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ADENOSINE INHIBITS AND POTENTIATES IgE-DEPENDENT HISTAMINE RELEASE FROM HUMAN LUNG MAST CELLS BY AN A_2-PURINOCEPTOR MEDIATED MECHANISM

PHILIP J. HUGHES*, STEPHEN T. HOLGATE† and MARTIN K. CHURCH*‡
*Clinical Pharmacology and †Medicine I, Southampton General Hospital, Southampton SO9 4XY, U.K.

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Abstract—Adenosine, at physiological concentrations, may modulate histamine release from mechanically dispersed human lung mast cells. Addition of adenosine to the dispersed mast cells at times up to 5 min before immunological challenge with anti-human IgE inhibited histamine release. When added after this time adenosine caused a small potentiation of immunological histamine release, maximum potentiation occurring with addition of adenosine 5 min after challenge, coincidental with the end of the rapid phase of histamine release. Both inhibition and potentiation of histamine release were more pronounced with low levels of immunological challenge. Theophylline, 8-phenyltheophylline, dipyridamole and analogues of adenosine were used to determine the site of action of adenosine on mast cell mediator release. Theophylline and 8-phenyltheophylline displaced the concentration–response lines for both inhibition and potentiation of mediator release by adenosine to the right whilst dipyridamole, 1 μM, was without significant effect. This suggests that both effects result from interaction of adenosine with cell surface receptors. This was confirmed by demonstrating that the P-site agonist 2′,5′-dideoxyadenosine produced only inhibition of histamine release, an effect which was inhibited by dipyridamole but not by theophylline. The rank potency order of adenosine analogues, NECA $\gg$ adenosine $\geq$ L-PIA $\geq$ D-PIA in both inhibiting and potentiating immunological histamine release suggests that both effects are mediated through activation of cell surface A_2-purinoceptors. Since adenosine is released into the circulation of asthmatic subjects following bronchial provocation with antigen, causes bronchoconstriction and has the ability to modulate mast cell histamine release, this nucleoside should be considered as an additional inflammatory mediator of allergic reactions.

Adenosine, a naturally occurring purine nucleoside formed by 5′-nucleotidase cleavage of 5′-adenosine monophosphate (AMP), exerts a modulating effect on a large number of cell systems [1]. At physiological concentrations it interacts with specific cell surface receptors which regulate intracellular levels of 3′,5′-cyclic adenosine monophosphate (cyclic AMP). Adenosine at nanomolar concentrations interacts with a high affinity A_1-purinoceptor (Ri-site) to inhibit adenylate cyclase activity. At micromolar concentrations the nucleoside interacts with a second receptor sub-type, the A_2-purinoceptor (Ra-site), to stimulate adenylate cyclase [2, 3] and increase intracellular levels of cyclic AMP. These distinct purinoceptors, which have been reported not to coexist on the same cell types [4], have been defined further by their differing sensitivities to adenosine analogues. At the A_1-receptor the rank order of potency is N^6-substituted analogues > adenosine > 5′-*N*-substituted analogues, whereas at the A_2-receptor the reverse order of potency is observed [4, 5]. In addition to actions on cell surface receptors, adenosine in the millimolar concentration range, may also interact with an intracellular P-site to inhibit adenylate cyclase activity [3]. P-site stimulation may be achieved more selectively by use of 2′,5′-dideoxyadenosine, an adenosine analogue which does not interact with either A_1 or A_2 cell surface receptors [4].

Adenosine has been shown to modulate mediator secretion from mast cells and basophils. In rat serosal mast cells and guinea-pig lung fragments, adenosine and analogues which act at cell surface receptors to elevate cyclic AMP levels, have been shown to enhance IgE- and calcium ionophore-induced mediator secretion [6–8]. In contrast, preincubation of human lung fragments, enzymatically dispersed lung mast cells and basophil leucocytes with adenosine has been shown to inhibit immunological mediator release [9–11]. We have recently reported that in human basophil leucocytes, preincubation with adenosine inhibits immunological mediator release whereas addition of the nucleoside after challenge potentiates release, both effects being mediated by A_2-purinoceptors [12].

The present study investigates the characteristics and mechanisms of adenosine modulation of immunological histamine release from mast cells mechanically dispersed from human lung.

MATERIALS AND METHODS

Materials. Adenosine and its analogues and antagonists were obtained from the following sources: adenosine (Sigma, Poole), 5′-*N*-ethylcarboxamide-adenosine (NECA, donated by Drs I. F. Skidmore

‡ To whom correspondence should be addressed.

and C. J. Vardey, Glaxo Group Research Ltd, Ware, Herts), L-N^6-phenylisopropyladenosine (L-PIA, Boehringer Mannheim, Boehringer Corporation (London) Ltd., Lewes, East Sussex), D-N^6-phenylisopropyladenosine (D-PIA, donated by Dr M. G. Collis, ICI Pharmaceuticals, Alderley Edge, Cheshire), 2′,5′-dideoxyadenosine (PL Biochemicals, Northampton), dipyridamole (C. H. Boehringer Sohn, Ingelheim Rhein, Germany), theophylline (Sigma, Poole) and 8-phenyltheophylline (Calbiochem, La Jolla, CA). All other chemicals and reagents were obtained from Sigma or BDH Chemicals, Poole. The composition of the HEPES buffered physiological saline was: sodium chloride 137 mM, potassium chloride 2.7 mM, sodium dihydrogen phosphate 0.4 mM, magnesium chloride 0.5 mM, calcium chloride 0.9 mM, glucose 5.5 mM, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid 10 mM, and human serum albumin 0.03%. The HEPES buffer was adjusted to pH 7.4 with sodium hydroxide immediately prior to use. NECA, L-PIA, D-PIA and 2′,5′-dideoxyadenosine were diluted from 10 mM stock solutions containing 1% dimethylsulphoxide immediately prior to use. Final concentrations of dimethylsulphoxide were always less than 0.1% and did not affect histamine release. 8-Phenyltheophylline was dissolved in 80% methanol in 0.02 M NaOH at a concentration of 10 mM and was diluted with HEPES buffer prior to use. Other drugs were freshly dissolved in HEPES buffer. Goat anti-human IgE was heat inactivated by incubation at 56° for 1 hr.

Dispersal and challenge of human lung mast cells. Mast cells were dispersed from fresh human lung tissue by a mechanical method previously described by Church *et al.* [13]. Microscopy of lung cells stained with Kimura's stain showed mast cells to comprise 1–8% of the total nucleated cells. For the estimation of histamine release, duplicate tubes containing 1 to 3×10^4 mast cells were prewarmed at 37° for 10 min before addition of drug or anti-IgE, and incubated for 15 min after challenge at a final volume of 1 ml. Release reactions were stopped by rapid centrifugation at 200 *g* for 10 min at 4° after which the supernatants were removed, acidified with trichloroacetic acid (final concentration 5%) and stored at −20° for histamine assay. Total cellular histamine was assessed in 8–10 replicate tubes in which the cells were disintegrated with 5% trichloracetic acid. Spontaneous histamine release was measured in tubes incubated in the absence of anti-IgE challenge. Histamine was assayed by automated spectrofluorimetry [13]. Net immunological histamine release is expressed as the percentage of total cellular histamine released into the supernatant corrected for spontaneous release.

Statistical analysis. The statistical significance of drug effects were analysed by comparing net histamine releases of control and drug treated cells using Student's *t*-test for paired data. Parallelism of concentration–response lines was confirmed by covariant analysis. Dose ratios and concentrations of drugs calculated to produce a specific inhibition or potentiation of histamine release were calculated from best fit linear regression lines. pA_2 Values were calculated from Arunlakshana and Schild plots [14].

RESULTS

Adenosine on IgE-dependent histamine release

The effect of varying the time of adenosine additions with respect to that of immunological challenge was examined in six experiments in which cells were challenged with a 1/100 dilution of anti-IgE (Fig. 1). Addition of adenosine at a final concentrations of 10 μM 15 min before challenge inhibited the net histamine secretion by 28.8 ± 7.8% (mean ± S.E.M.) (a decrease in the net histamine release from 15.4 ± 2.5% to 11.8 ± 3.0%, $P < 0.01$). As the preincubation time with adenosine was shortened the degree of inhibition of histamine secretion decreased. Following simultaneous addition of adenosine and anti-IgE there was a slight 3.9 ± 1.3% ($P < 0.01$) potentiation of histamine release. Addition of adenosine at increasing times after immunological challenge caused a progressive further potentiation of histamine release, reaching 19.7 ± 3.1% 5 min after challenge (an increase in the net histamine release from 15.4 ± 2.5% to 18.6 ± 2.9%, $P < 0.01$). In all subsequent experiments, inhibition and potentiation of histamine release were measured following additions of adenosine and related nucleosides 15 min before and 5 min after challenge respectively.

To establish the concentration-related effects of adenosine and the influence of the strength of immunological challenge upon this relationship, five experiments were performed in which mast cell preparations were challenged with 1/1000, 1/100 and 1/10 dilutions of anti-IgE (Fig. 2). Net histamine release produced by these dilutions of anti-IgE was 9.5 ± 1.6%, 16.1 ± 2.5% and 20.9 ± 3.0% respectively. Addition of adenosine, 0.1–100 μM, 15 min before or 5 min after challenge resulted in a concentration-related inhibition and potentiation of his-

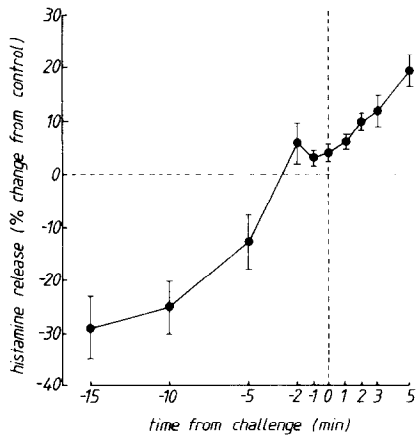

Fig. 1. Time–response relationship for adenosine on anti-IgE induced histamine release from mechanically dispersed human lung cells. Adenosine, 10 μM, was added at the stated time with respect to immunological challenge. Release reactions were stopped 15 min after challenge with a 1/100 dilution of anti-IgE in all cases. Results are the mean ± S.E.M. of six experiments in which net histamine release was 15.4 ± 2.5% and spontaneous histamine release was 17.8 ± 2.1%.

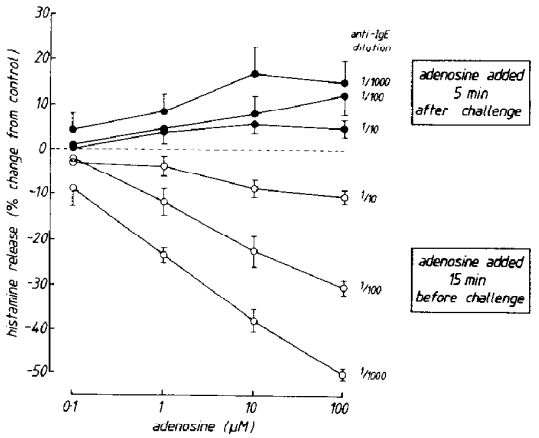

Fig. 2. Concentration–response relationship for adenosine against histamine release from mechanically dispersed human lung cells induced by 1/1000, 1/100 and 1/10 dilutions of anti-IgE. Adenosine was added either 15 min before (open symbols) or 5 min after (closed symbols) immunological challenge. Results are mean ± S.E.M. mean of five experiments. Net histamine release induced by 1/1000, 1/100 and 1/10 dilutions of anti-IgE were 9.4 ± 1.6%, 16.1 ± 2.5% and 20.9 ± 3.0% respectively. Spontaneous release was 9.3 ± 1.5%.

tamine release respectively at each of the anti-IgE dilutions used. However, the ability of adenosine to decrease or increase histamine secretation respectively was inversely related to the strength of immunological stimulation and net histamine release in untreated cells. Preincubation of cells with 100 μM adenosine resulted in a 46.0 ± 3.3% inhibition of histamine release in cells challenged with a 1/1000 dilution of anti-IgE compared to a 10.6 ± 2.1% inhibition in cells challenged with 1/10 dilution of anti-IgE. Potentiation of histamine secretion following addition of 100 μM adenosine 5 min after challenge was less marked. With a 1/1000 dilution of anti-IgE a 20.6 ± 3.6% ($P < 0.02$) potentiation was observed whereas with a 1/10 dilution of anti-IgE the degree of potentiation was only 6.7 ± 1.9% ($P < 0.05$).

Site of action of adenosine

To investigate whether adenosine modulated immunological histamine release from human lung mast cells by interacting with external cell surface receptors or by an intracellular mechanism, the effects of two methylxanthines, theophylline and 8-phenyltheophylline, an inhibitor of the facilitated uptake of adenosine, dipyridamole, and an adenosine analogue which acts at the internal P-site, 2',5'-dideoxyadenosine, were examined.

The effect of theophylline, a competitive inhibitor of the cell surface actions of adenosine [15], was examined in three experiments (Fig. 3a). Theophylline was added to the cell preparations, at a final concentration of 50 μM, 3 min before addition of adenosine. At this concentration theophylline had no effect on spontaneous or immunologically induced histamine secretion. Following adenosine addition 15 min before challenge, theophylline caused a significant ($P < 0.05$) parallel displacement of the inhibitory adenosine concentration response curve to the right. The geometric mean dose ratio for theophylline was 5.4 and the estimated pA_2 4.94. Similarly following adenosine addition 5 min after challenge theophylline produced a parallel displacement of the concentration–response curve for enhancement of histamine release, the geometric mean dose ratio being 5.7 and the estimated pA_2 4.97.

8-Phenyltheophylline, a theophylline analogue which has greater efficacy at adenosine receptors but is a weaker inhibitor of phosphodiesterase activity [16], was added to the mast cell preparations in concentrations of 1, 3 and 10 μM 3 min before adenosine. In these concentrations 8-phenyltheophylline had no effect on spontaneous or immunological histamine release. 8-Phenyltheophylline produced concentration related parallel shifts in both inhibitory and potentiatory adenosine concentration–response curves (Table 1). With respect to the inhibitory adenosine concentration–response curve, the pA_2 value for 8-phenyltheophylline was 6.33 and the slope of the Arunlakshana–Schild plot was 0.83. Following adenosine addition 5 min after challenge the pA_2 for 8-phenyltheophylline was 6.23 and slope of the Arunlakshana–Schild plot 0.87.

Dipyridamole, an adenosine uptake inhibitor [19] when added at a concentration of 1 μM 3 min before adenosine produced a slight, but not significant, enhancement of both adenosine-mediated inhibition and potentiation of immunological histamine release in three experiments (Fig. 3b).

The effect on immunological histamine release of 2', 5'-dideoxyadenosine, which acts at the intracellular P-site but does not interact with cell surface adenosine receptors [4] was examined in three experiments (Fig. 4). This adenosine analogue pro-

Table 1. Antagonism by 8-phenyltheophylline (8-PT) of adenosine mediated inhibition and potentiation of anti-IgE induced histamine release from mechanically dispersed human lung cells

	Time of adenosine addition	
	15 min pre-challenge	5 min post-challenge
Dose ratios for 8-PT concentrations of		
1 μM	3.3 (2.9–3.9)	2.9
3 μM	15.8 (11.4–20.0)	8.2
10 μM	31.9 (27.9–35.5)	27.4
pA_2	6.33 (6.26–6.41)	6.23
Slope of Arunlakshana–Schild plot	0.83 (0.80–0.85)	0.87

Data for the effect of 8-PT on the inhibitory effect of adenosine is expressed as the mean of three experiments performed in duplicate. Data for the effect of 8-PT on the enhancing effects of adenosine is expressed as the mean of a single experiment performed in duplicate. Figures in brackets are the range of the results obtained.

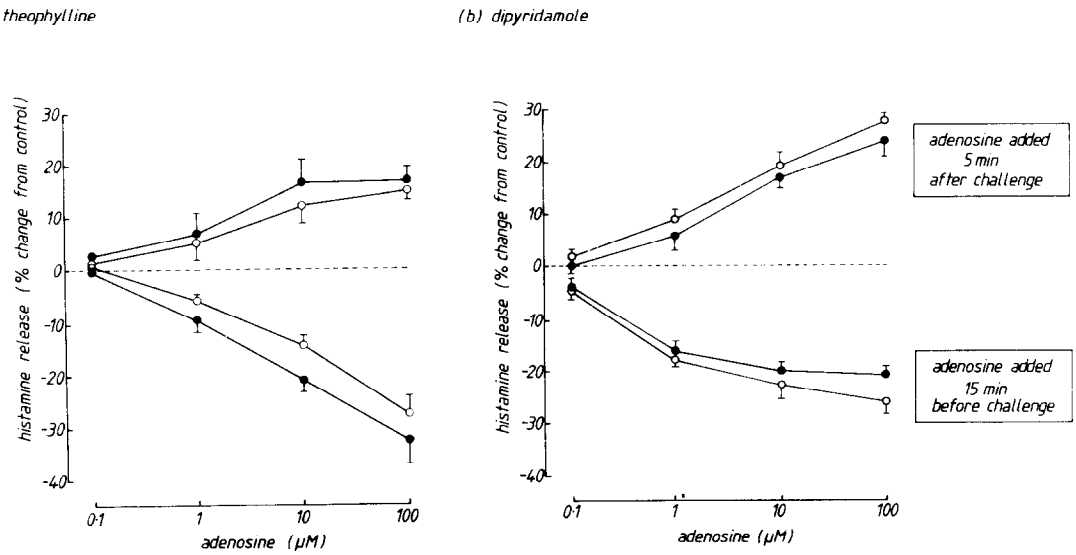

Fig. 3. Theophylline and dipyridamole on adenosine modulation of anti-IgE induced histamine release from mechanically dispersed human lung cells. (a) Theophylline, 50 μM, or (b) dipyridamole, 1 μM, was added 3 min before adenosine. Adenosine was added 15 min before challenge (lower sections) or 5 min after challenge (upper sections) with a 1/100 dilution of anti-IgE in three experiments. Results are shown as the mean ± S.E.M. for (●) adenosine alone or (○) adenosine plus (a) theophylline or (b) dipyridamole. In these experiments net histamine release was 15.5 ± 1.8%, and spontaneous release 9.0 ± 1.8%.

duced a concentration related inhibition of histamine release when added to the mast cell preparations either before or after immunological challenge. The intracellular site of action of 2′,5′-dideoxyadenosine was confirmed by three experiments in which dipridamole, 1 μM, but not theophylline, 50 μM, added 3 min before the nucleoside significantly ($P < 0.01$) reduced its inhibitory effect on immunological histamine release.

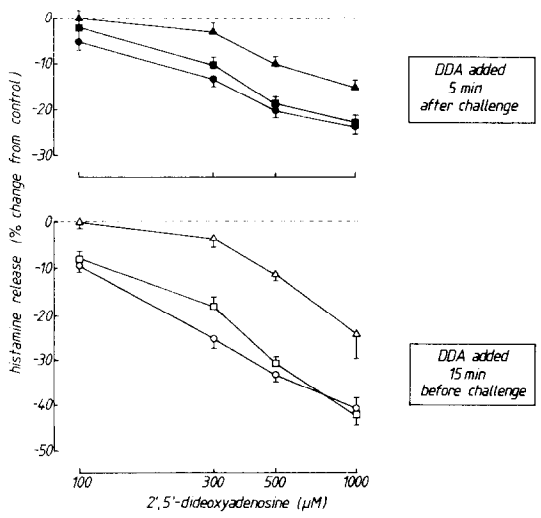

Fig. 4. 2′,5′-Dideoxyadenosine on anti-IgE induced histamine release from mechanically dispersed human lung cells and its modifications by theophylline and dipyridamole. 2′,5′-Dideoxyadenosine (DDA) was added either 15 min before challenge (○ in the lower section) or 5 min after challenge (● in the upper section) with a 1/100 dilution of anti-IgE. 50 μM Theophylline (□■) or 1 μM dipyridamole (△▲) was added 3 min before 2′,5′-dideoxyadenosine. All results are the mean ± S.E.M. of three experiments in which net histamine release was 25.0 ± 2.5% and spontaneous release 8.0 ± 1.1%.

Characterization of mast cell receptors for adenosine

The nature of the mast cell surface receptors mediating the modulatory actions of adenosine was investigated using adenosine analogues with differing specificities for A_1- and A_2- purinoceptors. Preincubation of lung mast cells with adenosine, NECA, L-PIA or D-PIA for 15 min prior to immunological challenge with a 1/100 dilution of anti-IgE inhibited histamine secretion in a concentration-dependent manner (Fig. 5). The rank order of inhibitory potencies was NECA ≫ adenosine ≥ L-PIA ≥ D-PIA, the concentrations calculated to inhibit histamine release by 25% (IC_{25}) being 2.1 (N = 6), 35 (N = 17), 328 (N = 10) and 767 (N = 2) μM respectively. The same nucleosides added to mast cell preparations 5 min after challenge produced a concentration-dependent potentiation of histamine release in all cases, the rank order of potencies being NECA ≫ adenosine = L-PIA = D-PIA. These results indicate that both inhibition and potentiation of immunological histamine release by adenosine and its analogues occur through an interaction with the purinergic A_2-receptor sub-type.

DISCUSSION

Adenosine, at physiological concentrations, modulated IgE-dependent mediator secretion from human lung mast cells. We have demonstrated that the direction of this modulation was dependent upon the time of exposure of the cells to the nucleoside

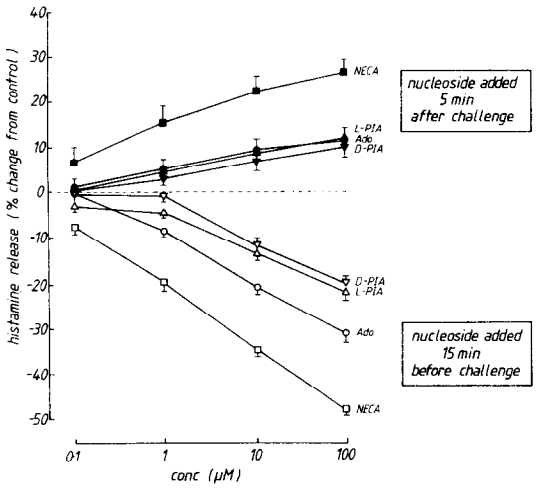

Fig. 5. Adenosine and adenosine analogues on anti-IgE induced histamine release from mechanically dispersed human lung cells. Adenosine (Ado ○, ●), L-PIA (△,▲), D-PIA (▽, ▼), and NECA (□, ■) were added either 15 min before challenge (open symbols) or 5 min after challenge (closed symbols) with a 1/100 dilution of anti-IgE. Results are mean ± S.E.M. of 17 experiments for adenosine in which net histamine release was 15.9 ± 1.1% and spontaneous release 9.1 ± 0.8%, 11 experiments for L-PIA in which net histamine release was 15.0 ± 6.0% and spontaneous release 8.7 ± 1.1%, two experiments for D-PIA in which net histamine release was 15.0 ± 7.5% and spontaneous release 6.6 ± 1.0% and seven experiments for NECA in which net histamine release was 17.1 ± 1.8% and spontaneous release 9.9 ± 0.8%.

with respect to immunological challenge. Addition of adenosine before challenge inhibited histamine release whereas addition after challenge potentiated release. In all experiments performed the inhibitory actions of adenosine and its analogues were more marked than the potentiatory effects. However, both effects were more pronounced with low levels of immunological stimulation. The use of adenosine analogues, methylxanthines and dipyridamole demonstrated that both inhibition and potentiation of histamine release result from an interaction of adenosine with cell surface A_2-purinoceptors.

Previous studies have reported that preincubation with adenosine inhibits immunological histamine release from human lung fragments [10] and human basophil leucocytes [9, 12], whereas in rat serosal mast cells [6, 7] and guinea-pig lung fragments [8] it potentiates both immunological and the calcium ionophore A23187-induced release. In basophil leucocytes, shortening of the preincubation period with adenosine before immunological challenge decreases its inhibitory capacity [9, 12] whilst it enhances potentiation in rat mast cells (unpublished data). Our studies show that preincubation of human lung mast cells for periods in excess of 5 min before immunological challenge leads to a progressive inhibition of histamine release. However, addition of adenosine between 2 min before and 5 min after challenge leads to a progressive potentiation of immunological histamine secretion. Maximum potentiation occurred when the addition of adenosine coincided with completion of the rapid phase of histamine release 5 min after challenge [13]. A similar pharmacological profile of adenosine and its relationship to the kinetics of mediator secretion has also recently been shown for human basophil leucocytes [12].

Experiments with methylxanthines, dipyridamole and adenosine analogues suggest that both potentiation and inhibition of mediator release are the consequence of interaction of adenosine with specific receptors on the mast cell surface. Theophylline is a competitive antagonist of the cell surface actions of adenosine at concentrations 10- to 100-fold lower than those required to inhibit cyclic AMP phosphodiesterase [2, 15, 20]. In human mast cells, theophylline, at a concentration which alone did not affect histamine secretion, reduced both inhibition and potentiation of immunological histamine release produced by adenosine. The parallel shifts of the concentration–response curves indicate competitive antagonism. Adenosine antagonism by theophylline was relatively weak, as demonstrated by the dose-ratios for inhibition and potentiation of 5.4 and 5.7 respectively, but was consistent with its ability to antagonize the receptor-mediated effects of adenosine in other systems. 8-Phenyltheophylline is a more potent adenosine antagonist yet is essentially free of phosphodiesterase inhibitory activity [16] and, therefore, is a more powerful tool with which to examine adenosine interaction with cell surface purinoceptors. The dose ratios and pA_2 values obtained for 8-phenyltheophylline in antagonizing adenosine mediated inhibition and potentiation of immunological histamine are consistent with those reported by Hillyard *et al.* for antagonism of NECA mediated inhibition of histamine and SRS-A release from human lung mast cells [10]. The pA_2 values also agree with those found for 8-phenyltheophylline in guinea pig left atria [16], guinea-pig trachea [17] and in brain slices [18].

In contrast to theophylline, dipyridamole did not reduce either inhibition or potentiation of histamine release by adenosine. Furthermore, L-PIA and NECA had similar qualitative effects to adenosine. Unlike adenosine, however, these analogues do not undergo facilitated uptake, are devoid of P-site activity and are not substrates for metabolism [4] indicating that these cellular events are therefore not prerequisites for the observed actions of adenosine on the mast cell. This was confirmed by comparison with 2′,5′-dideoxyadenosine, an analogue which interacts with intracellular P-sites but not with cell surface purinoceptors [4]. This nucleodise inhibited immunological histamine secretion either when added before or after challenge. In addition, its inhibitory effects were reduced by dipyridamole but not by theophylline, consistent with its proposed intracellular site of action.

By comparing the effects of adenosine analogues with varying specificities for A_1 and A_2 adenosine receptor subtypes, we have shown that both inhibition and potentiation of immunological histamine release from human lung mast cells are mediated by stimulation of the adenosine A_2-receptors. The rank orders of potency, NECA ≫ adenosine ≫ L-PIA ≥ D-PIA for inhibiting and NECA ≫ adenosine = L-

PIA = D-PIA for potentiating histamine release are consistent with A_2-receptor stimulation in a number of animal tissues and isolated cell preparations including modulation of basophil histamine release [5, 12]. Also, the observation that L-PIA was only marginally more potent than D-PIA agrees with ligand binding and isolated tissue studies where L-PIA is only 5-10-times more potent than D-PIA at A_2-receptors but 100-times more potent at A_1 receptors [21]. In basophil-rich leucocyte preparations [22], fibroblasts [15], heart slices [23] and lymphocytes [24] stimulation of adenosine A_2-receptors stimulates adenylate cyclase to cause a rise in intracellular cyclic AMP.

The findings that its modulatory effects were inversely related to the strength of immunological stimulation and histamine release are consistent with a cyclic-AMP-mediated action of adenosine. Inhibition of histamine release by stimulation of β-adrenergic receptors in human lung mast cells [25] and H_2-receptors in basophils [26] is similarly more pronounced at low levels of histamine release. However, the mechanism by which adenosine induced changes in cyclic AMP levels exert a dual effect on mediator secretion is not clear. Immunological challenge of mast cells and basophils stimulates adenylate cyclase to elevate intracellular levels of cyclic AMP and in this respect it is similar to A_2-receptor stimulation. In rat mast cells this leads to activation of cyclic-AMP-dependent protein kinases implicated in activation–secretion coupling [27]. In both human lung mast cells and basophils maximum potentiation of histamine release was obtained when adenosine was added to cell suspensions at the time when the rapid phase of secretion was nearing completion. This enhancement of release may, therefore, be a consequence of an A_2-receptor mediated rise in cyclic AMP and activation of protein kinases at a critical time in the mediator secretory process. Inhibition occurred when adenosine was added to human mast cells 10 or more minutes before the slowing of mediator secretion, i.e. 5 min before antigen challenge. A similar relationship of adenosine preincubation and release kinetics has been observed in human basophils [12]. It is possible, therefore, that preincubation of cells with adenosine causes premature activation of cyclic AMP-dependent protein kinases out of sequence with later IgE-dependent secretory events although alternative explanations should not be discounted.

Following the observation that bronchial challenge of asthmatic subjects with antigen raises plasma levels of adenosine by three- to fourfold [28], a modulatory role for the nucleoside on mast cell mediator release assumes potential clinical relevance. In asthma, where release of less than 2% of mast cell histamine is sufficient to cause marked bronchoconstriction [29], release of adenosine in the lung following challenge would not only contribute directly to bronchoconstriction [30], but may markedly facilitate mast cell mediator release thereby contributing indirectly to bronchoconstriction. The findings that theophylline and sodium cromoglycate selectively antagonize adenosine induced bronchoconstriction [31] and reduce adenosine potentiation of histamine release in rat mast cells [32] suggest that antagonism of adenosine in the lung may be of therapeutic benefit to patients with asthma.

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