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# Adenosine potentiates immunological histamine release from rat mast cells by a novel cyclic AMP-independent cell-surface action

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Adenosine enhanced histamine release and prolonged the adenosine 3':5'-cyclic monophosphate (cyclic AMP) response in purified rat peritoneal mast cells following immunological challenge. The effect on the cyclic AMP response, which was blocked by 8-phenyltheophylline, probably results from an interaction with $A_2$-purinoceptors. Enhancement of histamine release showed different characteristics. It was not inhibited by dipyridamole or hexobendine, thereby indicating an action at the cell surface. However, the relative potencies of adenosine analogues and nucleotides, together with the observation that this effect was not antagonized by 8-phenyltheophylline or theophylline, suggest that it is not mediated by a previously recognised purinoceptor. Thus, enhancement of histamine release may represent a novel cell surface action of adenosine which is independent of its effects on adenylate cyclase.

**Introduction** Immunological activation of mast cells and basophils induces a transient elevation of intracellular adenosine 3':5'-cyclic monophosphate (cyclic AMP) which precedes mediator secretion (Holgate *et al.*, 1980; Hughes *et al.*, 1983). Adenosine, acting at cell surface $A_2$-purinoceptors, activates adenylate cyclase to increase intracellular cyclic AMP (Londos & Wolff, 1977). In rat mast cells and human basophils, this is observed as an elevation and prolongation of the cyclic AMP response to immunological activation (Holgate *et al.*, 1980; Hughes *et al.*, 1983). Stimulation of human basophil $A_2$-purinoceptors by preincubation with adenosine decreases IgE-dependent histamine secretion (Church *et al.*, 1983). Although elevation of cyclic AMP is normally associated with decreased mediator secretion, in rat mast cells adenosine-induced elevation of cyclic AMP is accompanied by enhancement of immunological histamine release (Holgate *et al.*, 1980) suggesting that both result from $A_2$-purinoceptor stimulation. However, Vardey & Skidmore (1985) reported that 8-phenyltheophylline does not block adenosine enhancement of histamine release suggesting that this effect is not $A_2$-purinoceptor mediated. We have, therefore, examined the relationship between adenosine-induced cyclic AMP changes and enhancement of histamine release from rat mast cells and conclude that they are unrelated.

**Methods** Mast cells, harvested by peritoneal lavage of male Sprague-Dawley rats, were purified by centrifugation through Percoll, 1.09 g ml$^{-1}$, at 700 *g* for 15 min. The interface cells, containing >90% mast cells, were passively sensitized for 2 h at 37°C with 50 ng ml$^{-1}$ of mouse monoclonal IgE anti-dinitrophenol$_{13}$-human serum albumin (DNP-HSA, kindly donated by Dr T. Ishizaka) and suspended in HEPES buffered salt solution (HBSS, Church *et al.*, 1983) supplemented with gelatin 0.5 g l$^{-1}$, $MgCl_2$ 0.5 mM and $CaCl_2$ 1 mM. Duplicate cell aliquots were incubated for 10 min at 37°C with purinoceptor antagonists or uptake blockers before challenge with DNP-HSA (1 ng ml$^{-1}$). Adenosine or adenosine derivatives were added simultaneously with DNP-HSA. For histamine release experiments, reactions in $3-5 \times 10^4$ mast cells were terminated at 15 min by centrifugation at 4°C and histamine measured spectrofluorimetrically (Hughes *et al.*, 1983). For determination of cyclic AMP changes, reactions in $2-5 \times 10^5$ mast cells were terminated 15–300 s after challenge by addition of ice cold ethanol and cyclic AMP measured by radioimmunoassay (Hughes *et al.*, 1983).

**Results** Challenge of passively sensitized rat mast cells with DNP-HSA induced 11.2 ± 2.3% histamine release and a monophasic rise in intracellular cyclic AMP content 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 1). By 1 min, cyclic AMP levels had returned to baseline.

Adenosine (0.01–1000 μM) caused a concentration-related enhancement of histamine release which reached a maximum of 137 ± 21% above control. The concentration of adenosine calculated to enhance histamine release by 50% ($EC_{50}$) was 0.076 μM. Adenosine (100 μM) did not significantly potentiate the

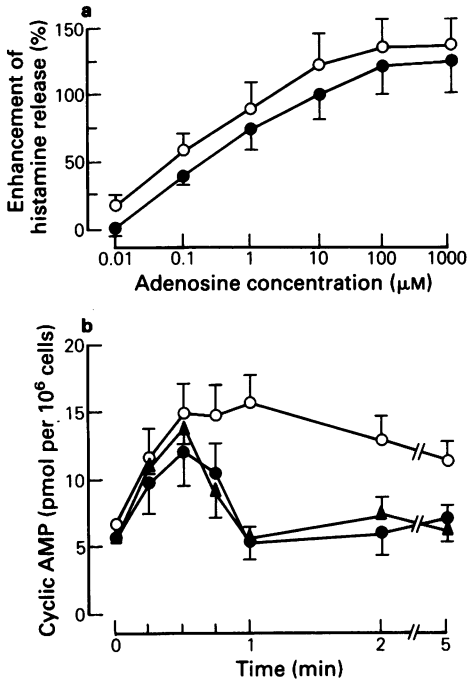


**Figure 1** The enhancement by adenosine and the effect of 8-phenyltheophylline (8-PT) on (a) histamine release and (b) cyclic AMP production induced by IgE-dependent stimulation of purified rat peritoneal mast cells. In five histamine release experiments, DNP-HSA 1 ng ml$^{-1}$ induced 11.2 ± 2.3% net histamine release with a 3.9 ± 0.6% spontaneous release. In three cyclic AMP experiments, baseline cyclic AMP levels were 5.7 ± 0.7 pmol per $10^6$ cells. Symbols ± s.e.mean are: (▲) DNP-HSA challenge; (○) challenge by simultaneous addition of adenosine (100 μM in b) and DNP-HSA; (●) 10 min preincubation with 8-PT (3 μM) before challenge with DNP-HSA and adenosine. Statistical analysis using Student's *t* test for paired data showed that 8-PT did not significantly reduce enhancement of histamine release at any concentration of adenosine. Cyclic AMP levels were significantly ($P < 0.05$) higher at 45 s and later in adenosine-treated cells compared with cells challenged with DNP-HSA alone or in the presence of 8-PT.

cyclic AMP rise during the first 30 s but significantly prolonged the response ($P < 0.05$, Figure 1). Preincubation of cells for 10 min with 8-phenyltheophylline (3 μM) abolished the effect of adenosine on the cyclic AMP response indicating effective blockade of $A_2$-purinoceptors. However, 8-phenyltheophylline (3 μM) failed to reduce significantly the enhancement of histamine release produced by adenosine. In three further experiments, the purinoceptor antagonist, theophylline (50 μM), and inhibitors of adenosine uptake, dipyridamole (1 μM) and hexobendine (5μM), also failed to reduce the effect of adenosine on histamine release.

To investigate the characteristics of purine-mediated enhancement of histamine release, the effects of adenosine analogues and nucleotides were examined. The potency ratios of these agents compared to adenosine ( = 1) were D-$N^6$-phenylisopropyladenosine 1.1, 5'-N-ethylcarboxamideadenosine 1.0, L-$N^6$-phenylisopropyladenosine 0.9, adenosine-5'-triphosphate 0.2, adenosine-5'-monophosphate 0.06 and adenosine-5'-diphosphate 0.002.

**Discussion** That adenosine both enhanced histamine release and prolonged the cyclic AMP response following immunological challenge of rat mast cells accords with previous observations (Holgate *et al.*, 1980). Abolition of its effect on the cyclic AMP response by 8-phenyltheophylline, a potent adenosine antagonist (Griffiths *et al.*, 1981), suggests an $A_2$-purinoceptor action of adenosine (Londos & Wolff, 1977). Adenosine enhancement of histamine release was not reduced by blockade of cellular uptake with dipyridamole or hexobendine (Turnheim *et al.*, 1978) suggesting cell surface action. However, the failure of 8-phenyltheophylline and theophylline to antagonize this effect suggests a site of action unrelated to classical $A_1$- or $A_2$-purinoceptors. This conclusion is further substantiated by the comparative potencies of adenosine analogues and nucleotides which do not correlate with reported activities either at $A_1$- or $A_2$-subtypes of $P_1$-(adenosine sensitive) purinoceptors or at $P_2$-(ATP sensitive) purinoceptors (Londos & Wolff, 1977; Burnstock, 1978; Vardey & Skidmore, 1985).

Our results suggest that adenosine enhancement of histamine release from rat mast cells is unrelated to its effects on cyclic AMP. Furthermore, the cell surface receptor mediating the effect on histamine release cannot be readily classified in terms of hitherto recognized purinoceptors. It may, therefore, represent a novel adenosine receptor whose properties warrant further investigation.

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