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Immunology

Role of adenylate cyclase in immunologic release of mediators from rat mast cells: Agonist and antagonist effects of purine- and ribose-modified adenosine analogs

(protein kinase/R and P sites/secretion/cyclic AMP)

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ABSTRACT **The initial monophasic rise in cyclic AMP beginning 5–15 sec after bridging of rat mast cell IgE-Fc receptors precedes the secretion of granule constituents, thereby implying a causal relationship. Direct evidence for a relationship between IgE-dependent transmembrane activation of adenylate cyclase and granule secretion was provided by the capacity of purine-modified (R site active) and ribose-modified (P site active) adenosine analogs, respectively, to augment and suppress mediator release while simultaneously increasing and decreasing the activity of adenylate cyclase. R site stimulation alone does not cause granule secretion but augments the rate and magnitude of IgE-Fc receptor-induced secretion, reflecting the coupled relationship of such receptors. Inhibition of adenylate cyclase at the P site attenuates the rise in cellular cyclic AMP and suppresses IgE-dependent mediator release in a parallel and superimposable dose–response fashion. Further, the relationship between the attenuation in the rise in cyclic AMP and the diminution in immunologic mediator release is linear with the regression line passing through the origin, indicating a direct relationship between the IgE-dependent activation of adenylate cyclase and preformed mediator release. Although not the only events in coupled mast cell activation–secretion, there is a sequential relationship among perturbation of IgE-Fc receptors, transmembrane activation of adenylate cyclase, elevation of cytoplasmic levels of cyclic AMP, activation of cyclic AMP-dependent protein kinase, and secretion of mast cell granules.**

The IgE-dependent stimulation of rat serosal mast cells initiates a series of biochemical events leading to the noncytotoxic release of preformed granule-associated mediators (1, 2) and the generation of secondary lipid mediators (3). Crosslinking of cell-bound IgE molecules with secondary bridging of IgE-Fc receptors apparently uncovers membrane-associated serine esterase(s) (4, 5) and stimulates the activity of two methyltransferases that sequentially convert phosphatidylethanolamine to phosphatidylcholine (6). Reorientation of phospholipids within the membrane is associated with the formation of calcium channels and an increase in calcium flux (6, 7). Other cellular events pertinent to coupled activation–secretion in mast cells include phosphorylation of intracellular proteins (8), energy utilization (9), organization of cytoskeletal contractile (10) and noncontractile (11) filaments, stimulation of Ca^{2+},Mg^{2+}-ATPase activity (12) and an apparent increase in perigranular membrane ion and water transport as inferred from swelling of the granule and partial solubilization of its matrix (13, 14).

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An additional early event in the activation of mast cells by IgE-Fc receptor bridging is a monophasic increase in cellular levels of cyclic AMP at 5–15 sec (15, 16). This increase is followed by a second monophasic increase in cyclic AMP at 3–5 min, which is suppressed by the cyclooxygenase inhibitor indomethacin (16) and is probably due to the generation of prostaglandins D_2 and I_2 (3). The early monophasic increase in cyclic AMP is not inhibited by indomethacin, precedes the release of preformed mediators (16), is synergistic in the presence of the methylxanthine phosphodiesterase inhibitor theophylline (17), and activates cytoplasmic cyclic AMP-dependent protein kinase (18). Thus, the transmembrane signal after IgE-Fc receptor bridging activates adenylate cyclase which, through the generation of cyclic AMP, could continue the activation–secretion response.

The functional significance of rat mast cell IgE-Fc receptor-dependent stimulation of adenylate cyclase has been further documented with purine- and ribose-modified analogs of adenosine to stimulate and inhibit, respectively, adenylate cyclase activity at the time of immunologic challenge. Adenosine and purine-substituted adenosine analogs stimulate adenylate cyclase by occupying a cell-surface receptor termed the R site, which, for activation, has an obligatory requirement for an intact ribose moiety (19, 20). High concentrations of adenosine through its metabolite 5′-AMP or 9-substituted adenine analogs inhibit adenylate cyclase activity by occupation of a receptor positioned on the inner surface of the plasma membrane, termed the P site because of an absolute requirement for an intact purine ring (19, 20). The requirement for activation of mast cell adenylate cyclase in immunologic mediator release was established by relating the cellular levels of cyclic AMP to the quantity of mediator release after immunologic challenge in the presence of adenosine or of one of its purine- or ribose-modified analogs.

MATERIALS AND METHODS

Materials. Porcine heparin, *p*-nitrophenol-β-D-2-acetamido-2-deoxyglucopyranoside, adenosine, and L-methionine (Sigma), DNase I (Calbiochem), gelatin (Difco), metrizamide (Accurate Chemical, Hicksville, NY), acetic anhydride and diethylamine (Mallinkrodt), Norit A decolorizing carbon (Fisher, Medford, MA), ^{125}I-labeled tyrosine methyl esters of

Abbreviations: TGD buffer, Tyrode's buffer containing 100 mg of gelatin and 1 mg of DNase per 100 ml; TGD^- buffer, calcium-free TGD buffer; ddAdo, 2′,5′-dideoxyadenosine; PIAdo, N^6-phenylisopropyladenosine; SQ 22536, 9-(tetrahydro-2-furyl)adenine.

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succinyladenosine and succinylguanosine 3′,5′-cyclic monophosphate (600 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels), cyclic nucleotide standards, rabbit cyclic nucleotide antisera, and normal rabbit serum (Collaborative Research, Waltham, MA), N^6-phenylisopropyladenosine (PIAdo) (Boehringer Manheim), 2′,5′-dideoxyadenosine (ddAdo) (P-L Biochemicals), and L-[*methyl*-^{3}H]methionine (15 Ci/mmol) (New England Nuclear) were obtained from the manufacturers. The 9-(tetrahydro-2-furyl)adenine (SQ 22536) was provided by D. N. Harris (Squibb). Rabbit IgG anti-rat IgE was prepared by ammonium sulfate fractionation and DEAE ion-exchange chromatography (21).

Mast Cell Activation. Suspensions of 5×10^5 purified mast cells (18) in 250 μl of Tyrode's buffer containing 100 mg of gelatin and 1 mg of DNase per 100 ml (TGD buffer) in the presence or absence of a pharmacologic agent were incubated with 10 μl of rabbit IgG anti-rat IgE or with buffer alone at 37°C for 0–5 min. The reactions were terminated by the addition of 1 vol of ice-cold calcium-free TGD buffer (TGD^- buffer) and the cells were sedimented at $400 \times g$ for 5 min at 4°C. The supernatant fractions were decanted, and the cell pellets were resuspended in 500 μl of TGD^- buffer and solubilized by sonication at 4°C. IgE-dependent mast cell release of β-hexosaminidase, which parallels that of histamine (2), was calculated by the following formula:

$$\text{Net \% release} = \frac{\text{stimulated release} - \text{unstimulated release}}{\text{stimulated release} + \text{residual} - \text{unstimulated release}} \times 100.$$

Cyclic nucleotide levels were measured in samples of 10^6 mast cells in 250 μl of TGD buffer carried in parallel with those assessed for β-hexosaminidase release. Reactions were terminated by the addition of 25 μl of 100% trichloroacetic acid and by freezing in dry ice/acetone. Duplicate reaction mixtures were extracted with ether to remove trichloroacetic acid, and after acetylation, cyclic AMP and cyclic GMP were quantified by radioimmunoassay (16, 22).

Changes in cyclic nucleotide levels and immunologic β-hexosaminidase release were analyzed by Student's *t* test for paired samples.

RESULTS

Effect of R Site Analogs on Mast Cell Immunologic Release of β-Hexosaminidase and Cyclic Nucleotide Levels. Cyclic nucleotide levels were assessed at 0, 5, 15, 30, 60, 120, and 300 sec after addition of PIAdo at a final concentration of 10^{-5} M or of buffer alone. PIAdo caused a rapid increase in mast cell cyclic AMP from 218 fmol/10^6 mast cells at time zero to a peak of 472 fmol/10^6 mast cells at 30 sec followed by a gradual decline to 312 fmol/10^6 mast cells at 5 min. There were no changes in cyclic GMP levels during the same 5-min period after the addition of PIAdo.

In three experiments, PIAdo was added to mast cells 15 sec before rabbit IgG anti-rat IgE so that their maximal effects on cyclic AMP would coincide 15 sec after IgE-Fc receptor perturbation (16). Immunologic release of β-hexosaminidase reached a maximum of 48 ± 5 (mean ± SEM) net percent. Stimulation of mast cells with 10^{-5} M PIAdo increased the rate of immunologic release between 5 and 15 sec by a factor of 4.5 ($P < 0.05$), with peak release at 60 sec being increased to 56% ± 2% ($P < 0.05$) (Fig. 1). IgE-dependent mediator release in buffer alone was preceded by an initial monophasic rise in cyclic AMP from a basal level of 119 ± 55 (mean ± SEM) to 179 ± 8 fmol/10^6 mast cells at 15 sec after challenge. PIAdo alone produced a similar rise in cyclic AMP to 173 ± 12 fmol/10^6 mast cells at this time. The combination of PIAdo stimulation of the R site and anti-IgE activation increased mast cell levels of cyclic AMP to 330 ± 10 and 390 ± 16 fmol/10^6 mast cells 15 and 30 sec after immunologic challenge, respectively. Thus, PIAdo accelerated and enhanced the release of a preformed mediator from mast cells and strikingly augmented the initial monophasic rise in the cellular level of cyclic AMP, the onset of which preceded mediator release.

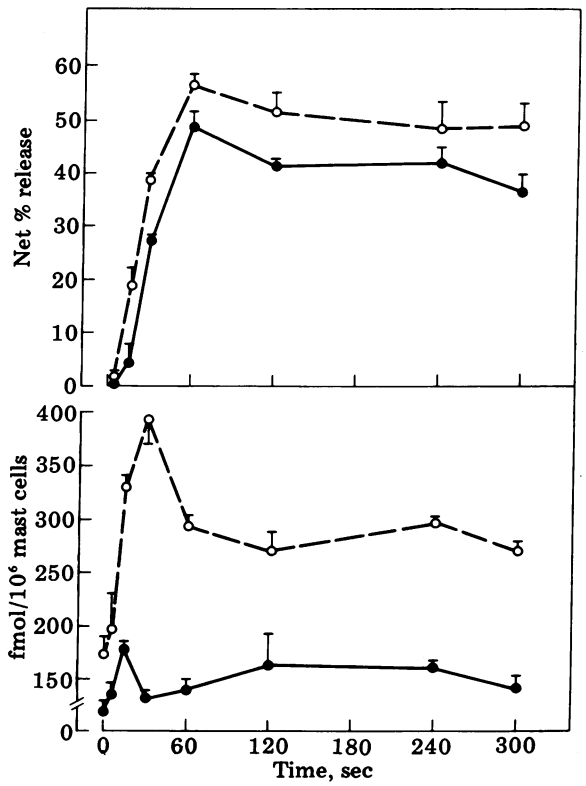

FIG. 1. Time course of net β-hexosaminidase release (*Upper*) and change in mast cell levels of cyclic AMP (*Lower*) induced by rabbit IgG anti-rat IgE in the absence (●—●) and presence (○- -○) of 10^{-5} M PIAdo. Bars represent the mean values ± SEM for three experiments carried out in duplicate. Spontaneous β-hexosaminidase release was 2.3% ± 0.8%.

The dose–response effects of two R site agonists, adenosine and PIAdo, introduced 15 sec before immunologic activation on mast cell mediator release at 2 min are depicted in Fig. 2 for five consecutive experiments. The dose–response effects of the agonists on the immunologic release of β-hexosaminidase were parallel and required similar concentrations to achieve half the maximal effect (ED_{50}, 2×10^{-7} M for PIAdo and 10^{-7} M for adenosine); however, the maximal augmentation of mediator release was less with PIAdo (39% ± 5%) than with adenosine (57% ± 4%) ($P < 0.005$). The almost identical dose–response curves for adenosine and PIAdo in increasing mast cell levels of cyclic AMP were parallel to those for enhancing β-hexosaminidase release but were shifted by 1 logarithm unit to the right (Fig. 2).

Effect of P Site Analogs on Mast Cell Immunologic Release of β-Hexosaminidase and Cyclic Nucleotide Levels. The 9-substituted adenine analogs, ddAdo and SQ 22536, were introduced 5 min before IgE-dependent mast cell activation in order to assess their effect on immunologic β-hexosaminidase release and on levels of cyclic nucleotides. In three kinetic experiments (Fig. 3), 500 μM ddAdo reduced the rate of β-hexosaminidase release 5.3-fold between 15 and 30 sec ($P < 0.01$) and 2.5-fold between 30 and 60 sec ($0.10 > P > 0.05$) and re-

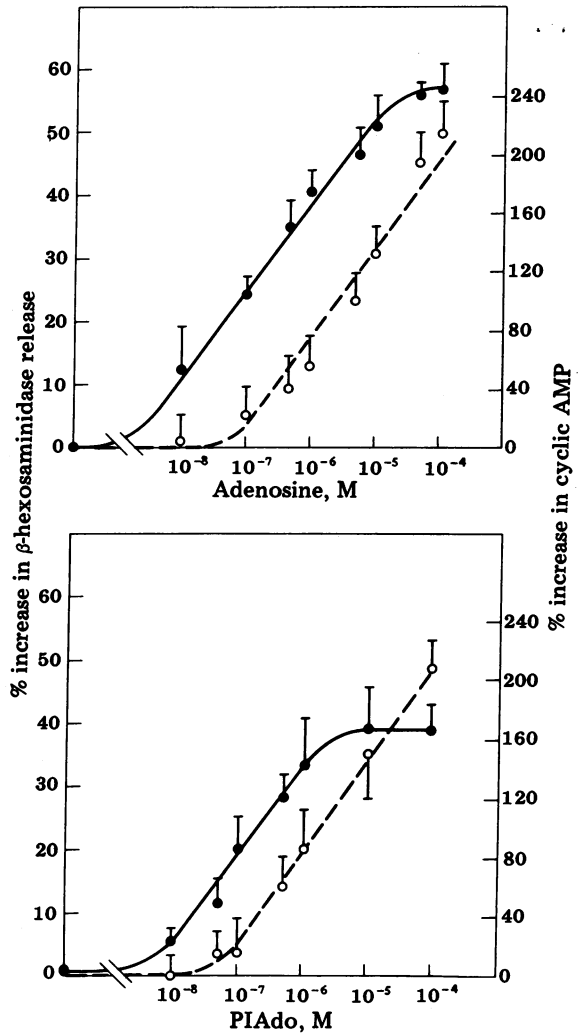

FIG. 2. Dose-related effects of adenosine (*Upper*) and PIAdo (*Lower*) on rat mast cell β-hexosaminidase release (●—●) and cyclic AMP levels (○- -○) after immunologic challenge of rat mast cells with rabbit IgG anti-rat IgE. Bars represent the mean values ± SEM for five experiments carried out in duplicate. Percent increase in β-hexosaminidase release (left ordinate) represents the increment of the mean net percent release relative to 22.6% ± 22% release in buffer alone; percent increase in cyclic AMP (right ordinate) represents the increment relative to 251 ± 89 fmol/10^6 mast cells challenged in buffer alone.

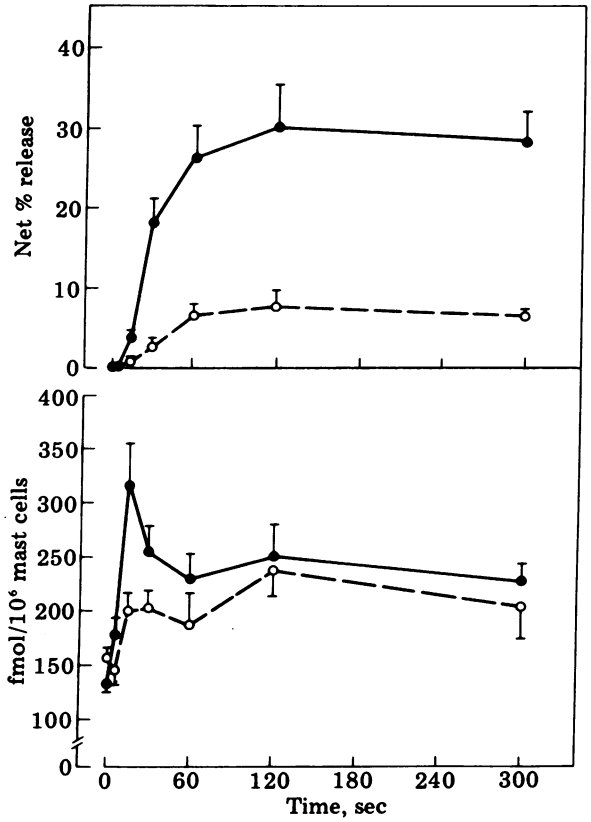

FIG. 3. Time course of net percent β-hexosaminidase release (*Upper*) and change in mast cell levels of cyclic AMP (*Lower*) induced by rabbit IgG anti-rat IgE in the absence (●—●) and presence (○- -○) of 500 μM ddAdo. Bars represent the mean values ± SEM for three experiments carried out in duplicate. Spontaneous β-hexosaminidase release was 1.8% ± 0.6%.

duced the peak net release at 120 sec from 30% ± 5% to 8% ± 2% ($P < 0.0005$). The initial monophasic rise in cyclic AMP from a base line of 139 ± 20 fmol/10^6 mast cells at time zero to 319 ± 74 fmol/10^6 mast cells at 15 sec was attenuated by the ddAdo to 200 ± 74 fmol/10^6 mast cells ($P < 0.05$). ddAdo had no effect on mast cell levels of cyclic GMP during the same 5-min period after immunologic activation. Thus, ddAdo both retarded and diminished the immunologic release of a preformed mast cell mediator and inhibited the initial monophasic rise in cyclic AMP.

The dose–response effects of ddAdo on the immunologic release of β-hexosaminidase from rat mast cells for five consecutive experiments are depicted in Fig. 4. ddAdo produced a dose-dependent inhibition of net β-hexosaminidase release from mast cells over a concentration range of 50–1000 μM. Within the same range of concentrations, ddAdo caused a parallel dose-dependent inhibition of the IgE-mediated initial rise in mast cell levels of cyclic AMP (Fig. 4). The values (ID_{50}) for the inhibition of both β-hexosaminidase release and the increase in cellular cyclic AMP induced by IgE-dependent mast cell activation were identical at 250 μM. The relationship between the net percent β-hexosaminidase release and the initial rise in cyclic AMP is linear, with the regression line passing through the origin (Fig. 4). In two dose–response experiments with SQ 22536, the ID_{50} values for the inhibition of net percent β-hexosaminidase release and the attenuation in the initial rise of cyclic AMP were 250 μM and 300 μM, respectively. In addition, the two dose–response curves were parallel to each other and to those with ddAdo. Over the range of concentrations used, neither ddAdo nor SQ 22536 was cytotoxic, as assessed by the release of the cytoplasmic enzyme lactate dehydrogenase (23).

To determine whether immunologic activation of mast cell adenylate cyclase is dependent upon phospholipid methylation, we observed the effect of ddAdo on plasma membrane methyltransferase activity. Fourteen million purified mast cells in 3.5 ml of TGD$^-$ buffer containing 2 μM L-[*methyl*-^{3}H]methionine were incubated at 37°C for 30 min, washed, resuspended in TGD buffer, and immunologically challenged with rabbit IgG anti-rat IgE under standard conditions in the presence of 0–1000 μM ddAdo. The mast cell reactions to be measured for phospholipid methylation were terminated after 15 sec by addition of 500 μl of ice-cold 10% trichloroacetic acid containing 10 mM L-methionine followed by freezing in dry ice/acetone. The tubes were centrifuged at 18,000 × *g* for 10 min at 4°C, and the precipitates, after being washed with 1 ml of 10% trichloroacetic acid, were individually extracted into 3 ml of chloroform/methanol, 2:1 (vol/vol). The extracted membrane phospholipid was assessed for methylation by liquid

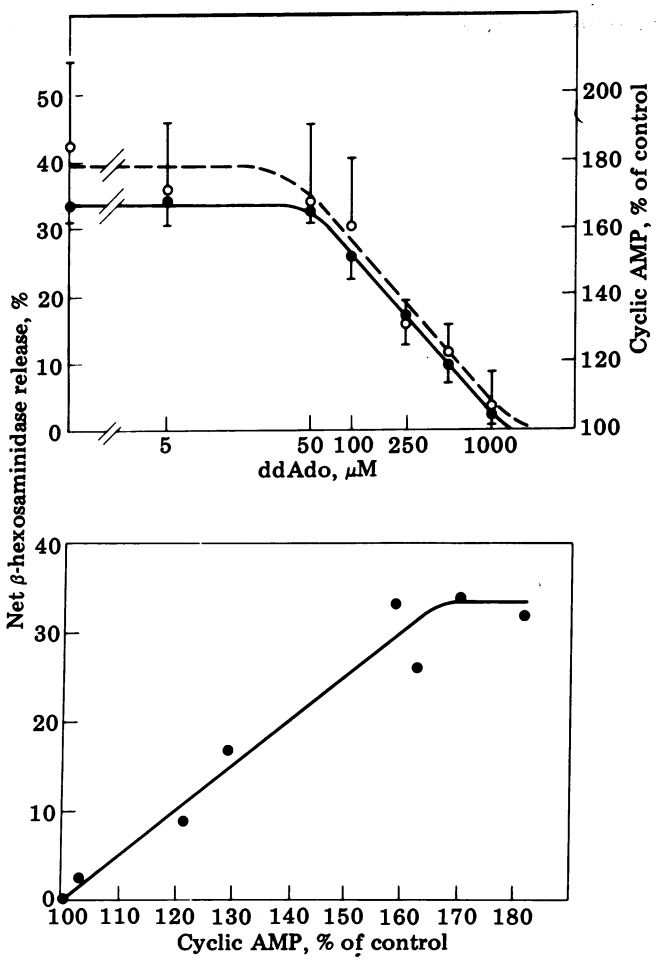

FIG. 4. Dose-related effects of ddAdo on rat mast cell net percent β-hexosaminidase release (●—●) and on percent increase in cellular levels of cyclic AMP (○- -○) after immunologic challenge with rabbit IgG anti-rat IgE (*Upper*). These data are replotted (*Lower*) as net percent β-hexosaminidase release against percent increase in cellular cyclic AMP. Spontaneous β-hexosaminidase release was 2.3% ± 1.1%; the cyclic AMP level in unchallenged cells was 139 ± 20 fmol/10^6 mast cells suspended in buffer alone.

scintillation spectrometry (6). In three experiments, IgE-dependent activation of rat mast cells gave a net 35% ± 2% (mean ± SEM) β-hexosaminidase release and increased [^{3}H]CH_3 incorporation into phospholipid from a basal level of 1116 ± 146 to 1540 ± 170 dpm (38% increase). Increasing concentrations of ddAdo between 50 μM and 1000 μM caused a dose-related inhibition of mediator release but had no significant effect on membrane phospholipid methylation.

DISCUSSION

The initial monophasic rise in cellular levels of cyclic AMP to twice the baseline level within 5–15 sec of perturbation of the IgE-Fc receptors of rat mast cells (15, 16) introduced the possibility that the receptor was directly linked to adenylate cyclase by a series of transmembrane proteins (24, 25). The transmembrane linkage of the IgE-Fc receptor to adenylate cyclase is consistent with the synergistic rise in cyclic AMP observed when receptor perturbation is coupled with the presence of theophylline, a methylxanthine phosphodiesterase inhibitor (17). The current finding that adenosine or an adenosine analog modified in the purine ring to specifically activate the R site of adenylate cyclase is more than additive with perturbation of the IgE-Fc receptor in raising levels of mast cell cyclic AMP provides additional evidence of the transmembrane linkage between the receptor for IgE and activation of adenylate cyclase (Figs. 1 and 2).

The recent finding that, with increasing concentrations of the immunologic stimulus, the mediator release was inversely related to the remaining mast cell concentration of cyclic AMP-dependent protein kinase suggested that a step between the elevation of cellular cyclic AMP and the release of mediators was the uncovering of cyclic AMP-dependent protein kinase activity (18). Direct evidence for a relationship between IgE-dependent activation of adenylate cyclase and the release of granule constituents is derived from the capacity of purine- and ribose-modified adenosine analogs, respectively, to augment and suppress mediator release while simultaneously increasing and decreasing the activity of adenylate cyclase.

The presence of an R site agonist during IgE-dependent mast cell activation increased the rate and peak amplitude of mediator release (Fig. 1). R site stimulation by PIAdo alone does not cause granule secretion, and the extent to which it augments IgE-dependent secretion reflects the coupling of R receptors via the regulatory protein of adenylate cyclase to its catalytic site (20). Although the dose–response relationships for the action of the R site agonists on increasing immunologic β-hexosaminidase release and cellular cyclic AMP levels were parallel, the efficiency was 10 times greater for mediator release than for cyclic AMP (Fig. 2), suggesting that only a limited fraction of the R receptors was coupled to the IgE receptors. The potentiating effects of R site agonists on ionophore A23187-induced release from rat mast cells are reported to be greater than for IgE-dependent release (26, 27), reflecting the absence of a requirement for coupling of the R receptor to a receptor required in the activation process. The capacity of adenosine to increase IgE-dependent β-hexosaminidase release at concentrations beyond those at which PIAdo is active may be related either to the ability of adenosine to stimulate more than one subclass of cell surface R site (28) or to the metabolism of adenosine to derivatives, such as inosine, which may in themselves facilitate the release reaction (26).

The most convincing evidence of a direct relationship between IgE-dependent activation of adenylate cyclase and mediator release was the capacity of 9-substituted adenine derivatives, which inhibit adenylate cyclase at the P site (19, 29, 30), to attenuate the increase in mast cell cyclic AMP and suppress mediator release in a parallel and superimposable dose–response fashion (Fig. 4). The relationship between the attenuation of the increase in cyclic AMP and the diminution in mediator release is linear, with the regression line passing through the origin (Fig. 4). This indicates that any measurable attenuation in the immunologic release of β-hexosaminidase is directly related to the inhibition of IgE-receptor-linked adenylate cyclase. It thereby follows that there is a sequential relationship among IgE-Fc receptor perturbation, transmembrane activation of adenylate cyclase, increase in cytoplasmic levels of cyclic AMP, activation of cyclic AMP-dependent protein kinase, and secretion of mast cell granules.

The finding that IgE-Fc receptor bridging on purified rat serosal mast cells to initiate mediator release is associated with an increase in the intracellular level of cyclic AMP appears to contradict the earlier studies showing that pharmacologically induced elevations in cyclic AMP levels suppress release. Theophylline and aminophylline, inhibitors of cyclic nucleotide phosphodiesterase, produce dose-dependent inhibition of rat mast cell mediator release which closely correlates with intracellular levels of cyclic AMP (17, 31–34). However, adrenaline, isoprenaline, albuterol (32, 35, 36), prostaglandin D_2, prostaglandin I_2 (17), and the phosphodiesterase inhibitor isobutyl-

methylxanthine (34, 37) not only fail to inhibit release of rat peritoneal mast cell mediators but in some cases cause enhancement. If the inhibitory action of theophylline on mediator release from purified rat mast cells is due to increased levels of cyclic AMP, the inability of prostaglandin D_2, β-adrenergic agonists, and isobutylmethylxanthine also to produce inhibition by increasing cyclic AMP suggests separate pools of cyclic AMP, only one of which decreases coupled activation–secretion. The mast cell, similar to other cells, contains two isoenzymes of cyclic AMP-dependent protein kinase which can be resolved by DEAE-cellulose chromatography (18), and their differential activation by stimuli acting via cyclic AMP could, in part, determine the physiologic effect of the stimuli. Although the inhibitory action of theophylline could be due to cyclic AMP-dependent phosphorylation so as to inactivate a critical protein in the reaction sequence, an alternative possibility is that competitive depletion of a cyclic AMP-dependent holoenzyme *per se* prevents progression of the activation–secretion process.

Both the initial monophasic increase in levels of cyclic AMP in rat mast cells and the stimulation of membrane phospholipid methylation occur within 15 sec of cell activation (6, 16), but nonetheless appear to be independent of one another. Inhibition of phospholipid methylation prevents mediator release but does not affect the early rise in cyclic AMP (5), and the inhibition of mast cell adenylate cyclase activity inhibits mediator release but has no effect on lipid methylation. The recent report (5) that diisopropyl fluorophosphate prevents the early increase in both cyclic AMP and phospholipid methylation with immunologic challenge of rat mast cells suggests that these events are each initiated by membrane serine esterase(s) which become activated by IgE-Fc receptor dimerization (4).

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1. Becker, E. L. & Henson, P. M. (1973) *Adv. Immunol.* 93–193.
2. Schwartz, L. B., Austen, K. F. & Wasserman, S. I. (1979) *J. Immunol.* **123,** 1445–1450.
3. Lewis, R. A., Holgate, S. T. & Austen, K. F. in *Biochemistry of the Acute Allergic Reactions,* Fourth International Symposium, eds. Becker, E. L. & Austen, K. F. (Liss, New York), in press.
4. Becker, E. L. & Austen, K. F. (1966) *J. Exp. Med.* **124,** 379–385.
5. Ishizaka, T., Hirata, F., Ishizaka, K. & Axelrod, J. in *Biochemistry of the Acute Allergic Reactions,* Fourth International Symposium, eds. Becker, E. L. & Austen, K. F. (Liss, New York), in press.
6. Ishizaka, T., Hirata, R., Ishizaka, K. & Axelrod, J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1903–1906.
7. Foreman, J. C., Hallet, M. B. & Mongar, J. L. (1977) *J. Physiol. (London)* **271,** 193–214.
8. Sieghart, W., Theoharides, T. C., Alper, J. E., Douglas, W. W. & Greengard, P. (1978) *Nature (London)* **275,** 329–331.
9. Diamant, B. (1975) *Int. Arch. Allergy Appl. Immunol.* **49,** 155–171.
10. Rohlich, P. (1975) *Exp. Cell Res.* **93,** 293–298.
11. Lagunoff, D. & Chi, E. Y. (1976) *J. Cell Biol.* **57,** 252–259.
12. Chakravarty, N. & Echetebu, Z. (1978) *Biochem. Pharmacol.* **27,** 1561–1569.
13. Lawson, D., Raff, M. C., Gomperts, B., Fewtrell, C. & Gibula, N. B. (1977) *J. Cell Biol.* **72,** 242–257.
14. Caulfield, J. P., Lewis, R. A., Hein, A. & Austen, K. F. (1980) *J. Cell Biol.* **85,** 299–312.
15. Sullivan, T. J., Parker, K. L., Kulczycki, A., Jr. & Parker, C. W. (1976) *J. Immunol.* **117,** 713–716.
16. Lewis, R. A., Holgate, S. T., Roberts, L. J., II, Maguire, J. F., Oates, J. A. & Austen, K. F. (1979) *J. Immunol.* **123,** 1663–1668.
17. Holgate, S. T., Lewis, R. A., Maguire, J. F., Roberts, L. J., II, Oates, J. A. & Austen, K. F. (1980) *J. Immunol.* **125,** 1367–1373.
18. Holgate, S. T., Lewis, R. A. & Austen, K. F. (1980) *J. Immunol.* **124,** 2093–2099.
19. Londos, C. & Wolff, J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5482–5486.
20. Fain, J. L. & Malbon, C. C. (1979) *Mol. Cell. Biochem.* **25,** 143–169.
21. Fahey, J. L. & Terry, E. W. (1973) in *Handbook of Experimental Immunology,* ed. Weis, D. M. (Blackwell, London), Vol. 1, p. 71.
22. Harper, J. F. & Brooker, G. (1975) *J. Cyclic Nucleotide Res.* **1,** 207–218.
23. Amador, E., Dorfman, L. E. & Wacker, W. E. C. (1963) *Clin. Chem.* **9,** 391–399.
24. Ross, E. M. & Gilman, A. G. (1977) *J. Biol. Chem.* **252,** 6966–6969.
25. Ross, E. M., Howlett, A. C., Ferguson, K. M. & Gilman, A. G. (1978) *J. Biol. Chem.* **253,** 6401–6412.
26. Marquardt, D. L., Parker, C. W. & Sullivan, T. J. (1978) *J. Immunol.* **120,** 871–878.
27. Welton, A. F. & Simko, B. A. (1980) *Biochem. Pharmacol.* **29,** 1085–1092.
28. Londos, C., Cooper, D. M. F. & Wolff, J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2251–2254.
29. Haslam, R. J., Davidson, M. L. & Desjardins, J. V. (1978) *Biochem. J.* **176,** 83–95.
30. Harris, D. N., Asaad, M. M., Philips, M. B., Goldenberg, J. J. & Antonaccia, M. J. (1979) *J. Cyclic Nucleotide Res.* **5,** 125–134.
31. Kaliner, M. & Austen, K. F. (1974) *J. Immunol.* **112,** 664–674.
32. Sullivan, T. J., Parker, K. L., Eisen, S. A. & Parker, C. W. (1975) *J. Immunol.* **114,** 1480–1485.
33. Fredholm, B. B., Guschin, I., Elwin, K., Schwab, G. & Üvnas, B. (1976) *Biochem. Pharmacol.* **25,** 1583–1588.
34. Norn, S., Geisler, A., Stahl Skov, P. & Klysner, R. (1977) *Acta Allergol.* **32,** 183–191.
35. Johnson, A. R., Moran, N. C. & Mayer, S. E. (1974) *J. Immunol.* **112,** 511–519.
36. Butchers, P. R., Fullarton, J. R., Skidmore, I. F., Thompson, L. E., Vardey, C. J. & Wheeldon, A. (1979) *Br. J. Pharmacol.* **67,** 23–32.
37. Norn, S., Geisler, A., Stahl Skov, P. & Klysner, R. (1979) *Agents Actions* **9,** 64–65.