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Desensitization of Histamine H_1 Receptor-Mediated Cyclic GMP Formation in Mouse Neuroblastoma Cells

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SUMMARY

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Histamine H_1 receptor-mediated cyclic GMP formation by intact mouse neuroblastoma cells (clone N1E-115) was attenuated by prolonged exposure to histamine. This desensitization was dependent upon the concentration of histamine and at 10 μM the half-time was $\simeq$9 min, whereas the half-time for resensitization in the absence of histamine was $\simeq$13 min. The order of potency for agonist-induced desensitization correlated with the order of potency for stimulating the H_1 receptor (histamine > 2-methylhistamine; 4-methylhistamine, no effect). Pyrilamine (50 nM) was more potent than metiamide (15 μM) in blocking desensitization by histamine. The ED_{50} for activation and for desensitization by histamine of the receptor-mediated response was approximately equal to its K_A for the H_1 receptor. Omission of Ca^{++} in the medium prevented cyclic GMP formation, but did not affect desensitization, suggesting that cyclic GMP formation was not required for the development of the desensitized state. Desensitization was temperature-dependent and marked inhibition of protein synthesis did not affect desensitization or its reversal. Finally, the ED_{50}'s for histamine-stimulated cyclic GMP formation in control and in partially desensitized cells were similar while the maximum response was reduced.

INTRODUCTION

Desensitization, which may be non-specific or specific, is the loss of sensitivity of a tissue to an agonist (e.g., a neurotransmitter) after prolonged exposure to this compound. The first reported example of non-specific desensitization was made by Cantoni and Eastman (1) who found that pre-incubation of guinea pig ileum with high concentrations of acetylcholine or histamine abolished the contractile response to a previously effective dose of either agonist. Specific desensitization was first reported by Barsoum and Gaddum (2), who found that pre-incubation of fowl rectal caecum with histamine rendered this tissue insensitive to histamine while the response to acetylcholine (as well as to other agents) was only slightly reduced. Subsequently, specific desensitization has been observed to occur for the nicotinic acetylcholine (3), α- and β-adrenergic (4, 5), insulin (6), thyroid-stimulating hormone (7), prostaglandin (8), and histamine H_2 receptors (9).

While many different mechanisms may be involved in non-specific desensitization, specific desensitization is generally considered to involve the agonist-receptor complex (10). In particular, recent *in vitro* stud-

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ies aided by radioligand binding techniques have shown that the desensitization of nicotinic acetylcholine receptors of *Torpedo marmorata* electroplaque (3) and β-adrenergic receptors of frog erythrocytes (4) both result from a decrease in the number of available receptors. In the first case the affinity of the remaining receptors for the agonist appeared to be increased, while in the latter it was unchanged.

Recent reports from this laboratory (11–13) and others[2] showed that histamine caused a rapid and large increase in cyclic GMP formation by mouse neuroblastoma cells (clone N1E-115). This response, which is mediated by histamine H_1 receptors, exhibited the phenomenon of specific desensitization since the sensitivity to muscarinic receptor-mediated cyclic GMP formation was not altered in histamine-desensitized cells (12). The molecular mechanisms associated with the loss of sensitivity to histamine stimulation have not been thoroughly investigated. In this communication we present data from a more extensive examination of the characteristics of this phenomenon in these cultured nerve cells.

MATERIALS AND METHODS

Materials. Histamine dihydrochloride and pyrilamine maleate were purchased from Sigma Chemical Co.; [^{3}H]guanine and [^{14}C]cyclic GMP from Amersham/Searle Corporation; fetal calf serum and D_1 concentrate from Colorado Serum; and AG50W-X2 (200–400 mesh) cation exchange resin from Bio-Rad Laboratories. Metiamide, 2-methylhistamine, 4-methylhistamine, and dibenamine were from Smith, Kline & French Labs.

Cell culture conditions. Mouse neuroblastoma cells (clone N1E-115) were grown at 37° in Dulbecco's modification of Eagle's medium which had been supplemented with 10% fetal calf serum (medium I) without antibiotics. The incubation atmosphere consisted of 10% CO_2 saturated with water vapor.

Histamine H_1 receptor-mediated cyclic GMP assay. The assay for receptor-mediated cyclic GMP formation utilizing radioactively-labeled precursor and intact cells is described in detail elsewhere (14). In brief, the cells were harvested for assay by aspirating off medium I and incubating for five minutes with a modified Puck's D_1 solution (medium II). The dissociated cells were collected by low-speed centrifugation (~250 × *g* for 5 min), washed twice and resuspended at a concentration of 8–10 × 10^6 cells/ml in a phosphate-buffered saline solution (medium III) containing 110 mM NaCl, 5.3 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.0 mM Na_2HPO_4, 25 mM glucose and 70 mM sucrose (titrated to pH 7.4 with 1 N HCl; osmolality 335–340 mOsm). The cell suspension was then placed in a 25 ml Erlenmeyer flask to which was added 10 μCi/ml of [^{3}H]guanine (1 μM final concentration) to radioactively label the intracellular stores of guanosine 5′-triphosphate (GTP) and rotated at 37° for 45 minutes at 80 rpm (Gyratory Shaker, New Brunswick Scientific Co.). After collection by centrifugation, the cells were washed three times with medium III and distributed (280 μl aliquots) into the wells of a multi-well tray (Disposo trays, FB16-24TC, Bellco Glass) at a concentration of approximately 2 × 10^5 cells per well. For desensitization cells were first radioactively labeled as above, diluted with medium III containing [^{3}H]guanine to give a density of 2.5–5 × 10^6 cells/ml and then distributed in one ml aliquots into 10 ml conical centrifuge tubes to which histamine was added (histamine had no effect on uptake of [^{3}H]guanine). After desensitization, the cells were washed and distributed into wells as described above. To test for histamine-stimulated cyclic GMP formation, the cells were exposed to histamine (20 μl) for 30 seconds at 37° and the incubation was terminated by the addition of 30 μl of 50% TCA. Each well was then sonicated for approximately five seconds with an Ultrasonic Cell Disrupter (Kontes Glass Co.). After the addition of 0.5 nCi of [^{14}C]cyclic GMP as an internal standard, the contents of each well were passed through a 0.8 × 8 cm AG50W-X2 ion exchange column which had been equilibrated with 5 ml of 0.1 N HCl. In addition, each well was washed with 0.50 ml of 5% TCA and this acid solution was added to the column. The column was washed with

[2] Study, R., T. Bartfai, X. O. Breakfield and P. Greengard, unpublished observations.

4.4 ml of 0.1 N HCl (eluate discarded), 1.0 ml of water (eluate discarded) and finally with 1.4 ml of water. To this last eluate, which contained 80–85% of the cyclic GMP, equal volumes (25 μl) of 3.2 M $ZnSO_4$ and 3.2 M Na_2CO_3 were added to further precipitate any residual GDP and GTP. After the precipitate had been removed by centrifugation, the supernatant was transferred to 10 ml of Quantafluor and the radioactivity determined in a Searle Isocap/300 liquid scintillation counter. All samples were corrected for the recovery of [^{14}C]cyclic GMP and quenching was determined by the external standard ratio technique.

Cell counts and protein assay. Protein was determined by means of a modification of the procedure reported by Lowry *et al.* (15) with bovine serum albumin as the standard. Cell counts were obtained with an electronic cell counter (Model Z_F), Coulter Electronics.

RESULTS

Kinetics of histamine-stimulated cyclic GMP formation. The exposure of the neuroblastoma cells to 100 μM histamine resulted in a rapid conversion of [^{3}H]GTP to [^{3}H]cyclic GMP (Fig. 1). The peak response, which occurred at 30 seconds, represented a seven-fold stimulation over the basal levels for this experiment. As with the muscarinic receptor-mediated response (16, 17), the degree of stimulation was variable from day to day and was dependent on the length of time that the cells were maintained in the stationary phase prior to assay. When cells were pre-incubated with the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine, and then stimulated with histamine, the rate of decay of [^{3}H]-cyclic GMP was significantly decreased (Fig. 1).

Determination of the equilibrium dissociation constant (K_A) for histamine. The K_A for histamine-stimulated cyclic GMP formation was determined according to the technique described by Furchgott (18, 19). This procedure, which utilizes the irreversible antagonist actions of dibenamine [N-(2-chloroethyl)dibenzylamine hydrochloride], has been used extensively to obtain estimates of K_A values and to detect the

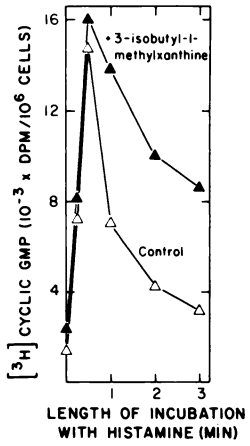

FIG. 1. *Time course of histamine H_1 receptor-mediated cyclic GMP formation*

Clone N1E-115 mouse neuroblastoma cells (subculture 19) were incubated with [^{3}H]guanine as described in MATERIALS AND METHODS; washed with medium III; incubated without or with 0.1 mM 1-methyl-3-isobutylxanthine (IBMX) for 15 min; and assayed in triplicate for basal and histamine-stimulated [^{3}H]-cyclic GMP formation. The concentration of histamine was 0.1 mM. There were approximately 2.5×10^5 cells and 0.6 mg protein per assay. The control (△) and IBMX (▲) curves differed significantly at one, two, and three minutes; the respective p values were <0.05, <0.001, and <0.001.

presence of "spare" receptors. In the presence of dibenamine the dose-response curve for histamine (Fig. 2A) had a reduced maximum but was not shifted to the right suggesting the absence of a receptor reserve. The K_A value calculated from the inverse plot of these data (Fig. 2B) was 8.3 μM, a value similar to that reported for histamine and its receptor in the guinea pig ileum (10 μM) (18).

The time course of desensitization and recovery of sensitivity. The development of insensitivity to histamine-stimulated cyclic GMP formation was a relatively rapid, first-order process and dependent on the concentration of histamine. When the cells were pre-incubated with 10 μM histamine, the half time for desensitization was approximately 9 min (including lag period); the half time was shorter at higher concentrations of histamine (Fig. 3).

The amount of desensitization at 10 min varied with the pre-incubation concentration of histamine (Fig. 4) and occurred in

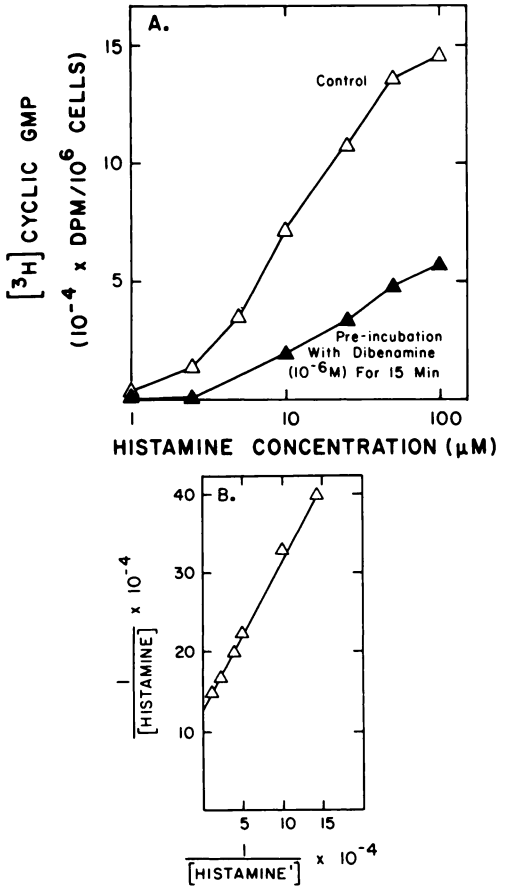

FIG. 2. *Determination of the equilibrium dissociation constant (K_A) for histamine H_1 receptor-mediated cyclic GMP formation*

Clone N1E-115 mouse neuroblastoma cells (subculture 12) were incubated with [^{3}H]guanine as described in the MATERIALS AND METHODS; washed with medium III; incubated without or with 0.01 mM dibenamine for 10 min; and assayed in duplicate for histamine-stimulated [^{3}H]cyclic GMP formation at the indicated concentrations of histamine (A). The concentrations of histamine giving equal stimulations of [^{3}H]cyclic GMP for control ([histamine]) and dibenamine-treated cells ([histamine']) were interpolated from the curves in A and their reciprocals were plotted against each other (B). The K_A was calculated according to the formula described by Furchgott (18, 19):

$$K_A = \frac{\text{slope} - 1}{\text{y intercept}}$$

Basal radioactivity which averaged 1300 and 1000 dpm/10^6 cells for the control and dibenamine-treated cells, respectively, has been subtracted from the data to give the values presented in A. There were approximately 1.8×10^5 cells and 0.45 mg of protein per assay.

the same concentration range as histamine stimulation of cyclic GMP formation (1 to 100 μM). The ED_{50} for desensitization was about 10 μM, which approximated the equilibrium dissociation constant (8 μM) for the histamine H_1 receptor complex of the mouse neuroblastoma cells (Fig. 2A, B).

The time course for recovery of responsiveness was prolonged (half-time ≃13 min), was first-order (Fig. 5), and was not dependent upon the concentration of histamine used to desensitize cells. One-hundred percent recovery of sensitivity was never observed in our experiments.

If one assumes that the recovery of sensitivity to histamine is described by a single exponential, then the instantaneous rate of this recovery is given by

$$\frac{dR}{dt} = k(R_\infty - R_t) \qquad (1)$$

where R_∞ is the response at infinite time (i.e., ≥120 min in Fig. 5). Integration of Eq. 1 yields

$$\ln \frac{R_\infty - R_o}{R_\infty - R_t} = k(t - t_o) = \frac{\ln 2}{T_{1/2}}(t - t_o) \qquad (2)$$

where $T_{1/2}$ is the half-time. A plot of the data of Fig. 5 according to Eq. 2 is linear (Fig. 5, inset), indicating that the recovery of sensitivity to histamine is adequately described by a process which is first order.

Specificity of desensitization. Previously we showed that desensitization of cells to histamine or carbamylcholine was associated with only a slight reduction in the ability of the other drug to stimulate cyclic GMP formation (12). Cells progressively desensitized by carbamylcholine showed a progressive shift to the right and a progressive decrease in the maximum of the dose-response curve for that drug (12). However, desensitization by carbamylcholine did not affect the ED_{50} value for the histamine response, although the magnitude of the response was slightly reduced at all concentrations of histamine (data not shown).

To further explore the specificity of the histamine-induced desensitization, separate cell samples were pre-incubated with various histamine-like agonists, washed free of agonist, and subsequently chal-

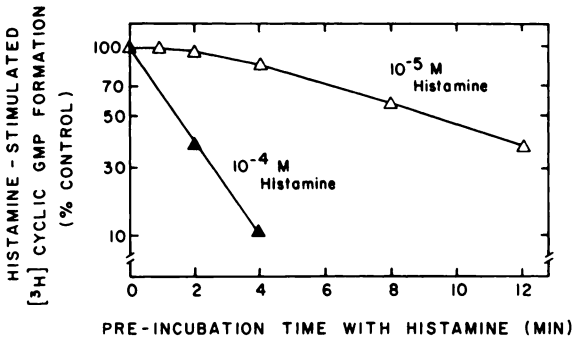

FIG. 3. *Semi-log plot of the time course of histamine-induced desensitization*

Clone N1E-115 mouse neuroblastoma cells (subculture 11) were incubated with [^{3}H]guanine as described in the MATERIALS AND METHODS; pre-incubated without (control) or with histamine for the indicated times; washed with medium III; and assayed in duplicate for basal and histamine-stimulated [^{3}H]cyclic GMP formation. Each point represents the mean of two independent experiments, each of which was determined in duplicate. △, 10^{-5} M histamine; ▲, 10^{-4} M histamine.

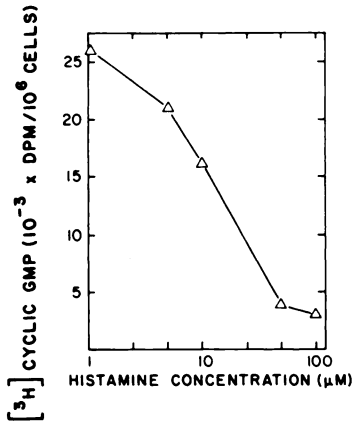

FIG. 4. *Concentration dependence of histamine-induced desensitization*

Mouse neuroblastoma clone N1E-115 cells (subculture 20) were incubated with [^{3}H]guanine as described in the MATERIALS AND METHODS; pre-incubated with the indicated concentrations of histamine for 10 min; washed with medium III; and assayed in duplicate for histamine (0.1 mM)-stimulated [^{3}H]cyclic GMP formation. These data are increases over basal levels of radioactivity which averaged 1000 dpm/10^6 cells (range: 650–1200). There were approximately 2.3×10^5 cells and 0.6 mg protein per assay.

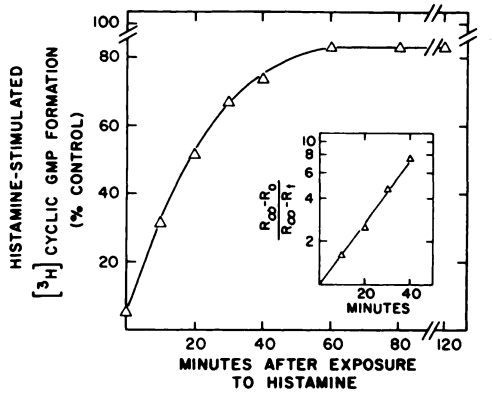

FIG. 5. *Time course for the reversal of histamine-induced desensitization*

Clone N1E-115 mouse neuroblastoma cells (subculture 11) were incubated with [^{3}H]guanine as described in MATERIALS AND METHODS. After pre-incubation without (control) or with histamine (0.1 mM) for 10 min, the control and histamine-treated cells were washed with medium III and assayed in duplicate for basal and histamine (0.1 mM) stimulated [^{3}H]cyclic GMP formation at the indicated times. Each point represents the mean of three independent experiments each of which was determined in duplicate. Inset: Semi-log plot of the data using Eq. 2 where R_∞ is the response at infinite time.

lenged with histamine (Table 1). Histamine was the most potent desensitizing agent followed by the selective H_1 agonist, 2-methylhistamine. Pre-incubation with the histamine H_2 receptor agonist, 4-methylhistamine, was virtually without effect in reducting the response of a subsequent histamine challenge. This rank order of potency was identical to that observed for H_1 receptor-mediated cyclic GMP formation (11).

These results suggested that desensitization directly involved the activation of histamine H_1 receptors, and therefore that the H_1 antagonist, pyrilamine, would be ex-

TABLE 1

Desensitization by histamine agonists of histamine H_1 receptor-mediated cyclic GMP formation

Mouse neuroblastoma clone N1E-115 cells (subculture 12) were incubated with [^{3}H]guanine as described in the MATERIALS AND METHODS; pre-incubated without (control) or with the indicated agonists (0.1 mM) for 10 min; washed free of the drugs; and assayed in triplicate for [^{3}H]cyclic GMP formation stimulated by histamine (0.1 mM) for 30 sec. The data are increases over the basal levels of radioactivity which averaged 1700 dpm/10^6 cells (range 1200–2200). There were approximately 1.5×10^5 cells and 0.5 mg protein per assay.

Agonist present (0.1 mM) during pre-incubation	Histamine-stimulated [^{3}H]-cyclic GMP formation	
	DPM/10^6 cells ± SEM	Percent control
None	8100 ± 800	—
Histamine	700 ± 300	9[a]
2-Methylhistamine	2200 ± 700	27[b]
4-Methylhistamine	7800 ± 1400	96

[a] Significantly different from control: $p < 0.001$.
[b] Significantly different from control: $p < 0.01$.

TABLE 2

Antagonism of histamine-induced desensitization

Mouse neuroblastoma clone N1E-115 cells (subculture 16) were incubated with [^{3}H]guanine as described in MATERIALS AND METHODS; pre-incubated for 30 min with either pyrilamine (50 nM) or metiamide (15 μM); and then incubated for an additional 10 min without (−) or with (+) 0.1 mM histamine. The cells were washed free of the drugs and assayed in triplicate for [^{3}H]cyclic GMP formation stimulated by histamine (0.1 mM) for 30 sec. The data are increases over the basal levels of radioactivity which averaged 1900 dpm/10^6 cells (range 1500–2100). There were approximately 2.9×10^5 cells and 1 mg of protein per assay.

Antagonist present during preincubation	Histamine (0.1 mM) present during 10 min incubation	Histamine-stimulated [^{3}H]-cyclic GMP formation	
		DPM/10^6 cells ± SEM	Percent control
None	−	31,000 ± 2000	—
	+	6,100 ± 800	20[a]
Metiamide	−	32,000 ± 3000	—
(15 μM)	+	10,000 ± 1000	31[b]
Pyrilamine	−	40,000 ± 5000	—
(50 nM)	+	34,000 ± 3000	85

[a] Significantly different from respective control: $p < 0.001$.
[b] Significantly different from respective control: $p < 0.01$.

pected to antagonize the development of the desensitized state. Pre-incubation of the cells for 30 min with 50 nM pyrilamine [25 times its K_D for H_1 receptors of these cells (11)] effectively antagonized the desensitization caused by histamine (0.1 mM), whereas the H_2 receptor antagonist, metiamide (15 μM [approximately 20 times its K_D for H_2 receptors of rat uterus (20)], was much less effective (Table 2). Neither pyrilamine nor metiamide alone caused desensitization.

Relationship between desensitization and cyclic GMP formation. The preceding experiments showed that desensitization of histamine-induced cyclic GMP formation required activation of the receptor by agonist. It was of interest to know, however, if the formation of cyclic GMP was necessary for desensitization. The experimental approach to this problem was based on previous results which demonstrated that histamine H_1 receptor-mediated cyclic GMP formation was dependent on the availability of extracellular calcium (11). In the present work we found that the omission of calcium from the pre-incubation medium had no effect on desensitization (data not shown), suggesting that desensitization was a consequence of receptor activation and not secondary to Ca^{++} influx and/or the formation of cyclic GMP. (The ability of histamine to cause desensitization was also unaffected by the omission of sodium, potassium or magnesium from the medium.)

The effect of temperature on desensitization. Decreasing the temperature of the pre-incubation medium greatly reduced the desensitization caused by histamine. When cells were pre-incubated with histamine (0.1 mM) for 10 min at 37°, nearly complete (95%) desensitization to histamine was achieved. However, pre-incubation at 22° and 4° resulted in 70% and 10% desensitization, respectively.

Dose-response curve changes associated with the desensitized state. The loss of sensitivity to histamine in the desensitized state appeared to involve the histamine H_1 receptor and could be a consequence of a change in its affinity for histamine, or a decrease in the population of active histamine receptors, or both. To investigate

these possibilities, the dose-response relations of histamine on cyclic GMP formation were compared in control and partially desensitized cells (Fig. 6). The desensitized cells responded differently from control only in a reduction in the maximum response to histamine stimulation. In both cases the ED_{50}'s for histamine were similar to the K_A for histamine and the H_1 receptor. One possible interpretation of these data is that this desensitization results from a decrease in the number of functional H_1 receptor sites with no change in the affinity of these sites for histamine.

The effect of inhibition of protein synthesis on desensitization and its reversal. Cycloheximide (10 μM), when incubated with the cells for 90 min, decreased the incorporation of [^{14}C]leucine into protein by 75%, but did not affect the degree or rate of desensitization or resensitization (data not shown). Higher concentrations of cycloheximide inhibited control responses to histamine.

DISCUSSION

In our previous studies on the desensitization of muscarinic receptor-mediated formation of cyclic GMP by mouse neuroblastoma cells (12), we showed that desensitization by histamine left largely unaffected the ability of carbamylcholine to stimulate cyclic GMP formation and the reverse. These results indicated that the agonist-induced desensitizations of these receptor-mediated responses are specific desensitizations and probably do not involve guanylate cyclase. Our data are compatible with the hypothesis that this desensitization is the result of agonist-induced changes

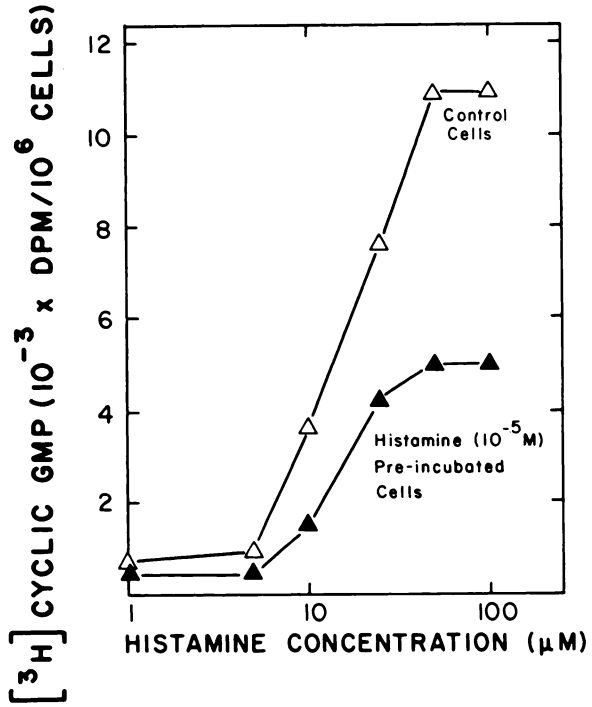

FIG. 6. *The effect of desensitization on the dose-response curve for histamine-stimulated cyclic GMP formation*

Mouse neuroblastoma clone N1E-115 cells (subculture 10) were incubated with [^{3}H]guanine as described in the MATERIALS AND METHODS; pre-incubated without (control) or with histamine (0.1 mM) for 10 min; washed with medium III; and assayed in duplicate for [^{3}H]cyclic GMP formation at the indicated concentrations of histamine. These data are increases over the basal levels of radioactivity which were in dpm/10^6 cells 1100 and 2400 for the control and histamine-treated cells, respectively. There were approximately 1×10^5 cells and 0.3 mg protein per assay.

at the level of the histamine H_1 receptor. The evidence in support of this hypothesis is briefly summarized as follows: Desensitization is 1) specific for H_1 receptor agonists whose potencies parallel their ability to activate H_1 receptors (the ED_{50} for stimulation and for desensitization by histamine is approximately equal to its K_A for the H_1 receptor); 2) antagonized by a specific H_1 receptor antagonist; 3) not dependent upon cyclic GMP; and 4) associated with a decreased maximum in the dose-response curve for H_1 receptor-mediated cyclic GMP formation.

Activation of muscarinic acetylcholine or histamine H_1 receptors of various cell types (e.g., smooth muscle, mouse neuroblastoma cells) causes similar effects (e.g., muscle contraction, cyclic GMP formation). In addition H_1 receptor antagonists (antihistamines) commonly have antimuscarinic properties as well (21, 22). These facts suggest functional and structural similarities between these two receptors. The time course for the stimulation of cyclic GMP formation is essentially the same after activation of either muscarinic acetylcholine or histamine H_1 receptors of mouse neuroblastoma clone N1E-115; and the dependence of this cyclic GMP synthesis on calcium ions in the external medium is also similar for the two receptors. The characteristics of the specific desensitization of the two receptor-mediated responses are also very similar. In addition, the time courses for the recovery of sensitivity to histamine and carbamylcholine in cells that were desensitized to the two agonists were essentially superimposable.[3] Thus far, the major difference between the two receptor-mediated responses is that "spare" receptors are involved in the muscarinic receptor-mediated response[4] but not in the H_1 receptor-mediated response in these mouse neuroblastoma cells.

If one uses the receptor occupancy theory, then at submaximal concentrations of agonist the cyclic GMP response is a direct function of the number of receptors occupied. Then, in the presence of "spare" receptors the agonist concentration giving half-maximal response (ED_{50}) is lower than the agonist concentration giving 50% receptor occupancy. In the absence of "spare" receptors the ED_{50} for the agonist is equal to the concentration of agonist giving 50% receptor occupancy. In the case of a system with "spare" receptors, a progressive inactivation of receptors would result in a progressive shift to the right of the dose response curve until the ED_{50} for the agonist is equal to the concentration of agonist giving 50% receptor occupancy (i.e., the point where "spare" receptors are no longer present). Further inactivation of receptors would result in a decline in the maximum response. Thus, our result with desensitization of the muscarinic receptor showing a shift to the right and a decreased maximum of the dose-response curve in partially desensitized cells (12) is compatible with the presence of "spare" receptors for the muscarinic response, while our result with the histamine H_1 receptor desensitization (Fig. 6) showing only a decreased maximum in the dose-response curve is compatible with the absence of "spare" receptors for this latter response. These conclusions were made on the basis of the results from the dibenamine experiments[3] (Fig. 2). Thus, the mechanism(s) involved in desensitization of the muscarinic acetylcholine and histamine H_1 receptors may be similar and independent of receptor number. The differences in dose-response relationships for the two receptors in partially desensitized states likely reflect the presence or absence of "spare" receptors.

There is considerable evidence to show that histamine may serve as a neurotransmitter (23, 24) and that both H_1 and H_2 receptors are present within the CNS[5] (25–27). While it is certain that the stimulation of histamine H_2 receptors in the CNS results in an activation of adenylate cyclase (27), it is not known whether the stimulation of H_1 receptors in the CNS results in an activation of guanylate cyclase. The evidence described in this and other studies (11–13)[1] using cultured nerve cells demon-

[3] Taylor, J. and E. Richelson, unpublished data.

[4] Richelson, E., unpublished observations.

[5] The abbreviation used is CNS, central nervous system.

strates that the activation of H_1 receptors results in the formation of cyclic GMP and that, like some receptors in the CNS (20), the sensitivity of histamine H_1 receptors can be acutely regulated in response to the degree of stimulation. In non-neural tissues, histamine stimulates cyclic GMP formation (presumably by activation of H_1 receptors) (29) and desensitizes its receptor (2, 30). Thus, our results very likely are relevant to studies on histamine H_1 receptors of non-neural tissues but the application of these results to CNS H_1 receptors remains to be demonstrated.

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