

## INHALED ADENOSINE AND GUANOSINE ON AIRWAY RESISTANCE IN NORMAL AND ASTHMATIC SUBJECTS

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- 1 The airway response to the inhaled nucleosides, adenosine ( $6.7 \times 10^{-4}$ – $6.7$  mg/ml) and guanosine ( $7.3 \times 10^{-4}$ – $1.4$  mg/ml) was studied in normal and asthmatic subjects. Airway response, measured in the body plethysmograph, was expressed as percentage change in specific airway conductance (sGaw) from baseline.
- 2 Inhaled adenosine caused no change in sGaw in normal subjects but produced a dose-dependent reduction in sGaw in both allergic and non-allergic asthmatic subjects (76 and 62% reduction respectively at 6.7 mg/ml).
- 3 Kinetics of adenosine induced bronchoconstriction were studied in 12 asthmatic subjects who inhaled a single concentration of adenosine. Bronchoconstriction was maximal within 5 min (42% reduction in sGaw) with partial recovery by 30 min.
- 4 The related nucleoside guanosine caused no change in sGaw in normal or asthmatic subjects.
- 5 Adenosine, but not guanosine, is a potent bronchoconstrictor in asthma suggesting that it may have a specific pharmacological effect.

### Introduction

Methylxanthines such as theophylline and its ethylenediamine salt, aminophylline, are widely used in the treatment of asthma. Following the discovery of adenosine 3',5'-cyclic monophosphate (cyclic AMP) as a second messenger mediating relaxation of airway smooth muscle, it has been generally accepted that methylxanthines act by inhibiting the breakdown of cyclic AMP by phosphodiesterases (PDE) (Butcher & Sutherland, 1962; Lancet, 1970). However, the high concentrations of theophylline required to effectively inhibit PDE activity of airway smooth muscle make it unlikely that this mechanism is their sole or main action in asthma (Stoclet, 1980; Lohmann *et al.*, 1977; Bergstrand, 1980). Theophylline also inhibits the receptor-mediated effects of adenosine, a naturally occurring purine nucleoside which is formed by the 5' nucleotidase cleavage of 5' adenosine monophosphate (5' AMP) (Ally & Nakatsu, 1976; Fain & Malbon, 1979). If adenosine antagonism is relevant to theophylline's effect on the airways in asthma, then adenosine should be a bronchoconstrictor (Fredholm, 1980a). In animal isolated tracheal and bronchial airway preparations whose tone has been artificially increased, adenosine causes relaxa-

tion (Fredholm *et al.*, 1979; Coleman, 1976; Farmer & Farrar, 1976) whereas, in preparations of guinea-pig trachea with normal intrinsic tone, adenosine causes a weak but consistent contraction (Fredholm *et al.*, 1979; Advenier *et al.*, 1982). To extend the observations to man, we have investigated the effect of adenosine and the related nucleoside, guanosine, on the airways of normal and asthmatic subjects *in vivo*.

### Methods

Six normal non-allergic, six allergic asthmatic and seven non-allergic asthmatic subjects, whose clinical details are summarised in Table 1, were studied. All subjects gave their informed consent and the study was approved by the local Ethical Committee. Asthmatic subjects omitted bronchodilators and sodium cromoglycate for 8 h prior to each visit. Forced expiratory volume in 1 s (FEV<sub>1</sub>) varied by less than 12% and specific airway conductance (sGaw) by less than 15% between study days. The purine nucleosides and saline were administered as

**Table 1** Details of normal and asthmatic subjects

	Number	Age (years) mean (range)	FEV <sub>1</sub> (l) (s.e. mean)	FEV <sub>1</sub> (% predicted)	sGaw (s <sup>-1</sup> kPa <sup>-1</sup> ) (s.e. mean)
Normal subjects	6	29 (21–40)	4.7 (0.31)	117	2.16 (0.23)
Allergic subjects	6	24 (18–31)	3.4 (0.29)	91	1.43 (0.11)
Non-allergic* subjects	7	47 (22–66)	2.8 (0.40)	89	1.41 (0.18)

\* Non-allergic subjects skin test negative to dermatophagoides pteronyssinus, aspergillus fumigatus, grass pollens, shrub pollens, house dust, cat fur, dog hair, feathers.

aerosols, generated at room temperature from disposable nebulisers (Mini-neb Inspiron) which were driven by compressed air at 8 l/min. Aerosols were inhaled from a face mask for 1 min using tidal respiration synchronised to 16 breaths/min with a metronome. Airway responses were measured in a whole body plethysmograph on line to a microprocessor and the results expressed as sGaw, the reciprocal of airway resistance corrected for thoracic gas volume. Solutions of the purine nucleosides, adenosine and guanosine (Rona Laboratories, Hitchin, Herts), were prepared in 0.9% saline to produce a range of concentrations from  $6.7 \times 10^{-4}$  to 6.7 mg/ml (pH 6.7) and  $7.3 \times 10^{-4}$  to 1.4 mg/ml (pH 6.5) respectively.

#### Dose-response studies

The normal and allergic asthmatic subjects inhaled repeated doses of 0.9% saline or increasing concentrations of adenosine and guanosine on separate days in a randomised blind crossover study. Plethysmograph measurements were made 1 and 4 min after each inhalation and followed immediately by the next inhalation. Since guanosine was less soluble than adenosine, the final inhalation (1.4 mg/ml) was given over 2.5 min to achieve the same cumulative molar dose as adenosine. The seven non-allergic asthmatic subjects were studied in a similar way with the exclusion of guanosine.

#### Single dose time course

To determine the kinetics of adenosine-induced bronchoconstriction, 12 asthmatic subjects (five allergic, seven non-allergic) inhaled for 1 min a single

concentration of adenosine which in previous dose-response studies had been shown to produce a 35–40% fall in sGaw from baseline. After the adenosine inhalation sGaw was measured at 1, 2, 4 and 5 min and thereafter at 2.5 min intervals for 30 min.

#### Statistical tests

Comparisons of FEV<sub>1</sub> between different subject groups, and for the same group on separate days, were analysed by Student's non-paired and paired *t*-test respectively. Resting values of sGaw were compared using Wilcoxon's signed rank test. Airway responses to the aerosols were expressed as percentage change in sGaw from baseline and Wilcoxon's signed rank test, used to assess the statistical significance of the change from baseline.

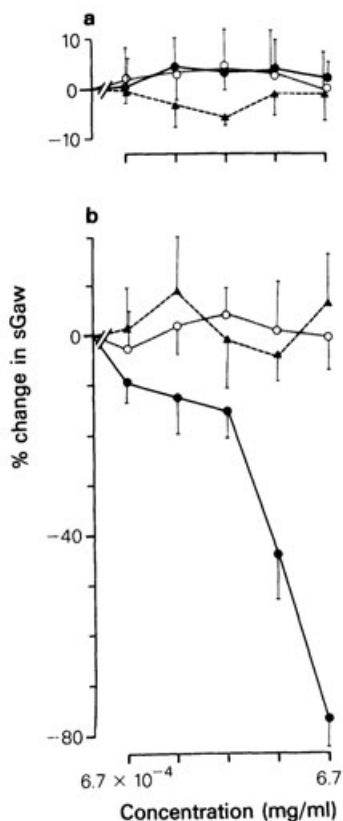
#### Results

Mean baseline data for FEV<sub>1</sub> and sGaw for the normal and asthmatic subjects is shown in Table 2. There was no significant difference between resting values of FEV<sub>1</sub> and sGaw on any of the study days for any of the three groups. Values for the asthmatic groups did not differ from each other but were significantly lower than those for the normal group ( $P < 0.05$ ). Since there was no difference in the sGaw recordings at 1 and 4 min after aerosol administration only the 4 min values were used for analysis.

Adenosine, guanosine and saline had no significant effect on sGaw in any of the normal subjects (Figure 1a). However, in the allergic asthmatic subjects adenosine nebulised in concentrations above  $6.7 \times$

**Table 2** Mean (s.e. mean) baseline values of FEV<sub>1</sub> and sGaw in the normal and asthmatic subjects

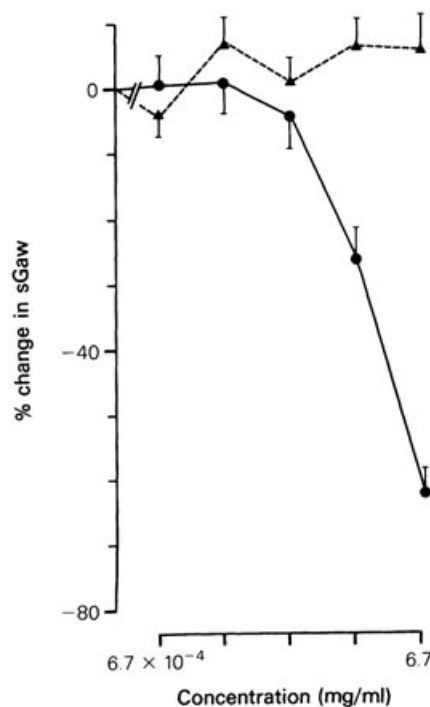
Subjects	FEV <sub>1</sub> (l)			sGaw (s <sup>-1</sup> kPa <sup>-1</sup> )		
	Adenosine	Guanosine	Saline	Adenosine	Guanosine	Saline
Normal	4.61 (0.31)	4.74 (0.34)	4.70 (0.30)	2.15 (0.25)	2.17 (0.29)	2.15 (0.21)
Allergic asthma	3.42 (0.30)	3.38 (0.32)	3.46 (0.27)	1.54 (0.15)	1.37 (0.10)	1.38 (0.12)
Non-allergic asthma	2.82 (0.39)	—	2.84 (0.40)	1.44 (0.20)	—	1.39 (0.16)



**Figure 1** The response of sGaw to inhalation of adenosine (●), guanosine (○) and saline (▲) in (a) normal and (b) allergic asthmatic subjects. Each point represents the mean ( $\pm$  s.e. mean) percentage change in sGaw from baseline for six subjects.

$10^{-2}$  mg/ml produced a statistically significant dose-dependent fall in sGaw while neither nebulised saline nor guanosine had any effect (Figure 1b). At 6.7 mg/ml the reduction in sGaw with adenosine was  $76 \pm 6\%$  ( $P < 0.01$ ). Adenosine also caused bronchoconstriction in the non-allergic asthmatic subjects with sGaw falling from 1.44 to  $0.56 \text{ s}^{-1} \text{ kPa}^{-1}$  (Figure 2) ( $P < 0.01$ ). In allergic and non-allergic asthma the calculated geometric mean adenosine concentrations required to produce a 40% fall in sGaw from baseline ( $PC_{40}$ ) were  $0.86 \pm 0.48$  and  $1.9 \pm 0.45 \text{ mg/ml}$  ( $\pm$  s.e. mean) respectively.

After inhalation of a single concentration of adenosine sGaw fell rapidly within 1 min from a mean value of  $1.24$  to  $0.81 \text{ s}^{-1} \text{ kPa}^{-1}$  ( $P < 0.01$ ) to reach a minimum at 5 min (sGaw 0.68) after which it slowly increased (Figure 3). The kinetics of bronchoconstriction were similar in both the allergic and non-allergic asthmatic subjects.

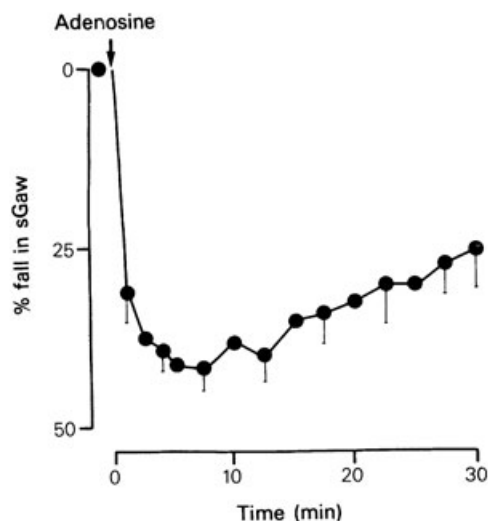


**Figure 2** The response of sGaw to inhalation of adenosine (●) and saline (▲) in non-allergic asthma. Each point represents the mean ( $\pm$  s.e. mean) percentage change in sGaw from baseline for seven subjects.

## Discussion

The present study clearly demonstrates that inhaled adenosine is a potent bronchoconstrictor in both allergic (Figure 1b) and non-allergic (Figure 2) asthma with a time course similar to that of histamine and methacholine (Laitinen, 1974). Adenosine had no effect on the airways of normal subjects (Figure 1a) even at a concentration which produced a mean 69% fall in sGaw in asthma. However, the poor solubility of adenosine prevented us from testing the effect of higher concentrations in normal subjects.

This is the first study to define the pharmacological activity of adenosine on human airways. There have been many *in vitro* studies to show that adenosine and its modified purine analogues are capable of relaxing gastrointestinal (Ally & Nakatsu, 1976), vascular (Herlihy *et al.*, 1976) and tracheal (Coleman, 1976) smooth muscle preparations. However, in asthma, rather than showing a relaxant response adenosine is a powerful bronchoconstrictor. This finding is in agreement with two recent *in vitro* studies demonstrating a contractile effect on guinea pig isolated airway smooth muscle under resting tone (Fredholm



**Figure 3** Time course of bronchoconstriction induced by a single inhalation of adenosine in asthma. Each point represents the mean ( $\pm$  s.e. mean) percentage change in sGaw from baseline for twelve subjects.

*et al.*, 1979, Advenier *et al.*, 1982) whereas most other workers have shown relaxant responses on precontracted tracheal preparations (Christie & Satchell, 1980; Coleman, 1976; Farmer & Farrar, 1976).

The mechanism of adenosine-induced bronchoconstriction in asthma is at present obscure. A non-specific irritant effect with reflex bronchoconstriction is unlikely since the pH of the inhaled solution was close to neutrality (pH 6.7) and inhalation of adenosine produced no subjective sensation of irritation or cough. Moreover the structurally similar nucleoside, guanosine (pH 6.5), over the same concentration range as adenosine, was ineffective, suggesting that bronchoconstriction produced by adenosine relates to a specific pharmacological activity.

In many tissues adenosine mediates its physiological effects by modulating levels of cyclic AMP via occupation of cell surface receptors which either stimulate (R<sub>a</sub>) or inhibit (R<sub>i</sub>) adenylate cyclase activity (Wolff *et al.*, 1981). Receptors for adenosine identified in guinea pig bronchial smooth muscle are of the R<sub>a</sub> type (Brown & Collis, 1982). Stimulation of these receptors would increase cellular levels of cyclic AMP, thereby explaining the smooth muscle relaxant response of adenosine observed in many of the *in vitro* studies. In asthma, airway calibre is highly dependent upon  $\beta$ -receptor sympathetic

tone (Richardson & Sterling, 1969). Adenosine may reduce this sympathetic tone to the airways by acting as a weak agonist and interfering with  $\beta$ -adrenoceptor adenylate cyclase coupling (Braun & Levitzki, 1979) or by inhibiting sympathetic neurotransmission via a specific presynaptic receptor (Verhaeghe *et al.*, 1977).

It is also possible that adenosine causes bronchoconstriction indirectly by augmenting inflammatory mediator release. *In vitro*, adenosine has been shown to potentiate IgE-dependent mediator release, both from rat isolated mast cells (Holgate *et al.*, 1980) and guinea pig lung fragments (Welton & Simko, 1980). Since adenosine is equipotent as a bronchoconstrictor in allergic and non-allergic asthmatic subjects in the present study, and the kinetics of bronchoconstriction are similar to those of histamine and methacholine rather than allergen induced bronchoconstriction, enhanced IgE-dependent release of mast cell associated mediators with secondary effects on the airways would seem an unlikely explanation.

Alternatively, a neurological reflex mechanism may be important since adenosine has been shown to stimulate afferent nerve endings (Bleehe & Keele, 1977) and might therefore produce its effects by increasing vagal activity.

The accepted hypothesis that methylxanthines produce bronchodilatation in asthma by inhibiting cyclic AMP phosphodiesterase activity has recently been questioned (Stoclet, 1980; Fredholm, 1980a). Concentrations of theophylline at the top of the therapeutic range in asthma produced only 10–12.5% inhibition of phosphodiesterase activity in broken cell preparations of human lung tissue (Polson *et al.*, 1978). In addition, more effective phosphodiesterase inhibitors such as dipyridamole and papaverine have no bronchodilator activity (Ruffin & Newhouse, 1981; Endres, 1978). If theophylline was acting by inhibiting phosphodiesterase, a synergistic interaction with  $\beta$ -adrenoceptor agonists in producing bronchodilatation would be expected, but studies both *in vitro* (Karlsson & Persson, 1981) and *in vivo* (Handslip *et al.*, 1981) have failed to show this. Since therapeutic concentrations of theophylline fall well within the range for adenosine antagonism (Fredholm, 1980b) and exogenous adenosine is a potent bronchoconstrictor in asthma, it is possible that at least part of the therapeutic response to theophylline in asthma may be through antagonism of endogenous adenosine. Further work is in progress to investigate whether this is a realistic possibility.

We thank Rona Laboratories Limited for providing adenosine and Mrs M. Dowling for typing the manuscript.

## References

- ADVENIER, C., BIDEET, D., FLOCH-SAINT-AUBIN, A. & RENIER, A. (1982). Contribution of prostaglandins and thromboxanes to the adenosine and ATP-induced contraction of guinea-pig isolated trachea. *Br. J. Pharmacol.*, **77**, 39–44.
- ALLY, A.I. & NAKATSU, K. (1976). Adenosine inhibition of isolated rabbit ileum and antagonism by theophylline. *J. Pharmacol. exp. Ther.*, **199**, 208–215.
- BERGSTRAND, H. (1980). Phosphodiesterase inhibition and theophylline. *Eur. J. resp. Dis.*, **61**, 37–44.
- BLEEHAN, T. & KEELE, C.A. (1977). Observations on the algogenic actions of adenosine compounds on the human blister base preparation. *Pain*, **3**, 367–377.
- BRAUN, S. & LEVITZKI, A. (1979). The attenuation of epinephrine-dependent adenylate cyclase by adenosine and the characteristics of the adenosine stimulatory and inhibitory sites. *Mol. Pharmacol.*, **16**, 737–748.
- BROWN, C.M. & COLLIS, M.G. (1982). Evidence for an A<sub>1</sub> purine receptor in the guinea-pig trachealis muscle. *Br. J. Pharmacol.*, **75**, 157P.
- BUTCHER, R.W. & SUTHERLAND, E.W. (1962). Adenosine 3',5'-phosphate in biological materials. *J. biol. Chem.*, **237**, 1244–1250.
- CHRISTIE, J. & SATCHELL, D.G. (1980). Purine receptors in the trachea: is there a receptor for ATP? *Br. J. Pharmacol.*, **70**, 512–514.
- COLEMAN, R.A. (1976). Effects of some purine derivatives on the guinea-pig trachea and their interaction with drugs that block adenosine uptake. *Br. J. Pharmacol.*, **57**, 51–57.
- ENDRES, P. (1976). Bronchialer Muskeltonus und zyklisches Adenosinmonophosphat im Plasma. *Prax. Pneumol.*, **30**, 751–763.
- FAIN, J.N. & MALBON, C.C. (1979). Regulation of adenylate cyclase by adenosine. *Mol. Cell. Biochem.*, **25**, 143–169.
- FARMER, J.B. & FARRAR, D.G. (1976). Pharmacological studies with adenine, adenosine and some phosphorylated derivatives on guinea-pig tracheal muscle. *J. Pharm. Pharmacol.*, **28**, 748–752.
- FREDHOLM, B.B. (1980a). Are methylxanthine effects due to antagonism of endogenous adenosine? *Trends in Pharmacol. Sci.*, **1**, 129–132.
- FREDHOLM, B.B. (1980b). Theophylline actions on adenosine receptors. *Eur. J. resp. Dis.*, **61**, 29–36.
- FREDHOLM, B.B., BRODIN, K. & STRANDBERG, K. (1979). On the mechanism of relaxation of tracheal muscle by theophylline and other cyclic nucleotide phosphodiesterase inhibitors. *Acta Pharmacol. Tox.*, **45**, 336–344.
- HANDSLIP, P.D.J., DART, A.M. & DAVIES, B.H. (1981). Intravenous salbutamol and aminophylline in asthma: a search for synergy. *Thorax*, **36**, 741–744.
- HERLIHY, J.T., BOCKMAN, E.L., BERNE, R.M. & RUBIO, R. (1976). Adenosine relaxation of isolated vascular smooth muscle. *Am. J. Physiol.*, **230**, 1239–1243.
- HOLGATE, S.T., LEWIS, R.A. & AUSTEN, K.F. (1980). Role of adenylate cyclase in immunologic release of mediators from rat mast cells and agonist and antagonist effects of purine- and ribose-modified adenosine analogs. *Proc. Nat. Acad. Sci., U.S.A.* **77**, 6800–6804.
- KARLSSON, J.A. & PERSSON, C.G.A. (1981). Influence of tracheal contraction on relaxant effect *in vitro* of theophylline and isoprenaline. *Br. J. Pharmacol.*, **74**, 73–79.
- LAITINEN, L.A. (1974). Histamine and methacholine challenge in the testing of bronchial reactivity. *Scand. J. resp. Dis., Suppl.* **86**, 1–48.
- LANCET (1970). Cyclic-AMP: the second messenger. *Lancet*, **ii**, 1119–1121.
- LOHMANN, S.M., MIECH, R.P. & BUTCHER, F.R. (1977). Effects of isoproterenol, theophylline and carbachol on cyclic nucleotide levels and relaxation of bovine tracheal muscle. *Biochim. Biophys. Acta*, **499**, 238–250.
- POLSON, J.B., KRZANOWSKI, J.J., GOLDMAN, A.L. & SZENTIVANYI, A. (1978). Inhibition of human pulmonary phosphodiesterase activity by therapeutic levels of theophylline. *Clin. exp. Pharmacol. Physiol.*, **5**, 535–539.
- RICHARDSON, P.S. & STERLING, G.M. (1969). The effect of beta-adrenergic receptor blockade on airway conductance and lung volume in normal and asthmatic subjects. *Br. med. J.*, **3**, 143–145.
- RUFFIN, R.E. & NEWHOUSE, M.T. (1981). Dipyridamole—is it a bronchodilator? *Eur. J. resp. Dis.*, **62**, 123–126.
- STOCLET, J.C. (1980). L'Amp cyclique intervient—il dans l'effet bronchodilatateur de la theophylline. *Bull. Physiopathol. Resp.*, **16**, 5–12.
- VERHAEGHE, R.H., VANHOUTTE, P.M. & SHEPHERD, J.T. (1977). Inhibition of sympathetic neurotransmission in canine blood vessels by adenosine and adenine nucleotides. *Circ. Res.*, **40**, 208–215.
- WELTON, A.F. & SIMKO, B.A. (1980). Regulatory role of adenosine in antigen-induced histamine release from the lung tissue of actively sensitised guinea-pigs. *Biochem. Pharmacol.*, **29**, 1085–1092.
- WOLFF, J., LONDOS, C. & COOPER, D.M.F. (1981). Adenosine receptors and the regulation of adenylate cyclase. *Adv. Cyclic Nucleotide Res.*, **14**, 199–214.

(Received May 26, 1982,  
accepted October 14, 1982)