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Adenosine inhibits and potentiates IgE-dependent histamine release from human basophils by an A_2-receptor mediated mechanism

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1 Adenosine added to human basophils before anti-IgE challenge inhibited histamine release, whereas addition after challenge potentiated release. Peak responses for the two effects occurred 15 min before and after challenge respectively.

2 The effects of adenosine on histamine secretion were dose-related over concentration ranges of 1–100 μM for inhibition and 0.01–1 μM for potentiation.

3 The capacity of adenosine to inhibit and potentiate histamine secretion was inversely related to the strength of immunological challenge.

4 The ability of theophylline (50 μM) to inhibit and dipyridamole (1 μM) to enhance slightly adenosine-induced responses, and the differing pharmacological effect of 2′,5′-dideoxyadenosine suggested that adenosine's effects on basophil histamine secretion were mediated by stimulation of cell surface adenosine receptors.

5 The order of potency of adenosine and its analogues L- and D- N^6-phenylisopropyladenosine (PIA) and 5′-N-ethylcarboxamideadenosine (NECA) in inhibiting and potentiating IgE-dependent histamine release from basophils indicated that both responses were mediated by stimulation of the adenosine A_2-receptor subtype.

6 The capacity of adenosine to cause a transient increase of cyclic AMP levels in 40–70% basophil-enriched leucocytes confirmed the association between stimulation of A_2-receptors and activation of adenylate cyclase.

Introduction

Immunological activation of rat serosal mast cells and human basophil leucocytes stimulates adenylate cyclase to induce an early rise in the intracellular level of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) which precedes the onset of mediator release (Sullivan, Parker, Kulczycki & Parker, 1976; Lichtenstein, Sobotka, Malveaux & Gillespie, 1978; Lewis, Holgate, Roberts, Maguire, Oates & Austen, 1979; Holgate, Lewis & Austen, 1980a; Ishizaka, Hirata, Sterk, Ishizaka & Axelrod, 1981; Hughes, Holgate, Roath & Church, 1983). This rise in cyclic AMP activates cytoplasmic cyclic AMP-dependent protein kinases as part of the coupling between IgE-dependent activation and mediator secretion (Holgate, Lewis & Austen, 1980b).

In a large number of cells the purine nucleoside, adenosine, may modulate intracellular levels of cyclic AMP following its interaction with cell surface purinoceptors (Burnstock, 1980). These receptors have been subdivided into two types, A_1- and A_2-receptors (Van Calker, Muller & Hamprecht, 1979). A_1-receptors have a high affinity for adenosine and are inhibitory to adenylate cyclase (Ri-site effect), whereas A_2-receptors have a low affinity for adenosine and activate adenylate cyclase (Ra-site effect) (Van Calker *et al.*, 1979; Londos, Cooper & Wolff, 1980). A_1- and A_2-receptors also differ in their sensitivity to stimulation by adenosine analogues, particularly N^6-substituted analogues such as N^6-phenylisopropyladenosine (PIA) and 5′-substituted analogues such as 5′-N-ethylcarboxamideadenosine (NECA). At the A_1-receptor the order of potency is PIA > adenosine > NECA, whereas at the A_2-receptor the reverse order of potency is observed (Wolff, Londos & Cooper, 1981; Daly, 1982). Furthermore, the two receptor sub-types show a differential stereospecificity for PIA, the potency ratio of L-PIA: D-PIA being 100:1 at the A_1-receptor and 5:1 at the A_2-receptor (Bruns, Daly & Snyder,

1980). No selective A_1 or A_2-receptor antagonists have yet been found; however, methylxanthines such as theophylline competitively antagonize adenosine at both receptor sub-types in concentrations lower than those required to inhibit cyclic-AMP phosphodiesterase (Fredholm, 1980).

In addition to stimulating cell surface receptors, adenosine may enter cells by facilitated uptake and inhibit adenylate cyclase by interacting with adenosine-sensitive P-sites on the inner surface of the membrane (Londos & Wolff, 1977). Facilitated transport of adenosine and therefore its action at P-sites is blocked by dipyridamole (Stafford, 1966).

Reports on the modification of mast cell and basophil histamine release by adenosine are inconsistent. In rat mast cells (Marquardt, Parker & Sullivan, 1978; Holgate *et al.*, 1980a) and guinea-pig lung tissue (Welton & Simko, 1980) adenosine and analogues which interact with cell surface A_1- and A_2-receptors but not those which interact with intracellular P-sites, potentiate immunological histamine release. Conversely, preincubation of human basophil leucocytes with adenosine inhibits IgE-dependent mediator release (Marone, Findlay & Lichtenstein, 1979). In this paper we show that adenosine may inhibit or potentiate histamine release from human basophils depending on the time of its addition with respect to immunological challenge and demonstrate that both effects are mediated through stimulation of adenosine A_2-receptors.

Methods

Fresh venous blood was anticoagulated with disodium edetate (EDTA), 0.5 ml of 0.5 M EDTA/20 ml blood and the leucocytes separated by dextran sedimentation (Lichtenstein & Osler, 1964). After washing in calcium- and magnesium-free HEPES buffered physiological saline (HPS^-), leucocytes were resuspended in complete HPS and aliquots distributed into duplicate tubes prior to stimulation.

For estimation of histamine release, leucocytes from healthy donors were used at a concentration of 1×10^7 nucleated cells ml^{-1}. Duplicate aliquots of 0.8 ml leucocytes were incubated at 37°C with drug or HPS for varying periods before challenge with 0.1 ml goat heat-inactivated anti-human IgE serum (1/1000 dilution unless otherwise stated). In experiments where drug was added after challenge, 0.8 ml aliquots of cells were challenged with 0.1 ml of anti-IgE diluted to give a constant strength of stimulus followed by 0.1 ml of drug added after challenge at the time stated. Reactions were terminated 45 min after challenge by centrifugation at 400 *g* for 10 min at 4°C. The supernatants were removed, acidified by addition of 0.1 ml 55% trichloroacetic acid (TCA) and histamine assayed spectrofluorimetrically. Histamine release was expressed as a percentage of total histamine, measured in duplicate tubes in which the cells had been lysed by addition of 0.1 ml 55% TCA and corrected for spontaneous release (Church, Pao & Holgate, 1982).

The effects of adenosine on cellular levels of cyclic AMP were assessed using leucocytes from a patient with a 40–70% basophilia separated from fresh venous blood as described above (Hughes *et al.*, 1983). Fifty microlitres of 1000 μM adenosine was added to duplicate 0.45 ml aliquots of leucocyte suspensions containing 3×10^6 nucleated cells and incubated at 37°C for the stated times. Reactions were terminated by addition of 0.5 ml of ice-cold ethanol and vortex mixing. The disrupted cell debris was precipitated by centrifugation at 400 *g* for 10 min at 4°C. The cyclic AMP content of duplicate 0.1 ml aliquots of the supernatant was assayed by radio-immunoassay of acetylated samples (Hughes *et al.*, 1983).

Materials

The following drugs were used: adenosine (Sigma), L-N^6-phenylisopropyladenosine (L-PIA, Boehringer-Mannheim), D-N^6-phenylisopropyladeno-

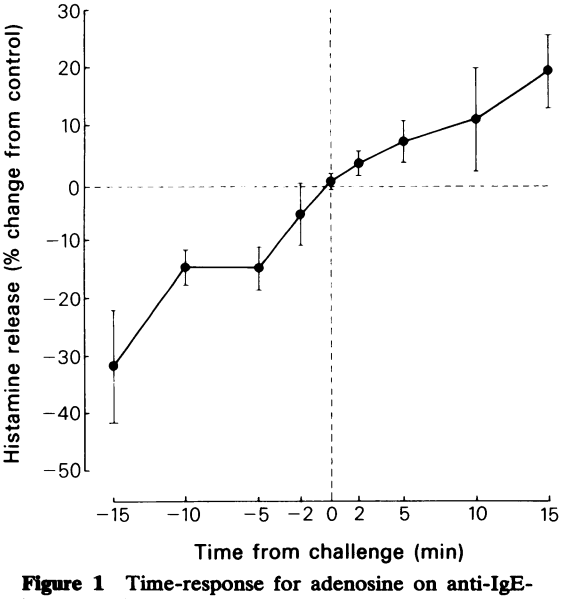

Figure 1 Time-response for adenosine on anti-IgE-induced histamine release from human basophils. Adenosine, 10 μM, was added between 15 min before and 15 min after challenge with a 1/1000 dilution of anti-IgE. Net histamine release determined at 45 min after challenge was 26.9 ± 6.2%. Results are expressed as the mean ± s.e.mean percentage change from control histamine release in four experiments.

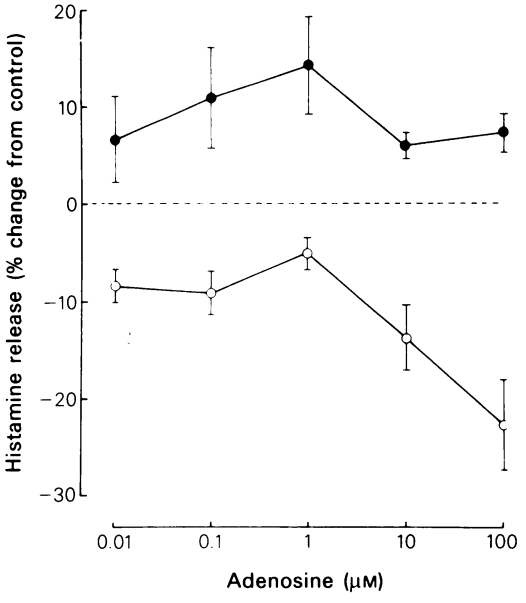

Figure 2 Concentration-related effects of adenosine on anti-IgE induced histamine release from human basophils. Adenosine was added either 15 min before (○) or 15 min after (●) challenge with a 1/1000 dilution of anti-IgE. Net histamine release determined 45 min after challenge was 51.7 ± 8.7%. Results are expressed as mean ± s.e.mean percentage change from control histamine release in four experiments.

sine (D-PIA, donated by Dr M. Collis, ICI Pharmaceuticals), 5'-N-ethylcarboxamideadenosine (NECA, donated by Dr C. Vardey, Glaxo Group Research), 2', 5'-dideoxyadenosine (DDA, P-L Biochemicals), theophylline (Sigma) and dipyridamole (Boehringer Ingelheim). All compounds were dissolved in HPS immediately before use with the exception of L-PIA and NECA which were diluted from a 1 mM stock solution in HPS containing 1% dimethylsulphoxide. Heat inactivated goat anti-human IgE was prepared as described by Church *et al.* (1982). Dextran used for sedimentation was Dextraven 110 (Fisons).

The composition of HEPES buffered physiological saline (HPS) was (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.0, $MgCl_2$ 0.5, NaH_2PO_4 0.4, glucose 5.5, HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid) 10 and human serum albumin 0.03%. The pH was adjusted to 7.4.

Results

The effect of adenosine on anti-IgE induced histamine release from human basophil leucocytes

In four experiments addition of adenosine to a final concentration of 10 μM, to leucocyte suspensions 15 min before anti-IgE challenge significantly

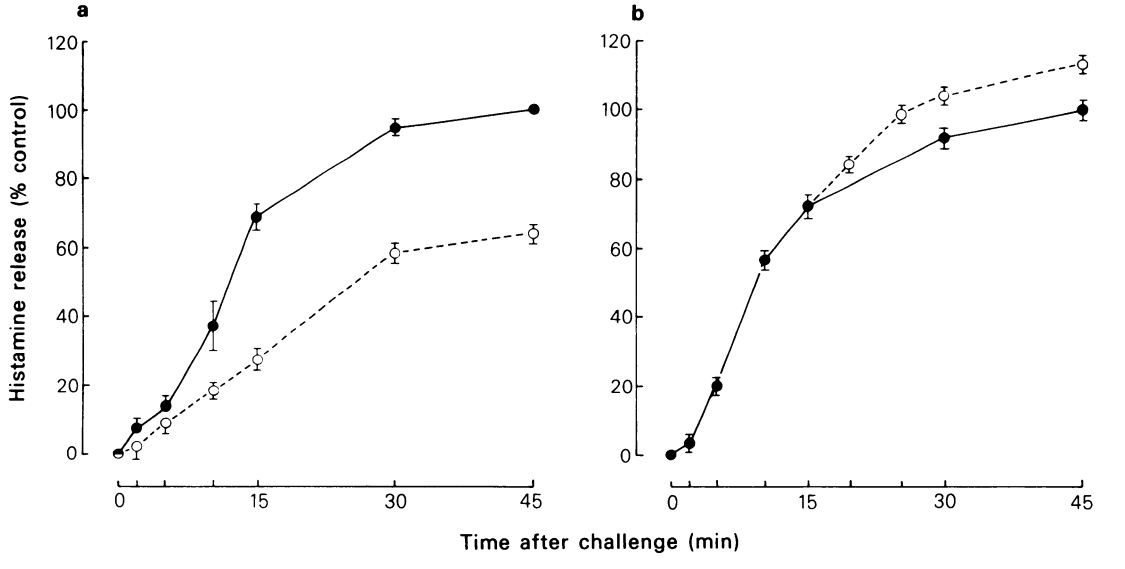

Figure 3 Kinetics of adenosine modulation of anti-IgE induced histamine release from human basophils. Adenosine, 10 μM, was added (a) 15 min before challenge, and (b) 15 min after challenge, with a 1/1000 dilution of anti-IgE. Reactions were stopped and net histamine release determined at 2–45 min after challenge. Each result is the mean histamine release expressed in terms of net control histamine release at 45 min = 100%, the absolute values of which were (a) 20.3 ± 4.9% in four experiments and (b) 34.0 ± 5.4% in two experiments; (●) control release and (○) release in the presence of adenosine 10 μM.

($P<0.01$) inhibited histamine release measured at 45 min by 31.5 ± 9.8% (mean ± s.e., Figure 1). As the preincubation time with adenosine was shortened the degree of inhibition of mediator release was reduced. Simultaneous addition of adenosine and anti-IgE produced no significant effect on histamine release. In contrast, addition of adenosine at increasing times after immunological challenge produced a progressive potentiation of histamine release which reached a maximum of 19.2 ± 7.3% when adenosine was added 15 min after challenge ($P<0.001$). In subsequent experiments, inhibition and potentiation of IgE-dependent histamine release by adenosine was assessed following drug addition 15 min before or after challenge respectively.

Concentration-related effects of adenosine, 0.01–100 μM, were assessed in four experiments (Figure 2). When added 15 min before challenge, inhibition of histamine was logarithmically related to the concentration of adenosine between 1 and 100 μM with the highest concentration producing 22.8 ± 5.2% inhibition ($P<0.01$). When added 15 min after challenge, adenosine potentiated histamine release by a maximum of 14.1 ± 5.1% at 1 μM. In the absence of immunological stimulation, adenosine neither induced histamine release nor suppressed spontaneous histamine release.

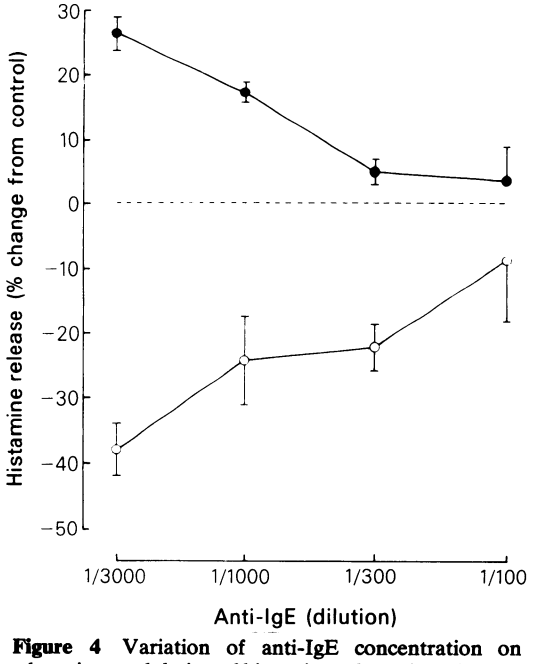

Figure 4 Variation of anti-IgE concentration on adenosine modulation of histamine release from human basophils. Adenosine, 10 μM, was added either 15 min before (○) or 15 min after (●) challenge with anti-IgE. Net histamine releases determined 45 min after challenge were 19.1 ± 6.2% at 1/3000, 29.6 ± 8.8% at 1/1000, 32.5 ± 10.2% at 1/300 and 30.5 ± 8.2% at 1/100 dilution of anti-IgE. Results are expressed as mean ± s.e.mean percentage change from control histamine release in four experiments.

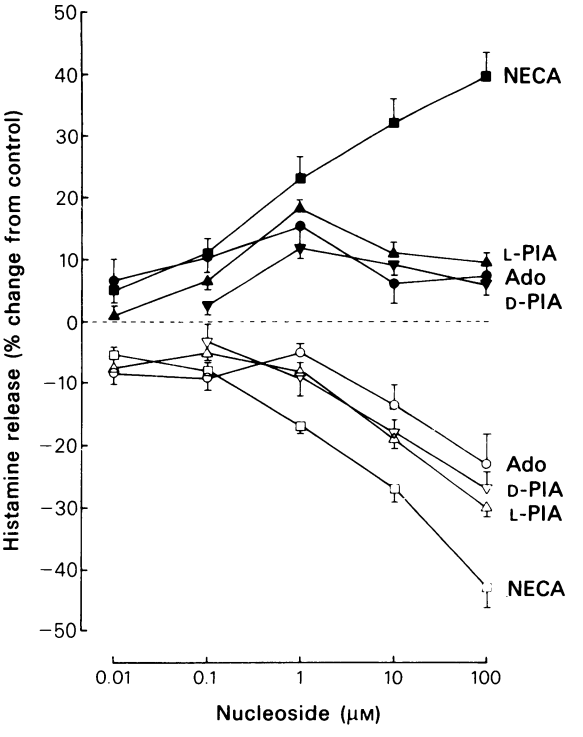

Figure 5 Effects of adenosine and ribose-modified analogues on anti-IgE induced histamine release from human basophils. Adenosine (Ado, (○●), L-N^6-phenylsiopropyladrenosine (L-PIA, △▲), D-PIA (▽▼) or 5′-N-ethylcarboxamideadenosine (NECA, □■) was added either 15 min before (open symbols) or 15 min after (closed symbols) challenge with a 1/1000 dilution of anti-IgE. Net histamine releases determined 45 min after challenge were 51.7 ± 8.7% in four experiments with adenosine, 33.3 ± 2.9% in all experiments with L-PIA, 32 ± 4.7% in four experiments with D-PIA and 35.0 ± 2.0% in seven experiments with NECA. Results are expressed as mean ± s.e.mean percentage change from control histamine releases.

The effect of preincubating leucocytes for 15 min with 10 μM adenosine on the time course of immunological histamine release was assessed in four experiments. Pre-incubation of basophils with adenosine reduced histamine release induced by anti-IgE at all time points, following challenge; at 45 min release was inhibited by 36.5 ± 6% ($P<0.01$, Figure 3a). Addition of adenosine to a final concentration of 1 μM 15 min after challenge prolonged the rapid phase of release; at 45 min histamine release was potentiated by 12.8 ± 1.1% ($P<0.02$, Figure 3b).

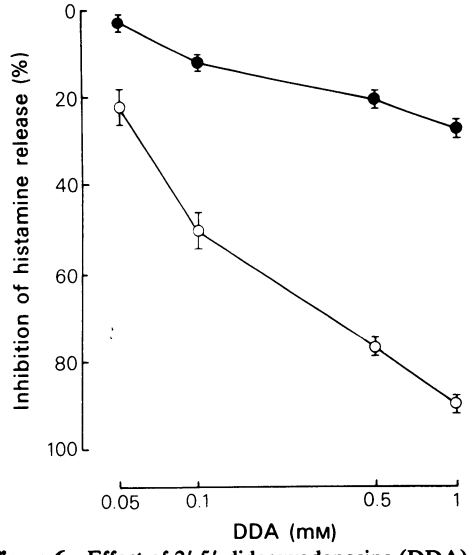

Figure 6 Effect of 2',5'-dideoxyadenosine (DDA) on anti-IgE induced histamine release from human basophils. DDA was added either 15 min before (○) or 15 min after (●) challenge with a 1/1000 dilution of anti-IgE. Net histamine release determined 45 min after challenge was 44.1 ± 8.5%. Results are expressed as mean ± s.e.mean percentage change from control histamine release in four experiments.

An inverse relationship between the strength of immunological stimulation and the ability of adenosine to modulate mediator release was demonstrated in four experiments (Figure 4).

Characterization of basophil adenosine receptors

Adenosine analogues Modulation of anti-IgE-induced histamine release was examined using three adenosine analogues which interact with A_1- and A_2-receptors (L-PIA, D-PIA and NECA) and one which interacts with intracellular P-sites (DDA).

Full dose-response curves for adenosine and its analogues could not be constructed because of limitations in drug solubility. When added 15 min before challenge, L-PIA, D-PIA and NECA all caused a concentration-related inhibition of histamine release (Figure 5). Drug potencies were derived from concentrations of the nucleosides required to produce 20% inhibition of histamine release (IC_{20}). For adenosine, D-PIA, L-PIA and NECA the geometric mean IC_{20} values were 130.6, 24.8, 11.5 and 1.5 μM respectively. When added 15 min post challenge 0.01–1.0 μM L-PIA, D-PIA, and NECA produced similar concentration-related potention of histamine release as that observed with adenosine (Figure 5). However, while the effects of adenosine, L-PIA and D-PIA in enhancing IgE-dependent histamine release plateaued or decreased at concentrations above 1 μM, the response to NECA continued to 100 μM.

In contrast, the ribose-modified adenosine analogue, DDA inhibited anti-IgE induced histamine release in a concentration-related manner when added either before or after immunological challenge (Figure 6). Inhibition of histamine release by 1 mM DDA was 89.6 ± 0.9% ($P < 0.001$) when added 15 min before and 27.5 ± 1.1% ($P < 0.001$) when added 15 min after anti-IgE challenge in four experiments.

Theophylline The effects of theophylline on the modulation of histamine release by adenosine ($n = 3$), L-PIA ($n = 4$) and NECA ($n = 3$) were examined. Theophylline 50 μM alone had no effect on anti-IgE-induced basophil histamine release. Theophylline, 50 μM, added 5 min before addition of adenosine antagonized both adenosine-induced inhibition and potentiation of histamine release (Figure 7a). With addition of adenosine before immunological challenge, theophylline caused a parallel displacement of the concentration-response curve to the right as assessed by analysis of covariance. Theophylline had a similar effect on the inhibitory responses observed with L-PIA and NECA. Geometric mean dose-ratios for theophylline in inhibiting responses by adenosine, L-PIA and NECA were 5.8, 8.8 and 4.3 respectively. Theophylline also inhibited the potentiation of histamine induced by adenosine, PIA and NECA but because the concentration-response curves for adenosine and L-PIA were bell shaped, it was impossible to calculate dose-ratios. However, theophylline significantly ($P < 0.05$) reduced potentiation of histamine release caused by 1 μM adenosine and L-PIA. The geometric mean dose-ratio for theophylline on NECA-induced potentiation was 5.1

Dipyridamole The inhibition of facilitated adenosine uptake by dipyridamole 1 μM when added 5 min before adenosine, caused a slight but statistically insignificant enhancement of both inhibition and potentiation of histamine release in four experiments (Figure 7b). When added alone, dipyridamole had no significant effect on histamine release.

Cyclic AMP response to adenosine The effect of adenosine, 100 μM, on the production of cyclic AMP was assessed in a leucocyte preparation from a patient with a 40–70% basophilia (Hughes *et al.*, 1983). In two experiments the resting cyclic AMP level of unstimulated cells was 7.8 ± 1.5 pmol 10^{-7} cells. Adenosine induced a rapid rise in cyclic AMP reaching a peak of 179 ± 26% above baseline 30 s after addition after which the levels rapidly returned to resting values (Figure 8).

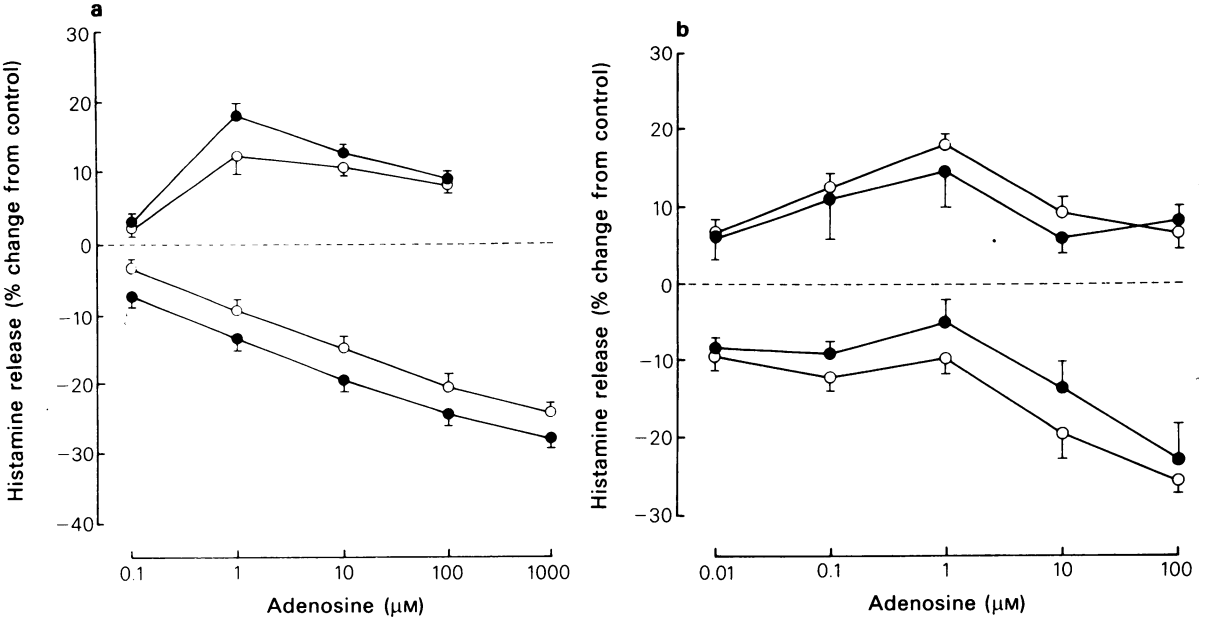

Figure 7 Effects of theophylline and dipyridamole on adenosine modulation of anti-IgE induced histamine release from human basophils. (a) Theophylline 50 μM, or (b) dipyridamole, 1 μM was added 5 min before adenosine. Adenosine was added either 15 min before (inhibition of control histamine release lower section in (a) and (b)) or 15 min after (potentiation of control histamine release upper section in (a) and (b)) challenge with a 1/1000 dilution of anti-IgE. Net histamine releases determined 45 min after challenge were 33.3 ± 2.4% in three experiments with theophylline and 51.4 ± 7.0% in four experiments with dipyridamole. Results are expressed as mean ± s.e.mean percentage change from control histamine release for (●) adenosine alone and (○) adenosine plus (a) theophylline, or (b) dipyridamole.

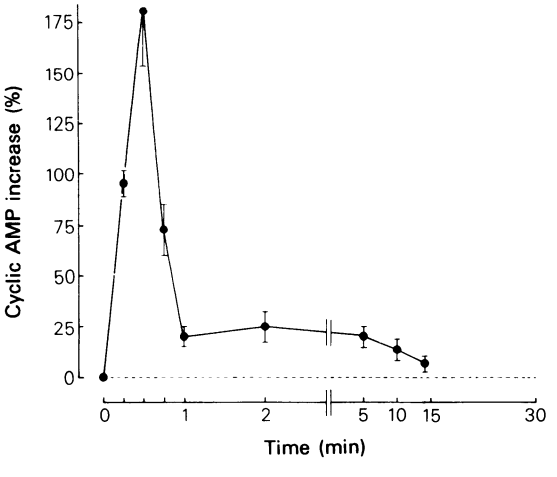

Figure 8 Cyclic AMP response to adenosine in basophil-rich human leucocytes. Results are expressed as the mean percentage increase in cyclic AMP production from control (7.8 ± 1.5 pmol 10^{-7} cells) at the indicated times after the addition of adenosine 100 μM in two experiments.

Discussion

We have demonstrated that adenosine may either inhibit or potentiate anti-IgE-induced histamine release from human basophil leucocytes depending upon the time of its addition with respect to immunological challenge. The effects of adenosine are concentration-related and more pronounced under conditions of mild challenge. We suggest that the majority of these effects result from an interaction of adenosine with cell surface A_2-receptors associated with stimulation of adenylate cyclase and a rise in basophil levels of cyclic AMP.

The inhibitory effect of adenosine on basophil histamine release confirms the observations of Marone *et al.* (1979). However, the capacity of adenosine to potentiate release when added after challenge has not been previously reported. Furthermore, this spectrum of activity is markedly different from rat and guinea-pig mast cells in which adenosine enhances release even when added before challenge (Marquardt *et al.*, 1978; Holgate *et al.*, 1980a; Welton & Simko, 1980).

Evidence that adenosine did not induce its effects

by an action at intracellular P-sites or following its metabolism was gained from the following observations. (1) Dipyridamole, which inhibits facilitated transport of adenosine (Stafford, 1966; Marone, Plaut & Lichtenstein, 1978) enhanced rather than reduced the effects of adenosine. (2) L-PIA and NECA had similar qualitative effects to adenosine but unlike adenosine are not substrates for facilitated uptake, are devoid of P-site activity and are not metabolised (Daly, 1982). (3) DDA, which undergoes facilitated uptake and interacts with intracellular P-sites to inhibit adenylate cyclase but not cell surface adenosine receptors, causes only inhibition of histamine release.

Evidence that the effects of adenosine result from stimulation of cell surface receptors accrued from the use of theophylline, a competitive antagonist of the effects of adenosine at A_1- and A_2-receptors but not its actions on other purine-sensitive sites (Burnstock, 1980). Theophylline, in a concentration that did not modify basophil histamine release in the absence of adenosine, antagonized both inhibition and potentiation of IgE-dependent histamine release caused by adenosine, L-PIA and NECA. These effects are likely to be due to competitive antagonism since the linear portion of the log dose-response curves were displaced to the right in a parallel fashion. We were unable to assess the effects of theophylline on maximal responses because of the low solubility of these nucleosides.

We suggest that the capacity of adenosine both to inhibit and potentiate immunological mediator secretion from human basophils occurs by stimulation of A_2-receptors. The concentrations of adenosine required to produce both effects were in the micromolar range, similar to those reported for A_2-receptor stimulation in other cell systems, whereas A_1-receptors are sensitive to nanomolar concentrations (Van Calker *et al.*, 1979; Londos *et al.*, 1980). (2) The 5'-substituted adenosine analogue NECA was more potent than adenosine or the 6-substituted analogues L-PIA and D-PIA in both inhibiting and potentiating histamine release. A similar order of potency has been observed for stimulating A_2-receptors linked to adenylate cyclase (Van Calker *et al.*, 1979; Londos *et al.*, 1980). Ligand binding studies and isolated tissues have shown L-PIA to be 100 times more potent than D-PIA on A_1-receptors but only 5 times more potent on A_2-receptors (Bruns *et al.*, 1980; Paton, 1981). In the present study L-PIA was as potent or only slightly more potent than D-PIA in potentiating and inhibiting basophil histamine release respectively. Incubation of basophil-rich human leucocytes with adenosine resulted in a rapid transient rise in cellular cyclic AMP levels. Stimulation of adenylate cyclase to produce this effect is an A_2-receptor mediated effect (Wolff *et al.*, 1981; Daly, 1982).

The finding that adenosine added 15 min before challenge reduced IgE-dependent histamine release at all time points after immunological challenge, confirms the modulatory role of cyclic AMP in basophil mediator release. Adenosine's effect in prolonging the rapid phase of mediator secretion when added 15 min after challenge may result from an enhanced early rise in cellular cyclic AMP levels at a crucial phase of mediator release (Holgate *et al.*, 1980a; Ishizaka *et al.*, 1980).

In conclusion, addition of adenosine before immunological challenge inhibits histamine release whereas addition after challenge potentiates release. The primary mechanism appears to be an interaction with cell surface A_2-receptors and stimulation of adenylate cyclase to increase basophil levels of cyclic AMP. Adenosine at physiological concentrations may therefore have a role in regulating IgE-dependent mediator secretion from human basophils *in vivo* through a cyclic AMP-dependent mechanism.

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