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# Characterization of the adenosine receptor responsible for the inhibition of histamine and SRS-A release from human lung fragments

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**1** The inhibitory effects of a range of natural and synthetic derivatives of adenosine on the antigen-induced release of histamine and slow reacting substance of anaphylaxis (SRS-A) from human lung has been studied.

**2** The nucleotides ATP, ADP and AMP appear to act by being converted to adenosine.

**3** The rank order of inhibitory potency of the synthetic analogues indicates that these compounds act at an extracellular $A_2/R_a$ purinoceptor.

**4** The xanthines, 1, 3-diethyl-8-phenylxanthine, 8-phenyltheophylline and theophylline antagonized the inhibitory action of N-ethyl-carboxamideadenosine competitively. Theobromine was inactive. This supports the view that the inhibitory receptor is of the A/R type.

**5** Hexobendine and dipyridamole, reported to antagonize the uptake of adenosine, failed to modify the response of human lung fragments to adenosine.

**6** The P site agonist 2′, 5′ dideoxyadenosine inhibited the release of histamine and SRS-A. This effect was not prevented by the inhibitors of uptake, hexobendine and dipyridamole, nor was it antagonized by 8-phenyltheophylline.

## Introduction

Extracellular receptors for adenosine and adenine nucleotides have been divided into two major subtypes (Burnstock, 1978). $P_1$ purinoceptors are more sensitive to adenosine than to ATP and the converse applies to $P_2$ purinoceptors. $P_1$ receptors have been subdivided into $A_1/R_i$ and $A_2/R_a$ subclasses on the basis of their different responses to adenosine and some of its synthetic analogues (Van Calker *et al.*, 1979; Londos *et al.*, 1980). Activation of $A_1/R_i$ receptors leads to inhibition of adenylate cyclase, whereas activation of $A_2/R_a$ receptors to stimulation of adenylate cyclase. At $A_1$ receptors the rank order of agonist potency is L-$N^6$-phenylisopropyladenosine (L-PIA) > adenosine > 5′-N-ethylcarboxamideadenosine (NECA), the reverse has been observed at $A_2$ receptors (Londos *et al.*, 1980). In addition at $A_1$ receptors, L-PIA is about 100 times more potent than D-PIA while at $A_2$ receptors, L-PIA is approximately three times more potent than D-PIA (Daly, 1982). Theophylline and certain other xanthines antagonize competitively the action of adenosine at both $A_1$ and $A_2$ receptors with equal potency (Daly, 1982). In addition, high concentrations of adenosine and ribose-modified analogues such as 2′,5′-dideoxyadenosine (DDA) can inhibit adenylate cyclase by interacting at an intracellular 'P' site (Londos & Wolff, 1977). This action is insensitive to theophylline but is prevented by agents such as hexobendine and dipyridamole which prevent the uptake of adenosine by cells (Daly, 1982).

Adenosine and several of its analogues enhance the release of histamine from rat serosal mast cells and fragments of guinea-pig lung stimulated by antigen or A23187 (Marquardt *et al.*, 1978; Welton & Simko, 1980) and theophylline has been reported to antagonize this enhancement. L-PIA, at concentrations that enhance the release of histamine from rat mast cells also causes a transient rise in the intracellular concentration of cyclic AMP (Holgate *et al.*, 1980). Evidence suggests, therefore, that rat serosal mast cells and perhaps also guinea-pig lung mast cells carry an $A_2/R_a$ receptor.

Although DDA has no effect on antigen-induced release of histamine from guinea-pig lung (Welton & Simko, 1980), DDA does inhibit release from rat mast cells (Holgate *et al.*, 1980). DDA also prevents

the early transient rise in intracellular cyclic AMP that occurs on antigenic stimulation of these cells and this has been taken as evidence that activation of adenylate cyclase is an integral part of the release mechanism (Holgate *et al.*, 1980).

In contrast adenosine inhibits the antigen-induced release of histamine from human basophils (Marone *et al.*, 1979) and human lung mast cells (Schulman *et al.*, 1982). In basophils this inhibition is antagonized by theophylline but not by dipyridamole. Inhibition in basophils is associated with elevation of intracellular cyclic AMP and it has been concluded that adenosine inhibits release by activating adenylate cyclase via interaction at a cell surface receptor for adenosine (Marone *et al.*, 1979).

In this study we show that adenosine and several of its analogues inhibit the antigen-dependent release of histamine and slow-reacting substance of anaphylaxis (SRS-A) from sensitized fragments of human lung and characterize the type of receptor involved.

## Methods

### *Sensitization, challenge and incubation of lung fragments*

Fragments of human lung were prepared, sensitized to and challenged with antigen according to the method of Butchers *et al.* (1979). Briefly, macroscopically normal lung parenchyma was cut into small pieces (2–3 $mm^3$) and incubated overnight at 20°C in human reaginic serum suitably diluted in bicarbonate-buffered Tyrode solution containing (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, $NaHCO_3$ 11.9, $NaH_2PO_4$ 0.4, D-glucose 5.6, pH 7.4. The fragments were then washed thoroughly, distributed into plastic tubes containing 500 μl Tyrode solution and incubated at 37°C for 15 min. Aqueous solutions of compounds (10 μl) or vehicle controls (10 μl) were added 10 min before challenge unless stated otherwise in the results. Challenge was with 50 μl of antigen solution (dialysed) extracts of $B_2$ grass pollen, cat fur or *Dermatophagoides pteronyssinus* (Bencard) at an experimentally determined dilution calculated to release not more than 40% of the total histamine of the fragment. Each incubation was performed at least in triplicate. Fifteen minutes after challenge the diffusates were removed from the lung fragments and assayed for released histamine and SRS-A. Distilled water (1.0 ml) was added to the residual fragments and boiled for 2 min after which the supernatants were assayed for histamine.

Unless otherwise stated all incubations contained hexobendine (5 μM) to prevent uptake of adenosine by cells (Huang & Daly, 1974). In experiments where xanthines were used as potential antagonists, the lung fragments were incubated with antagonists for 30 min at 37°C before addition of the agonist. Incubation was then continued for a further 10 min before challenge with antigen.

### *Assay of histamine and SRS-A*

Both histamine and SRS-A were assayed on guinea-pig isolated ileum using an automated superfusion technique and authentic standards of histamine and leukotriene $D_4$ ($LTD_4$). Samples were pumped onto the ileum using an autoanalyser manifold designed to dilute all samples by a factor of 20. For the assay of histamine the superfusion Tyrode solution was supplemented with atropine (0.3 μM) and FPL 55712 (sodium 7-[3 (4 - acetyl - 3 - hydroxy - 2 propylphenoxy) - 2 - hydroxy propoxy] - 4 oxo - 8 - propyl - 4H - 1 - benzopyran - 2 - carboxylate, 1 μM), for the assay of SRS-A the solution was supplemented with atropine (0.3 μM) and promethazine (1 μM). None of the experimental compounds, at any of the concentrations used, interfered with these assays.

### *Treatment of results*

Inhibition of the release of histamine was expressed as:

$$\text{percentage inhibition} = \frac{(a-b)}{a} \times 100$$

where a = percentage release of histamine in compound free controls, b = percentage release of histamine in treated samples.

Both a and b were corrected for spontaneous release of histamine. SRS-A released on challenge is synthesized *de novo* and is not released from intracellular stores. Accordingly, the release of SRS-A was related to the total histamine present in the lung fragment and expressed as $LTD_4$ equivalents released per unit total histamine.

Log concentration – effect curves were constructed for each compound and potencies calculated by least squares regression analysis using the linear portion of the curve. Agonist potencies were expressed as the negative logarithm of the molar concentration causing half maximal inhibition of the release of spasmogen ($pD_2$). Concentration-ratios, the ratio of the concentrations required to produce a given response in the presence and absence of antagonist, were calculated using $EC_{50}$ values obtained by regression analysis and $pA_2$ values were calculated for 8-phenyltheophylline (8-PT) and 1,3-diethyl-8-phenylxanthine (DPX) by the method of Arunlakshana & Schild (1959) using three concentrations of antagonist. For theophylline an approximate $pA_2$ value was estimated from the concentration-ratios observed at a single concentration (50 μM).

**Table 1** Inhibition of antigen induced histamine release from fragments of human lung by adenosine and adenine nucleotides

| | *Inhibition of histamine release* (%) | | | | |
|---|---|---|---|---|---|
| *Agonist* [μM] | 1 (*n*) | 10 (*n*) | 100 (*n*) | 1000 (*n*) | $pD_2$ |
| Adenosine | −0.7 ± 4.9 (17) | 8.5 ± 3.4 (21) | 48.6 ± 5.2 (21) | 70.2 ± 5.5 (14) | 4.16 ± 0.08 |
| AMP | 20.5 ± 13.5 (2) | 4.3 ± 5.9 (4) | 43.5 ± 11.4 (4) | 68.1 ± 14.6 (4) | 4.07 ± 0.08 |
| ADP | −3.4 ± 6.4 (3) | 12.3 ± 6.3 (4) | 51.4 ± 7.4 (4) | 89.6 ± 3.9 (4) | 4.17 ± 0.12 |
| ATP | 2.6 ± 6.0 (6) | −6.8 ± 13.9 (7) | 48.0 ± 8.3 (7) | 76.4 ± 11.8 (5) | 4.23 ± 0.19 |

Data are expressed as the means ± s.e.mean of (*n*) separate experiments each performed in triplicate.

**Table 2** Inhibition of SRS-A release from fragments of human lung by adenosine and adenine nucleotides

| *Agonist* [μM] | 1 (*n*) | 10 (*n*) | 100 (*n*) | 1000 (*n*) | $pD_2$ |
|---|---|---|---|---|---|
| Adenosine | −1.8 ± 6.3 (13) | 12.6 ± 8.6 (16) | 68.0 ± 5.2 (16) | 92.0 ± 4.5 (11) | 4.18 ± 0.09 |
| AMP | 26.2 ± 1.8 (2) | 12.0 ± 23.0 (2) | 74.3 ± 12.7 (2) | 89.9 ± 10.1 (2) | 4.50 ± 0.34 |
| ADP | −10.4 ± 13.9 (3) | −3.5 ± 2.1 (3) | 67.7 ± 11.9 (3) | 70.2 ± 4.1 (3) | 3.98 ± 0.06 |
| ATP | −0.4 ± 4.6 (6) | 20.8 ± 13.3 (6) | 59.5 ± 11.5 (6) | 64.2 ± 16.2 (3) | 4.42 ± 0.26 |

Data are expressed as the mean ± s.e. mean of (*n*) separate experiments each performed in triplicate.

*Compounds*

Compounds investigated as agonists were: adenosine, 2-chloroadenosine, ATP, ADP, AMP, inosine (all Sigma Chemical Company, London); L-PIA (Boehringer Mannheim), D-PIA, 5'N-cyclopropylcarboxamideadenosine, (NCPCA, both synthesized by Dr. C. Wallis, Glaxo Group Research Ltd., Ware), NECA (Dr. D. I. C. Scopes, Glaxo Group Research , Ware) α, β-methylene ATP and 2',5'dideoxyadenosine (DDA, both P-L Biochemicals Ltd.).

Theobromine, theophylline (Sigma), 8-phenyltheophylline (8-PT, Calbiochem Behring Corp.), 1,3-diethyl-8-phenylxanthine, (DPX, Dr. C Wallis) were tested as antagonists. Dipyridamole (Sigma) and hexobendine (Oesterreichische Stickstoffwerke) were used as uptake blockers. Adenosine deaminase was obtained from Boehringer Mannheim and promethazine from May & Baker Ltd. All compounds were used in fresh aqueous solution.

## Results

*Inhibition of the release of histamine and SRS-A by adenosine analogues*

Adenosine, AMP, ADP and ATP, when preincubated with human lung fragments for 10 min before the addition of antigen, all inhibited the release of histamine and SRS-A in a concentration-dependent manner and with similar potency (Tables 1 and 2). However, if adenosine deaminase (5 u $ml^{-1}$) was included in the incubation then the potency of all four compounds was greatly reduced. For example, deaminase reduced the inhibition caused by 100 μM ATP from 57.0 ± 6.7% to −1.2 ± 1.5% ($n = 3$). In addition, the non-selective A/R purine receptor antagonist 8-PT (10 μM) reduced the inhibition caused by 100 μM ATP from 53.2 ± 12.6% to 2.3 ± 7.5% ($n = 3$). The analogue of ATP, α,β-methylene ATP which is resistant to hydrolysis failed to inhibit the release of histamine giving 0.5 ± 13.5% ($n = 6$) enhancement of release at 300 μM.

A range of synthetic purine nucleosides structurally related to adenosine inhibited the release of histamine and SRS-A in a concentration-dependent manner when preincubated with sensitized lung for 10 min before the addition of antigen (Figures 1 and 2). The rank order of inhibitory potency was: NECA = NCPCA > 2-chloroadenosine > adenosine > L-PIA > D-PIA for inhibition of the release of histamine and NCPCA > NECA > 2-chloroadenosine > adenosine > L-PIA > D-PIA for inhibition of the release of SRS-A (Table 3). The 5'-substituted analogues NECA and NCPCA were much more potent than the $N^6$-substituted analogues L-PIA and D-PIA and there was little difference in potency between L-PIA and D-PIA. Inosine was ineffective at concentrations up to 1000 μM (Table 3).

Although most nucleosides were approximately equipotent as inhibitors of the release of histamine and SRS-A, the two most potent compounds NECA and NCPCA were two to four times more potent as

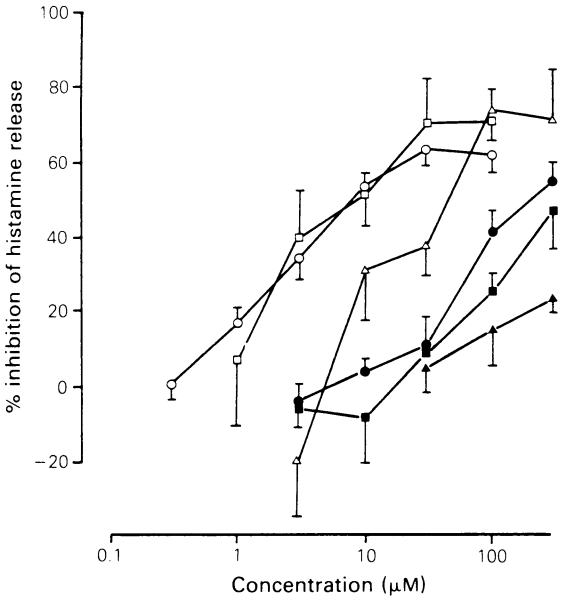


**Figure 1** Inhibition of histamine release by adenosine and analogues: relative potency. Data are expressed as the mean of (*n*) separate experiments each performed in triplicate; vertical lines indicate s.e.mean. (○) 5′-N-ethylcarboxamideadenosine (NECA) (35); (□) 5′-N-cyclopropylcarboxamideadenosine (NCPCA) (3); (△) 2-chloroadenosine (2 ClADO) (3); (●) adenosine (15); (■) L-$N^6$-phenylisopropyladenosine (L-PIA) (10); (▲) D-PIA (4).

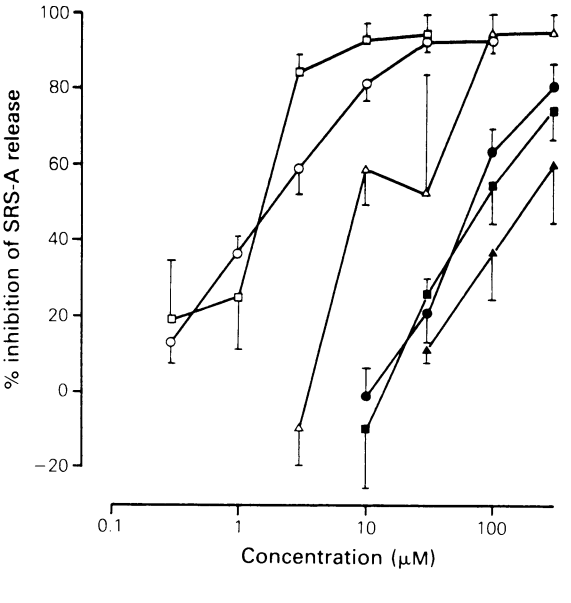


**Figure 2** Inhibition of SRS-A release by adenosine and analogues: relative potency. Data are expressed as the mean of (*n*) separate experiments each performed in triplicate; vertical lines indicate s.e.mean. (○) NECA (28); (□) NCPCA (3): (△) 2 ClADO (3); (●) adenosine (11); (■) L-PIA (8); (▲) D-PIA (5). For abbreviations, see legend to Figure 1.

inhibitors of SRS-A generation than as inhibitors of histamine release (Table 3).

In experiments where the length of preincubation was varied it was found that addition of NECA (1–100 μM) at the same time as the antigen produced inhibition of release but that there was a tendency towards greater inhibition as the time of preincubation was increased (Table 4).

**Table 3** Inhibition of antigen-induced release of histamine and SRS-A from fragments of human lung by adenosine and analogues (relative potency)

| | | *Histamine* | | | | *SRS-A* | | |
|---|---|---|---|---|---|---|---|---|
| *Agonist* | $pD_2$ | *Slope* | (*n*) | *EC* | $pD_2$ | *Slope* | (*n*) | *EC* |
| NECA | 5.40 ± 0.09 | 42.6 ± 3.3 | (35) | 1.0 | 5.80 ± 0.09 | 54.2 ± 4.3 | (28) | 1.0 |
| NCPCA | 5.40 ± 0.15 | 43.4 ± 15.3 | (3) | 1.0 | 5.92 ± 0.06 | 55.7 ± 9.2 | (3) | 0.8 |
| 2 ClADO | 4.71 ± 0.17 | 57.3 ± 12.3 | (3) | 4.9 | 4.80 ± 0.12 | 55.5 ± 6.8 | (3) | 10 |
| Adenosine | 4.16 ± 0.08 | 44.4 ± 4.7 | (15) | 17.4 | 4.18 ± 0.09 | 63.6 ± 7.5 | (11) | 41.7 |
| L-PIA | 3.87 ± 0.11 | 44.1 ± 9.7 | (10) | 33.9 | 4.09 ± 0.17 | 48.1 ± 6.1 | (8) | 51.3 |
| D-PIA | 3.44 ± 0.17 | 50.0 ± 12.3 | (4) | 91.2 | 3.62 ± 0.25 | 51.0 ± 14.5 | (5) | 151.4 |
| Inosine | < 3 | – | (3) | – | < 3 | – | (3) | – |

Data are expressed as the mean ± s.e. mean of (*n*) separate experiments each performed in triplicate.
EC = equipotent concentrations, 5′-N-ethylcarboxamideadenosine (NECA) = 1.0. NCPCA = 5′N-cyclopropylcarboxamideadenosine; 2ClADO = 2-chloroadenosine; L-PIA = L-$N^6$-phenylisopropyladenosine; D-PIA = D-$N^6$-phenylisopropyladenosine.

**Table 4** Inhibition of histamine and SRS-A release by 5'-N-ethylcarboxamideadenosine (NECA): effect of preincubation time

| *NECA concentration* | *Mediator* | *Preincubation time (min)* 0 | 1 | 3 | 10 |
|---|---|---|---|---|---|
| 1 μM | Histamine | −6.1 ± 2.0 | 0.6 ± 13.7 | 3.6 ± 12.7 | 7.0 ± 13.3 |
| | SRS-A | 16.5 ± 17.1 | 29.3 ± 9.4 | 19.6 ± 20.1 | 30.3 ± 4.6 |
| 10 μM | Histamine | 29.9 ± 3.2 | 32.6 ± 7.5 | 38.9 ± 1.7 | 45.9 ± 10.1 |
| | SRS-A | 70.6 ± 6.9 | 65.0 ± 12.0 | 68.3 ± 14.0 | 89.0 ± 11.0 |
| 100 μM | Histamine | 26.8 ± 10.5 | 26.9 ± 4.7 | 21.6 ± 1.9 | 44.0 ± 8.7 |
| | SRS-A | 72.8 ± 2.2 | 94.2 ± 5.9 | 89.3 ± 10.7 | 100 ± 0 |

Data are the means ± s.e.mean of 2 separate experiments each performed in triplicate.
Values are percentage inhibition of release.

Inclusion of the inhibitors of adenosine uptake, hexobendine (5 μM) and dipyridamole (5 μM), failed to modify the inhibitory action of adenosine or of NECA (hexobendine only, Table 5).

*Antagonism of the inhibitory action of NECA by substituted xanthines*

Four substituted xanthines; DPX, 8-PT, theophylline and theobromine were compared as antagonists of the inhibition of release by NECA (Table 6). 8-PT (1–10 μM) produced concentration-related parallel shifts in the concentration-inhibition curves for NECA (Figure 3) and from two separate experiments a $pA_2$ value was calculated (Table 6). DPX (0.3–10 μM) also antagonized the effects of NECA competitively and was about 5 times more potent than 8-PT. Theophylline at a concentration (50 μM) that alone had no stimulatory or inhibitory effects on the release of histamine and SRS-A was a competitive antagonist of NECA. At concentrations above 50 μM theophylline inhibited release in its own right and at concentrations below 50 μM concentration-ratios were too low to be reliable. A $pA_2$ value for theophylline was therefore estimated from the single concentration-ratio available (Table 6). Theobromine (50 μM) did not antagonize NECA.

*Inhibition by dideoxyadenosine*

The P site agonist DDA (30–300 μM) caused concentration-related inhibition of the release of histamine and SRS-A (Figure 4). Neither hexobendine (5 μM) nor dipyridamole (5 μM) modulated this effect (Table 7). The non-selective A/R receptor antagonist 8-PT (10 μM) failed to antagonize inhibition by DDA (Table 7).

## Discussion

In this paper we show that adenosine inhibits antigen-induced release of histamine and SRS-A from sensitized fragments of human lung. Adenosine and its phosphorylated derivatives AMP, ADP and ATP were all of similar potency as inhibitors. This is characteristic neither of actions at the $P_1$ or $P_2$ receptors of Burnstock (1978) nor of actions at the A/R receptor sites of Van Calker *et al.* (1979) and Londos *et al.* (1980). Three lines of evidence suggest that the

**Table 5** Inhibition of histamine and SRS-A release by adenosine and 5'-N-ethylcarboxamideadenosine (NECA): effect of hexobendine (5 μM) and dipyridamole (5 μM)

| *Agonist* | *Mediator* | *pD₂* *Control (n = 4)* | *+ Hexobendine (n = 4)* | *pD₂* *Control (n = 3)* | *+ Dipyridamole (n = 3)* |
|---|---|---|---|---|---|
| Adenosine | Histamine | 4.20 ± 0.15 | 4.12 ± 0.10 | 4.25 ± 0.17 | 4.14 ± 0.10 |
| | SRS-A | 4.23 ± 0.07 | 4.19 ± 0.07 | 4.41 ± 0.05 | 4.47 ± 0.10 |
| NECA | Histamine | 5.69 ± 0.20 | 5.71 ± 0.19 | – | – |
| | SRS-A | 5.67 ± 0.09 | 6.14 ± 0.10 | – | – |

Data are expressed as the mean ± s.e.mean of (*n*) separate experiments each performed in triplicate.

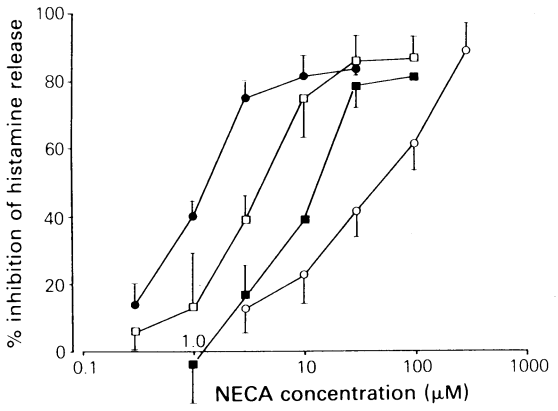


**Figure 3** Inhibition of histamine release by 5'-N-ethylcarboxamideadenosine (NECA): effect of 8-phenyltheophylline. Data are expressed as the mean of a single experiment performed in quadruplicate; vertical lines indicate s.e.mean. (●) Control; (□) 8-phenyltheophylline 1 μM; (■) 8-phenyltheophylline 3 μM; (○) 8-phenyltheophylline 10 μM.

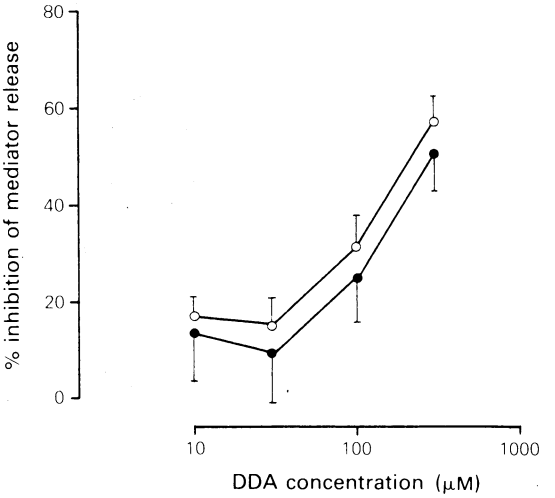


**Figure 4** Inhibition of mediator release from fragments of human lung by 2',5'dideoxyadenosine (DDA). Data are expressed as the mean of (*n*) separate experiments each performed in triplicate; vertical lines indicate s.e.mean. (○) Histamine (6); (●) SRS-A (6).

nucleotides are rapidly converted to adenosine and that the effect of each compound is exerted via adenosine acting at an A/R ($P_1$) receptor. Firstly, adenosine deaminase, which rapidly converts adenosine to inactive inosine (Londos & Wolff, 1977) but which does not deaminate the nucleotides, prevents the inhibitory action of all inhibitors. Secondly, 8-PT, which is a non-selective A/R antagonist (Smellie *et al.*, 1979) also antagonizes the response to ATP. Finally, α,β-methylene ATP, which is resistant to hydrolysis to the nucleoside, yet retains high $P_2$-purinoceptor agonist activity, (Maguire & Satchell, 1981), does not inhibit the release of histamine or SRS-A.

The rank order of inhibitory potency of the adenosine analogues indicate that the inhibitory receptor is of the $A_2/R_a$ subtype (Van Calker *et al.*, 1979; Londos *et al.*, 1980). This classification is based on the differential potency of the 5'-substituted and $N^6$-substituted analogues of adenosine. At $A_2/R_a$ receptors NECA is reported to be 10–50 times more potent than L-PIA whereas the reverse is true for $A_1/R_i$ receptors. The observation that L-PIA is only marginally more active than D-PIA

**Table 6** Antagonism by substituted xanthines of the inhibition of release by 5'-N-ethylcarboxamideadenosine (NECA)

| *Mediator* | | *DPX* | *8-PT* | *Theophylline* | *Theobromine* |
|---|---|---|---|---|---|
| Histamine | $pA_2$ | 6.90 (5.86–7.94) | 6.24 (6.13–6.36) | ~5* | Inactive + |
| | Slope | 1.09 (0.31–1.86) | 1.21 (1.05–1.38) | | |
| | | *n* = 4 | *n* = 2 | *n* = 12 | *n* = 3 |
| SRS-A | $pA_2$ | 7.19 (6.06–8.33) | 6.41 (5.97–6.84) | ~5* | Inactive + |
| | Slope | 0.96 (0.21–1.62) | 1.31 (0.72–1.88) | | |
| | | *n* = 3 | *n* = 2 | *n* = 9 | *n* = 2 |

Data are expressed as the mean of *n* separate experiments each performed in triplicate.
Values in parentheses are 95% confidence limits.
* $pA_2$ estimated from concentration ratios at 50 μM.
+ No antagonistic activity up to 50 μM.
DPX = 1, 3-diethyl-8-phenylxanthine; 8-PT = 8-phenyltheophylline.

**Table 7** Inhibition of the release of histamine and SRS-A by 2',5'dideoxyadenosine (DDA): Effects of hexobendine (5 μM), dipyridamole (5 μM), and 8-phenyltheophylline (8-PT, 10 μM)

| | $pD_2$ | | (n) | $pD_2$ | | (n) | $pD_2$ | | (n) |
|---|---|---|---|---|---|---|---|---|---|
| | *Control* | *Hexobendine* | | *Control* | *Dipyridamole* | | *Control* | *8-PT* | |
| Histamine | 4.12±0.18 | 3.89±0.20 | (3) | 4.16±0.30 | 3.89±0.13 | (3) | 3.99±0.10 | 4.16±0.23 | (4) |
| SRS-A | 3.38±0.21 | 3.51±0.25 | (3) | 4.12±0.39 | 3.87±0.08 | (3) | 3.96±0.02 | 3.98±0.10 | (2) |

Data are expressed as mean ± s.e. mean of (*n*) separate experiments each performed in triplicate.

is further evidence for the involvement of $A_2/R_a$ receptor (Daly, 1982). Interaction with $A_2/R_a$ receptors is associated with the activation of adenylate cyclase (Van Calker *et al.*, 1979) and this is consistent with the observation that other agents acting via receptors which activate adenylate cyclase, for example β-adrenoceptor agonists, also inhibit the release of histamine and SRS-A from fragments of human lung (Orange *et al.*, 1971; Butchers *et al.*, 1980).

As in other physiological systems the inhibitory receptor is probably a component of the outer leaflet of the cell membrane. Two lines of evidence support this. Firstly, both NECA and 2-chloroadenosine, which are reported to be poor substrates for the adenosine uptake mechanism (Daly, 1982), are effective inhibitors of release. Secondly, hexobendine and dipyridamole which inhibit the uptake of adenosine in some tissues (Daly, 1982) do not block the activity of adenosine. In some preparations the effects of adenosine are potentiated by hexobendine and dipyridamole (Coleman & Levy, 1974; Brown & Collis, 1982) suggesting that by blocking uptake the extracellular concentration of adenosine is increased. In contrast, inhibition of mediator release by adenosine was not enhanced by uptake blockers. Carrier-mediated uptake of adenosine in human lung may not be sensitive to these drugs or it may not be a significant contributor to the inactivation of adenosine by this tissue. The observation that DDA, which is thought to act at an intracellular P site (Londos & Wolff, 1977), inhibits the release process and is unaffected by hexobendine and dipyridamole, supports the view that uptake by this tissue may be abnormal.

At present there are no antagonists acting selectively at one adenosine receptor subtype. The rank order of potency of the non-selective antagonists DPX > 8-PT > theophylline > theobromine = 0 is consistent with effects at extracellular A/R receptors (Daly 1982). The $pA_2$ values obtained for the antagonism of NECA by 8-PT agree well with values obtained in brain slices (Smellie *et al.*, 1979), guinea-pig left atria (Griffith *et al.*, 1981) and guinea-pig trachea (Brown & Collis, 1982). Furthermore, the observation that DPX is a more potent antagonist than 8-PT is in agreement with ligand-binding studies showing that this antagonist has high affinity for both $A_1$ and $A_2$ receptors in bovine and guinea pig brain membranes (Bruns *et al.*, 1980; 1983). DPX also antagonizes the depressant effects of adenosine analogues on guinea-pig isolated atria (Evans *et al.*, 1982). In this tissue DPX (10 μM) caused 50–200 fold shifts of agonist concentration-effect curves, consistent with a $pA_2$ value of about 7. However, $pA_2$ values for DPX in other functional systems have yet to be reported.

Human lung parenchyma contains many cell types in addition to the mast cell. Thus our results could reflect an indirect action on the mast cell due to an effect of adenosine and its analogues on another cell type. However, a recent report (Schulman *et al.*, 1982) that adenosine inhibits the release of histamine from human purified lung mast cells indicates that the $A_2/R_a$ receptor probably resides on the mast cell itself.

Although the rank order of potency of adenosine analogues was almost identical for inhibition of the release of both histamine and SRS-A, it appeared that NECA and NCPCA were relatively more effective against the release of SRS-A. A similar observation has been made with both isoprenaline (Orange *et al.*, 1971) and with salbutamol (Butchers *et al.*, 1979). Although having a common trigger in the cross-linking of IgE receptors and occurring in tandem in challenged cells the processes of SRS-A generation and the release of histamine are very different, particularly as only the latter involves the intracellular structural changes of exocytosis. It is, therfore, quite possible that these two events have distinct control points that show differential sensitivity to agents that elevate intracellular cyclic AMP.

In contrast to our observations, others have shown that adenosine added after challenge with antigen causes a modest stimulation of the release of histamine from human blood basophils (Church *et al.*, 1982) and from mast cells isolated from human lung fragments (Church *et al.*, 1983) and claim that both inhibition and stimulation of release by adenosine are mediated via $A_2/R_a$ receptors. These findings show some similarities with those obtained in rat mast cells

and guinea-pig lung where pretreatment with adenosine enhances antigen-induced release of histamine (Marquardt *et al.*, 1978; Welton & Simko, 1980; Holgate *et al.*, 1980; Sydbom & Fredholm, 1982).

DDA inhibits the release of histamine and SRS-A from human lung fragments and the release of β-hexoseaminidase and histamine from rat mast cells (Holgate *et al.*, 1980; Burt & Stanworth, 1983). In the rat mast cell this inhibition is associated with abrogation of the initial rise in cyclic AMP that precedes release and has been taken as evidence that the rise in cyclic AMP is essential for the release process (Holgate *et al.*, 1980). However, the release of histamine induced by the calcium ionophore A23187 is not associated with an early rise in cyclic AMP and yet is inhibited by DDA (Burt & Stanworth, 1983). Furthermore, another P site agonist, 9-β-D-arabinofuranosyl adenine (Londos & Wolff, 1977; Welton & Simko, 1980) fails to inhibit release induced either by antigen or by A23187. Thus it appears that the inhibition of the release of histamine from human lung by DDA may be independent of any activity at intracellular P sites. The failure of 8-PT to antagonize the effects of DDA indicates that it does not act through an extracellular A/R receptor site.

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