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Specific Desensitization of Histamine H_1 Receptor-Mediated [^{3}H]Glycogen Hydrolysis in Brain Slices

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SUMMARY

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In slices from mouse cerebral cortex, histamine elicits in a concentration-dependent manner the hydrolysis of [^{3}H]glycogen previously synthesized from [^{3}H]glucose, a response mediated by H_1 receptors. Desensitization of this glycogenolytic response occurs when slices are incubated with 50 μM histamine and is characterized by a rightward shift of the concentration-response curve (EC_{50} = 32 ± 1 μM instead of 4 ± 1 μM) without significant change in the maximal glycogenolysis. Desensitization occurs with a half-time of about 20 min, and the initial responsiveness is gradually recovered within 1 hr. Recovery is not impaired in the presence of cycloheximide, a protein synthesis inhibitor. Desensitization to the glycogenolytic action of histamine seems to be a selective process: (a) it results from stimulation of H_1 receptors, since 2-thiazolylethylamine, an H_1-receptor agonist (but not dimaprit, an H_2-receptor agonist) shares the desensitizing action of histamine; (b) only the glycogenolytic response to histamine is desensitized, whereas responsiveness to other glycogenolytic agents (noradrenaline, serotonin, adenosine, dibutyryl cyclic AMP) is not modified. Following exposure of slices to histamine, the binding of [^{3}H]mepyramine to H_1 receptors is significantly modified. The change consists of a 20% decrease in the B_{max} of the [^{3}H]antihistamine without significant change in either its K_d or the K_i of histamine.

INTRODUCTION

The ability of target cells to attenuate their responsiveness to stimulating agents after prolonged exposure to these compounds, generally termed desensitization, is now a well-documented process. Desensitization was first observed regarding the HA[1]-elicited contraction of smooth muscles such as guinea pig ileum (1) or rat cecum (2), both effects being mediated by H_1 receptors. More recently, desensitization was reported regarding the HA-induced stimulation of cyclic GMP accumulation in a clone of neuroblastoma cells, also an H_1 receptor-mediated effect (3). On several biological responses mediated by nicotinic (4) or *beta*-adrenergic receptors (5, 6), desensitization seems to consist of changes occurring at the level of the receptor molecule itself, but it has not yet been established whether this is the case for H_1 receptors.

H_1 receptors have been also identified by various experimental approaches in the mammalian brain, where they could mediate some of the effects of HA released from putative histaminergic neurons on target cells (7). These receptors participate in the HA-induced stimulation of cyclic AMP formation in slices from guinea pig hippocampus, but in a rather indirect manner: the HA-sensitive adenylate cyclase has the pharmacological specificity of an H_2 receptor (8), and the H_1 receptor-mediated increase in nucleotide accumulation is observed only when H_2 receptors are stimulated and seems to involve a translocation of calcium ions (9, 10). Hence, in view of the complex connection between the effects mediated by the two classes of HA receptors, this experimental model does not seem well-suited to study desensitization mediated by H_1 receptors.

On the other hand, HA exerts a potent glycogenolytic action on brain slices that seems to depend not on cyclic AMP formation but on translocation of calcium ions (11). Hydrolysis of [^{3}H]glycogen occurs in this preparation in the presence of HA and histaminergic agonists in low concentrations and, being selectively mediated by H_1 receptors, this constitutes a good model with which to investigate desensitization.

The present report describes the decreased responsiveness to the glycogenolytic action of HA as a result of prior exposure of brain slices to the amine. Furthermore,

[1] The abbreviation used is: HA, histamine.

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studies with [^{3}H]mepyramine, a selective ligand of H_1 receptors (12–14), indicate that desensitization is accompanied by a change occurring at the level of H_1-receptor molecules.

MATERIALS AND METHODS

Animals. Male Swiss albino mice (18–20 g) (Lessieux, France) were housed in groups of 10 in a well-ventilated room maintained at a temperature of 22° and artificially illuminated (light between 8 a.m. and 8 p.m.); standard food (Usine d'Alimentation Rationnelle, Epinay-Sur-Orge, France) and water available ad libitum.

Preparation and incubation of brain slices. Animals were killed by decapitation. The cerebral cortex was quickly removed, and slices (250 × 250 μm thick) were prepared in a cold room (4°) with a McIlwain tissue slicer. Cortical slices from six animals were pooled in a slightly modified Krebs-Ringer bicarbonate medium (120 mM NaCl, 5 mM KCl, 2.6 mM $CaCl_2$, 0.67 mM $MgSO_4$, 1.2 mM KH_2PO_4, 3 mM glucose, and 27.5 mM $NaHCO_3$), previously gassed with O_2:CO_2 (95:5) until pH 7.4 was reached. In desensitization experiments, slices were incubated at 37° for 5 min; the desensitizing agent was then added and incubations were continued for various time intervals under a constant stream of O_2:CO_2 (95:5). At the end of the incubation period, slices were washed three times with Krebs-Ringer bicarbonate medium and prepared for evaluation of either [^{3}H]glycogen synthesis and hydrolysis or [^{3}H]mepyramine binding.

[^{3}H]Glycogen synthesis and hydrolysis. Slices were resuspended in fresh Krebs-Ringer medium, and 300-μl aliquots of the tissue suspension, corresponding to approximately 0.5 mg of protein, were distributed in incubation tubes. [^{3}H]Glucose solution (13.4 nmoles) (1 mCi/ml) was added in a volume of 10 μl. In some experiments 20 nmoles of [^{3}H]glucose was added. After 20 min, 10 μl of a solution of the glycogenolytic agent (or Krebs-Ringer medium) were added and the slices were further incubated for 15 min at 37°. The incubations were stopped by rapid centrifugation. The supernatant was discarded and replaced by 300 μl of fresh medium in which the slices were sonicated (Générateur d'Ultrasons, Annemasse, France, 30 KH, 80 W). A fraction of resulting homogenate was then immediately deproteinized by heating at 95° for 10 min, followed by a short centrifugation. The supernatant was sampled for [^{3}H]glycogen assay.

[^{3}H]Glycogen was isolated by ethanol precipitation using a filter paper technique described previously. Briefly stated, a 150-μl sample was spotted on a disc of filter paper which was successively dipped into different baths of ethanol-trichloroacetic acid and 66% ethanol in order to wash out [^{3}H]glucose, whereas [^{3}H]glycogen selectively remained on the disc, as identified with purified amylo-1-6-glucosidase.

[^{3}H]Mepyramine binding. Washed slices were resuspended in 40 volumes of fresh 50 mM Na-K phosphate buffer (pH 7.5) and homogenized with a glass-Teflon Potter-Elvevehjem homogenizer. The washing procedure and homogenization took 10 min. A portion (450 μl) of the homogenate, corresponding to 0.8 mg of protein, was incubated with 10 nM [^{3}H]mepyramine at 37° for 20 min, under a final volume of 500 μl. Incubations were stopped by the addition of 3 ml of ice-cold buffer, followed by rapid filtration onto a glass-fiber filter (Whatman GF/B); the samples were rapidly rinsed twice with 20 ml of cold buffer. Radioactivity trapped on the filters was counted in 14 ml of scintillation mixture [2,5-diphenyloxazole, 16 g; 1,4-bis[2-(5-phenyloxazolyl)]benzene, 0.45 g; toluene, 2000 ml; Triton X-100, 1000 g] in the presence of 2 ml of water, after 24-hr storage at 4°. Specific binding was defined as the difference between radioactivity bound in the absence and in the presence of 0.3 μM triprolidine, an H_1 antihistamine.

Protein concentrations were determined by the method of Lowry *et al.* (15) using bovine serum albumin as the standard.

Chemicals and drugs. 2-Thiazolylethylamine was generously provided by Dr. M. E. Parsons (The Research Institute, Smith Kline & French Laboratories, Welwyn Garden City, United Kingdom) and triprolidine by the manufacturer (Wellcome Laboratories, Research Triangle Park, N. C.). Histamine was obtained from Prolabo (Paris, France); serotonin from Calbiochem (San Diego, Calif.); noradrenaline from Sigma Chemical Company (St. Louis, Mo.); and adenosine from Koch-Light Laboratories (Colnbrook, Bucks., England).

Radioisotopes. [^{3}H]Glucose (500 mCi/mmole) and [^{14}C]histamine (290 mCi/mmole) were purchased from the Radiochemical Centre (Amersham, England); [^{3}H]-mepyramine (27 Ci/mmole) was obtained from New England Nuclear Corporation (Boston, Mass.).

Analysis of data. Concentration-response curves were fitted by hand or by employing a computer program (16). The inhibition constant (K_i) of HA regarding [^{3}H]mepyramine binding was calculated, assuming competitive inhibition, according to the equation (17)

$$K_i = \frac{IC_{50}}{1 + S/K_d}$$

where IC_{50} is the concentration of HA required to produce 50% inhibition of [^{3}H]mepyramine binding, S represents the concentration of ^{3}H-labeled ligand, and K_d is its dissociation constant.

RESULTS

Effects of incubation of cortical slices with HA or HA agonists on H_1 receptor-mediated [^{3}H]glycogen hydrolysis. The [^{3}H]glycogen accumulated in control slices is hydrolyzed by HA in a clearly concentration-dependent manner (Fig. 1) with an EC_{50} of 4 ± 1 μM, the maximal glycogenolytic effect being 70 ± 5% of basal levels (means ± standard error of the mean of four values from separate experiments). When slices were incubated for 20 min with 50 μM HA, the concentration-response curve to HA was significantly shifted to the right (EC_{50} = 32 ± 0.6 μM; $p < 0.005$) without modification of either the basal [^{3}H]glycogen level or the maximal glycogenolytic response. To explore further the specificity of this effect, slices were incubated for 20 min with various histaminergic agonists or with noradrenaline and subsequently challenged with HA. Incubation with 100 μM 2-thiazolylethylamine, an H_1-receptor agonist, also resulted in a shift of the concentration-response curve to HA to the right (Fig. 2). However, 2-thiazolylethylamine was less potent than HA in inducing desensitization, since the

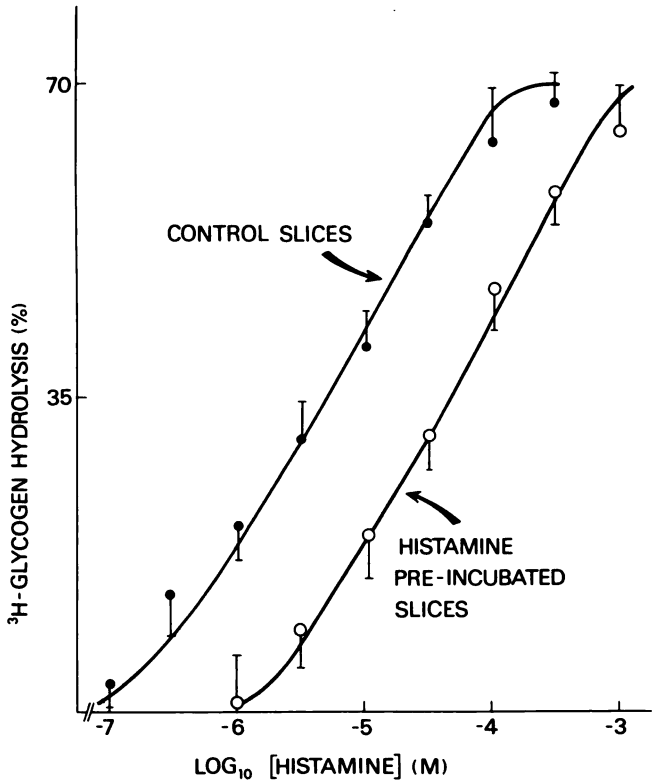

FIG. 1. *Effect of histamine incubation on histamine-induced hydrolysis of [^{3}H]glycogen in slices from mouse cortex*

Slices were incubated without (controls) or with 50 μM HA for 20 min and washed three times; [^{3}H]glycogen hydrolysis induced by HA at the indicated concentrations was then determined. Results are expressed as percentages of basal [^{3}H]glycogen levels: 20.2 ± 1.0 × 10^3 dpm/mg of protein for controls and 20.9 ± 1.0 × 10^3 dpm/mg of protein for HA-incubated slices. Each value represents the mean ± standard error of the mean of data from 20–30 separate incubations performed in four series of experiments.

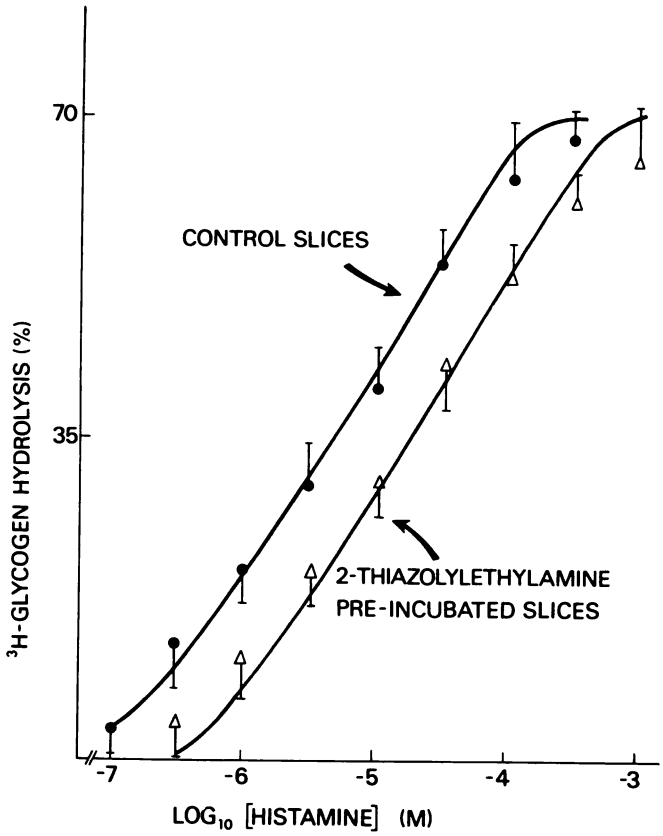

FIG. 2. *Effect of incubation in presence of 2-thiazolylethylamine, an H_1-receptor agonist, on histamine-induced glycogenolysis*

Slices from mouse cortex were incubated without (controls) or with thiazolylethylamine (100 μM) for 20 min and washed three times; [^{3}H]glycogen hydrolysis elicited by HA at the indicated concentrations was then determined. Results are expressed as percentages of basal [^{3}H]glycogen levels: 19.3 ± 0.8 × 10^3 dpm/mg of protein for control slices and 18.6 ± 1.1 × 10^3 dpm/mg of protein for 2-thiazolylethylamine-incubated slices. Each value represents the mean ± standard error of the mean of four to eight separate incubations.

rightward shift was less pronounced (EC_{50} was 15 μM as compared with 5 μM for the respective control slices), in spite of a 2-fold higher concentration of the former agent during the incubation. In contrast, incubation with 100 μM dimaprit, a pure histamine H_2-receptor agonist, or with 10 μM noradrenaline did not produce any significant change in the concentration-response curve to HA (Table 1).

Time-course and reversibility of desensitization. Slices were exposed to 50 μM HA for different periods (Fig. 3), washed three times to remove the excess of amine, and then challenged with HA in a fixed concentration (10 μM). Development of desensitization to HA occurred with a half-time of approximately 20 min. When the delay between first exposure and challenge of the slices with HA was increased, the responsiveness was progressively restored (Fig. 4). Total recovery of sensitivity to HA occurred within 1 hr after first exposure of the slices. Recovery of sensitivity to HA was not impaired when the recovery incubation was performed in the presence of 20 μM cycloheximide, an inhibitor of protein synthesis (Table 2).

Concentration-response relationship and specificity of the desensitization elicited by HA. The decrease in responsiveness to a test concentration of HA (10 μM) varied with the concentration of HA in the incubation medium (Fig. 5). In a range of 3–300 μM HA, a concentration-response relationship regarding desensitization was obtained with a threshold at 5 μM and a maximal effect at 300 μM. However, for the highest concentration of HA in the incubation medium, the subsequent synthesis of [^{3}H]glycogen was slightly impaired as shown by the significant decrease in basal level (Fig. 5).

The specificity of the desensitization process was investigated following a 20-min exposure of the slices to 50 μM HA. Among the various glycogenolytic agents tested, i.e., HA, 5-hydroxytryptamine, noradrenaline, adenosine, and dibutyryl cyclic AMP, only the response to HA was significantly diminished (Table 3).

[^{3}H]Mepyramine binding following incubation of slices with HA. The specific binding of [^{3}H]mepyramine to H_1 receptors (12) was determined by using 0.3 μM triprolidine to evaluate nonspecific binding, since this concentration corresponds to a plateau in its displacement curve (not shown). In homogenates prepared from slices incubated in the presence of HA, a small change in the specific binding of [^{3}H]mepyramine occurred as compared with homogenates from control slices (Fig. 6A).

TABLE 1

Effect of incubations in the presence of dimaprit, a histamine H_2-receptor agonist, or noradrenaline on histamine-induced hydrolysis of [^{3}H]glycogen in slices from mouse cortex

Slices from mouse cortex were incubated for 20 min with 100 μM dimaprit or 10 μM noradrenaline and washed three times; [^{3}H]glycogen synthesis and its hydrolysis elicited by HA at different concentrations were then determined. The basal level of [^{3}H]glycogen was not significantly modified under the various incubation conditions. Data represents mean ± standard error of the mean from 10–20 separate incubations performed in two series of experiments.

	Basal level of [^{3}H]glycogen	HA-induced glycogenolysis	
		EC_{50}	Maximal response
	10^3 dpm/mg protein	*μM*	*% basal level*
Control	18.8 ± 1.5	4.4 ± 1.0	72 ± 3
Dimaprit-incubated	24.2 ± 1.7	4.9 ± 2.7	69 ± 5
Noradrenaline-incubated	17.1 ± 1.0	7.0 ± 3.5	70 ± 3

Scatchard analysis of the saturation curves indicated that the K_d value was not significantly modified (5 nM instead of 6 nM), whereas the B_{max} was decreased by 21% (57 instead of 72 fmoles/mg protein). In homogenates from both HA-desensitized and control slices, Hill plot analyses of [^{3}H]mepyramine binding (not shown) indicated that values for the Hill coefficient were not significantly different from unity.

The change in maximal [^{3}H]mepyramine binding following desensitization was ascertained in a separate series of experiments. Sixteen distinct pools of slices were exposed to 50 μM HA and the radioreceptor assay was performed at 10 nM [^{3}H]mepyramine. This pre-treatment resulted in an 18% decrease ($p < 0.05$) in the specific binding of [^{3}H]mepyramine (35.6 ± 2.7 instead of 43.7 ± 2.9 fmoles/mg of protein).

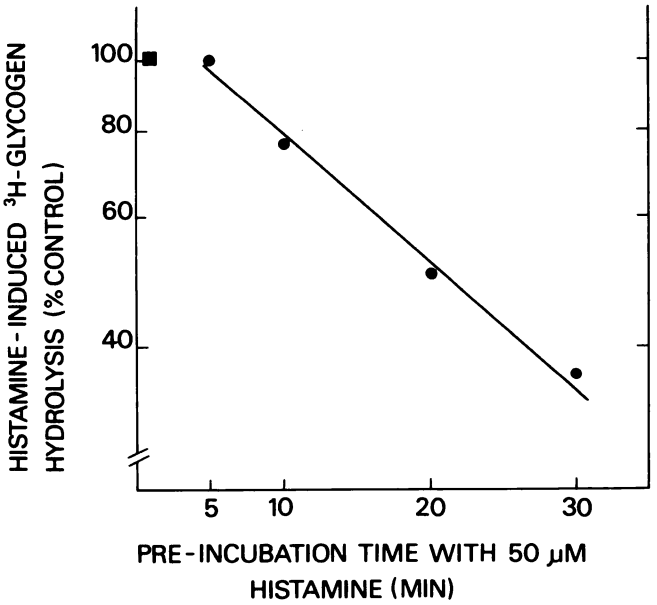

FIG. 3. *Time-course of histamine-induced desensitization*

Slices from mouse cortex were incubated with HA (50 μM) for the indicated times and washed three times; [^{3}H]glycogen hydrolysis elicited by HA (10 μM) was then determined. Results are expressed as percentages of [^{3}H]glycogen hydrolyzed by 10 μM HA. Basal [^{3}H]-glycogen levels (9.6 ± 0.4 × 10^3 dpm/mg of protein) were not signif cantly modified in slices incubated with HA. Each value represents th mean of 5–10 separate incubations.

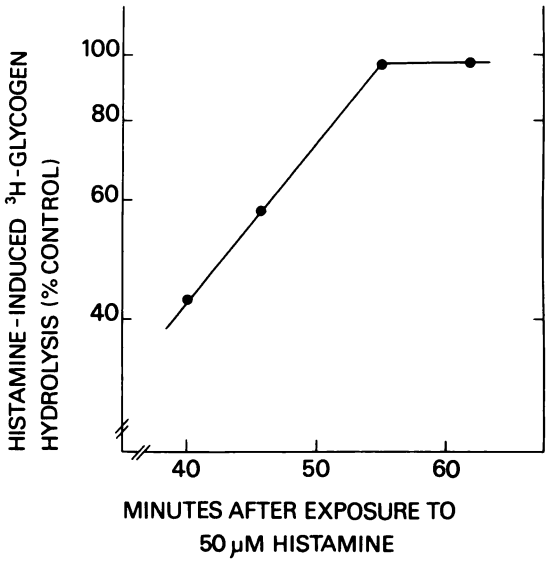

FIG. 4. *Time-course of the reversal of histamine-induced desensitization*

Slices from mouse cortex were incubated with HA (50 μM) for 20 min and washed three times; after the indicated times, [^{3}H]glycogen hydrolysis elicited by HA (10 μM) was determined. The shorter time interval between first exposure to HA and determination of HA-induced glycogenolysis was 40 min, which represents the time required for the [^{3}H]glycogen assay. Results are expressed as percentages of [^{3}H]glycogen hydrolyzed by HA (50 μM) in controls: 11.5 ± 0.7 × 10^3 dpm/mg of protein. Each value represents the mean of 5–10 separate incubations.

TABLE 2

Effect of cycloheximide, an inhibitor of protein synthesis, on the recovery from histamine-induced desensitization

Slices from mouse cortex were incubated for 20 min without or with 50 μM HA and washed three times. They were then resuspended in fresh Krebs-Ringer medium in the presence or absence of 20 μM cycloheximide and either tested immediately or after an additional "recovery" incubation of 20 min. [^{3}H]glycogen synthesis and its hydrolysis elicited by 10 μM HA were then evaluated. [^{3}H]Glycogen synthesis (i.e., the basal glycogen level) corresponded to 12.4 ± 1.2 × 10^3 dmp/mg of protein (no recovery incubation) and 8.0 ± 0.6 × 10^3 dpm/mg of protein (recovery incubation); no significant modification was observed in slices incubated in the presence of cycloheximide. Glycogenolysis is expressed as the percentage of corresponding basal levels. Data represent means ± standard error of the mean from 10 separate incubations for each condition.

Incubation condition	Recovery incubation	Glycogenolysis elicited by 10 μM histamine	
		−Cycloheximide	+Cycloheximide
		%	%
Krebs	No	42 ± 4	45 ± 3
Histamine	No	18 ± 4[a]	17 ± 4[a]
Krebs	Yes	36 ± 4	40 ± 5
Histamine	Yes	32 ± 2	36 ± 6

[a] $p < 0.05$ as compared with controls with no recovery incubations.

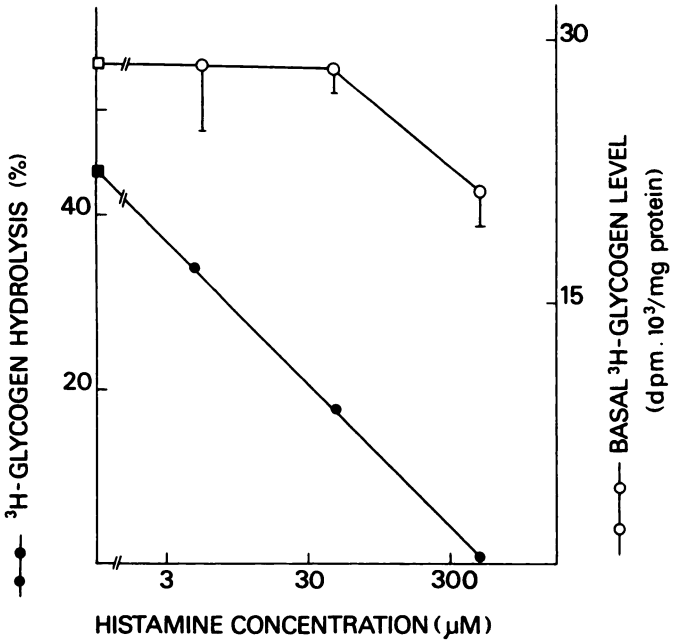

FIG. 5. *Concentration dependence of histamine-induced desensitization*

Slices from mouse cortex were incubated for 20 min with HA at the indicated concentrations and washed three times; [^{3}H]glycogen synthesis (basal levels) and its hydrolysis elicited by HA (10 μM) were then determined. [^{3}H]Glycogen hydrolysis is expressed as a percentage of basal [^{3}H]glycogen levels. Each value represents the mean of 10 separate incubations.

[^{3}H]mepyramine binding was already maximally decreased within 15 min of slice incubation, and control levels were restored when a 60-min time interval between incubation and homogenization of slices was observed (not shown).

The level of HA remaining in the radioreceptor assay following incubation of slices with 50 μM HA in spite of repeated washings was determined. For this purpose 44,000 dpm of [^{14}C]histamine were added at the beginning of the incubation, and after 20 min the slices were washed and homogenized; the radioactivity remaining in the homogenate usually submitted to the binding assay was found to be 880 ± 20 dpm. Taking into account the specific radioactivity of HA in the incubation medium, this corresponds to 1 μM HA in the binding assay medium (assuming that [^{14}C]HA is entirely in free form).

To determine whether the apparent loss of [^{3}H]mepyramine binding sites was due to HA remaining in the homogenate, HA (1 or 7 μM) was added to control slices just prior to homogenization, and a [^{3}H]mepyramine radioreceptor assay was performed. With 1 μM HA, [^{3}H]mepyramine saturation curves were not significantly changed (Fig. 6B). However, when the binding assay was performed in the presence of HA in higher concentration (7 μM), the K_d value was increased by about 2-fold, without modification in B_{max} (Fig. 6C).

The properties of [^{3}H]mepyramine binding sites following desensitization were determined by assessing the ability of HA to compete with the [^{3}H]antihistamine (Table 4). The apparent affinity of HA for H_1 receptors was not significantly modified, the K_i values being 25 ± 8 μM as compared with 40 ± 10 μM in controls (obtained by introducing K_d values of 5 and 6 nM, respectively, in the Cheng-Prussoff equation). The Hill coefficients were identical in both cases. The binding of [^{3}H]mepyramine maximally inhibited by HA (1 mM), corresponding to the specific binding as defined with 0.3 μM triprolidine, was decreased by 22% ($p < 0.01$), a value comparable to that reported in Fig. 6A.

TABLE 3

Specificity of histamine desensitization

Slices from mouse cortex were incubated without (controls) or with HA (50 μM) for 20 min and washed three times; they were then assayed for [^{3}H]glycogen synthesis and its hydrolysis by the different agents. Data represent means ± standard error of the mean of 5–10 separate incubations.

Glycogenolytic agents	[^{3}H]Glycogen content	
	Controls	HA-incubated
	10^3 dpm/mg protein	
None	32.2 ± 1.5	30.4 ± 1.7
Histamine (10 μM)	17.0 ± 0.3	23.4 ± 1.1[a]
5-Hydroxytryptamine (30 μM)	15.9 ± 1.0	16.2 ± 1.0
Noradrenaline (3 μM)	8.0 ± 0.6	9.3 ± 0.6
Adenosine (100 μM)	6.1 ± 0.2	6.5 ± 0.2
Dibutyryl cyclic AMP (10 μM)	19.2 ± 0.6	21.0 ± 0.8

[a] $p < 0.001$ relative to respective control.

DISCUSSION

Following exposure of slices from mouse cerebral cortex to HA, a clear decrease in responsiveness to the glycogenolytic action of the amine develops as shown by the significant rightward shift of the concentration-response curve (Fig. 1). This process is a progressive one depending both on the concentration of the amine during the incubation and on the duration of the exposure. Thus when slices were incubated in the presence of 50 μM HA, a concentration 10 times higher than the EC_{50} for glycogenolysis, desensitization developed with a half-time of about 20 min (Fig. 3), i.e., at a rate much slower than the glycogenolytic response to this amine (11). The process is an entirely reversible one since the responsiveness was totally restored 1 hr after first exposure of the slices (Fig. 4). These features are common to a variety of desensitization processes, particularly those involving H_1 receptor-mediated responses in guinea pig ileum (1) and in mouse neuroblastoma cells (3).

Furthermore, it is interesting to note that the time courses of both desensitization and recovery of the glycogenolytic response were similar to those observed in these two biological systems in which the intracellular events triggered by H_1 receptor stimulation also seem to involve a translocation of calcium ions. However, desensitization of the cyclic GMP response to HA in the neuroblastoma cells consisted of a decrease in maximal response, whereas desensitization of the glycogenolytic response in cerebral slices consisted of a rightward shift of the concentration-response curve. This does not imply that different mechanisms are implicated in the two desensitization processes, since the maximal response in

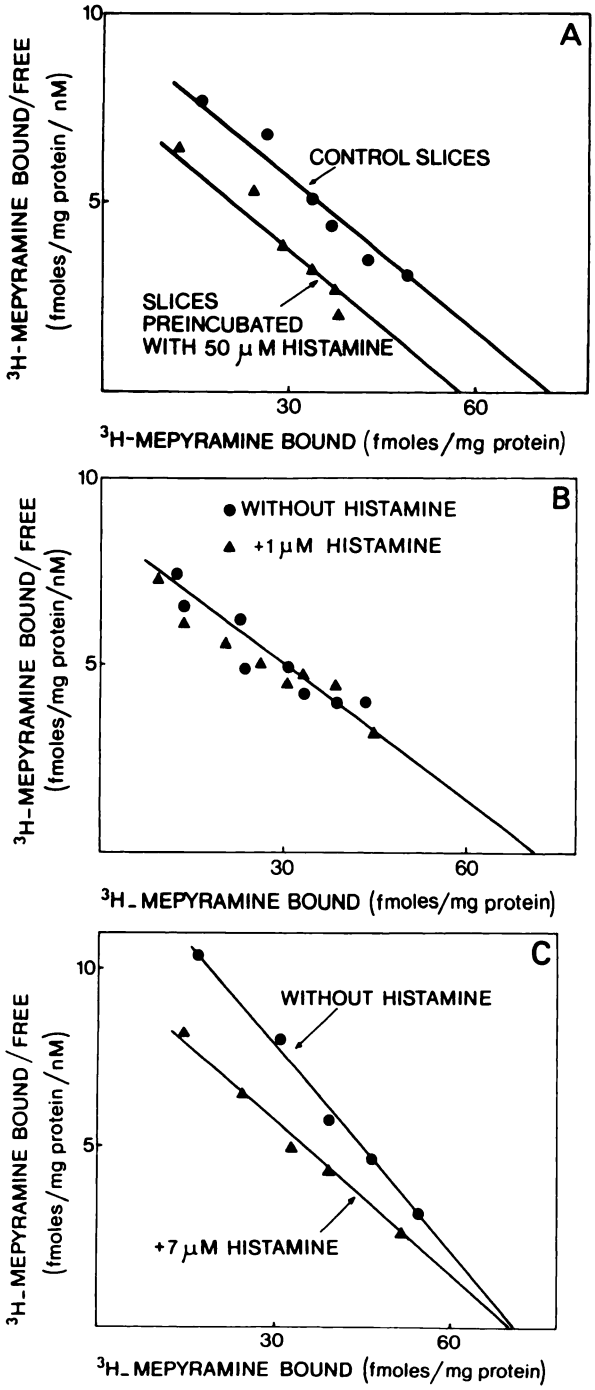

FIG. 6. *Scatchard analysis of the effects of histamine on specific binding of [^{3}H]mepyramine in brain homogenates*

A. Effects of incubation of slices in the presence of histamine on [^{3}H]mepyramine binding. Slices were incubated in the absence (control) or the presence of 50 μM HA for 20 min. The slices were then washed three times, homogenized in phosphate buffer, and [^{3}H]mepyramine binding was determined. Values shown are the means of nine determinations from three separate experiments. Parameters of [^{3}H]mepyramine binding were as follows: K_d = 6 nM, B_{max} = 72 fmoles/mg of protein (control slices), K_d = 5 nM, and B_{max} = 57 fmoles/mg of protein (desensitized slices).

B. Effects of 1 μM histamine added to brain homogenates on ^{3}H-mepyramine binding. Homogenates from mouse cortex were prepared and 1 μM HA was added in the incubation tubes just before [^{3}H]-mepyramine binding assays were performed. Data are presented from six incubations performed in two separate experiments. Parameters of [^{3}H]mepyramine binding were as follows: K_d = 6.7 nM, B_{max} = 70 fmoles/mg of protein (without HA), K_d = 7 nM, B_{max} = 72 fmoles/mg of protein (in the presence of 1 μM HA).

C. Effects of 7 μM histamine added to brain homogenates on [^{3}H]-mepyramine binding. Experiments were performed as described in B. Results shown are the means of six determinations from two representative experiments. Parameters of [^{3}H]mepyramine binding were as follows: K_d = 4.9 nM, B_{max} = 76 fmoles/mg of protein (without HA), K_d = 8.7 nM, and the B_{max} = 80 fmoles/mg of protein (in the presence of 7 μM HA).

TABLE 4

Inhibition of [^{3}H]mepyramine binding by histamine in homogenates prepared from desensitized slices

Slices were incubated for 20 min in the absence (control) or presence (desensitized) of 50 μM HA and were then washed three times with Krebs-Ringer medium and homogenized. Specific binding of [^{3}H]mepyramine was measured in the presence of 10 nM ligand and HA in increasing concentrations. Values are means ± standard error of the mean from three series of experiments. K_i values were calculated according to the Cheng-Prussof equation, IC_{50} values being obtained from the Hill equation plot using 8–10 concentrations of HA. Maximal inhibition = 45.07 ± 1.26 fmoles/mg of protein (controls) and 35.83 ± 1.53 fmoles/mg of protein (desensitized).

	K_i of histamine	Hill coefficient
	μM	
Control slices	40 ± 10	1.08 ± 0.09
Desensitized slices	25 ± 8	1.08 ± 0.11

the former system requires total H_1 receptor occupancy (3) whereas less than 10% occupancy seems to be required for maximal glycogenolysis (11).

Desensitization of the cortical slices by and to HA was a specific process in that it resulted from H_1 receptor stimulation, and only the glycogenolytic response mediated by this receptor was subsequently modified. Exposure of the slices to 2-thiazolylethylamine was also followed by desensitization to HA (Fig. 2). This compound is the most selective H_1 receptor agonist available, its potency being only about 0.3% that of HA on responses mediated by H_2 receptors but about 30% that of HA regarding responses mediated by H_1 receptors, including the glycogenolytic response in cortical slices (11, 18). The desensitization elicited by 100 μM 2-thiazolylethylamine was of slightly lower amplitude than that observed following exposure to 50 μM HA (compare Figs. 1 and 2), indicating that the relative potencies of this agent regarding desensitization and [^{3}H]glycogen hydrolysis are in the same range. In contrast with this H_1-receptor agonist, neither dimaprit, a highly selective H_2-receptor agonist (19) devoid of glycogenolytic activity (11), nor noradrenaline, a potent glycogenolytic agent acting via *beta*-adrenoreceptor stimulation (20), could induce desensitization to HA (Table 1). These results indicate that desensitization to the glycogenolytic action of HA is triggered by prior stimulation of H_1 receptors and not by a change occurring at the level of the chain of intracellular events leading to phosphorylase activation and glycogen hydrolysis.

This view is strengthened when considering the responses of the desensitized preparation to various glycogenolytic agents. The response to 5-hydroxytryptamine,

which, like that to HA, seems to involve calcium ions,[2] the response to noradrenaline, which involves cyclic AMP, and the response to dibutyryl cyclic AMP were not modified in slices pretreated with HA (Table 3). Taken together these observations suggest that the desensitization process is the result of agonist-induced changes occurring at the level of the H_1 receptor molecule itself. This hypothesis is supported by the observation that the binding of [^{3}H]mepyramine to the particulate fraction obtained from desensitized slices differs from that in a control preparation. Analysis of the saturation curve of this ligand indicated that desensitization was associated with a small but significant decrease in the number of binding sites (−18%) without a significant change in their apparent affinity for [^{3}H]antihistamine (Fig. 6A).

The apparent H_1 receptor loss observed following exposure of the slices to HA in relatively high concentration could have resulted from inhibition of [^{3}H]mepyramine binding by the amine remaining in the particulate fraction used in the radioreceptor assay in spite of the repeated washes of the slice preparations. In fact, the addition of [^{14}C]HA in tracer concentration indicated that the amine concentration remaining in the radioreceptor assay medium following exposure of the slices to 50 μM HA was about 1 μM. This cannot account for the decreased capacity in [^{3}H]mepyramine binding because (a) in the presence of 1 μM HA, [^{3}H]mepyramine binding was apparently not modified (Fig. 6B); (b) as could be expected, at a higher HA concentration (7 μM), only the apparent affinity of [^{3}H]mepyramine was decreased whereas the capacity was unchanged (Fig. 6C).

This apparent H_1 receptor loss raises two types of questions regarding its mechanism and its relationship with the desensitization of slices to the glycogenolytic action of HA. The decreased number of [^{3}H]mepyramine binding sites may not represent a real disappearance of a fraction of H_1 receptor molecules from the membrane but a transition of the latter to a state of low affinity for the [^{3}H]antagonist, since it is well established that the radioreceptor filtration assays can detect only binding sites with affinities in the nanomolar range. In agreement with this view, resensitization was observed to occur in slices in which protein synthesis was inhibited by cycloheximide (Table 2). One interesting speculation is that the desensitization process consists of a transition of the H_1 receptor to a "high-affinity" state in which it would very tightly bind HA or agonists but would not be functional in activating the glycogenolytic process and also would display decreased affinity for [^{3}H]mepyramine. Such a process has been proposed to be responsible for the desensitization of the responses of frog erythrocytes to *beta*-adrenergic agonists (21), and desensitization of the cholinergic receptor in membranes from *Torpedo marmorata* is also associated with a transition to a high-affinity state for agonists (22). High-affinity binding sites of [^{3}H]HA to membranes from rat brain, recently described in this laboratory (23, 24), could have been relevant, particularly in view of the weak inhibitory potency of mepyramine regarding these sites. However, since the regional distribution of [^{3}H]HA-binding sites markedly differs from that of [^{3}H]mepyramine-binding sites, it seems unlikely that the former represent H_1 receptors in a "desensitized" state.

The [^{3}H]mepyramine-binding sites remaining after exposure of the slices to HA were apparently not modified, since neither the apparent affinity of the ^{3}H-labeled ligand nor the inhibitory potency of HA (or its Hill coefficient) was significantly changed after desensitization (Table 4). The functional implication of the limited decrease in the number of [^{3}H]mepyramine-binding sites in the desensitization process remains doubtful. This decrease does not seem to account in itself for the important shift in concentration-response curve to HA: from data in Table 5 it can be calculated that the EC_{50} of HA in control slices (4 μM) corresponds to an occupation of 2 fmoles/mg of protein (4% of the total) in H_1 receptors, whereas it corresponds to an occupation of 14 fmoles/mg of protein (39% of the total) in the desensitized preparation in which half-maximal glycogenolysis is elicited by 32 μM HA. This difference suggests that the desensitization process might involve changes not only at the level of H_1 receptors themselves but also at the level of the events coupled to their activation by HA. It has recently been shown that desensitization of muscarinic receptors in neuroblastoma cells is accompanied by an inactivation of calcium channels to which they are coupled (25). A similar change might occur in desensitization to the glycogenolytic action of HA, which also seems to involve transmembrane fluxes of calcium ions (11). Further work is needed to assess whether this is the case and/or whether subtle conformational changes in the H_1 receptor molecule are involved.

[2] T. T. Quach, A.-M. Duchemin, C. Rose, and J.-C. Schwartz, in preparation.

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