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H_1-Histamine Receptors on Human Astrocytoma Cells

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SUMMARY

The H_1-histamine receptor antagonist [^{3}H]mepyramine bound with high affinity (K_d = 3–5 nM) to membranes derived from 1321N1 human astrocytoma cells. The H_1-receptor antagonists triprolidine and diphenhydramine inhibited [^{3}H]mepyramine binding with K_i values of 1–5 nM, whereas the K_i of the H_2-histamine receptor antagonist cimetidine was greater than 100 μM. Histamine also inhibited [^{3}H]mepyramine binding to 1321N1 cell membranes, and the histamine inhibition curve was shifted to the right and steepened in the presence of 1 μM guanosine 5′-*O*-(3-thiotriphosphate). Treatment of 1321N1 cells with pertussis toxin had no effect on the capacity of histamine to inhibit [^{3}H]mepyramine binding either in the absence or presence of guanosine 5′-*O*-(3-thiotriphosphate). Therefore, agonist-occupied histamine receptors in these cells apparently interact with a guanine nucleotide regulatory protein that is not the inhibitory guanine nucleotide regulatory protein of adenylate cyclase. Although adenylate cyclase activity was not affected by histamine in a cell-free preparation, incubation of 1321N1 cells with histamine resulted in an attenuation of cyclic AMP accumulation. Analysis of cyclic AMP degradation in the presence of histamine indicated that the effects of histamine on cyclic AMP accumulation are mediated through activation of phosphodiesterase. This idea was supported by the fact that the phosphodiesterase inhibitor 1-isobutyl 3-methylxanthine blocked attenuation of cyclic AMP accumulation by histamine in a noncompetitive manner. Histamine also markedly increased phosphoinositide breakdown and $^{45}Ca^{2+}$ efflux in 1321N1 cells. These histamine-induced effects apparently are mediated through H_1-receptors, since triprolidine, but not cimetidine, potently inhibited histamine action. As for histamine interaction with its receptor, pertussis toxin had no effect on histamine-induced phosphoinositide breakdown, $^{45}Ca^{2+}$ efflux, or attenuation of cyclic AMP accumulation. Taken together, these data indicate that 1321N1 human astrocytoma cells are a useful model system for the study of H_1-histamine receptors and the biochemical responses mediated through these receptors.

The autocoid and putative neurotransmitter histamine has been shown to interact with two different receptor subtypes, H_1- and H_2-histamine receptors (1, 2). Activation of adenylate cyclase by histamine occurs through H_2-receptors (3, 4), whereas accumulation of cyclic GMP in response to histamine occurs through H_1-receptors (5). Recently, it has been reported that activation of H_1-receptors also results in breakdown of phosphoinositides (6, 7). This observation is not surprising in light of the proposed association of phosphoinositide breakdown, mobilization of intracellular Ca^{2+}, and activation of guanylate cyclase (8).

Study of histamine receptors has been retarded by the lack of availability of model systems for examination of the responses mediated by these receptors. In the present study we report that 1321N1 human astrocytoma cells express an H_1-histamine receptor that when activated results in breakdown of phosphoinositides, mobilization of Ca^{2+}, and activation of a cyclic AMP phosphodiesterase. These responses to histamine are similar to those we have described previously for a muscarinic cholinergic receptor on these cells (9, 10). This similarity between the histamine and muscarinic receptors of 1321N1 cells is further underscored by the fact that, as with the muscarinic receptor (11–13), the histamine receptor of this cell line apparently interacts with a guanine nucleotide regulatory protein that, based on experiments with pertussis toxin, is not G_i.

Materials and Methods

DMEM, trypsin, and fetal calf serum were purchased from Grand Island Biological Co. (Grand Island, NY). Histamine, triprolidine, cimetidine, diphenhydramine, carbachol, atropine, isoproterenol, propranolol, IBMX, cyclic AMP, and prostaglandin E_1 were obtained from Sigma Chemical Co. (St. Louis, MO). Forskolin was purchased from

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ABBREVIATIONS: G_i, inhibitory guanine nucleotide regulatory protein of adenylate cyclase; DMEM, Dulbecco's modified Eagle's medium; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); QNB, quinuclidinyl benzilate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; TCA, trichloroacetic acid; IP_1, inositol 1-phosphate; IP_2, inositol 1,4-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; $K_{0.5}$, concentration required to obtain 50% of the maximal effect; IBMX,1-isobutyl 3-methylxanthine.

Calbiochem-Behring Corp. (San Diego, CA) and GTPγS was from Boehringer Mannheim (Indianapolis, IN). All chemicals and drugs were of reagent grade or the highest quality available. [^{3}H]Mepyramine (26 Ci/mmol), [^{3}H]QNB (39.4 Ci/ mmol), and [^{3}H]*myo*-inositol (16.5 Ci/mmol) were purchased from New England Nuclear (Boston, MA). [^{3}H]Adenine (25 Ci/mmol) and $^{45}Ca^{2+}$ were from ICN (Irvine, CA).

Cell culture. The 1321N1 human astrocytoma cells were grown as previously described (9). DMEM containing penicillin (50 units/ml) and streptomycin (50 μg/ml) was used in the experiments involving $^{45}Ca^{2+}$ efflux, phosphoinositide breakdown, or examination of the effects of pertussis toxin.

Membrane preparation. Membranes from 7-day cultures were prepared by aspirating the growth medium and swelling the cells by addition of 10 mM HEPES buffer containing 10 mM EDTA (pH 7.4). After 15 min on ice, the cells were scraped from the plate with a rubber spatula. Washed membranes were prepared by three centrifugations at 45,000 × *g* for 15 min and resuspension in 10 mM HEPES-10 mM EDTA. The final pellet was resuspended in 10 mM HEPES containing 5 mM $MgCl_2$ (pH 7.4). Membranes not used on the day of preparation were frozen at −80° in 0.32 M sucrose/5 mM $MgCl_2$/10 mM HEPES (pH 7.4).

[^{3}H]Mepyramine binding assay. [^{3}H]Mepyramine (5 nM, except where noted) was incubated at 30° with membranes (80–320 μg), 10 mM HEPES (pH 7.4), 5 mM $MgCl_2$, and drugs in a total volume of 0.4 ml. The reaction was started by addition of tissue and stopped by addition of 10 ml of ice-cold buffer (140 mM NaCl and 10 mM Tris, pH 7.4) Immediately after stopping the reaction, the sample was filtered through 25-mm glass fiber filters (Schleicher and Schuell #30), and each filter was washed with an additional 10 ml of buffer. Nonspecific binding was defined as the amount of [^{3}H]mepyramine bound in the presence of 2–10 μM triprolidine. Radioactivity retained by the filters was counted in a liquid scintillation counter at an efficiency of 40%. All assays were carried out in triplicate.

[^{3}H]QNB binding assay. [^{3}H]QNB (1 nM) was incubated at 37° for 90 min with tissue, 10 mM HEPES/5 mM $MgCl_2$ (pH 7.4), in a total volume of 1 ml (12). Nonspecific binding was defined as the amount of [^{3}H]QNB bound in the presence of 1 μM atropine.

Measurement of cyclic AMP accumulation. The accumulation of [^{3}H]cyclic AMP was measured as previously described (9) in cells usually grown in 12-well plates for 3 days. Briefly, the growth medium was aspirated and replaced with 1 ml of 25 mM HEPES-buffered Eagle's medium (pH 7.4) containing [^{3}H]adenine (1.0 μCi/ml). After incubation for 1 hr at 37°, the medium was aspirated and replaced with 0.95 ml of fresh medium without [^{3}H]adenine, and drugs were added in a volume of 50 μl. Drug challenges were usually for 5 min and were terminated by aspiration of the medium and the addition of 5% TCA containing 0.5 mM cyclic AMP. [^{3}H]ATP and [^{3}H]cyclic AMP were separated by chromatography on Dowex 50-X8 and alumina columns.

Adenylate cyclase assay. Adenylate cyclase activity was determined as previously described (14). Membranes (approximately 100 μg) were incubated in 5 mM $MgCl_2$, 1 mM EGTA, 8 mM creatine phosphate, 6 units/assay of creatine phosphokinase, 1 mM cyclic AMP (containing 30,000 cpm of [^{3}H]cyclic AMP), 0.4 mM ATP (containing 900,000 cpm of [^{32}P]ATP), 10 mM GTP, 1.4 mM dithiothreitol, 50 mM Tris (pH 7.4) at 30° for 12 min in a final volume of 150 μl. The reaction was terminated by addition of 0.8 ml of 5% TCA. [^{32}P]cyclic AMP was separated from [^{32}P]ATP by sequential column chromatography over Dowex 50-X8 and neutral alumina.

Unidirectional ^{45}Ca efflux. Unidirectional ^{45}Ca efflux was measured as described previously (10) with minor modifications. Cells were incubated with DMEM containing $^{45}CaCl_2$ (5 μCi/ml) for 18–20 hr. To initiate the efflux assay, the growth medium containing $^{45}Ca^{2+}$ was aspirated and the monolayers were rapidly washed twice with 2 ml of HEPES-buffered Eagle's medium without ^{45}Ca. HEPES-buffered Eagle's medium containing drugs was added, and the cells were incubated for 1 min. When antagonists were used, they were added to the $^{45}Ca^{2+}$-containing medium 5 min before starting the efflux assay. Incubations were terminated by rapidly washing the monolayers four times with 5 ml of 1.5% (w/v) bovine serum albumin, 150 mM NaCl, and 5 mM $LaCl_3$. Cell-associated $^{45}Ca^{2+}$ was determined after solubilizing the cells with 1 ml of 0.1 N NaOH and neutralizing the sample with 1 ml of 0.1 N HCl. The difference between the level of $^{45}Ca^{2+}$ retained by the cells in the absence and presence of a maximally effective concentration of histamine is given a value of 100%.

Assay of inositol phosphates. Phosphoinositide breakdown was monitored by measuring [^{3}H]inositol phosphates as previously described (10), with minor modifications. Phosphoinositides were labeled with DMEM containing [^{3}H]inositol (1 μCi/ml) for 18–20 hr. Just prior to the assay, the monolayers were washed twice with 1.5 ml of HEPES-buffered Eagle's medium and preincubated in the medium at 37° for 10 min. Assays were initiated by the addition of HEPES-buffered Eagle's medium containing drugs and 10 mM LiCl. To terminate the reaction, the monolayer was washed twice with 1.5 ml of HEPES-buffered Eagle's medium, and 0.5 ml of cold methanol was added. Cells were scraped and each dish was rinsed with an additional 0.5 ml of cold methanol. Chloroform (0.5 ml) and H_2O (0.4 ml) were added to the pooled methanol sample. The samples were vigorously mixed and an additional 0.5 ml each of chloroform and water was added prior to the centrifugation of the two-phase solution at 1500 × *g* for 10 min at 4°. The upper phase was transferred to a column containing 1 ml of bed volume of anion exchange resin (Bio-Rad AG 1-X8, 100–200 mesh, formate form). The columns were washed with 10 ml of water to remove [^{3}H]inositol. Ten-ml volumes of 50 mM ammonium formate then were added to remove glycerophosphoinositol. Total [^{3}H]inositol phosphates (IP_1, IP_2, and IP_3) were eluted with 8 ml of 1 M ammonium formate/100 mM formic acid. In some experiments, phosphoinositides were labeled by the addition of 5 μCi/ml of [^{3}H]inositol to inositol-free DMEM. The drug challenge was stopped by 5% TCA and, after TCA was removed by several ether washes, each inositol phosphate (IP_1, IP_2, and IP_3) was separated as follows. [^{3}H]IP_1 was eluted with 8 ml of 200 mM ammonium formate/100 mM formic acid, and the columns were washed with an additional 8 ml of this buffer. [^{3}H]IP_2 was eluted with 16 ml of 450 mM ammonium formate/100 mM formic acid, and the columns were washed with an additional 8 ml of this buffer. [^{3}H]IP_3 was then eluted with 8 ml of 1 M ammonium formate/100 mM formic acid.

Preparation of pertussis toxin. *Bordetella pertussis* (strain 165) was grown for 4 days as previously described (11). Pertussis toxin was partially purified according to the method of Sekura *et al.* (15).

ADP-ribosylation. To assess the capacity of pertussis toxin to inactivate G_i, membranes from cells pretreated with or without pertussis toxin were ADP-ribosylated by pertussis toxin in lysates using [^{32}P] NAD as described previously (11).

Protein assay. Protein concentration was determined by a protein-dye binding method (16) using bovine serum albumin as a standard.

Data analysis. Saturation isotherms were transformed using the method of Scatchard (17) and estimates of K_d and B_{max} were derived using weighted linear regression analysis of the transformed data. IC_{50} values were calculated from competition binding experiments, concentration response curves of cyclic AMP accumulation, ^{45}Ca efflux, and inositol phosphate accumulation. The IC_{50} values were transformed to apparent K_i values using the method of Cheng and Prusoff (18).

Results

Membranes from 1321N1 cells express a binding site for the H_1-receptor antagonist [^{3}H]mepyramine. Specific binding of [^{3}H]mepyramine, determined using 10 μM triprolidine, was 60–80% of total binding. Binding of [^{3}H]mepyramine (5 nM) attained steady state within 5–30 min and increased linearly with protein concentration up to at least 320 mg (data not shown). [^{3}H]Mepyramine binding was inhibited by the H_1-receptor antagonists triprolidine and diphenhydramine (Fig. 1). The H_2-receptor antagonist, cimetidine, was over 100,000 times less

potent than triprolidine, suggesting that interaction of [^{3}H] mepyramine is with an H_1-receptor.

[^{3}H]Mepyramine bound with high affinity (K_d = 3.3 ± 0.5 nM) to a single class of binding sites (Fig. 2). The estimated K_d for [^{3}H]mepyramine was similar to values reported previously for rat brain (19) and guinea pig small intestine (20). The kinetically derived dissociation constant ($K_d = k_2/k_1$) for [^{3}H] mepyramine in 1321N1 cell membranes was 5 nM (k_1 = 0.075 ± 0.008 nM^{-1} min^{-1}, k_2 = 0.41 ± 0.04 min^{-1}, n = 3; data not shown).

Subsequent to subculture at a density of 2500 cells/cm^2, the specific activity (pmol/mg of protein) of H_1-receptors progressively increased until 7 days of culture, after which there was a decrease in receptor specific activity (Fig. 3, *top panel*). These changes in H_1-receptor density reflect the fact that total receptors per culture dish increased until 7 days of culture, thereafter remaining constant, and the amount of protein (or cells) per dish continued to increase throughout the culture period. The amount of protein per cell did not change during a 2-week culture period. The growth-related expression of [^{3}H]mepyramine binding sites is similar to that previously described in detail for β-adrenergic receptors in this cell line (21). In contrast to H_1-receptors, the number of muscarinic cholinergic receptors per culture dish progressively increased throughout the culture period (Fig. 3, *bottom panel*).

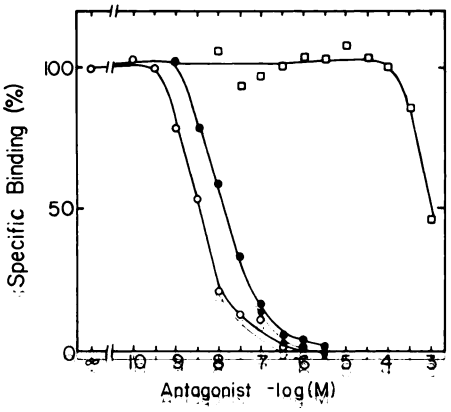

Fig. 1. Effects of triprolidine, diphenhydramine, and cimetidine on [^{3}H] mepyramine binding. Membranes (150 μg) were incubated with 5 nM [^{3}H] mepyramine in the presence of the indicated concentrations of triprolidine (○), diphenhydramine (●), or cimetidine (□). The data are plotted as percentage of specific binding observed in the presence of 2 μM triprolidine. Each *point* represents the mean value of triplicate assays, and the data are representative of three similar experiments.

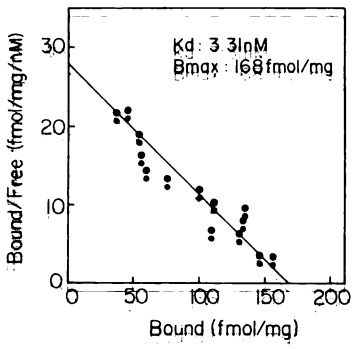

Fig. 2. Scatchard analysis of [^{3}H]mepyramine binding to 1321N1 cell membranes. Membranes (200 μg) were incubated with [^{3}H]mepyramine (0.2–20 nM) in the presence or absence of 2 μM triprolidine. The data are presented as a Scatchard plot and the values for K_d and B_{max} were determined as described in Materials and Methods. Each *point* represents the mean value of triplicate assays and the data are representative of three similar experiments.

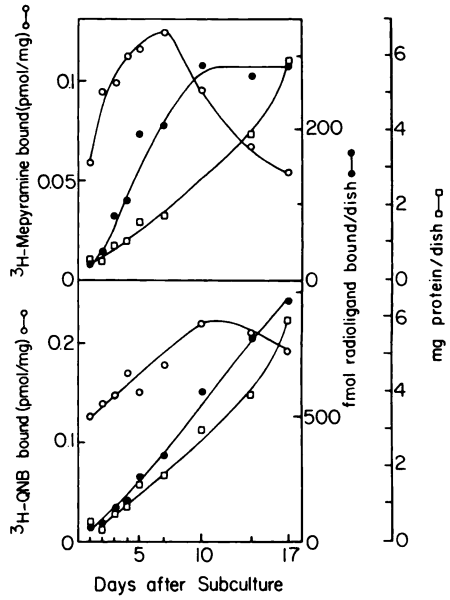

Fig. 3. Growth-related changes in histamine and muscarinic receptors. 1321N1 cells were subcultured at a density of 2.5 × 10^3 cells/cm^2 and growth medium was changed every 3–4 days thereafter. The density of histamine receptors was determined with 20 nM [^{3}H]mepyramine and the density of muscarinic receptors was determined with 1 nM [^{3}H]QNB after the indicated days in culture. The data are presented as receptor density (pmol/mg of protein; ——○——), as amount (fmol) of receptors per dish (——●——), and as the mg of protein per dish (——□——). Each *point* represents the mean value from triplicate dishes, and the results are representative of those obtained in two separate experiments.

Specific binding of [^{3}H]mepyramine was inhibited by histamine (Fig. 4). The histamine inhibition curve was shifted approximately 4-fold to the right and steepened in the presence of 1 μM GTPγS (Fig. 4), suggesting that H_1-receptors of 1321N1 cells interact with a guanine nucleotide regulatory protein. Pertussis toxin previously has been shown to ADP-ribosylate and inactivate G_i (22, 23), and to prevent GTP-sensitive high affinity binding of agonists to receptors that couple with G_i (12, 23). Pretreatment of 1321N1 cells with pertussis toxin (400 ng/ml) overnight had no effect on agonist binding to H_1-receptors. That is, GTPγS still steepened and shifted the histamine competition curve to the right (Fig. 4). Under these conditions, pertussis toxin-catalyzed incorporation of ^{32}P into the 41,000-M_r substrate, which has been shown to be the α-subunit of G_i, was reduced by 90% (Fig. 4, *inset*; Refs. 11 and 12).

Stimulation of muscarinic cholinergic receptors of 1321N1 cells results in attenuation of cyclic AMP accumulation (Table 1; Refs. 9 and 11), an increase in unidirectional $^{45}Ca^{2+}$ efflux (10, 13), and a breakdown of phosphoinositides (10, 13). We therefore examined the effects of histamine on these three responses. Histamine attenuated isoproterenol-stimulated [^{3}H] cyclic AMP accumulation in a concentration-dependent manner (Fig. 5A). Maximal attenuation of cyclic AMP accumulation by histamine ranged from 40 to 75%, and the attenuation observed with histamine was not additive with that observed with carbachol (Table 1). Furthermore, the attenuation of [^{3}H] cyclic AMP accumulation observed in the presence of histamine occurred irrespective of whether isoproterenol, prostaglandin

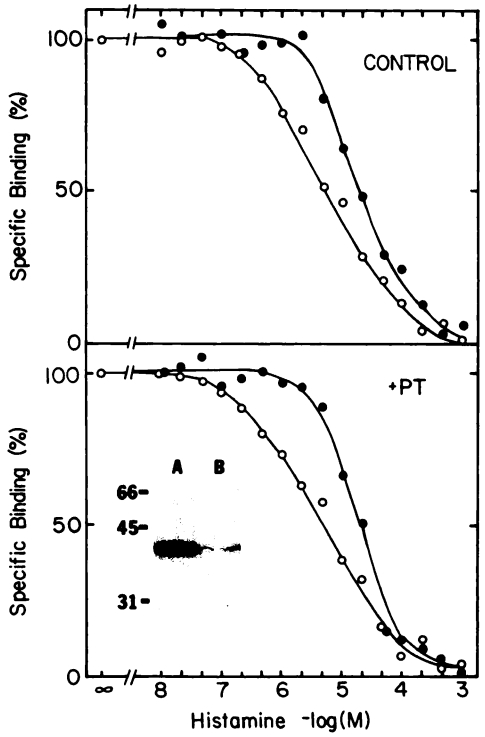

Fig. 4. Effect of pertussis toxin on high affinity, guanine nucleotide-sensitive agonist binding to H_1-histamine receptors. Cells were pretreated overnight with vehicle or pertussis toxin (400 ng/ml). Washed membranes from each condition were incubated with [^{3}H]mepyramine (5 nM) and the indicated concentrations of histamine in the presence (—●—) or absence (—○—) of GTPγS (1 μM). The Hill slopes for curves for control membranes were 0.71 and 0.92 in the absence and presence of GTPγS, respectively. The Hill slopes for curves for membranes from pertussis toxin-treated cells were 0.71 and 0.99 in the absence and presence of GTPγS, respectively. The data are plotted as a percentage of specific binding. Each *point* represents the mean value of triplicate assays and the data are representative of results obtained in four separate experiments. *Inset*, Membranes from control (A) or pertussis toxin-pretreated (B) cells were incubated with pertussis toxin and [^{32}P] NAD, and the samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiograms developed as described in Materials and Methods. The positions on the gels of known molecular weight markers are indicated.

E_1, or forskolin was the stimulatory agent (Table 1). The effect of histamine on [^{3}H]cyclic AMP accumulation is due to activation of phosphodiesterase since the rate of [^{3}H]cyclic AMP degradation in intact 1321N1 cells was enhanced in the presence of histamine (Fig. 6). IBMX blocked both histamine and muscarinic receptor-mediated attenuation of cyclic AMP accumulation (Table 1). The fact that the antagonism by IBMX of histamine-mediated effects was noncompetitive with histamine (Fig. 7) is consistent with the idea that the action of the inhibitor is at the level of phosphodiesterase rather than at the level of the histamine receptor. At high concentrations of IBMX, histamine increased, rather than decreased, cyclic AMP accumulation. This effect is similar to that previously observed with muscarinic receptor agonists or the divalent cation ionophore A23187 in the presence of high concentrations of IBMX (9), and is apparently due to a Ca^{2+}, calmodulin-mediated activation of adenylate cyclase.[2] Histamine had no effect on adenylate cyclase activity in cell-free preparations (Table 2).

The effect of histamine on Ca^{2+} mobilization was examined as previously described in detail for muscarinic cholinergic receptors of these cells (10) (see Materials and Methods). As illustrated in Fig. 5B, histamine stimulated the unidirectional efflux of $^{45}Ca^{2+}$ from $^{45}Ca^{2+}$-prelabeled cells. Histamine also stimulated the breakdown of phosphoinositides as measured by the accumulation of [^{3}H]inositol phosphates in [^{3}H]inositol-prelabeled 1321N1 cells (Fig. 5C). The effects of histamine on [^{3}H]mepyramine binding, attenuation of [^{3}H]cyclic AMP accumulation, $^{45}Ca^{2+}$ efflux, and inositol phosphate formation are summarized in Table 3. The $K_{0.5}$ for histamine-stimulated phosphoinositide breakdown was slightly higher than the $K_{0.5}$ values for Ca^{2+} mobilization or attenuation of [^{3}H]cyclic AMP accumulation. The binding affinity for histamine determined with [^{3}H]mepyramine in the presence of GTPγS and NaCl was similar to the $K_{0.5}$ value for histamine for stimulating phosphoinositide breakdown.

The effects of the H_1-receptor antagonist triprolidine and the H_2-receptor antagonist cimetidine on histamine-induced responses of intact 1321N1 cells are illustrated in Fig. 8. Triprolidine inhibited the histamine receptor-mediated attenuation of [^{3}H]cyclic AMP accumulation (Fig. 8A), stimulation of unidirectional $^{45}Ca^{2+}$ efflux (Fig. 8B), and stimulation of phosphoinositide breakdown (Fig. 8C) in a concentration-dependent manner. Cimetidine had no effect at the concentrations studied. As summarized in Table 2, the K_i values of triprolidine for antagonism of these three intact cell responses were very similar to the affinity of triprolidine determined in competition binding experiments with [^{3}H]mepyramine.

Muscarinic receptor-stimulated breakdown of phosphoinositides (13) and activation of phosphodiesterase (11) in 1321N1 cells are not affected by ADP-ribosylation and inactivation of G_i by pertussis toxin. However, it has been reported recently that phosphoinositide breakdown is inhibited by pertussis toxin treatment of rat mast cells (24) or human neutrophils (25, 26). Therefore, the effect of pertussis toxin treatment on histamine-receptor-stimulated breakdown of phosphoinositides was ex-

TABLE 1

Effects of histamine on [^{3}H]cyclic AMP levels in 1321N1 cells

1321N1 cells were incubated with the indicated combinations of isoproterenol (ISO, 10 μM), histamine (HA, 100 μM) carbachol (CARB, 100 μM), IBMX (100 μM), forskolin (FSK, 100 μM), and prostaglandin E_1 (PGE_1, 10 μM). Cyclic AMP accumulation was measured as described in Materials and Methods. The data are presented as mean ± SE of four experiments.

	Addition	% Conversion of [^{3}H]ATP to [^{3}H]cyclic AMP
A.	ISO	4.37 ± 0.22
	ISO + HA	1.80 ± 0.22[a]
	ISO + CARB	1.17 ± 0.08[a]
	ISO + HA + CARB	1.14 ± 0.06[a]
	ISO + IBMX	4.98 ± 0.23
	ISO + HA + IBMX	5.34 ± 0.12
	ISO + CARB + IBMX	5.57 ± 0.25
	ISO + HA + CARB + IBMX	6.48 ± 0.09[b]
B.	Basal	0.04 ± 0.01
	HA	0.04 ± 0.01
	ISO	6.65 ± 0.32
	ISO + HA	2.57 ± 0.04[c]
	FSK	4.29 ± 0.33
	FSK + HA	1.47 ± 0.02[c]
	PGE	2.33 ± 0.10
	PGE_1 + HA	0.81 ± 0.07[c]

[a] Significantly different ($p < 0.01$) from ISO alone.
[b] Significantly different ($p < 0.01$) from ISO + IBMX.
[c] Significantly different ($p < 0.01$) from accumulation with ISO, FSK, or PGE_1 alone.

[2] M. Liang and T. K. Harden, unpublished observations.

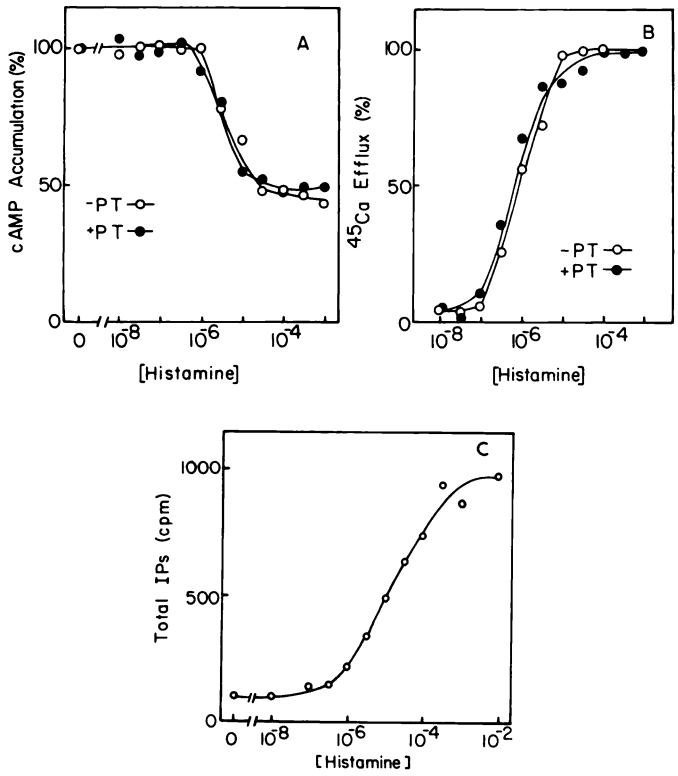

Fig. 5. Effects of histamine on cyclic AMP accumulation, $^{45}Ca^{2+}$ efflux, and inositol phosphate accumulation. A, Effect of histamine on isoproterenol (10 μM)-induced cyclic AMP accumulation in cells treated with (●) or without (○) pertussis toxin (400 ng/ml) overnight. Cyclic AMP accumulation in the presence of isoproterenol alone was taken as 100%. Each *point* represents the mean value of triplicate assays. B, Effect of histamine on $^{45}Ca^{2+}$ efflux in cells treated with (●) or without (○) pertussis toxin (400 ng/ml) overnight. $^{45}Ca^{2+}$ efflux was determined as described in Material and Methods. The amount of $^{45}Ca^{2+}$ retained by the cells after 1 min incubation was 6696 ± 318 cpm for control cells and 3957 ± 138 cpm for control cells incubated with histamine. The amounts of $^{45}Ca^{2+}$ retained after 1 min in pertussis toxin-treated cells were 6702 ± 126 cpm amd 4014 ± 159 cpm in the absence and presence of histamine, respectively. Each *point* represents the mean value of triplicate assays. C, Effect of histamine on the accumulation of inositol phosphates. Cells were incubated for 30 min with histamine at the indicated concentrations and total inositol phosphate accumulation was determined as described in Materials and Methods. Each *point* represents the mean value of triplicate assays.

amined in 1321N1 cells (Table 4). Both histamine and carbachol significantly increased the accumulation of IP_1, IP_2, and IP_3 in 1321N1 cells. Carbachol was routinely 2–5 times more efficacious than histamine for stimulation of phosphoinositide breakdown. As has been reported previously by Masters *et al.* (13) for carbachol-stimulated formation of IP_1 in 1321N1 cells, there was no significant effect of pertussis toxin on either histamine- or carbachol-stimulated formation of any of the inositol phosphates. In this and three similar experiments in which pertussis toxin had no effect on receptor-mediated inositol phosphate formation, toxin-catalyzed [^{32}P]ADP-ribosylation of a 41,000-M_r protein was reduced by >85% in toxin-treated cells. In addition, although toxin at concentrations as high as 3,000 ng/ml had no effect on receptor-mediated inositol phosphate formation (Fig. 9), 21.5 ng/ml of toxin completely blocked GTP-mediated inhibition of forskolin-stimulated adenylate cyclase activity in membranes from the same cells (control, 100 μM forskolin = 840 ± 36 pmol/min/mg of protein, 100 μM forskolin + 316 μM GTP = 419 ± 21 pmol/min/mg of protein; toxin-treated, 100 μM forskolin = 778 ± 22 pmol/min/mg of protein, 100 μM forskolin + 316 μM GTP = 764 ± 49 pmol/min/mg of protein, n = 4). Treatment of 1321N1 cells with pertussis toxin also had no effect on histamine-stimulated $^{45}Ca^{2+}$ mobilization (Fig. 5B) or on histamine receptor-mediated attenuation of cyclic AMP accumulation (Fig. 5A).

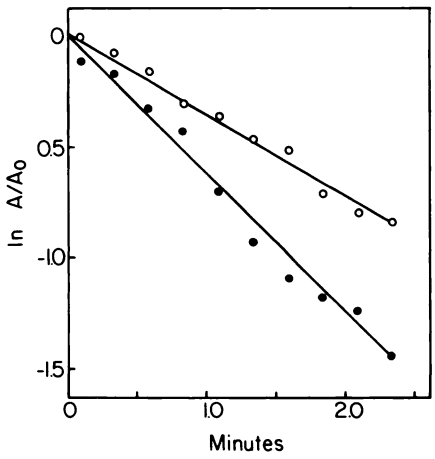

Fig. 6. Effect of histamine on cyclic AMP degradation. Cells were incubated in the presence of 10 μM isoproterenol (○) or 10 μM isoproterenol + 100 μM histamine (●). After 5 min, the synthesis reaction was stopped by the addition of propranolol to give a final concentration of 10 μM. The cells then were fixed at the indicated times by aspiration and addition of 1.0 ml of 5% TCA. [^{3}H]Cyclic AMP levels at each time (A) were divided by the initial level (A_0) of [^{3}H]cyclic AMP at the time of propranolol addition. The natural logarithm of A/A_0 is plotted versus time. The k_{deg} values averaged from six experiments in the presence and absence of histamine were 0.62 ± 0.02 min^{-1} and 0.32 ± 0.02 min^{-1}, respectively.

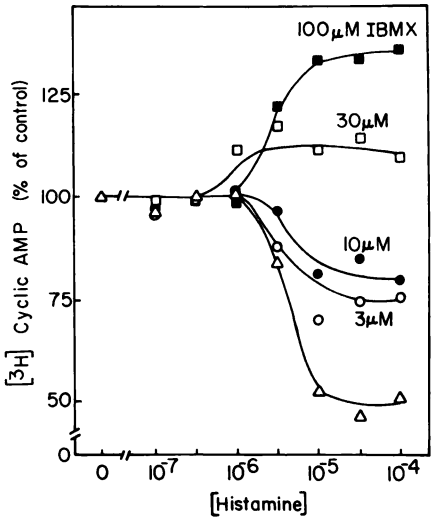

Fig. 7. Concentration-dependent antagonism by IBMX of the effects of histamine on cyclic AMP accumulation. Cells were incubated as described in Materials and Methods in the presence of isoproterenol (10 μM) and the indicated concentrations of histamine. Drug challenge was in the absence (—△—) or presence of 3 (—○—), 10 (—●—), 30 (—□—), or 100 (—■—) μM IBMX. The data are plotted as a percentage of the [^{3}H]cyclic AMP formation in the presence of isoproterenol and the absence of histamine for each IBMX concentration. These values were 5.55%, 5.14%, 5.72%, 5.85%, and 7.87% conversion of [^{3}H]ATP to [^{3}H]cyclic AMP for 0, 3, 10, 30, and 100 μM IBMX, respectively. The data are the mean of triplicate assays and are representative of results obtained in two separate experiments.

TABLE 2
Effect of histamine on adenylate cyclase activity in membrane preparations
Adenylate cyclase was measured as described in Materials and Methods. The data are the mean ± SE of four experiments.

Addition	Adenylate cyclase activity
	pmol/mg/min
None	21 ± 1
HA[a] (100 μM)	21 ± 1
ISO[b] (10 μM)	146 ± 2
ISO + HA	144 ± 1

[a] HA, histamine.
[b] ISO, isoproterenol.

TABLE 3
Comparison of affinities of histamine and triprolidine for histamine receptors and histamine receptor-mediated responses of 1321N1 cells
The affinities of histamine and triprolidine were determined in [^{3}H]mepyramine binding experiments and in assays of cyclic AMP accumulation, $^{45}Ca^{2+}$ efflux, and phosphoinositide breakdown. The [^{3}H]mepyramine binding experiments for histamine were carried out in the presence of 1 μM GTPγS and 140 mM NaCl. The values are the means ± SE of the indicated number of experiments (in parentheses).

Assay	Histamine	Triprolidine
	μM	*nM*
Attenuation of cyclic AMP accumulation	$K_{0.5}$ = 3.3 ± 1.3 (3)	K_i = 1.0 ± 0.7 (3)
$^{45}Ca^{2+}$ efflux	$K_{0.5}$ = 4.2 ± 1.2 (4)	K_i = 0.6 ± 0.4 (3)
Phosphoinositide breakdown	$K_{0.5}$ = 10.4 ± 1.7 (3)	K_i = 1.3 ± 0.6 (3)
Binding of [^{3}H]mepyramine	K_i = 11.1 ± 2.4 (4)	K_i = 1.0 ± 0.2 (3)

Discussion

The presence of H_1-histamine receptors on 1321N1 human astrocytoma cells is suggested by the following observations: (a) the H_1-receptor antagonist [^{3}H]mepyramine bound with high affinity to 1321N1 cell membranes; (b) triprolidine, an H_1-receptor antagonist, inhibited [^{3}H]mepyramine binding 100,000 times more potently than did cimetidine, an H_2-receptor antagonist; (c) histamine did not activate adenylate cyclase but markedly increased phosphoinositide breakdown, Ca^{2+} mobilization, and [^{3}H]cyclic AMP degradation; and (d) histamine-induced inositol phosphate formation, histamine-induced $^{45}Ca^{2+}$ efflux, and histamine-induced attenuation of [^{3}H]cyclic AMP accumulation were inhibited potently by triprolidine, but not by cimetidine.

To our knowledge, histamine-induced decreases in intracellular cyclic AMP accumulation have not been reported previously. The inhibitory response to histamine of 1321N1 cells is analogous to that observed with muscarinic receptors in these cells. No evidence of inhibitory coupling of histamine to adenylate cyclase has been obtained; as with muscarinic receptors the effect apparently is due solely to activation of phosphodiesterase. That is, phosphodiesterase inhibitors completely block the histamine-induced attenuation of cyclic AMP accumulation, and pertussis toxin has no effect on histamine action. Thus, the histamine receptor of 1321N1 cells can be added to a growing class of receptors that modulate cyclic AMP accumulation through effects on phosphodiesterase: the muscarinic receptor of this cell line (9, 11) and dog thyroid slices (27), the α_1-adrenergic receptor of rat heart cells (28), and the gonadotropin-releasing hormone receptor of cultured rat granulosa cells (29).

Although its role has not been confirmed in all of the cell types mentioned above, a Ca^{2+}/calmodulin-regulated phosphodiesterase is responsible for muscarinic receptor-stimulated increases in cyclic AMP degradation in 1321N1 cells (30). The coincident histamine-induced increases in phosphoinositide breakdown, Ca^{2+} mobilization, and cyclic AMP degradation

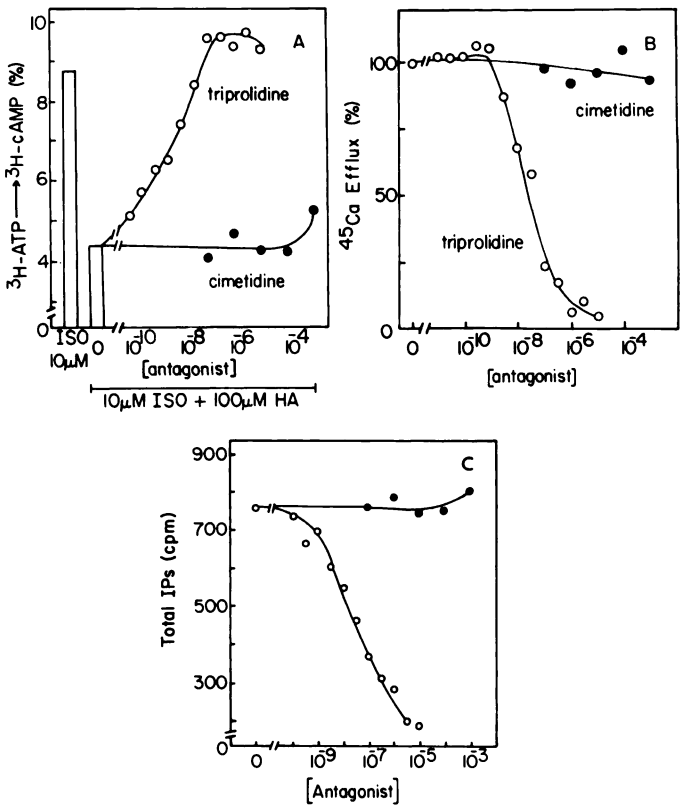

Fig. 8. Effect of triprolidine and cimetidine on histamine-induced attenuation of cyclic AMP accumulation, $^{45}Ca^{2+}$ efflux, and inositol phosphate formation. A, The conversion of [^{3}H]ATP to [^{3}H]cyclic AMP was determined in the presence of isoproterenol (*ISO*, 10 μM), histamine (*HA*, 100 μM), and the indicated concentrations of triprolidine (○) and cimetidine (●). Each *bar* and *point* represent the mean value of triplicate assays. B, Effects of triprolidine (○) and cimetidine (●) on histamine (100 μM)-induced $^{45}Ca^{2+}$ efflux. The amount of $^{45}Ca^{2+}$ retained by the cells after 1 min incubation was 6073 ± 271 and 3278 ± 163 in the absence and presence of 100 μM histamine, respectively. $^{45}Ca^{2+}$ efflux was determined as described in Materials and Methods. Each *point* represents the mean value of triplicate assays. C, Effects of triprolidine (○) and cimetidine (●) on histamine (100 μM)-induced inositol phosphate formation. Each *point* represents the mean value of triplicate assays.

TABLE 4
Effect of pertussis toxin on histamine- and carbachol-stimulated inositol phosphate formation
Phosphoinositides in 1321N1 cells were labeled by incubation with 5 μCi/ml of [^{3}H]inositol in inositol-free DMEM for 20 hr. Labeling was carried out in the absence or presence of 100 ng/ml of pertussis toxin. Following transfer to [^{3}H]inositol-free medium, the cells were challenged for 5 min with histamine (1 mM) or carbachol (1 mM) in the presence of 10 mM LiCl, and IP_1, IP_2, and IP_3 were separated as described in Materials and Methods. The data (cpm) are means ± SE of quadruplicate dishes and are representative of four similar experiments.

Addition	Pertussis toxin	IP_1	IP_2	IP_3
			cpm	
Basal	−	2905 ± 55	279 ± 11	595 ± 19
	+	2766 ± 124	288 ± 14	628 ± 15
Histamine	−	6008 ± 477[a]	538 ± 87[a]	867 ± 44[a]
	+	5468 ± 402[a]	497 ± 37[a]	772 ± 32[a]
Carbachol	−	13246 ± 492[a]	1321 ± 53[a]	1656 ± 44[a]
	+	13998 ± 168[a]	1318 ± 22[a]	1556 ± 43[a]

[a] Significantly different ($p < 0.01$) from each basal value.

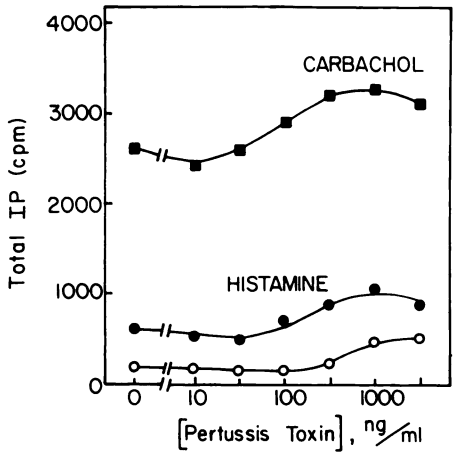

Fig. 9. Histamine- and carbachol-stimulated inositol phosphate formation following treatment of 1321N1 cells with various concentrations of pertussis toxin. Cells were treated with the indicated concentration of pertussis toxin overnight. Inositol phosphate formation in response to 1 mM histamine (—●—), 1 mM carbachol (—■—), or no drug (—○—) was measured. The results are presented as the mean of results obtained with three cultures for each condition.

therefore are not surprising. Receptor-stimulated breakdown of phosphatidylinositol 4,5-bisphosphate produces the second messenger IP_3 which in turn mobilizes intracellular Ca^{2+} (8). Elevation of intracellular Ca^{2+} then activates a cyclic AMP phosphodiesterase resulting in an enhanced degradation of cyclic AMP. This mechanism of regulation of cyclic AMP levels is probably a much more common and important component of hormone action than heretofore has been recognized. That is, in all cells where there are substantial levels of a Ca^{2+}/calmodulin-regulated phosphodiesterase, hormone receptors that mediate substantial production of IP_3 and, consequently, substantial elevation of cytoplasmic Ca^{2+} would be expected to increase cyclic AMP degradation.

Finally, the role of a guanine nucleotide regulatory protein in histamine action in 1321N1 cells should be discussed. Guanine nucleotide regulatory proteins have been shown to be crucial components of receptor-mediated activation and receptor-mediated inhibition of adenylate cyclase (31). A guanine nucleotide regulatory protein, transducin, also couples activation of rhodopsin by light to activation of a cyclic GMP phosphodiesterase in retinal rod outer segment (31). As has been reported previously by Chang and Snyder (32) for agonist binding to H_1-receptors in guinea pig brain membranes, histamine interacts with its receptor on washed 1321N1 membranes in a GTP-sensitive manner. Based on work with many receptor systems, these data can be taken as an indication of the interaction of H_1-receptors with a guanine nucleotide regulatory protein. Since there is no evidence that histamine regulates adenylate cyclase in 1321N1 cells and since pertussis toxin has no effect on histamine binding or its regulation by GTP, the involved guanine nucleotide regulatory protein is probably not G_i or the stimulatory guanine nucleotide regulatory protein of adenylate cyclase. The capacity of a series of muscarinic receptor agonists for formation of a GTP-sensitive high affinity binding complex is correlated with the efficacy of these agonists for stimulation of phosphoinositide hydrolysis in 1321N1 cells (33). Furthermore, we have demonstrated recently that muscarinic and histamine receptor agonists stimulate inositol phosphate formation in a guanine nucleotide-dependent manner in a membrane preparation from 1321N1 cells.[3] These data are consistent with several recent reports that also indicate that a guanine nucleotide regulatory protein is involved in receptor-mediated breakdown of phosphoinositides (34, 35). Although data have been presented suggesting that a guanine nucleotide regulatory protein that is a pertussis toxin substrate is important in regulation of polyphosphoinositide hydrolysis in human neutrophils (25, 26) and in mast cells (24), results from kidney (36), heart (13, 28), and 1321N1 astrocytoma cells (11–13) suggest that receptor-mediated increases in phosphoinositide hydrolysis do not involve G_i. Our prejudice is that another guanine nucleotide regulatory protein is involved, and it is this protein that couples histamine, muscarinic, and other receptors to phosphoinositide breakdown in 1321N1 cells and many other target tissues. The 1321N1 human astrocytoma cell should prove to be a useful model cell system to further characterize the interaction of H_1-histamine receptors with the phosphoinositide/Ca^{2+} signaling system.

References

1. Ash, A. S. F., and H. O. Schild. Receptors mediating some actions of histamine. *Br. J. Pharmacol.* **27**:427–439 (1966).
2. Black, J. W., W. A. M. Duncan, C. J. Durant, C. R. Ganellin, and E. M. Parsons. Definition and antagonism of histamine H_2-receptors. *Nature (Lond.)* **236**: 385–390 (1972).
3. Hegstrand, L. R., P. D. Kanof, and P. Greengard. Histamine-sensitive adenylate cyclase in mammalian brain. *Nature (Lond.)* **260**: 163–165 (1976).
4. Kanof, P. D., and P. Greengard. Pharmacological properties of histamine-sensitive adenylate cyclase from guinea pig cardiac ventricular muscle. *Mol Pharmacol.* **15**:445–461 (1979).
5. Richelson, E. Histamine H_1-receptor mediated guanosine 3′,5′-monophosphate formation by cultured mouse neuroblastoma cells. *Science* **211**:69–71 (1978).
6. Berridge, M. J., C. P. Downes, and M. R. Hanley. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. *Biochem. J.* **206**:587–595 (1982).
7. Daum, P. R., C. P. Downes, and J. M. Young. Histamine stimulation of inositol 1-phosphate accumulation in lithium-treated slices from regions of guinea pig brain. *J. Neurochem.* **43**:25–32 (1984).
8. Berridge, M. J. Inositol trisphosphate and diacylglycerol as second messengers. *Biochem. J.* **227**:933–937 (1985).
9. Meeker, R. B., and T. K. Harden. Muscarinic cholinergic receptor-mediated activation of phosphodiesterase. *Mol. Pharmacol.* **22**:310–319 (1982).
10. Masters, S. B., T. K. Harden, and J. H. Brown. Relationships between phosphoinositide and calcium responses to muscarinic agonists in astrocytoma cells. *Mol. Pharmacol.* **26**:149–155 (1984).
11. Hughes, A. R., M. W. Martin, and T. K. Harden. Pertussis toxin differentiates between two mechanisms of attenuation of cyclic AMP accumulation by muscarinic cholinergic receptors. *Proc. Natl. Acad. Sci. USA* **81**:5680–5684 (1984).
12. Evans, T., M. W. Martin, A. R. Hughes, and T. K. Harden. Guanine nucleotide-sensitive, high affinity binding of carbachol to muscarinic cholinergic receptors of 1321N1 astrocytoma cells is insensitive to pertussis toxin. *Mol. Pharmacol.* **27**:32–37 (1985).
13. Masters, S. B., M. W. Martin, T. K. Harden, and J. H. Brown. Pertussis toxin does not inhibit muscarinic receptor-mediated phosphoinositide hydrolysis and calcium mobilization. *Biochem J.* **227**:933–937 (1985).
14. Harden, T. K., A. G. Scheer, and M. M. Smith. Differential modification of the interaction of cardiac muscarinic cholinergic and beta-adrenergic receptors with a guanine nucleotide binding component(s). *Mol. Pharmacol.* **21**:570–580 (1982).
15. Sekura, R. D., F. Fish, C. R. Manclark, B. Meade, and Y.-L. Zhang. Pertussis toxin: affinity purification of a new ADP-ribosyltransferase. *J. Biol. Chem.* **258**:14647–14651 (1983).
16. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
17. Scatchard, G. The attractions of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51**:660–672 (1949).
18. Cheng, Y.-C, and W. H. Prusoff. Relationship between the inhibition constant (Ki) and the concentration of inhibitor which cause 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3108 (1973).
19. Tran, V. T., R. S. L. Chang, and S. H. Snyder. Histamine H_1 receptors identified in mammalian brain membranes with [^{3}H]mepyramine. *Proc. Natl. Acad. Sci. USA* **75**:6290–6294 (1978).
20. Hill, S. J., and J. M. Young. Characterization of [^{3}H]mepyramine binding to

[3] J. R. Hepler and T. K. Harden, submitted for publication.

the longitudinal muscle of guinea pig small intestine. *Mol. Pharmacol.* **19**:379–387 (1981).
21. Harden, T. K., S. J. Foster, and J. P. Perkins. Differential expression of components of the adenylate cyclase system during growth of astrocytoma cells in culture. *J. Biol. Chem.* **254**:4416–4422 (1979).
22. Katada, T., and M. Ui. ADP-ribosylation of a specific membrane protein of C6 cells by islet-activating protein associated with modification of adenylate cyclase activity. *J. Biol. Chem.* **257**:7210–7216 (1982).
23. Kurose, H., T. Katada, and M. Ui. Specific uncoupling by islet-activating protein, pertussis toxin, of negative signal transduction via adrenergic, cholinergic, and opiate receptors in neuroblastoma × glioma hybrid cells. *J. Biol. Chem.* **258**:4870–4875 (1983).
24. Nakamura, T., and M. Ui. Simultaneous inhibitions of inositol phospholipid breakdown, arachidonic acid release, and histamine secretion in mast cells by islet-activating protein, pertussis toxin. A possible involvement of the toxin-specific substrate in the Ca^{++}-mobilizing receptor-mediated biosignaling system. *J. Biol. Chem.* **260**:3584–3593 (1985).
25. Brandt, S., R. W. Dougherty, E. G. Lapetina, and J. E. Niedel. Pertussis toxin inhibits chemotactic peptide-stimulated generation of inositol phosphates and lysosomal enzyme secretion in human leukemic (HL-60) cells. *Proc. Natl. Acad. Sci. USA* **82**:3277–3280 (1985).
26. Smith, C. D., B. C. Lane, I. Kusaka, M. W. Verghese, and R. Snyderman. Chemoattractant receptor-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate in human polymorphonuclear leukocyte membranes. Requirement for a guanine nucleotide regulatory protein. *J. Biol. Chem.* **260**:5875–5878 (1985).
27. Dumont, J. E., F. Miot, C. Erneux, D. Couchile, P. Couchaux, C. Gervy-Decoster, J. Van Sande, and J. N. Wells. Negative regulation of cyclic nucleotide phosphodiesterase: the example of the dog thyroid. *Adv. Cyclic Nucleotide Protein Phosphorylation Res.* **16**:325–335 (1985).
28. Buxton, I. L. O., and L. L. Brunton. Action of the cardiac alpha$_1$-adrenergic receptor—activation of cyclic AMP degradation. *J. Biol. Chem.* **260**:6733–6737 (1985).
29. Ranta, T., M. Knecht, J.-M. Darbon, A. J. Baukal, and K. J. Catt. Calcium dependence of the inhibitory effect of gonadotropin-releasing hormone on luteinizing hormone-induced cyclic AMP production in rat granulosa cells. *Endocrinology* **113**:427–429 (1983).
30. Tanner, L. I., T. K. Harden, J. N. Wells, and M. W. Martin. Indirect identification of the phosphodiesterase isozyme regulated by muscarinic receptors in astrocytoma cells. *Fed. Proc.* **44**:1816 (1985).
31. Gilman, A. G. G-proteins and dual control of adenylate cyclase. *Cell* **36**:577–579 (1984).
32. Chang, R. S. L., and S. H. Snyder. Histamine H_1-receptor binding sites in guinea pig brain: regulation of agonist interactions by guanine nucleotides and cations. *J. Neurochem.* **34**:916–922 (1980).
33. Evans, T., J. R. Hepler, S. B. Masters, J. H. Brown, and T. K. Harden. Guanine nucleotide regulation of agonist binding to muscarinic cholinergic receptors: relation to efficacy for stimulation of phosphoinositide breakdown and Ca^{++} mobilization. *Biochem. J.* **232**:751–757 (1985).
34. Cockcroft, S., and B. D. Gomperts. Role of a guanine nucleotide binding protein in the activation of polyphosphoinositide phosphodiesterase. *Nature* **314**:534–536 (1985).
35. Litosch, I., C. Wallis, and J. N. Fain. 5-Hydroxytryptamine stimulates inositol phosphate production in a cell-free system from blowfly salivary glands: evidence for a role of GTP in coupling receptor activation to phosphoinositide breakdown. *J. Biol. Chem.* **260**:5464–5471 (1985).
36. Boyer, J. L., A. Garcia, C. Posadas, and J. A. Garcia-Sainz. Differential effect of pertussis toxin on the affinity state for agonists of renal alpha$_1$- and alpha$_2$-adrenoceptors. *J. Biol. Chem.* **259**:8076–8079 (1984).

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