol, bethanechol, oxotremorine and arecoline. While pD_2 values obtained for acetylcholine, carbachol, bethanechol and methacholine were similar, oxotremorine and arecoline were approximately 25 and 50 fold less potent respectively, than these other agonists. All of these agonists induced maximal or near maximal relaxation of Phe-induced tone (Table 1). Removal of the tracheal EpDIF donor preparations or the use of epithelium-denuded tracheal tubes as donor tissue, resulted in abolition of relaxation responses to histamine and methacholine.

In contrast, several agents failed to cause reductions in Phe-induced tone in rat aortic strips in co-axial assemblies. These

Table 1 Relaxant effects of histamine and various muscarinic agonists in co-axial assemblies

Agonist	pD_2	EC_{50}[a] (μM)	E_{max}[b] (%)	n
Histamine	5.04 ± 0.04	9.1	84 ± 5	15
Methacholine	5.69 ± 0.09	2.0	79 ± 6	14
Acetylcholine	5.52 ± 0.12	3.0	89 ± 5	10
Carbachol	5.76 ± 0.05	1.7	99 ± 7	10
Bethanechol	5.68 ± 0.08	2.1	90 ± 5	10
Oxotremorine	4.30 ± 0.33	50	88 ± 4	6
Arecoline	4.00 ± 0.09	100	105 ± 5	5

Data are expressed as mean ± s.e.mean. n = the number of preparations tested. [a] EC_{50} values are derived from mean pD_2 values. [b] E_{max} = maximal reduction in the level of tone induced by Phe (0.05 μM).

included nicotine (25 μM), DMPP (25 μM), calcium ionophore A23187 (0.5 μM), bradykinin (0.05–0.5 μM), substance P (5 μM), PAF (1–100 nM), LTC_4, LTD_4 and LTE_4 (0.1–10 nM), as well as urea (160 mM) and mannitol (160 mM). These agents were also found not to alter significantly Phe (0.05 μM)-induced tone in isolated endothelium-denuded aorta preparations in the absence of EpDIF donor tissue.

Effect of non-agonists in co-axial assemblies

Various enzyme inhibitors, receptor antagonists, radical scavengers and antioxidants were used in an attempt to modify the activity of EpDIF released in response to histamine or methacholine. The effect of these agents on the level of Phe-induced tone in endothelium-denuded rat aorta preparations alone, was also determined. BW 755C (150 μM), metyrapone (1 mM) and SKF 525A (25 μM) caused significant reductions in Phe-induced tone of between 25 and 45% (n = 12–14 preparations, $P < 0.05$, paired t test), while the remainder had no significant effect (n = 10–16 preparations, $P > 0.05$, paired t test).

The inhibitor of soluble guanylate cyclase, methylene blue (50 μM), abolished EDRF-induced relaxation, but failed to inhibit EpDIF activity induced by either histamine (100 μM) or methacholine (25 μM) (Table 2). Co-axial bioassay experiments were routinely performed in the presence of the cyclo-oxygenase inhibitor, indomethacin (5 μM). In addition, BW 755C (150 μM), phenidone (50 μM), NDGA (10 μM) and gossypol (30 μM) all failed to inhibit EpDIF activity (Table 2). The leukotriene receptor antagonist, FPL 55712 (10 μM), the β-adrenoceptor antagonist, propranolol (1 μM) and the PAF receptor antagonist, WEB 2086 (50 μM), all failed to inhibit EpDIF-induced vascular relaxation (Table 2). The cytochrome P-450 inhibitors, metyrapone (1 mM) and SKF 525A (25 μM), were also ineffective against EpDIF activity (Table 2).

In another series of experiments, concentration-effect curves to both histamine and methacholine were constructed in co-axial assemblies in the absence and presence of methylene blue (50 μM) or phenidone (50 μM) (Table 3). Methylene blue increased the potencies of histamine and methacholine by 3.1 fold (95% confidence limits: 1.9–4.9; n = 10 preparations) and 6.6 fold (95% confidence limits: 3.9–11.2; n = 9 preparations) respectively, while phenidone failed to alter the potencies of either agonist (Table 3). In addition, the maximal relaxant effects of histamine and methacholine (84 ± 7% and 76 ± 8%, respectively) were increased significantly in the presence of methylene blue (109 ± 14%, n = 10 and 115 ± 12%, n = 9, respectively; $P < 0.05$, paired t test). Phenidone however, was without significant effect in this regard.

Concentration-effect curves to histamine were also constructed in co-axial assemblies in the absence and presence of phosphoramidon (1 μM) to determine any effects of this neutral endopeptidase inhibitor on histamine potency and maximal relaxant effect. Potency values obtained to histamine in the absence and presence of phosphoramidon (5.02 ± 0.09 and 5.12 ± 0.07, respectively), were not significantly different ($P > 0.1$, paired t test; n = 9 preparations). Similarly, E_{max} values obtained to this agonist in the absence (84 ± 4) and presence (100 ± 10) of phosphoramidon were not significantly altered ($P > 0.1$).

In addition, superoxide dismutase did not significantly alter the relaxation response mediated by EpDIF. In co-axial assemblies, histamine (100 μM) caused mean relaxations of 66 ± 10% or 75 ± 15% in the absence or presence of superoxide dismutase (50 u ml^{-1}) respectively (n = 5 preparations, $P > 0.2$, paired t test).

Table 2 Effect of various agents on relaxant responses to histamine (100 μM) or methacholine (25 μM) in co-axial assemblies

	Histamine (100 μM)		*Methacholine (25 μM)*	
	Control	*Test (+ agent)*	*Control*	*Test (+ agent)*
Methylene blue (50 μM)	77 ± 7 (8)	71 ± 3†	104 ± 5 (10)	96 ± 11†
BW 755C (150 μM)	70 ± 11 (7)	84 ± 10†	73 ± 7 (6)	73 ± 8†
Phenidone (50 μM)	94 ± 20 (6)	108 ± 7†	93 ± 8 (6)	91 ± 4†
NDGA (10 μM)	90 ± 7 (6)	85 ± 7†	103 ± 10 (10)	93 ± 6†
Gossypol (30 μM)	74 ± 4 (6)	82 ± 5†	90 ± 7 (7)	101 ± 9†
FPL 55712 (10 μM)	80 ± 8 (8)	74 ± 6†	85 ± 10 (9)	92 ± 9†
Propranolol (1 μM)	97 ± 7 (5)	116 ± 8†	97 ± 7 (5)	99 ± 5†
WEB 2086 (50 μM)	74 ± 8 (6)	71 ± 7†	94 ± 7 (7)	94 ± 9†
Metyrapone (1 mM)	89 ± 7 (7)	83 ± 9†	98 ± 7 (5)	81 ± 5†
SKF 525A (25 μM)	86 ± 5 (7)	87 ± 6†	75 ± 9 (6)	74 ± 11†

Data are expressed as mean (± s.e.mean) % reductions in the level of Phe (0.05 μM)-induced tone. n = the number of paired observations.
† Relaxation response not significantly different from corresponding control response ($P > 0.05$, paired t test).

Table 3 Effect of methylene blue and phenidone on the relaxant potency (pD_2) of histamine and methacholine in co-axial assemblies

	Control	*pD_2*	*Test (+ agent)*	*Ratio¶*
Histamine				
Methylene blue (50 μM)	5.06 ± 0.06	(10)	5.55 ± 0.11*	3.1
Phenidone (50 μM)	4.99 ± 0.04	(5)	4.89 ± 0.08†	0.8
Methacholine				
Methylene blue (50 μM)	5.53 ± 0.07	(9)	6.35 ± 0.09*	6.7
Phenidone (50 μM)	5.45 ± 0.06	(6)	5.82 ± 0.18†	2.3

Data are expressed as mean ± s.e.mean. Numbers in parentheses indicate the number of paired observations.
¶ Ratio of EC_{50} values = EC_{50} Control: EC_{50} Test.
* pD_2 value significantly different from control value ($P < 0.05$, paired t test).
† pD_2 value not significantly different from control ($P > 0.05$, paired t test).

Discussion

In a co-axial bioassay system containing epithelium-intact guinea-pig trachea, histamine as well as the muscarinic agonists methacholine, acetylcholine, carbachol, bethanechol, oxotremorine and arecoline caused concentration-dependent relaxation of assay preparations via the release of EpDIF. Oxotremorine is a muscarinic agonist equal in potency to acetylcholine, but with no apparent nicotinic activity (Cho *et al.*, 1962). In contrast, arecoline has a higher intrinsic activity than acetylcholine, but is approximately 16 times less potent than acetylcholine in guinea-pig isolated ileum (Gloge *et al.*, 1966). In this study, however, oxotremorine and arecoline were found to be approximately 17 and 33 times less potent respectively than acetylcholine in inducing the release of EpDIF from guinea-pig trachea. Removal of the epithelium from EpDIF donor airway tissue resulted in abolition of this relaxation indicating that these responses were due to the release of EpDIF. Fernandes *et al.* (1989) showed that histamine- and methacholine-induced release of EpDIF was mediated via histamine H_1-receptors and muscarinic cholinoceptors respectively.

Prostaglandin E_2 is the major prostanoid produced by the epithelium in response to mediators such as major basic protein (Jacoby *et al.*, 1988). This prostanoid can reduce airway smooth muscle contraction (Barnett *et al.*, 1988; Manning *et al.*, 1989). In the present study however, PGE_2 caused concentration-dependent contraction of assay tissue and has previously been shown to induce further contraction of precontracted endothelium-denuded rat aorta preparations (Hay *et al.*, 1989), indicating that this prostanoid could not be the EpDIF detected in our co-axial bioassay.

The present studies were routinely performed in the presence of indomethacin (5 μM) in order to inhibit cyclo-oxygenase activity. In addition, BW 755C (150 μM), phenidone (50 μM), NDGA (10 μM) and gossypol (30 μM) did not inhibit EpDIF activity in this system, indicating that this inhibitory factor is neither a cyclo-oxygenase nor a lipoxygenase product and is apparently unaffected by radical scavengers and antioxidants.

The failure of methylene blue (50 μM) to inhibit relaxation responses to histamine (100 μM) and methacholine (25 μM) in co-axial assemblies, indicates that EpDIF does not act via stimulation of soluble guanylate cyclase. However, EpDIF-induced relaxation responses have been associated with increases in guanosine 3':5'-cyclic monophosphate levels, perhaps generated via membrane-bound guanylate cyclase (Hay *et al.*, 1989). The reasons for methylene blue-induced increases in the potencies and maximal relaxant effects of histamine and methacholine are unclear but presumably do not involve inhibition of soluble guanylate cyclase. The present study also showed that EpDIF was not a stimulant of leukotriene receptors, β-adrenoceptors or PAF receptors.

Superoxide dismutase (50 u ml^{-1}) caused enhancement of EDRF-induced relaxation responses of rat aorta but failed to alter those induced by EpDIF, indicating that the latter was apparently unaffected by superoxide radicals. Phosphoramidon, a neutral endopeptidase inhibitor was similarly ineffective in altering EpDIF activity in this system. If EpDIF was a peptide substrate for this enzyme, phosphoramidon might have been expected to alter its relaxant activity.

Removal of the epithelium has been shown to result in increased sensitivity of guinea-pig tracheal preparations to substance P (Tschirhart & Landry, 1986), LTC_4 and LTD_4 (Hay *et al.*, 1987; Hisayama *et al.*, 1988). While the increased sensitivity to substance P observed in epithelium-denuded guinea-pig tracheal preparations may be attributed to the loss of a metabolic site for this spasmogen (Fine *et al.*, 1989), it has been suggested that the leukotrienes may stimulate the production of an airway smooth muscle-sensitive inhibitory factor by the epithelium (Hay *et al.*, 1987; Hisayama *et al.*, 1988). However, stimulation of EpDIF release from guinea-pig trachea appears to be selective for some agonists including histamine and methacholine, whereas A23187 and hyperosmotic stimuli, stimulation of nicotinic cholinoceptors and receptors for bradykinin, substance P, PAF and the leukotrienes, all failed to induce EpDIF release that could be detected in this system. Furthermore, inhibition of cytochrome P-450 by metyrapone (1 mM) or SKF 525A (25 μM) failed to inhibit EpDIF-induced relaxation responses. Thus, while this enzyme system has been linked to epithelium-dependent modulation of smooth muscle contractility (Raeburn *et al.*, 1988), it was not involved in the production of EpDIF in the present system. These data suggest that the vascular smooth muscle-sensitive EpDIF detected with our co-axial bioassay was not the same factor as has been suggested to modulate airway smooth muscle tone.

It is important to note that several studies involving removal of the epithelium have shown that the potency of carbachol in isolated airway preparations was not significantly altered (Frossard & Muller, 1986; Goldie *et al.*, 1986; Tschirhart *et al.*, 1987; Hisayama *et al.*, 1988; Lundblad & Persson, 1988), suggesting that this agonist did not stimulate the production of an airway smooth muscle-sensitive EpDIF by the epithelium. However, the present study has demonstrated that carbachol induced EpDIF release that can be detected by a co-axial bioassay system. It may be that the co-axial bioassay is a more sensitive means of assessing the influence of cholinoceptor agonists on the epithelium than is the measurement of airway smooth muscle responsiveness before and after epithelium removal. Alternatively, the epithelium may secrete at least two inhibitory factors, one which selectively modulates airway smooth muscle tone (Vanhoutte, 1988) and one which modulates vascular smooth muscle tone (Vanhoutte, 1988; Fernandes *et al.*, 1989; the present study).

This study has evaluated an inhibitory factor released by guinea-pig tracheal epithelium in response to histamine and muscarinic agonists that is capable of relaxing vascular smooth muscle. The primary target for this, as yet unidentified, EpDIF may be the airway wall microcirculation. Increased bronchial blood flow might protect against severe bronchoconstriction by increasing the rate of removal of spas-

mogens from the airway wall. Reduction in EpDIF activity due to damage to the airway epithelium, as seen in asthma (Laitinen *et al.*, 1985; Dunnill, 1987) may therefore contribute towards bronchial hyperreactivity to spasmogens which is commonly associated with this disease (Boushey *et al.*, 1980; Jeffery *et al.*, 1989).

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