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Specific antagonism of adenosine-induced bronchoconstriction in asthma by oral theophylline

J. S. MANN* & S. T. HOLGATE
Medicine 1, Level D, Centre Block, Southampton General Hospital, Southampton, UK

1 The airway response to increasing concentrations of inhaled adenosine and histamine after oral theophylline or matched placebo was studied in nine asthmatic subjects. Changes in airway calibre were followed as sGaw and FEV_1 and concentration-response curves constructed.

2 Inhaled adenosine caused concentration-related bronchoconstriction and was four–five times less potent than inhaled histamine.

3 Theophylline, which achieved a mean plasma level of 15.9 and 16.6 μg/ml on the histamine and adenosine study days respectively, caused significant increases in FEV_1 (17%) and sGaw (41–53%) whereas placebo had no effect.

4 Theophylline also protected the airways against histamine-and adenosine-induced bronchoconstriction. However theophylline had a greater protective effect against adenosine (concentration-ratio 17.4 for FEV_1 and 12.8 for sGaw) than against histamine (concentration ratio 5.6 for FEV_1 and 5.4 for sGaw ($P < 0.05$)).

5 At therapeutic concentrations theophylline is a specific antagonist of the airway effects of adenosine in addition to being a bronchodilator and a functional antagonist.

Keywords adenosine theophylline bronchoconstriction

Introduction

Alkyl substituted xanthines, such as 3-methylxanthine (theophylline) and its diethylamine salt aminophylline, are effective agents for the treatment of acute and chronic asthma (Weinberger, 1984; Svedmyr *et al.*, 1977). Inhibition of cyclic 3′5′-adenosine monophosphate (cyclic AMP) phosphodiesterase (PDE) activity to increase airway smooth muscle cyclic AMP levels has provided an attractive biochemical mechanism which could explain the bronchodilator effect of methylxanthines in asthma (Butcher & Sutherland, 1962) and the reported synergism observed with β_2-adrenoceptor agonists in relaxing airway smooth muscle *in vitro* (Triner *et al.*, 1977). However, attempts to extend this pharmacological model to account for the therapeutic efficacy of methylxanthines in asthma have produced several discrepancies that require further explanation (reviewed in Cushley *et al.*, 1984).

An additional pharmacological activity exhibited by many substituted xanthines is the competitive antagonism of the physiological effects of adenosine, mediated through an interaction with specific cell surface purinoreceptors (Fredholm, 1980). These receptors have been divided into A_1 and A_2 sub-types according to whether their activation by adenosine or one of its analogues inhibits (A_1) or stimulates (A_2) adenylate cyclase to increase or decrease cellular levels of cyclic AMP respectively (Wolff *et al.*, 1981; Arch & Newsholme, 1978). The concentrations of theophylline optimal for adenosine antagonism, are lower than those required to inhibit cyclic AMP PDE and fall well within the

Correspondence: Dr J. S. Mann, Medicine 1, Level D, Centre Block, Southampton General Hospital, Tremona Road, Southampton, SO9 4XY, UK

range of therapeutic plasma concentrations (Fredholm, 1980; Fredholm *et al.*, 1979). We have recently shown that adenosine by inhalation caused bronchoconstriction in asthmatic but not normal subjects, whereas the related purine nucleosides guanosine (Cushley *et al.*, 1983a) and inosine (Cushley *et al.*, 1983b) were without effect. Theophylline administered by inhalation, was two–three times more effective in antagonising bronchoconstriction induced by inhaled adenosine than by histamine (Cushley *et al.*, 1984).

To investigate whether a similar mechanism is relevant to theophylline at therapeutic plasma concentrations in asthma, we have undertaken a study comparing the effects of orally administered theophylline on bronchoconstriction induced by inhaled adenosine and histamine. The study was specifically designed to separate the bronchodilator and non-specific anti-bronchoconstrictor effects of theophylline from any additional activity as a specific antagonist of adenosine.

Methods

Nine atopic asthmatic subjects, whose details are shown in Table 1, participated in the study. There were six males and three females and all were non-smokers. They had mild asthma which was being treated with inhaled steroids, and bronchodilators (Table 1). None of the subjects was taking theophylline preparations before the study and all abstained from xanthine-containing drinks and inhaled bronchodilators for 12 h before each study day, other medications being continued as usual. All subjects gave their informed consent and the protocol was approved by the Southampton University and Hospitals Ethics Committee.

Airway calibre was measured before and after bronchial provocation as the forced expiratory volume in one second (FEV_1) using a dry wedge spirometer (Vitalograph, Buckingham, UK) and as specific airways conductance (sGaw) measured over 10 panting breaths in a pressure compensated volume displacement body plethysmograph (Fenyves and Gut, Basle, Switzerland). Recordings of FEV_1 were made 1 min after sGaw with at least 5 min between each series of measurements, to minimise the effects of the forced expiratory manoeuvre on resting airway calibre (Orehek *et al.*, 1980). Despite this precaution, a forced expiratory manoeuvre repeatedly caused bronchoconstriction in one subject (No. 9) which did not recover over 10 min. In view of this the patient only performed measurements of sGaw throughout the study.

All inhaled drugs were administered from Mini-Neb disposable nebulisers (Inspiron, Bard, Sunderland, UK) as described previously (Cushley *et al.*, 1983a). Adenosine (Rona Laboratories, Hitchin, UK) and histamine acid monophosphate (BDH Chemicals, Poole, UK) were prepared in 0.9% saline to produce a range of concentrations from 0.0625 to 10 mg/ml for adenosine and 0.015 to 5 mg/ml for histamine.

Theophylline levels in plasma samples were measured by high performance liquid chromatography with an intra-assay coefficient of variation of 5% and an inter-assay coefficient of variation of 7% (James *et al.*, 1983).

The study was single-blind and randomised. Subjects attended the laboratory on four occasions having fasted for 12 h. After resting for 15 min, five baseline recordings of sGaw followed by three recordings of FEV_1 were

Table 1 Subject characteristics

Subject	*Sex*	*Age (years)*	*Height (cm)*	*(l)*	*FEV_1 (% pred)*	*PC_{s35}† histamine*	*Medication**
1	M	21	178	4.43	101.1	0.04	S
2	M	22	183	4.47	98.7	0.17	—
3	M	28	175	3.91	96.3	0.29	S
4	F	22	168	3.48	103.9	0.10	S
5	M	22	177	2.74	63.4	0.02	S, B
6	M	25	177	3.54	83.9	0.32	S
7	M	22	171	3.20	78.0	0.03	S, B
8	F	22	157	1.75	57.2	0.23	S, B
9	F	26	163	2.10	67.5	0.07	S
Mean		23.3	172	3.29	83.3	0.09†	
(s.e. mean)		(0.8)	(2.7)	(0.32)	(5.9)		

*S, Inhaled salbutamol; B, inhaled beclomethasone diproprionate
†PC_{s35} geometric mean

made (baseline 1). Subjects were then given either theophylline 500 mg as 125 mg tablets of micronised theophylline (Riker Laboratories, Loughborough, UK) or matched placebo. Since one subject (no. 9, Table 1) developed nausea and vomiting following the initial 500 mg theophylline, the dose for this subject was reduced to 375 mg throughout the study. Ninety minutes after the oral medication, three further measurements of FEV_1 and sGaw were made (baseline 2) and 10 ml venous blood were removed and transferred to a heparin tube for measurement of plasma theophylline levels.

On completion of the post-drug baseline, subjects inhaled 0.9% saline for 1 min followed by two further measurements of sGaw and FEV_1 (baseline 3). Nebulised adenosine or histamine was then administered and 2 min after each inhalation measurements of sGaw and FEV_1 were made. This was repeated with doubling concentrations of aerosol until sGaw had fallen to 40% of the value recorded as baseline 3. On completion of the study bronchoconstriction was reversed with inhaled salbutamol, 200 μg.

Baseline values of FEV_1 and sGaw within and between study days were compared using Student's *t*-test for paired data and Wilcoxon's signed rank test respectively. Bronchoconstriction induced by histamine and adenosine was expressed as concentration response curves which were constructed as the logarithm of the concentration nebulised against the percentage change in FEV_1 or sGaw from baseline 3. Concentration-response lines were derived by unweighted linear regression and compared for slope and position by covariant analysis. The concentrations of histamine or adenosine causing a 15% fall in FEV_1 from baseline 3 (PC_{f15}) or 35% fall in sGaw (PC_{s35}) were determined from the concentration-response curves, and geometric mean values compared by Student's paired *t*-test. The protective effect of theophylline against adenosine- and histamine-induced bronchoconstriction, as reflected by parallel displacement of the concentration-response curves to the right, was expressed as the concentration ratio calculated as the ratio of PC_{f15} and PC_{s35} after theophylline to the corresponding value after placebo. Concentration ratios were compared using Student's paired *t*-test.

Results

Baseline values of FEV_1 and sGaw did not differ significantly on any of the study days. Mean values (baseline 1) ranged from 3.42 ± 0.31 to 3.49 ± 0.34 l (± s.e. mean) for FEV_1 and 0.122 ± 0.033 to 0.145 ± 0.022 s^{-1} kPa^{-1} for sGaw.

Ninety minutes after theophylline, mean plasma levels reached 16.6 ± 1.1 (range, 14.6–21.0) μg/ml on the adenosine study day and 15.9 ± 1.0 (range, 12.9–21.4 μg/ml) on the histamine study day, which were not significantly different. Following oral theophylline mean FEV_1 (baseline 2) increased significantly to 3.94 ± 0.2 l (17%) and 3.97 ± 0.3 l (17%) ($P < 0.005$) and sGaw to 1.91 ± 0.36 (41%) and 1.89 ± 0.26 (53%) ($P < 0.01$) on the adenosine and histamine study days respectively, whereas placebo had no effect on airway calibre.

After taking the oral placebo, both inhaled adenosine and histamine caused concentration-related decreases in sGaw and FEV_1. The positions of the concentration-response lines expressed as the PC_{f15} and PC_{s35} values, were 0.49 (range 0.07 to 1.05) and 0.61 (range 0.07 to 1.24) mg/ml for adenosine, and 0.12 (range 0.02 to 0.25) and 0.09 (range 0.02 to 0.32) mg/ml for histamine respectively.

The concentration-response regression lines calculated for histamine and adenosine after placebo and theophylline did not depart significantly from parallel. Theophylline displaced the FEV_1 and sGaw concentration-response lines for adenosine and histamine to the right in all subjects (Figures 1 and 2). The geometric mean PC_{f15} values calculated for histamine increased from 0.12 mg/ml after placebo to 0.65 (range 1.49 to 11.64) mg/ml after theophylline ($P < 0.001$) and similarly the mean PC_{s35} increased from 0.09 to 0.52 (range 0.02 to 1.8) mg/ml ($P < 0.001$). When compared to placebo, theophylline increased the geometric mean PC_{f15} for adenosine from 0.49 to 8.47 (range 1.13 to 36.22) mg/ml ($P < 0.001$) and PC_{s35} from 0.61 to 7.82 (range 1.01 to 32.83) mg/ml ($P < 0.001$).

The geometric mean concentration-ratio for the protective effect of theophylline on adenosine-induced bronchoconstriction was 17.4 when airway calibre was measured as FEV_1 and 12.8 when measured as sGaw. These values were significantly higher than the corresponding concentration ratios for theophylline's effect on histamine-induced bronchoconstriction, being 5.6 for FEV_1 ($P < 0.05$) and 5.4 for sGaw ($P < 0.02$). There was no relationship between the effect of theophylline in increasing baseline FEV_1 and sGaw or between plasma theophylline concentrations and protection against adenosine and histamine induced bronchoconstriction when analysed by least squares linear regression. Similarly no relationship could be established between the protective effect of theophylline against bronchoconstriction induced by adenosine and that induced by histamine.

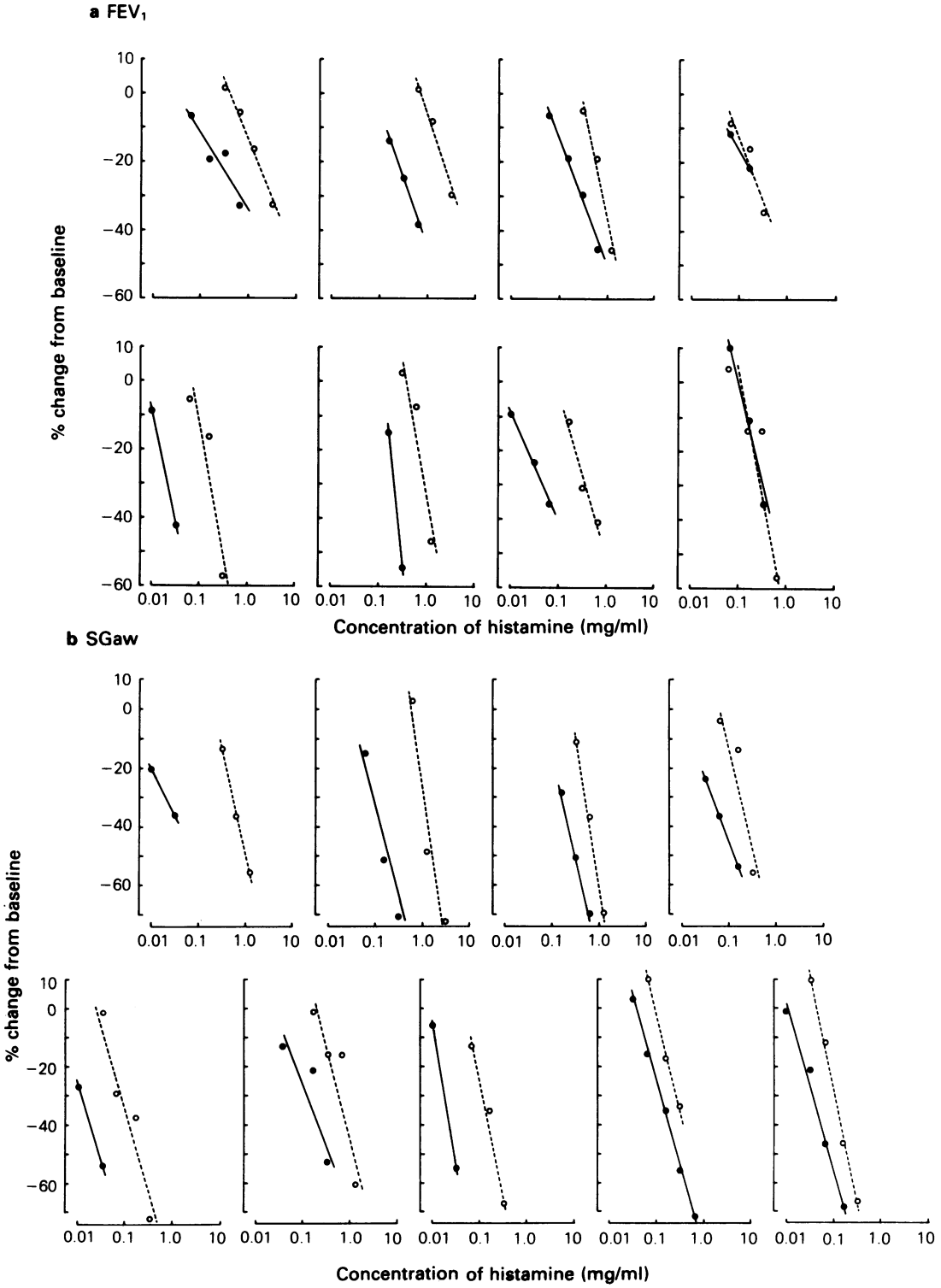

Figure 1 The individual regression lines of the response of FEV_1 (a) and sGaw (b) to inhalation of histamine preceded by oral placebo, (●—●) and by oral theophylline (○-----○).

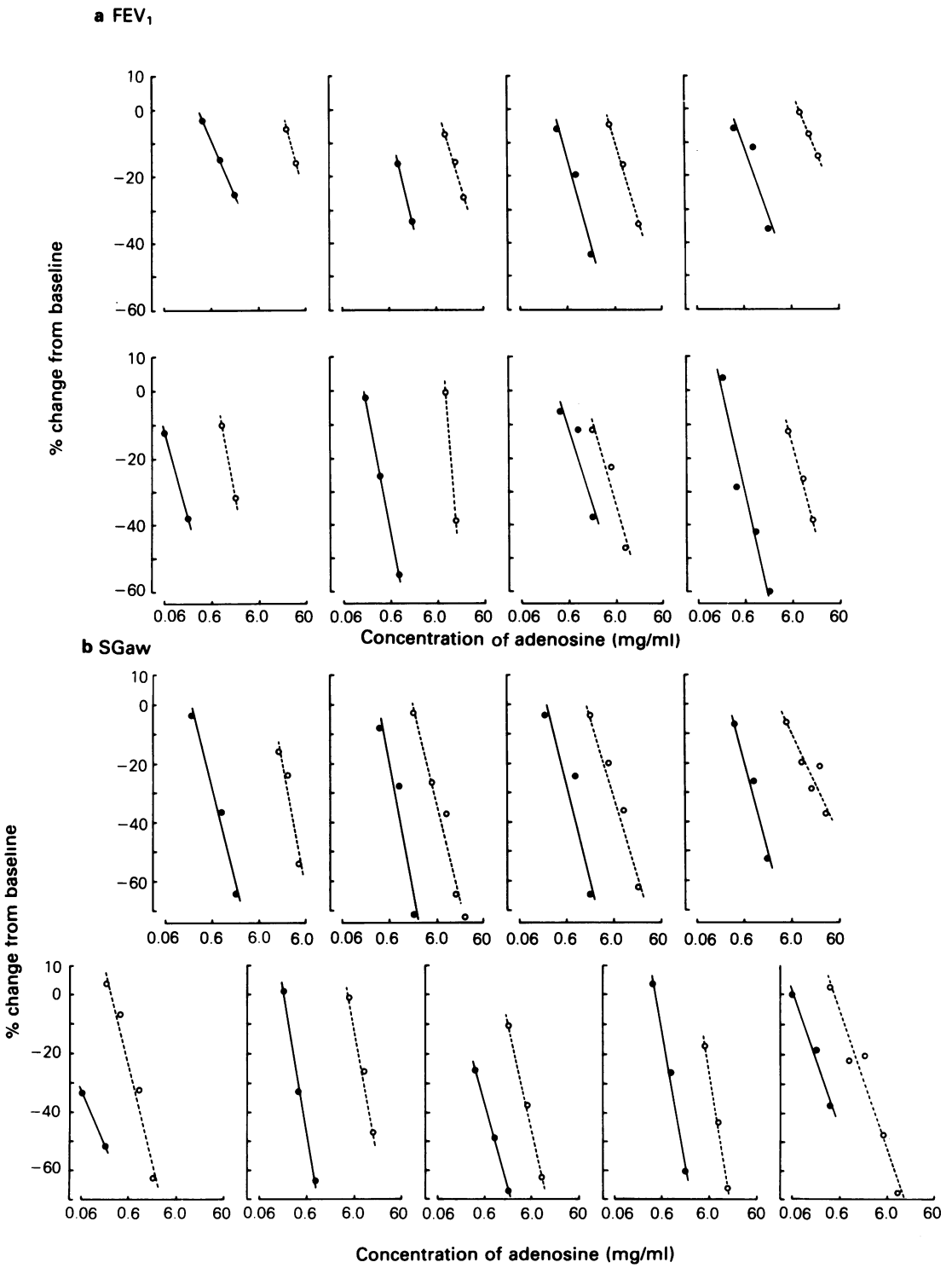

Figure 2 The individual regression lines of the response of FEV_1 (a) and sGaw (b) to inhalation of adenosine by oral placebo, (●—●) and by oral theophylline (○-----○).

Discussion

The findings of this study confirm our previous experience that inhaled adenosine is a bronchoconstrictor agent in patients with asthma, being four–five times less potent than histamine. Theophylline, administered as a single oral dose to produce plasma levels which fell within the accepted therapeutic range (10–20 μg/ml), caused significant bronchodilatation and antagonised bronchoconstriction induced by both inhaled histamine and adenosine. However, comparison of concentration-ratios demonstrated that theophylline was two–three times more effective in protecting the airways against adenosine compared to histamine. These data suggest that theophylline, at therapeutic concentrations, is a selective antagonist of adenosine-induced bronchoconstriction in addition to being a bronchodilator and functional antagonist of bronchoconstriction.

In the present study oral theophylline, which achieved a mean plasma concentration on the two study days of 15.9 and 16.6 μg/ml 90 min after administration, caused a 40–50% increase in sGaw and a 17% increase in FEV_1. This bronchodilatation probably represents both a direct effect of theophylline on constricted airway smooth muscle (Triner *et al.*, 1977; Bergstrand, 1980) and an indirect action through the release of adrenaline from the adrenal medulla (Higbee *et al.*, 1982; Mackay *et al.*, 1983).

In order to compare the bronchoconstrictor effects of adenosine and histamine after taking oral placebo and theophylline, concentration response curves were constructed in which changes in airway calibre were followed as FEV_1 and sGaw following serial inhalations of increasing concentrations of bronchoconstrictor agonists. Chung *et al.* (1982) have recently reported that when airway calibre is measured as sGaw, the starting baseline value influences the subsequent slope of a bronchoconstrictor concentration-sGaw response curve. An increase in baseline airway calibre reduces the slope of the concentration-response line, whereas a reduction of baseline airway calibre has the reverse effect. If the changes in airway calibre induced by a bronchoconstrictor agent are corrected for baseline values by expressing the airway response in the form of percentage change from baseline, then the concentration-response curves are parallel (Chung *et al.*, 1982; Mann *et al.*, 1984). Despite theophylline's bronchodilator action inhaled adenosine and histamine still caused bronchoconstriction. When the airway responses to the inhaled agonist after placebo and theophylline were expressed as percentage change in FEV_1 or sGaw from the post-drug baseline, the concentration-response lines were parallel (Figures 1 and 2). This enabled the positions of the concentration-response lines to be compared as the provocative concentrations of inhaled agonist causing a 15% reduction in FEV_1 or 35% reduction in sGaw, and the relative potencies of theophylline as an antagonist of histamine and adenosine to be calculated as concentration-ratios.

Application of this approach demonstrated that therapeutic plasma concentrations of theophylline not only produced bronchodilatation but also antagonised the bronchoconstrictor effect of inhaled histamine, as indicated by concentration ratios of 5.6 and 5.4 when airway calibre was measured as FEV_1 and sGaw respectively. This is in contrast to our previous finding that theophylline administered by inhalation had minimal bronchodilator activity and also exerted little protection against inhaled histamine (Cushley *et al.*, 1984). Theophylline by inhalation has a weak short-lived bronchodilator effect in asthma (Cushley & Holgate, 1985), which probably reflects its rapid transit from the lung into the pulmonary circulation (Thompson *et al.*, 1982). Since theophylline is not known to be an H_1-histamine or a muscarinic cholinergic receptor antagonist, its ability to protect against histamine-induced bronchoconstriction indicates functional antagonism similar to that reported for other bronchoconstrictor stimuli (Jones *et al.*, 1984; Olsson & Persson, 1976). We have, however, been unable to establish a relationship between the ability of theophylline to cause bronchodilatation and protect against histamine-induced bronchoconstriction, possibly suggesting that these two pharmacological activities may have differing mechanisms. The bronchodilator activity of β_2-adrenoceptor agonists has also been divorced from their anti-bronchoconstrictor effect (Salome *et al.*, 1983; Bandouvakis *et al.*, 1981).

The present study has demonstrated that theophylline was two–three times more effective in protecting asthmatic airways against the bronchoconstrictor effect of adenosine when compared to that of histamine. No correlation could be established between the relative potency of theophylline's protective effect on histamine and adenosine, suggesting that in addition to functional antagonism, theophylline exerts a specific action in antagonising the constrictor effect of adenosine. We have previously reported that theophylline by inhalation, at concentrations which have no effect on baseline airway calibre, afforded a three–four fold greater protection against the airway effect of adenosine compared to that of histamine (Cushley *et al.*, 1984). The preferential action of theophylline,

when administered by two different routes, against the airway effects of adenosine suggest an effect of theophylline mediated at the level of cell surface adenosine receptors (Fredholm, 1980).

Adenosine is released from animal lung tissue under hypoxic conditions and with antigen challenge (Mentzer *et al.*, 1975; Fredholm, 1981). Adenosine release has also been reported from purified preparations of rat serosal and mouse cultured bone marrow derived mast cells upon IgE-dependent challenge (Marquardt *et al.*, 1984). These observations, together with our recent finding of three–four fold increases in circulating levels of adenosine in parallel with bronchoconstriction following antigen bronchial provocation (Mann *et al.*, 1983), indicate that local release of this nucleoside in the airways may contribute to bronchoconstriction in asthma. If this occurs in clinical asthma, then at least some of the therapeutic effect of theophylline may be mediated by antagonism of adenosine's constrictor effect, though further research will be required to define whether this action is therapeutically significant.

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