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THE EFFECT OF RIBOSE AND PURINE MODIFIED ADENOSINE ANALOGUES ON THE SECRETION OF HISTAMINE FROM RAT MAST CELLS INDUCED BY IONOPHORE A23187

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Abstract—The purine nucleosides adenosine and 2′,5′-dideoxyadenosine (2′,5′ddAdo) enhance and inhibit respectively the anti-IgE-induced secretion of histamine and transient rise in cellular levels of cyclic AMP in rat mast cells. These findings have provided evidence for a role for cyclic AMP in the activation of mast cell secretion. It has been generally accepted that the nucleosides mediate their effects on mast cells by altering adenylate cyclase activity. We have investigated the effect of various purine and ribose modified analogues of adenosine on secretion of histamine from rat mast cells induced by ionophore A23187 for which there is no associated elevation in cyclic AMP and no evidence for the activation of adenylate cyclase in its mechanism of action. Adenosine and N^6, phenylisopropyladenosine (0.01–1000 μM) (activators of adenylate cyclase in many tissues) enhanced the secretion of histamine induced by ionophore A23187 and anti-IgE. Two inhibitors of adenylate cyclase had differential effects on secretion. 2′,5′ddAdo (100–1000 μM) inhibited both A23187- and anti-IgE-mediated secretion; whilst 9-β-D-arabinofuranosyladenine had no effect on secretion. These results suggest that the ability of these nucleosides to modulate histamine secretion is unrelated to their effects on adenylate cyclase.

In common with many other physiological processes, the secretion of histamine from rat mast cells can be regulated by adenosine and adenine nucleotides [1, 2]. There is evidence to suggest that in many systems adenine nucleotides mediate their effects by altering the activity of adenylate cyclase [3, 4]. Modulation of adenylate cyclase activity by adenosine occurs at stimulatory (Ra) or inhibitory (Ri) receptors on the plasma membrane and at an intracellular inhibitory site (P-site) [3, 5, 6]. The Ra- and Ri-type receptors have chemical specificities for adenosine analogues with an unaltered ribose group, whilst the P-site has a chemical specificity for analogues of adenosine with an unaltered purine moiety.

The briding of IgE–Fc receptors on the mast cell plasma membrane results in an early transient elevation in intracellular levels of cyclic AMP [7–9] occurring as a result of adenylate cyclase activation [10, 11], which accompanies mediator secretion. Holgate *et al.* [12] showed that the early transient rise in cyclic AMP and secretion of mediators were inhibited in a parallel manner by 2′,5′ddAdo* (P-site agonist [5, 6]), whilst adenosine and N^6, phenylisopropyladenosine (PIA) (Ra-receptor agonist [6]) potentiated the rise in cyclic AMP and enhanced mediator release. These findings suggested that adenosine and adenine nucleotides mediate their effects on mast cell secretion via the modulation of adenylate cyclase. Furthermore, on the basis of these results it has been proposed that cyclic AMP acts as a second messenger in activation–secretion coupling in mast cells [13]. Recent experiments in our laboratory have suggested that cyclic AMP may not be an essential component of the mechanism of mast cell activation; since for a variety of immunological and peptide stimuli, the transient accumulation of mast cell cyclic AMP occurs simultaneously with, or subsequent to, the release of mediators [9].

Selective secretion of mast cell histamine can also be elicited by the calcium ionophore A23187 [14]; however, unlike immunological triggers, this stimulus bypasses many of the initial biochemical changes associated with ligand–receptor interactions on the mast cell membrane [15]. Furthermore, triggering of mast cells by ionophore A23187 does not result in an increase in cellular levels of cyclic AMP [1, 9, 16] (and there is no evidence for the involvement of adenylate cyclase in its mechanism of action). In order to investigate further the mode of action of 2′,5′ddAdo and other analogues of adenosine on mast cell secretion, we describe in this report the effects of these substances on secretion of histamine from rat mast cells induced by the ionophore A23187.

MATERIALS AND METHODS

Male Wistar rats were obtained from Bantin and Kingman Ltd. (Hull, U.K.). Bovine serum albumin (grade V), adenosine (free base) and 9-β-D-arabinofuranosyladenine were from the Sigma Chemical Co. (Poole, Dorset, U.K.). N^6, Phenylisopropyladenosine was obtained from Boehringer–Mannheim (F.R.G.) and 2′,5′-dideoxyadenosine was

* Abbreviations: 2′,5′ddAdo, 2′,5′-dideoxyadenosine; Ara-A, 9-β-D-arabinofuranosyladenine; PIA, N^6, phenylisopropyladenosine; cyclic AMP, adenosine 3′,5′-cyclic monophosphate; anti-IgE, rabbit anti-rat IgE antiserum; 3-deaza-SIBA, 5′-deoxy-5′-isobutylthio-3-deazaadenosine.

supplied by P. L. Biochemicals Ltd. (Northampton, U.K.). Tris-(hydroxymethyl)-methylamine, D-glucose and Triton X-100 were from British Drug Houses. Gelatin was obtained from the Gelatin and Glue Research Co. The calcium ionophore A23187 was a gift from Dr. W. E. Brocklehurst, Lilly Research Centre, Windlesham, U.K. Rabbit anti-rat IgE antiserum was produced as previously described [17].

Mast cell isolation and challenge. Mast cells were harvested in a Tris (25 μM)-buffered mast cell medium containing 123 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 5.6 mM D-glucose and 0.1% gelatin, pH 7.5. Peritoneal and pleural cell washings were obtained from male Wistar rats (200–250 g) by the injection of 12 and 6 ml medium, respectively, into each cavity. Animals were massaged and medium was aspirated using a plastic Pasteur pipette. The pooled cell suspension was then centrifuged at 700 *g* for 1 min in a fixed angled rotor and the resulting cell pellet washed twice in medium. Mast cells in this preparation consisted of between 2 and 10% of the total cell population as determined by toluidine blue staining [18]. All cell incubations were performed at 37° in mast cell medium adjusted to pH 7.5 and supplemented with 1 mM $CaCl_2$. Washed cell suspensions (900 μl) containing approximately 1×10^4 mast cells/test were either preincubated with various concentrations of nucleoside for 5 min prior to challenge for 10 min with stimulus (100 μl), or challenged simultaneously with drug and stimulus for 10 min. Cell suspensions were immediately centrifuged at 700 *g* for 1 min and supernatants decanted into 1 ml aliquots of 0.8 N perchloric acid. To each cell pellet was added 2 ml of 0.4 N perchloric acid. Precipitated protein was sedimented by centrifugation and histamine levels in each cell and supernatant fraction were measured by automated spectrofluorometry [19]. Histamine release was expressed as a percentage of the total cell content corrected for spontaneous release and was calculated as follows:

$$\% \text{ release} = \frac{\text{histamine in supernatant}}{\text{histamine in cells + histamine in supernatant}} \times 100$$

Adenosine and 2′,5′-dideoxyadenosine were freely soluble in mast cell medium at the concentrations required. Stock solutions of 9-β-D-arabinofuranosyladenine and N^6, phenylisopropyladenosine at concentrations of 100 mM were prepared in dimethyl sulphoxide (DMSO) and diluted in medium to the required concentration. Calcium ionophore A23187 was prepared from a stock solution of 19.1 mM in ethanol. Neither DMSO nor ethanol alone at the final concentrations used in the assays affected secretion of histamine from mast cells induced by either the anti-IgE or the ionophore stimuli. None of the drugs at the concentrations used interfered with the automated assay of histamine.

RESULTS

Adenosine and PIA in the concentration range 0.01–100 μM enhanced the secretion of histamine induced by anti-IgE and ionophore A23187 in a dose-dependent manner (Fig. 1). Both nucleosides were consistently more effective as enhancers of the A23187-mediated secretory mechanism. Adenosine at a concentration of 100 μM augmented the release of histamine induced by anti-IgE to 133 ± 6.4% of the control release, whilst a similar degree of

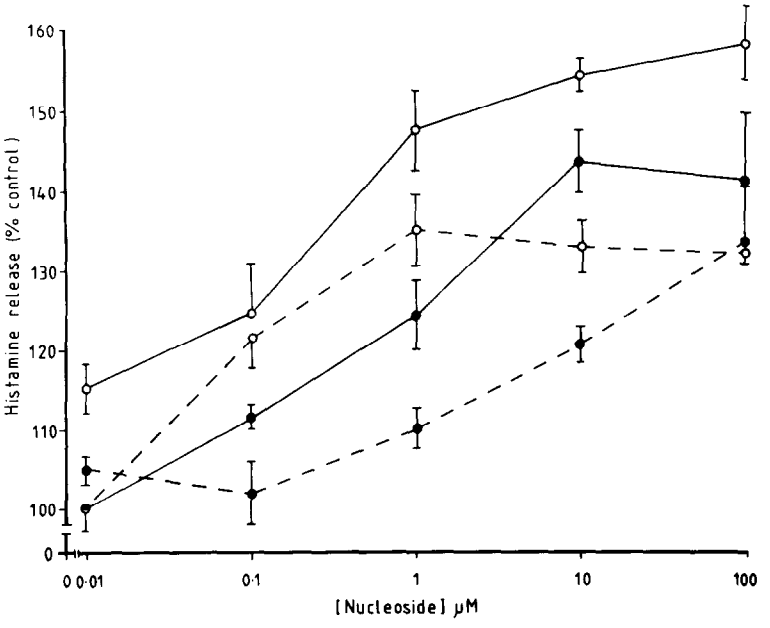

Fig. 1. Effect of adenosine and PIA on histamine secretion. Cells were challenged with adenosine (●) or PIA (○) and anti-IgE (1 in 1000) (broken line) or A23187 (0.4 μM) (solid line). Each point represents the mean ± S.E.M. for four determinations. The net percentage histamine release in the controls for anti-IgE and A23187 was 23.7 ± 0.8 and 45.5 ± 0.5%, respectively; spontaneous release was 4.2 ± 0.9 and 1.8 ± 0.4%, respectively.

Table 1. Effect of 2',5'ddAdo on release of histamine induced by Triton X-100 and Tween 20

Stimulus	Percentage net histamine release (mean ± S.E.M., $n = 3$) No inhibitor	500 μM 2',5'ddAdo	
Anti-IgE (1 in 100)	56.9 ± 2.3	24.1 ± 1.3	($P < 0.001$)
Triton X-100 (0.06 μl/ml)	19.0 ± 1.9	16.2 ± 0.5	($P < 0.025$)
Tween 20 (0.3 μl/ml)	20.5 ± 0.8	17.2 ± 0.9	(N.S.)

Cells were preincubated with 500 μM 2',5'ddAdo before challenge with Triton X-100 (0.06 μl/ml), Tween 20 (0.3 μl/ml) or anti-IgE (1 in 100). The mean percentage spontaneous release was 3.2 ± 0.8%. The statistical significance of differences between the drug-treated and non-drug-treated samples was determined using Student's *t*-test for non-paired comparisons.

enhancement of A23187-induced secretion of histamine (132.6 ± 8%) was achieved with 10 μM adenosine. PIA (100 μM) augmented the secretion of histamine induced by anti-IgE and ionophore A23187 to 132.5 ± 4.6 and 158.1 ± 3.2% respectively of the untreated controls.

The ability to enhance anti-IgE- and A23187-induced secretion of histamine was restricted to adenine nucleosides with an unaltered ribose moiety since 2',5'ddAdo and Ara-A, both ribose modified analogues of adenosine, showed no enhancement (Fig. 2). Anti-IgE-mediated secretion of histamine was inhibited by 2',5'ddAdo in the concentration range 100–1000 μM (Fig. 2). Maximum inhibition was reached at 1000 μM 2',5'ddAdo, giving a reduction in secretion to 32.9 ± 4% of the untreated control. Ionophore A23187-induced secretion of histamine was also inhibited by 2',5'ddAdo in the same concentration range. In the presence of 1000 μM 2',5ddAdo, release of histamine due to ionophore A23187 stimulation was approximately halved (Fig. 2). In this study, 2',5'ddAdo appeared to be more potent as an inhibitor of secretion mediated by anti-IgE than ionophore A23187. However, this may be due to the higher net percentage release of histamine for cells stimulated with ionophore A23187 (57.1 ± 4.6%) compared to those challenged with anti-IgE (23.9 ± 2.3%) in the absence of nucleoside.

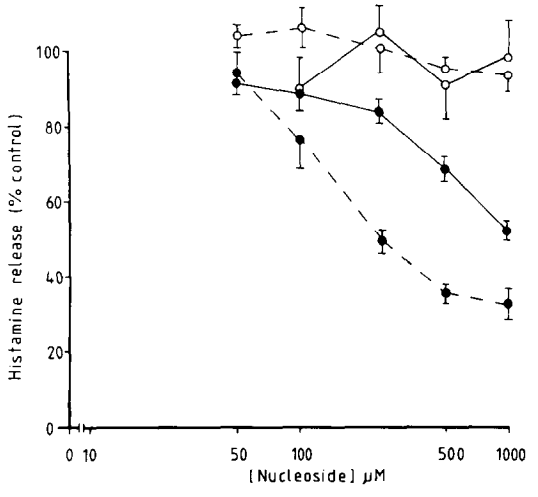

Fig. 2. Effect of 2',5'ddAdo and Ara-A on histamine secretion. Cells were preincubated for 5 min with 2',5'ddAdo (●) or Ara-A (○) before challenge with anti-IgE (1 in 1000) (broken line) or ionophore A23187 (0.47 μM) (solid line). Each point represents the mean ± S.E.M. for four determinations. The net percentage histamine release in the controls for anti-IgE and A23187 was 23.9 ± 2.3 and 51.7 ± 4.6%, respectively; spontaneous release was 6.7 ± 2 and 2.6 ± 3.7%, respectively.

The 9-β-D-arabinofuranosyl derivative of adenine—Ara-A—is a potent inhibitor of adenylate cyclase in many tissues [6], however, Ara-A failed to inhibit either the anti-IgE- or ionophore A23187-induced secretion of histamine at concentrations identical to those at which 2',5'ddAdo was found to be effective (Fig. 2).

In order to determine whether the inhibitory effect of 2',5'ddAdo was due to some specific action on biochemical processes within the cell rather than as a result of a generalized stabilizing effect on the mast cell membrane, the effect of 2',5'ddAdo on lytic release of histamine was investigated. Table 1 shows that 2',5'ddAdo (500 μM) had no significant effect on the release of histamine induced by Tween 20 and only marginally reduced the release mediated by Triton X-100. However, anti-IgE-induced secretion of histamine was markedly reduced to 42.3 ± 1.3% of the original value in the presence of 500 μM 2',5'ddAdo.

DISCUSSION

In this study we have shown that the secretion of histamine from rat mast cells induced by ionophore A23187 is augmented by adenosine and PIA, and inhibited by 2',5'ddAdo at concentrations identical to those at which these nucleosides enhance and inhibit the anti-IgE-induced secretion of mediators and transient elevation in cellular levels of cyclic AMP [12]. However, since the triggering of mast cells by ionophore A23187 is not associated with the activation of adenylate cyclase and transient rise in cyclic AMP levels [1, 9, 16], these findings suggest that the nucleosides used in this study do not mediate their effects on secretion via the modification of adenylate cyclase activity. This proposal is also supported by data published by Marquardt *et al.* [1] showing that the enhancement of ionophore A23187-mediated secretion of histamine by adenosine is not associated with increased levels of mast

cell cyclic AMP. Furthermore, the ability of adenosine to enhance the secretion of histamine induced by a wide range of stimuli including compound 48/80 [1] which also shows no requirement for the activation of adenylate cyclase in its mechanism of action [20]) is consistent with our proposal.

In this present study, 2',5'ddAdo and Ara-A, two 'P-site' inhibitors of adenylate cyclase [6], had differential effects on the secretion of histamine. This disparity is unlikely to be due to differences in the capacities of the nucleosides to inhibit adenylate cyclase. Ara-A is between three and ten times less potent than 2',5'ddAdo as an inhibitor of adenylate cyclase in most tissues [2, 5]. At this level of activity Ara-A would be expected to inhibit the secretion of histamine induced by anti-IgE to some degree if adenylate cyclase activation was an essential part of the triggering mechanism. A more plausible explanation for the differential activities shown by the two nucleosides on histamine secretion is that the inhibitory effect of 2',5'ddAdo is unrelated to the ability of this nucleoside to suppress adenylate cyclase activity.

A recent report has suggested that many inhibitors of mast cell secretion may mediate their effects by causing a general stabilization of the mast cell membrane [21]. However, this does not appear to be the case for 2',5'ddAdo since this nucleoside had little effect on the release of histamine induced by detergents. Our data suggest that 2',5'ddAdo inhibits secretion by acting at a biochemical step subsequent to the initial stimulus–membrane interaction common to both anti-IgE- and ionophore A23187-mediated secretory mechanisms.

Apart from their effects on adenylate cyclase, adenine nucleotides are known to modify the activity of phosphodiesterase [22], protein kinase [23] and *S*-adenosylhomocysteine hydrolase [24], enzymes which may be involved in the control of secretion of mast cell mediators [25–27]. Also, 3-deaza-SIBA—a ribose and purine-modified analogue of adenosine—has been shown to inhibit phospholipid biosynthesis in some tissues [28]. This nucleoside also inhibits both the anti-IgE- and ionophore A23187-mediated secretion of histamine from rat mast cells [29] as we have shown in this report for 2',5'ddAdo. It is possible that 2',5'ddAdo and 3-deaza-SIBA have common mechanisms of action.

In conclusion, our data suggest that adenine nucleosides do not mediate their effects on secretion via the modulation of adenylate cyclase activity. Furthermore, our results shed serious doubt on the experimental evidence that has led to the proposal that the activation of adenylate cyclase and transient elevation in cyclic AMP are causally related to mast cell mediator secretion.

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