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Studies on the receptor mediating cyclic AMP-independent enhancement by adenosine of IgE-dependent mediator release from rat mast cells

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1 Adenosine produced a concentration-related enhancement of antigen-induced 5-hydroxytryptamine (5-HT) release from rat serosal mast cells. This potentiation was maximal following the simultaneous addition of adenosine with antigen.

2 Enhancement of 5-HT release was accompanied by potentiation of the adenosine 3′:5′-cyclic monophosphate (cyclic AMP) response to challenge. The cyclic AMP response, which was antagonized by 8-phenyltheophylline, was characterized as an A_2-purinoceptor-mediated effect by the use of 5′-N-ethylcarboxamideadenosine (NECA) and L-N^6-phenylisopropyladenosine (L-PIA).

3 Enhancement of 5-HT release, conversely, was not blocked by 8-phenyltheophylline suggesting it to be mediated by a cyclic AMP-independent mechanism.

4 The effect of adenosine on 5-HT release was not reduced by the inhibition of the facilitated uptake of adenosine with dipyridamole, hexobendine or *p*-nitrobenzylthioguanosine, therefore, suggesting it to be mediated by a cell surface receptor.

5 The receptor mediating enhancement of 5-HT does not appear to belong to the P_2-purinoceptor subtype as adenosine was more potent than both adenosine monophosphate (AMP) and adenosine diphosphate (ADP) and α,β-methylene ATP was inactive. Furthermore, the effects of AMP were blocked by α,β-methylene ADP, which inhibits the conversion of AMP to adenosine.

6 Adenosine, NECA, L- and D-PIA were all of equal potency in enhancing 5-HT release. Inosine and 3-deazaadenosine were also active. The rank order of potency of these adenosine analogues is not consistent with an effect at A_1- or A_2-purinoceptors.

7 There appear to be two adenosine receptors on rat mast cells, an A_2-purinoceptor which stimulates adenylate cyclase and a separate purinoceptor, stimulation of which produces enhancement of mediator release by an unknown mechanism. The effects mediated by these receptors appear to be independent of each other.

Introduction

Adenosine enhances IgE-dependent mediator release from rat serosal mast cells (Marquardt *et al.*, 1978; Holgate *et al.*, 1980; Burt & Stanworth, 1983; Nishibori *et al.*, 1983; Church & Hughes, 1985; Vardey & Skidmore, 1985). This effect has been suggested to result from the interaction of adenosine with cell surface A_2-purinoceptors to activate adenylate cyclase (Van Calker *et al.*, 1979; Londos *et al.*, 1980). Evidence in support of this suggestion is derived from the following observations: (1) adenosine analogues which interact with cell surface purinoceptors also enhance mediator release (Holgate *et al.*, 1980; Burt & Stanworth, 1983; Nishibori *et al.*, 1983); (2) enhancement by L-N^6-phenylisopropyladenosine (L-PIA) is paralleled by its ability to potentiate the early transient rise in adenosine 3′:5′-cyclic monophosphate (cyclic AMP) induced by immunological challenge (Holgate *et al.*, 1980); (3) the 'P-site', inhibitor of adenylate cyclase, 2′,5′-dideoxyadenosine (2′,5′-DDA), reduces mediator secretion in parallel with its ability to attenuate the IgE-dependent cyclic AMP response (Holgate *et al.*, 1980).

Adenosine also modulates histamine release from human mast cells and basophils (Church *et al.*, 1983; Hughes *et al.*, 1984; Hillyard *et al.*, 1984) but the characteristics of the effect are different from those of

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the rat. In rat mast cells, preincubation with adenosine potentiates release whereas in human mast cells and basophils it inhibits, enhancement only occurring with the addition of adenosine at or after the time of challenge. As this suggests different mechanisms for the effect of adenosine in rat and human cells, we have re-examined the adenosine-induced enhancement of mediator release from rat serosal mast cells, particularly the nature of the receptor mediating this effect, and the relationship between cyclic AMP and the enhancement of release.

Methods

Rat serosal mast cells were obtained by peritoneal and pleural lavage of male Sprague-Dawley rats and the cells were suspended in calcium- and magnesium-free HEPES buffered salt solution ($HBSS^-$) of the following composition (mM): NaCl 137, KCl 2.7, NaH_2PO_4 0.4, glucose 5.5, N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) 10, foetal calf serum 1% (v/v) and heparin 5 u ml^{-1}, pH 7.4. The mast cells were purified to >90% homogeneity by centrifugation through Percoll (1.09 g ml^{-1}) at 500 *g* for 15 min. Cells were passively sensitized by incubation for 2 h at 37°C with 1 ml of either 50 ng ml^{-1} monoclonal anti-dinitrophenol$_{13}$-human serum albumin IgE (anti-DNP-HSA, kindly donated by Dr T. Ishizaka, Baltimore, U.S.A.) or 20% rat anti-dinitrophenol-ovalbumin serum (anti-DNP-ovalbumin) in HBSS ($HBSS^-$ supplemented with $CaCl_2$ 1.0 mM, $MgCl_2$ 0.5 mM and deoxyribonuclease 50 ng ml^{-1}). During this time mast cells were also labelled with [^{3}H]-5-hydroxytryptamine (5-HT) by inclusion of 5-[1,2-^{3}H(N)]-hydroxytryptamine, 5 μCi per 10^6 cells. After thorough washing, duplicate cell aliquots were incubated with drugs under test for various times before challenge with either DNP-HSA, 1 ng ml^{-1}, or DNP-ovalbumin, 1–10 ng ml^{-1}.

For measurement of 5-HT or histamine release, reactions in $3-5 \times 10^{-4}$ mast cells in a final volume of 0.5 ml were terminated, 15 min after challenge, by centrifugation at 7500 *g* for 30 s at 4°C and aliquots of the supernatant taken for [^{3}H]-5-HT determination by scintillation spectrometry, or histamine release by automated spectrofluorimetry (Hughes *et al.*, 1983). Net percentage release was calculated by reference to spontaneous release into the supernatant from unstimulated cells and total 5-HT or histamine obtained by disintegration of parallel cell aliquots. For measurement of cyclic AMP changes, reactions in $2-5 \times 10^5$ mast cells in a final volume of 0.25 ml were terminated 15–300 s after challenge by addition of 0.25 ml of ice-cold ethanol. Cyclic AMP was measured by radioimmunoassay of the acetylated nucleotide (Hughes *et al.*, 1983).

All compounds were dissolved in HBSS immediately before use with the exception of 5'-N-ethylcarboxamideadenosine (NECA) L-PIA and D-PIA, which were diluted from 1 mM stock solutions in 1% dimethylsulphoxide, and 8-phenyltheophylline which was diluted from a 10 mM stock solution of 0.02 M NaOH in 80% ethanol. None of these solvents affected 5-HT release when diluted.

Statistics and calculations

Best-fit concentration-response lines were calculated by least squares regression analysis. Agonist potencies, together with 95% confidence limits, are expressed as the molar concentrations of drug required to produce a 50% enhancement of antigen-induced mediator release (EC_{50}). Parallelism of concentration-response lines was examined by co-variant analysis. Results of individual drug concentrations are expressed as mean ± s.e.mean. The significance of differences between 5-HT release in the presence or absence of drugs was calculated from raw data using Student's *t* test for paired data or analysis of variance. $P < 0.05$ was taken as the lowest level of significance.

Materials

The following compounds were tested as adenosine receptor agonists: adenosine, adenosine 5'-monophosphate (AMP), adenosine 5'-diphosphate (ADP), adenosine 5'-triphosphate (ATP), inosine, hypoxanthine and adenine (all Sigma Chemical Co.), L-N^6-phenylisopropyladenosine (L-PIA Boehringer-Mannheim), D-N^6-phenylisopropyladenosine (D-PIA, donated by Dr M.G. Collis, ICI Pharmaceuticals), 5'-N-ethylcarboxamideadenosine (NECA, synthesized by Dr D.I.C. Scopes, Glaxo Group Research, Ware), 3-deazaadenosine (3-DZA, Southern Research Institute), α,β-methylene ATP (P.L. Biochemicals). The following compounds were tested as potential adenosine antagonists: theobromine, theophylline (Sigma), 8-phenyltheophylline (8-PT, Calbiochem), 1,3-diethyl-8-phenylxanthine (DPX, synthesized by Dr C. Wallis, Glaxo Group Research), enprofylline (AB Draco). The following compounds were used as purine uptake blockers; dipryridamole, *p*-nitrobenzylthioguanosine (Sigma) and hexobendine (Oesterreichische, Stickstoffwerke). The following compounds were used as adenosine deaminase or 5'-nucleotidase inhibitors: erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA, Burroughs Wellcome), 2'-deoxycoformycin (Sigma) α,β-methylene ADP (P-L. Biochemicals). Isobutylmethylxanthine (IBMX) was obtained from the Sigma Chemical Company. All reagents used with the cyclic AMP assay were obtained from sources listed in Hughes *et al.* (1983). S-[1,2-^{3}H(N)]-hydroxytryptamine was purchased from NEN. Foetal calf

serum was obtained from Flow Laboratories and Percoll from Pharmacia. All other chemicals were of analytical grade and purchased from Sigma or BDH.

Results

Effect of time of addition of adenosine relative to antigen challenge

The effect on 5-HT release of adenosine (100 μM) added to rat serosal mast cells between 15 min before, and 5 min after, antigen challenge was assessed in four experiments (Figure 1). Maximum enhancement of 118 ± 24% ($P < 0.02$) was achieved with simultaneous addition of adenosine and antigen. When added more than two min before, or more than one min after challenge, no significant enhancing effect was observed. Similar time courses of activity were obtained with the purinoceptor agonists NECA and L-PIA. In all further experiments adenosine analogues were added to mast cell suspensions simultaneously with antigen challenge.

Concentration-related effects of adenosine

Enhancement of 5-HT release from mast cells by addition of adenosine (0.01 – 1000 μM) simultaneously with antigen was assessed in nineteen experiments (Figure 2). The maximum potentiation of 5-HT release was 125 ± 13% at 1000 μM. The EC_{50} value (with 95% confidence limits, see Methods section for calculation) for adenosine was 1.47 (0.89–2.04) μM. Histamine release was similarly enhanced by adenosine in six experiments in which maximum potentiation by 1000 μM adenosine was 114.8 ± 14.3%. In these experiments the EC_{50} value was 2.31 (1.27–4.91) μM.

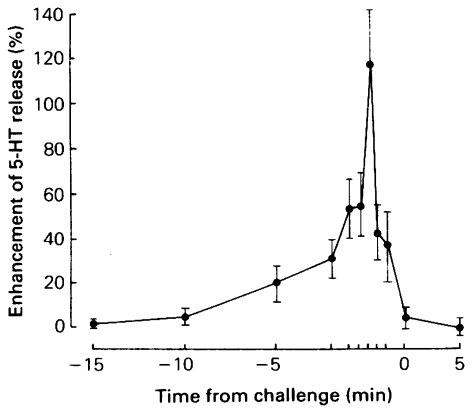

Figure 1 Effect of time of addition of adenosine (100 μM) with respect to antigen challenge on enhancement of 5-hydroxytryptamine (5-HT) release from rat mast cells. Results are mean (vertical lines represent s.e.mean) from four experiments in which DNP-HSA induced 5-HT release was 16.6 ± 4.9% and spontaneous release 3.1 ± 1.7%.

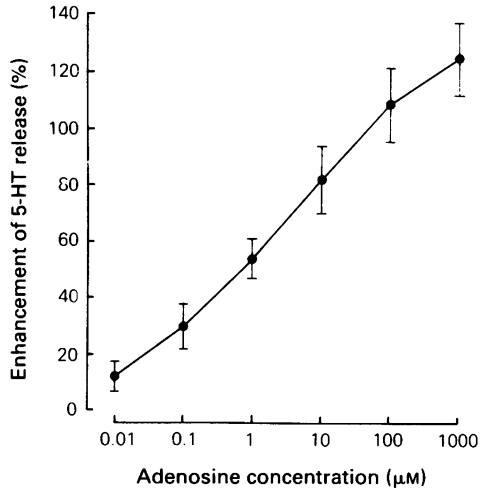

Figure 2 Concentration-related enhancement by adenosine of antigen-induced 5-hydroxytryptamine (5-HT) release from rat mast cells. Results are mean (vertical lines represent s.e.mean) of 19 experiments in which adenosine was added simultaneously with DNP-HSA challenge. Antigen-induced release was 11.4 ± 1.2% and spontaneous release 5.4 ± 0.6%.

Relationship between enhancement of 5-HT secretion and cyclic AMP

Following antigenic stimulation in three experiments, 5-HT was released rapidly during the first 5 min, after which time there was no significant further release (Figure 3a). Maximum release, assessed 15 min after challenge, was 15.0 ± 2.1%. This was accompanied by a rapid monophasic rise of intracellular cyclic AMP levels from a baseline of 5.7 ± 0.7 pmol per 10^6 cells to a maximum of 13.8 ± 1.6 pmol per 10^6 cells 30 s after challenge (Figure 3b). Addition of adenosine (100 μM) simultaneously with antigen significantly increased 5-HT release but did not alter the shape of the time-release curve. At 15 min release was 23.6 ± 2.9%, 58% above control ($P < 0.002$). Although adenosine did not significantly enhance the cyclic AMP response during the first 30 s, it prolonged the duration of cyclic nucleotide elevation, levels at one min and later being significantly ($P < 0.05$) higher than with antigen alone. Preincubation of mast cells with the P_1-purinoceptor antagonist 8-phenyltheophylline (8-PT, 3μM) for 10 min before challenge did not inhibit the enhan-

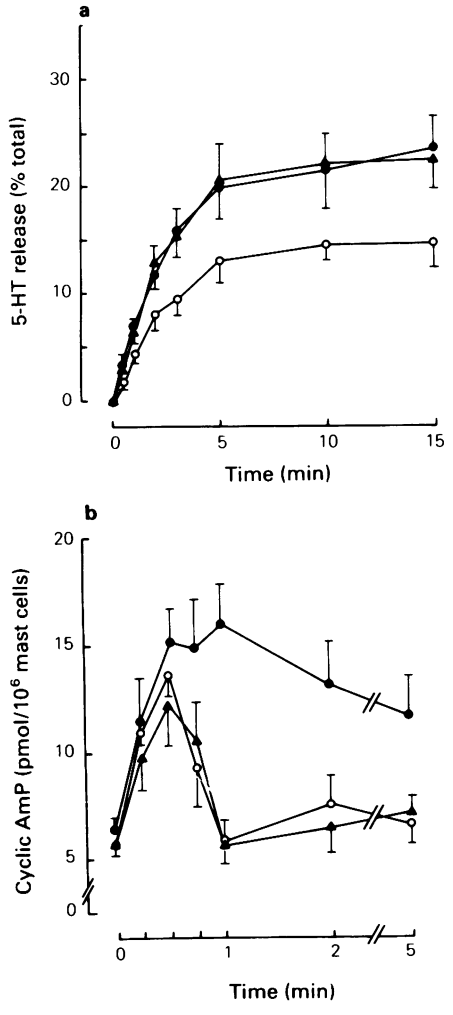

Figure 3 Effect of adenosine on the time course of (a) 5-hydroxytryptamine (5-HT) release and (b) cyclic AMP response in rat mast cells and its inhibition by 8-phenyltheophylline. Results are mean (vertical lines show s.e.mean) of three experiments in which cells were challenged with DNP-HSA (○), DNP-HSA simultaneously with 100 μM adenosine (●), or with DNP-HSA and adenosine following 10 min preincubation with 3 μM 8-phenyltheophylline (▲). Spontaneous 5-HT release in these experiments was 3.3 ± 1.7% assessed at the time of antigen challenge and 3.0 ± 0.7% at 15 min.

cement of 5-HT release by adenosine. It did, however, abolish the adenosine-induced change of the cyclic AMP response, restoring it almost exactly to that observed with antigen challenge alone (Figure 3a). These results suggest that enhancement of mediator secretion and prolongation of the cyclic AMP response are independent effects of adenosine.

Characterization of the purinoceptor mediating cyclic AMP accumulation in rat mast cells

To assess the effects of adenosine analogues on cyclic AMP accumulation, rat mast cells were preincubated with isobutylmethylxanthine (IBMX, 50 μM) for 10 min before stimulation of adenylate cyclase with adenosine, L-PIA and NECA, and the cyclic AMP content of the cell preparations measured 5 min later. Under these conditions IBMX had no significant effect on the basal cyclic AMP content of the mast cell preparations which was 1.38 ± 0.05 pmol per 10^6 mast cells. Adenosine, NECA and L-PIA all produced concentration-related increases in cyclic AMP. The mean concentrations of adenosine, NECA and L-PIA required to produce a doubling of cyclic AMP content over baseline were 1.16, 0.14 and 9.08 μM respectively (Figure 4).

Characterization of the receptor mediating enhancement of antigen-induced 5-HT release

To examine the possibility that adenosine may potentiate 5-HT release by an intracellular mechanism the

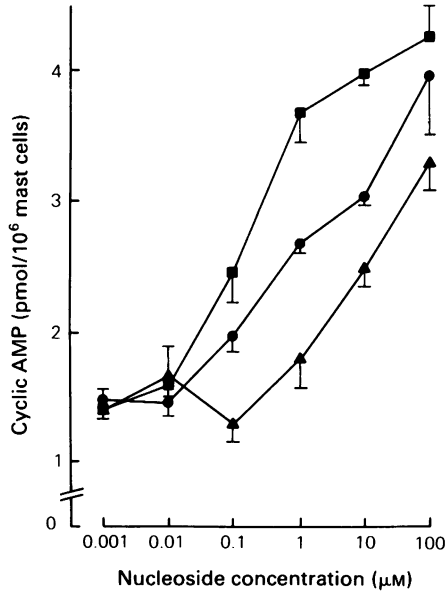

Figure 4 The effects of adenosine (●), 5'-N-ethylcarboxamide (NECA; ■) and L-N^6-phenylisopropyladenosine (L-PIA; ▲) on the cyclic AMP content of rat mast cells. All experiments were performed in cell preparations treated with isobutylmethylxanthine (IBMX; 50 μM) for 10 min. At this concentration IBMX itself did not have any significant effect on the basal cyclic AMP content which was 1.38 ± 0.05 pmol per 10^6 mast cells. Cyclic AMP levels were assessed 5 min after the addition of the nucleosides. Results are expressed as the mean (and vertical lines show s.e.mean) of four determinations.

effect of inhibition of facilitated uptake of adenosine was assessed. Cells were preincubated for 10 min with dipyridamole (1 μM), hexobendine (5 μM) or *p*-nitrobenzylthioguanosine (5 μM). In three experiments, these agents did not significantly shift the concentration-related enhancement of 5-HT release caused by adenosine (0.01–1000 μM). In the absence of uptake inhibitors, the EC_{50} for adenosine was 1.17 (0.11–14.2) μM. Adenosine EC_{50} values in the presence of inhibitors were: dipyridamole 1.12 (0.17–11.7) μM, hexobendine 0.37 (0.14–9.81) μM and *p*-nitrobenzylthioguanosine 1.07 (0.77–6.91) μM. These results suggest that the enhancement of 5-HT release was produced by the interaction of adenosine with an external cell surface purinoceptor.

To examine the possibility that adenosine was acting through a P_2-purinoceptor to enhance antigen-stimulated 5-HT release the effects of AMP, ADP and ATP were compared with adenosine in three experiments (Figure 5a). The EC_{50} for adenosine was 1.62 (0.41–6.61) μM. The adenine nucleotides were substantially less active. The EC_{50} for AMP was 20.4 (5.49–78.85) μM whilst the EC_{50} for ADP was well in excess of 1000 μM. Calculation of an EC_{50} for ATP was not possible as it induced 5-HT secretion in the absence of immunological stimulation in concentrations above 10 μM, a finding which confirms previous observations (Diamant, 1969). Inhibition of ecto-5'-nucleotidase activity by preincubation of cells for 10 min with α,β-methylene ADP (100 μM) significantly ($P<0.05$) reduced the enhancing effect of AMP, whilst not affecting the responses to NECA. Furthermore, α,β-methylene ATP, a non-hydrolysable ATP analogue which is selective for P_2-purinceptors (Maguire & Satchell, 1979; Burnstock & Meghi, 1981), did not enhance 5-HT release (Figure 5b). Thus suggesting that the effects of the adenine nucleotide were produced following its metabolic conversion to adenosine.

The effects of NECA, L-PIA and D-PIA, P_1-purinoceptor agonists used to discriminate between A_1- and A_2-receptor subtypes (Daly, 1982), were compared with adenosine in six experiments. All produced potent concentration-related enhancement of 5-HT release (Figure 6a) with EC_{50} values of: NECA 0.39 (0.31–0.49) μM, L-PIA 0.11 (0.06–0.20) μM, D-PIA 0.41 (0.27–0.64) μM and adenosine 1.44 (1.04–1.99) μM. There were no significant differences between these values.

To examine whether the actions of adenosine were subsequent to its deamination to inosine, cells were preincubated for 10 min with the adenosine deaminase

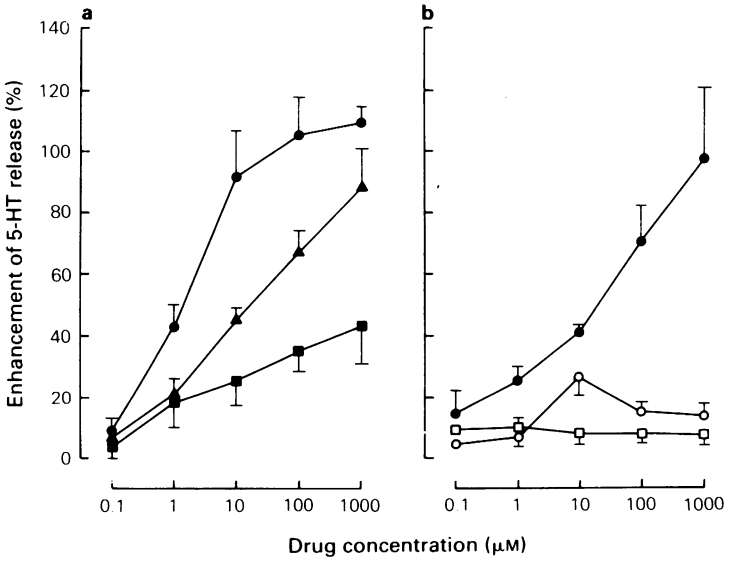

Figure 5 Enhancement of antigen-induced 5-hydroxytryptamine (5-HT) release from rat mast cells by P_2-purinoceptor agonists. (a) Effects of AMP (▲), ADP (■) and adenosine (●) added simultaneously with DNP-HSA challenge. Results are expressed as the mean of three experiments in which antigen-induced 5-HT release was 14.0 ± 0.9% and spontaneous release was 2.8 ± 0.8%. (b) Effects of AMP (●) and α,β-methylene ATP (○) added simultaneously with antigen and modification of AMP induced enhancement by incubation for 10 min with 100 μM α,β-methylene ADP (□). Results are expressed as the mean of three experiments (vertical lines indicate s.e.mean) in which DNP-HSA induced release was 14.4 ± 1.5% and spontaneous release was 4.0 ± 0.8%.

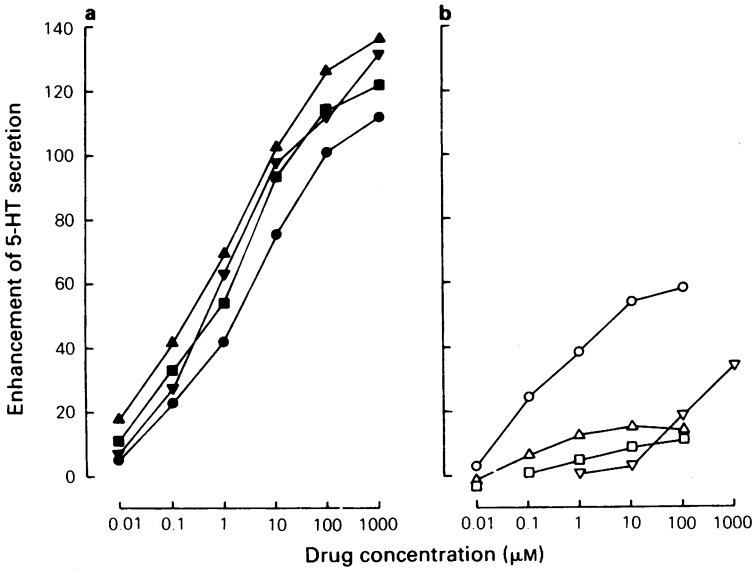

Figure 6 Enhancement of antigen-induced 5-hydroxytryptamine (5-HT) release from rat mast cells by P_1-purinoceptor agonists. (a) Effects of adenosine (●), 5′-N-ethylcarboxamideadenosine (NECA; ■), L-N^6-phenylisopropyladenosine (L-PIA; ▲) and D-PIA (▼) added simultaneously with DNP-HSA challenge. Results are expressed as the mean of six experiments in which antigen-induced 5-HT release was 12.2 ± 1.3% and spontaneous release was 3.2 ± 0.5%. (b) Effects of inosine (○), 3-deazaadenosine (▽), hypoxanthine (△) and adenine (□) added simultaneously with DNP-HSA challenge. Results are expressed as the mean of three experiments in which antigen-induced 5-HT release was 14.3 ± 1.6% and spontaneous release 4.6 ± 2.3%.

inhibitor, erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA, 100 μM) before the addition of adenosine and antigen simultaneously. In three experiments, EHNA did not affect the enhancement of 5-HT release, the EC_{50} value for adenosine in the presence of EHNA being 0.57 (0.19–3.25) μM and in its absence 0.44 (0.12–1.93) μM. Exogenous inosine did, however, enhance IgE-dependent 5-HT release in three experiments, but its EC_{50}, 9.33 (5.88–14.79) μM, showed it to be some 10 times less potent than adenosine. Inosine, which has been found not to stimulate P_1-purinoceptors (Daly, 1982), may act indirectly by inhibiting adenosine deaminase thereby increasing endogenous levels of adenosine (Welton & Simko, 1980). However, this is unlikely to be its mechanism in potentiating 5-HT release because its addition simultaneously with challenge, would not allow time for the accumulation of adenosine. Furthermore, two inhibitors of adenosine deaminase, EHNA (0.1–100 μM) and 2′-deoxycoformycin (DCF, 0.1–100 μM), (Agarwal, 1982) failed to alter significantly 5-HT release when added simultaneously with antigen.

The deribosylated adenosine metabolites, adenine and hypoxanthine produced no significant effects on 5-HT release (Figure 6b) suggesting that an intact ribose moiety is required for activity.

3-Deazaadenosine (3-DZA), which inhibits S-adenosylmethionine (SAM) – dependent methylation reactions upon prolonged incubation with mast cells (Hirata *et al.*, 1979), enhanced 5-HT release when added simultaneously with antigen (Figure 6b). The maximum effect of 3-DZA, 35.8 ± 5.2% enhancement at 1000 μM, showed it to be considerably less potent than other adenosine analogues. Preincubation of mast cells for 60 min with homocysteine thiolactone (100 μM) did not shift the concentration-response line for 3-DZA in three experiments suggesting that its effect was not related to its ability to inhibit SAM-dependent methylation. Furthermore, homocysteine thiolactone (100 μM), added simultaneously with antigen, did not affect 5-HT release.

Effects of purinoceptor antagonists on adenosine-mediated enhancement of antigen-induced 5-HT release

In five experiments, preincubation of mast cells for 10 min with the P_1-purinoceptor antagonist 8-PT (1–10 μM) failed to shift significantly the concentration-enhancement curve for adenosine (Figure 7). 8-PT also failed to affect significantly the adenosine – mediated enhancement of histamine secretion in two experiments. In three similar experiments, 8-PT (10 μM) and theophylline (50 μM) failed to produce

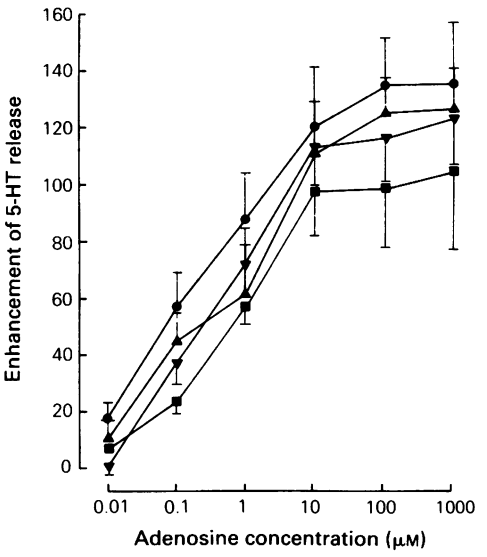

Figure 7 Effect of 8-phenyltheophylline (8-PT) on enhancement by adenosine of antigen-induced 5-hydroxytryptamine (5-HT) release from rat mast cells. Cells were preincubated for 10 min with 8-PT before simultaneous addition of DNP-HSA and adenosine in five experiments. Results are mean (vertical lines show s.e.mean) for control (●), 1 μM 8-PT (▲), 3 μM 8-PT (▼) or 10 μM 8-PT (■). Antigen-induced 5-HT release was 11.2 ± 2.3% and spontaneous release 3.9 ± 0.7%.

significant shifts in NECA and L-PIA concentration-response curves. Furthermore 1,3-diethyl-8-phenylxanthine (10 μM), theobromine (50 μM) and enprofylline (50 μM) also failed to shift significantly the concentration-response curves of NECA (0.01–100 μM) in two experiments.

Tachyphylaxis to adenosine

To examine whether the loss of activity of adenosine on prolongation of the preincubation time before challenge was due to its inactivation or to the development of tachyphylaxis to its effects, mast cells were incubated for 30 min with adenosine (100 μM). This produced no significant enhancement of 5-HT release in four experiments (Figure 8). Adenosine (100 μM), NECA (100 μM) and inosine (100 μM) produced a significant ($P<0.05$) potentiation of release when added to untreated cells. When added to adenosine pretreated cells, their effects were significantly ($P<0.02$) reduced, NECA and inosine producing no significant enhancement. Tachyphylaxis to adenosine and cross-tachyphylaxis with NECA and L-PIA suggest that all three compounds enhance release by a common mechanism.

Discussion

The observation that adenosine enhanced IgE-dependent secretion of mediator release from rat serosal mast cells confirms previous reports, (Marquardt *et al.*, 1978; Holgate *et al.*, 1980; Burt & Stanworth, 1983; Nishibori *et al.*, 1983). Also the finding that enhancement of mediator release was accompanied by a potentiation of the cyclic AMP response to challenge confirms the study of Holgate *et al.* (1980). That potentiation of the cyclic AMP response but not enhancement of mediator release was blocked by the methylxanthine purinoceptor antagonist 8-phenyltheophylline (8-PT) provides evidence for the suggestions, made by Burt & Stanworth (1983) and Leoutsakos *et al.* (1985), that adenosine-mediated enhancement of mediator release is not dependent upon changes in cyclic AMP. The cyclic AMP response is likely to be mediated through a cell surface A_2-purinoceptor. Experiments to determine the site of action of adenosine in enhancing 5-HT release suggest that it acts at hitherto uncharacterized cell surface receptors.

Cell surface purinoceptors have been classified into P_1- and P_2- receptors by their sensitivity to agonists. P_1-purinoceptors are preferentially stimulated by adenosine and its analogues whereas at P_2-purinoceptors AMP, ADP and ATP are more active (Burnstock, 1980). P_1-purinoceptors have been subclassified by Van Calker *et al.* (1979) and Londos *et al.* (1980) on the ability of agonists to inhibit or stimulate adenylate cyclase. At A_1-purinoceptors, which inhibit adenylate

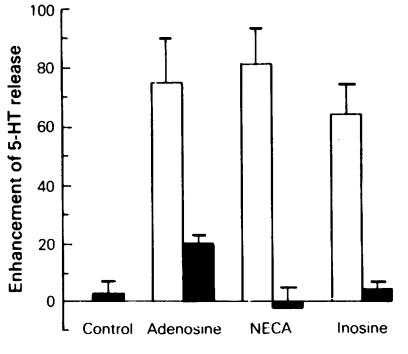

Figure 8 Tachyphylaxis to enhancement by adenosine of antigen-induced 5-hydroxytryptamine (5-HT) release from rat mast cells. Mast cells were preincubated with 100 μM adenosine (solid columns) or buffer (open columns) for 30 min before stimulation of 5-HT release with DNP-HSA alone or simultaneously with adenosine (100 μM), 5′-N-ethylcarboxamideadenosine (NECA; 100 μM) or inosine (100 μM). Results are mean (vertical lines show s.e.mean) of four experiments in which antigen-induced 5-HT release was 16.9 ± 1.6% and spontaneous release was 5.3 ± 1.5%.

cyclase, the potency order of adenosine analogues is L-PIA > adenosine > NECA whereas at A_2-purinoceptors, which activate adenylate cyclase, the order is NECA > adenosine > L-PIA. Furthermore, A_1-purinoceptors show marked stereoselectivity for PIA isomers (L-PIA being 50–100 times more active than D-PIA), whilst A_2-purinoceptors show little stereoselectivity (Bruns *et al.*, 1980). Both A_1- and A_2-purinoceptors are competitively antagonized by xanthine derivatives (Bruns, 1981; Griffiths *et al.*, 1981; Collis, 1983).

In the experiments in which the changes in cyclic AMP content were assessed following stimulation of the mast cells with adenosine analogues, NECA was more active than both adenosine and L-PIA. These observations suggest that rat mast cells possess an A_2-purinoceptor that is functionally linked to adenylate cyclase. However, the failure of 8-PT to block enhancement of 5-HT release in parallel with its blockade of the cyclic AMP response suggests that the effect of adenosine on 5-HT release is neither dependent on the enhanced cyclic AMP response nor mediated by A_2-purinoceptor activation.

As it has been demonstrated that the enhancement of antigen-stimulated 5-HT release from rat mast cells by adenosine is not mediated by cyclic AMP an alternative mechanism of action for adenosine must be sought. As well as acting at cell surface P_1- or P_2-purinoceptors, adenosine can be taken up into cells by facilitated diffusion and act at P-sites or modify cellular biochemistry.

An intracellular action of adenosine and its analogues is unlikely for the following reasons. Adenosine, NECA and L-PIA were all maximally effective when added to mast cells simultaneously with antigen. Also, NECA and L-PIA are not substrates for the facilitated uptake system of adenosine (Daly 1982). Adenosine-induced enhancement of mediator release was not reduced by preincubation of mast cells with dipyridamole, hexobendine or *p*-nitrobenzylthioguanosine, all inhibitors of the facilitated uptake of adenosine (Turnheim *et al.*, 1978). Furthermore, NECA and L-PIA are devoid of P-site activity (Daly, 1982) while 2′,5′-dideoxyadenosine inhibits mediator release from rat mast cells (Holgate *et al.*, 1980; Burt & Stanworth, 1983). Inhibition of S-adenosylmethionine-dependent methylation reactions by adenosine or 3-DZA required prolonged preincubation in whole cell preparations. Also, this action results in inhibition of IgE-dependent histamine release from rat mast cells (Hirata *et al.*, 1979).

As adenosine was approximately 16 times more potent than AMP, and AMP was more potent than ADP, this suggests that the receptor mediating enhancement of 5-HT release from rat mast cells is unlikely to be of the P_2-type. This is supported by the finding that α,β-methylene ATP, a non-hydrolysable ATP-analogue which acts solely at P_2-purinoceptors (Maguire & Satchell, 1979; Burnstock & Meghji, 1981), did not enhance mediator release. As inhibition of ecto-5′-nucleotidase activity by α,β-methylene ADP (Bruns, 1980) significantly reduced the ability of AMP to enhance 5-HT release, it is likely that the activity of adenosine nucleotides results from their metabolism to adenosine which then produces the response.

The use of adenosine analogues and metabolites failed to allow ready classification of the enhancing effect on mediator release of adenosine in terms of previously recognised cell surface purinoceptors. Adenosine, NECA, L-PIA and D-PIA were all of similar potency and efficacy. Inosine and 3-DZA, both reported to be devoid of agonist activity at purinoceptors, also enhanced mediator release. Evidence that the action of adenosine was not subsequent to its catabolism was obtained from experiments in which inhibition of adenosine deaminase activity by EHNA or 2′-deoxycoformycin (Agarwal, 1982) did not influence its action. Furthermore, the cross-tachyphylaxis between adenosine and its analogues NECA and inosine suggest all these agents enhance mediator release by a common mechanism. These findings add weight to the growing amount of evidence that the activity of adenosine analogues in producing physiological responses do not necessarily conform to the A_1/A_2 classification defined using inhibition or activation of adenylate cyclase as an indicator of activity. For example, the inhibitory potencies of D-PIA and L-PIA at the frog sartorius neuromuscular junction suggest an A_2-receptor-mediated effect, whereas the relative activities of other analogues suggest an A_1- receptor type (Ribeiro & Sebastiao, 1985).

A further deviation from previously characterized A_1/A_2-purinoceptors is the insensitivity of adenosine-induced enhancement to antagonism by xanthines. Our experiments showed that 8-PT caused an apparent small shift in the adenosine concentration-response curve, but this was not statistically significant. Similar results were obtained with theophylline, 1,3-diethyl-8-phenylxanthine, theobromine and enprofylline. This effect was not restricted to 5-HT release as 8-PT also failed to reduce adenosine-induced enhancement of histamine release.

Although the majority of studies on the cell surface actions of adenosine have tried to associate its actions with an effect on adenylate cyclase, alteranative mechanisms of action have been suggested. Kuroda (1983) has proposed the existence of adenosine receptors which modulate calcium influx or its intracellular metabolism. An alternative explanation may lie in the ability of adenosine to increase the activity of other guanine nucleotide binding proteins in addition to that comprising the regulatory sub-unit of adenylate cyclase. Recently it has been demonstrated that the introduction of guanosine triphosphate into per-

meabilized rat mast cells induced mediator release by guanine nucleotide binding protein-dependent activation of phosphatidylinositol-4,5-diphosphodiesterase (Gomperts, 1983; Cockcroft & Gomperts, 1985).

In conclusion, there appears to be at least two types of cell surface receptor for adenosine on the rat mast cell. One type is probably an A_2-purinoceptor, stimulation of which elevates intracellular cyclic AMP. This receptor is not involved in enhancement of mediator release by adenosine. The second receptor type, stimulation of which elevated IgE-dependent mediator release, is xanthine resistant and does not meet the criteria for classification of A_1- or A_2-subtypes of P_1-purinoceptors. The mechanism by which stimulation of this receptor type enhances mediator release is still unclear, but it is unlikely to involve adenylate cyclase activation.

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