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INHIBITION BY ADENOSINE OF HISTAMINE AND LEUKOTRIENE RELEASE FROM HUMAN BASOPHILS*

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Abstract—Adenosine inhibited the release of histamine and leukotriene C_4 (LTC_4) from immunologically-activated basophils in a dose-dependent manner. Structural congeners of adenosine also attenuated the elaboration of these two mediators from stimulated basophils and a rank order of potency for the inhibition was observed following the sequence 2-chloroadenosine ≥ *N*-ethylcarboxamidoadenosine (NECA) > adenosine ≥ *R*-phenylisopropyladenosine (*R*-PIA) ≥ *S*-PIA. These same nucleosides modulated the generation of LTC_4 more potently than the release of histamine. A number of methylxanthines, which are antagonists of cell surface adenosine receptors, reversed the inhibition by adenosine and its congeners of the release of both histamine and LTC_4 to varying extents. Dipyridamole and nitrobenzylthioinosine (NBTI), agents that block the intracellular uptake of adenosine, antagonized the inhibition of histamine release by adenosine (and 2-chloroadenosine) but failed to reverse the attenuation of LTC_4 generation by the nucleoside. These same uptake blockers were unable to antagonize the inhibitory effects of NECA on either histamine or LTC_4 release. In purified basophils, NECA and *R*-PIA, and in that order of decreasing reactivity, increased total cell cyclic adenosine monophosphate (cAMP) levels and inhibited the stimulated release of mediators. In total, these results suggest that the basophil possesses a cell surface adenosine receptor which, on the basis of both pharmacological and biochemical criteria, most closely conforms to an A_2/R_a-like receptor. However, in addition to an interaction at the cell surface, studies with agents that block the intracellular uptake of adenosine suggest that the nucleoside may also exert intracellular effects when countering the release of histamine (but not LTC_4).

Studies of the effects of adenosine on various biological processes have taken some prominence in recent years [1]. The interest provoked is warranted since the nucleoside exists naturally and has been implicated in the pathophysiology of a number of disease states [2] and as a modulator of the activity of various inflammatory cell types [3].

Two cell surface adenosine receptors, namely the A_1R_i and A_2/R_a sites, and an intracellular P site for adenosine have been described [4–6]. These sites are considered to be intimately associated with the adenylate cyclase complex, and their classification has been established on the grounds of both pharmacological and biochemical criteria. The use of various structurally-modified analogues of adenosine has proven useful in this context [5–7]. Thus, the modulation of cell function by nanomolar concentrations of adenosine and the demonstration that modified analogues display an order of reactivity following the series *R*-phenylisopropyladenosine (*R*-PIA§) > *N*-ethylcarboxamidoadenosine (NECA) is suggestive of an interaction at an A_1/R_i receptor site. Activation of an A_1/R_i receptor is usually associated with a reduction in adenylate cyclase activity. In a situation where micromolar concentrations of adenosine activate adenylate cyclase and NECA is more potent than *R*-PIA, the presence of an A_2/R_a receptor is indicated. The antagonism of an adenosine effect by low concentrations of methylxanthines is indicative of a process mediated at cell surface adenosine receptors [8]. Unfortunately, subtype-specific cell surface receptor antagonists are not currently available. Responses to adenosine in the millimolar range, which are attenuated by uptake inhibitors such as dipyridamole, imply an intracellular interaction [9]. In cases where dideoxyadenosine mimics the adenosine effect and reduces adenylate cyclase activity, then a P-site interaction may be operative [4].

Previous studies have demonstrated that adenosine inhibits mediator release from human basophils [10–13]. Although this inhibitory effect most probably involves an A_2/R_a-like interaction, a more recent report indicates that adenosine may also

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§ Abbreviations: cAMP, cyclic adenosine monophosphate; DPX, 1,3-diethyl-8-phenylxanthine; DMSO, dimethyl sulfoxide; HSA, human serum albumin; IgE, immunoglobulin E; ^{125}I.Sc. TME cAMP, iodinated succinyltyrosine methylester derivative of cyclic adenosine monophosphate; LTC_4, leukotriene C_4; NECA, *N*-ethylcarboxamideadenosine; NBTI, nitrobenzylthioinosine; PIPES, piperazine-*N*,*N*′-bis ethanesulfonic acid; *R*-PIA and *S*-PIA, *R*-and *S*-phenylisopropyladenosine; 8-PT, 8-phenyltheophylline; and 8-SPT, 8-*p*-sulfophenyltheophylline.

possess intracellular effects [13]. The present study was undertaken to evaluate more closely the nature of the regulation by adenosine of basophil function.

MATERIALS AND METHODS

Buffers. PAG buffer contained: piperazine-*N, N'*-bis 2-ethanesulfonic acid (PIPES), 7.6 g/L; NaCl, 6.4 g/L; glucose, 1 g/L; KCl, 0.37 g/L; 10 N NaOH, 4.2 ml/L; and human serum albumin (HSA), 30 mg/L. The pH was adjusted to 7.3. PAGCM is PAG with $CaCl_2 \cdot 2H_2O$, 0.14 g/L, and $MgCl_2 \cdot 6H_2O$, 0.20 g/L.

PBS buffer contained (g/L): NaCl, 8; Na_2HPO_4, 1.15; KCl, 0.2; KH_2PO_4, 0.2; $CaCl_2 \cdot 2H_2O$, 0.14; $MgCl_2 \cdot 6H_2O$, 0.20; glucose, 1; and HSA, 0.03. The pH was titrated to 7.3.

Basophil isolation and purification. Basophils were partially purified (3–12% purity) from the venous blood of both atopic and non-atopic individuals employing discontinuous Percoll gradients as previously described [14]. This procedure removes neutrophils from the cell preparation. Neutrophils have been shown not only to generate adenosine *in vitro* [15] but may also influence the stimulated generation of leukotriene C_4 (LTC_4) from basophils (unpublished observations). Alternatively, basophils were purified (75–92%) from "buffy coat" cell packs from healthy volunteers undergoing hemapheresis. Purification was performed according to a modification of the method described by De Boer and Roos [16]. In brief, white blood cell enriched packs were reconstituted in PAG, and loaded onto a countercurrent elutriator (model J2-21, Beckman Instruments Inc., Fullerton, CA). Several fractions were collected, and those fractions containing basophils in large numbers and of improved purity (>5%), as determined by alcian blue staining [17], were further purified over discontinuous Percoll gradients.

Histamine release. Histamine release experiments were performed in either PAGCM (impure basophils) or PBS (purified cells). Histamine release was initiated immunologically (anti-IgE or ragweed antigen E) and secretion was allowed to proceed for 45 min at 37° after which time the cells were pelleted by centrifugation (1000 *g*, room temperature, 3 min). Histamine released into the supernatant fraction was determined by the automated fluorometric method of Siraganian [18] and, when appropriate, an aliquot of the supernatant fraction was removed and stored frozen for LTC_4 analysis. In experiments with dipyridamole, histamine measurements of the supernatant fraction could not be performed, since it has been shown that dipyridamole interferes with the fluorometric [10] assay. To circumvent this, the supernatant fraction was discarded, and residual cell-associated histamine was determined by lysing the pellet with 1.6% perchloric acid. Histamine release into the supernatant fraction could then be calculated by subtracting the residual histamine associated with the cell pellet from the total histamine content. Total histamine content was determined by lysing aliquots of the cells with 1.6% perchloric acid. Cells incubated in buffer alone served as a measure of spontaneous histamine release (<6% in impure basophils, 3–13% in purified basophils). Histamine release was thus expressed as a percentage of the total histamine content after subtracting the spontaneous histamine release. All experiments were performed in duplicate.

Nucleosides were incubated at 37° for 15 min with cells before the addition of stimulus unless otherwise stated in the text. When either the methylxanthines or the adenosine uptake blockers were used to investigate a potential antagonism of a nucleoside-related effect, cells were preincubated for 5 min with either class of antagonist prior to coincubation with nucleoside for a further 15 min and then challenged.

LTC_4 assay. LTC_4 was determined by radioimmunoassay by a method described previously [19, 20].

Preparation of samples for cAMP analysis. Measurements of total cyclic adenosine monophosphate (cAMP) were performed in purified (75–92%) basophil preparations in PBS buffer and in parallel with mediator release determinations. In brief, samples were processed as follows. Cells ($0.75–1 \times 10^5$) were incubated at 37° in buffer or in the presence of a nucleoside and/or antagonist for the appropriate time interval (as indicated in the text) in a total volume of 100 μl. The reaction was terminated by the addition of ice-cold acidified ethanol, and the mixture was vortexed vigorously (briefly) and snap frozen in liquid nitrogen (Bay State, Hagerstown, MD). After thawing, cellular debris was pelleted in a microfuge (15,000 *g*, 4°, 3 min) and the supernatant fraction was removed and evaporated to dryness under reduced pressure (Savant Instruments Inc., Farmingdale, NY). The dried sample was reconstituted in assay buffer and stored frozen until the assay was performed. Total cell cAMP measurements were performed by a double antibody radioimmunoassay with prior acetylation of samples as previously described [21]. The competing ligand was the iodinated succinyltyrosine methylester derivative of cAMP (^{125}I.Sc.TME cAMP, specific activity 2200 Ci/mmol). Since adenosine cross-reacts (and NECA, very slightly) in the cAMP assay when samples are acetylated, on occasion the standard cAMP assay was performed which required approximately ten times as many cells (1×10^6) per sample. All cAMP determinations were performed in duplicate.

Materials. The following items were purchased: NECA, *R*-PIA and *S*-PIA (Boehringer Mannheim, Indianapolis, IN); HSA (CalBiochem, LaJolla, CA); PIPES, theophylline, 8-phenyltheophylline (8-PT), dipyridamole, nitrobenzylthioinosine (NBTI), dimethyl sulfoxide (DMSO), deoxycoformycin, 2-chloroadenosine and adenosine (Sigma, St. Louis, MO); ^{125}I.Sc.TME cAMP (New England Nuclear, Boston, MA); Percoll (Pharmacia, Piscataway, NY); 1,3-diethyl-8-phenylxanthine (DPX) and 8-*p*-sulfophenyltheophylline (8-SPT) (Research Biochemicals Inc., Natick, MA).

The following items were gifts: goat anti-human IgE (IgG fraction, Dr. D. W. MacGlashan, Jr., Johns Hopkins University); LTC_4 standard (Dr. J. Rokach, Merck-Frosst), anti-LTC_4 (Dr. E. Hayes, Merck Institute); and anti-cAMP (Dr. T. Ishizaka, Johns Hopkins University).

Adenosine, 2-chloroadenosine, NECA, theophylline and 8-SPT were prepared fresh daily by straight

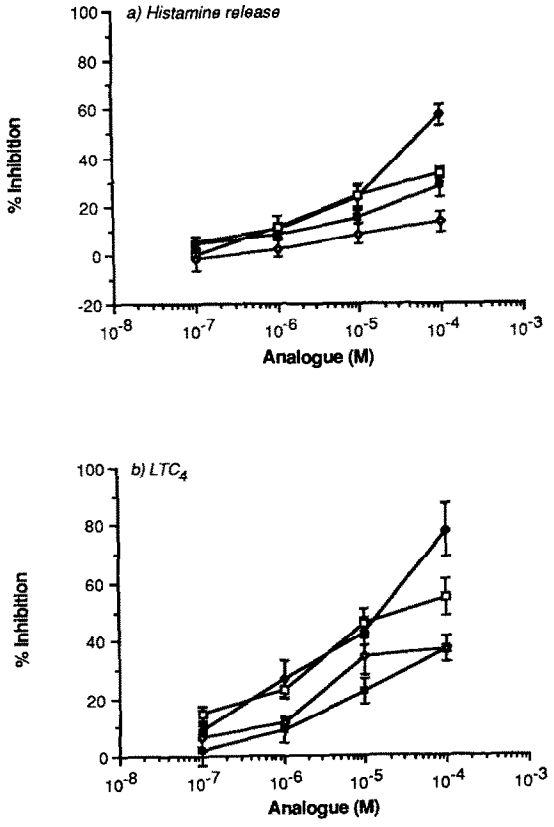

Fig. 1. Effect of 2-chloroadenosine (◆), NECA (□), adenosine (■) and *R*-PIA (◇) on the release of histamine (a) and LTC_4 (b) from basophils activated with antigen E (0.3 ng/ml). Basophils 5 ± 1% purity were preincubated for 15 min with nucleosides before challenge. Control releases were 54 ± 8% for (a) and 70 ± 4 ng/10^6 basophils for (b), Values are means ± SE, N = 4.

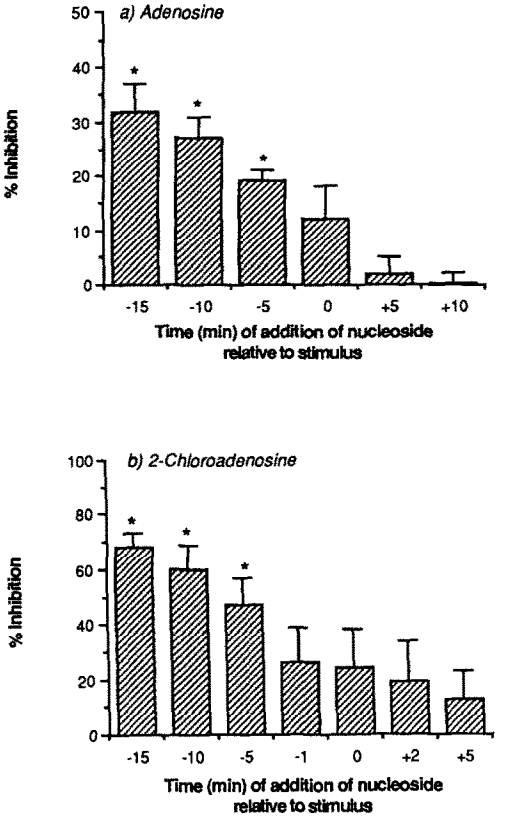

Fig. 2. Effect of incubation time on the inhibition of basophil histamine release by adenosine (100 μM, a) and 2-chloroadenosine (300 μM, b). The nucleosides were incubated with leukocytes either before (−) or after (+) addition of anti-IgE (0.1 μg/ml) or introduced simultaneously (0) with the stimulus. Control histamine releases were 34 ± 6% for (a) and 41 ± 2% for (b). Statistically significant levels of inhibition ($P < 0.05$ vs control) are indicated by an asterisk. Values are means ± SE, N = 4 for (a) and for (b).

dissolution into buffer as 1 mM solutions (NECA with vigorous vortexing). 8-PT (10 mM), dipyridamole (10 mM), DPX (10 mM), NBTI (100 mM), *R*-PIA (100 mM) and *S*-PIA (100 mM) were dissolved in DMSO and prepared fresh daily. In preliminary experiments, the concentrations of DMSO in working dilutions used experimentally had no effect on either histamine release or the LTC_4 assay.

Statistics. The statistical significance of nucleoside-related effects was analyzed by comparing control and treated cells using Student's *t*-test for paired data. Values were considered significant at the $P < 0.05$ level.

RESULTS

Inhibition by adenosine and analogues of mediator release from basophils. The release of the preformed mediator histamine (Fig. 1a) and the *de novo* generation of LTC_4 (Fig. 1b) from leukocyte preparations activated with ragweed antigen E (0.3 ng/ml) were inhibited by adenosine and its analogues (10^{-7}–10^{-4} M). The inhibition observed was dose dependent, and the nucleosides were rather more active in modulating the production of LTC_4 than the release of histamine. The extent to which secretion was inhibited by the nucleosides (and by adenosine in particular) varied among leukocyte preparations and was inversely related to the magnitude of the control release (data not shown). A rank order of potency for the inhibition of mediators was evident as determined from approximate IC_{25} (that concentration of nucleoside required to inhibit either histamine (HR) or LTC_4 release by 25%) values following the series: 2-chloroadenosine (IC_{25}^{HR}; IC_{25}^{LTA}: 11 μM; 0.9 μM) ≥ NECA (13; 1.2) > adenosine (50; 16) ≥ *R*-PIA (>100; 4). In a similar series of experiments (N = 6), in which anti-IgE (0.03 μg/ml) was used as the stimulus instead of antigen, the following rank order of potency was established (control histamine release was 33 ± 8%): 2-chloroadenosine (IC_{25}^{HR}; 14 μM) > NECA (37) > adenosine (80) > *R*-PIA (>100) ≥ *S*-PIA (>100). It should be stressed that the estimations of IC_{25} values are necessarily approximated because, especially in the cases of adenosine, *R*-PIA and *S*-PIA, maximal inhibition was not attained rendering any conclusions concerning rank order of

Table 1. Effect of deoxycoformycin (1 μM) on adenosine inhibition of histamine release*

Adenosine (μM)	% Inhibition of histamine release	
	−Deoxycoformycin	+Deoxycoformycin
10	17 ± 4	23 ± 5
30	25 ± 2	25 ± 4
100	26 ± 4	29 ± 5
300	33 ± 7	33 ± 4

* Values are the percent inhibition of the control histamine release which was 25 ± 4% of total histamine both in the presence and absence of deoxycoformycin. Basophils of 8 ± 1% purity were incubated with or without deoxycoformycin for 5 min and then for a further 15 min with adenosine prior to challenge with anti-IgE (0.03 μg/ml). Values are means ± SE and are based on four experiments.

potency somewhat tenuous. Despite these caveats, the data are, as a whole, concordant with the view that the basophil possesses an A_2/R_a-like cell surface adenosine receptor [5, 6].

Incubation time and inhibition of secretion. Prolonging the incubation time period (5, 10 or 15 min at 37°) of leukocytes with adenosine (100 μM; Fig. 2a) or 2-chloroadenosine (300 μM; Fig. 2b) prior to activation with anti-IgE (0.1 μg/ml) led to progressive increases in the inhibition of histamine release that were statistically significant ($P < 0.05$). The simultaneous addition of 2-chloroadenosine or adenosine with stimulus or the introduction of either nucleoside after the application of the stimulus led to modest but not statistically significant levels of inhibition ($P > 0.05$). NECA (30 μM) also displayed incubation-dependent characteristics similar to those of adenosine and 2-chloroadenosine (data not shown, N = 2).

Effects of uptake blockers and deoxycoformycin on the inhibition of secretion by adenosine analogues. That adenosine and 2-chloroadenosine may be inhibiting histamine release, in part, by intracellular mechanisms was revealed by studies with uptake blockers. The adenosine uptake blocker dipyridamole (1 μM) antagonized the inhibition by adenosine of histamine release from leukocytes challenged with ragweed antigen E (0.3 ng/ml), the antagonism being particularly evident at higher concentrations of adenosine (Fig. 3a). Dipyridamole failed to reverse the inhibition by adenosine of LTC_4 generation, however (Fig. 3b). Similar results for the antagonism by dipyridamole (1 μM) of the inhibition by 2-chloroadenosine (Fig. 4a) and adenosine (Fig. 4c) of histamine release induced by anti-IgE (0.1 μg/ml) were observed. In contrast, dipyridamole (data not shown, N = 4) and NBTI (Fig. 4d) failed to reverse the inhibition by NECA (an agonist not known to possess intracellular effects) of either the stimulated release of histamine or LTC_4 (data not shown, N = 3) when, in these same experiments, both antagonists were found to reverse the inhibitory effects of either adenosine or 2-chloroadenosine. Furthermore, NBTI (1 μM) reversed the inhibition by 2-chloroadenosine of anti-IgE-induced histamine release (Fig. 4b). Higher concentrations (3 or 10 μM) of NBTI failed to improve significantly upon the antagonism observed for 1 μM NBTI (data not shown, N = 4).

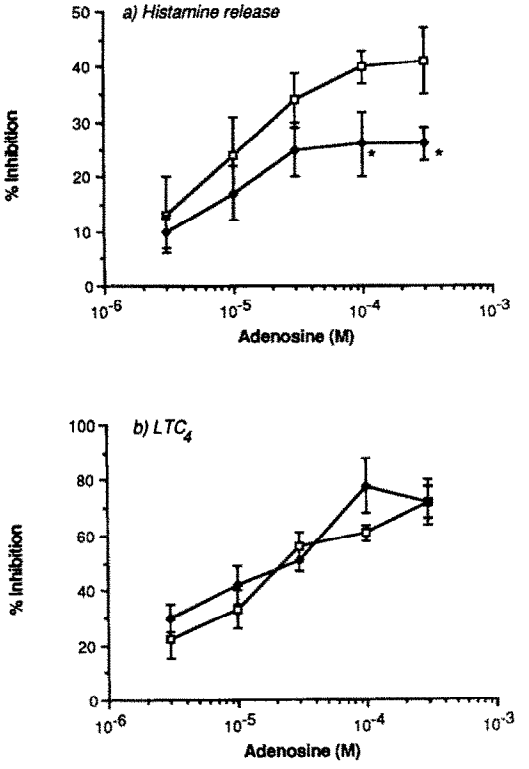

Fig. 3. Effect of dipyridamole (1 μM) on the adenosine-mediated inhibition of the release of histamine (a) and LTC_4 (b) from basophils activated with antigen E (0.3 ng/ml). Basophils of 5 ± 1% purity were incubated with (◆) or without (□) dipyridamole for 5 min and then for a further 15 min with adenosine before challenge. (a) Control histamine releases were (□) 43 ± 9%) and (◆), 45 ± 9%. (b) Control LTC_4 releases were (□) 22 ± 5 ng/10^6 basophils; and (◆) 21 ± 6 ng/10^6 basophils. Those points on the dipyridamole curve that were significantly different ($P < 0.05$) statistically from control inhibition are indicated by an asterisk. Values are means ± SE, N = 5.

The irreversible inhibitor of adenosine deaminase, deoxycoformycin (1 μM), was found not to influence significantly the inhibition of histamine release by adenosine (Table 1).

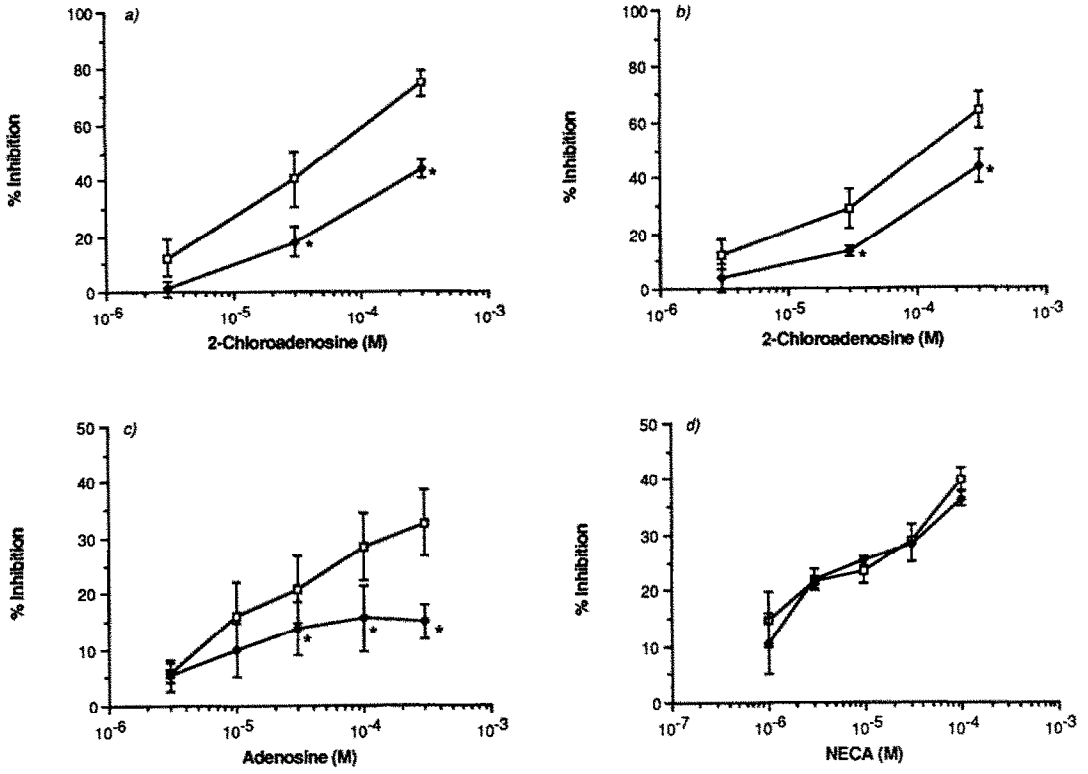

Fig. 4. Effects of NBTI (1 μM) and dipyridamole (1 μM) on the nucleoside-mediated inhibition of histamine release from basophils activated with anti-IgE (0.1 μg/ml). Basophils of 5–8% purity were incubated with (◆) or without (□) either uptake blocker for 5 min and then for a further 15 min with a nucleoside before challenge. (a) Effect of dipyridamole on the inhibition by 2-chloroadenosine. Control histamine releases were (□) 32 ± 7%; and (◆) 30 ± 5%. (b) Effect of NBTI on the inhibition by 2-chloroadenosine. Control histamine releases were (□) 25 ± 10%; and (◆) 27 ± 10%. (c) Effect of dipyridamole on the inhibition by adenosine. Control histamine releases were (□) 36 ± 6%; and (◆) 38 ± 7%. (d) Effect of NBTI on the inhibition by NECA. Control histamine releases were (□) 45 ± 1%; and (◆) 46 ± 3%. Those points on the antagonist curves that were statistically ($P < 0.05$) different from control inhibition are indicated by an asterisk. Values are means ± SE, N = 4, 4, 6 and 3 for (a), (b), (c) and (d) respectively.

Effects of methylxanthines on the inhibition of secretion by adenosine analogues. Attempts to reverse the nucleoside inhibition of IgE-triggered mediator release with cell surface adenosine receptor antagonists (methylxanthines) met with variable results (Table 2). Theophylline (10 μM) was moderately more effective as an antagonist of the nucleoside-associated inhibition of antigen-induced histamine release than secretion initiated by anti-IgE (Table 2). Attempts to antagonize further nucleoside inhibition by employing higher concentrations of theophylline (30 or 100 μM) were thwarted because the methylxanthine possessed inhibitory properties of its own at these higher concentrations (data not shown, N = 4). The more specific and potent cell surface adenosine antagonists 8-PT and DPX [22] were more active than theophylline (Table 2). Thus, 8-PT antagonized, in a competitive fashion, the inhibition by 2-chloroadenosine of the antigen-driven release of both histamine (Fig. 5a) and LTC_4 (Fig. 5b). This was reflected by an approximately 3-fold rightward shift of the linear portions of the 2-chloroadenosine dose-inhibition plots in the presence of 8-PT. The polar and, presumably, exclusively extracellular antagonist, 8-SPT [7], was also a very effective antagonist of the 2-chloroadenosine inhibition of histamine release (Table 2).

Effects of nucleosides on total cell cAMP levels. The analogues (30 μM) NECA and *R*-PIA raised total cell cAMP levels in purified preparations of basophils. Approximately half-maximal elevations in cAMP were observed 1 min after the addition of nucleoside and maximal increases were observed at 5 or 15 min and, in four out of five experiments, these increased levels in cyclic nucleotide were maintained elevated for up to 30 min (data not shown).

Elevations in cAMP correlated well with the inhibition of mediator release. Thus, NECA, which induced more pronounced increases in cyclic nucleotide than *R*-PIA (Fig. 6a), was a more effective inhibitor of mediator release (Fig. 6b). In a separate series of experiments (N = 4), adenosine (100 μM) elevated cAMP (66 ± 10% enhancement over basal levels) and inhibited the release of histamine and LTC_4 (32 ± 7 and 53 ± 8% inhibition respectively) in basophils (purity, 82 ± 4%) stimulated with anti-IgE (0.1 μg/ml).

Table 2. Effects of methylxanthines on the nucleoside inhibition of histamine release*

	IC_{25} values (μM)						
	Adenosine/ Theoph†	Adenosine/ Theoph	Adenosine/ 8-PT	NECA/ Theoph†	NECA/ DPX	2-Chloroad/ 8-PT†	2-Chloroad/ 8-SPT
Agonist	10 ± 2	113 ± 30	100 ± 33	11 ± 4	14 ± 2	5 ± 2	11 ± 3
Agonist + Antagonist	16 ± 3	137 ± 50	171 ± 15	14 ± 5	40 ± 20	16 ± 1	68 ± 7
P	NS	NS	<0.05	NS	NS	<0.025	<0.005
N	5	5	4	4	3	4	4

* IC_{25} values (μM) for the inhibition of histamine release are expressed as means ± SE of the indicated number of experiments. Basophils of 4–10% purity were incubated with or without either theophylline (10 μM), DPX (3 μM), 8-PT (1 μM) or 8-SPT (10 μM) for 5 min and then for a further 15 min with nucleosides (0.3 to 300 μM) and then activated with anti-IgE (0.03 or 0.1 μg/ml) except in those experiments indicated by a dagger (†) where antigen E (0.3 ng/ml) was used. To establish whether the inhibition by a nucleoside in the presence of a methylxanthine was statistically different from control, P values were determined (NS means not significant).

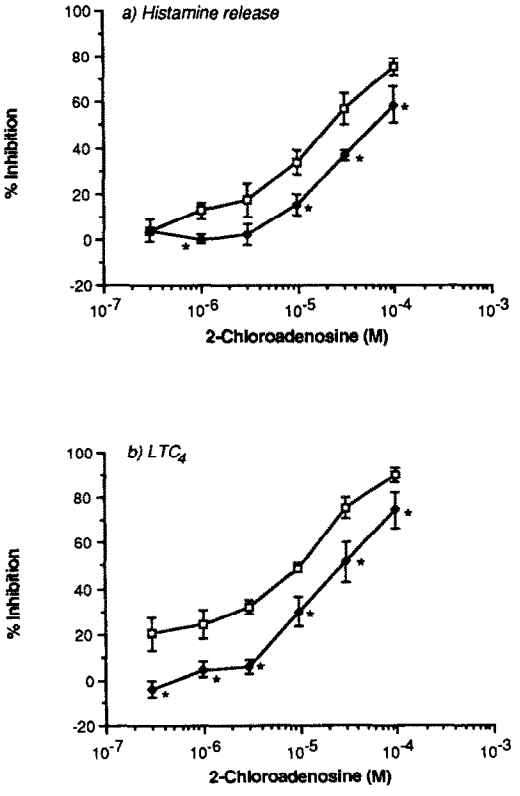

Fig. 5. Effect of 8-PT (1 μM) on the 2-chloroadenosine-mediated inhibition of the release of histamine (a) and LTC_4 (b) from basophils activated with antigen E (0.3 ng/ml). Basophils of 8 ± 2% purity were incubated with (◆) or without (□) 8-PT for 5 min and then for a further 15 min with adenosine before challenge. (a) Control histamine releases were (□) 37 ± 4%; and (◆) 34 ± 3%. (b) Control LTC_4 releases were (□) 33 ± 12 ng/10^6 basophils; and (◆) 30 ± 10 ng/10^6 basophils. Those points on the 8-PT curve that were statistically ($P < 0.05$) different from control inhibition are indicated by an asterisk. Values are means ± SE, N = 4.

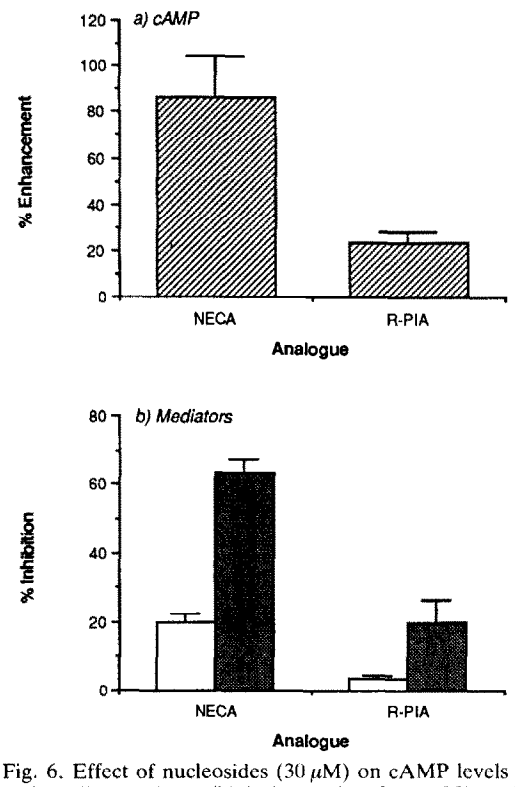

Fig. 6. Effect of nucleosides (30 μM) on cAMP levels (a) and mediator release (b) in basophils of 81 ± 2% purity. (a) Total cell cAMP levels were measured in basophils following a 15-min exposure to either NECA or *R*-PIA. Values reflect the percent enhancement in cAMP over the basal cAMP level which was 0.7 ± 0.2 pmol/10^6 cells. (b) Basophils were preincubated with either nucleoside for 15 min and then activated with anti-IgE (0.1 μg/ml). Inhibition of the release of histamine (open bars) and LTC_4 (shaded bars) is shown. Control histamine release was 35 ± 7% and control LTC_4 release was 12 ± 2 ng/10^6 basophils. Values are means ± SE, N = 8 for (a) and N = 6 for (b). In two of the eight cell preparations mediator release did not occur.

DISCUSSION

In its more recently defined capacity as an autacoid, adenosine has been variously described as an endogenous regulator of a large number of biological processes [1, 23]. This multifarious activity is, perhaps, not surprising since adenosine is distributed widely and various metabolic pathways exist for its synthesis and degradation [7]. Adenosine has been implicated in a number of pathophysiological conditions, most notably bronchoconstriction and hypertension [2] and the nucleoside has also been found to modulate the activity of a number of inflammatory cell types [3].

In accord with previous findings [10–13], adenosine was found to inhibit, in a dose-dependent manner, the release of histamine from basophils stimulated immunologically. There was, however, substantial variation in the inhibitory activity of adenosine among different leukocyte preparations. The inhibition was also largely influenced by the magnitude of the control release; lower levels of secretion were attenuated more effectively by the nucleoside. Moreover, histamine release initiated by antigen was more susceptible to the inhibitory effects of adenosine (and its analogues) than that induced by anti-IgE. This enhanced sensitivity of the antigenic stimulus to modulation by adenylate cyclase agonists has been recognized previously [24] and is consistent with the notion that antigen and anti-IgE constitute mechanistically similar but distinct stimuli [25]. The IgE-mediated generation of LTC_4 was also attenuated by adenosine. The elaboration of this newly-synthesized mediator was more potently modulated than that of the pre-formed mediator, histamine—a finding that suggests that the biochemical processes (e.g. activation of phospholipase A_2, lipoxygenase) leading to the products of arachidonate metabolism may be more sensitive to regulation by the nucleoside. A series of structurally-modified analogues of adenosine were also active and an approximate rank order of potency for the inhibition of the stimulated release of mediators was established following the series, 2-chloroadenosine $\geqslant$ NECA $>$ adenosine $\geqslant$ *R*-PIA. The fact that NECA was at least four times more active than *R*-PIA suggests that the basophil possesses an A_2/R_a-like receptor [5, 6].

A previous report [11] has shown that preincubation of leukocytes with adenosine before challenge inhibits mediator release from basophils, whereas addition of the nucleoside shortly after stimulation leads to modest levels of potentiation. In the present study, no such disparate time-related responses with incubations post-challenge were observed for adenosine, 2-chloroadenosine or NECA. Rather, the nucleosides either inhibited slightly or had no effect on mediator release when added after challenge. The discrepancy in results could possibly be related to experimental design. Prolonged incubation of basophils in the presence of calcium can decrease the sensitivity of the cell to subsequent immunological activation. In a series of pilot studies, where secretion was initiated with anti-IgE either immediately or after a 15-min incubation in the presence of calcium, histamine release was found to be 43 ± 2 and 32 ± 3% respectively. Thus, if the experimental design were such that cells incubated in each condition did not receive the stimulus at approximately the same time, then a small change in histamine release in the presence of nucleoside could be interpreted as an inhibition or potentiation of secretion dependent on which control release was employed as the index for change.

The adenosine uptake blocker, dipyridamole, partially antagonized the adenosine inhibition of the stimulated release of histamine, implying that part of the activity of the nucleoside is mediated at an intracellular locus. Similar results have been reported by Hughes *et al.* [13] although in their study a longer incubation (60 min) period of adenosine with cells was required before an antagonism of the inhibition by dipyridamole could be observed. Of all the analogues tested, 2-chloroadenosine might be expected to mimic the effects of adenosine most closely since it too may possess intracellular effects, unlike NECA and *R*-PIA which are considered to be exclusively surface receptor agonists [4]. The inhibition of histamine release by 2-chloroadenosine was reversed by both dipyridamole and NBTI whereas the inhibitory effects of NECA were not modified by either antagonist. That high concentrations of NBTI failed to abolish completely the 2-chloroadenosine inhibition suggests that the agonist does not impede histamine release via mechanisms that are solely intracellular in nature. A finding of considerable interest is that dipyridamole did not antagonize the inhibition of LTC_4 generation by adenosine, suggesting that the modulation of peptidoleukotriene release by the nucleoside can be accounted for entirely by surface-related interaction.

As indicated above, the inhibitory activity of adenosine was found to vary appreciably from one leukocyte preparation to another. This suggested that the complicating presence of adenosine deaminase in the incubation medium may influence the activity of adenosine. This, however, was not found to be the case since the irreversible inhibitor of adenosine deaminase, deoxycoformycin, at a concentration expected to inactivate the enzyme completely [26], did not modify the adenosine inhibition of histamine release.

In the present study, theophylline was a poor antagonist of the inhibitory effects of adenosine analogues. The apparent lack of activity for theophylline may result from the need to use high concentrations of adenosine to produce an effective inhibition. This notion is supported by the following evidence. When the inhibitory effects of adenosine are optimized by using sub-optimal concentrations of ragweed antigen E and basophil activation is "staged" (see Ref. 10), theophylline is a more effective antagonist of adenosine. In the present study, attempts to demonstrate a more pronounced reversal by increasing the concentration of theophylline ($>10\,\mu M$) were uninterpretable since the methylxanthine possessed inhibitory properties of its own at these higher concentrations, presumably resulting from an inhibition of phosphodiesterase activity. It should be noted that theophylline is not considered a good cell surface adenosine receptor antagonist [22], and a number of investigators have experienced problems when

attempting to antagonize a nucleoside interaction at a putative A_2/R_a receptor with the methylxanthine [27, 28]. When the more potent and specific antagonist 8-PT was employed [26], a compound which is thought to be without any significant phosphodiesterase activity [29], a more pronounced reversal of the inhibition by nucleosides of LTC_4 generation and histamine release was observed whether anti-IgE or antigen was used as the stimulus. The polar adenosine antagonist 8-SPT, which is unlikely to penetrate the cell membrane and interact with intracellular phosphodiesterase [7], was also a very effective antagonist of the 2-chloro-adenosine inhibition of histamine secretion.

Studies with purified cell preparations demonstrate that nucleosides increase total cell cAMP levels in a manner consistent with the basophil possessing an A_2/R_a receptor. Thus, NECA was more effective that *R*-PIA at both elevating cAMP levels and inhibiting the release of LTC_4 and histamine. Previous studies have shown that the extent to which secretion is impeded is related to the ability of an adenylate cyclase agonist to increase cAMP and to maintain those elevated levels [30]. The mechanism by which the parent compound, adenosine, inhibits mediator release would appear to be less obvious. Adenosine also elevated cAMP levels, a finding consonant with activation of an A_2/R_a receptor. In addition to this interaction, studies with the purinergic transport inhibitors in impure cell preparations imply that the inhibitory effects of adenosine are exerted intracellularly, in part. Since both the extracellular and intracellular interactions of adenosine are inhibitory, the latter interaction is unlikely to be at the P-site since this would lead to an inhibition of adenylate cyclase and counteract the activation of adenylate cyclase associated with an A_2/R_a-directed process. Whether the intracellular properties of adenosine are also mediated by cAMP, as has been suggested by a recent report [13], is not known. Certainly, appropriate experimental maneuvers, such as an excess of adenosine receptor antagonist or uptake inhibitor, may allow a more careful evaluation of the intracellular or extracellular processes, respectively, that mediate the effects of certain adenosine analogues.

To conclude, adenosine inhibited mediator release from stimulated basophils. Whilst a large body of evidence suggests that the basophil possesses an A_2/R_a-like receptor, the adenosine regulation of basophil function may involve interactions not only at this cell surface receptor but at some undetermined intracellular locus as well.

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