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^{3}H-Glycogen Hydrolysis Elicited by Histamine in Mouse Brain Slices: Selective Involvement of H_1 Receptors

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SUMMARY

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^{3}H-Glycogen synthesized in mouse cortical slices from ^{3}H-glucose is hydrolyzed in a concentration-dependent manner by histamine with an EC_{50} of $3 \cdot 10^{-6}$ M. This effect is selectively mediated by H_1 receptors. Thus 10^{-5} M metiamide, a H_2-receptor antagonist, did not affect the histamine-induced glycogenolysis, whereas mepyramine, a H_1-receptor antagonist, at increasing concentrations progressively shifted the concentration–response curve to the right. Schild plot analysis gives a straight line with a slope close to unity and a pA_2 value of 8.0 for mepyramine. In the same way a variety of H_1-receptor antagonists, including (+)-chlorpheniramine but not the (−) isomer, at low concentrations progressively inhibited histamine action. On the other hand, dimaprit, a selective H_2-receptor agonist, did not elicit any glycogenolytic response even at $3 \cdot 10^{-4}$ M. Other histamine agonists (2-methylhistamine, 2-thiazolylethylamine, and 4-methylhistamine) produced a glycogenolytic effect with relative potencies consistent with stimulation of H_1 receptors. When the effects of histamine on the glycogenolytic response and the inhibition of ^{3}H-mepyramine binding are compared, it appears that histamine is substantially weaker in competing with ^{3}H-mepyramine for binding (K_i, $4.5 \cdot 10^{-5}$ M) than in inducing ^{3}H-glycogen hydrolysis (EC_{50}, $3 \cdot 10^{-6}$ M), suggesting that the maximal response is elicited when H_1 receptors are only partially occupied. The glycogenolytic response to histamine was markedly reduced when the extracellular calcium ion concentration was reduced to $0.3 \cdot 10^{-3}$ M. The possibility is raised that the glycogenolytic response to histamine involves calcium as a second messenger, as other responses mediated by H_1 receptors.

INTRODUCTION

Although there is little doubt that histamine (HA)[1] exerts a neurotransmitter function (1), there are still few *in vitro* models to study HA receptors in the mammalian brain.

The discovery that the amine strongly stimulates the accumulation of 3′,5′-cyclic AMP in brain slices (2) has provided a useful model, but with some limitations. First, for reasons still poorly understood, a clear stimulation of cyclic AMP synthesis can be demonstrated only on some brain regions of the guinea pig or rabbit but not of the rat or the mouse. Second, this model does not allow an easy study of the two classes of HA receptors. Whereas the stimulation of cyclic AMP accumulation in guinea pig brain slices appears to be mediated by both H_1 and H_2 receptors (3), a HA-sensitive adenylate cyclase has been identified in broken cell preparations which is selectively coupled to H_2 receptors (4, 5). H_1 receptors are indeed present in the mammalian brain, as indicated by binding studies utilizing the antagonist ^{3}H-mepyramine as a ligand (6, 7; Quach, T. T., *et al.*, in preparation), but their mode of participation in the effect of HA on cyclic AMP accumulation in brain slices is poorly understood and is not easily investigated because it requires a simultaneous activation of the adenylate cyclase, mediated by H_2 receptors (8). Finally, the participation of the cyclic AMP generating system in the alleged synaptic actions of HA remains doubtful because following interruption of histaminergic inputs to telencephalic areas, the unmodified responsiveness of this system contrasts with the clear denervation hypersensitivity to iontophoretically applied HA in the brain of the same animals (9).

On the other hand, HA stimulates cyclic GMP formation in a clone of neuroblastoma cells (10) and in the bovine sympathetic ganglion (11), an action selectively mediated by H_1 receptors in both preparations, but clear

[1] Abbrevations used: HA, histamine; cAMP, adenosine 3′,5′-monophosphate; cGMP, guanosine 3′,5′-monophosphate; IBMX, 3-isobutyl-1-methylxanthine; TEA, 2-thiazolylethylamine; 2-MHA, 2-methylhistamine; 4-MHA, 4-methylhistamine.

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evidence for a HA-induced stimulation of guanylate cyclase in mammalian brain is still lacking.

Thus an *in vitro* model allowing the assessment of HA responses mediated by H_1 receptors in brain tissues would be useful. We have recently devised a simple technique to evaluate the action of agents on the ^{3}H-glycogen content of mouse brain slices previously incubated in the presence of ^{3}H-glucose and shown that, like noradrenaline, HA is a potent glycogenolytic effector (12). In the present report we provide evidence that this effect is selectively mediated by H_1 receptors and strongly depends on the external concentration of calcium ions.

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°C and artificially illuminated (light between 0800 and 2000 h). Standard food (U.A.R., France) and water were available *ad libitum*.

Preparations and incubations 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°C) with a MacIlwain tissue slicer. Pooled cortical slices from six animals were preincubated for 15 min at 37°C 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 $NaHCO_3$).

At the end of this period, the slices were washed once with fresh medium, 300-μl aliquots of the tissue suspension corresponding to 0.5 mg of protein were distributed in incubation tubes, and 10 μl of ^{3}H-glucose solution (1 mCi/ml) representing 20 nmol was added. After a 30-min incubation, 10 μl of a solution of the test agents (or Krebs–Ringer medium) was then added and the slices were further incubated, usually for 20 min at 37°C. 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 kHz, 80 W) during 7 ± 1 s. A fraction of the resulting homogenate was then immediately deproteinized by heating at 95°C for 10 min, followed by a short centrifugation. The supernatant was sampled for ^{3}H-glycogen assays.

^{3}H-Glycogen assays. ^{3}H-Glycogen was isolated by ethanol precipitation using a filter-paper technique described previously (13). Briefly a 150-μl sample was spotted on a disk of filter paper which was successively dipped into different baths of ethanol–trichloroacetic acid and of 66% ethanol in order to wash out ^{3}H-glucose, whereas ^{3}H-glycogen selectively remained on the disk as identified with purified amylo-1,6-glucosidase (12).

^{3}H-Mepyramine binding. Mouse cortex was homogenized into 30 vol of ice-cold 50 mM Na-K phosphate buffer (pH 7.5) with a glass-Teflon Potter homogenizer. After centrifugation (250*g* for 5 min), the supernatant was diluted 10-fold and centrifuged (15,000*g* for 15 min). The resulting pellet was washed by resuspension into the same volume of fresh buffer and centrifuged again. The final pellet was resuspended into the original volume of cold buffer with a Dounce homogenizer.

^{3}H-Mepyramine and unlabeled substances were added to 0.45 ml of membrane suspension. Incubation (final volume 0.5 ml) was carried out at 30°C for 30 min and ended by the addition of 3 ml of ice-cold buffer, followed by a rapid filtration onto a glass-fiber filter (Whatman GF/B). The filter was rapidly washed with 3 × 20 ml of cold buffer. Radioactivity trapped on the filters was counted in 14 ml of scintillation mixture (PPO, 16 g; POPOP, 0.45 g; toluene, 2000 *ml*; Triton X-100, 1000 g) in the presence of 2 ml water, after 24 h storage at 4°C.

Specific binding was defined as the difference between radioactivity bound in the absence and in the presence of 2 μM triprolidine, a H_1 antihistamine.

Proteins were determined by the method of Lowry *et al.* with bovine serum albumin as the standard.

Radioisotopes. ^{3}H-Glucose (500 mCi/mmol) was purchased from the Radiochemical Centre (Amersham, England) and ^{3}H-mepyramine (28.5 Ci/mmol) from New England Nuclear (Boston, Mass.).

Chemicals and drugs. Dimaprit, 4-methylhistamine, 2-methylhistamine, 2-thiazolylethylamine, and metiamide were generously provided by Dr. M. E. Parsons (The Research Institute, Smith, Kline and French Laboratories, U.K.). The H_1-receptor antagonists mepyramine, triprolidine, and promethazine were generously provided by the manufacturers (Wellcome, Specia). (+)-Chlorpheniramine and (−)-chlorpheniramine were kindly given by Dr. Palacios (Baltimore, Md.). 3-Isobutyl-1-methylxanthine and noradrenaline were obtained from Sigma Chemicals.

Alprenolol was given by Lematte and Boinot Laboratories. $CaCl_2$, KCl, and histamine were obtained from Prolabo, Rhône-Poulenc; serotonin creatine sulfate was from Calbiochem, methysergide from Sandoz, and phentolamine from Ciba-Geigy.

Analysis of data. Concentration–response curves were fitted by hand or by employing the computer method of Parker and Waud (14).

Inhibition constants (K_i) of histamine antagonists were calculated, assuming competitive inhibition, according to the equations (15):

$$K_i = IC_{50}/(1 + S/K_a),$$

where IC_{50} is the concentration of antagonist required to produce a 50% inhibition of the glycogenolytic effect of HA, S represents the concentration of HA, and K_a is the concentration of HA required to produce half-maximal ^{3}H-glycogen hydrolysis; and

$$K_i = I/[(K'_a/K_a)-1],$$

where K_a and K'_a are the concentrations of histamine required to produce half-maximal ^{3}H-glycogen hydrolysis in the absence and presence of antagonist, respectively, and I is the concentration of antagonist.

RESULTS

Effects of HA on ^{3}H-glycogen level in slices. The ^{3}H-glycogen accumulated in the slices increased linearly with time up to 25 min, after which a plateau was maintained during at least 30 min (Fig. 1). The addition of 100 μM HA resulted in a rapid fall of the ^{3}H-glycogen

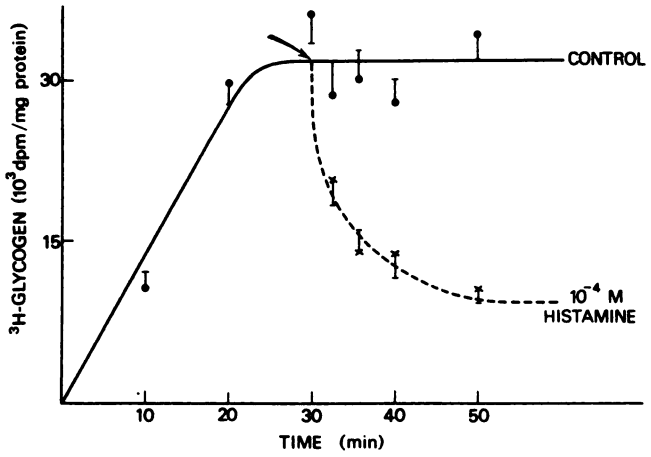

FIG. 1. *Time course of ^{3}H-glycogen synthesis and histamine-induced hydrolysis in slices from mouse cortex*

Slices were incubated in the presence of ^{3}H-glucose (33 μCi/ml). After 30 min (arrow) 10^{-4} M histamine was added to the incubation medium. Mean ± SEM from four or five separate incubations.

content which reached, within 15 min, a plateau, representing 25 ± 5% of the level in control slices.

The ^{3}H-glycogen hydrolysis elicited by HA is clearly concentration related (Fig. 2) and the concentration–response curve seems to follow Michaelis–Menten kinetics as shown by the Hill coefficient, not significantly different from unity (n_H = 0.86 ± 0.16; mean ± SEM of data from seven distinct sets of experiments). The Eadie–Hofstee plot of the data gives an EC_{50} of 3.4 ± 0.7 μM and a maximal glycogenolytic effect of 78 ± 3% of basal levels. In the presence of noradrenaline or serotonin the maximal glycogenolysis was also approximately 80%, and when these amines were added at supramaximal concentration together with HA, no further ^{3}H-glycogen hydrolysis was observed. In contrast, $5 \cdot 10^{-2}$ M potassium totally depleted the ^{3}H-glycogen content (Table 1). The possible involvement of cAMP in the glycogenolytic action of HA was investigated by studying the effects of 3-isobutyl-1-methylxanthine, a potent phosphodiesterase inhibitor. Although at 0.7 μM IBMX alone had no significant action on the ^{3}H-glycogen content, it slightly but significantly potentiated the response to 3 μM HA (Table 2). The effect of the inhibitor at higher concentrations was not tested because it elicits by itself a glycogenolytic response (12).

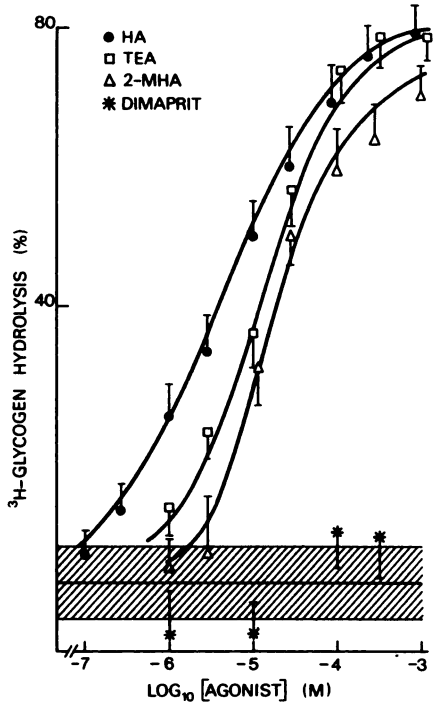

FIG. 2. *^{3}H-Glycogen hydrolysis induced by histamine and histamine agonists in slices from mouse cortex*

After a 30-min preincubation in the presence of ^{3}H-glucose, slices were incubated with increasing concentrations of histamine (HA), of 2-thiazolylethylamine (TEA) and 2-methylhistamine (2-MHA), two H_1-receptor agonists, or of dimaprit, a selective H_2-receptor agonist. Results are expressed as percentages of the ^{3}H-glycogen hydrolyzed. Basal ^{3}H-glycogen level, 36.2 ± 3.0 dpm·10^3/mg protein. Means ± SEM of 8–24 separate incubations.

TABLE 1

Effects of various amines at supramaximal concentrations and of K^+ on ^{3}H-glycogen content in slices from mouse cortex

Each value represents the mean ± SEM of data from 5 to 10 separate incubations.

Agents	^{3}H-Glycogen content	
	dpm·10^3/mg protein	%
None	48.1 ± 5.6	100
Histamine ($5 \cdot 10^{-4}$ M)	10.6 ± 0.5	22
Noradrenaline (10^{-5} M)	11.5 ± 1.0	24
Serotonin ($3 \cdot 10^{-4}$ M)	9.6 ± 0.5	20
Histamine ($5 \cdot 10^{-4}$ M) + noradrenaline (10^{-5} M)	11.1 ± 0.7	23
Histamine ($5 \cdot 10^{-4}$ M) + serotonin ($3 \cdot 10^{-4}$ M)	8.5 ± 0.5	18
K^+ ($5 \cdot 10^{-2}$ M)	1.3 ± 0.2	3

When slices were incubated in the presence of a lower calcium concentration ($0.4 \cdot 10^{-3}$ instead of $2.6 \cdot 10^{-3}$ M in the usual Krebs–Ringer medium), the glycogenolytic response to HA was strongly modified (Fig. 3), whereas the basal ^{3}H-glycogen level was not significantly changed. The maximal glycogenolysis was markedly reduced (49.2 ± 2.7 as compared to 76.5 ± 3.0% in controls; $P < 0.005$) without significant modification of the EC_{50} ($6.2 \pm 2.0 \cdot 10^{-6}$ instead of $3.8 \pm 1.8 \cdot 10^{-6}$ M in controls). In addition, it was observed that the response to a fixed concentration of HA ($6 \cdot 10^{-6}$ M) progressively diminished when the level of external calcium ions was decreased between $2.6 \cdot 10^{-3}$ and $0.3 \cdot 10^{-3}$ M. In a calcium-free medium the basal ^{3}H-glycogen level represented less than 20% that of controls and no glycogenolytic response to HA could be seen (not

TABLE 2

Effect of isobutylmethylxanthine (IBMX), a phosphodiesterase inhibitor, on ^{3}H-glycogen hydrolysis induced by histamine in mouse cortex slices

Means ± SEM of 5–10 separate incubations.

Agents	^{3}H-Glycogen content	
	dpm·10^3/mg protein	%
None	46.9 ± 2.9	100
IBMX ($7 \cdot 10^{-7}$ M)	46.0 ± 3.8	98
HA ($3 \cdot 10^{-6}$ M)	32.2 ± 1.7	69
HA ($3 \cdot 10^{-6}$ M) + IBMX ($7 \cdot 10^{-7}$ M)	25.5 ± 1.8*	54

* $P < 0.05$ as compared to incubations with histamine alone.

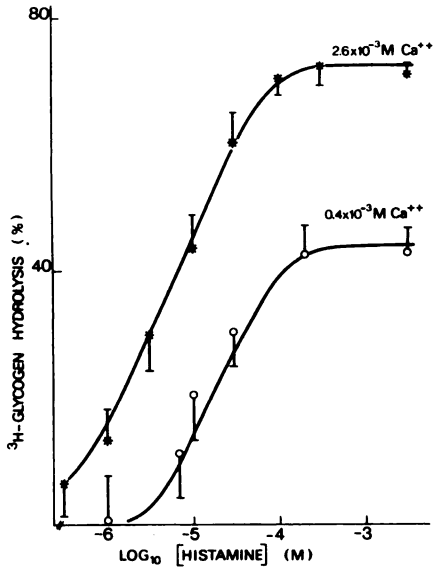

FIG. 3. *Histamine-induced glycogenolysis in normal Krebs–Ringer ($2.6 \cdot 10^{-3}$ M Ca^{2+}) and in the presence of $0.4 \cdot 10^{-3}$ M Ca^{2+}*

After a 30-min preincubation with ^{3}H-glucose in the presence of either the normal or the low-calcium medium, histamine was added and incubation continued for 20 min. Results are expressed as percentages of the basal ^{3}H-glycogen level: 38.5 ± 1.0 dpm·10^3/mg protein in normal medium and 43.2 ± 2.3 dpm·10^3/mg protein in low-calcium medium. Means ± SEM from four to eight separate incubations.

shown). The addition of 5 μM phentolamine, an α-adrenergic antagonist, 10 μM methysergide, a serotonin antagonist, or 10 μM alprenolol, a β-adrenergic antagonist neither modified the basal ^{3}H-glycogen level nor antagonized the glycogenolytic response to HA, as shown in experiments I, II, and III, respectively (Table 3).

Hydrolysis of ^{3}H-glycogen elicited by various histaminergic agonists. The predominantly H_1-receptor agonists (16, 17) 2-thiazolylethylamine and 2-methylhistamine induced a concentration-dependent hydrolysis of ^{3}H-glycogen with EC_{50} values of $9 \cdot 10^{-6}$ and $2.6 \cdot 10^{-5}$ M, respectively (Fig. 3).

Dimaprit, a selective histamine H_2-receptor agonist (18), did not elicit a glycogenolytic response even at concentrations as high as $3 \cdot 10^{-4}$ M. In the presence of high concentrations of 4-methylhistamine, a predominantly H_2-receptor agonist (16), a small glycogenolytic response occurred (30% maximal decrease at $5 \cdot 10^{-3}$ M), allowing an approximate estimation of the EC_{50} ($3 \cdot 10^{-4}$ M).

A comparison of the relative potencies of different H_1- and H_2-receptor agonists on the hydrolysis of ^{3}H-glycogen in slices from mouse cortex and on other biological systems is presented in Table 4.

Effects of histaminergic antagonists on HA-induced hydrolysis of ^{3}H-glycogen. Metiamide, a selective H_2-receptor antagonist (19), did not modify, at concentrations as high as 10^{-5} M, the glycogenolytic action of HA since the EC_{50} of the amine was $3 \cdot 10^{-6}$ M, as in its absence (Fig. 4). In contrast, the concentration–response curve to HA was progressively shifted to the right in the presence of increasing concentrations of mepyramine, a H_1-receptor antagonist (Fig. 5). Curves were analyzed by the computer method to determine the EC_{50}, and a Schild plot of the data gives a straight line with a slope close to unity (0.88 ± 0.28) and a pA_2 value of 8.01. Analysis of the same data by the Cheng Prussoff equation (see Materials and Methods) gives a K_i = 11 ± 2 nM, corresponding to a pA_2 value of 7.96 ± 0.12.

The glycogenolytic action of a fixed concentration of HA ($5 \cdot 10^{-5}$ M) is also progressively antagonized in the presence of various other H_1-receptor antagonists at increasing concentrations (Fig. 6). In the case of chlorpheniramine the inhibitory potency of the dextro isomer contrasted with the lack of effect of the levo isomer (Fig. 7). It was checked that the basal ^{3}H-glycogen level was not

TABLE 3

Effects of serotonin and noradrenaline antagonists on ^{3}H-glycogen hydrolysis induced by histamine in mouse cortex slices

Means ± SEM of 5–10 separate incubations.

Agents	^{3}H-Glycogen content	
	dpm·10^{-3}/mg protein	
Experiment I		
None	47.1 ± 4.3	100
Phentolamine ($5 \cdot 10^{-6}$ M)	47.4 ± 5.7	101
HA ($5 \cdot 10^{-5}$ M)	24.9 ± 1.8	53
Phentolamine ($5 \cdot 10^{-6}$ M) + HA ($5 \cdot 10^{-5}$ M)	24.1 ± 3.5[a]	51
Experiment II		
None	43.4 ± 1.5	100
Methysergide (10^{-5} M)	38.6 ± 0.7	90
HA ($5 \cdot 10^{-5}$ M)	14.3 ± 0.8	33
Methysergide (10^{-5} M) + HA ($5 \cdot 10^{-5}$ M)	19.2 ± 2.4[a]	44
Experiment III		
None	65.2 ± 3.9	100
Alprenolol (10^{-5} M)	66.1 ± 2.0	101
HA (10^{-5} M)	31.9 ± 1.8	49
Alprenolol (10^{-5} M) + HA (10^{-5} M)	37.0 ± 2.5[a]	56

[a] No significant modifications have been observed as compared to incubations with HA alone.

TABLE 4

Comparison of the "relative potencies" of histamine agonists on the hydrolysis of ^{3}H-glycogen in cortical slices and other systems

Relative potencies were calculated according to the equation, R. P. = (EC_{50} histamine/EC_{50} agonist) × 100. The EC_{50} values for agonists were calculated by Eadie–Hofstee plot. Other data from (a) Green *et al.* (5), (b) Black *et al.* (16), (c) Parsons *et al.* (18), (d) Durant *et al.* (17), and (e) Palacios *et al.* (8).

Agonist	Cyclic AMP accumulation in hippocampal slices, guinea pig	Adenylate cyclase in hippocampal homogenates, guinea pig	Gastric secretion, rat (H_2)	Ileum contraction, guinea pig (H_1)	Hydrolysis ^{3}H-glycogen in cortical slices, mouse
Histamine	100[e]	100[a]	100[b]	100[b]	100
Dimaprit	67[e]	219[a]	19.5[c]	0.0001[c]	0.0001
4-Methylhistamine	67[e]	58[a]	39[b]	0.3[b]	1
2-Methylhistamine	12[e]	12[a]	2[b]	16.5[b]	12
2-Thiazolylethylamine	7[e]	—	0.3[d]	26[d]	30

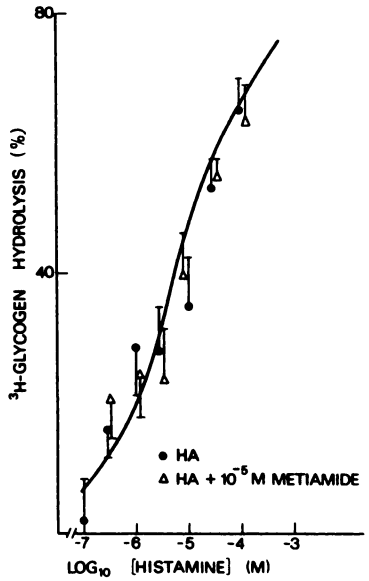

FIG. 4. *Histamine-induced hydrolysis of ^{3}H-glycogen in slices from mouse cortex and the effects of metiamide, a H_2-receptor antagonist*

Following a 30-min preincubation in the presence of ^{3}H-glucose, histamine was added at increasing concentrations and the incubation continued for 20 min. Metiamide added 2 min before histamine. Basal ^{3}H-glycogen level, 45.1 ± 3.2 dpm·10^3/mg protein. Means ± SEM of 5–10 separate incubations.

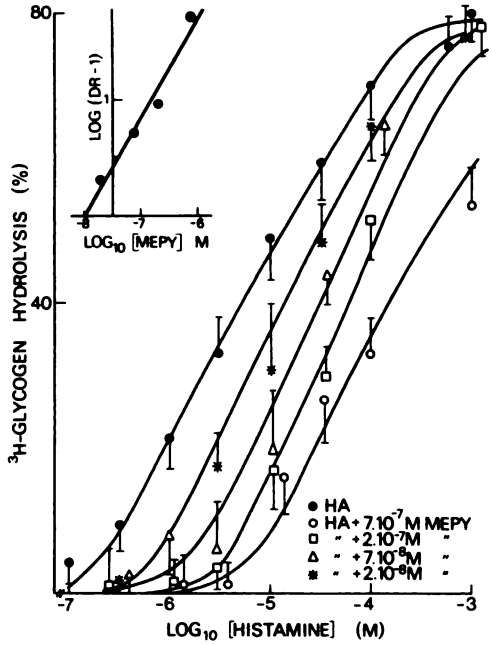

FIG. 5. *Inhibition by mepyramine, a H_1-receptor antagonist, of the histamine-induced glycogenolysis in slices from mouse cortex*

Slices were preincubated for 30 min in the presence of ^{3}H-glucose and incubated for 20 min following the addition of HA alone or with mepyramine at various concentrations. Results are expressed as percentages of the basal ^{3}H-glycogen level (37.9 ± 3.1 dpm·10^3/mg protein). Means ± SEM from 8–24 separate incubations. The concentration–response curve for HA alone is constructed by averaging data obtained in five distinct series of experiments. The inset represents the Schild plot of the same data in which the dose ratios (DR) are evaluated by comparison with the EC_{50} of HA alone within the same series of experiments. The pA_2 value, determined by linear regression, is 8.01.

altered by any of these agents (10^{-5} M). For each drug the concentration required for half-maximal inhibition of ^{3}H-glycogen hydrolysis was determined and the inhibition constants (K_i) were calculated, assuming competitive inhibition (Table 5).

^{3}H-Mepyramine binding. ^{3}H-Mepyramine binds rapidly and in a saturable fashion to membranes from mouse cortex. Equilibrium is reached in less than 15 min at 0.8 nM ^{3}H-mepyramine. Analysis of the saturation curve by an iterative program based on least squares (Malfroy, B., in preparation) leads to the following parameters: K_d = 3.0 ± 1.2 nM; capacity = 90 fmol/mg protein. The Hill coefficient, not significantly different from unity, suggests the absence of cooperative interaction (Fig. 8). The inhibition of the binding of 0.8 nM ^{3}H-mepyramine by HA at increasing concentrations is shown in Fig. 9. The K_i of HA in inhibiting ^{3}H-mepyramine binding is $4.5 \cdot 10^{-5}$ M. From the comparison of the two concentration–response curves in Fig. 9, it appears that HA is substantially weaker in competing for ^{3}H-mepyramine binding than in inducing ^{3}H-glycogen hydrolysis (EC_{50}, $3 \cdot 10^{-6}$ M).

DISCUSSION

The present study confirms that HA exerts a powerful glycogenolytic effect on brain slices. Among cerebral amines, the glycogenolytic action of noradrenaline mediated by β-adrenergic receptors is well established (12, 20–22) and that of serotonin has been recently described (12, 22). The lack of additive effects of the various amines (Table 1) suggests that the ^{3}H-glycogen hydrolysis elicited by HA might occur in the same pool(s) as that elicited by noradrenaline and serotonin. However, it is clear that the action of HA is mediated by receptors which are distinct from those mediating the ^{3}H-glycogen hydrolysis elicited by noradrenaline or serotonin. This is indicated by the lack of inhibitory potency of α- and β-adrenergic receptor blockers, as well as of methysergide, an antiserotoninergic agent, toward the response to HA (Table 3). Furthermore, the action of HA appears to be selectively mediated by typical H_1 receptors as demonstrated by the action of agonists and antagonists.

Regarding agonists, the sole highly selective compound which can be used to differentiate the two classes of HA receptors is dimaprit, a H_2-receptor agonist, almost inactive at H_1 receptors (18). No glycogenolytic effect could be observed even in the presence of $2 \cdot 10^{-4}$ M dimaprit (Fig. 3). In contrast, a clear glycogenolytic response, of the same amplitude as that elicited by HA (Fig. 3), is observed with 2-thiazolylethylamine and 2-methylhistamine, two predominantly H_1-receptor agonists. Furthermore, the relative potencies of these agents agree well with the corresponding values of responses mediated by typical H_1 receptors (Table 4).

That H_1 receptors are selectively involved in the glycogenolytic response to HA is further shown by the action of selective antagonists. Metiamide, a H_2-receptor antagonist, did not, at 10^{-5} M, modify the responses to HA at various concentrations (Fig. 4). In contrast, mepyramine, a H_1-receptor antagonist, at low concentrations inhibited the HA-induced glycogenolysis. As shown by the parallel shift of the concentration–response curve without modification of the maximal response, this antagonism is of the competitive type (Fig. 5). Thus, Schild

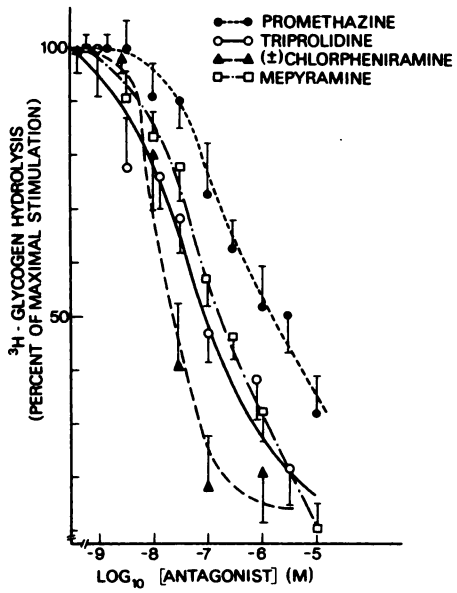

FIG. 6. *Effects of various H_1-receptor antagonists on the histamine-induced glycogenolysis in slices from mouse cortex*

After a 30-min preincubation in the presence of ^{3}H-glucose, slices were incubated for 20 min following the addition of HA ($5 \cdot 10^{-5}$ M) alone or in the presence of increasing concentration of various H_1-receptor antagonists. The stimulation elicited by $5 \cdot 10^{-5}$ M HA in the absence of antagonists is defined as 100%, and responses in the presence of each antagonist are expressed relative to this value. ^{3}H-Glycogen levels were 45.2 ± 3.3 and 14.3 ± 1.1 dpm·10^3/mg protein in the absence and presence of HA, respectively. Means ± SEM from four to eight separate incubations.

plot analysis of these data gives a straight line with a slope not different from unity and leads to a pA_2 value of 8.01, corresponding to an apparent K_i of 9.7 nM. This is in reasonable agreement with the affinity constants of mepyramine in typical H_1-receptor systems from peripheral tissues: Thus in guinea pig ileum a pA_2 value of 9.4

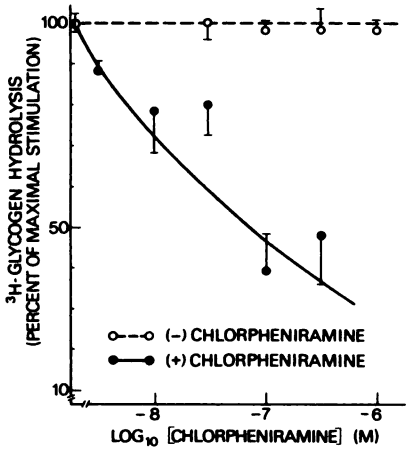

FIG. 7. *Inhibition of histamine-induced glycogenolysis by (+)- and (−)-chlorpheniramine*

Slices were incubated with HA ($5 \cdot 10^{-5}$ M) alone or in the presence of increasing concentrations of (+)- and (−)-chlorpheniramine. The response elicited by $5 \cdot 10^{-5}$ M HA in the absence of antagonist is defined as 100%, and responses in the presence of each stereoisomer are expressed relative to this value. ^{3}H-Glycogen levels were 55.0 ± 5.2 and 15.9 ± 0.7 dpm·10^3/mg protein in the absence and presence of HA, respectively. Means ± SEM of four to eight separate incubations.

TABLE 5

Comparison of K_i values of various H_1-receptor antagonists on the ^{3}H-glycogen hydrolysis induced by histamine in cortical slices and on other systems

Antagonist	K_i (M)		
	Hippocampal slices of guinea pig[a]	^{3}H-Mepyramine binding of brain membranes[b]	^{3}H-Glycogen hydrolysis in slices from mouse brain[c]
Mepyramine	$2 \cdot 10^{-9}$	$4.5 \cdot 10^{-9}$	$4.7 \cdot 10^{-9}$
(+)-Chlorpheniramine	—	$8 \cdot 10^{-9}$	$2 \cdot 10^{-9}$
Promethazine	$2.5 \cdot 10^{-8}$	$3 \cdot 10^{-9}$	$5 \cdot 10^{-9}$
Triprolidine	10^{-9}	$5.6 \cdot 10^{-9}$	$5.6 \cdot 10^{-9}$

[a] Data from Palacios *et al.* (8).
[b] Data from Chang *et al.* (24).
[c] K_i values were calculated from data of Fig. 6 using the equation, $K_i = IC_{50}/(1+S/K_a)$ (see Analysis of Data).

is generally reported, but when determined after only a short preincubation in the presence of the drug, the pA_2 value is 8.4 (23). In the same way the hydrolysis of ^{3}H-glycogen elicited by 50 μM HA is progressively inhibited by a variety of H_1-receptor antagonists including mepyramine; K_i values calculated by assuming a competitive antagonism (Table 5) are close to those reported for these compounds, regarding biological actions mediated by typical H_1 receptors (23). Finally, the involvement of the latter is also strongly substantiated and a stereospecific recognition of the HA molecule is suggested by the large difference in potency of the two chlorpheniramine stereoisomers (Fig. 7). Whereas (+)-chlorpheniramine has a potency similar to that of mepyramine on the HA-induced contractions of the guinea pig ileum, a classical test for H_1 receptor-mediated actions of HA, the (−) isomer is approximately 500-fold less potent (23).

In order to analyze the relationship between receptor occupancy and the glycogenolytic effect, we have used ^{3}H-mepyramine as a selective ligand of H_1 receptors (6,

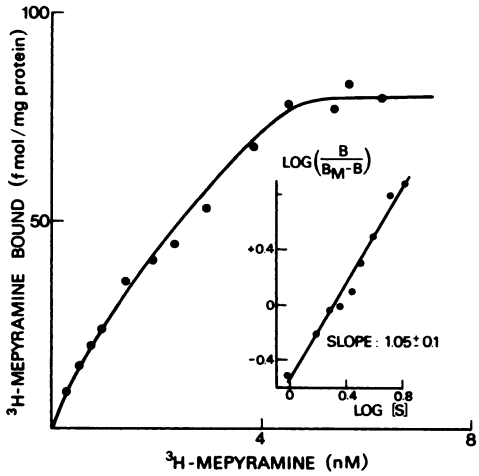

FIG. 8. *^{3}H-Mepyramine binding to mouse cortex membranes as a function of concentration of the ligand*

Plot of specific binding, i.e., the difference between the total binding and that determined in the presence of $2 \cdot 10^{-6}$ M triprolidine, a H_1-receptor antagonist. The inset represents the Hill plot of the same data. Means ± SEM from six determinations.

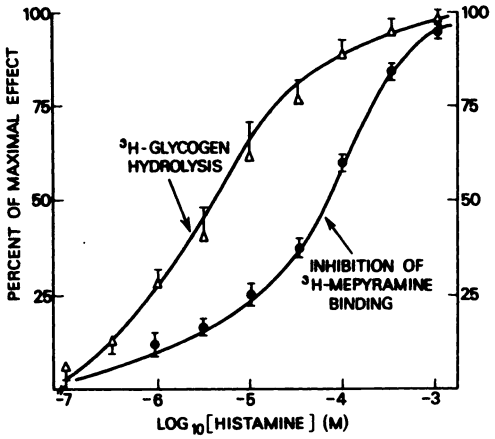

FIG. 9. *Comparison of concentration–response curves to HA regarding ^{3}H-glycogen hydrolysis and inhibition of ^{3}H-mepyramine binding in mouse cortex*

^{3}H-Mepyramine binding measured in the presence of 0.8 nM ligand. Basal level of ^{3}H-glycogen was 37.9 ± 3.1 dpm·10^3/mg protein. Means ± SEM from 8–24 separate incubations performed in three or four series of experiments. The EC$_{50}$ of HA regarding ^{3}H-glycogen hydrolysis was $3 \cdot 10^{-6}$ M, and its K_i value regarding ^{3}H-mepyramine binding was $4.5 \cdot 10^{-5}$ M.

7). As described in brain preparations from other animal species, the binding of ^{3}H-mepyramine to mouse cortical membranes is saturable (capacity is about 90 fmol/mg protein) and apparently occurs on a single population of sites without evidence for cooperative interactions (n_H = 1.05 ± 0.10). Interestingly, the K_d of ^{3}H-mepyramine (3.0 ± 1.2 nM) regarding the binding process is close to its apparent K_i values (9.7 nM from data of Fig. 5 and 4.7 nM from data of Fig. 6) evaluated from the competitive antagonism of HA-induced glycogenolysis. In the same way, the apparent K_i values of various H_1-receptor antagonists, estimated from their inhibitory potency on HA-induced glycogenolysis (Table 5), agree well with their inhibitory potency toward ^{3}H-mepyramine binding as reported by others (6, 24). These findings further support the idea that the same receptors are involved in the recognition of ^{3}H-mepyramine and in the initiation of the glycogenolytic response to HA. However, when the effects of HA at increasing concentrations on the glycogenolytic response and the inhibition of ^{3}H-mepyramine binding, respectively, are compared, a clear difference appears (Fig. 9). Significantly lower amine concentrations are required for the former effect (EC$_{50}$, $3 \cdot 10^{-6}$ M) than for the latter (K_i, $46 \cdot 10^{-6}$ M). It is unlikely that this variation results from the slightly different incubation conditions (temperature, duration) under which, in both cases, a steady state was reached. This apparent discrepancy can be explained by the assumption that the maximal glycogenolytic response is elicited when only a fraction of the H_1 receptors is occupied by HA. This explanation, i.e., the existence of a "receptor reserve," would be consistent with the high sensitivity of the glycogenolytic system in brain slices to the action of various effectors: For instance, the maximal glycogenolytic effect of noradrenaline in brain slices occurs at much lower amine concentrations than those required for stimulation of cyclic AMP accumulation in the same preparation (12). Nevertheless, it cannot be excluded that the difference in potency of HA toward glycogenolysis and ^{3}H-mepyramine binding arises from the differences in tissue preparations used in the two assays, i.e., intact cells and membranes, respectively.

What is the mechanism of the glycogenolytic action of HA? The small but significant potentiation observed in the presence of a phosphodiesterase inhibitor suggests a priori the involvement of cyclic nucleotides. Although HA stimulates a guanylate cyclase (10) in astrocytoma cells also through activation of H_1 receptors, it is unlikely that cyclic GMP is involved in the HA-induced hydrolysis of ^{3}H-glycogen because both the dibutyryl derivative of this nucleotide (in contrast with dibutyryl cyclic AMP) and cholinergic agonists are devoid of glycogenolytic potency (12). This would suggest that the HA effect might be mediated by cyclic AMP. Indeed the relationship between the level of cyclic AMP and either the percentage of glycogen phosphorylase in the active form or the glycogen content observed in liver and brain (25) has led to the cascade theory for cyclic AMP-dependent phosphorylase activation (26). In guinea pig brain a H_1 receptor-mediated increase in the accumulation of cyclic AMP into slices has been demonstrated (3, 8). However, it is unlikely that this effect is involved in the HA-induced glycogenolysis because it requires a simultaneous stimulation of adenylate cyclase mediated by H_2 receptors, which is not the case for the hydrolysis of ^{3}H-glycogen, as shown, for instance, by the lack of effect of metiamide (Fig. 4). It can also be remarked that the potentiation by IBMX is smaller for HA (Table 1) than for noradrenaline (12) and that the specificity of this agent as a phosphodiesterase inhibitor has been discussed (27).

On the other hand, it appears that the glycogen content can also be controlled by a mechanism which does not involve cyclic AMP: Much evidence indicates that Ca^{2+} ions play an important role in the α-adrenergic activation of glycogenolysis in rat liver and that stimulation of α receptors increases the transmembrane flux of Ca^{2+} ions (28). Furthermore, the activation of phosphorylase *b* kinase by Ca^{2+} ions has been demonstrated to occur in brain tissues (29).

The observation that a decrease in the extracellular level of Ca^{2+} ions results in a reduction by more than 40% of the maximal glycogenolytic response to HA (Fig. 3) would suggest that this response involves an increased calcium flux. Interestingly, a variety of other intracellular responses to HA mediated by H_1 receptors also appears to involve translocation of Ca^{2+} ions: This is, indeed, the case for smooth muscle contraction, as also for stimulation of cyclic GMP accumulation in neuroblastoma cells (10) or bovine cervical ganglia (11) and for stimulation of cyclic AMP accumulation in slices from guinea pig hippocampus (Barbin, G., *et al.*, in preparation). Taken together, these observations suggest that in a variety of biological systems H_1 receptors might be coupled with a calcium channel.

Whereas additional work is required to analyze the mechanism of HA-induced glycogenolysis, the present finding that this action is selectively mediated by H_1 receptors might have importance when considering the marked sedative properties of most antihistamines, pos-

sibly resulting from modifications of carbohydrate metabolism in cerebral tissues.

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