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Histamine-Stimulated Phosphoinositide Hydrolysis in Developing Rat Brain

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SUMMARY

Histamine stimulation of phosphoinositide breakdown has been studied in brain cortex from developing rats by measuring the accumulation of [^{3}H]inositol monophosphate in lithium-treated slices prelabeled with [^{3}H]inositol. Histamine stimulated phosphoinositide hydrolysis as early as day 3 after birth. The maximal response increased about 3-fold up to day 15 and remained essentially at the same level until adulthood. This developmental pattern contrasted with the 8-fold increase in [^{3}H]mepyramine binding to histamine H_1 receptors which takes place from the first to the end of the fourth week of life. Nonlinear regression analysis of concentration-effect curves for histamine generated at 6, 10, and 15 days of age revealed the presence of two components in the response, whereas a single component was found in adults. A high potency component showed EC_{50} values increasing from 6.4 μM at day 6 to 26.5 μM at day 15, and its contribution to the maximal response augmented from 65.8% to 78.9% during the same period. In 6-day-old animals, the H_1 receptor agonists N^α-methylhistamine and N^α,N^α-dimethylhistamine produced single-component responses with maximal effects similar to that estimated for the high potency component for histamine, and their relative potencies were consistent with an H_1 receptor-mediated response. Inhibition constants for mepyramine, calculated from the parallel displacement of the concentration-response curves for N^α-methylhistamine and the high potency component for histamine (6.3 nM and 10.8 nM, respectively) closely agreed with the reported affinity of rat brain H_1 receptors for this compound. The low potency component of the response (EC_{50} = 175 μM at day 6 and EC_{50} = 260 μM at day 15) did not involve histamine-H_2, serotonin 5-HT_2, α_1-adrenergic, or muscarinic receptors. Pretreatment of 6-day-old cortical slices with the irreversible antagonist phenoxybenzamine, at concentrations that completely abolish [^{3}H]mepyramine binding, left about 25% of the response to 1 mM histamine unaffected. This portion of the response, which was, moreover, insensitive to 1 μM mepyramine, was of a magnitude similar to that of the low potency component. Thus, at early stages of rat brain development, histamine induces phosphoinositide hydrolysis in part through a mechanism different from activation of H_1 receptors. The loss of histamine potency and efficacy at H_1 receptors throughout development is discussed and the natural occurrence of H_1 receptor subsensitivity suggested.

HA is a neurotransmitter in the central nervous system that exerts its actions through interaction with at least two types of receptors, named H_1 and H_2 (1, 2). Whereas H_2 receptors are positively coupled to adenylate cyclase (3), stimulation of H_1 receptors results in enhanced phosphoinositide hydrolysis (4, 5) a response that has been proposed as the membrane-transducing mechanism for these and other Ca^{2+}-mobilizing receptors (6).

The study of the ontogeny of the histaminergic system in rat brain has been hindered by the double compartmentation of HA in neuronal and non-neuronal (i.e., mast cell) stores (7, 8). Thus, the highest levels of HA are found in the newborn rat brain, where the major part of the amine seems to be non-neuronal, according to the relatively high number of mast cells present (9). On the other hand, the activity of the HA-synthesizing enzyme, L-histidine decarboxylase (L-histidine carboxylase, EC 4.1.1.22), considered a more specific marker for histaminergic neurons, increases gradually with age and reaches adult values during the fourth week of life, following a pattern that probably reflects the establishment of histaminergic synapses (10).

The ontogenic development of HA H_1 receptors labeled with [^{3}H]mepyramine in various regions of rat brain has been reported to parallel that of L-histidine decarboxylase (11, 12). These receptors were found to be functional by measuring the stimulation, by intracisternally administered HA, of the rate of incorporation of [^{33}P]orthophosphate into brain phospholipids (13). However, this is an indirect method of measuring an H_1

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ABBREVIATIONS: HA, histamine; IP, inositol monophosphate; N^α-mHA, N^α-methylhistamine; N^α,N^α-dimHA, N^α,N^α-dimethylhistamine; 2-mHA, 2-methylhistamine; PEA, 2-pyridylethylamine; TEA, 2-thiazolylethylamine; KH buffer, Krebs-Henseleit buffer.

receptor-mediated effect, and no detailed characterization of the response was reported.

In the present work, we have used a more direct method to examine the development of HA H_1 receptor functionally in rat brain during ontogeny. We have characterized the stimulation by HA of phosphoinositide hydrolysis in developing rat brain cortical slices by measuring the formation of [^{3}H]IP in [^{3}H]inositol-prelabeled tissue samples (14). Using this method we have found an earlier development of the response compared to that of [^{3}H]mepyramine binding. We have also observed the presence of a non-H_1 receptor-mediated component of the response at early stages of development and a significant loss of agonist potency with age.

Materials and Methods

Phosphoinositide hydrolysis. The method used to measure agonist-stimulated [^{3}H]IP accumulation was essentially as described previously (5). Briefly, cerebral cortices from Sprague-Dawley rats of different postnatal ages were dissected on an ice-cooled plate and cross-chopped in 350 × 350 μm slices. The tissue was preincubated in KH buffer [in mM: 116 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 2.5 $CaCl_2$, 25 $NaHCO_3$, 11 glucose, pH 7.4, equilibrated with O_2/CO_2 (95:5)] at 37° for 30 min, washed twice, and incubated in KH buffer containing 0.2 μM [^{3}H]inositol (16.5 Ci/mmol) at 37° for 120 min. Labeled slices were extensively washed and allowed to settle for 5 min, and 50-μl aliquots were incubated with agonists in a final volume of 250 μl of KH buffer containing 10 mM LiCl. When present, antagonists were added to the slices 15 min before the agonists. Samples were routinely incubated at 37° for 60 min except that, when characterizing the response in 6-day-old animals, the incubation was extended to 90 min, at which time [^{3}H]IP accumulation was still linear. Reactions were terminated by adding 0.94 ml of chloroform/methanol (1:2, v/v). After 15 min on ice, 0.31 ml of water and 0.31 ml of chloroform were added and the phases separated by centrifugation. Aqueous phases were chromatographed through Dowex 1X8 anion exchange columns and [^{3}H]IP was eluted as described (5). Lipid labeling was monitored by counting aliquots of the organic phases. [^{3}H]IP accumulation is expressed as percentage of total label incorporated into lipids in order to correct for variations in [^{3}H]inositol incorporation and sample size. When the effect of pretreatment with phenoxybenzamine was studied, [^{3}H]inositol-labeled slices were incubated in KH buffer with different phenoxybenzamine concentrations for 45 min, extensively washed, and processed as above.

[^{3}H]Mepyramine binding. Binding of [^{3}H]mepyramine (2 nM) to particulate fractions (50,000 × *g*) from rat brain cortex (0.7 mg of protein) was assayed in 0.5 ml of 50 mM Na_2/potassium phosphate buffer, pH 7.5, at 25° for 45 min, by the filtration method previously described (12). Nonspecific binding was determined in the presence of 0.6 μM promethazine. When the effect of pretreatment with phenoxybenzamine on [^{3}H]mepyramine binding was studied, rat brain cortices were cross-chopped and slices incubated in KH buffer with different concentrations of phenoxybenzamine for 45 min. After extensive washings, slices were homogenized and binding assays performed in the particulate fraction.

Analysis of data. Concentration-effect curves were routinely transformed to linear Hill equations in order to estimate EC_{50} values and Hill coefficients. When Hill coefficients were found to be lower than unity, the curves were fitted either to a single or a double hyperbola, using the BMDP 3R nonlinear regression iterative program (15) implemented on a VAX 11/785 system. Each point was weighted by the reciprocal of the variance associated with it. The best fit was taken to be the one associated with the lowest residual sum of squares and its goodness was assessed by comparing the residual mean square of the two alternative models using the *F* test. The equations fitted were:

$$E = \frac{100 \cdot A}{EC_{50} + A}$$

for a single component, and

$$E = \frac{N_1 \cdot A}{K_1 + A} + \frac{N_2 \cdot A}{K_2 + A}$$

for two components, where E is the percentage of maximal effect, A is the agonist concentration, K_1 and K_2 are the EC_{50} values for the two components, and N_1 and N_2 are the maximal stimulations reached by each component, assuming $N_1 + N_2 = 100$.

Inhibition constants for mepyramine were obtained from the dextral shift of the concentration-effect curves for agonists using the relationship

$$(EC_{50}'/EC_{50}) - 1 = [\text{mepyramine}]/K_i$$

where EC_{50} and EC_{50}' are the agonist concentrations giving half-maximal effect in the absence and presence of antagonist, respectively, and K_i the inhibition constant for the antagonist.

Chemicals. *myo*-[2-^{3}H]Inositol (16.5 Ci/mmol) and [^{3}H]mepyramine (24.1 Ci/mmol) were from New England Nuclear, Dowex 1X8 (100–200 mesh, formate form) was from Bio-Rad, and histamine, promethazine, and mepyramine were from Sigma. Dimaprit, cimetidine, metiamide, phenoxybenzamine, N^{α}-mHA, N^{α},N^{α}-dimHA, 2-mHA, PEA, and TEA were generous gifts from Smith, Kline and French. All batches of [^{3}H]inositol were purified free of anionic species just after receipt by passing the solution through a Dowex 1X8 column.

Results

Ontogenic development of HA-stimulated phosphoinositide hydrolysis and [^{3}H]mepyramine binding to H_1 receptors in rat brain cortex. Under our experimental conditions, incorporation of [^{3}H]inositol into lipids of brain cortical slices was linear with time for at least 120 min at all ages studied. At this incubation time, the rate of lipid labeling was 4 times higher in the 3-day-old animal than in the adult (Fig. 1), reflecting a faster metabolism of phosphoinositides in the brain of newborn animals. Taking into account these differences in lipid labeling, [^{3}H]IP accumulation was routinely normalized by expressing the results as dpm of [^{3}H]IP per 100 dpm of ^{3}H-lipids.

All through development, HA stimulated the accumulation of [^{3}H]IP in [^{3}H]inositol-prelabeled slices in the presence of lithium. As can be seen in Fig. 2a, while the response in unstimulated samples showed little variation with age, the net stimulation induced by 1 mM HA increased 3-fold during the first 2 weeks of life and remained at approximately the same level until adulthood. In contrast, specific binding of [^{3}H]mepyramine (2 nM) to cortical membranes, which was very low during the first week, increased about 8-fold during the second and third weeks and reached adult levels by the end of the fourth week after birth (Fig. 2b). This profile represents an increase of H_1 receptor density, since there was no change in receptor affinity (not shown). Thus, the maturation of the response seemed to precede the attainment of the adult number of H_1 receptors.

In order to investigate whether the HA-stimulated phosphoinositide hydrolysis in developing rat cortex was actually an H_1 receptor-mediated effect, we first studied the inhibition of the response to 1 mM HA by the H_1 antagonist mepyramine

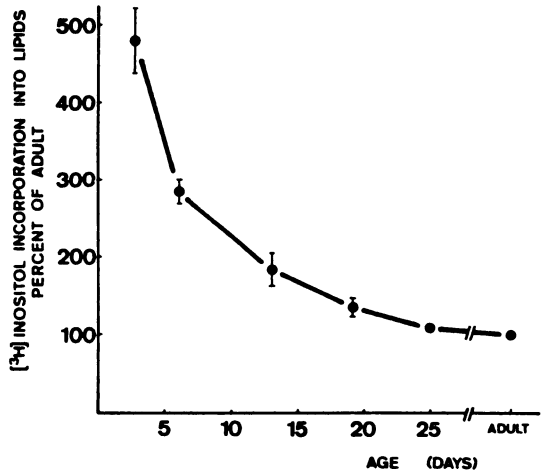

Fig. 1. Incorporation of [^{3}H]inositol into lipids of rat brain cortical slices during development. Slices obtained from rat brain cortices of different postnatal ages were incubated in KH buffer with 0.2 μM [^{3}H]inositol for 120 min and washed. Lipids were extracted with chloroform/methanol as described in Materials and Methods and radioactivity was measured in aliquots of the organic phases. Results were corrected for protein content and expressed as percentage of the adult value (16,500 ± 1,040 dpm/mg of protein). Data are means ± standard errors of three to five separate determinations performed in quadruplicate.

at a concentration (1 μM) high enough to abolish the same response in adult rat brain (5). As shown in Fig. 3, the extent of mepyramine inhibition of the response varied with age, increasing from only 25.8 ± 8.8% in the 3-day-old animal to around 100% at day 25 and onward.

To test the possibility that the low antagonistic activity of 1 μM mepyramine early in development was due to the higher agonist potencies, concentration-response curves for HA were generated at 6, 10, and 15 days of age. Linear Hill transformation of the data allowed the calculation of EC_{50} values and slope factors (n_H). As shown in Table 1, the potency of HA in the 6-day-old animal (EC_{50} = 19.6 ± 3.4 μM) was 5-fold higher than in the adult (EC_{50} = 94.7 ± 11.8 μM). On the other hand, only in adult animals was the slope factor not different from unity, whereas the curves corresponding to younger ages were better fitted to a two-component model, and high and low potency components could be resolved. The parameters estimated for these components, i.e., EC_{50} values (K_1 and K_2) and percent contribution to the total response (N_1 and N_2), were found to vary during development (Table 1). The EC_{50} for the high potency component increased from 6.4 μM at day 6 to 26.5 μM at day 15 and its contribution to the total response augmented from 65.8% to 78.9% during the same period. The EC_{50} for the low potency component also seemed to increase with age, although in this case the parameter estimates were subject to large errors due to the low contribution of this component to the total response.

Pharmacological characterization of the HA-induced phosphoinositide hydrolysis in the 6-day-old rat cortex. For the purpose of elucidating the nature of the different components involved in the response to HA in neonatal brain, a detailed characterization of this effect was performed in 6-day-old rat cortical slices. Initial experiments showed that HA H_2 receptors were not implicated in the response since dimaprit (1 mM) and impromidine (0.1 mM) had no effect and metiamide and cimetidine (100 μM) did not antagonize the effect elicited by 1 mM HA. The response was also unaffected by 1 μM

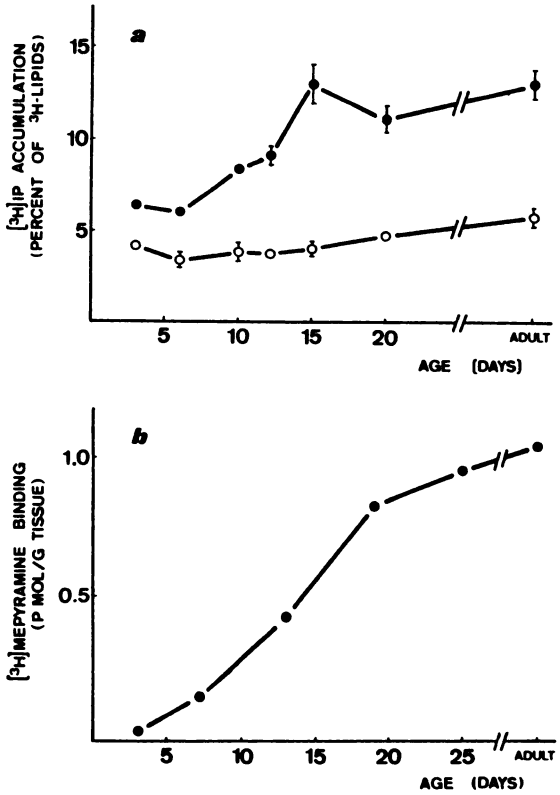

Fig. 2. Ontogenic development of HA-stimulated [^{3}H]IP accumulation and [^{3}H]mepyramine-specific binding in rat brain cortex. a. [^{3}H]Inositol-prelabeled cortical slices from developing rats were incubated for 60 min with (●) and without (○) 1 mM HA, and the accumulation of [^{3}H]IP was measured as described in Materials and Methods. Data are expressed as percentage of the radioactivity found in the organic phases and are means ± standard errors of three to five separate experiments performed in quadruplicate. b. Particulate fractions (0.7 mg of protein) from rat brain cortex of different postnatal ages were incubated with [^{3}H]mepyramine (2 nM) as described in Materials and Methods. Speficic binding was defined as that inhibited by 0.6 μM promethazine. Data are means of triplicate determinations.

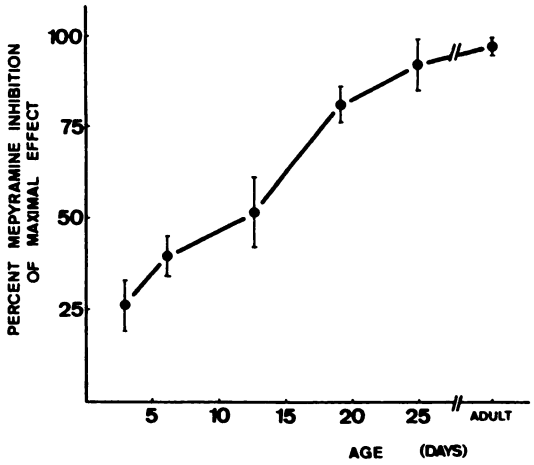

Fig. 3. Inhibition by mepyramine of the HA-stimulated [^{3}H]IP accumulation in cortical slices from developing rats. [^{3}H]Inositol-labeled slices were incubated for 60 min under basal conditions, with 1 mM HA or 1 mM HA plus 1 μM mepyramine. Results are expressed as percentage inhibition of the HA-induced response at each age. Data are means ± standard errors of three different experiments performed in quadruplicate.

TABLE 1

Parameters estimated for HA stimulation of [^{3}H]IP accumulation in developing rat brain cortex

Concentration-effect curves for HA at different postnatal ages were generated as indicated in Fig. 4 and the results analyzed by linear Hill transformation of the data. Slope factors (n_H) and EC_{50} values are means ± standard errors of the values obtained in the number of experiments given in parentheses. At each age the combined data from the different experiments were fitted either to a single or a double hyperbola as described in Materials and Methods. The best fit was considered to be that associated with the lowest residual sum of squares and its goodness assessed by using the *F* test. The parameters estimated (value ± standard deviation) from the two-component model are the EC_{50} values for each component (K_1 and K_2) and their percent contribution to the total response (N_1 and N_2).

	Age			
	6 Days (9)	10 Days (3)	15 Days (3)	Adult (9)
n_H	0.70 ± 0.05	0.80 ± 0.06	0.86 ± 0.02	1.04 ± 0.05
EC_{50} (μM)	19.6 ± 3.4	26.4 ± 2.2	47.5 ± 2.6	94.7 ± 11.8
Best fit components	2[a]	2[b]	2[b]	1
N_1 (%)	65.8 ± 1.4	80.1 ± 5.8	78.9 ± 6.6	
K_1 (μM)	6.4 ± 0.2	11.6 ± 2.8	26.5 ± 4.9	
N_2 (%)	34.2 ± 1.4	19.9 ± 5.8	21.1 ± 6.6	
K_2 (μM	175.0 ± 17.1	374.9 ± 59.3	259.9 ± 21.5	

[a] $p < 0.005$.
[b] $p < 0.05$.

concentrations of serotonin (methysergide), and muscarinic (atropine) and α_1-adrenergic (prazosin) receptor antagonists.

Concentration-effect curves for several H_1 receptor agonists are shown in Fig. 4 together with that for HA. All of the agonists tested induced the accumulation of [^{3}H]IP in the 6-day-old cortex in a concentration-dependent manner. However, only the curves for N$^\alpha$-mHA and N$^\alpha$,N$^\alpha$-dimHA allowed a clear definition of a maximal response, which in both cases was lower (77.6 and 67.8%, respectively) than that found for HA. The curves for these two H_1 receptor agonists showed n_H values not significantly different from unity, suggesting the interaction with a homogeneous population of receptors. Table 2 shows the potencies and maximal responses of these two H_1 receptor agonists compared to the same parameters estimated for the high potency component for HA. Maximal stimulations elicited by N$^\alpha$-mHA and N$^\alpha$,N$^\alpha$-dimHA were very close to that induced by HA with high potency, and their relative potencies were in accord with those expected for H_1 receptor mediated effects (16), suggesting the involvement of this receptor in the high potency component of the response to HA. The other H_1 receptor agonists tested, PEA, TEA and 2-mHA, did not show a well defined maximal response preventing the estimation of dose response parameters. Anomalous behavior of certain H_1 receptor agonists at high concentrations also has been observed when measuring the same response in guinea pig ileum, where a non-H_1 receptor component of the HA-induced phosphoinositide hydrolysis has been reported (17).

Further support for the involvement of H_1 receptors of the high potency component of the response to HA was obtained from the study of mepyramine inhibition of HA- and N$^\alpha$-mHA-induced responses. Mepyramine (1 μM) produced a large parallel displacement to the right of the N$^\alpha$-mHA concentration-response curve (Fig. 5b) and the inhibition constant (K_i) calculated from the dose ratio gave a value of 6.3 ± 1.4 nM, closely similar to that obtained from the inhibition of the response to HA in adult rat brain (5) and from binding studies in adult and newborn rat brain (11, 12, 18, 19). On the other hand, the concentration-response curve for HA was also right-shifted but

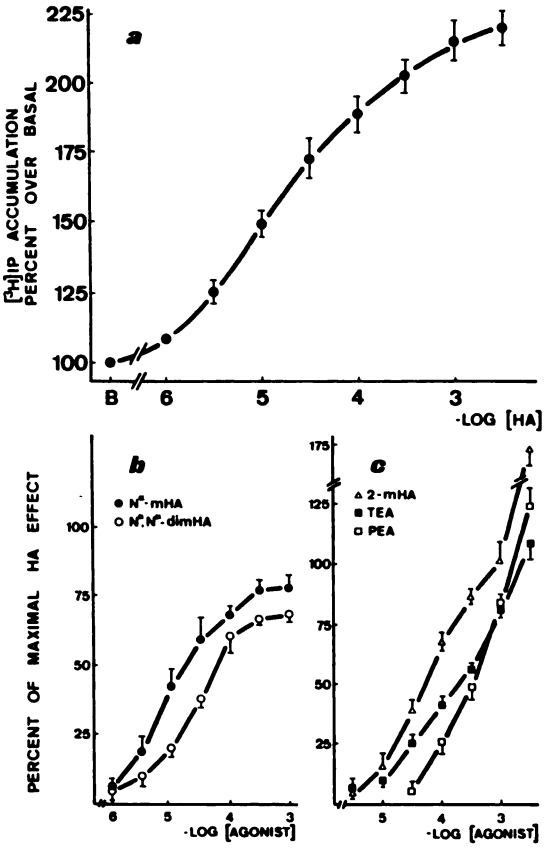

Fig. 4. Concentration-effect curves for agonist-induced [^{3}H]IP accumulation in cortical slices from 6-day-old rats. [^{3}H]Inositol-labeled slices were incubated for 90 min with the indicated concentrations of agonist. a. Concentration-response curve for HA represented as percentage stimulation over control (3.41 ± 0.25 dpm/100 dpm lipids). Results are means ± standard error of nine separate experiments performed in quadruplicate. b and c. Concentration-effect curves for H_1 receptor agonists, represented as percentage of the effect produced by 3 mM HA. Results are means ± standard errors of three independent experiments performed in quadruplicate.

TABLE 2

Concentration-response parameters for H_1 receptor agonist stimulation of [^{3}H]IP accumulation in brain cortex from 6-day-old rats

Slope factors (n_H) and EC_{50} values for N$^\alpha$-mHA and N$^\alpha$,N$^\alpha$-dimHA were calculated from linear Hill transformation of the concentration-response curves represented in Fig. 4. These parameters and maximal stimulations, expressed as percentage of the response induced by 3 mM HA, are means ± standard errors of three separate experiments performed in quadruplicate. Parameters for the high potency component for HA are from Table 1 and are presented here for comparison.

Agonist	EC_{50}	n_H	Maximal stimulation	Relative potency
	μM		%	
HA (high potency component)	6.4 ± 0.2	1	65.8 ± 1.4	100
N$^\alpha$-mHA	11.4 ± 0.8	0.96 ± 0.02	77.6 ± 3.3	56.1
N$^\alpha$,N$^\alpha$-dimHA	15.9 ± 1.2	0.95 ± 0.05	67.8 ± 3.0	50.3

to a lesser extent and in a non-parallel manner, with an apparent K_i value for mepyramine of 60.5 ± 12.0 nM. The mepyramine-displaced concentration-response for HA was fitted to a double hyperbola fixing the percent contribution of the low and high potency components to the total response, and alternatively, each of the EC_{50} values of the two components or none. The best fit was obtained when the EC_{50} value of the

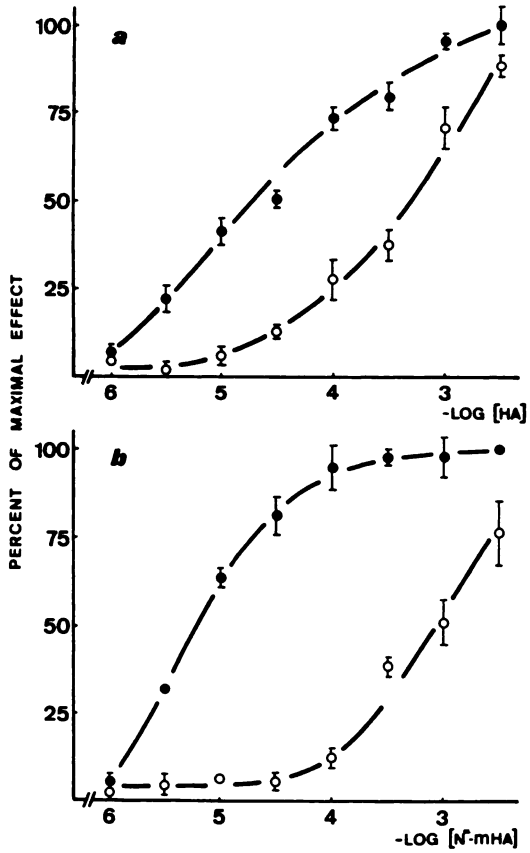

Fig. 5. Effect of mepyramine on the accumulation of [^{3}H]IP elicited by HA (a) and N$^{\alpha}$-mHA (b). Concentration-effect curves were generated in the absence (●) and presence (○) of 1 μM mepyramine. Results are expressed as means ± standard errors of three independent experiments performed in quadruplicate.

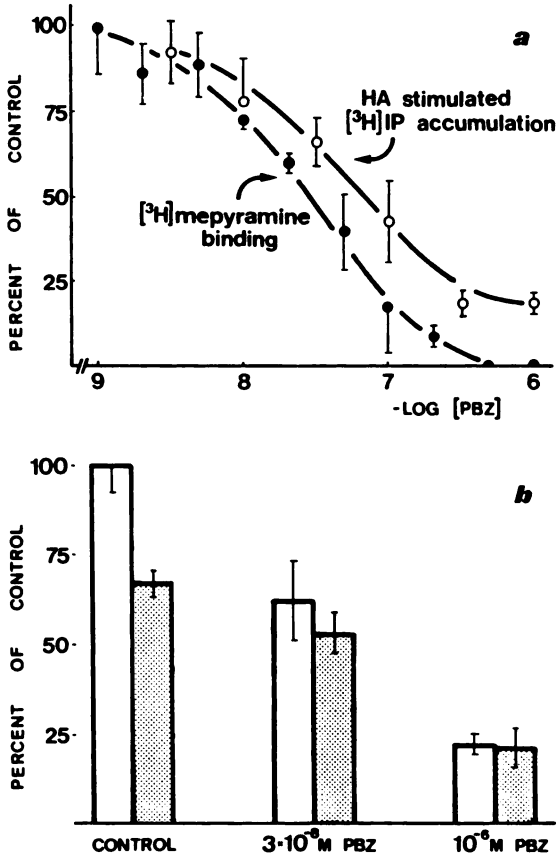

Fig. 6. Effect of pretreatment with phenoxybenzamine on [^{3}H]mepyramine binding and HA-stimulated [^{3}H]IP accumulation in 6-day-old rat brain cortex. a. Unlabeled and [^{3}H]inositol-labeled slices were incubated with the indicated concentrations of phenoxybenzamine (*PBZ*) for 45 min, washed, and assayed for [^{3}H]mepyramine (2 nM)-specific binding and HA (1 mM) stimulation of [^{3}H]IP accumulation, respectively. b. [^{3}H]Inositol-labeled slices were incubated with the indicated concentrations of phenoxybenzamine (*PBZ*) for 45 min and assayed for HA (1 mM) stimulation of [^{3}H]IP accumulation in the absence (□) or presence (□) of 1 μM mepyramine. In both panels, results are expressed as percentage of slices not treated with phenoxybenzamine and are means ± standard errors of three independent experiments.

low potency component was fixed at its value in the absence of antagonist (175 μM). In this case, the estimated EC_{50}' for the high potency component was 600.4 ± 91.4 μM and the inhibition constant for mepyramine became 10.8 ± 2.1 nM which is close to that found for the inhibition of the N$^{\alpha}$-mHA-induced response.

Blockade of H_1 receptors with phenoxybenzamine in the 6-day-old rat cortex. We previously showed (5) that the alkylating agent phenoxybenzamine irreversibly inactivates HA H_1 receptors in rat brain without altering their affinity for [^{3}H]mepyramine or the potency of HA in stimulating phosphoinositide hydrolysis. Searching further support for the contention that part of the effect of HA in newborn rat brain is not mediated by H_1 receptors, we studied the effect of preincubation of 6-day-old rat cortical slices with phenoxybenzamine on the HA-induced response and receptor density.

Phenoxybenzamine caused a concentration-dependent decrease in [^{3}H]mepyramine-specific binding which was totally abolished by exposure to concentrations of 0.5 μM and higher (Fig. 6a). The same treatment also caused a concentration-related decrease of the HA induced response without altering the basal values. In this case, however, μM concentrations of the irreversible antagonist left approximately 25% of the total response unaltered. Thus, a small portion of the effect was still found under conditions in which all of the [^{3}H]mepyramine specifically labeled sites had been irreversibly blocked. Furthermore, when the ability of 1 μM mepyramine to inhibit the response in untreated slices and in slices with totally or partially inactivated H_1 receptors was examined, it was shown that the percentage of the response remaining after pretreatment with 1 μM phenoxybenzamine was also uninhibited by mepyramine (Fig. 6b). Therefore, this portion of the response is not mediated by typical H_1 receptors and its magnitude is similar to the estimated contribution of the low potency component of the HA-induced response.

Discussion

The results obtained in this study show that a functional coupling between HA H_1 receptors and HA-induced phosphoinositide hydrolysis is already present in newborn rat brain cortex. We have also observed significant changes in the characteristics of the response during the postnatal maturation of rat brain cortical tissue. First, at early stages of development a small component of the response was not mediated by H_1 receptors. Second, the potency of HA for the H_1 receptor-specific component was found to gradually decrease with age. Third, the efficacy of HA in the newborns seemed to be higher than in the adult, as suggested by the earlier developmental

increase in the response with respect to the number of H_1 receptors.

An unexpected feature of the HA-induced phosphoinositide hydrolysis in newborn rat brain cortex was the presence of a non-H_1 receptor-mediated component of the response. Nonlinear regression analysis of concentration-effect curves for HA performed at different postnatal ages allowed the resolution of high and low potency components in the response. The high potency component, which accounted for 65–80% of the total effect during the first 2 postnatal weeks, with EC_{50} values increasing from 6.4 to 26.5 μM, showed characteristics of H_1 receptors. Thus, in 6-day-old rat cortex, the H_1 receptor agonists N^{α}-mHA and N^{α},N^{α}-dimHA induced maximal responses similar in magnitude to that of the high potency component for HA, and their potencies relative to that of HA were in agreement with those reported for other H_1 receptor-mediated responses (6). Inhibition by mepyramine of the response induced by N^{α}-mHA resulted in the expected parallel shift to the right of the concentration-effect curve, yielding a K_i for the antagonist of 6.3 nM, similar to that obtained from inhibition of the same response in adult rat brain (5) and from [^{3}H]mepyramine binding to H_1 receptors in adult and newborn rat brain (11, 12, 18, 19). On the other hand, if the agonist used was HA, the K_i for mepyramine, estimated from the observed dose ratio, was much higher (60 nM). However, when nonlinear regression analysis of the concentration-response curve obtained in the presence of mepyramine was performed assuming that only the high potency component for HA was inhibited, a K_i value of 10 nM was calculated, which is of the same order of magnitude as the reported affinity of H_1 receptors for this compound. Finally, pretreatment of cortical slices from 6-day-old rats with increasing concentrations of phenoxybenzamine, which we have previously shown to irreversibly block H_1 receptors in adult rat brain (5), inhibited [^{3}H]mepyramine-specific binding and the HA-induced response with similar potency but to a different extent. Concentrations high enough to completely abolish specific radioligand binding failed to inhibit approximately 25% of the response. This remaining effect, which was insensitive to mepyramine, was of magnitude similar to that accounted for by the low potency component of the response to HA. These results support the hypothesis that only that portion of the response to HA corresponding to the high potency component is mediated by H_1 receptors. The other portion of the response does not seem to be induced through stimulation of HA H_2 receptors since dimaprit (1 mM) and impromidine (0.1 mM) had no effect and metiamide and cimetidine (0.1 mM) were unable to inhibit the effect elicited by 1 mM HA. Contributions to the response from other receptors also coupled to enhanced phosphoinositide hydrolysis (20) such as serotonin 5-HT_2, α_1-adrenergic, and muscarinic receptors, which could be stimulated if HA was inducing the release of the corresponding endogenous amines, were ruled out by the lack of effect of specific antagonists. A non-H_1 receptor component of HA-induced phosphoinositide hydrolysis has also been reported in guinea pig ileum (17), suggesting the occurrence of more than one mechanism for that HA-stimulated effect. However, the relevance of this component for the physiological role of HA seems uncertain, given the high agonist concentrations needed to induce it.

The potency of HA was found to decrease during postnatal development as revealed by the gradual increase in EC_{50} values for both, the total response, and the high potency component. In experiments not shown, we found that HA inhibits [^{3}H]mepyramine binding to membrane preparations from 6-day-old rat brain cortex with a potency similar to that found in the adult (5). However, given the low specific binding present in the newborn tissue, it was difficult to perform an accurate measurement of the affinity for HA, and the possibility of ontogenic changes in the characteristics of agonist binding should not be ruled out.

In agreement with a previous report (11), the specific binding of [^{3}H]mepyramine to membranes from rat cortex increased about 8-fold from the first to the fourth postnatal week, at which time adult levels were reached. In contrast, phosphoinositide hydrolysis induced by 1 mM HA, the concentration eliciting maximal responses at all ages studied, was, during the first postnatal week, only 3-fold lower than in the adult. The response increased up to day 15 and the net stimulation by HA remained essentially the same thereafter. Even correcting the HA-induced response for the contribution of the non-H_1 receptor component observed to be present during the first 2 weeks of life, the developmental profiles for the H_1 receptor number and H_1 receptor-mediated response do not coincide. These differences could be explained if spare receptors appeared during development. However, this possibility is ruled out by our demonstration of the absence of a receptor reserve in adult rat brain (5). Our results are in contrast with those of Subramanian *et al.* (13) who reported similar ontogenic profiles for the specific binding of [^{3}H]mepyramine to membranes from whole rat brain and for the *in vivo* increase in [^{33}P]orthophosphate incorporation to brain lipids induced by intracisternal administration of HA. However, measurement of [^{33}P] orthophosphate incorporation into lipids, which only in part reflects phosphoinositide resynthesis after agonist-induced hydrolysis, is an indirect method of assessing an H_1 receptor-mediated response, and secondary effects may be masked.

The different ontogenic profiles observed in this work for [^{3}H]mepyramine binding and the H_1 receptor-mediated response might result from developmental changes in the size and/or specific labeling of a neuromodulator-sensitive phosphoinositide pool. Since the concentration of brain phosphoinositides increases with age (21), the higher rate of [^{3}H]inositol incorporation into lipids found in newborn rat brain cortex (Fig. 1) is indicative of a higher specific labeling. If an HA-sensitive phosphoinositide pool was labeled to a higher specific activity at earlier stages of development, an overestimation of the actual response to HA would result, even correcting the [^{3}H]IP accumulation for differences in total lipid labeling. This interpretation has been suggested by Orellana and Brown (22) to explain the differential ontogeny of muscarinic receptors and charbacol-stimulated phosphoinositide hydrolysis in chick heart, where a notable decrease in the response occurs during a period of embryonic development (22) when receptor number remains relatively constant (23).

A tentative explanation for the apparent decrease in HA efficacy after day 15 and for the loss of HA potency with age would arise from considering hypothetical developmental changes in the coupling between H_1 receptors and the effector system. Although little is known about the nature of that coupling, there is increasing evidence on the participation of a guanine nucleotide-regulatory protein (G protein) in agonist-induced phosphoinositide hydrolysis (24). That this would also

apply to H_1 receptors is suggested by the reported sensitivity of agonist binding to guanine nucleotides (25). Variations in the relative proportion of the components of the system, i.e., the receptor molecule, the putative G protein and the phosphoinositide-specific phospholipase C, and/or in the membrane environment where the interactions among these components take place, could be responsible for the changes in the characteristics of the HA-induced response during development.

Besides the molecular mechanism implicated, it is worth noting that the ontogenic rise in the response to HA extends only up to day 15 when the highest rate of increase in the activity of L-histidine decarboxylase takes place. Since in rat brain cortex the developmental pattern of the enzyme activity presumably represents the appearance of histaminergic innervation (10), the observed loss in HA potency and efficacy might reflect a naturally occurring form of desensitization. Receptor down-regulation as a possible consequence of ontogenic appearance of sympathetic innervation has been reported for α_1-adrenergic receptors in mouse heart (26) and rat pineal gland (27), and receptor subsensitivity and down-regulation for β-adrenergic receptors in embryonic chick ventricle (28). That this could be the case for rat cortical H_1 receptors is supported by recent findings from our laboratory. In two preparations of rat nervous tissue lacking physiological histaminergic innervation, i.e., astroglial cells in primary culture[1] and denervated rat brain cortex,[2] we have found that HA stimulates phosphoinositide hydrolysis with maximal responses and agonist potencies higher than those found in adult brain cortex and that these differences do not result from higher receptor densities. Further detailed work is needed to assess this appealing hypothesis.

Finally, the lack of correlation observed here between HA-induced phosphoinositide hydrolysis and [^{3}H]mepyramine binding could be sustained if, as recently suggested by Carswell and Young (29), the HA-induced [^{3}H]IP accumulation measured in the presence of lithium after long incubations does not solely reflect the initial receptor-coupled hydrolytic step.

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[2] J. García-Ladona, A. García, and F. Picatoste, manuscript in preparation.

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