**Effect of cholera toxin on histamine release from bone marrow-derived mouse mast cells**

(HUONIUS NUCLEOTIDE BINDING REGULATORY PROTEINS/INSOSOL PHOSPHOLIPIDS/QUIN-2 MEDIATOR RELEASE)

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**ABSTRACT** Bone marrow-derived mouse mast cells were sensitized with monoclonal mouse IgE antibody and treated with cholera toxin (CT), which ADP-ribosylated the α-subunit of the stimulatory guanine nucleotide-binding regulatory protein G_{s}, prior to challenge with either antigen or thrombin. The CT treatment increased intracellular cAMP levels, but neither enhanced nor inhibited antigen-induced histamine release or arachidionate release. The same treatment of the sensitized bone marrow-derived mouse mast cells with CT markedly enhanced thrombin-induced histamine release without affecting arachidionate release. The CT treatment failed to affect antigen-induced and thrombin-induced generation of inositol trisphosphate and of diacylglycerol or mobilization of intracellular Ca^{2+}. The results indicate that G_{s} in bone marrow-derived mouse mast cells is not involved in the transduction of the antigen-induced or thrombin-induced triggering signal to phospholipase C, which initiates the enhancement of phosphatidylinositol turnover. The enhancement of thrombin-induced histamine release by CT treatment with the observations that thrombin-induced histamine release was inhibited by pretreatment of the cells with pertussis toxin suggest that the involvement of a guanine nucleotide-binding regulatory protein in thrombin-induced biochemical events is an event distal to Ca^{2+} mobilization.

Mast cells and basophils release inflammatory mediators through the reaction of cell-bound IgE antibodies with multivalent antigen (1) and through various IgE-independent stimuli (2). Analysis of biochemical events involved in triggering of mediator release revealed that bridging of IgE receptors on mast cells and basophils results in the activation of various membrane-associated enzymes (3-5), induces mobilization of intracellular calcium (6, 7), and an enhancement of phosphatidylinositol (PtdIns) turnover (8, 9). These results suggested that guanine nucleotide-binding regulatory protein(s) [G protein(s)] are involved in the transduction of receptor-mediated signals to the enzymes. However, our studies have revealed that pretreatment of rodent mast cells and human basophils with pertussis toxin (PT), which ADP-ribosylates the G protein G_{i} and other G proteins (10, 11), failed to affect the IgE-mediated histamine release (12). The same PT treatment of rodent mast cells inhibited histamine release induced by thrombin or compound 48/80, and the treatment of human basophils resulted in the inhibition of histamine release by fMet-Leu-Phe in a dose-dependent manner. The present experiments were undertaken to determine the effect of cholera toxin (CT), which ADP-ribosylates the stimulatory G protein G_{s} (13, 14), on the histamine release. To analyze the effects of the CT treatment on the biochemical events induced by IgE-dependent and IgE-independent stimuli, we employed interleukin 3 (IL-3)-dependent, bone marrow-derived mouse mast cells (BMMC) in which both IgE-dependent and thrombin-induced histamine release were observed (12).

**MATERIALS AND METHODS**

**Cell Preparations.** BMMC were obtained from bone marrow of CBA/J mice (The Jackson Laboratory) in suspension culture containing mouse IL-3. The cells were cultured for 4 weeks in RPMI 1640 culture medium (GIBCO), supplemented with 10% (vol/vol) fetal calf serum, 50 mM 2-mercaptoethanol, 2 mM L-glutamine, and antibiotics and enriched with 5% (vol/vol) S15 72F-D11 cell conditioned medium (15) containing mouse IL-3. The cell line was kindly supplied by N. Ari (DNAX Institute of Molecular Biology, Palo Alto, CA). More than 95% of nonadherent cells in the culture were mast cells, and their viability was >99%.

**IgE Antibodies and Antigen.** Purified monoclonal mouse IgE anti-DNP antibody and dinitrophenyl derivatives of human serum albumin (DNP-HSA) were the same preparations as those described (5). The conjugate contained 13 dinitrophenyl groups per human serum albumin molecule.

**Passive Sensitization and Histamine Release.** BMMC were sensitized by incubation overnight with monoclonal mouse IgE antibody at 10 μg/ml at 37°C. After washing, the cells were resuspended in Tyrode solution (pH 7.4) containing 124 mM NaCl, 4 mM KCl, 0.64 mM Na_{2}HPO_{4}, 1.6 mM CaCl_{2}, 1 mM MgCl_{2}, 10 mM NaHCO_{3}, 5.5 mM glucose, 10 mM Hepes, 5 mM Mes, and 0.1% gelatin. A suspension of the sensitized cells (5 × 10^{5} to 10^{6} cells per ml) was incubated at 37°C with an optimal concentration (0.01 μg/ml) of DNP-HSA for maximum histamine release (12). Under this condition, 40-70% of histamine was released within 5 min. The same cell suspensions were also incubated with optimal concentration (0.5 unit/ml) of thrombin (bovine plasma, Boehringer Mannheim), which gave 10–30% histamine release. Histamine content in the medium supernatant was measured by the automated technique of Siraganian (16). Intracellular cAMP levels in the cells were determined by radioimmunoassay as described (17).

**Treatment of Mast Cells with CT and Identification of CT-Mediated ADP-Ribosylated Membrane Protein.** A suspension of sensitized BMMC in Tyrode solution was incu-

Abbreviations: CT: cholera toxin; PT: pertussis toxin; PtdIns, phosphatidylinositol; BMMC, bone marrow-derived mouse mast cells; DNP-HSA, 2,4-dinitrophenyl derivatives of human serum albumin; InsP, inositol 1-phosphate; InsPL, inositol 1,4,5-triphosphate; InsPL_{2}, inositol 1,4-triphosphate; acyl_{2}G_{3}r, 1,2-diacyl-an-glycerol; IL-3, interleukin 3; Δ_{1}G_{3}r, arachidonic acid; PGE_{1}, prostaglandin E_{1}; 8BrCAMP, 8-bromoadenosine 3',5'-cyclic phosphate; G protein, guanine nucleotide-binding regulatory protein; G_{i}, inhibitory and stimulatory G proteins, respectively.

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bated with CT at 0.1 ng/ml to 1 μg/ml at 37°C for 3 hr. ADP-ribosylation of the G protein by the CT treatment was determined as described (12). Briefly, the CT-treated and untreated cells were homogenized to obtain the particulate fraction. G protein in the fraction was then ADP-ribosylated in the presence of [α-32P]NAD and 100 μM GTP by CT that had been activated with 20 mM dihydrothreitol and 2 mM Mg-ATP. After incubation for 60 min at 30°C, the particulate fraction was dissolved in NaDodSO4 and analyzed by NaDodSO4/PAGE. ADP-ribosylation by activated PT was carried out as described (12). ADP-ribosylated proteins were detected by autoradiography of the gels (18).

Arachidonic Acid (Δ酰Ach) Release. Sensitized BMMC were labeled with [14C]Arachidonic Acid (Δ酰Ach) (58.2 mCi/mmol; 1 Ci = 37 GBq; Amersham) by the method of Nitta and Suzuki (19), with slight modifications (12). After washing, 0.5 ml of the cell suspension containing 2 × 10⁵ BMMC was incubated at 37°C with either DNP-HSA or thrombin. Cells were recovered by centrifugation, and lipids in both cell lysate and medium supernatant were extracted with cold ethyl acetate at pH 3.5. Aliquots of the extracts were dried under N₂ and dissolved in Aquasol (New England Nuclear), and their radioactivity was measured. The proportion of [14C]Δ酰Ach released from the cells was calculated by ([cpm in medium supernatant]/[cpm in medium supernatant + cpm in cell sediment]).

Determination of [3H]Inositol Phosphates. Fifteen million BMMC were suspended in 7 ml of culture medium that contained mouse anti-dinitrophenol (anti-DNP) IgE antibody at 20 μg/ml and 100 μCi of myo-[2-3H]inositol (16.9 Ci/mmol, Amersham), and the suspension was incubated for 12–20 hr at 37°C in a humid atmosphere of 5% CO₂/95% air. After washing, a 200-μl aliquot of the cell suspension (0.5–1 × 10⁵ cells per ml) in Tyrode solution was challenged with either DNP-HSA or thrombin. The reaction was terminated by the addition of 750 μl of chloroform/methanol, 1:2 (vol/vol). The aqueous phase was separated as described (12), and various [3H]inositol phosphates were separated by anion-exchange chromatography on Dowex-1 formate resin (Sigma) as described by Berridge et al. (20) and Beaven et al. (8). Dried procedures and separation of various inositol phosphates have been described (12). As the standard for inositol 1,4,5-trisphosphate (Ino₃P₃), an appropriate dose of myo-[2-3H]Ino₃P₃ (91.0 Ci/mmol, Amersham) was applied to the Dowex column and eluted in the same sequences as that employed for the separation of inositol phosphates.

Determination of 1,2-Diacylglycerol (acylₓGro). BMMC was incubated at 37°C overnight in culture medium containing [2-3H]glycerol (1 Ci/mmol, Amersham), at 100 μCi per 10⁶ cells per ml and mouse anti-DNP IgE antibody at 10 μg/ml. Cells were challenged with either DNP-HSA or thrombin, and the reaction was stopped by the addition of ice-cold chloroform/methanol, 2:1 (vol/vol). Lipids were extracted by the method of Bligh and Dyer (21), and analyzed by TLC on Silica G plates (Analtech, Newark, DE) with dichlороethane/ethanol, 98:2 (vol/vol). Radioactivity of the acylₓGro spot was measured in a scintillation spectrometer.

Measurement of Quin-2 Fluorescence. Sensitized BMMC was loaded with quin-2 tetraakis(acetoxymethyl) ester as described by Tsien et al. (22), with slight modifications (7). Quin-2 fluorescence was recorded by using an SLM 8000 photon-counting spectrofluorometer with a temperature-controlled cuvette (37°C) and a magnetically driven stirrer. Fluorescence excitation and emission wave lengths were 339 nm and 492 nm, respectively. Spectra were processed by using a Hewlett-Packard HP 85 desk computer.

Reagents. CT and CT B subunit were purchased from List Biological (Campbell, CA). 1,2-Dioleoyl-rac-glycerol, prostaglandin E₁ (PGE₁), and forskolin were purchased from Sigma. 1,2-Dichloroethane was purchased from Fisher. d-myo-[2-3H]InsP₃, myo-[2-3H]inositol, [14C]glycerol, and [14C]-Δ酰Ach were purchased from Amersham.

RESULTS

ADP-ribosylation of the Stimulatory G Protein G₄ by CT Treatment of BMMC. To determine whether CT could induce ADP-ribosylation of G protein in the cell membrane of BMMC, aliquots of a suspension of BMMC were incubated for 3 hr at 37°C with CT at 0.1 ng/ml, 10 ng/ml, or 1 μg/ml. The particulate fraction of untreated and CT-treated cells was incubated with activated CT in the presence of [α-32P]NAD, and ADP-ribosylated proteins were analyzed by NaDodSO₄/PAGE. As shown in Fig. 1, the activated CT (lane 2) predominately ADP-ribosylated a 43-kDa protein and an additional 48-kDa protein in untreated BMMC. The molecular weights of the proteins ADP-ribosylated by CT were identical to those of the α chain of G₄ in many types of cells (13, 14, 23) and distinct from α subunit of the inhibitory G protein Gᵢ, i.e., a 41-kDa protein, that was ADP-ribosylated by treatment of the same particulate fraction with activated PT (lane 3). If the same cells had been treated with CT at 0.1 ng/ml (lane 4), 10 ng/ml (lane 5), or 1 μg/ml (lane 6), the quantity of both the radiolabeled 43-kDa and 48-kDa proteins in the particulate fraction diminished in a dose-dependent manner. Radioactivity of each band was measured to calculate the percentage of the proteins ADP-ribosylated by the pretreatment. The degree of diminution of radioactivity of the bands indicated that 95% of the 43-kDa protein and 70% of the 48-kDa protein had been ADP-ribosylated by preincubation of the cells with CT at 1 μg/ml.

Effects of CT on Histamine Release and Δ酰Ach Release. To determine the possible effects of CT on the antigen-induced and thrombin-induced histamine release, sensitized BMMC were incubated with various concentrations of CT. The cells were washed three times and challenged with either DNP-HSA or thrombin. As shown in Fig. 2, pretreatment of BMMC with CT resulted in a dose-dependent enhancement of thrombin-induced histamine release but failed to affect the antigen-induced histamine release. The enhancement of thrombin-induced histamine release increased with preincubation period with CT. In three cell preparations, pretreatment with CT at 10 ng/ml for 10 min, 30 min, 60 min, and 120 min, and 180 min enhanced the thrombin-induced histamine release.

FIG. 1. ADP-ribosylation of BMMC membranes. Membranes prepared from untreated BMMC (lanes 1-3) and BMMC treated with CT at 0.1 ng/ml (lane 4), 10 ng/ml (lane 5), and 1 μg/ml (lane 6) were incubated with [α-32P]NAD alone (lane 1), in presence of activated PT (lane 3) or in the presence of activated CT (lanes 2, 4, 5, and 6). Arrows indicate positions of bovine serum albumin, 66.2 KDa; ovalbumin, 45 KDa; carbonic anhydrase, 31 KDa; and soybean trypsin inhibitor, 21.5 KDa. The arrow at 41 KDa indicates the α subunit of the inhibitory G protein Gᵢ. Repeated experiments of the same design with another cell preparation gave comparable results.
release by 27.8 ± 13.1%, 71 ± 20%, 89 ± 12.6%, 95 ± 16.7%, and 101.7 ± 10%, respectively. In contrast, the effect of the CT treatment on antigen-induced histamine release was not significant. Even after 3 hr incubation with CT at 1 μg/ml, an average histamine release by an optimal concentration of antigen (10 ng/ml) from six cell preparations increased from 60.0 ± 3.6% to 65.3 ± 3.4% (Fig. 2). It was confirmed that CT treatment neither enhanced nor inhibited 20–30% of the histamine release induced by a suboptimal concentration (0.1 ng/ml) of DNP-HSA.

Incubation of BMMC with CT resulted in a dose-dependent increase in intracellular cAMP (Fig. 2). The increase in cAMP also depends on incubation period with CT. In the same three cell preparations described above, which were incubated with CT at 100 ng/ml for 3 hr, intracellular cAMP levels increased from 2.9 ± 1.1 pmol per 10^6 cells to 7.3 ± 1.6 pmol per 10^6 cells, and this level was maintained at least 30 min after removal of CT.

To prove that the enhancement of the thrombin-induced histamine release by CT treatment depends on the ADP-ribosyltransferase activity of CT, we determined the effect of the B subunit of CT, which binds to the cells but lacks the enzyme activity (24). Preincubation of BMMC with the B subunit at 1 μg/ml for 3 hr resulted in only a 30% enhancement of thrombin-induced histamine release. When the same cell suspension was incubated with various concentrations of CT, preincubation with CT at 0.1 ng/ml exerted an enhancement comparable to the treatment with B subunit at 1 μg/ml. A slight enhancement of histamine release by a high concentration of B subunit is probably due to contamination of the preparation with either A subunit or CT.

Since our experiments have shown that sensitized BMMC released not only histamine but also Δ_ACh and its metabolites upon challenge with either DNP-HSA at 0.01 μg/ml or thrombin at 0.5 unit/ml (12), we determined the possible effect of the treatment of BMMC with CT at 1 μg/ml on antigen-induced and thrombin-induced Δ_ACh release. All quotes of the treated and untreated cells were labeled with [14C]Δ_ACh and then challenged with either antigen or thrombin. In three separate experiments, the stimulation of sensitized BMMC with an optimal concentration of DNP-HSA resulted in the release of 18.5 ± 1.4% of the [14C]Δ_ACh, while the spontaneous release from unstimulated cells was 0.73 ± 0.22%. Incubation of the same cells with thrombin at 0.5 unit/ml induced the release of only 1.96 ± 0.13% of the [14C]Δ_ACh as compared with the spontaneous release of 0.73 ± 0.20%. Nevertheless, preincubation of the same cells with CT at 1 μg/ml affected neither the antigen-induced [14C]Δ_ACh release nor thrombin-induced [14C]Δ_ACh release (results not shown).

Effect of CT Treatment on Thrombin-Induced and Antigen-Induced Inositol Phospholipid Hydrolysis. To determine the possible role of the stimulatory G protein Gs in thrombin-induced histamine release, we determined the effects of CT treatment on thrombin-induced PtdIns turnover. The experiments were designed based on the kinetics of the generation of InsP_2, inositol bisphosphate (InsP_3), and inositol phosphate (InsP) after the addition of thrombin at 0.5 unit/ml to sensitized BMMC (12). Thus, a suspension of sensitized BMMC was divided into three portions, and an aliquot of the suspension was labeled with myo-[3H]inositol for the analysis of InsP_2, InsP_3, and InsP. The second portion of the cells was labeled with [3H]glycerol for the determination of acyl_2,Gro, and the remainder was used for histamine release. Each of the three cell suspensions was incubated with various concentrations of CT for 3 hr. After washing, both the CT-treated and untreated cells were challenged with thrombin at 0.5 unit/ml. Since the generation of InsP_2 and InsP_3 after exposure to thrombin was transient, reached maximum at 15 sec and then declined, while InsP generation reached plateau at 1 min (12), the reaction was stopped at 15 sec for the determination of InsP_2 and InsP_3, and at 1 min for the determination of InsP. Based on reported data that the kinetics of the thrombin-induced acyl_2,Gro formation parallel histamine release (12), acyl_2,Gro and histamine were measured at 10 min. The results of three experiments are summarized in Fig. 3. It is apparent that pretreatment of BMMC with CT at 10 ng/ml to 1 μg/ml neither enhances nor inhibits the thrombin-induced generation of InsP_2, InsP_3, InsP, or acyl_2,Gro. Similar experiments were carried out to determine possible effects of CT treatment on antigen-induced PtdIns turnover. As expected, preincubation of the cells with CT at 1 μg/ml for 3 hr did not affect the antigen-induced generation of InsP_2, InsP_3, InsP, or acyl_2,Gro (results not shown).

Failure of CT treatment to affect the PtdIns turnover was confirmed by determination of quin-2 signal. In the experiments shown in Fig. 4, sensitized BMMC were incubated with CT at 1 μg/ml or in Tyrode solution for 3 hr, and the cells were loaded with quin-2 tetrakis(acetoxymethyl) ester. Addition of either thrombin at 0.5 unit/ml or DNP-HSA at 0.01 μg/ml increased quin-2 fluorescence. It is apparent in Fig. 4 that neither the thrombin-induced quin-2 signal nor the antigen-induced quin-2 signal was affected by pretreatment of the cells with CT.

Effect of Intracellular cAMP on Histamine Release. Since the treatment of BMMC with CT increased intracellular cAMP, we determined the effect of the elevation of cAMP levels on histamine release. Preincubation of three sensitized BMMC preparations with 0.25 mM to 2 mM 8-bromoadenosine 3',5'-cyclic phosphate (8BrcAMP) for 1 hr, and the presence of the reagent during challenge enhanced thrombin-induced histamine release in a dose–response fashion; the cells treated with 2 mM 8BrcAMP released twice as much histamine (34.7 ± 0.5%) compared with untreated cells (17.6 ± 2.9%). However, preincubation with, and the presence of, 8BrcAMP did not change the percentage of antigen-induced
concentrations of ml of InsP2, InsP3, and acyl2Gro. BMMC were preincubated with various concentrations of CT or culture medium for 3 hr. After washing, 0.5 ml of ice-cold acidic ethanol was added to the pellet of an aliquot of the unlabeled cells for cAMP assay (□). The rest of unlabeled cells and those of myo-[3H]inositol-labeled cells were washed and then challenged with thrombin at 0.5 unit/ml. The reaction was stopped at 15 sec for the determination of InsP3 (△) and of InsP2 (○), at 1 min for InsP (■), and at 10 min for acyl2Gro (◇). Each point represents an average ± SEM of three sets of experiments. In the untreated cells, thrombin-induced generation of InsP3, InsP2, InsP, and acyl2Gro was 1046 ± 194 cpm per 10^7 cells (basal level, 348 ± 53 cpm), 990 ± 126 cpm per 10^7 cells (basal level, 287 ± 45 cpm), 2715 ± 229 cpm per 10^7 cells (basal level, 1745 ± 250 cpm), and 1390 ± 296 cpm per 10^6 cells (basal level, 957 ± 142 cpm), respectively.

histamine release. It was also found that 8BrcAMP failed to affect the generation of InsP3, InsP2, InsP, and acyl2Gro induced by either thrombin or antigen. Thus, the effects of 8BrcAMP on BMMC were similar to those obtained by CT treatment. However, pretreatment with PGE1 and forskolin, which also increased intracellular CAMP level, gave entirely different effects on histamine release. Intracellular CAMP levels in three BMMC preparations increased from 1.6 ± 0.6 pmol per 10^7 cells to 3.0 ± 0.9 pmol per 10^7 cells by incubation of the cells with 30 μM PGE1 for 15 min, but the pretreatment with PGE1 neither enhanced nor inhibited antigen-induced histamine release or thrombin-induced histamine release. As shown in Fig. 5, incubation of BMMC with 50–200 μM forskolin for 15 min increased intracellular CAMP levels in a dose–response fashion, and resulted in the inhibition of not only antigen-induced histamine release but also the generation of InsP3, InsP2, InsP, and acyl2Gro induced by antigen.

**DISCUSSION**

Data presented in this paper show that treatment of BMMC with CT at 1 μg/ml, which ADP-ribosylated 95% of α subunit of the stimulatory G protein Gs, neither inhibited nor enhanced IgE-mediated histamine release or ΔAch release. These findings with our observations that treatment of BMMC or IL-3-dependent mouse mast cell line PT-18 cells (25) with PT, which ADP-ribosylates >97% of the α subunit of the inhibitory G protein Gi, failed to affect the IgE-mediated histamine and ΔAch release (12), suggest strongly that neither Gi nor Gs is involved in the process of IgE-dependent mediator release. In contrast, histamine release from BMMC by thrombin, an IgE-independent secretagogue, was markedly enhanced by pretreatment of the cells with CT (Fig. 2) and inhibited by pretreatment with PT (12). It should be noted, however, that neither the CT treatment nor PT treatment affected the thrombin-induced ΔAch release. It...
has been believed that biochemical processes for histamine release and those for \( \Delta_4 \)ACh release may share common triggering events. Discrepancies in the effect of CT (and PT) treatment between thrombin-induced histamine release and thrombin-induced \( \Delta_4 \)ACh release suggest that G protein(s) may be involved in the reactions in the degranulation process that are not shared by \( \Delta_4 \)ACh release. Indeed, neither CT treatment (Fig. 3) nor PT treatment (12) of BMMC affected the thrombin-induced generation of InsP$_3$, and acylGlo or mobilization of intracellular Ca$^{2+}$. These findings suggest that CT-sensitive G protein as well as PT-sensitive G protein may be involved in the biochemical processes distal to the increase in intracellular Ca$^{2+}$. This is in agreement with the hypothesis by Fernandez et al. (26) who demonstrated that introduction of guanosine 5'-[\gamma-thio]triphosphate and MgATP into mast cells caused degranulation. They suggested that stepwise increase in the cell membrane capacitance during the degranulation process of mast cells is due to the fusion of individual secretory granules with the plasma membrane and that G protein(s) are involved in the control of this process.

Since CT treatment of BMMC induces an increase in intracellular cAMP, the possibility was considered that an enhancement of thrombin-induced histamine release was a consequence of an increase in cAMP levels. Indeed, 8Br-cAMP mimicked the effect of CT by enhancing thrombin-induced histamine release without affecting the IgE-mediated histamine release or thrombin-induced PtdIns turnover. However, a 2-fold increase of cAMP levels in BMMC by PGE$_2$ failed to affect both thrombin-induced and antigen-induced histamine release. Furthermore, treatment with forskolin, which also increased intracellular cAMP by 2- to 3-fold, inhibited antigen-induced histamine release and PtdIns turnover. It is not known why these reagents, all of which increased cAMP levels, exerted entirely different effects on mediator release from BMMC. Nevertheless, the results did not provide definitive evidence that the enhancement of thrombin-induced histamine release by CT treatment is the consequence of an elevation of cAMP levels.

Evidence suggests that G proteins are involved in the receptor-mediated signal transduction in various types of cells, such as neutrophils. Preincubation of neutrophils with PT resulted in the inhibition of ligand-mediated PtdIns turnover and mobilization of intracellular Ca$^{2+}$ (27, 28). In both IgE-dependent mediator release and thrombin-induced mediator release from BMMC, however, neither CT treatment nor PT treatment (12) of the cells affected the enhancement of PtdIns turnover or mobilization of intracellular Ca$^{2+}$. The results indicate that neither CT-sensitive G protein (G$_s$) nor PT-sensitive G protein (G$_q$ or G$_\alpha$) is involved in the transduction of triggering signals to phospholipase C, which initiates the enhancement of PtdIns turnover. However, it is still possible that a distinct G protein in mast cells/basophils is involved in signal transduction for mediator release. Gomperts (29) demonstrated that introduction of nonhydrolyzable GTP analogues into rat mast cells caused histamine release in response to extracellular Ca$^{2+}$.

It has been shown that IgE-dependent histamine release from human basophils and rat mast cells was inhibited by pretreatment of the cells with reagents that increased intracellular cAMP (30, 31) Lichtenstein et al. (32) reported that IgE-dependent histamine released from human basophils was inhibited by pretreatment of the cells with CT and speculated that the level of intracellular cAMP regulated histamine release. Indeed, we found that preincubation of cultured human basophils (33) with CT at 1 ng/ml to 1 \( \mu \)g/ml for 3 hr resulted in a dose-dependent increase in cAMP and inhibition of IgE-mediated histamine release. Since other reagents that induce elevation of intracellular cAMP, such as forskolin, also inhibited IgE-mediated histamine release from human basophils, inhibition of IgE-mediated histamine release by CT treatment of human basophils may be a consequence of an increased cAMP. It is not known why an increase in cAMP induced by CT treatment inhibited IgE-mediated histamine release from human basophils but not the IgE-mediated histamine release from BMMC. Nevertheless, the findings in human basophils do not provide the evidence that the stimulatory G protein G$_s$ is involved in the transduction of IgE-mediated triggering signal for mediator release.

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