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# Effects of bronchoconstrictors and bronchodilators on a novel human small airway preparation

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**1** Human lung bronchiolar segments (about 2 mm long and with a diameter of 0.6–1.5 mm) were dissected and circular muscle tension recorded. Airways were identified by histology and in some preparations by relaxant responses to noradrenaline (0.1–10 $\mu$M).

**2** Adenosine (1–100 $\mu$M) produced only very weak contractions, whereas carbachol ($EC_{50}$ = 0.40 $\mu$M), histamine ($EC_{50}$ = 0.63 $\mu$M), prostaglandin $D_2$ ($EC_{50}$ = 0.50 $\mu$M), substance P ($EC_{50}$ = 4.6 $\mu$M) and ATP (1–100 $\mu$M) produced much greater ones.

**3** The contractions generally developed rapidly and were stable. The mean maximum increase in tension achieved with the most efficient constrictor, carbachol, was 0.5 g. ATP was the least efficient producing only about 40% of carbachol's maximum.

**4** Terbutaline, theophylline and enprofylline relaxed carbachol (2.0 $\mu$M = $EC_{70}$)-contracted preparations. Terbutaline (3–3000 nM) relaxed 4 out of 11 bronchioles. Theophylline (10–4000 $\mu$M) and enprofylline (1–400 $\mu$M) consistently relaxed the bronchiolar preparations including those exhibiting little responsiveness to the $\beta_2$-adrenoceptor agonist.

**5** Since enprofylline (which does not block adenosine receptors) was a five times more potent relaxant than theophylline and since adenosine produced only weak contractions, antagonism of adenosine receptors is probably not involved in relaxation of the small airways.

**6** It is suggested that the present data, which apparently differ from those obtained with lung parenchymal strips, are of relevance for human small airways responsiveness.

## Introduction

In chronic airway obstruction, emphysema, and chronic bronchitis the increased resistance to airflow seems to be located principally in small airways with a diameter of less than 2 mm. The obstruction may depend on mucus plugging, inflammatory changes including loss of tissue elastic recoil, and on bronchiolar contraction (Bignon *et al.*, 1969; 1970; Thurlbeck *et al.*, 1970; Butler, 1982; Lopez-Vidriero & Reid, 1983). Also in asthma, and particularly during early pathological changes (Hogg *et al.*, 1968), a considerable degree of resistance to flow is localized to small airways (Despas *et al.*, 1972; McFadden *et al.*, 1977). McFadden & Ingram (1979) suggested that small airways narrowing in asthma may be due to rapid and reversible changes in the contractile state of the smooth muscle.

Using human and animal lung specimens, Persson & Ekman (1976) dissected tubal segments of small airways (about 1 mm in diameter) and showed them to be responsive to mediators and drugs with concentration-dependent tension changes (Persson & Ekman, 1976; Persson, 1980). Lulich *et al.* (1976), on the other hand, excised strips of subpleural lung parenchyma which also were demonstrated to be responsive to pharmacological stimuli. Presumably due to the ease by which animal and human lung parenchymal strips can be prepared, this latter type of preparation has since been frequently used in experimental pharmacology and physiology. The results obtained have generally been interpreted as reflecting changes in peripheral airway smooth muscle tension. This is unfortunate because lung parenchymal strips contain several components with contractile properties. In addition to airway smooth muscle, vascular smooth muscle, interstitial cells (Kapanci *et al.*, 1974) and alveolar duct smooth muscle (Miller, 1921) would be able to participate in the contractile activity. Further-

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more, the relative proportions of these cellular components have been shown to be highly variable in individual lung parenchymal strips (Bertram *et al.*, 1983; Finney *et al.*, 1984).

In the present study the technique described by Persson & Ekman (1976) has been further developed and effects of proposed asthma mediators on bronchiolar rings examined. Part of these results have been presented to the British Pharmacological Society (Finney *et al.*, 1983).

## Methods

Lung tissue, taken from patients undergoing surgery for localized lung lesions, was examined by a pathologist and macroscopically normal tissue was selected for use in these experiments. Within 1 h of resection, the tissue was immersed in Krebs solution (composition in mM: NaCl 118.0, KCl 4.6, $CaCl_2$ 2.5, $MgSO_4$ 1.15, $NaHCO_3$ 24.9, $KH_2PO_4$ 1.15 and glucose 5.5; pH = 7.4) aerated with 5% $CO_2$ and 95% $O_2$ and immediately transported to the laboratory. Tissue was usually stored overnight at 4°C for use the following day. Bronchiolar rings from eleven patients were studied. The mean age of the patients was 62.5 yr (s.d. 8.8 yr) and nine patients were male. All patients had carcinoma of the lung but none with a known history of other airway diseases or airway hyperreactivity.

Airways were initially identified macroscopically and a flexible plastic tube (outside diameter 0.6 mm) was gently inserted as far as possible into the airway lumen. The airway was then carefully dissected free of surrounding tissue (the plastic tube ensuring correct identification to the airway's distal end) yielding isolated bronchioles of lumen 0.6–1.5 mm. These were cut into 2 mm long tubular segments and each segment slid over two very fine wire prongs in a 2.5 ml organ bath (Högestätt *et al.*, 1983), containing Krebs solution (37°C) and gassed with 5% $CO_2$ and 95% $O_2$. One prong was connected to a force-displacement transducer (Grass FT 03) and the other to an adjustable sled. By use of a micrometer screw, the sled was adjusted to give the desired degree of tension in the bronchiole. Isometric tension was recorded on a Grass polygraph model 5 and resting tone established at 0.5 g. The preparations were allowed to equilibrate for 1 to 2 h and during this time the Krebs solution was changed at 20 min intervals. (This procedure should minimize the amount of any premedication drug in the bronchiolar tissue.)

Cumulative concentration-response (C/R) curves to contractile and relaxant drugs were obtained. Drugs were added to the organ bath in approximately 3 fold increments and responses were allowed to stabilize (3–10 min) between additions. When studying contractile agents a maximally-effective concentration of carbachol (100 μM) was always added on top of each curve to obtain a defined maximal response.

Since preparations did not usually develop tone spontaneously, the bronchioles were contracted by 2.0 μM carbachol (corresponding to 70% of the maximum carbachol contraction) before relaxant responses to bronchodilators were evaluated. In seven rings from seven different patients, one or more concentrations of noradrenaline (0.1–10 μM) were added to the bath before starting other studies.

At the conclusion of each experiment, the bronchiolar rings were stored in saline formalin (10% formalin, NaCl 154 mM) for subsequent histological examination. Paraffin embedded specimens were stained with haematoxylin and eosin and after sectioning to 0.8 μm, examined by light microscopy. Microscopic examination of stained sections revealed airways of morphology usually classified as terminal bronchioles, that is, a near or total absence of cartilage and the presence of the highly characteristic longitudinally folded mucosa (Bloom & Fawcett, 1968, Figure 1).

For each ring the change in tension in response to each cumulative addition of drug was measured. Generally each specific drug evaluation was made in more than one preparation from the same subject yielding a mean result which was used for the calculations. Thus, $n$ equals numbers of subjects studied. C/R curves were constructed by plotting the molar concentration of drug versus contractile or relaxant response expressed as a percentage of its own maximal response. From this plot the $EC_{50}$ value (concentration of drug producing 50% of its own maximal effect) was determined. Geometric mean $EC_{50}$ values (mean of individual log $EC_{50}$ values) were calculated for the drugs. Student's $t$ test for non-correlated data was used to test the probability of differences between geometric mean $EC_{50}$ values ($P < 0.05$ was considered to be significant).

### *Drugs*

Solutions of carbamylcholine chloride (Sigma), dipyridamole (Persantine, Boehringer Ingelheim), histamine chloride (Apoteksbolaget) and prostaglandin $D_2$ ($PGD_2$; Upjohn) were prepared daily by dilution from (frozen) stock solutions with Krebs solution. Adenosine (Sigma), adenosine 5'-triphosphate (ATP; Sigma), substance P (Sigma or Beckman), and (−)-noradrenaline bitartrate (Sigma) were dissolved in 154 mM NaCl solution immediately before use and kept on ice (ascorbic acid, 20 μg $ml^{-1}$, was added to the noradrenaline solution). Theophylline (Draco), enprofylline (Draco) and terbutaline sulphate (Draco) were diluted from stock solutions daily.

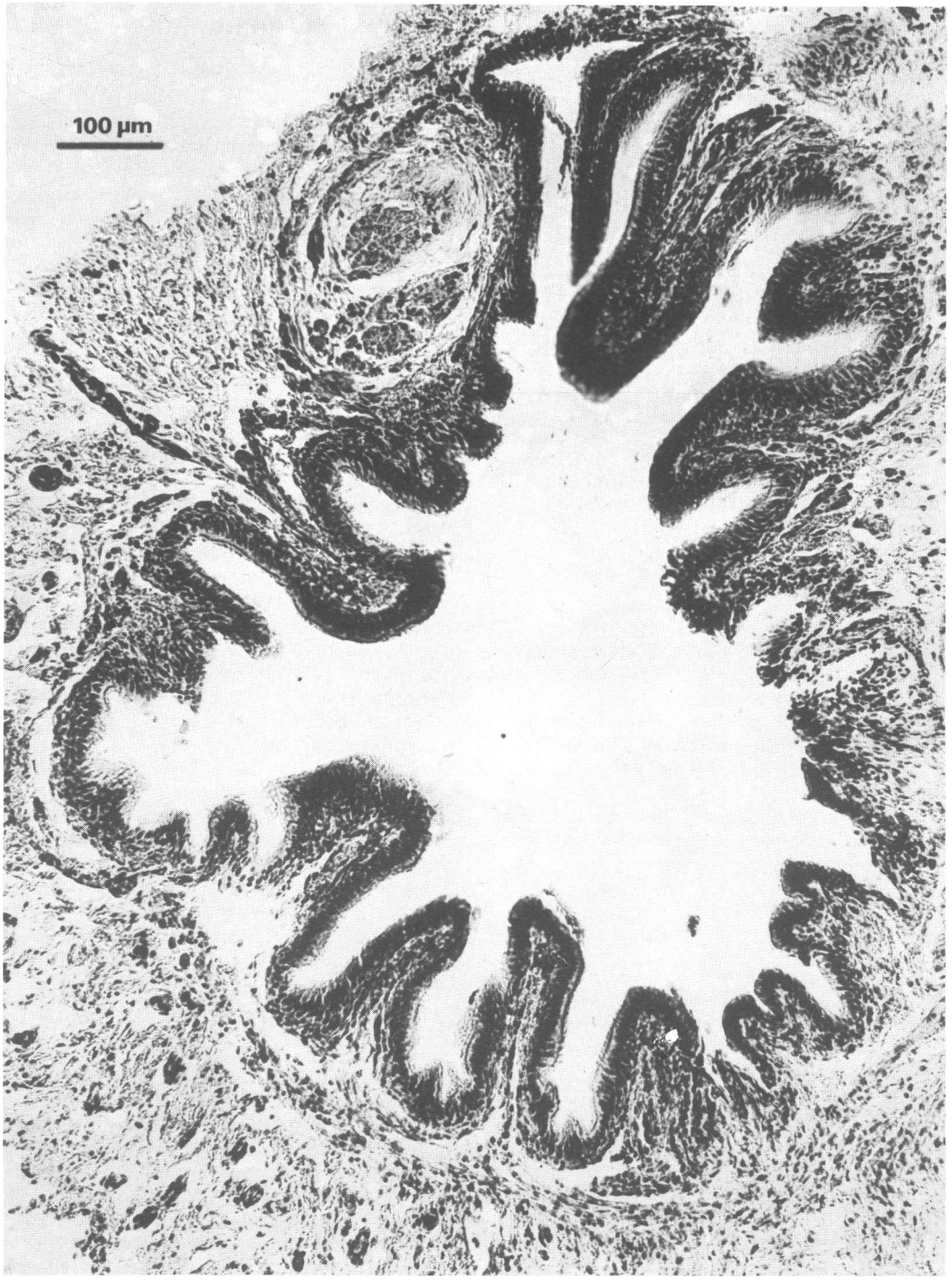


**Figure 1** Photomicrograph of a cross-section of one of the bronchiolar preparations examined. The bronchiole has a maximal lumen diameter of about 0.6 mm. The preparation was stained with haematoxylin and eosin (see Methods).

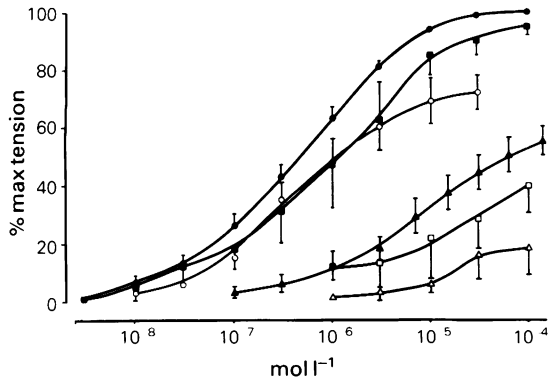


**Figure 2** Contractile concentration-response curves to carbachol (●, $n = 5$), histamine (■, $n = 4$), prostaglandin $D_2$ (○, $n = 3$), substance P (▲, $n = 5$), adenosine (△, $n = 3$) and adenosine 5'-triphosphate (□, $n = 3$) obtained in human isolated bronchioles. The substances were added cumulatively and the response is expressed as a percentage of a maximum contraction to carbachol ($10^{-4}$ M). Mean results are shown and vertical lines indicate s.e.mean.

## Results

C/R curves could be obtained from 36 rings, confirmed to be of terminal bronchioles by histology. Four preparations exhibited a spontaneous activity of a slow wave-like appearance. These rings were still responsive to drugs, but the unstable baseline prevented a quantitative evaluation of drug actions, and were not included in this study.

**Table 1** Negative log molar $EC_{50}$ values of agents that contract or relax human isolated bronchioles

| *Bronchoconstrictors* | *− log $EC_{50}$* | *% maximum carbachol contraction* | n |
|---|---|---|---|
| Carbachol | 6.40 ± 0.09 | | 5 |
| Histamine | 6.20 ± 0.29 | 96 ± 3.1 | 4 |
| Prostaglandin $D_2$ | 6.39 ± 0.12 | 69 ± 7.7 | 3 |
| Substance P | 5.34 ± 0.20 | 57 ± 5.9 | 5 |
| *Bronchorelaxants* | | *% inhibition* of carbachol contraction* | |
| Theophylline | 3.90 ± 0.08 | 127 ± 20.3 | 5 |
| Enprofylline | 4.59 ± 0.13 | 125 ± 31.7 | 6 |
| Terbutaline | 6.70 ± 0.13 | 117 ± 23.4 | 4 |

*n* equals number of subjects studied
* A value > 100% means that the trachea was relaxed below the basal tone that existed before the addition of carbachol.

*Effects of bronchoconstrictors*

Carbachol produced a concentration-dependent contraction of the human bronchioles (Figure 2) with a mean maximal tension increase of 460 ± 135 mg (mean ± s.e.mean, $n = 5$). The geometric mean $EC_{50}$ value is shown in Table 1. Responses were stable and reached a plateau 3 to 5 min after addition of each concentration. C/R lines to carbachol were reproducible when repeated 3 to 4 times in each preparation. These carbachol-mediated contractions were completely blocked by pretreatment (15 min) with atropine (1 μm; $n = 3$). Bronchioles also contracted concentration-dependently to histamine (3 nM-0.1 mM). The contractions were stable and the maximal contraction obtained was only slightly smaller than that induced by carbachol (Figure 2). The mean $EC_{50}$ value is shown in Table 1.

Contractile responses were produced by $PGD_2$ and substance P (Figure 2). $PGD_2$ was equipotent with carbachol and histamine (Table 1) whereas substance P was approximately ten times less potent (Table 1). $PGD_2$ produced 68.7 ± 7.7% ($n = 3$) of a maximum carbachol contraction. The C/R curve for substance P apparently did not reach its maximum within the limit of its solubility (100 μM). However, the response to 100 μM was used as a maximum in calculations of the $EC_{50}$ of substance P (Table 1).

The purine nucleoside adenosine (100 μM, in the presence or absence of 2 μM of the adenosine uptake-blocking drug dipyridamole) produced only very weak contractions (mean < 20% of a maximum carbachol contraction, $n = 3$) of human bronchioles with a basal tone (Figure 2). The phosphorylated derivative, ATP, induced a larger constriction (Figure 2). Since only small contractions were produced, mean $EC_{50}$ values were not calculated for the purines. Neither adenosine nor ATP relaxed preparations with a basal tone or contracted by carbachol (2.0 μM).

*Effects of bronchodilators*

Preparations with a basal tone could be relaxed by the xanthines but the quantitative C/R relationships were studied only in carbachol-contracted (2.0 μM, corresponding to 70% of carbachol's maximum) bronchioles. These were concentration-dependently relaxed by theophylline (10–4000 μM) and enprofylline (1–400 μM) (Figure 3). Both xanthines were able to inhibit completely the carbachol-induced tone (Table 1). $EC_{50}$ values are shown in Table 1. Enprofylline was approximately 5 times more potent ($P < 0.01$) than theophylline.

Relaxant responses to the $\beta_2$-adrenoceptor

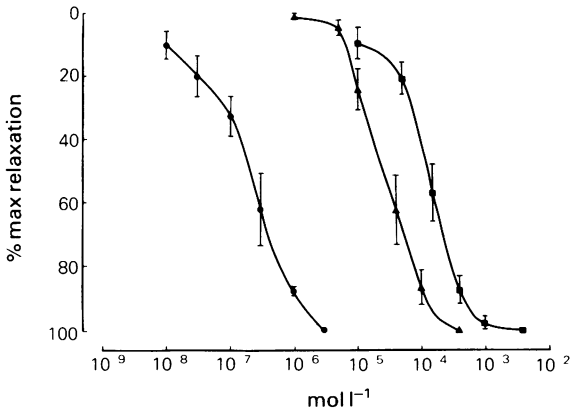


**Figure 3** Relaxant concentration-response curves to terbutaline (●, $n = 4$), enprofylline (▲, $n = 6$) and theophylline (■, $n = 5$) obtained in human isolated bronchioles contracted by carbachol ($2 \times 10^{-6}$ M = $EC_{70}$). The drugs were added cumulatively and the response is expressed as a percentage of its own maximum effect. Mean results are shown and vertical lines indicate s.e.mean. Note: not all preparations relaxed in response to terbutaline (see text).

stimulant terbutaline in carbachol (2.0 μM)- contracted preparations were variable. Of eleven preparations studied, only four (from four different subjects) responded with concentration-dependent relaxations (Figure 3 and Table 1). Four bronchioles totally failed to relax to terbutaline at an otherwise almost maximally effective concentration (1.0 μM) yet theophylline and enprofylline relaxed these rings completely. C/R curves to the xanthines in terbutaline-unresponsive preparations were not different from those obtained in other preparations ($P > 0.05$). The remaining three rings responded to terbutaline in degrees between these two extremes.

Responses to one or more concentrations of noradrenaline (0.1–10 μM) were examined in seven preparations with spontaneous tone only. Noradrenaline always produced some degree of relaxation. Only this qualitative aspect of noradrenaline was evaluated and noradrenaline was not examined in the presence of carbachol.

## Discussion

This study showed that isolated bronchioles from human lung responded in a sensitive and reproducible way to drugs and possible mediators of asthma. Thus constrictor agents produced stable contractions which could be relaxed by bronchodilator drugs. In contrast to the lung parenchymal strip preparation, where many types of contractile tissues are involved, these isolated bronchioles should, indeed, reflect small airway smooth muscle reactivity. The tension changes were immediate and reversible, which is compatible with the view that peripheral airways narrowing in lung disease may in part be due to rapid changes in the contractile state of the smooth muscle (McFadden & Ingram, 1979).

The adrenergic neurotransmitter noradrenaline frequently contracts the human lung parenchymal strip preparation via an action on α-adrenoceptors (Goldie *et al.*, 1982; Bertram *et al.*, 1983), and in small airways of ferrets relatively large numbers of α-adrenoceptors are present (Barnes *et al.*, 1983). It has also been shown that α-adrenoceptor blocking drugs may possess some anti-asthmatic actions (Dyson *et al.*, 1980; Barnes *et al.*, 1981; Marlin *et al.*, 1981). However, the isolated bronchioles responded only with relaxation to noradrenaline. This observation does not support a role for this amine in small airways constriction. Rather, it is likely that tissues other than small airways, as for example vascular smooth muscle, mediated the contractile response to noradrenaline in lung strip preparations (Black *et al.*, 1981; Goldie *et al.*, Bertram *et al.*, 1983). It is possible that the relaxant effect or lack of contraction by noradrenaline may demonstrate that the preparation under study is a small airway and not a blood vessel.

Carbachol was found to be a potent constrictor of human bronchioles. It acted via muscarinic receptors, and mimicked the cholinergic neurotransmitter acetylcholine, since the contractions were blocked by atropine. Nerve fibres with immunoreactivity to the smooth muscle constrictor peptide substance P are present in human airways (Lundberg *et al.*, 1984). Substance P contracted human bronchioles, but it was about ten times less potent than carbachol and its maximum effect was small. It is conceivable that some other, more potent, bronchoconstrictor tachykinin peptide is also present in the lung and is a more likely candidate than substance P in non-cholinergic airway constriction (Karlsson *et al.*, 1984).

The lung mast cell is generally recognized as a major source of non-neurally derived putative mediators of asthma (see Lagunoff, 1983). $PGD_2$ is the quantitatively predominant cyclo-oxygenase product released from sensitized human lung tissue (probably from mast cells) after challenge *in vitro* with antigen (Schulman *et al.*, 1981). The present study has demonstrated that it is almost as effective as the traditional asthma mediator, histamine, in contracting human small airways. Also, inhaled $PGD_2$ has recently been shown to be a potent bronchoconstrictor in man (Hardy *et al.*, 1984). Thus $PGD_2$ may well be as important as histamine for small airways constriction in obstructive airway diseases.

It has been suggested that the purine compounds adenosine and ATP are released from neural or non-

neural tissues and modulate airway tone (Fredholm *et al.*, 1979; Cushley *et al.*, 1983; 1984). Given by inhalation, both adenosine and ATP produced a marked bronchoconstriction in asthmatic subjects (Cushley *et al.*, 1983; 1984). In the present study, adenosine produced only weak and inconsistent contractions whereas ATP was a slightly more effective constrictor. In larger human bronchi (> 2 mm) adenosine (< 300 μM) was completely without effects on tone (unpublished observations). In animal studies, adenosine had variable but predominantly relaxant responses (Coleman, 1976; Farmer & Farrar, 1976; Karlsson *et al.*, 1982) but ATP produced bronchoconstriction (Farmer & Farrar, 1976; Lundblad *et al.*, 1984). Since the anti-asthmatic drug theophylline is a potent adenosine antagonist, the possibility that adenosine is an asthma mediator has received much interest. The poor response found in this study does not suggest that this purine is important in small airways contraction.

It may be argued that bronchiolar smooth muscle from asthmatics would have responded differently from our preparations that were obtained from patients with lung carcinoma and without asthma. However, this argument has at present little support, since studies of contractile mediators have not been able to separate airway reactivity in isolated bronchi (and parenchymal strips) from normals and asthmatics (Vincenc *et al.*, 1983; Finney *et al.*, 1983; Schellenberg & Foster, 1984; Roberts *et al.*, 1984; Paterson *et al.*, 1984). Thus, the present observations agree with the possibility that histamine, $PGD_2$, substance P, ATP, and acetylcholine (mimicked by carbachol) participate in bronchiolar constriction. Although these agents are known to be present in the lung, their roles are difficult to assess since the relevant concentrations of these agents at target cells like the bronchiolar smooth muscle cell are not known.

It is of interest for the evaluation of *in vitro* findings that drugs with acute and significant effects on the signs and symptoms of asthma are known to be active in patients within certain plasma (and tissue) levels. Systemic treatment with β-adrenoceptor stimulants like terbutaline and salbutamol results in bronchodilatation at 10–100 nM in plasma (e.g. van den Berg *et al.*, 1984). However, it was reported that 0.1–1000 μM of salbutamol was needed to relax human isolated bronchi with a basal tone and that salbutamol was almost without effect in histamine-contracted preparations ($EC_{50}$ = 1 mM) (Davis *et al.*, 1980). We were particularly interested in the possibility suggested by Persson & Ekman (1976) that human bronchiolar strips could respond to low concentrations of a β-adrenoceptor stimulant and we considered those preparations unresponsive that did not relax to 1 μM terbutaline. Only four out of 11 carbachol-contracted bronchioles were sensitive to terbutaline suggesting that $\beta_2$-adrenoceptor stimulants are potent relaxants of small airways at least in some human subjects.

In contrast to the effect of $\beta_2$-adrenoceptor stimulation the bronchiolar relaxation induced by xanthines was consistent and a significant portion of the response was obtained within clinically relevant concentrations of theophylline and enprofylline. The finding that xanthines also relaxed those preparations that were poorly responsive to terbutaline suggests that the inability of this agent to relax bronchiolar rings was due to some limitation in the $\beta_2$-adrenoceptor function rather than to relaxant properties of the isolated bronchiolar smooth muscle. The elevated tension (induced by carbachol) *per se* could have contributed to the difference since xanthines but not β-receptor agonists, readily relax highly contracted guinea-pig trachealis muscle (Karlsson & Persson, 1981). The *in vivo* experiences with $\beta_2$-adrenoceptor stimulants showing pronounced anti-asthma effects quite comparable to xanthines is at variance with the present findings.

Enprofylline has been characterized as a xanthine which does not block adenosine receptors whereas theophylline is an effective antagonist (e.g. Persson, 1982). The observation that enprofylline was more potent than theophylline as a human bronchiole relaxant *in vitro* agrees with bronchodilator potencies observed in human large airways *in vitro* (Persson *et al.*, 1981) and in asthmatic subjects *in vivo* (e.g. Lunell *et al.*, 1982). It also gives further support to the view that adenosine-antagonism does not explain the anti-asthmatic lung actions of xanthine derivatives (Persson *et al.*, 1982; Persson, 1985).

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