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Adenosine receptor-mediated contraction and relaxation of guinea-pig isolated tracheal smooth muscle: effects of adenosine antagonists

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1 The effects of several adenosine analogues and antagonists on guinea-pig isolated trachea have been examined.

2 5'-N-ethylcarboxamidoadenosine (NECA), 5'-N-methylcarboxamidoadenosine (MECA) and adenosine (in the presence and absence of dipyridamole) elicited concentration-dependent tracheal relaxation.

3 The **R**(−)- and **S**(+)-enantiomers of N^6-(2-phenylisopropyl)adenosine (**R**-PIA and **S**-PIA respectively), N^6-cyclohexyladenosine (CHA) and 2-chloroadenosine (CADO) caused contractions at low concentrations (0.05–2.0 μM), whereas at higher concentrations, relaxation resulted.

4 For tracheal relaxation, the adenosine analogues exhibited the following rank order of potency: NECA > CADO > **R**-PIA = MECA > **S**-PIA > adenosine. The rank order of potency for inducing contractions was **R**-PIA > CHA > CADO > **S**-PIA. These data suggest that relaxation is mediated by adenosine A_2-receptors, whereas contraction is the result of activation of A_1-receptors.

5 8-Phenyltheophylline (8-PT), aminophylline, the triazoloquinazoline CGS 15943A and NPC205 (1,3-di-*n*-propyl-8-(4-hydroxyphenyl)xanthine) each inhibited the **R**-PIA-induced contractile response, whereas enprofylline was without effect. NPC205, aminophylline and 8-PT were competitive antagonists, but CGS15943A was non-competitive.

6 That the most potent antagonist was the A_1-selective agent, NPC205 (pA_2 = 7.80), further suggests that the contraction is mediated by A_1-receptors. Moreover, NPC205 was 13 times more potent as an antagonist of **R**-PIA-induced contractions (A_1) than of NECA-induced relaxations (A_2).

7 The antagonists were also found to relax the trachea by an unknown mechanism. That enprofylline did not antagonize the **R**-PIA-induced contractions, but was 3–4 times more potent a tracheal relaxant than aminophylline, further suggests that a direct effect on airway smooth muscle, rather than antagonism of endogenous adenosine, is more relevant to the bronchodilator effect of alkylxanthines in the treatment of asthma.

Introduction

Of the many mediators implicated in the pathogenesis of bronchial asthma, one of the most recent is the ubiquitous purine nucleoside, adenosine (Holgate *et al.*, 1987). Adenosine is a potent bronchoconstrictor in allergic and non-allergic asthmatic subjects (Cushley *et al.*, 1983). Moreover, allergen-induced bronchospasm in asthmatics is associated with elevated plasma levels of adenosine (Mann *et al.*, 1986).

The bronchoconstrictor effect of adenosine appears to be mediated by specific receptors, since neither inosine, an adenosine metabolite, nor guanosine are active. Adenosine monophosphate (AMP) and adenosine diphosphate (ADP), both of which are degraded in the body to form adenosine, are as effective as adenosine as bronchoconstrictors (Cushley *et al.*, 1983). In addition, the adenosine receptor antagonist theophylline inhibits adenosine-induced bronchoconstriction (Cushley *et al.*, 1984). Indeed, it has been suggested (Holgate *et al.*, 1987) that the bronchodilator effect of theophylline is due to antagonism of endogenous adenosine. There are several possible mechanisms whereby adenosine may cause bronchoconstriction. For example, the purine

may increase release of mast cell mediators (Church et al., 1985; Church & Hughes, 1985). Alternatively, adenosine may have a direct effect on the airway smooth muscle of asthmatics. The latter possibility, however, has been difficult to test due to a lack of animal models. In fact, canine (Krzanowski et al., 1987), feline (Ito & Takeda, 1982) and guinea-pig (Brown & Collis, 1982) airway smooth muscles relax in response to adenosine. In guinea-pig trachea, Holroyde (1986) demonstrated that, following epithelium removal, low concentrations of adenosine elicited contractions, but this could not be confirmed by others (Farmer et al., 1986).

There are a few reports of adenosine inducing contraction of airway smooth muscle. Finney et al. (1985) demonstrated that adenosine and ATP contract human isolated bronchiolar smooth muscle, and Pauwels & Van der Straeten (1987) recently showed that adenosine analogues cause bronchoconstriction in anaesthetized rats. Fredholm et al. (1979) and Advenier et al. (1982) found that adenosine contracts guinea-pig tracheae with low basal tone, but relaxes those which have been precontracted. The relaxation of guinea-pig trachea to adenosine may be mediated by A_2-adenosine receptors (Brown & Collis, 1982). Caparrotta et al. (1984) found that the stable adenosine analogue, R-phenylisopropyladenosine (R-PIA), at concentrations lower than 1 μM, elicited a contractile response in guinea-pig trachea, an effect which was blocked by indomethacin. Since R-PIA is relatively selective for adenosine A_1-receptors (Daly, 1982; Burnstock & Buckley, 1985), the contractile response may be mediated by this receptor subtype.

The purpose of the present study was to examine the effect of several adenosine analogues on guinea-pig isolated trachea. In addition, we have tested several agents for their ability to inhibit the responses.

Methods

Male, Duncan-Hartley guinea-pigs (300–400 g; Hazelton, Denver, PA) were stunned, exsanguinated and the trachea removed and placed in modified Krebs-Henseleit solution (composition mM: NaCl 113, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, glucose 5.5). Following removal of fat and connective tissue, the trachea was slit open along its longitudinal axis. Transverse strips consisting of two adjacent cartilage rings were prepared and suspended in 10 ml organ chambers containing Krebs solution at 37°C and gassed with 95% O_2 and 5% CO_2. Preparations were connected to Gould force-displacement transducers for measurement of isometric tension responses, which were displayed on a Gould RS3800 chart recorder. Before each experiment the tissues were equilibrated for 60 min under a resting tension of 1 g, and washed with fresh solution every 15 min.

To ascertain tissue viability, a contraction to acetyl-β-methylcholine (MCh, 2 μM) was obtained before each experiment. Following washout, and recovery of basal tone, increasing concentrations of adenosine analogues were added cumulatively to the bath. Since responses to these agonists are slow to develop, only one concentration-response curve was obtained from each tissue. The results are expressed as a percentage of the maximum contraction or relaxation to each agonist, with pD_2 values ($-\log EC_{50}$) determined from linear regression of the concentration-response curves.

Antagonists were added to the bath 30 min before the addition of agonists. Although only one antagonist concentration was examined in each preparation, the effects of different concentrations of antagonists were tested in different tissues from the same animal. The pA_2 values for antagonists were calculated as described by Arunlakshana & Schild (1959). Schild plot linearity and slope were determined from regression analysis, and pA_2 values obtained from the intercept on the abscissa scale.

Since the antagonists caused tracheal relaxation, we examined the concentration-dependence of this phenomenon in tissues with basal tone, and in tissues precontracted with MCh (0.1 μM). When the maximum effect was attained, sodium nitroprusside (SNP, 30 μM) was added to the bath to relax the tissue maximally. The relaxant effect of each antagonist was expressed as a pD_2 value (the $-\log$ of the concentration of drug producing 50% of the maximum relaxation to SNP). Data are expressed as mean $\pm$ s.e.mean of n determinations.

Drugs

MCh (Sigma) and SNP (Sigma) were each dissolved in 0.9% w/v NaCl solution (saline), while dipyridamole (Sigma) was prepared as a 20 mM stock solution in absolute ethanol. The following adenosine receptor agonists were dissolved as 25 mM stock solutions in acidified saline: adenosine hemisulphate (Sigma), 5'-N-ethylcarboxamidoadenosine (NECA, Research Biochemicals Inc.), 5'-N-methylcarboxamidoadenosine (MECA, Research Biochemicals Inc.), 2-chloroadenosine (CADO, Research Biochemicals Inc.), the R(−)- and S(+)-enantiomers of N^6-(2-phenylisopropyl)adenosine (R-PIA and S-PIA respectively, Research Biochemicals Inc.), and N^6-cyclohexyladenosine (CHA, Research Biochemicals Inc.). Enprofylline (Research Biochemicals Inc.), 8-phenyltheophylline (8-PT, Calbiochem), aminophylline (Sigma) and NPC205 (1,3-di-*n*-propyl-8-(4-

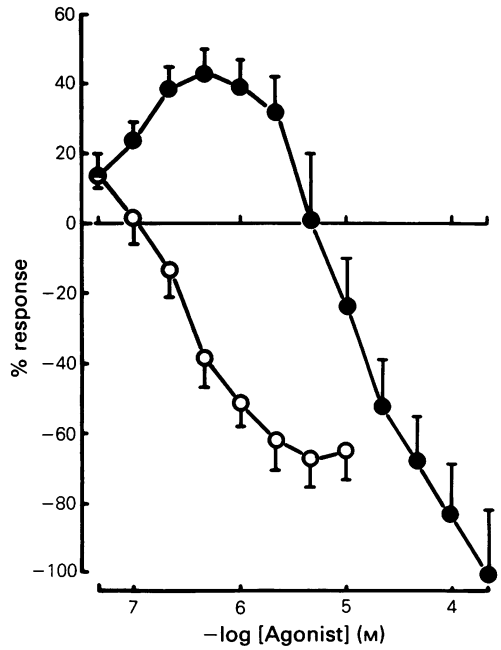

Figure 1 Concentration-response curves for 5'-N-ethylcarboxamidoadenosine (NECA; ○)and R(−)-N^6-(2-phenylisopropyl)adenosine (R-PIA; ●) in guinea-pig isolated trachea. Responses are expressed as a % of a reference contractile response to methacholine (2 μM).

hydroxyphenyl)xanthine, synthesized at Nova) were prepared freshly as 1 mM stock solutions in water containing 4% ethanol and 2% 1 N KOH. The adenosine antagonist CGS15943A (a 2-furanyltri-

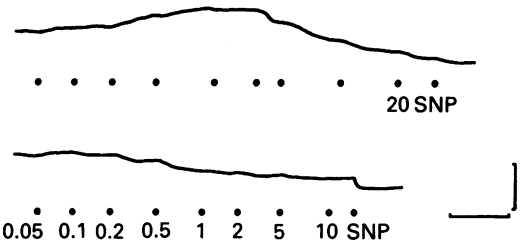

Figure 2 Representative tracing of responses of guinea-pig isolated trachea to R(−)-N^6-(2-phenylisopropyl)adenosine (R-PIA, top) and 5'-N-ethylcarboxamidoadenosine (NECA, bottom). Dots indicate concentrations (μM) of R-PIA or NECA in the bath, and SNP indicates the addition of sodium nitroprusside (30 μM) at the end of the experiment. Calibration lines represent 10 min and 1 g.

azoloquinazoline derivative, kindly supplied by Dr M. Williams, Ciba-Geigy Corporation, New Jersey, for structure, see Williams *et al.*, 1987) was dissolved in DMSO as a 20 mM stock solution.

Results

Dual effect of adenosine analogues

Depending on the particular adenosine analogue, and its concentration, qualitatively different tracheal responses were elicited (Figure 1). At concentrations of less than 2 μM, R-PIA produced relatively weak (40% of the response to MCh) contractions (Table 1), whereas at higher concentrations, it caused relaxation, the maximal response being similar to that produced by SNP (30 μM). In these experiments, R-PIA was over 300 times more potent in causing

Table 1 Effects of adenosine analogues on guinea-pig tracheal smooth muscle

	Relaxation		*Contraction*	
Analogue	pD_2	*Max**	pD_2	*Max**
Adenosine	3.69 ± 0.09	556 ± 75 (9)	N/A	N/A
Adenosine + DP[1]	4.43 ± 0.24	476 ± 93 (6)	N/A	N/A
NECA	6.37 ± 0.08	350 ± 100 (6)	N/A	N/A
MECA	4.60 ± 0.26	400 ± 58 (6)	N/A	N/A
R-PIA	4.62 ± 0.06	518 ± 41 (9)	7.12 ± 0.12	160 ± 22 (10)
S-PIA	4.08 ± 0.03	297 ± 54 (6)	6.01 ± 0.06	175 ± 42 (7)
CADO	5.24 ± 0.12	660 ± 140 (6)	6.79 ± 0.08	80 ± 21 (5)
CHA	4.41 ± 0.12	440 ± 79 (5)	6.98 ± 0.09	219 ± 79 (6)

Responses to each analogue were determined in preparations with basal tone.
* Denotes maximum relaxant or contractile response in mg.
[1] Denotes adenosine in the presence of dipyridamole (DP, 0.5 μM).
N/A denotes no contraction was elicited.
Numbers in parentheses are number of observations. Abbreviations used: NECA, 5'-N-ethylcarboxamidoadenosine; MECA, 5'-N-methylcarboxamidoadenosine; R-PIA, R(−)-N^6-(2-phenylisopropyl)adenosine; CADO, 2-chloroadenosine; CHA, N^6-cyclohexyladenosine.

contractions than relaxations (Table 1). Qualitatively similar data were obtained with CHA, CADO and S-PIA (Table 1), with these agonists producing contractions at lower concentrations, but relaxations at higher ones.

Adenosine itself generally elicited only relaxations, though in one preparation a small contraction (60 mg) occurred at a concentration of 1 μM. Adenosine was nearly 9 fold less potent than **R**-PIA in causing relaxation, though the maximum relaxation was similar to that produced by SNP (Table 1). In the presence of dipyridamole (0.5 μM), the concentration-response curve for adenosine was shifted to the left such that it became approximately equipotent to **R**-PIA as a relaxant (Table 1). Dipyridamole did not affect the maximum response to adenosine and no contractions were seen at any concentration of the purine.

The major response to NECA was relaxation (Figure 1), although two preparations contracted at the lowest concentration (0.01 μM). NECA was around sixty times more potent a tracheal relaxant than **R**-PIA (Table 1), but it did not produce as great a maximum relaxation. The only response elicited by MECA was relaxation, with its potency in this response being similar to **R**-PIA, and approximately sixty times less than NECA (Table 1). Figure 2 shows typical responses of guinea-pig trachea to **R**-PIA and NECA.

Agonist potency-ratios

The adenosine analogues exhibited the following rank order of potency for inducing tracheal relaxation: NECA > CADO > **R**-PIA = MECA = adenosine + dipyridamole > **S**-PIA > adenosine. The rank order of potency for inducing contractions was **R**-PIA > CHA > CADO > **S**-PIA (Table 1).

Effects of antagonists

Table 2 summarizes the effect of antagonists on **R**-PIA-induced contractions, and it can be seen that while enprofylline was without effect, NPC205 and CGS15943A were both potent antagonists. Whereas NPC205 (Figure 3) and 8-PT were competitive antagonists of the **R**-PIA-induced contractions, CGS 15943A was non-competitive, since the Schild plot had a slope greater than 1 (Table 2). In addition, CGS15943A, at a concentration of 1 μM, reduced the maximum response to **R**-PIA from 159 ± 13 mg (n = 10) to 72 ± 18 mg (n = 4). Because of its limited solubility, it was not possible to study CGS15943A at concentrations > 1 μM. It was not possible to obtain a Schild plot for aminophylline since, at the concentrations used, 0.1 μM, 1 μM and 10 μM, only the latter concentration produced a dextral shift in the

Table 2 Effects of antagonists on **R**(−)-N^6-(2-phenylisopropyl)adenosine (**R**-PIA)-induced contractions of guinea-pig isolated tracheal smooth muscle.

Drug	pA_2	*Schild plot slope*
Enprofylline	no effect	—
8-PT	6.46 ± 0.19 (5)	−0.77 ± 0.14
Aminophylline	5.45 ± 0.08 (5)*	—*
NPC205	7.80 ± 0.38 (5)	−1.02 ± 0.34
CGS15943A	7.00 ± 0.08 (4)	−1.83 ± 0.21[1]

[1] Denotes significantly different from unity ($P < 0.05$), as determined by Duncan's Multiple Range Test.
* pA_2 was estimated from the dextral shift, produced by aminophylline (10 μM), in the **R**-PIA concentration-response curve. See text for explanation. Numbers in parentheses are number of observations. 8-PT, 8-phenyltheophylline.

R-PIA concentration-response curve. However, aminophylline is a well-documented competitive antagonist at adenosine receptors. In addition, in the present study, aminophylline (10 μM) had no effect on the maximum response to **R**-PIA (control maximum, 159 ± 13 mg (n = 10); in the presence of aminophylline, 152 ± 20 mg, (n = 5)). The pA_2 value (5.45 ± 0.08, n = 5) was estimated from the shift produced (4.2 ± 0.5 fold) in the **R**-PIA curve by 10 μM aminophylline.

We also examined the effects of NPC205 and CGS15943A on responses to NECA, the most potent relaxant. NPC205, with a pA_2 of 6.68 ± 0.06 (n = 5), was over 13 times less potent as an antagonist of NECA-induced relaxation than of **R**-PIA-induced contractions (Table 2). Effects of NPC205 on NECA-induced relaxation were often difficult to assess, since the xanthine derivative itself caused tracheal relaxation. Indeed, the maximal relaxation to NECA in controls (574 ± 55 mg) was reduced to 452 ± 23 mg, 430 ± 34 mg and 256 ± 30 mg, respectively, in the presence of 0.1 μM, 1 μM and 10 μM NPC205. When the NPC205-induced relaxation was taken into account, however, the maximum relaxation to NECA, from original baseline, was unchanged (data not shown). Antagonism of the relaxant effect of NECA by NPC205 was competitive, since the Schild plot was linear, with a slope of −0.76 ± 0.15 (n = 4), a value which is not significantly different from unity.

CGS15943A was as potent as NPC205 as a NECA antagonist, displaying a pA_2 value of 6.79 ± 0.15 (n = 6). However, CGS15943A was approximately equipotent as an antagonist of NECA-induced relaxation and of **R**-PIA-induced contractions. Antagonism of NECA by CGS15943A

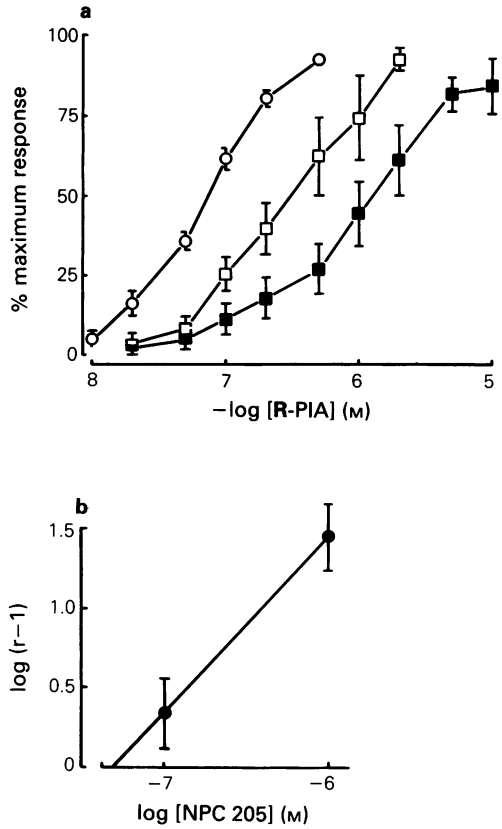

Figure 3 (a) Concentration-response curves for the contractile effect of R(−)-N^6-(2-phenylisopropyl)adenosine (R-PIA) on guinea-pig isolated trachea. (○), Control; (□) in presence of 0.1 μM NPC205, and (■) 1 μM NPC205. Each point represents the mean of 5 observations and vertical lines indicate s.e.mean. (b) Schild plot for NPC205. Concentration-ratios (r) were calculated from the mean EC_{50} values for R-PIA in the presence and absence of NPC205. Slope (−1.02 ± 0.34) and pA_2 (7.80 ± 0.38) were determined from regression analyses.

was competitive since the slope of the Schild plot (−0.91 ± 0.21, $n = 5$) was not different from unity. Moreover, CGS15943A was without effect on the maximum response (control maximum, 493 ± 67 mg, $n = 6$; in the presence of 1 μM CGS 15943A, 483 ± 115 mg, $n = 6$).

Further experiments were undertaken to examine the antagonist-induced relaxations, with the results indicating that NPC205 was the most potent agent in this regard, being some twenty times more potent than aminophylline (Table 3). The rank order of potency in preparations with basal tone was NPC205 > 8-PT > enprofylline > aminophylline.

Table 3 Direct effects of antagonists on guinea-pig tracheal tone

Drug	*Relaxation (basal)** pD_2	*Relaxation (MCh)** pD_2
Enprofylline	4.86 ± 0.09 (5)	4.77 ± 0.08 (5)
8-PT	5.19 ± 0.17 (5)	4.99 ± 0.10 (6)
Aminophylline	4.43 ± 0.12 (4)	4.32 ± 0.09 (4)
NPC205	5.76 ± 0.08 (7)	4.89 ± 0.06 (6)

Data are expressed as the −log concentration of each drug required to elicit 50% (pD_2) of the tracheal maximum relaxation to sodium nitroprusside (30 μM).
* Relaxations were obtained in preparations with basal tone or which were precontracted with methacholine (MCh, 0.1 μM).
Numbers in parentheses are number of observations. 8-PT, 8-phenyltheophylline.

In preparations precontracted with MCh (0.1 μM), the rank order of potency was similar, NPC205 = 8-PT > enprofylline > aminophylline (Table 3). It is interesting that, whereas 8-PT, enprofylline and aminophylline were as potent relaxants of tracheae with basal tone as they were of precontracted tracheae, NPC205 was five times more potent a relaxant of tissues with basal tone (Table 3). CGS15943A caused small tracheal relaxations, but EC_{50} values could not be determined since, as described, this agent was insoluble in Krebs solution at concentrations greater than 1 μM. At a concentration of 1 μM, CGS15943A caused relaxations of approximately 25% of the maximum response to SNP.

Discussion

Tracheal relaxation to adenosine analogues

In guinea-pig trachea, Brown & Collis (1982) proposed that the adenosine-induced relaxation is mediated by A_2-receptors. This conclusion was based on the finding that the rank order of potency of a series of adenosine analogues was 5'-N-cyclopropylcarboxamidoadenosine (NCPCA) > NECA > CADO > R-PIA > adenosine > S-PIA, and is clearly supportive for the tracheal relaxation being mediated by A_2-receptors (Daly, 1982; Burnstock & Buckley, 1985). Furthermore, there was relatively little stereoselectivity for the enantiomeres R-PIA and S-PIA (Brown & Collis, 1982), again suggesting an A_2-receptor effect (Burnstock & Buckley, 1985). In the present study, the rank order of potency for causing relaxation was similar with NECA > CADO > R-PIA = MECA > S-PIA > adenosine. Both studies demonstrated that dipyridamole, an

inhibitor of adenosine uptake (Daly, 1982), potentiates the relaxant effect of adenosine, indicating an action on cell surface receptors. The other analogues are not subject to the uptake mechanism, and are therefore unaffected by dipyridamole (Brown & Collis, 1982). In the present study, like that of Brown & Collis, R-PIA was only 3.5 fold more potent a tracheal relaxant than S-PIA. This relative lack of stereoselectivity and the relative potencies of the analogues (Burnstock & Buckley, 1985) further support the hypothesis that adenosine-induced tracheal relaxation is mediated by A_2-receptors.

Bruns and colleagues (1986), utilizing receptor-ligand binding studies in rat striatum, showed MECA to be approximately seven times less potent than NECA at the A_2-receptor. In the present study MECA was sixty times less potent than NECA, but approximately equipotent with R-PIA as a tracheal relaxant (A_2). MECA, however, unlike R-PIA, did not cause contraction (A_1). This suggests that MECA, although A_2-selective, is not a particularly potent agonist at the tracheal A_2-receptor.

Tracheal contraction to adenosine analogues

In most studies of the relaxant actions of bronchodilator agents, concentration-response curves are usually obtained when the tone has been raised by an excitatory agonist (although this may be unnecessary since guinea-pig trachea has inherent tone). This may explain why many investigators fail to observe the weak, purine-induced contraction. Caparrotta *et al.* (1984) were the first to show that R-PIA has a dual effect on guinea-pig trachea. At concentrations greater than 1 μM, R-PIA caused relaxation, but at less than 1 μM, a slow, well-maintained contractile response was observed. This contraction was inhibited by indomethacin, suggesting that it is mediated by cyclo-oxygenase metabolites of arachidonate. When the preparation was precontracted with carbachol (0.5 μM), R-PIA no longer caused contraction. These results support the suggestion that others may not have observed adenosine-induced contractions because the tracheal preparations had been precontracted. Recently, the studies of Caparrotta *et al.* (1984) have been extended to show that other adenosine analogues contract the guinea-pig trachea by a mechanism involving neither acetylcholine, noradrenaline nor histamine (Ghai *et al.*, 1987). However, in neither of these studies was the effect of adenosine itself examined. The rank order of potency for producing contraction was R-PIA > CADO = N^6-cyclopentyladenosine (CPA) = CHA. Stereoselectivity in the contraction to PIA could not be assessed since S-PIA (as well as CHA and CPA) elicited very small responses (Ghai *et al.*, 1987).

In the present study the rank order of potency for contractions was R-PIA > CHA > CADO > S-PIA. In contrast to the study by Ghai *et al.* (1987), we found R-PIA to be nearly 13 fold more potent than S-PIA, although the maximum responses to the enantiomers were similar (Table 1). The relative potencies of the analogues and the stereoselectivity for the PIA enantiomers suggests (Burnstock & Buckley, 1985) that there are A_1-receptors on guinea-pig tracheal smooth muscle which mediate contraction. This assertion is strengthened by the observation that NPC205, which has previously been shown to be A_1-selective (Schwabe *et al.*, 1985; Kaplita *et al.*, 1986), was more potent as an antagonist of R-PIA-induced contractions than of NECA-induced relaxations.

It is puzzling that adenosine itself does not consistently cause contraction, although it is a reliable relaxant. It may be that the response to adenosine reflects a balance between A_2-mediated relaxation and A_1-mediated contraction. Where contractions have been obtained (Coleman & Levy, 1974; Farmer & Farrar, 1976; Kamikawa & Shimo, 1976; Caparrotta *et al.*, 1984), they occurred at lower concentrations than required for relaxations. This may reflect the fact that A_1-receptors have a higher affinity for adenosine than do A_2-receptors (Daly, 1982). It would be interesting to determine whether adenosine-induced relaxation is converted to contraction in the presence of an, as yet unavailable, A_2-selective antagonist.

Ghai and colleagues did not observe relaxations to R-PIA, probably because they did not examine concentrations greater than 1 μM (see Figure 3, Ghai *et al.*, 1987). They did, however, note that CADO elicited a contraction at lower concentrations ($\leqslant 0.2\,\mu$M) and relaxation at higher concentrations. Responses to CADO were inhibited in a concentration-dependent manner by the A_1-receptor antagonist 1,3-dipropyl-8-(2-amino-4-chlorophenyl) xanthine (PACPX). These authors also showed that contractions to CADO were not affected by the less selective and less potent adenosine antagonist theophylline (1 and 3 μM). In the present study, the pA_2 value for aminophylline (the ethylene diamine salt of theophylline) was estimated to be 5.45, indicating that 3.6 μM aminophylline is required to produce only a 2 fold dextral shift in the R-PIA concentration-response curve. This contrasts with pA_2 values of 6.46 for 8-PT, and 7.80 for NPC205, demonstrating that these drugs are 10 and 220 fold more potent, respectively, than aminophylline. The present results are similar to those of Caparrotta *et al.* (1984), who demonstrated that theophylline (20 μM) and 8-PT (5 μM) caused rightward shifts in the concentration-response curve to R-PIA. They also showed that, in the presence of these antago-

nists, the magnitude of the contraction to R-PIA was enhanced, whereas the relaxation component was decreased. Nevertheless, as we and others (Fredholm *et al.*, 1979; Kolbeck *et al.*, 1979; Persson & Gustafsson, 1986) have shown, xanthines themselves cause tracheal relaxation. Therefore, the enhanced maximum contractile response to R-PIA (Caparrotta *et al.*, 1984) may simply have been due to the fact that, in the presence of theophylline or 8-PT, the tracheal tone was reduced. A reduction in basal tone produced by the xanthines could also explain their observation that the maximum relaxation to R-PIA was decreased. Indeed, in the present study, the NPC205-induced relaxation resulted in an apparent reduction in the maximum relaxation produced by NECA.

The potency of CGS15943A as an R-PIA antagonist was similar to that of the A_1-selective antagonist NPC205. This is surprising since, in radioligand binding studies with brain tissue, Williams *et al.* (1987) found CGS 15943A to be approximately 7 times more potent at A_2-receptors than at A_1-receptors. However, the drug, though a competitive A_1 antagonist, was non-competitive at A_2-receptors. In contrast, in the present study, CGS15943A was a non-competitive A_1 antagonist (Schild plot slope, -1.83), but a competitive A_2 antagonist (Schild plot slope, -0.91). At a concentration of $1\,\mu M$, CGS15943A caused an approximately 50% reduction of the maximal response to R-PIA. Thus, although potent, with an apparent pA_2 of 7.00, this drug is not a competitive antagonist of R-PIA-induced contraction. Furthermore, CGS 15943A, like NPC205, was a slightly more potent antagonist of R-PIA-induced contractions (A_1) than of NECA-induced relaxations (A_2). Again, this is in contrast to binding studies where CGS15943A was shown to be A_2-selective (Williams *et al.*, 1987).

Direct effects of antagonists

Recently, the role of adenosine as a mediator of bronchial asthma has been questioned (Persson *et al.*, 1986). Doubts are based largely on the fact that enprofylline, which does not appear to be an adenosine antagonist (Persson *et al.*, 1981), and does not antagonize adenosine-induced bronchoconstriction in asthmatics, is approximately five times more potent as a bronchodilator *in vitro* and *in vivo* than theophylline (see references in Persson *et al.*, 1986). Theophylline, however, is a much more potent adenosine antagonist than enprofylline (Persson *et al.*, 1981). Similarly, in the present study, enprofylline was approximately 3 fold more potent than aminophylline as a tracheal relaxant. Conversely, aminophylline antagonized the contraction to R-PIA, whereas enprofylline was without effect. This is, perhaps, further evidence that the bronchodilatation produced by alkylxanthines in man may be due to a direct effect(s) on airway smooth muscle rather than to adenosine antagonism.

There was a weak correlation between the potencies as antagonists of R-PIA-induced contraction and potencies as relaxants. Thus, both as R-PIA antagonists and tracheal relaxants, NPC205, 8-PT and aminophylline exhibited the same rank order of potencies. However, whereas enprofylline was about 3 times more potent than aminophylline in relaxing the trachea, the former, unlike the latter, was inactive as an R-PIA antagonist. Assuming these antagonists have the same mechanism of action, it is unlikely that either adenosine antagonism or phosphodiesterase (PDE) inhibition are involved. 8-PT, a relatively potent adenosine antagonist, is not a PDE inhibitor (Smellie *et al.*, 1979; Scotini *et al.*, 1983), whereas enprofylline is not an adenosine antagonist (Persson *et al.*, 1981). From the present study, it is not possible to assess the mechanism of action of the xanthines. However, since the potency of NPC205, but not that of 8-PT, aminophylline or enprofylline, was reduced when the trachea was precontracted (Table 3), it is possible that they cause relaxation by differing mechanisms.

In conclusion, there appear to be two subtypes of adenosine receptor in guinea-pig trachea; A_1- receptors mediating contraction and A_2-receptors, relaxation. The contractile response was inhibited by adenosine antagonists, but not by enprofylline. Since enprofylline was more potent as a tracheal relaxant than aminophylline, this is further evidence that direct effects on airway smooth muscle, rather than adenosine antagonism, explain the clinical usefulness of xanthines as bronchodilators in the treatment of asthma.

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