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Effects of eicosanoids, neuromediators and bioactive peptides on murine airways

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Abstract

The effects of several mediators including prostanoids, neuromediators, bioactive peptides and leukotrienes were investigated on the trachea, upper bronchi, lower bronchi and lung parenchyma of selected strains of mice mounted in a cascade superfusion system. The upper airways (trachea, upper bronchi) responded with greater maxima than lower airways (lower bronchi, lung parenchyma). Acetylcholine, carbachol, serotonin and 9,11-dideoxy-9α,11α-epoxymethano-prostaglandin $F_{2\alpha}$ (U-44069) contracted the mouse airways (acetylcholine > carbachol > serotonin ≥ U-44069). However, the rank order of potency for these compounds was U-44069 > carbachol > serotonin ≥ acetylcholine. Prostaglandins E_2, $F_{2\alpha}$ and D_2 relaxed the carbachol precontracted isolated mouse airways achieving > 90% relaxation in some cases. The rank order of potency for the prostaglandins was $E_2 \geq F_{2\alpha} > D_2$ with the exception of the lower bronchi on which prostaglandins had the following order of potency: $F_{2\alpha} \geq E_2 > D_2$. The effects of prostaglandins were similar in four commonly used strains of mice (CD-1, BALB/c, C57BL/c6 and C3H) with some variations in efficacy. Iloprost was a weak mouse airway relaxant. It had the greatest effect on the trachea and bronchi of BALB/c and C57BL/c6 mice, whereas it had little or no effect on the airways of the CD-1 and C3H mouse strains. Vasoactive intestinal peptide potently relaxed the carbachol and precontracted the mouse trachea and bronchi. However, vasopressin, another bioactive peptide, potently and efficaciously contracted the mouse trachea and upper bronchi but had little effect on the lower bronchi. Vasopressin was the most potent and efficacious contractile agonist tested in this study. Contractions were observed with endothelins-1, -2 and -3 on mouse trachea and bronchi, but marked tachyphylaxis was present. Sarafotoxin s6c followed the same pattern suggesting the presence of endothelin ET_B receptors on the mouse airways. Of all leukotrienes assayed (B_4, C_4, D_4 and E_4) only leukotriene C_4 weakly contracted the mouse trachea and upper bronchi, but tachyphylaxis was most evident.

Keywords: (Mouse); Airway; Prostaglandin; Leukotriene; Neuromediator; Endothelin

1. Introduction

Asthma is a disease characterized by recurrent episodes of bronchoconstriction, non-specific bronchial hyperreactivity, cellular migration in the airways, bronchial desquamation and airway oedema (Djukanovic et al., 1990). These characteristic features are related to the release of several inflammatory mediators by lung cells. For instance, eicosanoids (prostaglandins, thromboxane A_2 and leukotrienes) have been shown to be, among many others, potent myotropic agents acting either directly or indirectly on airway smooth muscles. Depending on the species studied and the experimental conditions, prostaglandins have been shown to elicit either contractile or relaxing effects on isolated airway tissues whereas thromboxane A_2 was a potent bronchoconstrictor in most tissues. Prostaglandin E_2 relaxes the guinea pig trachea under basal tone but all other prostanoids contract the tissue (Schneider and Drazen, 1980). Moreover, under low tonal level (in the presence of a cyclooxygenase inhibitor), all naturally occurring prostanoids contract the guinea pig trachea (Gardiner, 1986). At high tonal level where a submaximal contraction is induced by a non-eicosanoid spasmogen (usually carbachol), the true relaxing effect of prostaglandins was revealed (Coleman and Kennedy, 1980; Schneider and Drazen, 1980). Prostanoids have also been studied in many other species, such as ferret, sheep, rabbit,

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pig, chick and frog trachea or bronchus. Prostaglandin E_2 reduced either basal or precontracted tone of the trachea or bronchus of the aforementioned species (Main, 1964; Chand and Eyre, 1978; Esculenta et al., 1983). However, prostaglandin $F_{2\alpha}$ was a contractile agonist on ferret, chick and frog trachea and bronchus.

In studies using human airway tissues, prostaglandins $F_{2\alpha}$, D_2 and E_2 have been shown to be contractile agonists on the inherent tone (Sweatman and Collier, 1968; Dawson et al., 1974; Hedqvist et al., 1978; Karim et al., 1980; Black et al., 1986). In some cases, however, prostaglandins $F_{2\alpha}$ and E_2 have been shown to relax these tissues (Collier and Gardiner, 1974). Other prostanoids such as prostaglandin E_1 and, to a lesser extent, prostaglandin I_2 relax the inherent tone and tone induced by other agonists (carbachol, histamine and prostaglandin $F_{2\alpha}$) (Sheard, 1968; Sweatman and Collier, 1968; Collier and Gardiner, 1974; Gardiner, 1975; Strandberg and Hedqvist, 1977; Karim et al., 1980; Hage-Legrand et al., 1986). Prostaglandin I_2 is also capable of dual effects. Compound U-46619 (thromboxane A_2 mimetic) is a potent contractile agonist on human bronchial muscles (McKenniff et al., 1988).

Leukotrienes, like the prostaglandins, have also been studied in many species and are known to be potent contractile agents of the airway smooth muscles (Gardiner, 1989). In man, leukotriene D_4 and leukotriene C_4 have been shown to be approximately 1200 times more potent than histamine (Barnes et al., 1984). Many other mediators, such as serotonin, acetylcholine, histamine and selected peptides (bradykinin, vasoactive intestinal peptide, vasopressin) are active on airways and have been postulated to be involved in asthma. The present study aims at exploring the effects (contractile or relaxing) of eicosanoids and selected mediators on four different levels of the murine airways (trachea, upper and lower bronchi, and lung parenchyma). In this study we show the effects of the above mentioned mediators and give the results of calculations of pharmacological parameters such as ED_{50} and E_{max} for these substances. In the case of prostaglandins, four commonly used strains of mice were used to verify that the effects of these compounds are not particular to one specific strain. Furthermore, due to the lack of such data for mouse airways, the basic results presented in this study may be useful in future studies related to asthma in mice.

2. Materials and methods

2.1. Animals

All animals referred to in this study were used and cared for according to the Canadian Council on Animal Care and the Guide to the Care and Use of Experimental Animals.

2.2. Preparation of isolated airway tissues

Male CD-1 mice weighing 20–25 g (Charles River, St-Constant, PQ, Canada) were anaesthetized with pentobarbitone sodium 50 mg kg^{-1} i.p. (May and Baker, Montreal, Canada) and exsanguinated by cutting the abdominal aorta. The thoracic cavity was thereafter opened, the lungs were removed and immediately put into an oxygenated (95% O_2 and 5% CO_2) Krebs–Henseleit solution (mM composition: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.18, $NaHCO_3$ 25, $MgSO_4$ 7, H_2O 1.18 and glucose 11.1), pH 7.0. The trachea, upper and lower bronchi, and

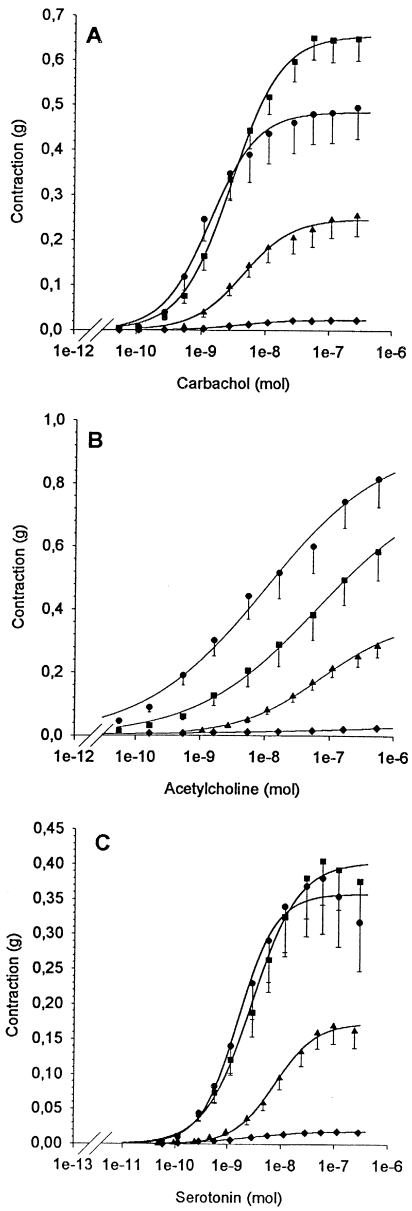

Fig. 1. Effect of carbachol (A), acetylcholine (B) and serotonin (C) on mouse airways. These compounds were administered in a dose range of 2×10^{-11}–4.8×10^{-6} on trachea (●), upper bronchi (■), lower bronchi (▲) and lung parenchyma (♦) to obtain contractile dose–response curves. Results are mean ± S.E.M. of 11 to 18 experiments.

parenchyma were dissected and cleaned of remaining connective tissue and fat. In this study, upper and lower bronchi will be defined as follows: the upper bronchus (main bronchus) extends from the trachea to the parenchymal lobe and the lower bronchus will be considered as being the part extending through the parenchymal lobe. Tracheal and bronchial rings and strips of left lung parenchymal lobe were mounted onto Grass isometric force-displacement transducers (FTO3C) coupled to a Grass 7D polygraph. The tissues were put under an initial tension of 0.5 g (bronchi and parenchyma) and 1.0 g (trachea) and superfused at a rate of 5 ml min^{-1} with oxygenated Krebs–Henseleit buffer (37°C) in a cascade superfusion system. Tissues were equilibrated for 60 min.

2.3. Experimental protocol

After a 60-min equilibration period, the responsiveness of the tissues was tested with carbachol (2 μg bolus; approximate ED_{50} for carbachol). Bolus injections of each agonist were then administered to the tissues under basal tone to obtain sequential dose–response curves. All compounds were initially treated as contractile agents before being tested as relaxing agents. For agonists that did not elicit an increase in tension under basal tone, tracheal and bronchial preparations were pre-contracted with an infusion of 15 and 30 ng ml^{-1} carbachol, respectively, via a syringe infusion pump (Harvard Apparatus, St-Laurent, PQ, Canada). Bolus injections (5–500 ng) of prostaglandins were administered after tissues reached a steady state of contraction before assessing relaxation. When the effect of the selected prostaglandin was completed, the infusion of carbachol was stopped and the tissues were left to stabilise before the next pre-contraction. In all cases, vehicle was tested for a response, but no effect was observed.

2.4. Drugs used

The following drugs were used: Carbachol, acetylcholine hydrochloride, serotonin (5-HT), vasopressin, noradrenalin, histamine from Sigma, Mississauga, Ontario, Canada; 9,11-dideoxy-9α,11α-epoxymethano-prostaglandin $F_{2\alpha}$ (U-44069), prostaglandin E_2, prostaglandin $F_{2\alpha}$ from Upjohn, Kalamazoo, MI, USA; prostaglandin D_2 from Cayman, Ann Arbor, MI, USA; Iloprost from Schering, Berlin, Germany. Leukotrienes were supplied by Merck-Frosst Montreal, PQ, Canada. Endothelins and sarafotoxin s6c were from American Peptide, Sunnyvale, CA, USA. Vasoactive intestinal peptide (VIP) was a generous gift from Dr. Alain Fournier. Bradykinin was synthesised locally.

2.5. Statistics and data analysis

All ED_{50} values, the effective dose causing 50% of maximal response, were obtained from the calculation of ED_{50} for each individual representative curve and then averaged ± S.E.M.

Sigmoidal curves were obtained by fitting the dose–response curve data by the logistic equation:

$$\text{Effect} = \frac{E_{\max} \times [\text{A}]^n}{[\text{A}]^n + [\text{A}_{50}]^n}$$

where $E_{\max}$ is the maximal effect, $[A]$ is the agonist concentration, and n is the slope parameter. For experiments with agonists that induced contractile responses, $[A_{50}]$ represents the dose that induced 50% of the maximal contraction. For experiments with relaxing agonists, $[A_{50}]$ is the dose that induced 50% of the maximal relaxation.

All results marked with an asterisk are estimated ED_{50} or $E_{\max}$ from curve fitting parameters.

3. Results

3.1. Effects of neuromediators on murine airways

Acetylcholine, carbachol and serotonin induced dose-dependent contractions of murine airways (Fig. 1). Carbachol, a synthetic muscarinic receptor agonist (not a neuro-

Table 1
ED_{50} and $E_{\max}$ values for neuromediators on mouse airways (C, contraction; R, relaxation)

Agonist	Tissue	Effect	ED_{50} (mol)	S.D. (mol)	$E_{\max}$ (g)	S.D. (g)
Serotonin	Trachea	C	1.6×10^{-9}	2.6×10^{-10}	0.36	0.011
	Upper bronchi	C	2.9×10^{-9}	3.4×10^{-10}	0.40	0.010
	Lower bronchi	C	7.9×10^{-9}	6.2×10^{-10}	0.17	0.004
	Lung parenchyma	C	2.0×10^{-9}	3.8×10^{-10}	0.02	0.0006
Acetylcholine	Trachea	C	5.9×10^{-9}	2.1×10^{-9}	1.00	0.052
	Upper bronchi	C	6.3×10^{-8}	1.1×10^{-8}	0.85	0.034
	Lower bronchi	C	7.3×10^{-8}	6.4×10^{-9}	0.38	0.009
	Lung parenchyma	C	4.2×10^{-7}	2.3×10^{-7}	0.04	0.004
Carbachol	Trachea	C	1.1×10^{-9}	1.2×10^{-10}	0.48	0.009
	Upper bronchi	C	2.7×10^{-9}	1.5×10^{-10}	0.66	0.008
	Lower bronchi	C	4.0×10^{-9}	5.0×10^{-10}	0.25	0.008
	Lung parenchyma	C	4.2×10^{-9}	2.8×10^{-10}	0.02	0.0004

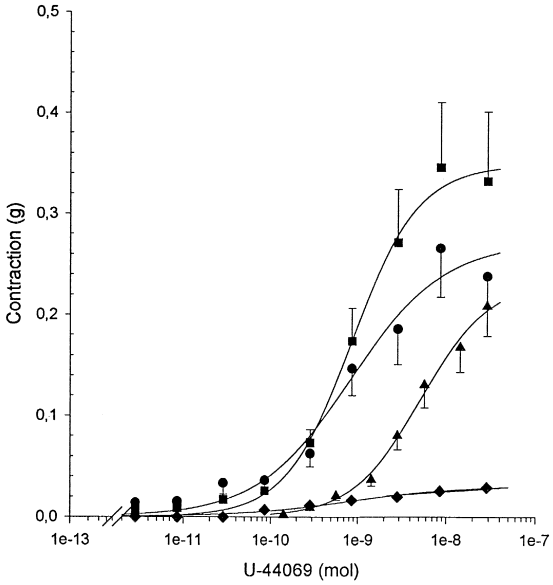

Fig. 2. Effect of compound U-44069 (2.85×10^{-10} to 2.85×10^{-8} mol), a thromboxane A_2 mimetic, on mouse airways. Contractile dose–response curves to U-44069 on CD-1 mouse trachea (●), upper bronchi (■), lower bronchi (▲) and lung parenchyma (♦). Points are means ± S.E.M. of 9 to 16 experiments.

mediator) will be discussed in this section. All three agonists were very potent in contracting in this order; the trachea, the upper bronchi, the lower bronchi and the lung parenchyma. Carbachol, in a dose range of 2×10^{-11}–4×10^{-7} mol was the most potent. Acetylcholine (6×10^{-10} to 4.8×10^{-6} mol), though less potent than carbachol, was a stronger contractile agonist than serotonin (7×10^{-11} to 4×10^{-7} mol) on lung tissues (Fig. 1, Table 1). Conversely, another neurotransmitter, norepinephrine, relaxed the tracheal smooth muscles (data not shown). Histamine induced contractions of the murine trachea only at very high concentrations (data not shown). The ED_{50} and E_{max} values of serotonin were 1.6×10^{-9} mol and 0.36 g on the trachea, 2.9×10^{-9} mol and 0.4 g on the upper bronchi, 7.9×10^{-9} mol and 0.17 g on the lower bronchi, and 2×10^{-9} mol and 0.02 g on lung parenchyma. Threshold doses for achieving initial responses varied from 1.5×10^{-10} to 1.5×10^{-9} mol through the airways from trachea to lung parenchyma, respectively (Fig. 1). On the other hand, contractions induced by acetylcholine gave greater E_{max} values but with lesser potency. The ED_{50} and E_{max} values of acetylcholine were 5.9×10^{-9} mol and 1.0 g on the trachea, 6.3×10^{-8} mol and 0.85 g on the upper bronchi, 7.3×10^{-8} mol and 0.38 g on the lower bronchi, and 4.2×10^{-7} mol and 0.04 g on lung parenchyma. The maximum was not obtained on lung parenchyma and the values have been extrapolated. ED_{50} and E_{max} values of carbachol on lung tissues were comparable to those obtained with serotonin. Carbachol was more potent but less efficacious than acetylcholine in contracting the airway tissues. ED_{50} and E_{max} values of carbachol were 1.1×10^{-9} mol and 0.48 g on the trachea, 2.7×10^{-9} mol and 0.66 g on the upper bronchi, 4×10^{-9} mol and 0.25 g on the lower bronchi, and 4.2×10^{-9} mol and 0.02 g on lung parenchyma.

Carbachol was also used as a contractile agent in precontraction experiments where an elevated tone was needed. Carbachol was infused at 15 and 30 ng ml^{-1} on the trachea and bronchi, respectively, leading to a sustained contraction of 0.75 to 1.0 g. All compounds having relaxing properties were injected after the precontraction had reached a stable plateau. The relaxations induced by the selected compounds were calculated as a percentage of the associated precontraction.

3.2. Effects of prostanoids on murine airways

Compound U-44069 (a thromboxane A_2 mimetic) in a dose range of 2.85×10^{-10}–2.85×10^{-8} mol caused marked dose-dependent contractions of the murine airway preparations (Fig. 2). Increases in tension were greater on the upper airways (trachea and upper bronchi), whereas the lower airways showed lesser reactivity to this compound. U-44069 induced a maximal contraction (E_{max}) of 0.26 g

Table 2
ED_{50} and E_{max} values for prostanoids on mouse airways (C, contraction; R, relaxation)

Agonist	Tissue	Effect	ED_{50} (mol)	S.D. (mol)	E_{max} (g or %)	S.D. (g or %)
Thromboxane A_2	Trachea	C	8.8×10^{-10}	2.6×10^{-10}	0.26	0.020
	Upper bronchi	C	9.1×10^{-10}	9.9×10^{-11}	0.35	0.011
	Lower bronchi	C	5.2×10^{-9}	1.2×10^{-9}	0.24	0.026
	Lung parenchyma	C	8.3×10^{-10}	4.6×10^{-10}	0.03	0.005
Prostaglandin D_2	Trachea	R	1.6×10^{-10}	2.3×10^{-11}	91.1%	1.23
	Upper bronchi	R	1.6×10^{-10}	7.0×10^{-12}	92.8%	1.67
	Lower bronchi	R	6.1×10^{-10}	3.0×10^{-11}	81.3%	2.01
Prostaglandin $F_{2\alpha}$	Trachea	R	3.9×10^{-11}	5.0×10^{-12}	83.9%	1.30
	Upper bronchi	R	6.6×10^{-11}	5.2×10^{-12}	73.2%	1.12
	Lower bronchi	R	4.9×10^{-11}	3.5×10^{-12}	93.4%	1.79
Prostaglandin E_2	Trachea	R	1.4×10^{-11}	2.5×10^{-12}	92.1%	1.33
	Upper bronchi	R	2.9×10^{-11}	3.0×10^{-12}	89.0%	1.02
	Lower bronchi	R	1.2×10^{-10}	8.1×10^{-12}	88.4%	1.54

of the trachea with an ED_{50} of 8.8×10^{-10} mol. On the upper bronchi, the E_{max} of U-44069 was 0.35 g with an ED_{50} of 9.1×10^{-10} mol. The maximal contraction of the lower bronchi to U-44069 was 0.24 g with an ED_{50} of 5.2×10^{-9} mol. The E_{max} and ED_{50} values of this compound on the lung parenchyma were 0.03 g and 8.3×10^{-10} mol, respectively (Table 2).

Prostaglandins E_2, $F_{2\alpha}$, and D_2 caused dose-dependent relaxations of precontracted trachea and bronchi (Fig. 3), but did not affect basal tone. Lung parenchyma could not be sufficiently precontracted to visualize relaxations and was therefore left out of this set of experiments. Prostaglandin E_2 and prostaglandin $F_{2\alpha}$ showed similar potencies in relaxing the airways, whereas prostaglandin D_2 was consistently less potent than the latter two (Table 2, Fig. 3). As far as trachea and bronchi were concerned, a maximal response of 73% to 93% relaxation could be achieved by doses ranging from 5 to 200 ng of the three prostaglandins studied (Fig. 3). It is noteworthy that threshold doses to achieve initial relaxation were different (Fig. 3). Iloprost (prostaglandin I_2 analog) induced week relaxations only at very high doses (> 1 μg; $> 2.77 \times 10^{-9}$ mol) compared to the effect of other prostaglandins (5–200 ng; 1.42×10^{-11}–5.67×10^{-10} mol). ED_{50} values for prostaglandin E_2, prostaglandin $F_{2\alpha}$ and prostaglandin D_2 on the trachea were 1.4×10^{-11}, 3.9×10^{-11} and 1.6×10^{-10} mol, respectively. Similar results were obtained on the upper bronchi. ED_{50} values of prostaglandin E_2, prostaglandin $F_{2\alpha}$ and prostaglandin D_2 on this tissue were 2.9×10^{-11}, 6.6×10^{-11} and 1.6×10^{-10} mol, respectively. ED_{50} values of prostaglandin E_2, prostaglandin $F_{2\alpha}$ and prostaglandin D_2 on the lower bronchi were 1.2×10^{-10}, 4.9×10^{-11} and 6.1×10^{-10} mol.

3.3. Effects of eicosanoids on selected mouse strains

Since prostaglandin $F_{2\alpha}$ and prostaglandin D_2 have contractile activities in the airways of some species (Gardiner, 1989), the observed relaxing effect of these prostaglandins in our experiments was tested on selected strains of mice. A fixed dose of each prostaglandin giving an appreciable response in CD-1 mice, was used to verify strain dependent variance of responsiveness in Balb/c, C57BL/c6, C3H and CD-1 mice.

Prostaglandin $F_{2\alpha}$ and prostaglandin D_2 induce relaxations in all four strains of mice studied. However, these effects were variable in potency depending on the strain (Fig. 4). Prostaglandin D_2 (200 ng) showed similar potency in tracheae of all four strains (Fig. 4A). The effect of prostaglandin $F_{2\alpha}$ (20 ng) on the trachea showed a tendency to decrease in the following order: C3H > C57BL/c6 > Balb/c = CD-1 (Fig. 3B). Interestingly, iloprost (prostaglandin I_2) had no effect on CD-1 mouse tracheae but relaxed the tracheae of the other strains (Balb/c > C57BL/c6 > C3H) at a dose of 1 μg (Fig. 4C). Prostaglandin D_2 (200 ng; 5.67×10^{-10} mol) induced similar responses on the upper bronchi of CD-1 and C3H strains with a slightly lower response in Balb/c and slightly higher response in C57BL/c6 (Fig. 4D). Prostaglandin $F_{2\alpha}$ (20 ng; 4.21×10^{-11} mol) induced

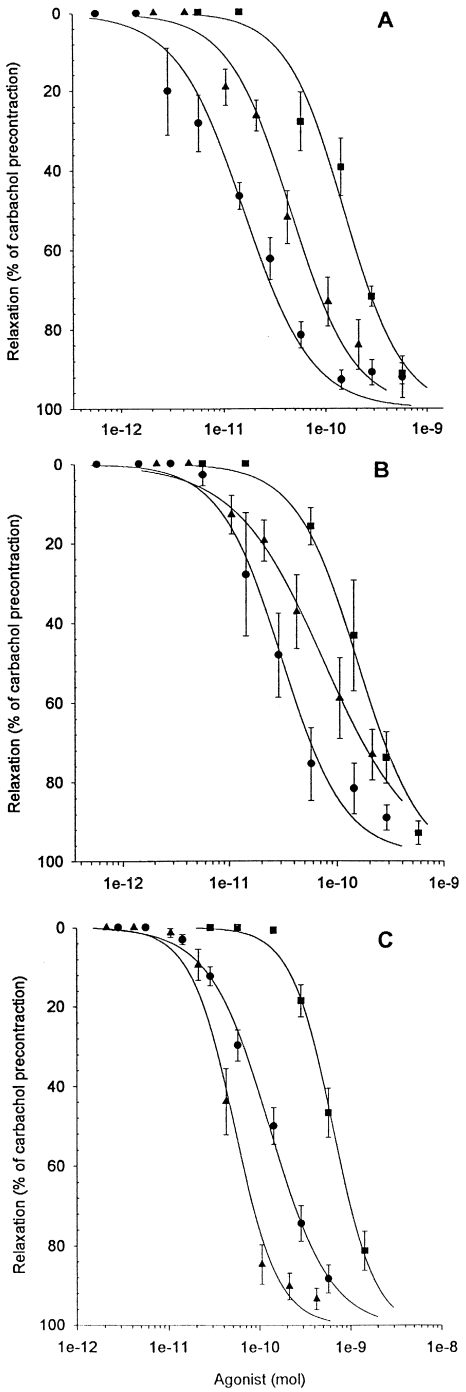

Fig. 3. Effects of prostaglandin E_2, prostaglandin D_2 and prostaglandin $F_{2\alpha}$ on mouse airways. Tissues were precontracted with an infusion of carbachol. Bolus doses of prostaglandins were given after a stable plateau was reached. Relaxation dose–response curves to prostaglandin E_2 (●), prostaglandin $F_{2\alpha}$ (▲) and prostaglandin D_2 (■) are shown on (A) trachea, (B) upper bronchi and (C) lower bronchi. Points are means ± S.E.M. of 7 to 12 experiments.

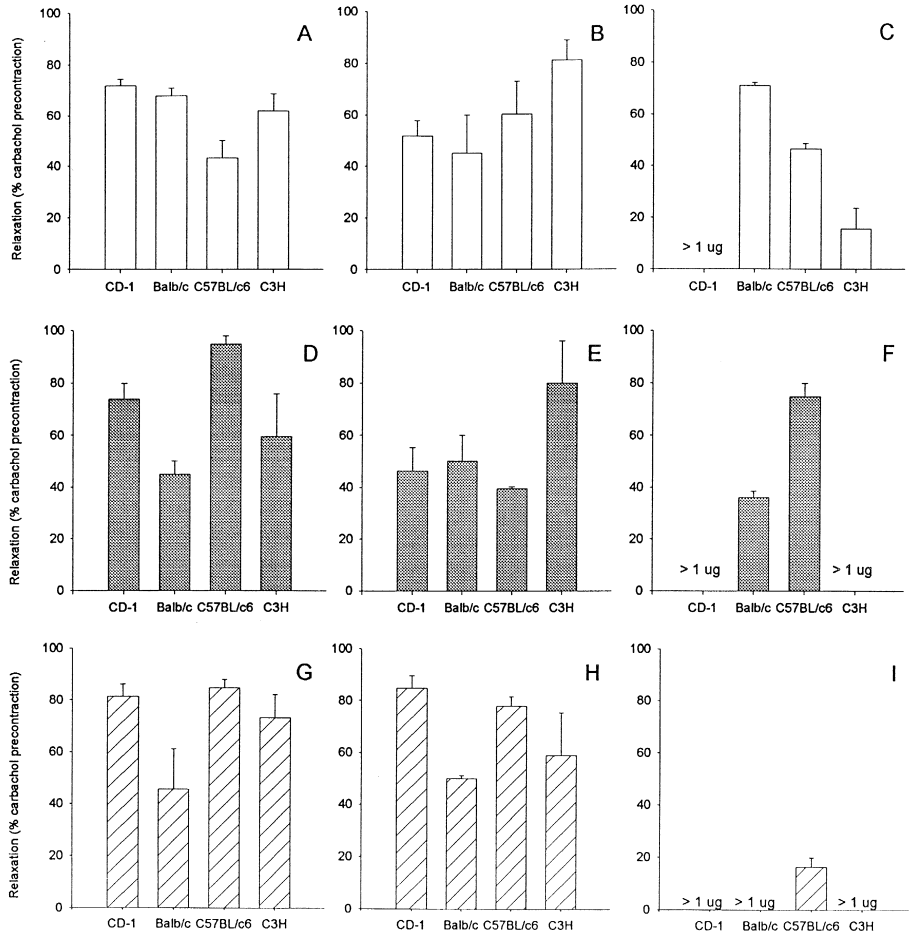

Fig. 4. Comparative effects of prostaglandins on CD-1, Balb/c, C57BL/c6 and C3H mouse strains. The effects of 200 ng (5.67×10^{-12} mol) prostaglandin D_2 (A and D), 500 ng (1.42×10^{-9} mol) prostaglandin D_2 (G), 20 ng (4.21×10^{-12} mol) prostaglandin $F_{2\alpha}$ (B and E), 50 ng (1.05×10^{-10} mol) prostaglandin $F_{2\alpha}$ (H) and 1 μg (2.77×10^{-9} mol) iloprost (C, F, and I) are shown on the trachea (open columns), upper bronchi (closed columns) and lower bronchi (cross-hatched columns). Shown are relaxations as percentage of precontraction. Points are means ± S.E.M. of 6 to 10 experiments.

equipotent relaxations in CD-1, Balb/c and C57BL/c6 mice but a greater response was seen in the C3H strain (Fig. 4E). Iloprost (1 μg; 2.77×10^{-9} mol) relaxed the upper bronchi of C57BL/c6 and Balb/c mice but the compound had no effect on CD-1 and C3H strains (Fig. 4F). The effect of prostaglandin D_2 (500 ng; 1.42×10^{-9} mol) on lower bronchi was similar in CD-1, C57BL/c6 and C3H mice with a slightly lower potency on the lower bronchi of Balb/c mice (CD-1 = C57BL/c6 = C3H > Balb/c) (Fig. 3G). The same tendency was observed for prostaglandin $F_{2\alpha}$ (50 ng; 1.05×10^{-10} mol) (Fig. 4H). However, iloprost (1 μg; 2.77×10^{-9} mol) had no effect on the lower bronchi of CD-1, Balb/c or C3H mice and had a small effect on C57BL/c6 mice (Fig. 3I).

3.4. Effects of bioactive peptides on murine airways

Vasopressin induced dose-dependent contractions of the trachea and upper and lower bronchi (Fig. 5). The ED_{50} and E_{max} values of vasopressin were 5.4×10^{-11} mol and 0.85 g on trachea, 5.8×10^{-11} and 0.72 g on upper bronchi, and 1.3×10^{-10} mol and 0.04 g on lower bronchi (Table 3). Vasoactive intestinal peptide induced, in contrast, a dose-dependent relaxation of the trachea, upper and lower bronchi (Fig. 5B). The ED_{50} values were 1.4×10^{-10} mol on trachea, 1.4×10^{-10} mol on upper bronchi and 8.8×10^{-11} mol on lower bronchi. Maximal responses ranging from 78% to 92% were obtained. Endothelins-1, -2, -3 and sarafotoxin s6c contracted the mouse trachea and upper bronchi, but these contractions were not dose-dependent in consecutive doses on the same tissue (data not shown). Another peptide, bradykinin, contracted mouse airway smooth muscle preparations only at high (non-physiological) doses.

3.5. Effects of leukotrienes on murine airways

The effects of leukotrienes (leukotriene B_4, leukotriene C_4, leukotriene D_4 and leukotriene E_4) were assayed on all mouse airway tissue preparations (trachea, upper bronchi, lower bronchi and lung parenchyma) of the four strains

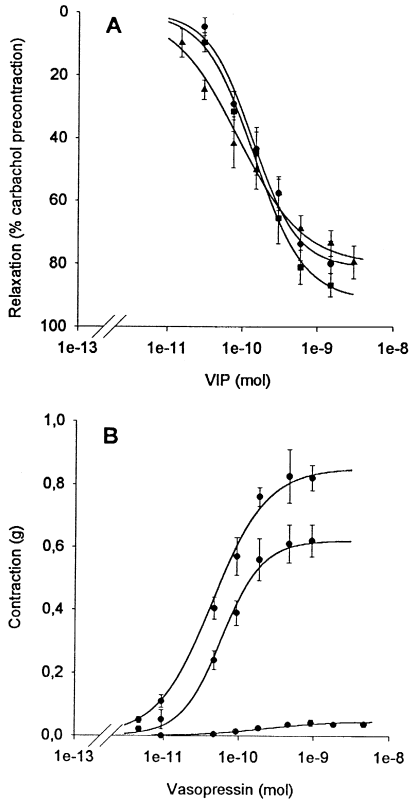

Fig. 5. Effects of vasoactive intestinal peptide (A) and vasopressin (B) on mouse trachea (●), upper bronchi (■) and lower bronchi (▲) in a dose range of 1.5×10^{-11}–3×10^{-9} and 9.22×10^{-12}–4.61×10^{-9} mol, respectively. Vasopressin was administered at the tissue basal tone (contractile dose–response curves) and vasoactive intestinal peptide was given to tissues precontracted with an infusion of carbachol (15 and 30 ng ml^{-1} for trachea and bronchi, respectively) (relaxation dose–response curves). Results are mean ± S.E.M. of 12 to 17 experiments.

used in this study. The maximal dose used was 5×10^{-10} mol. In most cases, leukotrienes had no effect on mouse airways, but in a few cases, very weak contractions to leukotriene C_4 (5×10^{-10} mol) were observed on trachea and upper bronchi of CD-1 mice. This effect on trachea and upper bronchi was very tachyphylactic. Each dose of leukotriene C_4 was given at 90-min intervals. Between two leukotriene C_4 administrations, four evenly spaced carbachol (2 μg) injections were given to assess the contractility of the tissue preparation.

4. Discussion

A number of in vitro studies have been done in airway tissues of species such as guinea pig, rat, dog, human, etc. (Gardiner, 1989), but very few were done with murine trachea due to the small size of this preparation. Generally, there are two methods of airway tissue preparation; the first is to cut the tissue into a spiral and the other is to cut rings from the tissue. Efforts have been made to use murine trachea prepared as a spiral, but this preparation was very unreliable. It has therefore been concluded that mouse airway tissues (when possible) should be prepared as rings. Such a preparation avoids excessive manipulation and reduces the risk of damage to the tissue.

In the present investigation we have studied the reactivity of trachea (rings), bronchi (rings) and lung parenchyma (strips) to several mediators which may play a role in asthmatic and inflammatory reactions. Maximal responses to contractile agonists were more marked on the upper (trachea and upper bronchi) than in the lower airways (lower bronchi and lung parenchyma). This pattern is likely due to the amount of smooth muscle present in the preparation. Lung parenchyma, which is a preparation containing various smooth muscles of vascular and airway origins was the least reactive of the preparations. Our results showed that, in contrast to the myotropic response of guinea pig airways (Gardiner, 1989), the basal tone of the mouse trachea and bronchi was not maintained by mediators such as prostaglandins, since injections of exogenous prostaglandins (prostaglandin E_2, prostaglandin D_2, prostaglandin $F_{2\alpha}$ and iloprost) and/or incubation with indomethacin (a cyclooxygenase inhibitor) did not alter the baseline. A spasmogen-induced tone is required in order to assess relaxations by exogenous prostaglandins in our model. It is suggested that in a mouse model of asthma, prostaglandins could act as physiologic antagonists in constricted airways.

The parasympathetic nervous system, which is important in the regulation of airway tone, may be the main mediator of neuronal bronchoconstriction (Nadel and Davis, 1980). In our model, acetylcholine (a parasympathetic mediator) and its analog carbachol induced contractions of murine airways with decreasing efficacy from the

Table 3
ED_{50} and E_{max} values for bioactive peptides on mouse airways. C, contraction; R, relaxation

Agonist	Tissue	Effect	ED_{50} (mol)	S.D. (mol)	E_{max} (g or %)	S.D. (g or %)
VIP	Trachea	R	1.4×10^{-10}	1.2×10^{-11}	81.4%	2.34
	Upper bronchi	R	1.4×10^{-10}	7.2×10^{-12}	91.7%	1.77
	Lower bronchi	R	8.8×10^{-11}	2.7×10^{-12}	78.1%	1.05
Vasopressin	Trachea	C	5.4×10^{-11}	5.5×10^{-12}	0.85	0.027
	Upper bronchi	C	5.8×10^{-11}	6.0×10^{-12}	0.72	0.025
	Lower bronchi	C	1.3×10^{-10}	2.2×10^{-11}	0.04	0.019

upper to the lower airways. In guinea pigs and rats, acetylcholine and carbachol was also shown to induce dose-dependent airway contractions (Joos et al., 1994; Watts et al., 1994). In human bronchi, Aizawa et al. (1988) showed concentration dependent contractions to acetylcholine with an EC_{50} value of 3.5×10^{-5} M and an E_{max} of 1.4 ± 0.4 g.

Our results also showed that the thromboxane A_2 mimetic U-44069 induced marked contractions of mouse airways. In guinea pig, U-46619, another thromboxane A_2 mimetic, was shown to be a very potent and highly effective contractile agonist of the lung strip (Schneider and Drazen 1980; Coleman and Kennedy, 1985; Gardiner et al., 1987), but was somewhat less effective on the trachea under inherent tone (Schneider and Drazen 1980). This is explained by the fact that the basal tone of the guinea pig trachea is likely maintained by local prostanoid synthesis whereas the lung strip is not. McKenniff et al. (1988) also demonstrated U-46619 as a potent contractile agonist on the human bronchial muscle. Since the basal tone of mouse airway tissues was unaffected by indomethacin, exogenous thromboxane A_2 mimetic administration generated more potent contractions. In guinea pig airways, prostaglandins cause either contractions or relaxations of the trachea depending on the tonal level of the tissue. Under basal tone, prostaglandin D_2 > prostaglandin $F_{2\alpha}$ > prostaglandin I_2 (in this order) were contractile agonists (Schneider and Drazen, 1980) and in some cases prostaglandin E_2 contracted the guinea pig trachea (Gardiner and Collier, 1980). When the guinea pig trachea was at low tone, prostaglandins were potent contractile agonists with the following potency, prostaglandin E_2 = U-46619 > prostaglandin $F_{2\alpha}$ > prostaglandin I_2 > prostaglandin D_2. Under high tonal level, prostaglandin E_2 produced potent relaxations and prostaglandin I_2, prostaglandin D_2 and prostaglandin $F_{2\alpha}$ had variable but low relaxant activities (Gardiner, 1989). In contrast with the guinea pig airways, neither exogenous administration of prostaglandins, nor incubation of the mouse tissues with indomethacin affected the basal tone. However, mouse airway tissues relaxed in the presence of prostaglandins when they were precontracted with a spasmogen. The rank order of potency of relaxation was prostaglandin E_2 > prostaglandin $F_{2\alpha}$ > prostaglandin D_2 ≫ prostaglandin I_2 on trachea and upper bronchi, and prostaglandin $F_{2\alpha}$ ≥ prostaglandin E_2 > prostaglandin D_2 ≫ prostaglandin I_2, on lower bronchi. These findings are in agreement with the data obtained with the cat trachea which has no inherent tone and did not contract to any of the prostaglandins (Main, 1964; Horton and Main, 1965; Lulich et al., 1976; Apperley et al., 1979). When the cat tissue was precontracted with non-eicosanoid, all of the prostanoids except thromboxane A_2 or U-46619 produced dose-dependent relaxations with the following rank of potency, prostaglandin E_2 > prostaglandin I_2 > prostaglandin D_2 > prostaglandin $F_{2\alpha}$ (Gardiner, 1989). In human airway tissues, prostaglandins D_2 and $F_{2\alpha}$ were shown to be contractile agonists, whereas prostaglandins E_2 and I_2 were capable of dual actions, i.e., contractions or relaxations (Sheard, 1968; Sweatman and Collier, 1968; Collier and Gardiner, 1974; Dawson et al., 1974; Gardiner, 1975; Strandberg and Hedqvist, 1977; Hedqvist et al., 1978; Karim et al., 1980; Black et al., 1986; Hage-Legrand et al., 1986).

Our results also showed that relaxations of airway tissues induced by prostaglandins do not depend on the mouse strain but on the varied potency from one strain to the other. This finding was most evident for the effect of iloprost (prostaglandin I_2 analog). The main mouse strain used in this study (CD-1) showed only very weak tracheal and bronchial relaxations at very high doses of iloprost (> 1 μg; 2.77×10^{-9} mol) whereas in other mouse strains such as Balb/c and C57BL/c6, Iloprost was more potent. Prostaglandin D_2 and prostaglandin $F_{2\alpha}$ induced marked relaxations in all mouse strains studied. These findings suggest the presence of prostaglandin and thromboxane A_2 receptors in mouse airways. It is likely that the number of receptors present on the tissues accounted for the strain differences in responsiveness.

Our results also showed that murine airways contracted in the presence of neuromediators such as acetylcholine, carbachol and serotonin. Guinea pig trachea has been shown to contract to carbachol and serotonin (Watts and Cohen, 1992; Watts et al., 1994), the latter producing much lesser maximal contractions. EC_{50} values for carbachol and 5-HT were, respectively, around 1×10^{-7} M on the trachea whereas EC_{50} values ranged between 1.6 to 2.2×10^{-7} M for carbachol and 1.02 to 1.05×10^{-6} M for 5-HT in the rat (Joos et al., 1994). As in guinea pig and rat, our results show that in mouse airways serotonin produced lesser maximal contractions than carbachol, but both compounds have ED_{50} values around 10^{-9} mol. Garssen et al. (1990) have also reported contractions of the mouse trachea to carbachol and serotonin, with EC_{50} values of 3.9×10^{-7} and 5.2×10^{-3} M, respectively. The differences with our results could probably be explained by the different organ bath systems used in each study. Gardiner and Stock (1984) have demonstrated such differences in tissue reactivity between organ bath systems where, for instance, they showed that leukotriene D_4 was 304 times more potent than histamine in the cascade system but was 2187 times more potent than histamine in the isolated tissue bath system. We also demonstrated that the neuromediators studied at present, a similar rank order of potency in all tissues, but the following rank order of efficacy in contracting each tissue: acetylcholine > carbachol > 5-HT. Contractions to 5-HT were also observed in human parenchymal strips by Bertram et al. (1983), but they conclude that these contractions may be in large part due to the contractions of blood vessels present in the preparations.

Bioactive peptides such as vasoactive intestinal polypeptide, vasopressin and endothelins were also active

on mouse airway tissues. Vasoactive intestinal peptide, for instance, relaxed mouse airways as previously shown with guinea pig airways. Palmer et al. (1986) showed the relaxing capabilities of VIP in human isolated airways (EC_{50} 1.7×10^{-8} M). Vasopressin, which is a potent vasoconstrictor, was a bronchoconstrictor in mouse airways. Amongst the contractile agonists used in this study, vasopressin was the most potent (vasopressin > carbachol > 5-HT > acetylcholine), and one of the most efficacious in contracting the mouse trachea and upper bronchi. Lower bronchi responded very weakly to vasopressin. Endothelins which have been widely studied in guinea pig airways are known as very strong contractile agonists (Maggi et al., 1989; Filep et al., 1990). Endothelin-1, -2 and -3 induced marked contractions of mouse trachea and upper bronchi but these were markedly tachyphylactic; no dose–response curves could be obtained. Endothelins were also tested in a classical isolated tissue bath system but cumulative concentration–response curves were also impossible to obtain. Mouse trachea and upper bronchi also contracted in presence of sarafotoxin s6c, a specific endothelin ET_B receptor agonist. These contractions were similar to those induced by endothelins. This finding suggests the presence of endothelin ET_B receptors on the murine trachea and upper bronchi.

In conclusion, several agonists including neuromediators such as acetylcholine, carbachol, serotonin, prostanoids such as thromboxane A_2 (U-44069), prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostaglandin D_2 and in some cases Iloprost (prostaglandin I_2 analog), and bioactive peptides such as vasopressin, vasoactive intestinal peptide and endothelins were shown to have myotropic effects on mouse airways. The basal tone of mouse airways did not appear to be dependent on prostanoids. We have also shown that the relaxing properties of prostaglandins on mouse airways are not dependent on the strain of mouse used although variations in the relaxing efficacies of prostaglandins were noted in the selected strains. Leukotrienes were not active (or very weak) on mouse airways. Our experiments on the contractile activities of the endothelins on murine airways suggested the presence of endothelin ET_B receptors.

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