Biochemical Pharmacology, Vol. 37, No. 20, pp. 4019–4025, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

ALTERATION OF MAST CELL RESPONSIVENESS TO ADENOSINE BY PERTUSSIS TOXIN*

DIANA L. MARQUARDT†‡ and LINDA L. WALKER
University of California, San Diego School of Medicine, San Diego, CA, U.S.A.

(*Received* 9 *November* 1987; *accepted* 12 *April* 1988)

Abstract—Adenosine potentiates mouse bone marrow-derived mast cell mediator release by a mechanism that appears to involve cell surface adenosine receptors. In an attempt to explore possible interactions between G proteins and adenosine receptors, mast cells were incubated with activated pertussis toxin, an agent that ADP-ribosylates and inactivates some G protein subtypes, prior to challenge with specific antigen or the calcium ionophore A23187. Mast cells preincubated with 10 ng/ml pertussis toxin for at least 2 hr exhibited an inhibition of antigen-induced β-hexosaminidase and leukotriene C_4 release. The ability of adenosine to potentiate β-hexosaminidase release was attenuated to an even greater degree by pertussis toxin. A23187-stimulated mediator release was not altered by pertussis toxin, although a modest inhibition of the ability of adenosine to enhance A23187-induced β-hexosaminidase release was observed in pertussis toxin-treated mast cells. Although up to 24-hr exposure to 100 ng/ml pertussis toxin did not alter resting mast cell cyclic AMP levels, the ability of adenosine to elevate cell cyclic AMP concentrations was diminished markedly by doses of the toxin higher than those required to affect mediator release. Neither antigen-stimulated intracellular free calcium level augmentation alone nor the additional potentiation of these levels by adenosine was changed by pertussis toxin treatment. Inositol trisphosphate was generated by mast cells stimulated by IgE-mediated mechanisms, but a preincubation with pertussis toxin did not influence its generation. In summary, adenosine appeared to produce some of its alterations in mast cell biochemical events by a mechanism that was partially inhibited by pertussis toxin. The nature of the G protein linked to the mast cell adenosine receptor is yet to be determined.

Adenosine potentiates stimulated mediator release from both connective tissue and mucosal-type mast cells [1, 2], but its mechanism of action is only partially understood. Adenosine appears to act primarily at cell surface receptors that can be up-regulated by chronic exposure to adenosine receptor antagonists [3] and down-regulated by preincubation with adenosine analogs [4]. However, the subtype(s) of G protein(s) that mediates the effects of adenosine on mast cells is uncertain at present. In some cell types, an enhancement of adenylate cyclase activity induced by hormones is associated with a stimulation of a G_s protein and an inhibition of adenylate cyclase activity is associated with the stimulation of a G_i protein [5]. In the mast cell, adenosine rapidly augments intracellular cyclic AMP concentrations [2] and potentiates mediator release from stimulated cells, but an increase in mast cell cyclic AMP levels induced by other agents is associated with an inhibition of mast cell mediator release [6], raising doubts as to the importance of cyclic AMP metabolism in the regulation of mast cell secretion.

Guanine nucleotide binding proteins (G proteins) appear to function in signal transduction between cell surface receptors and adenylate cyclase activity or polyphosphoinositide phosphodiesterase activity [5], among others. Several subtypes of G proteins have been identified, and numerous agents that interact with these proteins and alter their activities have been described [5]. However, many of the agents that have been shown to ADP-ribosylate a particular G protein subunit may also influence other G protein subtypes, resulting in complex biochemial responses [7]. Pertussis toxin has been shown to ADP-ribosylate a putative G_i in mast cells [8], to inhibit mast cell secretion under some experimental conditions [9], and to fail to affect mast cell secretion under other conditions [8].

To begin to explore the mast cell–adenosine receptor–G protein–catalytic subunit interactions involved in the potentiation of preformed mast cell mediator release, pertussis toxin was used as an initial probe to ascertain its effects on adenylate cyclase activity, inositol trisphosphate formation, mediator release, and calcium fluxes in the absence and presence of exogenous adenosine. Information regarding the sometimes conflicting reports [8, 9] of the ability of pertussis toxin to alter mast cell mediator release was also obtained.

MATERIALS AND METHODS

Chemicals. The following were purchased from the manufacturers indicated: adenosine, 2-mercaptoethanol, *N*-acetyl-β-D-glucosaminide (Sigma

* This work was supported by National Institutes of Health Grants AI-21504, AI-17268, and the Hartford Foundation.

† Dr. Marquardt is the recipient of a John A. and George L. Hartford Foundation fellowship.

‡ Correspondence address: Diana L. Marquardt, M.D., c/o UCSD Medical Center, H-811-G, 225 Dickinson St., San Diego, CA 92103.

Chemical Co., St. Louis, MO); ^{125}I-labeled cAMP radioimmunoassay kit, [^{3}H]myoinositol (Amersham, Arlington Heights, IL); [^{3}H]LTC_4* radioimmunoassay kit (New England Nuclear, Boston, MA); calcium ionophore A23187 (Calbiochem, La Jolla, CA); RPMI 1640, penicillin/streptomycin, L-glutamine, MEM nonessential amino acids, fetal calf serum (GIBCO, Grand Island, NY); pertussis toxin (List Biological Laboratories, Campbell, CA); and fura-2 (Molecular Probes, Inc., Eugene, OR).

The following were donated: mouse hybridoma anti-dinitrophenyl (DNP) IgE antibody, dinitrophenyl–bovine serum albumin (DNP–BSA) antigen (18 DNP molecules/BSA), and [^{125}I]-labeled IgE (Drs. Futong Liu and David Katz, La Jolla, CA).

Culture of mouse bone marrow-derived mast cells. Bone marrow from femurs from BALB/c mice (Simonsen Laboratories, Gilroy, CA) was cultured in 20% WEHI-3 supernatant fraction [10] and 90% RPMI supplemented with 10% fetal calf serum, 50 μM 2-mercaptoethanol, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 100 units/ml penicillin and 100 μg/ml streptomycin. Cells were passaged weekly, and by 21 days of tissue culture were more than 90% viable, pure mast cells.

β-Hexosaminidase and LTC_4 releases from mast cells. Cultured mast cells were washed free of medium three times, passively sensitized with a saturating dose of anti-DNP IgE (1 μg/10^6 cells) where appropriate for 30 min at 37° in 1 ml Tyrode's buffer lacking divalent cations, exposed to pharmacologic agents or buffer for the times indicated, and challenged with DNP–BSA antigen (200 ng/3×10^5 cells in 400 μl) or the calcium ionophore A23187 (1 μg/ml) in complete Tyrode's buffer for 10 min at 37°. Supernatant fractions and pellets were separated by centrifugation at 200 *g* for 10 min, and each was assayed for β-hexosaminidase activity as described elsewhere [11]. β-Hexosaminidase is a preformed, granule-associated mast cell mediator whose release generally parallels that of histamine [12].

In separate experiments, supernatant aliquots were stored at −20° for up to 4 weeks and assayed for immunoreactive LTC_4 concentrations using a [^{3}H]LTC_4 radioimmunoasay. Although there is some cross-reactivity in the assay between LTC_4, LTD_4, and LTE_4, mouse bone marrow-derived mast cells primarily synthesize LTC_4 by lipoxygenation of arachidonic acid, and the assay compares favorably with high performance liquid chromatography in this system [13].

Assessment of mast cell cyclic AMP concentrations. To quantitate intracellular cyclic AMP concentrations, washed mouse bone marrow-derived mast cells (4×10^5 cells/180 μl) were warmed to 37° in Tyrode's buffer, treated with the appropriate agent or buffer alone for the times indicated, and further stimulated with adenosine or buffer for 15 sec. Reactions were terminated by the addition of 20 μl of ice-cold 100% TCA and freezing in dry ice and acetone. Reaction mixtures were later thawed, sonicated, centrifuged at 250 *g* for 20 min, extracted three times with water-saturated ether, lyophilized, and frozen at −20°. Samples were resuspended in 50 mM acetate buffer, acetylated, and assayed utilizing a commercially-available ^{125}I-labeled cyclic AMP radioimmunoassay kit. This assay is useful for cyclic AMP concentrations as low as 0.0025 pM and exhibits negligible cross-reactivity with AMP, ADP, ATP, and cyclic GMP.

Assessment of intracellular free calcium levels. Bone marrow-derived mast cells (5–10 $\times$ 10^6 cells) were incubated in complete medium at 37° for 2 hr in the absence or presence of activated pertussis toxin. Cells were washed twice with Hanks' buffer containing 1 mM Ca^{2+} and 0.5 mM Mg^{2+} and resuspended in 0.5 ml Hanks' buffer and 5 μl of 1 mM fura-2 for 20 min at 37°. The suspension was diluted with buffer to 5 ml, and anti-DNP IgE (2 μl/10^6 cells) was added for 40 min, followed by two more washes. Cells were finally resuspended in a concentration of 10^6 cells/ml, and 2-ml aliquots were placed into a cuvette at 37° with constant stirring. Cells were excited at 340 nm wavelength and emission was measured at 505 nm. Ca^{2+} concentrations were determined by the formula:

$$[Ca^{2+}] = 224 \times 10^{-9}\ M \cdot \frac{(F - F_{min})}{(F_{max} - F)}$$

where F is the fura-2 fluorescence reading obtained, F_{max} is the reading after the addition of 20 μl of 10% Triton X-100, F_{min} is the reading after the addition of 20 μl of 100 mM $MnCl_2$, and 224×10^{-9} M is the K_D for fura-2/Ca^{2+} as determined in this system.

Generation of inositol phosphates. Mast cells (10^7 cells/ml culture medium) were incubated with 100 μCi myo[2-^{3}H]inositol, 10 μl anti-DNP IgE, and either 10 ng/ml pertussis toxin or medium alone for 2 hr at 37° in a CO_2 incubator. Cells were washed twice with Tyrode's buffer lacking divalent cations and containing 10 mM inositol and resuspended in 10 ml of 10 mM inositol in Tyrode's buffer for 30 min at 37°. Cells were again washed with Tyrode's buffer, resuspended in a volume of 5×10^6 cells/ml, warmed to 37° for 5 min, and challenged with 200 ng DNP-BSA antigen/10^6 cells for the times shown. Reactions were stopped by adding 200 μl of ice-cold 15% TCA and placing mixtures on ice for 10 min, centrifuging at 200 *g* for 10 min, and collecting supernatant fractions for multiple ether extractions and neutralization with 5.6 mM borax. Labeled inositol phosphates were separated using Dowex columns as detailed elsewhere [14].

Quantitation of mast cell IgE receptors. IgE receptors on mouse bone marrow-derived mast cells were examined by [^{125}I]-labeled IgE binding to the cells in the presence of known concentrations of cold IgE by the method of Kulczycki and Metzger [15].

Statistical analysis of data. Statistical significance was determined using the paired, two-tailed Student's *t*-test. Results are expressed as means ± standard errors (SE) unless otherwise specified.

RESULTS

Effect of pertussis toxin on IgE- and A23187-stimulated granule-associated mediator release from mouse

* Abbreviations: LTC_4, leukotriene C_4; DNP–BSA, dinitrophenyl-bovine serum albumin; IP_3, inositol trisphosphate; and TCA, trichloroacetic acid.

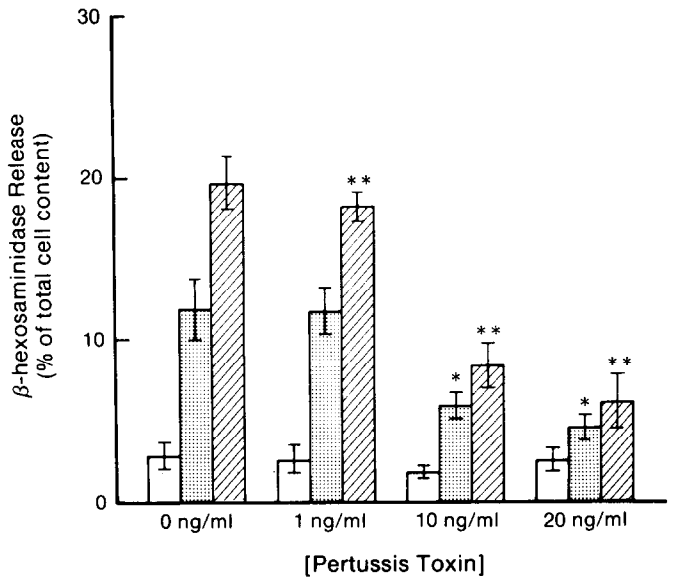

Fig. 1. Antigen-stimulated β-hexosaminidase release from pertussis toxin-treated mast cells. Mouse bone marrow-derived mast cells were incubated in the concentrations of pertussis toxin shown for 2 hr, sensitized, and challenged for 10 min in the absence (▨) or presence (▨) of 10 μM adenosine. β-hexosaminidase released into the supernatant fraction is expressed as a perdent of total cell β-hexosaminidase content. Total cell β-hexosaminidase measured spectrophotometrically at 410 nm averaged 1.22 ± 0.09 (mean ± SE, N = 12) O.D. units/3 × 10^5 cells. Depicted are means ± SE of values from six experiments performed in duplicate. Key: (*) indicates significantly different from controls ($P < 0.005$); and (**) indicates significantly different from controls, and that adenosine potentiation was significantly less than in control cells ($P < 0.05$).

bone marrow-derived mast cells. Mouse bone marrow-derived mast cells sensitized with anti-DNP IgE and challenged with DNP–BSA antigen released approximately 12% of the total cell β-hexosaminidase content, and this release nearly doubled if challenges were performed in the additional presence of 10 μM adenosine. Mast cells incubated with activated pertussis toxin for 2 hr prior to challenge exhibited a dose-dependent inhibition of antigen-stimulated β-hexosaminidase release and an even greater relative inhibition of mediator release induced by antigen and adenosine together (Fig. 1). When concentrations of pertussis toxin ≥100 ng/ml were incubated with cells overnight, some variable cytotoxicity was evidenced by occasional increased spontaneous release of β-hexosaminidase and marked depletion of preformed mast cell mediators. The number of cell surface IgE receptors on control and pertussis toxin-treated cells was approximately 1 × 10^5 receptors/cell in each population.

Mast cells similarly treated with pertussis toxin responded differently when the calcium ionophore A23187 was used as the secretagogue (Fig. 2). Up to 20 ng/ml of pertussis toxin for 2 hr failed to induce an inhibition of A23187-stimulated β-hexosaminidase release, nor did 100 ng/ml pertussis toxin overnight when cytotoxicity was not excessive. However, a modest, statistically significant inhibition of the ability of 10 μM adenosine to potentiate A23187-induced mediator release occurred at 10 and 20 ng/ml pertussis toxin concentrations. Thus, pertussis toxin seemed to display a greater effect on the adenosine-induced potentiation of mediator release than on antigen-stimulated mediator release alone and no measurable effect on ionophore-stimulated degranulation.

Pertussis toxin effects on mast cell leukotriene C_4 generation. Mouse bone marrow-derived mast cells sensitized with anti-DNP IgE and challenged with DNP–BSA antigen generated approximately 70 ng $LTC_4/10^6$ cells, and even greater amounts of LTC_4 when A23187 was the stimulus (Fig. 3). Previous studies have demonstrated that exogenous adenosine fails to potentiate LTC_4 production in these cells [3]. A 2-hr preincubation with activated pertussis toxin induced a modest, dose-dependent inhibition of antigen-stimulated mast cell LTC_4 generation but did not alter significantly the production of LTC_4 induced by A23187 (Fig. 3). Thus, the ability of pertussis toxin to block antigen- but not ionophore-stimulated mediator release appears to hold for both preformed and generated mast cell mediators.

Pertussis toxin effects on mast cell cyclic AMP levels. By ADP-ribosylating the G_i protein and perhaps others as well, pertussis toxin is thought to alter cyclic AMP concentrations in some cell types. Mouse bone marrow-derived mast cells exhibited a rapid enhancement of intracellular cyclic AMP concentrations induced by adenosine and a more modest, inconsistent enhancement of cyclic AMP in the presence of isoproterenol. Cells incubated for 2 hr with 10 ng/ml pertussis toxin demonstrated a very modest attenuation of the ability of adenosine

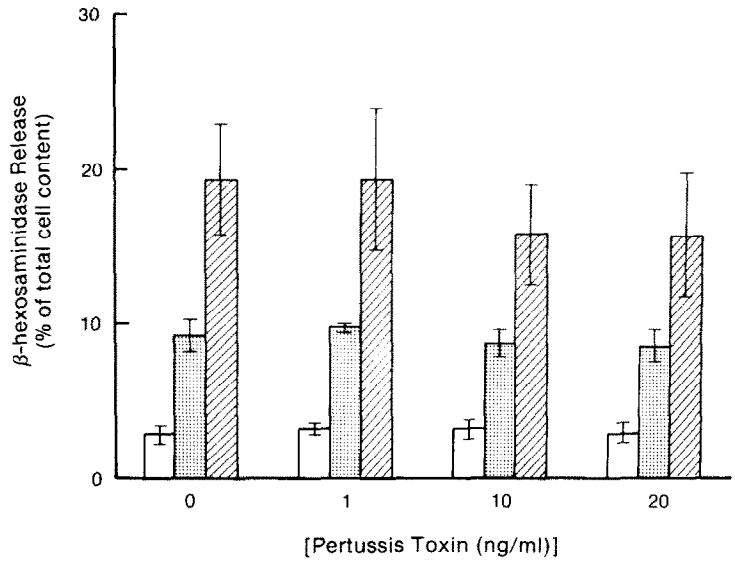

Fig. 2. A23187-stimulated β-hexosaminidase release from pertussis toxin-treated mast cells. Mouse bone marrow-derived mast cells incubated for 2 hr with pertussis toxin (1–20 ng/ml) or buffer alone were challenged with A23187 (▨) or A23187 and 10 μM adenosine (▨). β-hexosaminidase released into the supernatant fraction is expressed as a percentage of total cell β-hexosaminidase content. Shown are means ± SE derived from eight experiments performed in duplicate. A significant inhibition of the ability of adenosine to augment β-hexosaminidase release was observed at pertussis toxin concentrations of 10 and 20 ng/ml ($P < 0.05$).

to enhance cyclic AMP (Fig. 4), even though this concentration clearly inhibited mediator release. Resting cyclic AMP levels were nearly identical in controls and cells treated with all concentrations of pertussis toxin studied. A higher but non-toxic dose of pertussis toxin (100 ng/ml for 2 hr) provoked a significant ($P < 0.01$) diminution in adenosine-induced cyclic AMP formation that was similar to

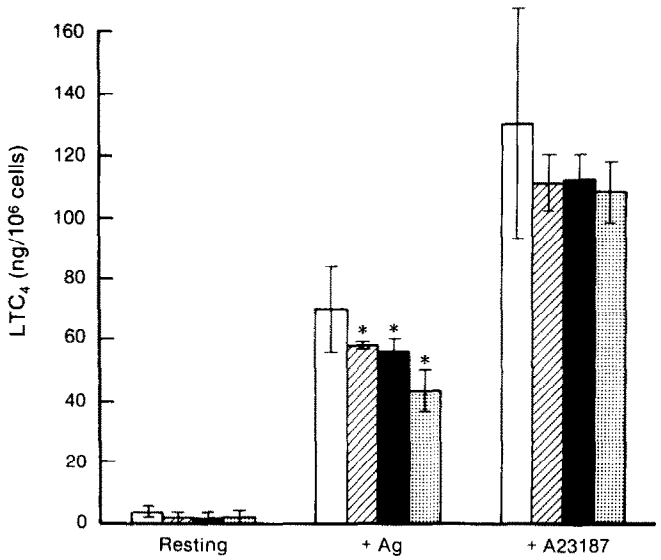

Fig. 3. Effects of pertussis toxin on mast cell leukotriene C_4 generation. Mouse bone marrow-derived mast cells were incubated for 2 hr with buffer alone (□), or the following concentrations of pertussis toxin: 1 ng/ml (▨), 10 ng/ml (■), 20 ng/ml (▨), followed by sensitization and challenge with either antigen or A23187. LTC_4 concentrations in supernatant fractions were assessed by radioimmunoassay and are expressed as means ± SE of values from three or more experiments performed in duplicate.
Key: (*) significantly different from control values in the absence of pertussis toxin ($P < 0.05$).

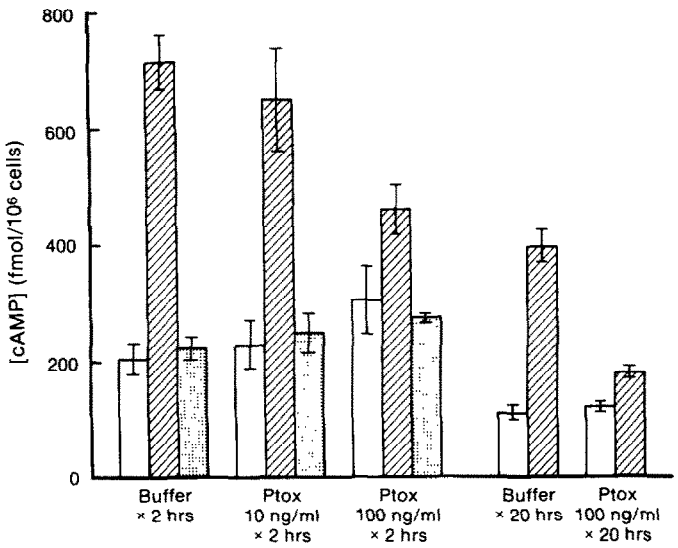

Fig. 4. Effects of pertussis toxin on resting and stimulated mast cell cyclic AMP concentrations. Mouse bone marrow-derived mast cells incubated in buffer or pertussis toxin for either 2 hr or overnight were stimulated with buffer alone (□), 10 μM adenosine (▨), or 10 μM isoproterenol (▩) for 15 sec, and intracellular cyclic AMP levels were assessed. Shown are means ± SE of values from seven experiments in duplicate. Cells treated with 100 ng/ml pertussis toxin for either time period displayed cyclic AMP concentrations significantly different from control values in the presence of adenosine ($P < 0.01$).

the inhibition of the effects of adenosine observed in cells cultured overnight in 100 ng/ml pertusis toxin (Fig. 4). These data seem to indicate that mediator release in the absence or presence of adenosine is inhibited more easily by pertussis toxin than is cyclic AMP production.

Pertussis toxin effects on resting and stimulated mast cell intracellular calcium concentrations and inositol phosphates in the absence and presence of adenosine. Whether pertussis toxin is capable of blocking the linkage between mast cell secretagogues and calcium fluxes or phosphatidylinositide hydrolysis is controversial and appears to differ considerably in different cells and species [8, 9, 16]. Sensitized mast cells stimulated with specific antigen exhibited a nearly 2-fold increase in intracellular free calcium as measured by fura-2 fluorescence (Fig. 5). An additional potentiation of intracellular free calcium

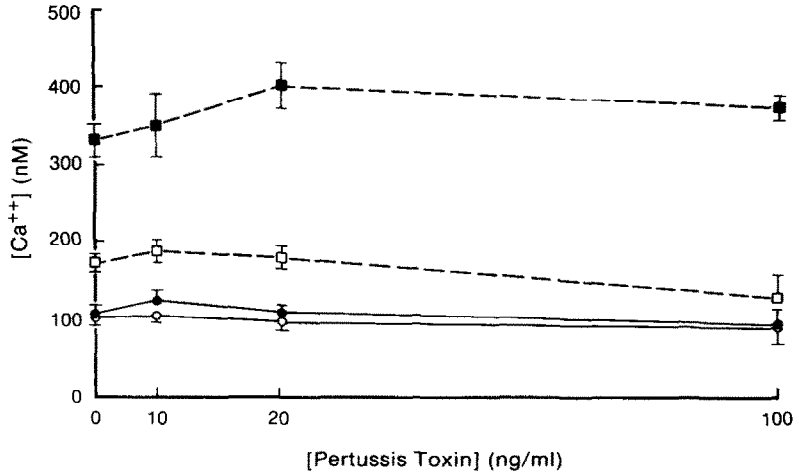

Fig. 5. Effects of pertussis toxin on mast cell intracellular free calcium concentrations. Fura-2 was used to measure Ca^{2+} concentrations in cells incubated with buffer or pertussis toxin for 2 hr and challenged with buffer alone (○—○), 10 μM adenosine alone (●—●), DNP-BSA antigen (□--□), or DNP-BSA antigen +10 μM adenosine (■--■). Depicted are means ± SE of values from six experiments performed in duplicate.

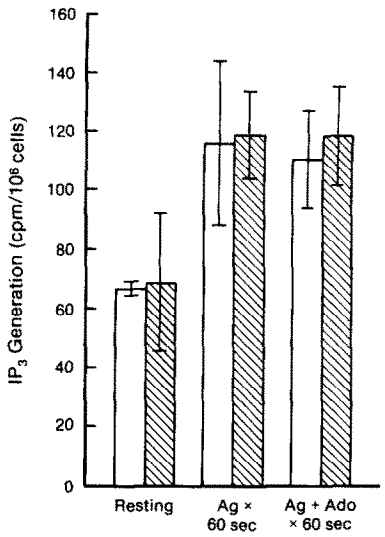

Fig. 6. Inositol trisphosphate (IP_3) generation in pertussis toxin-treated mast cells. Mouse bone marrow-derived mast cells pre-loaded with myo[2-^{3}H]inositol were sensitized with anti-DNP IgE and challenged with DNP–BSA antigen for 60 sec with or without exogenous 10 μM adenosine (□). Parallel groups of cells were incubated with 10 ng/ml pertussis toxin for 2 hr before challenge (▨). IP_3 generation, as determined by ^{3}H counts per minute in the appropriate column fraction, is shown as mean ± SE of values from four experiments performed in duplicate.

levels was observed in antigen-stimulated cells in the presence of exogenous adenosine, although adenosine alone had no effect on calcium. Up to 100 ng/ml pertussis toxin for 2 hr failed to alter the intracellular free calcium concentrations of resting or stimulated mast cells, suggesting that the ability of pertussis toxin to limit IgE-mediated mediator release and adenosine's potentiation of this release was not due to an inhibition of calcium fluxes.

Antigen challenge caused a marked enhancement of IP_3 generation measured at 60 sec, but exogenous adenosine failed to alter this IP_3 generation. Preincubation with pertussis toxin had no identifiable effect on IP_3 levels in resting, antigen-stimulated, or adenosine and antigen-stimulated mast cells (Fig. 6), consistent with previous reports of the lack of effect of pertussis toxin on IP_3 generation by mast cells [8].

DISCUSSION

Mouse bone marrow-derived mast cell adenosine receptors appear to be linked to a GTP-binding protein whose function is at least partially inhibited by exposure to pertussis toxin. The ability demonstrated herein of low concentrations of pertussis toxin to inhibit DNP–BSA antigen-stimulated mouse bone marrow-derived mast cell β-hexosaminidase release (Fig. 1) is in contrast to the finding of Saito and colleagues [8] where such inhibition was not demonstrated but in agreement with Nakamura and Ui [9] who observed a significant inhibition of IgE-mediated histamine release from rat peritoneal mast cells incubated with 10 ng/ml pertussis toxin for 2 hr. Our finding that the A23187-stimulated mast cells were more resistant to the inhibition of degranulation by pertussis toxin (Fig. 2) than cells stimulated with other secretagogues also parallels previous studies in rat peritoneal mast cells [9]. Furthermore, we have demonstrated that these differences cannot be accounted for by changes in IgE-Fc receptor expression. However, not all of the conflicting data can be explained by differences in species, mast cell source, or experimental conditions, so many questions remain. The source of pertussis toxin was different in our studies, and the possibility that some impurities could directly affect the cells cannot be excluded. Of perhaps greater interest is the demonstration that the ability of exogenous adenosine to augment mediator release was inhibited by pertussis toxin to a greater degree than the inhibition of IgE-mediated secretion alone (Figs. 1 and 2), suggesting an interaction between adenosine receptors and the G protein affected here by pertussis toxin.

The precise nature of the G protein(s) ADP-ribosylated by pertussis toxin and the subcellular events mediated by mast cell G proteins are just beginning to be explored. Pertussis toxin ADP-ribosylates a 41,000 molecular weight protein in mouse bone marrow-derived mast cells [8], and the pertussis toxin employed in our studies produced a similar pattern, but whether this is truly G_i or another yet unidentified G protein that is susceptible to pertussis toxin is unknown [5]. Some insight into this question may be obtained indirectly by assessing the ability of pertussis toxin to alter mast cell cyclic AMP levels. Because the G_i protein classically mediates an inhibition of adenylate cyclase, its ADP-ribosylation by pertussis toxin should abolish this inhibition, and if G_i were active in resting mast cells, cyclic AMP levels could increase. However, pertussis toxin concentrations shown to completely ADP-ribosylate G_i in mast cells [8] did not alter mast cell cyclic AMP concentrations (Fig. 4), consistent with minimal activity of G_i in the resting cell. The ability of exogenous adenosine to markedly augment mast cell cyclic AMP levels was not altered by 10 ng/ml pertussis toxin, a concentration that consistently inhibited IgE-induced mediator release. A 10-fold higher concentration of pertussis toxin inhibited adenosine-stimulated cyclic AMP formation, however. Thus the amount of pertussis toxin required to decrease mast cell secretion in the absence or presence of adenosine was much less than that required to inhibit adenosine-induced cyclic AMP production, demonstrating another lack of correlation between cyclic AMP levels and mast cell mediator release. One could also postulate that the G protein linking mast cell adenosine receptors to adenylate cyclase may not be the same G protein involved in adenosine's potentiation of mast cell mediator release, although there is no direct evidence of this.

Leukotriene C_4 generation by mast cells was inhibited by pertussis toxin treatment when the secretagogue was antigen but not A23187 (Fig. 3). This is in keeping with the fact that A23187 bypasses several early steps in the mast cell secretory process, and perhaps one of these early steps may be responsible for the ability of pertussis toxin to modulate mast cell mediator generation and release. Because

adenosine preferentially potentiates preformed mediator release as compared to arachidonic acid metabolism in mouse bone marrow-derived mast cells [3], the effects of adenosine and pertussis toxin on LTC_4 production were not studied.

An increase in intracellular free calcium has been observed early in the course of mast cell activation [17, 18]. Exogenous adenosine enhanced intracellular free calcium concentrations in stimulated but not resting cells (Fig. 5), but pertussis toxin apparently does not exert its effects there. Up to 100 ng/ml of the toxin during a 2-hr incubation did not appreciably alter the ability of antigen alone or antigen plus adenosine to augment calcium levels, a finding consistent with that of Saito and colleagues who found that, in a system where mast cell mediator release is inhibited by pertussis toxin, changes in quin-2 fluorescence are not demonstrated [8]. Similarly, IP_3 generation was not inhibited by preincubation with concentrations of pertussis toxin that consistently inhibited mediator release.

Taken together, these studies indicate that pertussis toxin preferentially inhibited antigen-induced mast cell mediator release and the ability of adenosine to potentiate ongoing mediator release by a mechanism that may involve cyclic AMP but does not clearly involve inositol trisphosphate generation or calcium fluxes as measured utilizing fura-2. Since the classic G_i protein responsible for an inhibition of adenylate cyclase is unlikely to be the sole link between mast cell adenosine receptors and subcellular events (because exogenous adenosine alone stimulates adenylate cyclase), it is likely that one or more different G proteins are affected by mast cell pertussis toxin exposure or alternatively, that the biochemical activity of pertussis toxin involves more than ADP-ribosylation of G proteins. Further studies of key G protein linkages should begin to answer these questions.

Acknowledgements—The authors thank Dr. Stephen Wasserman and Dr. Futong Liu for helpful advice and assistance.

REFERENCES

1. D. L. Marquardt, C. W. Parker and T. J. Sullivan, *J. Immun.* **120**, 871 (1978).
2. D. L. Marquardt, L L. Walker and S. I. Wasserman, *J. Immun.* **133**, 932 (1984).
3. D. L. Marquardt, H. E. Gruber and S. I. Wasserman, *J. Allergy clin. Immun.* **78**, 462 (1986).
4. D. L. Marquardt and L. L. Walker, *Biochem. Pharmac.* **36**, 4297 (1987).
5. L. Strye and H. R. Bourne, *A. Rev. Cell Biol.* **2**, 391 (1986).
6. P. E. Alm and G. D. Bloom, *Life Sci.* **30**, 213 (1982).
7. C. Jacquemin, H. Thibout, B. Lambert and C. Correze, *Nature, Lond.* **323**, 182 (1986).
8. H. Saito, F Okajima, T. F. P. Molski, F. I. Sha'afi, M. Ui and T. Ishizaka, *J. Immun.* **138**, 3927 (1987).
9. T. Nakamura and M. Ui, *Biochem. Pharmac.* **32**, 3435 (1983).
10. E. Razin, J. N. Ihle, D. Seldin, J. M. Mencia-Huerta, H. R. Katz, P. A. LeBlanc, A. Hein, J. Caulfield, K. F. Austen and R. L. Stevens, *J. Immun.* **132**, 1479 (1984).
11. D. L. Marquardt and S. I. Wasserman, *J. Immun.* **131**, 934 (1983).
12. L. B. Schwartz, K. F. Austen and S. I. Wasserman, *J. Immun.* **125**, 1445 (1979).
13. E. Razin, J. M. Mencia-Huerta, R. L. Stevens, R. A. Lewis, F. Lui, E. J. Corey and K. F. Austen, *J. exp. Med.* **157**, 189 (1983).
14. M. J. Berridge, M. C. Dawson, C. P. Downes, J. P. Heslop and R. F. Irvine, *Biochem. J.* **212**, 473 (1983).
15. A. Kulczycki, Jr. and H. Metzger, *J. exp. Med.* **140**, 1676 (1974).
16. J. A. Warner, I. D. Lawrence, K. B. Yancey, L. M. Lichtenstein and D. W. MacGlashan, Jr., *Fedn Proc.* **46**, 930 (1987).
17. T. Ishizaka, F. Hirata, K. Ishizaka and J. Axelrod, *Proc. natn. Acad. Sci. USA* **77**, 1903 (1980).
18. J. R. White, T. Ishizaka, K. Ishizaka and R. I. Sha'afi, *Proc. natn. Acad. Sci. USA* **81**, 3978 (1984).