Biochemical Pharmacology, Vol. 29, pp. 2269-2272.

0006-2952/800815-2269$02.00/0

SEPARATE BINDING SITES FOR HISTAMINIC DRUGS IN RAT CEREBRAL CORTEX

Stephen I. Kandel,* Glenn H. Steinberg,* James W. Wells,*
Marianne Kandel† and Allan G. Gornall†

*Faculty of Pharmacy and †Department of Clinical Biochemistry,
University of Toronto, Toronto, Ontario, Canada M5S 1A1

(Received 11 February 1980; accepted 3 June 1980)

Saturable binding in brain has been reported for the H_1 antihistamine [^{3}H]mepyramine in several mammalian species [1-4], the H_2 antihistamine [^{3}H]cimetidine in guinea pig [5], and [^{3}H]histamine itself in rat [6]. A comparison of the sites occupied by these probes is rendered difficult, however, by differences both in species and in experimental conditions among the different laboratories. Comparative studies with mepyramine, for example, have shown that a seven-fold difference in affinity exists between guinea pig and rat [3,4]. To probe the relationships among these sites, we have measured the binding of all three radiolabelled drugs in the same subcellular fraction from rat cerebral cortex. The results indicate that each site is distinct and raise the possibility that H_2 antihistaminic effects do not arise from simple occlusion of a receptor otherwise accessible to an agonist.

MATERIALS AND METHODS

All assays were carried out on a crude synaptosomal preparation similar to that described elsewhere [7]: P_2 pellets were prepared from the pooled cerebral cortices of 20-40 male rats (Charles River Wistar, 176-225 g) and suspended in a modified Krebs-Henseleit buffer at pH 7.5. Protein concentration was estimated by the Lowry method using bovine serum albumin as the standard. Radioligands were purchased from either New England Nuclear ([^{3}H]-mepyramine, 28.5 and 27.0 Ci/mmol; [^{3}H]histamine, 8.13 and 8.80 Ci/mmol) or Amersham Corporation ([^{3}H]cimetidine, 17.0 Ci/mmol).

Binding was measured as described by Birdsall *et al.*[7]; reaction mixtures containing the drugs and the synaptosomal suspension (1.0-1.7 mg protein per ml) were allowed to equilibrate for 45 min at 30° and assays were performed in quintuplicate. In competitive experiments, the specific component of binding constituted approximately 20% of total binding for [^{3}H]histamine, 26% for [^{3}H]cimetidine, and 60% for [^{3}H]mepyramine at the concentrations of radioligand used. Full inhibition of the specific component was taken as a region of the competition profile in which binding was independent of the concentration of unlabelled ligand. Concentrations in this region were chosen to define the non-specific component when binding was measured directly; that is, when the amount of radiolabelled ligand was varied. For each radiolabelled probe, the ratio of specific to non-specific binding was the same with several unlabelled histaminic drugs. Free and bound radioactivity were separated by rapid filtration through fibreglass filters (Whatman GF/F) in competitive experiments with [^{3}H]-mepyramine, and by microcentrifugation in all other experiments.

Direct binding curves typically contained 10-14 data points and competition curves 14-20 data points; good fits of the data were obtained with a rectangular hyperbola and the Hill equation, respectively. Standard errors on each point generally were less than 2% with [^{3}H]-histamine and [^{3}H]mepyramine, and less than 1% with [^{3}H]cimetidine. Parametric values were estimated using the non-linear, iterative procedure of Marquardt [8].

RESULTS AND DISCUSSION

Two experimental approaches have been used to confirm that the binding data are internally consistent for the three ligands available in tritiated form. With [^{3}H]mepyramine and [^{3}H]histamine, good agreement is obtained between affinities measured directly (K_d, Table 1) and by dilution with the non-radiolabelled analogue (K_c, Table 2). For these two ligands, specific binding was measurable at concentrations of the radiolabel sufficient to occupy more than 60% of the sites. Capacity thus was determined simultaneously with affinity when fitting a rectangular hyperbola to the direct binding data. With [^{3}H]cimetidine, however, the relatively low affinity precludes direct measurements above 45% occupancy and restricts competitive studies to lower levels where the ratio of specific to total binding is more favourable. The affinity of this drug thus was determined from cimetidine/[^{3}H]cimetidine competition profiles obtained at two concentrations of the radioligand: 2.2 nM and 23 nM. Assuming only that binding is to a single population of sites, the fitting procedure yielded a value of 6.86 for $-\log K_d$ (Table 1) that was in excellent agreement with all of the data. The capacity for cimetidine then was determined by assigning this value to $-\log K_d$ when fitting a rectangular hyperbola to the direct binding data. With all three probes, competitive profiles obtained by dilution with the unlabelled analogue were characterised by Hill coefficients near one.

Several lines of evidence indicate that the three probes label different sites in the suspension. (a) Whereas absolute capacities differed markedly among groups of 20-40 animals (Table 1), relative capacities within any particular preparation yielded a constant ratio of approximately 1:2:11 for [^{3}H]histamine, [^{3}H]mepyramine, and [^{3}H]cimetidine, respectively. (b) A series of histaminic drugs including three antagonists, six agonists, and histamine itself all exhibit different competitive potencies *versus* each of the three, radiolabelled probes (Table 2). (c) The affinities of histamine, mepyramine, and cimetidine permit each site to be saturated selectively; that is, each of the three drugs can be added at a concentration that saturates its site of highest affinity while occupying only a negligible fraction of the other two sites. A series of competitive experiments has demonstrated that the affinity of any one drug measured via the progressive dilution of its radiolabelled analogue is not affected by selective saturation of either of the other two sites (data not shown).

Good agreement exists for H_1 antihistamines between binding affinity at the [^{3}H]-mepyramine site and H_1 pharmacological potency in the guinea pig ileum [1,2]. Similarly, the affinities of cimetidine and metiamide at the [^{3}H]cimetidine site recall their H_2 pharmacological potencies in the guinea pig right atrium (Table 2). Among H_1 agonists, however, there is no apparent agreement at any of the three sites between binding affinity and H_1 pharmacological potency in the ileum, although the similarity of affinities introduces some uncertainty into the rank order (Table 2). At the [^{3}H]mepyramine site, for example, there is only a 1.2-fold difference in K_c between 2-pyridylethylamine and 2-methylhistamine. In contrast, H_2-active agonists apart from histamine exhibit a strong correlation ($P < 0.05$) and a marked numerical similarity between affinity at the [^{3}H]histamine site and H_2 pharmacological potency in the atrium (Table 2). No such agreement is found for these agonists at either of the other two sites. With the H_2 antagonists cimetidine and metiamide, affinities measured at the [^{3}H]-histamine site are 6-26 times lower than reported pharmacological potencies in the atrium (Table 2).

The present data describe a paradox wherein agreement between binding affinity and H_2 pharmacological potency occurs at one site for H_2 antagonists and at a separate and mutually independent site for H_2 agonists. No interconversion of these sites has been observed under the conditions of the binding studies. While this pattern appears to argue in favour of

Table 1. Direct binding of histaminic ligands*

[^{3}H]Ligand	Capacity† (pmol/g protein)	$-\log K_d$
[^{3}H]Histamine	39 ± 3 to 84 ± 8 (5)	7.84 ± .04 (5)
[^{3}H]Mepyramine	74 ± 7 to 154 ± 8 (4)	8.43 ± .09 (4)
[^{3}H]Cimetidine	592 ± 21 to 918 ± 27 (3)	6.86 ± .04

* Concentrations of unlabelled ligands used to define non-specific binding were as follows: 2 μM triprolidine or promethazine with [^{3}H]-mepyramine, 32 μM metiamide or cimetidine with [^{3}H]cimetidine, 10 μM histamine with [^{3}H]histamine. Parametric values were determined as described in the text and are presented as mean ± S.E.M. The number of independent experiments is shown in parentheses. Where the number of experiments exceeds one, the error reflects the variability among experiments; where only one experiment was made, the error reflects the fit of the model to the data.

† Values indicate the range found for the number of experiments shown.

Table 2. Competitive binding and reported pharmacological activity of histaminic ligands*

Ligand	$-\log K_c$†			$-\log EC_{50}$	
	[^{3}H]Mepyramine	[^{3}H]Cimetidine	[^{3}H]Histamine	Guinea pig atrium	Guinea pig ileum
Histamine	3.68 ± .16 (1)	3.76 ± .05 (1)	8.15 ± .08 (8)	5.96 [9]	7.25§
Mepyramine	8.19 ± .08 (3)	3.25 ± .05 (1)	5.18 ± .34 (2)		
Cimetidine	3.25 ± .08 (1)	6.74 ± .08 (4)	4.89 ± .22 (2)	6.53‖	
Metiamide	3.14 ± .10 (1)	6.47 ± .09 (1)	5.44 ± .35 (2)	6.19¶	
2-Methylhistamine	3.90 ± .07 (1)	3.29 ± .07 (1)	4.94 ± .26 (3)	4.70 [9]	6.47§
2-Thiazolylethylamine	3.85 ± .05 (1)	<3.0 (1)	4.07 ± .08 (2)	**	6.67§
2-Pyridylethylamine	3.81 ± .05 (1)	3.10 ± .14 (1)	4.34 ± .27 (2)	**	6.00§
4-Methylhistamine	3.10 ± .14 (1)	3.87 ± .09 (1)	5.43 ± .03 (2)	5.51 [9]	4.61§
Impromidine	4.71 ± .07 (1)	5.75 ± .06 (1)	7.48 ± .10 (2)	7.60 [10]	<3.0 [10]
Dimaprit	3.52 ± .08 (1)	<3.0 (1)	6.07 ± .15 (5)	5.74 [11]	††

* Concentrations of the radiolabelled ligand in competitive binding experiments were as follows: [^{3}H]mepyramine, 0.7-0.9 nM; [^{3}H]cimetidine, 2-23 nM; [^{3}H]histamine, 5-10 nM. Further experimental details are described in the text and in the legend to Table 1.

† The molar concentration of unlabelled ligand required to inhibit the specific component of binding by 50% was obtained by fitting the Hill equation to the competition profile; this value was corrected by the factor $(1+[[^3H]Ligand]/K_d)$ to yield K_c.

§ Potency was calculated from the reported percentage activity [12] relative to that of histamine. The value shown for histamine represents the mean (S.E.M. = 0.20) from five published dose-response curves [13,14].

‖ Value of pA_2 measured against impromidine [10].

¶ Value of pA_2 measured against dimaprit [11].

** Reported to exhibit less than 1% the activity of histamine [12].

†† Reported to exhibit less than 0.0001% the activity of histamine [11].

separate sites for agonists and antagonists in controlling H_2 responses *in vivo*, it must be noted that a correlation does not in itself establish that a binding site is indeed a receptor. Caution particularly is required in the case of agonists and when the comparisons are between observations in different tissues. Moreover, histamine itself exhibits a range of pharmacological potencies, often the result of both H_1 and H_2 effects, that generally fall between its binding affinities at the [^{3}H]histamine and the [^{3}H]cimetidine sites.

Acknowledgements: The authors are grateful to the following companies for their generosity in providing histaminic drugs: Burroughs Wellcome Limited (triprolidine), Merck Frosst Laboratories (mepyramine), Smith Kline and French Canada Limited (cimetidine, metiamide, impromidine, dimaprit, 4-methylhistamine, 2-methylhistamine, 2-thiazolylethylamine, 2-pyridylethylamine), and Poulenc Limited (promethazine). Miss Marybeth Chen is thankfully acknowledged for typing the manuscript. This investigation was supported by the Medical Research Council of Canada (Grants MT3057 and MT369) and by the Banting Research Foundation. J.W.W. is a Scholar of the Canadian Heart Foundation.

REFERENCES

1. S.J. Hill, P.C. Emson and J.M. Young, *J. Neurochem.* 31, 997 (1978).
2. V.T. Tran, R.S.L. Chang and S.H. Snyder, *Proc. natn. Acad. Sci. U.S.A.* 75, 6290 (1978).
3. R.S.L. Chang, V.T. Tran and S.H. Snyder, *J. Neurochem.* 32, 1653 (1979).
4. S.J. Hill and J.M. Young, *Br. J. Pharmac.* 66, 93P (1979).
5. W.P. Burkard, *Eur. J. Pharmac.* 50, 449 (1978).
6. J.-M. Palacios, J.-C. Schwartz and M. Garbarg, *Eur. J. Pharmac.* 50, 443 (1978).
7. N.J.M. Birdsall, A.S.V. Burgen, E.C. Hulme and J.W. Wells, *Br. J. Pharmac.* 67, 371 (1979).
8. D.L. Marquardt, *J. Soc. indust. appl. Math.* 11, 431 (1963).
9. J.W. Black, W.A.M. Duncan, C.J. Durant, C.R. Ganellin and E.M. Parsons, *Nature* 236, 385 (1972).
10. G.J. Durant, W.A.M. Duncan, C.R. Ganellin, M.E. Parsons, R.C. Blakemore and A.C. Rasmussen, *Nature* 276, 403 (1978).
11. M.E. Parsons, D.A.A. Owen, C.R. Ganellin and G.J. Durant, *Agents and Actions* 7, 31 (1977).
12. G.J. Durant, C.R. Ganellin and M.E. Parsons, *J. med. Chem.* 18, 905 (1975).
13. D.A. Cook, *Br. J. Pharmac.* 43, 197 (1971).
14. T.P. Kenakin and D.A. Cook, *Can. J. Physiol. Pharmacol.* 54, 386 (1976).