

Bronchodilator-mediated relaxation of normal and ovalbumin-sensitized guinea-pig airways: lack of correlation with lung adenylate cyclase activation

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1 Isoprenaline, vasoactive intestinal peptide (VIP), prostaglandin E₂ (PGE₂) and forskolin caused a dose-dependent relaxation of normal and ovalbumin-sensitized guinea-pig tracheal spirals and lung parenchymal strips *in vitro*. There was no difference in magnitude of relaxation or sensitivity to these relaxants between normal and sensitized tissues. The rank order of potency (concentration of each drug at which 50% of the maximum is obtained) for these relaxants on both trachea and parenchyma was VIP > isoprenaline > PGE₂ > forskolin, although the parenchyma was more sensitive than the trachea.

2 The rank order of efficacy of the drugs used in relaxing both the trachea and lung parenchyma was isoprenaline (10 μM) > forskolin (30 μM) > VIP (0.1 μM) > PGE₂ (10 μM). PGE₂ at concentrations greater than 1 μM sometimes contracted the lung strip.

3 Pretreatment with indomethacin (8.5 μM), a cyclo-oxygenase inhibitor, reduced the resting tone of tracheal spirals, but did not significantly affect the tone of lung strips. Indomethacin-pretreatment did not affect drug-induced relaxations of either normal or sensitized tracheal spirals. However, both normal and sensitized indomethacin-pretreated lung strips relaxed significantly less ($P < 0.05$) to isoprenaline, PGE₂ and forskolin. Indomethacin-pretreatment did not affect sensitivity of normal and sensitized trachea or parenchyma to the relaxant drugs.

4 All the relaxant drugs used stimulated adenylate cyclase activity in normal or sensitized lung parenchyma membrane preparations. The rank order of efficacy (maximal activation) was forskolin > isoprenaline = VIP > PGE₂. There was no difference in response between normal and sensitized lungs. Adenylate cyclase activity of normal lung was stimulated as follows: forskolin (100 μM), 500.0 ± 50.0%; isoprenaline (100 μM), 186.0 ± 29.0%; VIP (10 μM), 213.0 ± 19.0% and PGE₂ (100 μM), 155.0 ± 23.0% of basal activity. Similar values were obtained for sensitized lung parenchyma.

5 Indomethacin-pretreatment did not significantly affect normal or sensitized lung adenylate cyclase stimulation by isoprenaline, VIP, forskolin or PGE₂.

6 It was concluded that: (a) Immunological sensitization to ovalbumin does not induce hypoactivity of relaxant drug receptors and/or the adenylate cyclase system of the airway tissues of the guinea-pig. (b) There is an apparent lack of correlation between tissue relaxation *in vitro* and adenylate cyclase activity since the rank order of the efficacy of a range of relaxants was different for the two effects and furthermore indomethacin-treatment of airway tissues yielded differential results.

Introduction

Human asthmatic airways contract to a greater degree and at lower concentrations of bronchoconstrictors than normal airways (Hargreave *et al.*, 1981; Orehek, 1982). This asthmatic airway hyper-reactivity helps establish the diagnosis of bronchial asthma (Boushey *et al.*, 1980). The suggestion by

Szentivanyi (1968) that hypoactive β-adrenoceptors induce airway hyper-reactivity initiated further studies on this hypothesis. Briefly, this hypothesis suggested that the β-adrenoceptor, which influences bronchial smooth muscle relaxation, was defective and incapable of exerting its full relaxant influence on

the airways. Hence hyper-reactivity to constricting stimuli ensues (for review, see Kaliner *et al.*, 1982). More recent experiments have corroborated this hypothesis. β -adrenoceptors in various tissues from asthmatics show reduced β -adrenoceptor receptor density (Meurs *et al.*, 1982; Sano *et al.*, 1983). Furthermore, a guinea-pig model of asthma shows reduced lung β -adrenoceptor density (Barnes *et al.*, 1980, Cheng & Townley, 1982).

Another important modulatory hormone, vasoactive intestinal peptide (VIP), relaxes the airways of a variety of species including man and guinea-pig (Kitamura *et al.*, 1980; Richardson, 1981; Said, 1982). This influence is exerted via the non-adrenergic, non-cholinergic inhibitory nervous system which innervates the airways of man and guinea-pig (Richardson, 1981; Said, 1982).

The β -adrenoceptor and the VIP-receptor are both linked to adenylate cyclase (Robberecht *et al.*, 1981; Lefkowitz & Michel, 1983) which catalyzes the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cyclic AMP). Studies on a canine model of asthma (Rinard *et al.*, 1979) and on human asthmatic tissues (Meurs *et al.*, 1982) have originated the hypothesis that hormone stimulated adenylate cyclase is defective and hence produces less cyclic AMP than normal. Since cyclic AMP may promote smooth muscle relaxation (Sutherland & Rall, 1960) and inhibit mediator release, a defect would lead to less airway relaxation and enhanced mediator release (Said, 1982). A canine model of asthma has been shown to exhibit airway hyper-reactivity and depressed cyclic AMP levels (Rinard *et al.*, 1979).

We showed previously that airway tissues from ovalbumin-sensitized guinea-pigs can synthesize leukotrienes (LTs) C₄ and D₄ (Burka & Saad, 1984), which are products of lipoxygenation of arachidonic acid (AA). These LTs are bronchoconstrictive mediators important in the pathogenesis of human asthma (Hanna *et al.*, 1981). In order to determine whether hypoactivity to bronchodilators exists in this model of asthma we examined the effects of isoprenaline, VIP, prostaglandin E₂ (PGE₂) and forskolin on relaxations of airway smooth muscle from this model and from normal control animals.

The above agents occupy β -adrenoceptors and receptors for VIP or PGE. Forskolin is a diterpene which activates the catalytic unit of adenylate cyclase (Seamon & Daly, 1981). Forskolin is also a potent relaxant of various smooth muscle preparations (Muller & Baer, 1983).

We also measured adenylate cyclase activation after stimulation with the above drugs, thus allowing us to compare an event distal to receptor occupation (i.e. adenylate cyclase activation) with an end effect (i.e. smooth muscle relaxation).

Finally, since products of AA are released from airway tissues and are biologically active in these tissues (Hyman *et al.*, 1978), we used indomethacin, an inhibitor of prostaglandin synthesis (Vane 1971), in order to determine a role for prostaglandins in airway smooth muscle relaxation and adenylate cyclase activation of lung tissue induced by isoprenaline, VIP, PGE₂ and forskolin.

Methods

Tissue preparation for in vitro study

Male English short-hair guinea-pigs (200–250 g) (Connaught Laboratories, Toronto, Ontario) were sensitized with ovalbumin (OA:Sigma, grade II), 100 mg subcutaneously and 100 mg intraperitoneally and used 3 weeks later. Normal animals were untreated. Animals were killed by stunning and exsanguination. The trachea was removed, placed in Krebs-Henseleit solution (KHS), spirally cut (Constantine, 1965), and divided into four equal segments. The lung was removed and treated in one of two ways. For measurement of tissue relaxation, parenchymal strips were prepared from the distal edges of each lobe (Lulich *et al.*, 1979), and for measurement of adenylate cyclase activation, the lung was treated as described later under lung membrane preparation. The composition (mM) of the KHS was: NaCl, 118, KCl 4.7, MgSO₄·7H₂O 1.2, CaCl₂ 2.2, KH₂PO₄ 1.2, NaHCO₃ 24.9, (+)-glucose 11.1. The tissues were placed in 10 ml jacketed organ baths containing KHS maintained at 37°C and bubbled with 95% O₂ and 5% CO₂. The tissues were attached by silk threads to force displacement transducers (Grass FTO3C) at a resting tension of 1 g and the responses displayed on Grass polygraphs (Model 7D). Following equilibration (2 h) the tissues were challenged with histamine (100 μ M) until responses became constant.

Concentration-relaxation curves

For establishment of concentration-relaxation (C-R) curves, the trachea was pre-contracted with carbachol (1 μ M), a concentration which produces 70–80% of the maximum contraction of trachea (Saad & Burka, 1983). Parenchymal strips were not pre-contracted, but mechanically adjusted to maintain 1 g base line tension. When tension of trachea and parenchyma became constant, isoprenaline, VIP, PGE₂ or forskolin were added cumulatively to the tissues. When the response to a dose plateaued, the next higher dose was added. After plateau of the response to the highest concentration of each drug had occurred, isoprenaline (10 μ M) was added to

each tissue to obtain maximal relaxation. Results were expressed as a percentage of the maximal response to isoprenaline ($10\ \mu\text{M}$) obtained on each tissue. We also expressed results as mg relaxation per mg dry weight of the tissue (dried at 65°C for 8 h after the experiment). The data expressed in this way were similar to the data expressed as a % of the maximal response to isoprenaline. Hence only the latter data are presented in this paper. Two relaxant drugs were studied on tissues from one animal. One tissue acted as a paired time control, the other was used to determine the effect of indomethacin ($8.5\ \mu\text{M}$) on the relaxations induced by isoprenaline, VIP, PGE₂ or forskolin.

Effect of cyclo-oxygenase inhibition

After establishment of the initial C-R curves, the tissues were washed with KHS until tone became constant again at 1 g tension. Subsequently, of the two tissues originally relaxed with a given drug, one was chosen randomly and treated with indomethacin ($8.5\ \mu\text{M}$) for 30 min. The other tissues served as a paired time and vehicle control. The indomethacin-treated trachea exhibited a 30–40% reduction in tone. These tissues were mechanically readjusted back to 1 g tension, carbachol ($0.1\text{--}1\ \mu\text{M}$) was then added to the trachea in order to achieve the same degree of tension developed before generation of the initial C-R curve. Parenchymal strips were maintained at 1 g tension and C-R curves re-established; each tissue yielded an initial control C-R curve and subsequently either a C-R curve generated after indomethacin-treatment or a C-R curve after vehicle (i.e. paired time and vehicle control). The responses of tissues after the initial C-R curve were expressed as a percentage of the maximum isoprenaline response obtained during the initial C-R curve.

Sensitivity

The concentration of a relaxant required to decrease tension by 50% of the maximum attained to each drug were called the EC₅₀ and converted into a negative logarithm. The data were determined for the initial control, the indomethacin-treated, and the paired time and vehicle control tissues.

Adenylate cyclase activation

Preparation of lung membranes Only normal and sensitized lung parenchyma were used for this set of experiments due to the inherent difficulty in obtaining hormonally-sensitive adenylate cyclase from guinea-pig tracheal smooth muscle. Lungs from two guinea-pigs were used for each membrane preparation. The lungs were initially perfused through the

pulmonary vein with cold KHS until the perfusate was free of blood. The lungs were then chopped (excluding bronchi, fat and adhering tissue) on ice into small fragments (10 mg). The chopped lung was then divided equally into two portions, placed in 30 ml oxygenated KHS and incubated at 37°C . One portion was treated with indomethacin ($8.5\ \mu\text{M}$) and the other with vehicle for 30 min. Hence experiments were done on a paired basis. The lung portions were then placed in 10 volumes of homogenization buffer of the following composition: Tris-HCl (50 mM), dithiothreitol (1 mM) and sucrose (0.25 M) pH, 7.5 at 4°C , and homogenized using a glass homogenizer and a motor driven Teflon pestle. The homogenate was initially centrifuged at 500 g for 10 min, the supernatant collected and centrifuged at 15,000 g for 20 min at 4°C . The resulting pellet was washed with homogenization buffer and resuspended in a small amount of the same buffer. Aliquots of 400 μl were frozen and stored until use (less than four weeks) under liquid nitrogen. Protein was determined by the method of Lowry *et al.* (1951).

Adenylate cyclase assay The method of Baer (1975) was used to determine adenylate cyclase activity. This method uses polyethyleneimine thin layer chromatography to separate radioactive cyclic AMP. Assays were done in duplicate in a final volume of 0.05 ml. Each tube contained the following: 25 mM 4-(2-hydroxyethyl)-1-piperazineethane sulphonic acid (HEPES, pH 7.4), 5 mM MgCl₂, 1 mM cyclic AMP, 20 mM creatine phosphate, 0.2 mg ml⁻¹ creatine kinase, 0.1 mM [α -³²P]-ATP (800,000 cpm), and 25–50 μg membrane protein. Drugs were added as indicated in results.

Incubations were carried out at 37°C for 3 min and the reaction was stopped by adding 10 μl of a solution containing sodium salts of cyclic AMP, ATP, AMP and EDTA, each at 25 mM.

Statistics

Student's *t* test for paired and unpaired data was used to assess the significance of the difference between groups. Differences were considered to be statistically significant when $P < 0.05$.

Materials

Histamine dihydrochloride, carbamylcholine chloride, vasoactive intestinal peptide, prostaglandin E₂, isoprenaline, and ovalbumin (grade II for sensitization and grade V for challenge) were purchased from Sigma (St. Louis, MO). Forskolin was purchased from Calbiochem-Behring, San Diego, CA, USA. Indomethacin was a gift from Dr Wm. D. Dorian, Merck Frosst Laboratories (Pointe Claire-Dorval,

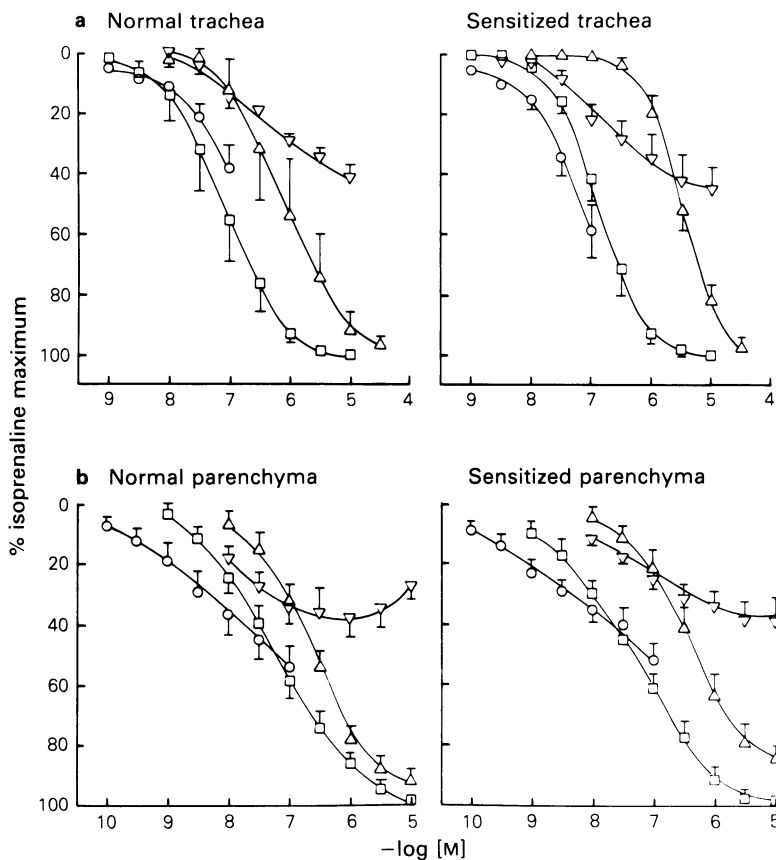


Figure 1 The relaxant responses of normal and ovalbumin-sensitized guinea-pig tracheal spirals (a) and lung parenchymal strips (b). Results are expressed as a % of the maximum response to isoprenaline ($10 \mu\text{M}$) and are the mean of five experiments; s.e. mean shown by vertical lines. Symbols indicate the following: (\square) isoprenaline, (Δ) forskolin, (\circ) vasoactive intestinal peptide and (∇) prostaglandin E_2 . Tracheal spirals were precontracted with carbachol ($1 \mu\text{M}$) and lung parenchymal strips were mechanically adjusted to 1 g baseline tension.

Quebec). All other drugs were of reagent quality. Forskolin was dissolved in dimethylsulphoxide and diluted in water. Isoprenaline was dissolved in water containing $100 \mu\text{M}$ ascorbic acid and made up fresh

daily. Dimethylsulphoxide and ascorbic acid at the concentrations resulting from drug additions did not alter smooth muscle tone or affect adenylate cyclase activity.

Table 1 Sensitivity^a of airway tissues from normal and sensitized animals to relaxant agents *in vitro*.

Agonist	Trachea		Parenchyma	
	Normal	Sensitized	Normal	Sensitized
Isoprenaline	7.11 ± 0.27^b	6.80 ± 0.13	7.19 ± 0.17	7.30 ± 0.11
Forskolin	6.10 ± 0.30	5.44 ± 0.13	6.66 ± 0.09	6.40 ± 0.13
VIP	7.66 ± 0.06	7.59 ± 0.05	8.51 ± 0.18	8.73 ± 0.14
PGE ₂	6.54 ± 0.15	6.83 ± 0.28	7.62 ± 0.24	7.37 ± 0.21

^a = Negative logarithm of the concentration of each drug required to achieve 50% of the maximum response to that drug.

^b Mean \pm s.e. mean of 5 experiments

Results

Relaxant responses to bronchodilators

Isoprenaline, VIP, PGE₂ and forskolin caused a dose-dependent relaxation of the guinea-pig trachea and the lung parenchymal strips (Figure 1). There was no difference between normal and sensitized tissues in the relaxant responses to all four drugs used. The rank order of potency of these agents on the airways was VIP > isoprenaline > PGE₂ >

forskolin (Table 1). The parenchyma was more sensitive than the trachea to these drugs. Normal and sensitized tissues exhibited similar sensitivity to these agents. Isoprenaline was the most efficacious drug used on the trachea causing the most relaxation (Figure 1, Table 2). Forskolin was the next most efficacious drug with VIP and PGE₂ being equivalent. PGE₂ relaxed airways in low concentrations. However, at higher concentrations (> 1 μM) this agent sometimes contracted the lung parenchymal strip (Figure 1).

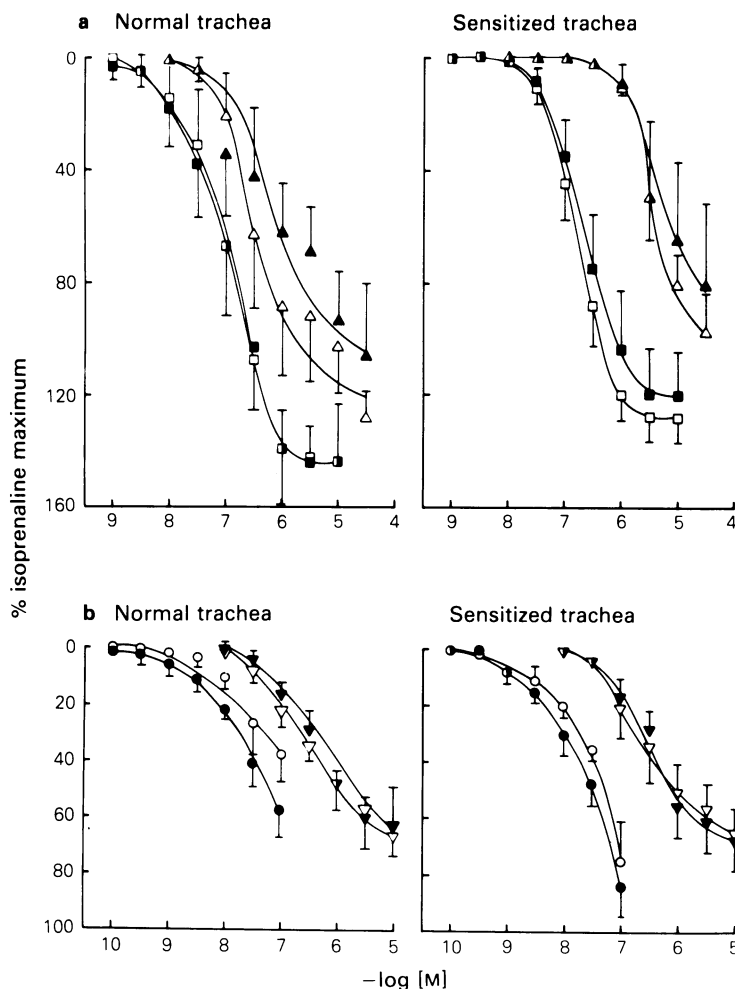
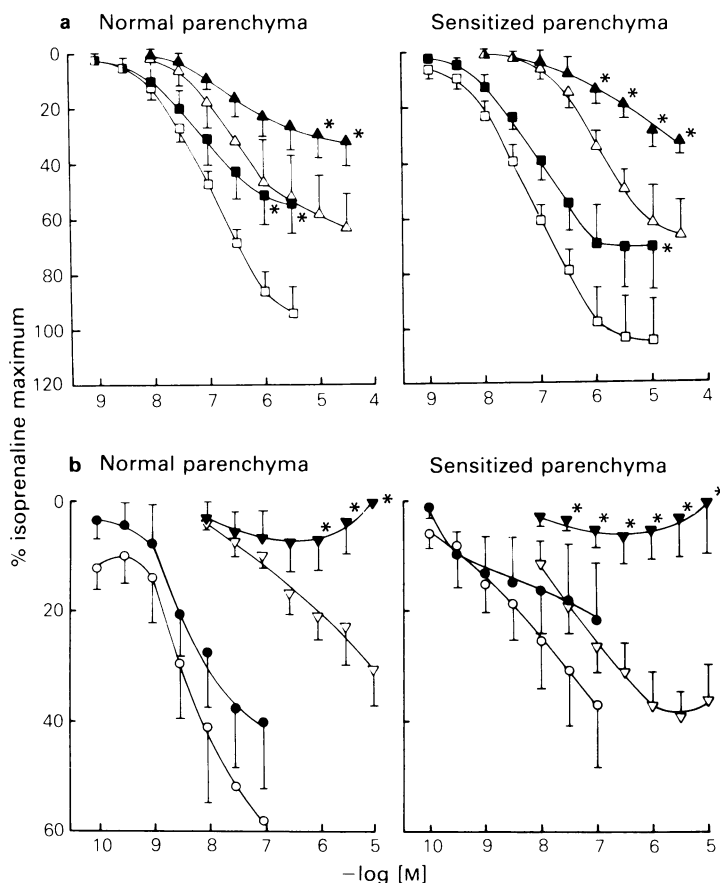


Figure 2 Effects of indomethacin on the relaxant responses of normal and ovalbumin-sensitized guinea-pig tracheal spirals to isoprenaline (□, ■) forskolin (△, ▲) (a) and vasoactive intestinal peptide (○, ●), and prostaglandin E₂ (▽, ▼) (b). Open symbols represents the responses of the time and vehicle control tissues and the closed symbols represent the responses of the paired indomethacin (8.5 μM)-treated tissues. Results are expressed as a % of the maximum relaxant response to isoprenaline (10 μM) obtained in the absence of any modulatory treatment and are the mean of five experiments; s.e. mean shown by vertical lines.

Table 2 Maximum control relaxations (mg force mg⁻¹ tissue dry weight) of airway tissues from normal and ovalbumin-sensitized guinea-pigs after addition of relaxant drugs *in vitro*

Agonist	Trachea		Parenchyma	
	Normal	Sensitized	Normal	Sensitized
Isoprenaline (10 μM)	120.8 ± 21.4*	111.9 ± 18.5	7.9 ± 0.7	9.3 ± 1.1
Forskolin (30 μM)	106.7 ± 17.8	86.8 ± 10.2	10.8 ± 1.4	11.7 ± 0.8
VIP (0.1 μM)	35.7 ± 10.3	69.7 ± 18.8	3.3 ± 0.5	4.6 ± 1.0
PGE ₂ (trachea: 30 μM) or (parenchyma: 10 μM)	39.2 ± 8.3	55.9 ± 16.0	2.6 ± 0.8	3.1 ± 0.7

* = Mean ± s.e. mean of 5 experiments

**Figure 3** Effects of indomethacin on the relaxant responses of normal and ovalbumin-sensitized guinea-pig lung parenchymal strips to isoprenaline (□, ■), forskolin (△, ▲) (a) and vasoactive intestinal peptide (○, ●), and prostaglandin E₂ (▽, ▼) (b). Open symbols represent the responses of the time and vehicle control tissue and the closed symbols represent the responses of the paired indomethacin (8.5 μM)-treated tissue. Results are expressed as a % of the maximum relaxant response to isoprenaline (10 μM) obtained in the absence of any modulatory treatment and are the mean of five experiments; s.e. mean shown by vertical lines. * Significantly different (*P* < 0.05) from the time and vehicle control tissue responses.

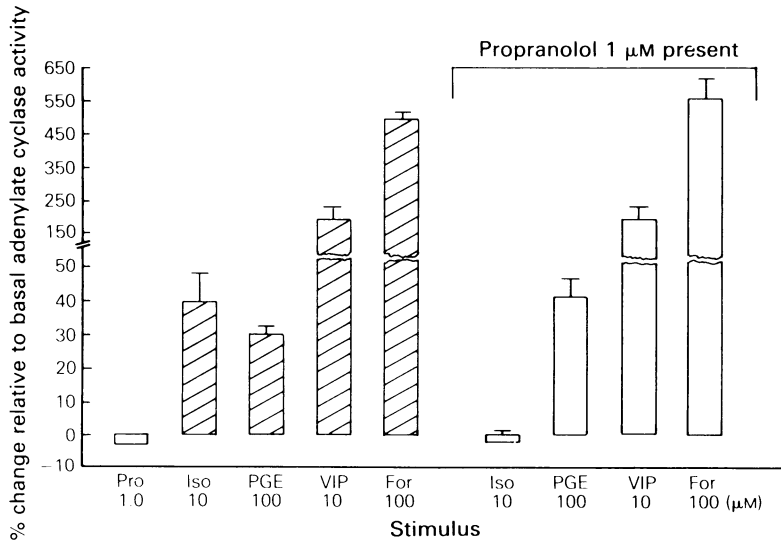


Figure 4 Hormonal specificity of the responses of guinea-pig lung parenchymal adenylate cyclase. Activation of adenylate cyclase by isoprenaline (Iso), prostaglandin E_2 (PGE), vasoactive intestinal peptide (VIP) and forskolin (For) in the absence and presence of propranolol ($1 \mu\text{M}$) is shown. The assays, which were carried out in duplicate, are the mean of results from 2 preparations. Hatched bars are responses in the absence of propranolol and open bars are responses with propranolol (Pro, $1 \mu\text{M}$) present.

Effects of indomethacin-pretreatment on drug-induced relaxations

Indomethacin ($8.5 \mu\text{M}$)-pretreatment did not significantly change the efficacy of isoprenaline, VIP, PGE_2 or forskolin in causing relaxations of the normal or sensitized trachea (Figure 2). Furthermore the sensitivity of the tissues to the drugs (expressed as the negative logarithm of the EC_{50}) was unchanged (data

not shown). All comparisons were relative to the paired time and vehicle control tissue.

Indomethacin ($8.5 \mu\text{M}$)-pretreatment significantly ($P < 0.05$) reduced the magnitude of the relaxation of parenchyma induced by high concentrations of isoprenaline ($1-10 \mu\text{M}$), forskolin ($1-10 \mu\text{M}$) and PGE_2 ($0.1-10 \mu\text{M}$) on normal and sensitized lung parenchyma (Figure 3). However the EC_{50} of each drug was unchanged when compared to control. As

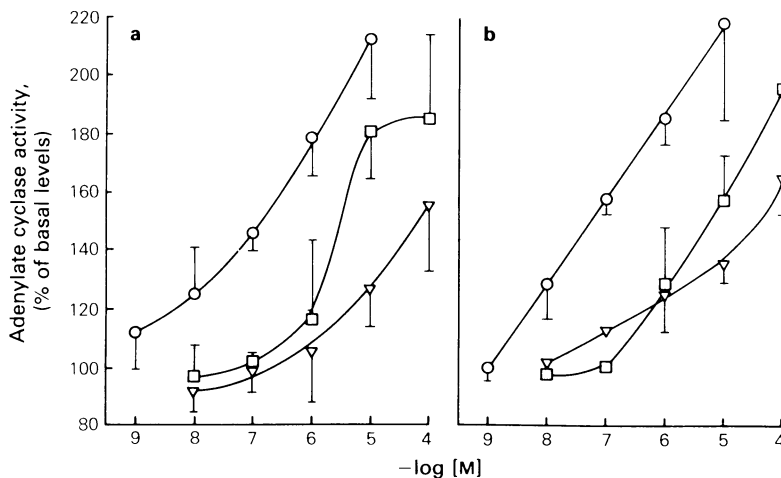


Figure 5 Activation of normal (a) and ovalbumin-sensitized (b) guinea-pig lung adenylate cyclase by isoprenaline (\square), vasoactive intestinal peptide (O) and prostaglandin E_2 (∇). Assays were carried out in duplicate and the results are the mean of experiments using 4 different preparations each of normal and sensitized lung; s.e. mean shown by vertical lines. Adenylate cyclase activity is expressed as a % of the basal activity measured in the absence of any drug.

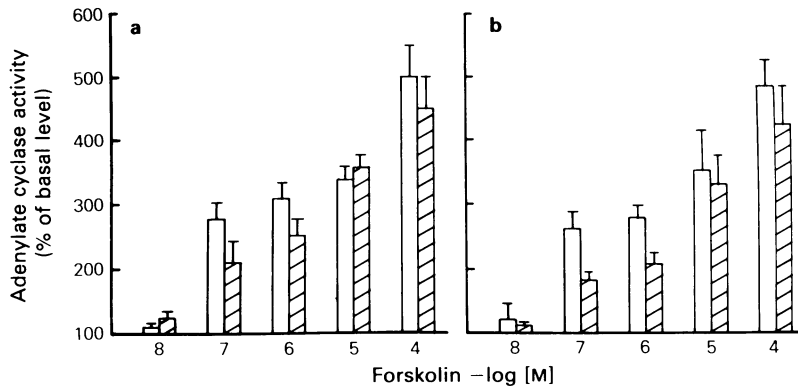


Figure 6 Effects of indomethacin ($8.5 \mu\text{M}$)-pretreatment on normal (a) and sensitized (b) guinea-pig lung adenylate cyclase activation induced by forskolin. Open bars are responses without, and hatched bars are responses with indomethacin ($8.5 \mu\text{M}$)-pretreatment. Assays were carried out in duplicate and the results are the mean of experiments using 3 different preparations each of normal and sensitized lung; s.e.mean shown by vertical lines. Adenylate cyclase activity is expressed as a % of the basal activity measured in the absence of any drug.

with the trachea, all comparisons are relative to the paired time and vehicle control tissue.

Adenylate cyclase activation

The adenylate cyclase of normal and sensitized guinea-pig lung had basal activities (measured in the absence of any drugs) of 54.8 ± 9.6 and $60.3 \pm 8.2 \text{ pmol cyclic AMP mg}^{-1} \text{ protein min}^{-1}$ respectively. Responses of lung adenylate cyclase to isoprenaline were hormone-specific (Figure 4) since they were abolished by the β -adrenoceptor antagonist propranolol ($1 \mu\text{M}$), whereas the responses to PGE_2 , VIP and forskolin were unaffected by propranolol. Isoprenaline, VIP, PGE_2 and forskolin stimulated normal and sensitized guinea-pig lung adenylate cyclase activity. There was no difference in the magnitude of the stimulation between normal and sensitized lung (Figures 5 and 6).

The stimulation of adenylate cyclase by the above drugs was concentration-dependent, and occurred at low concentrations (Figures 5 and 6). The rank order of efficacy of these drugs on lung adenylate cyclase activation was forskolin $>$ isoprenaline = VIP $>$ PGE_2 . Forskolin was the most efficacious drug on lung adenylate cyclase. Basal lung adenylate cyclase activity was increased to $500.0 \pm 50.0\%$ of basal activity with a forskolin concentration of $100 \mu\text{M}$ (Figure 6).

Effect of indomethacin-pretreatment on adenylate cyclase activation

Indomethacin ($8.5 \mu\text{M}$)-pretreatment of guinea-pig lung parenchyma did not significantly change basal activity or drug-stimulated activity of the enzyme.

The effects of indomethacin-pretreatment on the forskolin-induced activation of adenylate cyclase of normal and sensitized lung are shown in Figure 6.

Discussion

In this work, we have used isoprenaline, VIP, forskolin and PGE_2 to induce bronchodilatation *in vitro* of normal and ovalbumin-sensitized guinea-pig trachea and lung parenchyma. Since these agents exhibited similar potency and efficacy on both normal and sensitized airway tissues (Figure 1, Tables 1 and 2), we have demonstrated that bronchodilator hypoactivity does not exist in this animal model of asthma as a consequence of mere immunological sensitization. A major criticism of the hypoactive bronchodilator theory of asthma is that hypoactivity arises as a consequence of long term treatment with bronchodilators such as β -adrenoceptor stimulants (Connolly & Greenacre, 1976) or as a consequence of the stresses of the disease itself (Meurs *et al.*, 1982). Our results tend to support the above criticisms, since the model we used in this work was immunologically sensitized but unchallenged. Other workers have used sensitized guinea-pigs that have been challenged. In fact, some of these animals do exhibit decreased β -adrenoceptor binding density concomitant with increased bronchoconstrictor receptor binding density (Barnes *et al.*, 1980). In other models of asthma, such as natively allergic canine models used by Rinard *et al.* (1979), hypoactivity to isoprenaline does exist. However, there may be genetic differences in the canine species which might account for the observed hypoactivity to β -adrenoceptor agonists.

Adenylate cyclase is intimately linked with a varie-

ty of hormone receptors and is one of the links between receptor occupancy and the biological end effect (Lefkowitz & Michel, 1983). It can thus be used as a direct index of bronchodilator receptor activation. In our experiments isoprenaline, PGE₂, VIP and forskolin caused significant stimulation of this enzyme (Figures 5 and 6). Again, normal and sensitized lungs responded similarly to these relaxants. Hence we can assume that the receptor linkage with the enzyme, and the enzyme itself, are not affected by immunological sensitization.

Although forskolin caused slightly less relaxation of the airways than isoprenaline (Figure 1, Table 2), the diterpene caused significantly greater stimulation of pulmonary adenylate cyclase than did isoprenaline. Although it has not been conclusively proven, it is widely accepted that certain types of smooth muscle relaxation are mediated by increased intracellular levels of cyclic AMP (Scheid *et al.*, 1979). Since forskolin stimulates the synthesis of cyclic AMP to a much greater extent than isoprenaline, we would have expected forskolin to cause significantly greater relaxation of the airways than we demonstrated in this work. A possible explanation of this apparent discrepancy is that in the membrane preparation we used, the forskolin receptor, possible the catalytic unit of adenylate cyclase (Schmidt *et al.*, 1984), is much more stable and retains its homogeneity even after the somewhat disruptive procedures needed to prepare membranes from the lung. It is known that hormone receptors are extremely labile and it is entirely possible that during membrane preparation part of the receptor pool for a given relaxant would be inactivated. Furthermore, it is possible that airway smooth muscle relaxation may not be directly related to cyclic AMP accumulation which follows activation of the adenylate cyclase. Drugs like nitroglycerine and sodium nitroprusside can relax vascular smooth muscle (Kukovetz *et al.*, 1979) and concomitantly increase levels of cyclic guanine monophosphate (cyclic GMP) (Kukovetz *et al.*, 1979; Lewicki *et al.*, 1982). Hence there are other candidates besides cyclic AMP that mediate smooth muscle relaxation.

Similar dissociation between airway smooth muscle relaxation and cyclic AMP accumulation has been observed by Tipton *et al.* (1981). These authors used isoprenaline to induce a subsensitivity of guinea-pig airway smooth muscle relaxation. However, during the same time period cyclic AMP accumulation was normal. Hence our results support a discordance between airway smooth muscle relaxation and cyclic AMP levels.

Another point worth considering is that the lung is a very heterogeneous tissue made up of structural, secretory, and contractile cell types. Hence forskolin may activate the adenylate cyclase of non-smooth

muscle cell types, whereas isoprenaline, VIP or PGE₂ may not.

Indomethacin, a cyclo-oxygenase inhibitor (Vane, 1971), did not affect tracheal relaxation induced by isoprenaline, VIP, PGE₂ or forskolin (Figure 2). However indomethacin-pretreatment reduced the magnitude of lung parenchymal relaxations to isoprenaline, PGE₂ and forskolin. The data for VIP were too variable to obtain statistical significance. The reduction of bronchodilator-induced relaxations of the lung strip by indomethacin is hard to explain but different hypotheses may be advanced. Indomethacin, by inhibiting the cyclo-oxygenase enzyme in the lung, would cause a diversion of endogenous arachidonic acid metabolism to the lipoxigenase pathway, (Piper *et al.*, 1979, Burka & Saad, 1984) resulting in enhanced synthesis of potent bronchoconstrictor leukotrienes which would cause the lung strip to be more resistant to a relaxant influence. Indomethacin might also exert a physicochemical action which may affect receptor activation. Since the EC₅₀ of each bronchodilator in relaxing the lung strip did not change after indomethacin-pretreatment, it was assumed that drug binding to the receptor would not have been affected. Indomethacin has been shown by other workers to suppress completely PGE₂-induced bronchodilatation in human subjects (Walters *et al.*, 1982). Furthermore indomethacin has been shown to inhibit the relaxant effects of prostaglandins (Yamaguchi *et al.*, 1976; Burka & Paterson, 1980).

Many drugs including prostacyclin (MacDermot & Barnes, 1980) and VIP (Robberecht *et al.*, 1981) have been shown to activate adenylate cyclase of lung tissue. We have extended these findings to include forskolin. This agent apparently binds to specific sites located on adenylate cyclase itself (Schmidt *et al.*, 1984). We used it as an agent which might reveal differences in adenylate cyclase of lung from normal and sensitized animals. As shown in Figure 6, forskolin-induced activation of the lung adenylate cyclase was similar in normal and sensitized animals. Hence, we assume that the adenylate cyclase system of normal and sensitized lungs is identical. Furthermore indomethacin-pretreatment did not significantly affect forskolin-induced activation of cyclase (Figure 6), nor activation induced by isoprenaline, PGE₂ or VIP, in normal or sensitized lungs.

In conclusion, mere immunological sensitization does not affect bronchodilator receptors nor the adenylate cyclase system of the lungs of guinea-pigs. Finally the efficacy of the relaxant agents used in this study differed depending upon the effect studied, and indomethacin-treatment reduced relaxation of the lung strip but did not affect lung adenylate cyclase activation by the same agents at similar concentrations.

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