Biochemical Pharmacology, Vol. 38, No. 13, pp. 2175–2180, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00
Pergamon Press plc

HIGH AFFINITY, SATURABLE [^{3}H]MEPYRAMINE BINDING SITES ON RAT LIVER PLASMA MEMBRANE DO NOT REPRESENT HISTAMINE H_1-RECEPTORS

A WARNING

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(*Received* 7 *October* 1988; *accepted* 4 *January* 1989)

Abstract—Rat liver plasma membrane contains a saturable, high affinity binding site for the labelled histamine H_1-antagonist [^{3}H]mepyramine. Nonlinear regression analysis of the performed saturation experiments revealed an equilibrium dissociation constant (K_d) of 7.7 ± 0.4 nM and a maximal binding capacity (B_{max}) of 70.4 ± 9.5 pmol/mg protein. Specific binding could be inhibited completely by several histaminergic ligands. However, the affinities of the tested H_1-antagonists other than mepyramine for this binding site were quite low and the known stereospecificity displayed by the histamine H_1-receptor for the enantiomers of chlorpheniramine and pheniramine was not found. Moreover, the H_2-selective agonist 4-methylhistamine (K_i = 412 μM) was even more potent than its H_1-selective 2-methylderivative (K_i = 772 μM). Since several ethylenediamines were also very potent in displacing [^{3}H]mepyramine we suggest the presence of an ethylenediamine recognition site on rat liver plasma membrane which is unrelated to the histamine H_1-receptor. It is stressed that a proper pharmacological characterization of a reported binding site is needed, since we show in this study that [^{3}H]mepyramine, which is frequently used in studies concerning the H_1-receptor, labels non-H_1-receptor binding sites in rat liver plasma membrane.

Histamine is one of the physiological mediators, which is possibly implicated in the regulation of the metabolic activity of the liver. Recently, Garcia-Sainz *et al.* showed that histamine stimulated three major metabolic pathways (glycogenolysis, gluconeogenesis and ureagenesis) in isolated rat hepatocytes [1]. These effects were suggested to be mediated via both the Ca^{2+}-mobilizing H_1- and the c-AMP generating H_2-receptor, since the effects were partially stimulated by the H_1-agonist 2-thiazolylethylamine and the H_2-agonist impromidine and partially inhibited by the H_1-antagonist mepyramine and the H_2-antagonist cimetidine.

Another approach for investigating receptor-mediated processes is the use of labelled compounds to study drug–receptor interaction or to study alterations in receptor densities and/or affinities after processes like desensitization or membrane damage. However, as recently stated by Laduron, receptor binding has led to a controversial multiplication of receptor subtypes [2]. He summarized several criteria for receptor sites. Besides a high affinity, a reversibility, a saturability, a stereospecificity and a displacement of binding with drugs of a different chemical class, a functional correlation between binding data and pharmacological activity is an absolute requirement for a receptor site [2].

The most commonly used radioligand for the H_1-receptor is [^{3}H]mepyramine. In 1977 Hill *et al.* described saturable, high affinity binding of [^{3}H]mepyramine to H_1-receptors in homogenates of the longitudinal muscle preparation of guinea-pig ileum [3]. The specific binding could be displaced by several non-labelled H_1-antagonists and data obtained in these studies showed a good correlation with results obtained from isolated organ studies. In radioligand binding studies concerning the H_1-receptor [^{3}H]mepyramine is currently considered as first choice as radiolabel.

Previously Imoto *et al.* [4] showed the presence of high affinity, saturable binding of [^{3}H]mepyramine and [^{3}H]cimetidine to rat liver plasma membrane preparation. According to the authors these observations suggested the presence of both H_1- and H_2-receptors on rat liver plasma membranes. A remarkable result of this study was the huge density (pmol amounts/mg protein) of [^{3}H]mepyramine binding sites in this preparation. Thereafter the same group of investigators extended the study of [^{3}H]mepyramine binding to isolated rat hepatocytes and found again a saturable, high affinity [^{3}H]mepyramine binding site with an extremely high maximal binding capacity [5]. However, in both studies a proper pharmacological identification of this [^{3}H]mepyramine binding site was not provided [4, 5].

In the present study we identify this [^{3}H]mepyramine binding-site on rat liver plasma membranes as a non-H_1-receptor site, using stereoselective H_1-antagonists, several mepyramine analogues and selective histaminergic agonists.

MATERIALS AND METHODS

Chemicals

Mepyramine hydrochloride and triprolidine

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hydrochloride were purchased from Sigma Chemical Co. Ltd (St Louis, MO, U.S.A.). Histamine dihydrochloride was obtained from Janssen Pharmaceutica (Beerse, Belgium). 2-Methylhistamine dihydrochloride and 4-methylhistamine dihydrochloride were gifts of Smith Kline & French (Herts, U.K.). The stereoisomers of chlorpheniramine maleate and pheniramine maleate were gifts from Dr A. Beld (K. U. Nijmegen, the Netherlands). VUF 7621, 7628 and 7658 (all hydrochlorides) were synthesized by Dr H. J. Eggelte as essentially described by Hutrer *et al.* [6]. This synthesis was slightly modified by using NaH instead of $NaNH_2$ as base. Identification of the compounds was performed using a Varian EM360 60 MHz NMR and a Finnigan MAT (MAT90) mass spectrometer:

N-*phenyl*- N- (4-*methoxybenzyl*)- N',N'- *dimethylethylenediamine hydrochloride, VUF* 7621. Yield: 81%, m.p.: 184.5–186.0°, ^{1}H NMR ($CDCl_3$, NaOH/D_2O): δ = 2.25 ppm (singlet, N'-$\underline{CH_3}$, 6H), δ = 2.53 ppm (triplet, J = 7.0 Hz, $\underline{CH_2}$-N'$(CH_3)_2$, 2H), δ = 3.50 ppm (triplet, J = 7.0 Hz, $\underline{CH_2}$-*N*-phenyl-*N*-(4-methoxybenzyl), 2H), δ = 3.73 ppm (singlet, O$\underline{CH_3}$, 3H), δ = 4.45 ppm (singlet, $\underline{CH_2}$-4-methoxyphenyl, 2H), δ = 6.43–7.27 ppm (multiplet, phenyl- + benzyl H, 9H). Base *m/e*: calculated: 284.189; found: 284.184.

N-*phenyl*- N- (4-*methoxyphenyl*)- N',N'-*dimethylethylenediamine hydrochloride, VUF* 7628. Yield: 60%, m.p.: 202.0–203.5°, ^{1}H NMR ($CDCl_3$, NaOH/D_2O): δ = 2.25 ppm (singlet, N'-$\underline{CH_3}$, 6H), δ = 2.53 ppm (triplet, J = 7.0 Hz, $\underline{CH_2}$-N'$(CH_3)_2$, 2H), δ = 3.73 ppm (triplet, J = 7.0 Hz, $\underline{CH_2}$-*N*-phenyl-*N*-(4-methoxyphenyl), 2H), δ = 3.80 ppm (singlet, O$\underline{CH_3}$, 3H), δ = 6.50–7.30 ppm (multiplet, phenyl H, 9H). Base *m/e*: calculated: 270.173; found: 270.171.

N-(2-*pyridyl*)-N-(4-*methylbenzyl*)-N',N'-*dimethylethylenediamine hydrochloride, VUF* 7658. Yield: 80%, m.p.: 156.0–157.0°, ^{1}H NMR ($CDCl_3$, NaOH/D_2O): δ = 2.23 ppm (singlet, N'-$\underline{CH_3}$, 6H, δ = 2.30 ppm (singlet, $\underline{CH_3}$-benzyl, 3H), δ = 2.50 ppm (triplet, J = 7.0 Hz, $\underline{CH_2}$-N'$(CH_3)_2$, 2H), δ = 3.67 ppm (triplet, J = 7.0 Hz, $\underline{CH_2}$-*N*-(2-pyridyl)-*N*-(4-methylbenzyl), 2H), δ = 4.70 ppm (singlet, $\underline{CH_2}$-4-methylphenyl, 2H), δ = 6.43–7.27 ppm (multiplet, 2-pyridyl + benzyl H, 7H), δ = 8.13 ppm (doublet, J = 4.0 Hz, H_6 2-pyridyl, 1H). Base *m/e*: calculated: 269.189; found: 269.189.

All other reagents were of analytical grade.

Plasma membrane preparation

Male Wistar rats (200–240 g, Harlan CPB, Zeist, the Netherlands) were killed by decapitation. Livers were quickly removed and homogenized in 3 vol. ice-cold 0.25 M sucrose, 50 mM Tris–HCl (pH = 8.0 at 4°) by three 10-sec bursts of a Polytron homogenizer and a subsequent homogenization with a glass–Teflon homogenizer. This homogenate was layered on top of 20 ml 2.0 M sucrose, 50 mM Tris–HCl (pH = 8.0 at 4°) and centrifuged for 60 min at 80,000 *g* at 4°. Plasma membranes were obtained from the interface of the two sucrose solutions and homogenized in 50 mM Tris–HCl, 10 mM $MgCl_2$ (pH = 7.4 at 4°). After centrifugation for 30 min at 80,000 *g* at 4° membranes were homogenized in 50 mM Tris–HCl, 10 mM $MgCl_2$ (pH = 7.4 at 37°), frozen in liquid nitrogen and stored at −80° until further use. Plasma membrane purity was assessed by measuring 5'-nucleotidase- and Na^+/K^+-ATPase activity and usually yielded a four-fold enrichment of enzyme activity (data not shown).

[^{3}H]*Mepyramine binding studies*

Membrane protein (200 μg/ml) was incubated with the radioligand for 20 min at 37° in 50 mM Tris–HCl, 10 mM $MgCl_2$ (pH = 7.4 at 37°) in a total volume of 200 μl. In the saturation experiments increasing concentrations of [^{3}H]mepyramine (0.1–40 nM) were incubated in the presence or absence of 10 μM *l*-chlorpheniramine to determine the non-specific binding. In the displacement studies approximately 1 nM [^{3}H]mepyramine was incubated with increasing concentrations of non-labelled compounds. Incubations were performed in triplicate and started by the addition of 50 μl protein. The incubations were terminated by the addition of 3 ml ice-cold 50 mM Tris–HCl, 10 mM $MgCl_2$ (pH = 7.4 at 4°) and rapid filtration under vacuum through glass filters (Whatman GF/C, Whatman International Ltd, Maidstone, U.K.). The tubes were rinsed once with another 3 ml buffer and the filters were subsequently washed twice with 3 ml buffer. Filters were placed in 20 ml polyethylene scintillation vials, dried for 20 min at 60° whereafter 5 ml Dynagel scintillation fluid (J. T. Baker Chemicals B.V., Deventer, the Netherlands) was added. All samples were counted for 10 min in a Mark III scintillation spectrometer (model 6880, Searle Analytic Inc., Des Plaines, U.S.A.) with an efficiency of 45–50%.

The binding data were evaluated by using the non-linear curve fitting program LIGAND [7] on a Zenith Z-110 microcomputer. This program is based on the first-order mass action law for multiple binding sites and provides a correction for free ligand concentration and non-specific binding. Fits for multiple binding sites were considered significantly best when the P-value for a binding site was smaller than 0.05. Binding data from the displacement studies were evaluated by simultaneous fitting of several independent experiments.

Tissue bath studies

Male guinea-pigs (350–450 g) were killed by a blow on the head. Ileal segments (± 10 mm long) were mounted in 20 ml Krebs-buffer (117.5 mM NaCl, 5.6 mM KCl, 1.18 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.28 mM NaH_2PO_4, 25 mM $NaHCO_3$, 5.5 mM glucose), continuously gassed with 95% O_2–5% CO_2 and maintained at 37°. Contractions were recorded isotonically under 0.4 g tension. Antagonists were equilibrated for 10 min whereafter a new histamine dose–response curve was obtained.

Protein determination

The protein determinations were performed according to Bradford [8], using bovine serum albumin as standard.

RESULTS

When rat liver plasma membranes were incubated

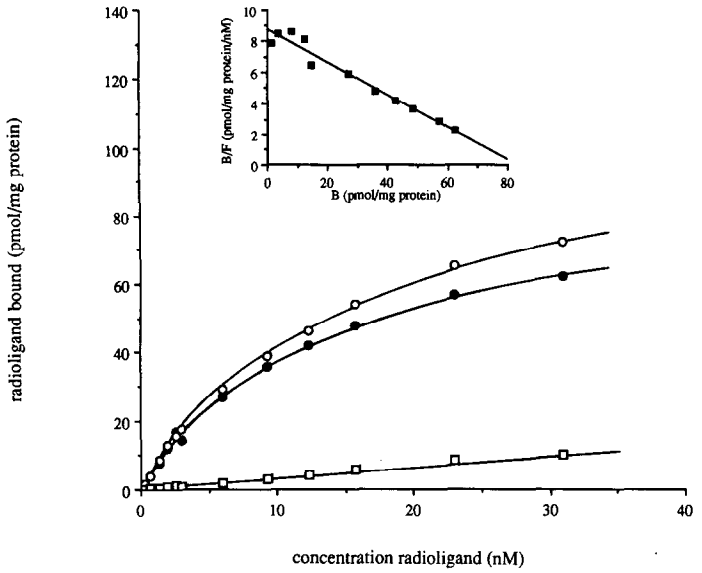


Fig. 1. Saturation experiment for [^{3}H]mepyramine binding to rat liver plasma membrane at 37°. Non-specific binding (□) was determined in the presence of 10 μM *l*-chlorpheniramine. Specific binding (●) was determined by subtracting the non-specific binding from the total binding (○). A typical experiment out of three is shown. Inset shows the transformation of the data into a Scatchard plot.

with increasing amounts of [^{3}H]mepyramine in the absence or presence of 10 μM *l*-chlorpheniramine to define non-specific binding, the obtained specific binding was saturable and showing a high affinity and high capacity for [^{3}H]mepyramine (Fig. 1). Analysis of the binding data by nonlinear regression revealed the presence of a single binding site for [^{3}H]mepyramine with an equilibrium dissociation constant (K_d) of 7.7 ± 0.4 nM and a maximal binding capacity (B_{max}) of 70.4 ± 9.5 pmol/mg protein (SE, N = 3). Fits of the saturation experiments according to a two binding site model were not significantly better ($P > 0.05$). Transformation of the specific binding into a Scatchard plot resulted in a linear relationship (inset Fig. 1) with a K_d-value of 9.3 ± 0.9 nM and a B_{max}-value of 76.1 ± 7.7 pmol/mg protein (SE, N = 3). Analysis of the data using a Hill plot gave a Hill coefficient close to unity (n_H = 0.99 ± 0.01), also indicating an interaction according to a one binding site model.

In order to characterize this [^{3}H]mepyramine high affinity, high capacity binding site on rat liver plasma membranes we performed some displacement studies with several H_1-antagonists and histaminergic H_1- and H_2-agonists. Specific binding of 1 nM [^{3}H]-mepyramine was completely inhibited by the studied H_1-antagonists (Fig. 2A) and the histaminergic agonists (Fig. 2B). Analysis of the displacement curves of both antagonists and agonists revealed Hill-coefficients not significantly different from unity (n_H = 0.91–1.14), indicating an interaction with a single binding site. The IC_{50} values obtained from the simultaneous fit of independent competition experiments were transformed into the corresponding inhibition constants (K_i). Table 1 shows that specific binding of [^{3}H]mepyramine is potently inhibited by non-labelled mepyramine (K_i = 4.9 nM). However, the potent H_1-antagonist triprolidine is less effective in displacing

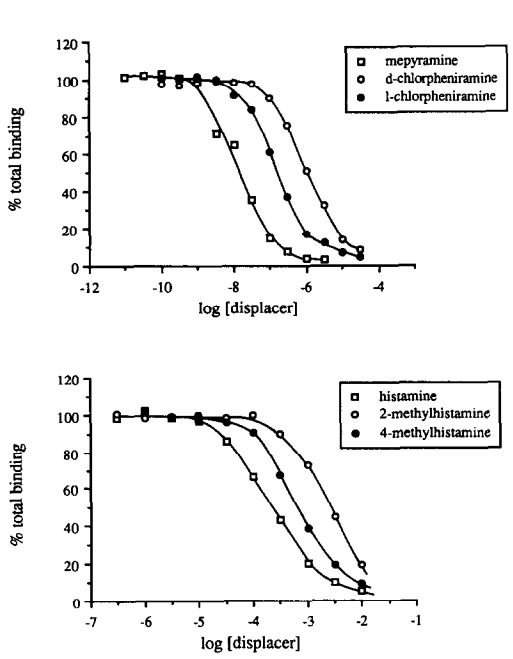
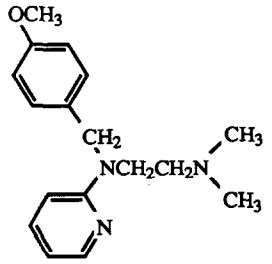


Fig. 2. Displacement of the total binding of 1 nM [^{3}H]mepyramine to rat liver plasma membranes at 37°. (A) Competition experiments of some H_1-antagonists. (B) Data from the displacement studies of some histaminergic agonists. Data are the mean of three independent competition experiments.

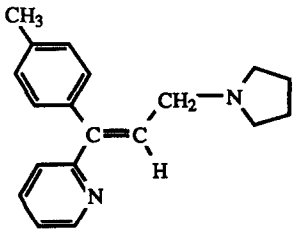
[^{3}H]mepyramine from rat liver plasma membranes (K_i = 208 nM). This is in marked contrast to the activity of triprolidine in other H_1-receptor preparations (Table 1). This discrepancy is not limited to

Table 1. Potencies of various histaminergic drugs for displacing [^{3}H]mepyramine from rat liver plasma membrane (this study), rat brain membranes [9] and porcine tracheal membranes [10] and for inhibiting histamine-induced contraction of guinea-pig ileum [11]

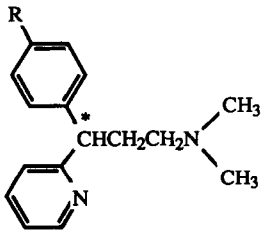
Compound	K_i (nM)			K_B (nM)
	Liver	Brain	Trachea	
Mepyramine	4.9 ± 0.3	4.5	12	0.03
trans-Triprolidine	208 ± 14	5.6	44	0.09
d-Chlorpheniramine	1050 ± 130	8.0	10	0.50
l-Chlorpheniramine	75.8 ± 5	700	3270	31.6
d-Pheniramine	7060 ± 497	—	—	11.0
l-Pheniramine	2050 ± 121	—	—	183
Histamine	123,000 ± 6830	40,000	42,000	—
2-Methylhistamine	772,000 ± 155,000	—	107,000	—
4-Methylhistamine	412,000 ± 51,800	—	1,010,000	—



mepyramine



triprolidine



R = H pheniramine
R = Cl chlorpheniramine

K_i-values (± SD) on liver plasma membrane were determined by simultaneous fitting of three independent experiments and were calculated from the respective IC_{50}-values using the formula $K_i = IC_{50}/(1 + [L]/K_d)$, in which $[L]$ is the concentration [^{3}H]mepyramine and K_d is the dissociation constant of [^{3}H]mepyramine. K_B-values on the guinea-pig ileum were obtained by transformation of the pA_2-values to geometric values.

triprolidine but is also displayed by the other tested H_1-antagonists *d*- and *l*-chlorpheniramine and *d*- and *l*-pheniramine (Table 1, Fig. 2A). Besides their low affinity for this [^{3}H]mepyramine binding site the lack of a proper stereospecificity for the two pair of enantiomers is remarkable. As can be seen in Fig. 2A the *l*-isomer of chlorpheniramine is more potent in displacing [^{3}H]mepyramine than the *d*-isomer, whereas on H_1-receptor preparations *d*-chlorpheniramine is usually a factor 100 more potent than its corresponding *l*-isomer. The same phenomenon is apparent for the two isomers of the analogue pheniramine (Table 1).

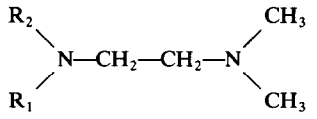
We also tested several structural analogues of mepyramine for their inhibitory activity in the [^{3}H]mepyramine binding assay. All tested compounds were able to displace [^{3}H]mepyramine and the obtained displacement curves all showed Hill-coefficients not significantly different from unity ($n_H = 0.94$–1.11). As can be seen in Table 2 the methyl-analogue of mepyramine (VUF 7658) is very potent in displacing [^{3}H]mepyramine ($K_i = 3.2$ nM). VUF 7621, in which the 2-pyridyl ring of mepyramine is replaced by a phenyl-ring, is also a very active displacer ($K_i = 15.0$ nM), whereas VUF 7628, in which not only the 2-pyridyl ring is replaced by a phenyl-ring but the *p*-methoxybenzyl has been replaced by the corresponding phenyl group, shows a much weaker potency ($K_i = 140$ nM). In case of the mepyramine analogues the potency order for the inhibition of [^{3}H]mepyramine binding to liver plasma membrane is qualitatively the same as for the inhibition of histamine induced contraction of guinea-pig ileum, although it is remarkable that in the case of VUF 7628 a selectivity towards the hepatic [^{3}H]mepyramine binding site is encountered for the first time.

Besides H_1-antagonists, histaminergic agonists were tested too for their inhibitory potency to displace [^{3}H]mepyramine binding. Figure 2B shows the obtained displacement curves for histamine, the H_2-selective agonist 4-methylhistamine and the H_1-selective agonist 2-methylhistamine. It is clear that none of these compounds is very potent in displacing [^{3}H]mepyramine binding; the K_i-values are in the range of 0.1–1 mM (Table 1). Histamine is the most potent of the histamine analogues, whereas the H_2-selective agonist 4-methylhistamine is slightly more potent than 2-methylhistamine.

DISCUSSION

In the present study we investigated the binding characteristics of the previously reported [^{3}H]mepyramine binding site on rat liver plasma membrane. In 1977 [^{3}H]mepyramine was introduced by Hill as a radioligand for the study of the histamine H_1-receptor [3]. The specific binding in homogenate of guinea-pig ileal smooth muscle was found to be saturable, with a high affinity and a good correlation with data from organ studies was found; the estab-

Table 2. Inhibitory potencies of some mepyramine analogues for displacing [^{3}H]mepyramine on rat liver plasma membranes (K_i) and inhibiting histamine-induced contraction of guinea-pig ileum (K_B)



Compound	R_1	R_2	K_i (nM)	K_B (nM)
Mepyramine	2-pyridyl	*p*-methoxybenzyl	4.9 ± 0.3	0.83 ± 0.25
VUF 7658	2-pyridyl	*p*-methylbenzyl	3.2 ± 0.3	0.79 ± 0.26
VUF 7621	phenyl	*p*-methoxybenzyl	15.0 ± 0.8	4.30 ± 1.70
VUF 7628	phenyl	*p*-methoxyphenyl	140 ± 7	1698 ± 435

K_i-values (± SD) on liver plasma membrane were determined by simultaneous fitting of three independent experiments and were calculated from the respective IC_{50}-values using the formula $K_i = IC_{50}/(1 + [L]/K_d)$, in which $[L]$ is the concentration [^{3}H]mepyramine and K_d is the dissociation constant of [^{3}H]mepyramine. K_B-values on the guinea-pig ileum are the mean ± SD of three independent determinations and were obtained by transformation of the pA_2-values to geometric values.

lished stereospecificity of the [^{3}H]mepyramine binding sites towards the isomeric chlorpheniramines in several tissue preparations (e.g. porcine airways [10] and guinea-pig and rat brain [9, 12]) further convinced workers in the histamine research field that [^{3}H]mepyramine was a suitable ligand for labelling histamine H_1-receptors. Currently [^{3}H]mepyramine is the most commonly used radioligand for the H_1-receptor.

In 1985 Imoto *et al.* described the presence of two binding sites for [^{3}H]mepyramine on rat liver plasma membrane [4]; a high affinity (K_d = 4.2 nM) binding site with a remarkably high binding capacity (B_{max} = 4.7 pmol/mg protein) and a secondary, low affinity binding site (K_d = 21.3 nM, B_{max} = 16 pmol/mg protein). Two years later this study was extended to isolated rat hepatocytes by Tsuchie *et al.*, resulting in the confirmation of the results obtained with plasma membrane [5]. Moreover, recently this [^{3}H]-mepyramine binding site was even purified and solubilized from rat liver membranes [13]. In this study only one binding site (K_d = 19 nM) could be solubilized and target size analysis of this binding protein revealed a molecular mass of 162 kDa, confirming the previous results of Wang *et al.* [14]. These authors observed a molecular mass of hepatic [^{3}H]mepyramine binding sites of various species to range from 107–162 kDa [14]. The observed value for the rat hepatic [^{3}H]mepyramine binding site (162 kDa [13, 14]) is close to the previously reported value of 155 kDa and 157 kDa for H_1-receptors in human and bovine cerebral cortex, respectively [15]. However, it should be stated that those above-mentioned values for molecular mass were all determined using target size analysis. Recently Ruat *et al.* used a different approach [16]. By using the photoaffinity label [^{125}I]-iodoazidophenpyramine these authors showed that the ligand binding domain of the H_1-receptor of guinea-pig cerebellar membranes probably resides within a subunit of 56 kDa, whereas the native H_1-receptor probably has a molecular mass of 350–400 kDa [16].

Classical H_1-antagonists were quite inactive in displacing [^{3}H]mepyramine from the solubilized hepatic binding site and the authors suggested the presence of a H_1-receptor subtype on rat liver plasma membrane [13]. Since we are interested in the use of isolated rat hepatocytes as a model system for the study of H_1-receptor dynamics we tried to characterize this reported [^{3}H]mepyramine binding site on rat liver plasma membrane, using stereoselective H_1-antagonists and some histaminergic agonists.

The results of the performed saturation experiments on rat liver plasma membrane in this study confirm the reported findings of Imoto, Tsuchie *et al.* [4, 5]; a high affinity (K_d = 7.73 nM) [^{3}H]-mepyramine binding site with a high maximal binding capacity (B_{max} = 70.4 pmol/mg protein). The difference in B_{max}-value can be explained by the use of another protein determination, whereas also a different way of preparation of plasma membranes might result in this higher B_{max}-value. This later suggestion is supported by the fact that we were not able to find the reported secondary, low affinity [^{3}H]mepyramine binding site in our plasma membrane preparation.

However, results of displacement studies with several H_1-antagonists clearly show that the binding characteristics of [^{3}H]mepyramine on rat liver plasma membrane do not meet the commonly accepted characteristics for [^{3}H]mepyramine binding to H_1-receptors. As shown in Table 1 triprolidine is less active than mepyramine in displacing [^{3}H]mepyramine from rat liver plasma membrane. This is in marked contrast to its activity on H_1-receptor preparations. For the inhibition of [^{3}H]-mepyramine binding to guinea-pig cerebellar membranes by triprolidine K_i-values similar to mepyramine are found, whereas for the inhibition of histamine induced contractions of guinea-pig ileum triprolidine is even more potent than mepyramine (Table 1). This dissimilarity between the potencies in the different test systems is also apparent for the tested pheniramines. Moreover, the stereospecificity towards the pheniramine enantiomers displayed by the hepatic [^{3}H]mepyramine binding site is not com-

parable to the one found for the H_1-receptor. Whereas in H_1-receptor systems *d*-chlorpheniramine is usually a hundred times more potent than the *l*-isomer in inhibiting H_1-receptor responses, the *l*-isomer is more potent in displacing [^{3}H]mepyramine binding on rat liver plasma membranes, suggesting completely different stereochemical demands.

The observations obtained with the H_1-antagonists were further supported by the data found with the histaminergic agonists. All agonists tested were relatively inactive as displacer and the H_2-selective agonist 4-methylhistamine was even more potent than the H_1-selective agonist 2-methylhistamine. These results strongly suggest the presence of a non-H_1-receptor binding site (*vide infra*) or a H_1-receptor subtype on rat liver plasma membrane.

Since one of the main structural differences between mepyramine (ethylenediamine), triprolidine (alkene) and pheniramine (alkane) is the connection of the aromatic head to the dimethylamino group (Table 1), we used some mepyramine analogues to study the possibility of an ethylenediamine binding site on rat liver plasma membrane. Table 2 shows that all tested compounds were more potent in displacing [^{3}H]mepyramine than triprolidine and that structural manipulation of the aromatic head in the molecule can lead to changes in displacing activity. In contrast to the other H_1-antagonists the displacing activity of the VUF-compounds parallels the inhibitory action on the guinea-pig ileum, although in the case of VUF 7628 selectivity towards the hepatic [^{3}H]mepyramine binding site is obtained.

These results might suggest that on rat liver plasma membrane [^{3}H]mepyramine labels an ethylenediamine binding site which is distinct from the H_1-receptor. Such a phenomenon has also been shown for [^{3}H]cimetidine, which has been shown to label an imidazole binding site instead of the H_2-receptor [17]. The affinity of histamine for this [^{3}H]-mepyramine binding site is very low (K_i = 0.12 mM), whereas Garcia-Sainz *et al.* reported EC_{50}-values for histamine of approximately 0.2 μM for stimulation of glycogenolysis, glyconeogenesis and ureagenesis in isolated rat hepatocytes [1]. Therefore we do not consider this [^{3}H]mepyramine binding site as a novel histaminergic receptor, although the presence of a receptor reserve for histamine for stimulation of glycogenolysis, glyconeogenesis and ureagenesis cannot be excluded. The presence of a receptor reserve would mean that the K_i-value is expected to be lower than the EC_{50}-value.

In conclusion, results of this study confirm the presence of a saturable, high affinity [^{3}H]mepyramine binding site on rat liver plasma membrane but show that it is unrelated to the classical H_1-receptor, since it does not fulfil the criteria described by Laduron [2]. This conclusion is based on the finding that this binding site has a low affinity for several structural non-related H_1-antagonists, has a high affinity for several ethylenediamine derivatives, shows stereochemical requirements different from the H_1-receptor and has a very low affinity for histaminergic agonists. Therefore, although histamine H_1-receptors, involved in the regulation of metabolic activity, seem to be present on rat hepatocytes [1] [^{3}H]mepyramine is not a suitable radioligand for labelling these receptors.

Acknowledgements—The authors wish to thank Ms G. P. H. Biewenga and Ms J. F. de Vlieger for technical assistance.

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