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HISTAMINE H_1-RECEPTORS IN THE BRAIN OF THE GUINEA-PIG AND THE RAT: DIFFERENCES IN LIGAND BINDING PROPERTIES AND REGIONAL DISTRIBUTION

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1 The equilibrium dissociation constant, K_d, for mepyramine binding to a particulate fraction from rat brain, 9.1 nM, determined from inhibition of the binding of 1 nM [^{3}H]-mepyramine, was distinctly higher than that, 0.83 nM, measured on an equivalent preparation from guinea-pig brain.

2 In rat brain the dissociation constant for mepyramine, determined from the binding of [^{3}H]-mepyramine sensitive to inhibition by 2×10^{-6} M promethazine, was higher than the constant obtained from the inhibition of the binding of 1 nM [^{3}H]-mepyramine by non-radioactive mepyramine. This suggests that the promethazine-sensitive binding of [^{3}H]-mepyramine includes a lower affinity non-receptor component, which becomes apparent at higher concentrations of [^{3}H]-mepyramine.

3 In the guinea-pig the dissociation constant for mepyramine determined from inhibition of [^{3}H]-mepyramine binding was in good agreement with the value obtained from inhibition of the contractile response of intestinal smooth muscle to histamine. No similar comparison was possible in the rat. Rat ileum was much less sensitive to histamine and the contraction produced was not inhibited by 10^{-6} M mepyramine, indicating that it is not mediated by H_1-receptors.

4 Low levels of promethazine-sensitive [^{3}H]-mepyramine binding were present in membrane fractions prepared from the longitudinal muscle from rat small intestine, but the characteristics of this binding suggest that it may be largely to lower affinity, non-receptor sites.

5 Promethazine was practically equipotent as an inhibitor of [^{3}H]-mepyramine binding in rat and guinea-pig brain. Chlorpheniramine showed stereospecificity in the rat as in the guinea-pig, although the potency of the (+)-isomer in the rat was only a tenth of that in the guinea-pig. Histamine had nearly the same IC_{50} in both species.

6 The evidence suggests that the high-affinity [^{3}H]-mepyramine binding sites in rat brain can be described as H_1-receptors, but that these differ structurally from H_1-receptors in the guinea-pig.

7 The regional distribution of [^{3}H]-mepyramine binding in rat brain was not the same as that in guinea-pig brain, the most notable difference being the very much lower level in rat cerebellum compared to guinea-pig cerebellum.

Introduction

[^{3}H]-mepyramine binds selectively to histamine H_1-receptors in homogenates of the longitudinal muscle of guinea-pig ileum (Hill, Young & Marrian, 1977) and has been used to provide evidence for the presence of H_1-receptors in the brain of the guinea-pig (Hill & Young, 1978; Hill, Emson & Young, 1978) and the rat (Chang, Tran & Snyder, 1978; Tran, Chang & Snyder, 1978). In the guinea-pig there is in general a good quantitative correlation between the binding affinities of H_1 antagonists deduced from inhibition of [^{3}H]-mepyramine binding and those obtained from inhibition of the contractile response of ileal smooth muscle to histamine (Hill *et al.*, 1977; Hill *et al.*, 1978). However, the affinities of antagonists determined from inhibition of [^{3}H]-mepyramine binding in the rat do not all agree well with the values in the guinea-pig, although in most respects the characteristics of [^{3}H]-mepyramine binding are those expected for selective labelling of H_1-receptors (Chang *et al.*, 1978; Tran *et al.*, 1978). These observations suggest that histamine H_1-receptors in the rat may not be structurally identical with those in the guinea-pig and in this paper we present further evidence in support of this view. Some of these results have been presented to the British Pharmacological Society (Hill & Young, 1979).

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Methods

[^3H]-ligands

[^3H]-*mepyramine*, 21.2 Ci/mmol, was synthesized and purified as described previously (Marrian, Hill, Sanders & Young, 1978).

[^3H]-*quinuclidinyl benzilate*, 13 Ci/mmol, was purchased from the Radiochemical Centre, Amersham, and used without further purification.

Preparation of membrane fractions

Brain Guinea-pig (Hartley strain, either sex, 400 to 600 g) or rat (Sprague–Dawley, males, 200 to 400 g) whole brain was homogenized in 5 vol of 50 mM Na-K phosphate buffer (37.8 mM Na_2HPO_4, 12.2 mM KH_2PO_4), pH 7.5, treated with a Polytron blender at setting 5 for 15 s and centrifuged at 6000 *g* for 20 min. The pellet was resuspended in 8 ml 50 mM Na-K phosphate, pH 7.5, centrifuged at 8700 *g* for 1 min in a Beckman microfuge B and the resulting pellet suspended in 8 ml of the same buffer. The last centrifugation ensures that all the protein sediments in the microfuge assay. The protein concentration of the final suspension was measured by the method of Lowry, Rosebrough, Farr & Randall (1951). The suspension was either used immediately or stored frozen at −10°C for up to 4 days. There was no evidence for any deterioration in the binding properties over this period.

Ileum Longitudinal muscle strips were prepared from guinea-pig or rat small intestine essentially as described by Rang (1964). The muscle was cut into small pieces with scissors and homogenized with the Polytron blender (setting 5) for 3 periods of 20 s at 2 min intervals, the suspension being cooled throughout this treatment in an iced water bath. The membrane fraction was isolated from the homogenate as described for brain, except that the final centrifugation in the microfuge was for 30 s.

Binding measurements

Aliquots of the membrane suspension (50 µl, 0.5 to 0.8 mg protein) in 1.95 ml 50 mM Na-K phosphate buffer, pH 7.5, were incubated with various concentrations of [^{3}H]-mepyramine in the presence or absence of 2×10^{-6} M promethazine for 60 min at 30°C. Aliquots (0.4 ml) were centrifuged at 8700 *g* for 1 min in the microfuge and the pellet washed superficially twice with 0.1 ml ice-cold phosphate buffer. The bottom of the microfuge tube was cut off into a scintillation vial, 10 ml scintillation mix (butyl PBD-ethoxyethanol-toluene, 0.6:33:67, w/v/v) added and the pellet freed from the tube by vigorous shaking. Quadruplicate determinations were made on duplicate incubations at each [^{3}H]-mepyramine concentration. Radioactivity as ct/min measured by liquid scintillation counting was converted to d/min by the channels ratio method, using an external radioactive source.

For experiments on the inhibition of [^{3}H]-mepyramine binding, incubations were as above except that the concentration of [^{3}H]-mepyramine was fixed at 1 nM and the concentration of inhibitor varied. The equilibrium dissociation constant, K_d, of the inhibitor was calculated from the concentration of drug, IC_{50}, required for 50% inhibition of the receptor-specific binding of [^{3}H]-mepyramine, using the relationship $K_d = IC_{50}/(1 + M/K_{mep})$, where M is the concentration of [^{3}H]-mepyramine and K_{mep} its dissociation constant. In the special case of non-radioactive mepyramine this expression simplifies to $K_{mep} = IC_{50} - M$, assuming that the substitution of one atom of tritium for hydrogen has no effect on the dissociation constant. These formulae assume that the inhibitor competes with [^{3}H]-mepyramine for a single set of binding sites.

Where the experimental data were sufficient, IC_{50} was taken from a weighted best-fit curve to the experimentally measured variation of % of uninhibited binding of [^{3}H]-mepyramine with concentration of inhibitor, A. The only assumption made was that the binding of the inhibitor could be described by a Hill equation, i.e. fractional receptor occupancy $= A^n/(A^n + K)$, where n is the Hill coefficient, and the equation fitted was:

$$\begin{matrix}\%\text{ of uninhibited} \\ \text{binding of} \\ [^3\text{H}]\text{-mepyramine}\end{matrix} = \frac{100 - NS}{A^n/K + 1} + NS$$

with n, K and NS (non-specific, i.e. inhibitor insensitive binding) as unknowns. K is the apparent binding dissociation constant of the inhibitor, since the effect of competition with [^{3}H]-mepyramine will be a shift of the curve to higher inhibitor concentrations by a factor of $(M/K_{mep} + 1)$. Each point was weighted according to the reciprocal of the variance associated with it. A modified Marquardt method, as implemented in the Harwell Library routine VB01A was used to obtain the best-fit values of the parameters and their estimated standard errors.

A particular advantage of this approach is that no assumption is made about the level of non-specific binding. However, where the foot of the curve was insufficiently well-defined for this approach, the level of non-specific binding was taken to be the percentage of the binding of 1 nM [^{3}H]-mepyramine insensitive to 2×10^{-6} M promethazine.

The same non-linear minimisation procedure, VB01A, was used to fit a single hyperbola ([^{3}H]-mepyramine bound = capacity × $M/(M + K_{mep})$) or

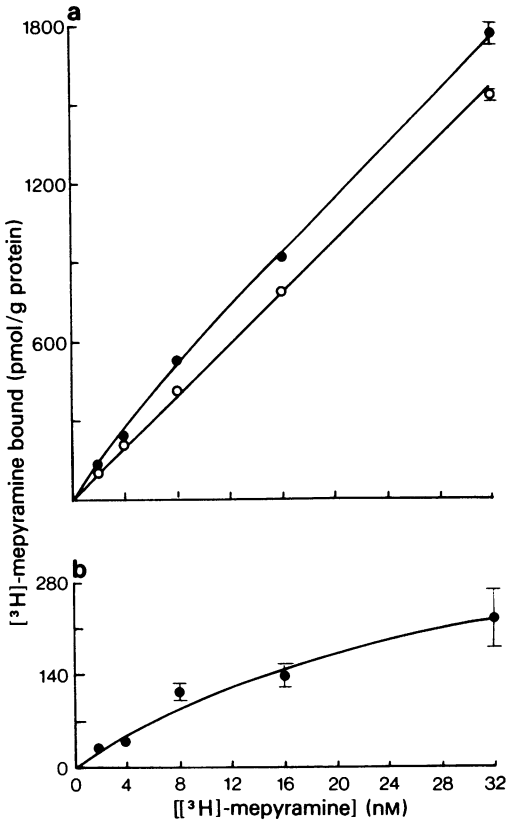

Figure 1 Binding of [^{3}H]-mepyramine to rat brain. (a) Binding of [^{3}H]-mepyramine in the presence (○) or absence (●) of 2×10^{-6} M promethazine. Where no error bar is shown the standard error was smaller than the symbol. (b) Promethazine-sensitive binding of [^{3}H]-mepyramine. The curve drawn is the best-fit hyperbola obtained by the method of Wilkinson (1961).

a hyperbola + linear component ([^{3}H]-mepyramine bound = capacity of receptor site $\times$ $M/(M + K_{mep})$ + $b.M$, where b is the slope of the linear component) to the data describing the variation of the promethazine-sensitive binding of [^{3}H]-mepyramine to rat brain membranes with the concentrations of [^{3}H]-mepyramine. Each point was again weighted by the reciprocal of the variance associated with it.

Regional distribution of [^{3}H]-mepyramine binding in rat brain

Rat brain was dissected into individual areas and the binding of 2 nM [^{3}H]-mepyramine measured on unfractionated homogenates exactly as described previously for guinea-pig brain (Hill *et al.*, 1978). There was again no evidence, as indicated by measurements on posterior cortex, for any decline in the binding of [^{3}H]-mepyramine to the homogenate on freezing or on storage at -10°C for up to 3 days.

Drugs

Drugs were obtained from the following sources: mepyramine maleate, promethazine hydrochloride and chlorpromazine hydrochloride (May & Baker); carbachol chloride and histamine dihydrochloride (BDH). Gifts of (+)- and (−)-chlorpheniramine maleate (Schering) and methapyrilene hydrochloride (Lilly) are gratefully acknowledged.

Results

Binding of [^{3}H]-mepyramine to rat brain membranes

The binding of [^{3}H]-mepyramine to rat brain membranes in the presence or absence of 2×10^{-6} M promethazine, a potent H_1 antagonist, is shown in Figure 1a. The data in Figure 1 are taken from a single experiment, but each of four independent experiments (5 to 8 pairs of points in each) showed the same general features, although in two of them the highest concentration of [^{3}H]-mepyramine employed was 16 nM. The proportion of promethazine-insensitive binding of [^{3}H]-mepyramine is high and consequently limits the accuracy with which the promethazine-sensitive binding (Figure 1b), presumed to represent binding to histamine H_1-receptors, can be determined. The combined promethazine-sensitive binding data from all four experiments is shown in Figure 2. Combination of the data necessitates the assumption that the maximum amount of binding is the same in each case, but the smooth curve which results and the reasonably small standard error associated with those points obtained by combining data from 2 to 4 experiments (open circles) suggest that this condition is reasonably well met. In guinea-pig brain, where the proportion of promethazine-insensitive binding is lower, the mean dissociation constants, K_d, for [^{3}H]-mepyramine binding, determined from similar experiments, was 1.6 ± 0.2 nM (Hill *et al.*, 1978), but it is apparent that in the rat (Figure 1b and 2) half-maximal saturation is not achieved at this concentration and consequently that the apparent K_d is greater. The difference between the rat and the guinea-pig is shown clearly by the difference in slopes of Scatchard plots (Figure 3) of two guinea-pig (filled symbols) and two rat (open symbols) experiments.

The value of K_d obtained from the promethazine-sensitive binding of [^{3}H]-mepyramine in each individual experiment with rat brain, analysed as a single hyperbola, depended on the method used to fit the data, although the data shown in Figure 1b yielded

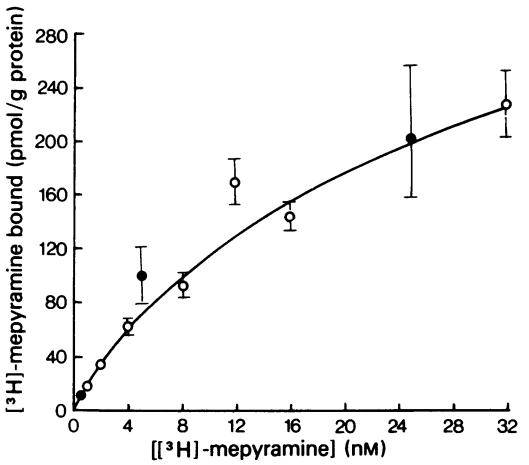

Figure 2 Promethazine-sensitive binding of [^{3}H]-mepyramine to rat brain: combined data from 4 independent experiments. Error bars indicate ±s.e. The curve drawn was fitted (see Methods for details) assuming that the binding is the sum of a saturable component and a component increasing linearly with concentration. (●) Points derived from one experiment only; (○) combined data from 2 to 4 experiments.

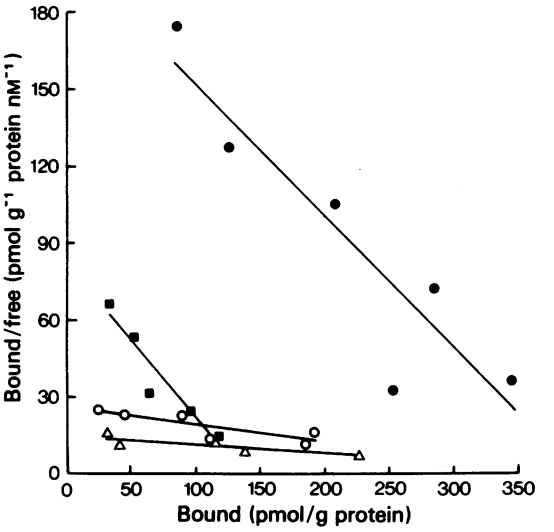

Figure 3 Scatchard plots of promethazine-sensitive [^{3}H]-mepyramine binding from two experiments with guinea-pig (●, ■) and two experiments with rat (○, △) brain.

similar values whether analysed by weighted non-linear regression (see Methods), by the method of Wilkinson (1961) or by unweighted linear regression (y on x) of a Scatchard plot (K_d = 32, 26 and 29 nM, respectively). In the other experiments the estimates varied more widely, but if each curve was analysed individually by a given method and the four estimates of K_d then combined, the resultant mean value was practically the same whichever method was employed (26 ± 9, 28 ± 12 and 27 ± 4 nM, methods in same order as above). If all the points from the 4 experiments were first combined, giving more points for analysis but with the reservation noted above, and then analysed as a single hyperbola by each of the three methods, the best-fit estimates of K_d were somewhat lower, but again in good agreement (16 ± 3, 15 ± 3 and 17 ± 4 nM). However, it was notable that if K_d was calculated using only binding data obtained at concentrations at [^{3}H]-mepyramine < 10 nM or the first 4 points of each curve, then the estimates were consistently lower, except for one Wilkinson and one Scatchard determination, whichever method was used, than those made including the data at higher concentrations of [^{3}H]-mepyramine. The mean K_d from the individual curves fell to 15 ± 3 nM, including values ranging down to 5 nM, and similarly for the combined data (Figure 2) the mean K_d fell to 12 ± 3 nM. This suggests the possibility that the promethazine-sensitive binding of [^{3}H]-mepyramine has more than one component, the binding of lower-affinity sites only becoming an appreciable fraction of the total at high concentrations of [^{3}H]-mepyramine. In this case the values obtained by fitting a single hyperbola are misleading, since the further the binding curve is extended to higher concentrations of [^{3}H]-mepyramine the higher the apparent K_d and the apparent capacity. The curve drawn through the points in Figure 2 has been calculated on the assumption that in addition to a saturable high-affinity component there is a second low-affinity component, which in the concentration range studied appears to be linear. Further evidence for this second component is discussed below.

Inhibition of [^{3}H]-mepyramine binding by non-radioactive mepyramine

In an attempt to obtain an equilibrium constant for mepyramine under conditions where the binding of [^{3}H]-mepyramine to high-affinity sites will be favoured, if lower-affinity sites are also present, we have measured the inhibition of the binding of a low concentration, 1 nM, of [^{3}H]-mepyramine to rat brain membranes by non-radioactive mepyramine (Figure 4). For comparison the curve obtained in guinea-pig brain under the same conditions is also shown (lower curve, Figure 4). The high proportion of the binding of 1 nM [^{3}H]-mepyramine insensitive to non-radioactive mepyramine, coupled with the relatively small amount bound at this [^{3}H]-mepyramine concentration (18 pmol mepyramine-sensitive sites/g protein), again make it difficult to obtain accurate experi-

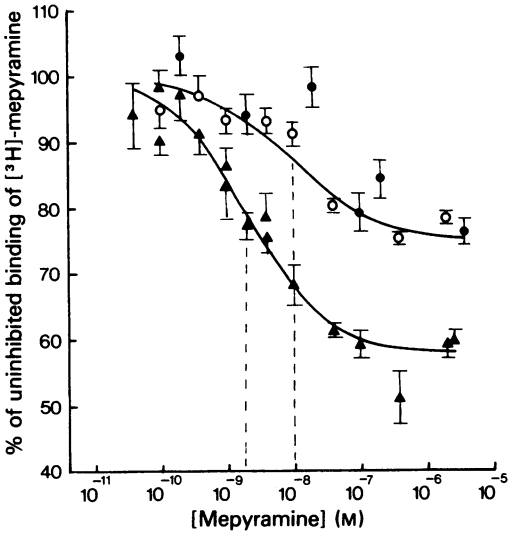

Figure 4 Inhibition of the binding of 1 nM [^{3}H]-mepyramine by non-radioactive mepyramine in guinea-pig and in rat brain. (▲) Guinea-pig brain; (○, ●) rat brain. A total of 31 measurements were made on the rat brain preparation, but where two or more measurements were made at the same inhibitor concentration the data have been combined (○). The weighted best-fit curves were calculated as described under Methods. Error bars represent ±s.e. The vertical broken line indicates the IC_{50}. Note that the ordinate scale does not start at zero.

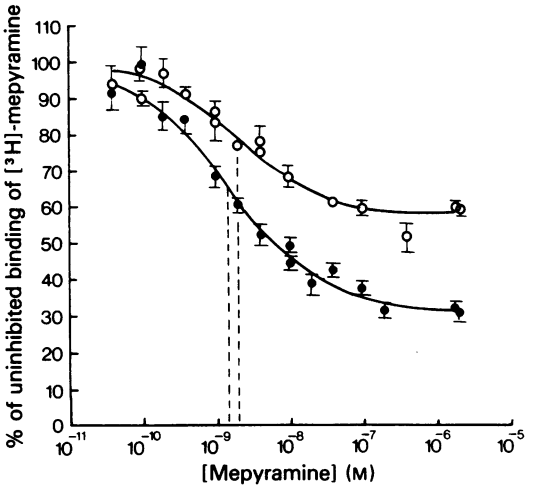

Figure 5 Inhibition of the binding of 1 nM [^{3}H]-mepyramine by non-radioactive mepyramine in comparable membrane fractions from guinea-pig brain and ileum. (○) Brain; (●) ileum. The weighted best-fit curves were calculated as described under Methods. Error bars represent ±s.e. and the vertical broken line indicates the IC_{50}.

mental data with rat brain. The line drawn is the weighted best-fit curve obtained from non-linear regression (see Methods), with the percentage of mepyramine-insensitive binding fitted as one of the unknowns. The proportion of mepyramine-insensitive binding, 74 ± 2%, was closely similar to the percentage insensitive to 2×10^{-6} M promethazine, 75 ± 1. The concentration of non-radioactive mepyramine required for 50% inhibition (IC_{50}) of specific [^{3}H]-mepyramine binding to rat brain membranes is 10 nM, whereas with the equivalent guinea-pig preparation the IC_{50} is 1.8 nM.

The IC_{50} of 10 nM in the rat is smaller than the mean apparent K_d, 27 nM, calculated from [^{3}H]-mepyramine binding curves, but is nearer to the values, 12 to 15 nM, determined omitting the points at high [^{3}H]-mepyramine concentrations. This is consistent with the presence of lower-affinity promethazine-sensitive binding sites, which become apparent at high concentrations of [^{3}H]-mepyramine. However, the difference between the IC_{50} in the rat and the guinea-pig measured with 1 nM [^{3}H]-mepyramine provides evidence that the binding properties of high affinity [^{3}H]-mepyramine binding sites in rat brain differ from those in the guinea-pig.

H_1-receptors in guinea-pig and rat intestinal smooth muscle

The difference between the affinity of mepyramine for H_1-receptors in the guinea-pig and the rat raises the question whether the binding in rat brain is to H_1-receptors. In the guinea-pig the IC_{50} for inhibition of the mepyramine-sensitive binding of 1 nM [^{3}H]-mepyramine was similar in comparable membrane fractions from brain and from longitudinal muscle strips from the small intestine (Figure 5) and the equilibrium dissociation constants, 0.6 and 0.8 nM, respectively, deduced from the IC_{50} values are in good agreement with the value obtained for [^{3}H]-mepyramine, 0.6 nM, from antagonism of the contractile response of muscle strips to histamine (Hill *et al.*, 1977). This gives some confidence that in the guinea-pig the binding is to H_1-receptors, although there is no indication whether they are all coupled to a physiological response.

No similar comparison could be made in the rat, since, as earlier authors have noted (Parrot & Thouvenot, 1966), the rat ileum is relatively insensitive to histamine. High doses of histamine, usually $> 10^{-4}$ M, were necessary to produce a response, which almost always showed a marked tachyphylaxis. Neither 10^{-6} M mepyramine nor 10^{-4} M cimetidine, either separately or in combination, had any significant inhibitory effect, but 10^{-5} M indomethacin, in the two experiments in which it was applied, abolished the response

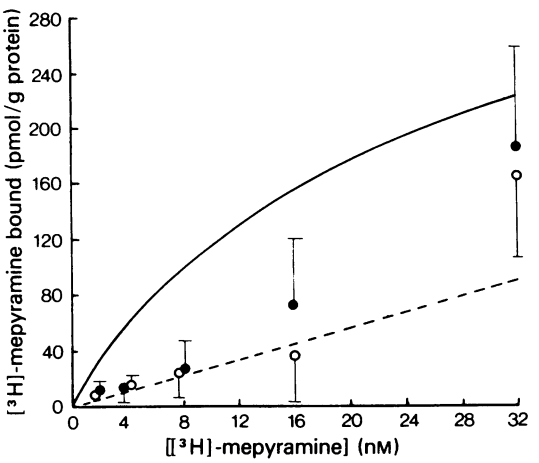

Figure 6 Comparison of the promethazine-sensitive binding of [^{3}H]-mepyramine to rat intestinal muscle with that to rat brain. Intestinal muscle: (●) measured on a fraction stored frozen at −10°C for 24 h; (○) measured on a freshly prepared membrane fraction. Only the two points at 32 nM [^{3}H]-mepyramine and one point (○) at 2 nM [^{3}H]-mepyramine were significantly different from zero ($P < 0.05$). The unbroken curve drawn is the best-fit line to the points in Figure 2, assuming that the promethazine-sensitive binding of [^{3}H]-mepyramine is the sum of a saturable component and a component increasing linearly with concentration. The linear component is represented by the broken line.

to histamine. It thus seems probable that the response of rat longitudinal muscle strips to histamine is not mediated by H_1-receptors and may well involve prostaglandin intermediates.

Binding of [^{3}H]-mepyramine to homogenates of rat intestinal smooth muscle

The absence of any clear H_1 contractile response in rat longitudinal muscle strips suggests, making the simplest assumption, that there are few H_1-receptors and hence that there should be little promethazine-sensitive binding of [^{3}H]-mepyramine, unless non-receptor sites are present.

The amount of promethazine-sensitive binding of [^{3}H]-mepyramine to a membrane fraction prepared from longitudinal muscle strips from rat intestine by the same procedure as that for rat brain was small and was very similar whether measurement was made on a freshly prepared homogenate or one stored frozen overnight (Figure 6). The proportion of the total binding of [^{3}H]-mepyramine insensitive to 2×10^{-6} M promethazine was high and ranged up to 90% at 32 nM [^{3}H]-mepyramine. The promethazine-sensitive binding gives no indication of any saturation (Figure 6) and the points appear to fit reasonably well, at least up to 16 nM [^{3}H]-mepyramine, to a simple linear relationship, which could represent the foot of a low affinity, high capacity binding curve. These characteristics are also those expected for the low-affinity non-receptor component which seems, on the evidence presented above, to be present in [^{3}H]-mepyramine binding to rat brain homogenates and the curve through the combined promethazine-sensitive binding data from rat brain (unbroken line, Figure 6) has been fitted assuming that the binding can be described by the sum of a high-affinity saturable site and a linear component. The best-fit parameters with their estimated errors (weighted non-linear regression) are: K_d: 10 ± 6 nM; capacity, 176 ± 124 pmol/g protein (both for the saturable component) and 2.8 ± 3.0 pmol g^{-1} protein nM^{-1} for the slope of the linear component. The limitations of the experimental data for fitting this three parameter system are indicated by the size of the estimated errors, but it is notable that the linear component (broken line, Figure 6) is a reasonably good fit to the rat muscle points and that the best fit value of K_d for the saturable site in rat brain, 10 nM, corresponds well with the value, 9 nM, determined from mepyramine inhibition of [^{3}H]-mepyramine binding.

The amount of promethazine-sensitive [^{3}H]-mepyramine binding to rat intestinal muscle is much less than to an equivalent membrane fraction from guinea-pig intestinal smooth muscle, where the mean H_1-receptor binding capacity from 14 measurements was 184 ± 23 pmol/g protein, with a K_d of 0.8 nM. To obtain some evidence that the low level of promethazine-sensitive [^{3}H]-mepyramine binding in rat muscle is not simply a consequence of excessive proteolysis, the binding of [^{3}H]-quinuclidinyl benzilate ([^{3}H]-QNB), a muscarinic receptor ligand (Yamamura & Snyder, 1974), was measured simultaneously on one of the rat muscle preparations. The binding of 7.7 nM [^{3}H]-QNB sensitive to 1 μM methylatropinium, 451 ± 37 pmol/g protein, was less than a factor of 2 different from the amount of methylatropinium-sensitive [^{3}H]-QNB binding in guinea-pig muscle, 823 ± 52 pmol/g protein (4 measurements). This result is comparable with our earlier observations that the number of muscarinic receptors labelled by [^{3}H]-propylbenzilylcholine mustard in intact longitudinal muscle strips is very similar in guinea-pig and rat (Taylor, Cuthbert & Young, 1975) and is in line with the similar sensitivities of both muscles to muscarinic agonists.

Inhibition of [^{3}H]-mepyramine binding in rat brain by other H_1 antagonists

In addition to mepyramine, we have examined a limited number of other H_1 antagonists as inhibitors

of [^{3}H]-mepyramine binding to rat brain membranes. A [^{3}H]-mepyramine concentration of 1 nM was employed in order to make the measurements strictly comparable with those reported previously in guinea-pig brain (Hill *et al.*, 1978), even though in the rat this means working at low occupancy with the attendant problem of low counts. The potency of the inhibitors examined is given (Table 1) in terms of the IC_{50} and the apparent dissociation constant in each species. The calculation of the dissociation constants has been made on the assumption that the antagonist and [^{3}H]-mepyramine compete for a single set of high-affinity binding sites, although examination of the slopes of inhibition curves in guinea-pig brain suggests that this assumption may not be justified for all antagonists (Hill *et al.*, 1978). The large experimental error inherent in the measurements on rat brain at low [^{3}H]-mepyramine occupancy, at which the high-affinity sites will be preferentially labelled, has made it difficult to test the assumption of homogeneity of these sites, but it is interesting to note that the best fit value of the Hill coefficient to the mepyramine inhibition curve shown in Figure 4, is 0.65 ± 0.15, similar to the value 0.73 ± 0.10 observed in the guinea-pig (Hill *et al.*, 1978). The constants calculated must consequently be regarded as apparent affinities, but the values obtained (Table 1) indicate that because the receptor occupancy is apparently much lower in the rat than the guinea-pig the difference in affinities between the two species will be greater than the difference in IC_{50} values.

Clearly not all H_1 antagonists have a much lower affinity in the rat than in the guinea-pig, as inhibitors of [^{3}H]-mepyramine binding. Promethazine is practically equipotent in the two species and the difference with chlorpromazine and histamine is small. (+)-Chlorpheniramine is an order of magnitude less potent in the rat than the guinea-pig, but shows the same stereospecificity in both animals.

Regional distribution of [^{3}H]-mepyramine binding in rat brain

The near equipotency of promethazine as an inhibitor of [^{3}H]-mepyramine binding in rat and guinea-pig and the stereospecificity of chlorpheniramine gives some ground for identifying sites bound preferentially by low concentrations of [^{3}H]-mepyramine in rat brain as H_1-receptors and consequently for believing that measurement of the binding of [^{3}H]-mepyramine in different brain regions reflects the distribution of H_1-receptors. Measurements were made with 2 nM [^{3}H]-mepyramine to minimize binding to the non-receptor sites apparently revealed at higher concentrations, but working at low occupancy does have the disadvantage that if the affinity of [^{3}H]-mepyramine varies between brain regions, then comparison of the amounts bound at 2 nM [^{3}H]-mepyramine will not reflect accurately the relative binding capacities of different regions. The distribution observed is set out in Table 2.

Discussion

There is a marked species variation in almost all aspects of the histamine system and on the evidence presented above this must be extended to include the structure of [^{3}H]-mepyramine binding sites and their regional distributions in brain.

The difference in [^{3}H]-mepyramine binding site structure between rat and guinea-pig is demonstrated most clearly by the lower affinity of mepyramine in the rat and this raises the question whether the high-affinity binding sites in rat brain can be identified as H_1-receptors. In the absence of an affinity constant for mepyramine derived from antagonism of some physiological response requiring H_1-receptor function, only indirect evidence is available. Promethazine

Table 1 Inhibition of [^{3}H]-mepyramine binding in rat brain

	IC_{50} (M)		*Apparent* K_d (M)	
	Guinea-pig	*Rat*	*Guinea-pig*	*Rat*
Mepyramine	1.8×10^{-9}	1.0×10^{-8}	8.3×10^{-10}	9.1×10^{-9}
Promethazine	3.1×10^{-9}	2.0×10^{-9}	1.4×10^{-9}	1.8×10^{-9}
(+)-Chlorpheniramine	1.8×10^{-9}	1.0×10^{-8}	8.3×10^{-10}	9.1×10^{-9}
(−)-Chlorpheniramine	4.4×10^{-7}	5.5×10^{-7}	2.0×10^{-7}	5.0×10^{-7}
Chlorpromazine	5.1×10^{-9}	7.9 ± 10^{-9}	2.3×10^{-9}	7.1×10^{-9}
Methapyrilene	1.0×10^{-8}	2.5×10^{-8}	4.5×10^{-9}	2.3×10^{-8}
Histamine	7.6×10^{-5}	6.3×10^{-5}	3.4×10^{-5}	5.6×10^{-5}

The IC_{50} is the concentration of inhibitor required for 50% inhibition of the receptor-specific binding of 1 nM [^{3}H]-mepyramine. The incubation conditions, method of definition of receptor-specific binding and calculation of dissociation constants are given under Methods. The data for guinea-pig brain are taken from Hill *et al.* (1978).

is practically equipotent in the two species and chlorpheniramine shows stereospecificity in the rat as in the guinea-pig, even though in the rat the potency of the (+)-isomer is a tenth of that in the guinea-pig. It thus seems likely, although the evidence is very limited, that high affinity [^{3}H]-mepyramine binding sites in the rat can be identified as H_1-receptors. The apparent affinity of histamine itself for the receptor is similar in both species and although the value obtained is difficult to interpret in the absence of an understanding of receptor mechanisms, the equipotency suggests that functionally the H_1-receptors in rat brain need be no less sensitive to the natural agonist than those of the guinea-pig.

The determination of an accurate binding affinity for [^{3}H]-mepyramine in the rat is complicated by the likely presence of promethazine-sensitive, but non-receptor, binding. Two pieces of evidence point to the presence of such a component. First, the binding of [^{3}H]mepyramine to brain membranes is not obviously saturable in the range over which it has been measured and estimates of both K_d and the maximum binding capacity appear to increase the further the curve is taken to higher concentrations of [^{3}H]-mepyramine. Second, fitting a single hyperbola to the combined data from four experiments gives a K_d of 16 nM (average of the values from the three methods of analysis), somewhat lower than the mean estimate from individual curves, 27 nM, but still higher than the value, 9 nM calculated from the inhibition of the binding of 1 nM [^{3}H]-mepyramine by non-radioactive mepyramine. At 1 nM [^{3}H]-mepyramine binding will be preferentially to high affinity sites and consequently the value, 9 nM, obtained in this way should be a more reliable estimate of the K_d for the presumed H_1-receptor. It is striking that this value is closely similar to the best-fit value of K_d, 10 nM, obtained by fitting a two component model to the combined binding data.

The only piece of evidence that might seem to be at variance with the presence of secondary binding sites is that Scatchard plots of the promethazine-sensitive binding in rat brain are not obviously non-linear. However, inspection of Scatchard plots of binding data generated using the best-fit parameters to the two-component model reveals that non-linearity only becomes obvious at concentrations of [^{3}H]-mepyramine above about 15 nM and if the uncertainty associated with each point is not small then the deviation from linearity will not be readily apparent. With guinea-pig brain, where the affinity of mepyramine is nearer 1 nM and where binding curves are consequently not usually measured above 10 to 15 nM [^{3}H]-mepyramine, a second promethazine-sensitive site, presuming it existed, would not be apparent.

In the absence of any indication of the nature of the low-affinity promethazine-sensitive [^{3}H]-mepyramine binding sites in rat brain, there is no certainty that similar sites should be found in other tissues. However, it seems likely that much, if not all, of the rela-

Table 2 Regional distribution of [^{3}H]-mepyramine binding sites in rat and guinea-pig brain

	[^{3}H]-mepyramine bound (pmol/g protein)		Mean ratio relative to posterior cortex	
	Rat 1	*Rat 2*	*Rat*	*Guinea-pig**
Amygdala	70 ± 5	17 ± 3	1.2	0.9
Hypothalamus	61 ± 6	14 ± 1	1.0	1.5
Posterior cortex	56 ± 3	15 ± 1	1.0	1.0
Thalamus	34 ± 6	14 ± 1	0.8	1.3
Brain stem	37 ± 4	14 ± 1	0.8	0.4
Colliculi	40 ± 5	11 ± 2	0.7	1.6
Anterior cortex	38 ± 4	11 ± 2	0.7	1.1
Hippocampus	42 ± 7	9 ± 2	0.7	1.3
Caudate nucleus	37 ± 9	5 ± 2	0.5	0.4
Cerebellum	23 ± 6	6 ± 1	0.4	2.5
Spinal cord	25 ± 5	N.S.	0.2	0.5

Values are means ±s.e. of the binding of 2 nM [^{3}H]-mepyramine sensitive to inhibition by 2×10^{-6} M promethazine. Measurements were made on unfractionated homogenates in two independent experiments. Note that since the apparent dissociation constant for [^{3}H]-mepyramine binding in rat brain is 9.1 nM (Table 1), the fractional occupancy of receptor sites by 2 nM [^{3}H]-mepyramine will be approx 0.2 and consequently values given need to be multiplied by a factor of 5 to obtain an estimate of the maximum binding capacity. This estimate cannot be compared directly with the capacity determined from binding curves, since the proportion of the total protein sedimenting in the last stage of the assay will differ widely between the two preparations.

* Data taken from Hill *et al.* (1978).

tively low levels of promethazine-sensitive [^{3}H]-mepyramine binding to the particulate fraction from rat intestinal smooth muscle could be accounted for by such non-receptor sites. This would be consistent with our failure to find any evidence for an H_1 response in this muscle and it is notable, though not conclusive, that the linear non-receptor binding component extracted from the brain binding data fits the experimental muscle points quite well.

The apparent affinities of H_1 antagonists in rat brain determined from inhibition of [^{3}H]-mepyramine binding are in broad agreement with those reported by Snyder's group (Chang *et al.*, 1978; Tran *et al.*, 1978), who have independently come to the conclusion that histamine H_1-receptors in rat brain differ from those in the guinea-pig (Chang, Tran & Snyder, 1979). The differences in preparation of the membrane fraction and the method of assay could well explain, at least in part, the rather lower proportion of H_1 antagonist-insensitive binding in their experiments. They reported no evidence for secondary binding sites, but since their binding curve was extended only to 8 nM [^{3}H]-mepyramine these would not have been apparent.

In view of the weak response of peripheral tissues of the rat to H_1 actions of histamine, the apparent presence in rat brain of appreciable numbers, approx 180 pmol/g protein, of H_1-receptors is particularly interesting, although what function they might perform remains unknown. Much attention has been given to a neurotransmitter role for histamine (reviewed by Schwartz, 1977), but there are few indications in any species of actions mediated by H_1-receptors. The best evidence seems to be the inhibition of a component of the histamine-sensitive adenylate cyclase from guinea-pig hippocampal slices by mepyramine and other H_1 antagonists (Palacios, Garbarg, Barbin & Schwartz, 1978), but the affinity constants reported are all somewhat lower than the values deduced from inhibition of the contractile response of guinea-pig ileum. In the rat the evidence for H_1 actions of histamine is sparse. Hegstrand, Kanoff & Greengard (1976) found little evidence for a histamine-stimulated adenylate cyclase in the areas of rat brain they examined and the high concentrations of mepyramine necessary to inhibit the histamine-sensitive adenylate cyclase from rat hypothalamus (Portaleone, Pagnini, Crispino & Genazzani, 1978) make it doubtful whether this is an H_1 action. However, in view of the evidence for an H_1-sensitive histamine-stimulated guanylate cyclase in mouse neuroblastoma cells, K_i for mepyramine 2 nM (Richelson, 1978), it would be interesting to know whether a similar enzyme is present in the brain of the rat and other species.

The regional distribution of [^{3}H]-mepyramine binding sites in rat brain is similar to that reported by Chang *et al.* (1978), but differs from that in the guinea-pig (Hill *et al.*, 1978; Tran *et al.*, 1978), most strikingly in the very much smaller numbers of H_1-receptors in rat cerebellum (mean value, 15 pmol/g protein specific binding of 2×10^{-9} M [^{3}H]-mepyramine in rat cerebellum (Table 1), compared with approximately 150 pmol/g protein in guinea-pig cerebellum, Hill *et al.*, 1978). In both species some of the receptor material may be associated with vascular tissue, but even in the rat, where the distribution is more even than in the guinea-pig, the difference in receptor numbers between amygdala or hypothalamus and spinal cord or cerebellum makes it very unlikely that the differences could merely reflect differing degrees of vascularization.

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