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RE-EXAMINATION OF [^{3}H]MEPYRAMINE BINDING ASSAY FOR HISTAMINE H_1 RECEPTOR USING QUININE

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[^{3}H]Mepyramine, a potent antagonist of the histamine H_1 receptor, has been widely used as a radioligand binding assay for the H_1 receptor. Previously, we purified a mepyramine binding protein (MBP) from rat liver, but found that its partial amino acid sequences were very similar to those of debrisoquine 4-hydroxylase isozymes (P450 db1 and db2), which are members of the superfamily of cytochrome P450. Using cloned histamine H_1 receptor cDNA, we found that [^{3}H]mepyramine could bind only the H_1 receptor and did not bind MBP in the presence of 10^{-5} M quinine, an inhibitor of debrisoquine 4-hydroxylase isozymes. We developed a method to determine the contents of the H_1 receptor and MBP separately using [^{3}H]mepyramine and quinine and found that MBP is abundant in certain areas of bovine brain.

Histamine receptors can be classified into three subtypes, H_1, H_2 and H_3 receptors (1). The histamine H_1 receptor is involved in neurotransmission in the central nervous system and in allergic reactions in peripheral tissues (2). [^{3}H]Mepyramine, a potent H_1 receptor antagonist, has been widely used as a ligand to label the H_1 receptor (3). Pharmacological studies have indicated the existence of the H_1 receptor in many tissues. Liver also contains a large amount of mepyramine binding sites (4, 5). We solubilized and purified the mepyramine binding protein (MBP) from rat liver (6, 7), but partial amino acid sequences of this MBP were very similar to those of rat debrisoquine 4-hydroxylase isozymes (P450 db1 and db2), members of the superfamily of cytochrome P450 (7, 8). These findings indicated that mepyramine recognizes not only the H_1 receptor but also MBP. Recently, we cloned a cDNA for the bovine histamine H_1 receptor (9), and found no homologies between the amino acid sequence of its product and that of debrisoquine 4-hydroxylase. This finding indicated the necessity for study of the relationship between the H_1

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receptor and MBP, and re-examination of the [^{3}H]mepyramine binding assay for the histamine H_1 receptor. In this paper, we characterized the [^{3}H]mepyramine binding sites of the H_1 receptor and MBP and developed a method for separate determinations of the H_1 receptor and MBP. We demonstrated considerable amounts of MBP in several areas of bovine brain.

MATERIALS AND METHODS

Materials

[^{3}H]Mepyramine (23.9 Ci / mmole) was purchased from New England Nuclear. Unlabeled mepyramine was from Sigma Chemical Co. Quinine was from Nacalai Tesque Co. Triprolidine was generous gift from Wellcome Japan.

Transfection and stable expression of bovine H_1 receptor cDNA in rat C6 glioma cells

Rat glioma C6 cells were grown in Ham's nutrient mixture F-10 (GIBCO) supplement with 15 % horse serum and 2.5 % fetal bovine serum in a humidified incubator under 5 % CO_2 in air at 37°C. pEF-BOSH1 (9), which contains the bovine H_1 receptor cDNA sequence under the control of the promoter of the polypeptide chain elongation factor 1 α gene was co-transfected into the C6 cells with pSV2neo carrying the neomycin resistance gene by the calcium phosphate precipitation method (10). Cells were grown in the presence of the neomycin analog G418 (210 μg / ml), and a G418-resistant colony expressing the bovine H_1 receptor was isolated.

Preparation of membrane fractions from liver, brain and C6 cells

Bovine liver and brain were obtained from a slaughterhouse. Membrane fractions of these tissues were prepared as described previously (5, 6). C6 cells were suspended in 50 mM Na, K-phosphate buffer (pH 7.4) and disrupted in a sonicator (Branson Sonifier 250).

[^{3}H]Mepyramine binding assay

The binding assay was carried out as described previously (6). Samples containing about 0.15 mg of protein were incubated with [^{3}H]mepyramine and drugs in the absence (total binding) or presence (non-specific binding) of 20 μM triprolidine at 25°C for 60 min in a final volume of 500 μl. Data were analyzed as described previously (6). Values obtained from at least three independent experiments are expressed as means ± S.D.

Assay of protein

Protein concentration was determined by the method of Smith *et al.* (11) with a Piece BCA Protein Assay Kit and bovine serum albumin as a standard.

RESULTS AND DISCUSSION

Previously, we purified MBP from rat liver and determined its partial amino acid sequences (7), but found that this partial amino acid sequences were very similar to those of rat debrisoquine 4-hydroxylase isozymes (P450 db1 and db2) (7, 8). However, no homologies were found between the amino acid sequences of MBP and that of the histamine H_1 receptor (9). Thus MBP must be a member of the debrisoquine 4-hydroxylase subfamily. As both the H_1 receptor and MBP can bind

mepyramine, their binding sites for mepyramine may be quite similar. Therefore, we studied the effects of various inhibitors or substrates of debrisoquine 4-hydroxylase on [^{3}H]mepyramine binding to membranes from liver and brain (data not shown). In this experiment, we found quinine is strongest to inhibit [^{3}H]mepyramine binding to membranes from liver (Ki value, 1.41 nM), but weak to brain membranes (Ki value, 40.5 μM). For precise study of the effects of quinine on the H_1 receptor, we stably expressed the cloned bovine H_1 receptor in rat glioma C6 cells. C6 cells alone show no detectable [^{3}H]mepyramine binding. C6 cells expressing the bovine H_1 receptor (C6-H_1R) contained a saturable [^{3}H]mepyramine binding site (data not shown). We compared the inhibition profiles of mepyramine and quinine on [^{3}H]mepyramine binding to the membranes of C6-H_1R, bovine cerebral cortex and liver (Fig. 1). The affinity of quinine to the H_1 receptor was much weaker than that of mepyramine, the Ki values of mepyramine and quinine to C6-H_1R being 0.9 nM and 25.5 μM, respectively (Fig. 1A). On the other hand, the curves for inhibitions by mepyramine and quinine of [^{3}H]mepyramine binding to the membranes from bovine liver almost overlapping, their Ki values for [^{3}H]mepyramine binding being 26.1 nM and 44.2 nM, respectively (Fig. 1C), indicating that mepyramine and quinine have almost the same affinity for MBP. With membranes from cerebral cortex, the Ki values of mepyramine and quinine were 17.5 nM and 21.9 μM, respectively (Fig. 1B). This Ki value of mepyramine to cerebral cortical membranes (17.5 nM) is much higher than that to the expressed histamine H_1 receptor (0.9 nM). As MBP shows low affinity for mepyramine, these results indicate that MBP may be present in the brain. In the presence of 10^{-5} M quinine, which should completely inhibit [^{3}H]mepyramine binding to MBP (Fig. 1C), the Ki value for mepyramine was 3.7 nM (Fig. 1B), which is almost the same as that to the expressed H_1 receptor (Fig. 1A). So far, we have obtained the following results on the relationships of the histamine H_1 receptor, MBP and debrisoquine 4-hydroxylase db1 and db2: (a) mRNA for the histamine H_1 receptor was not detectable in bovine liver by Northern blot analysis using H_1 receptor cDNA as a probe (9). (b) Partial amino acid sequences of MBP purified from rat liver are very similar to those of debrisoquine 4-hydroxylases, db1 and db2, indicating that MBP is a member of the debrisoquine 4-hydroxylase subfamily. This finding is supported by recent reports of others (12 - 14). (c) The H_1 receptor and MBP both have high affinities for mepyramine (Ki values, 0.9 nM and 26.1 nM, respectively). 10^{-5} M quinine, an inhibitor of debrisoquine 4-hydroxylase, inhibited [^{3}H]mepyramine binding to MBP but not to the H_1 receptor (Fig. 1). These findings indicate that the actual H_1 receptor can be assayed by [^{3}H]mepyramine binding in the presence of 10^{-5} M quinine.

Results for bovine cerebral cortex shown in Fig. 1B suggest the presence of MBP in the brain. We measured the amounts of MBP in various areas of the brain (Table 1). The [^{3}H]mepyramine bindings to various regions of the brain in the absence of quinine were essentially consistent with

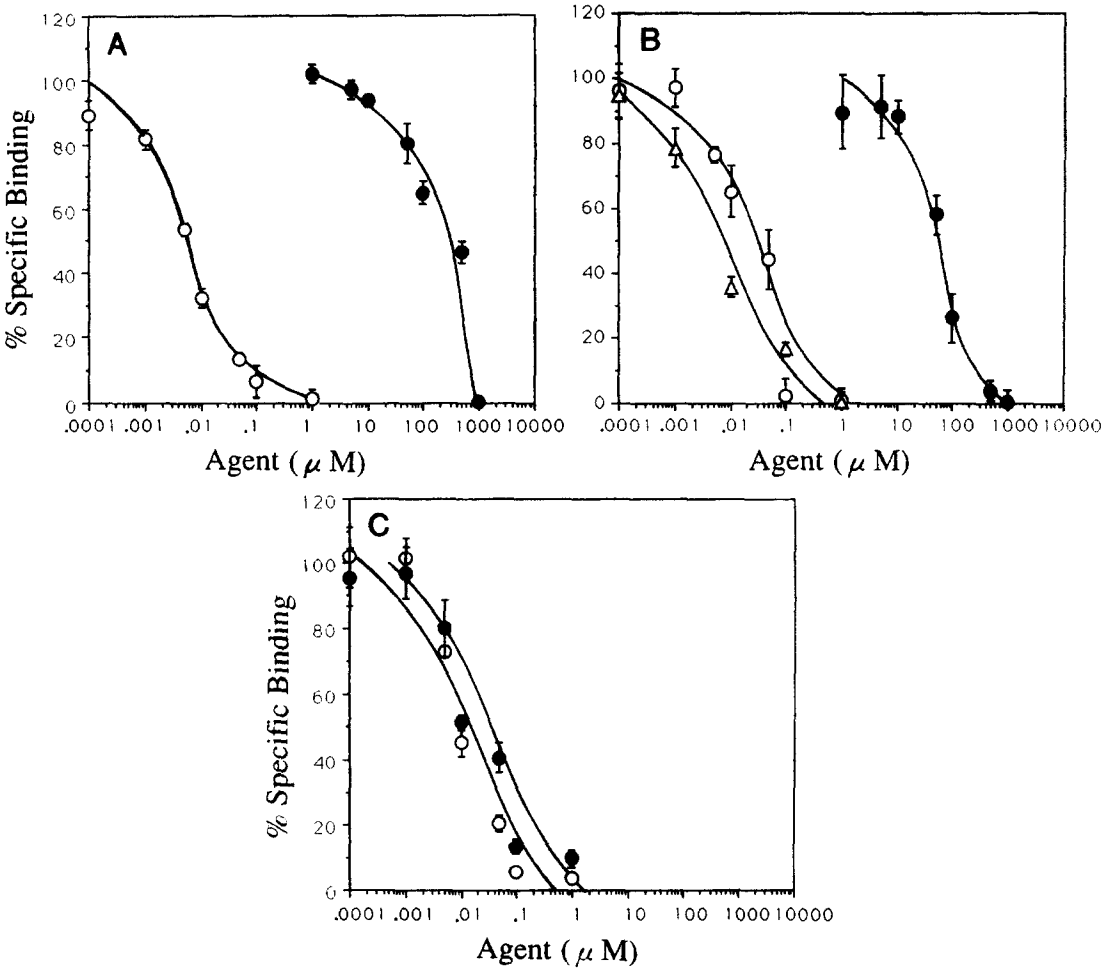

Fig.1. Inhibition of [^{3}H]mepyramine binding to membranes from C6 cells, bovine cerebral cortex and liver by unlabeled mepyramine and quinine. Membranes from C6 cells expressing bovine histamine H_1 receptor (C6-H_1R) (A), bovine cerebral cortex (B) and bovine liver (C) were incubated with 5 nM [^{3}H]mepyramine in the presence or absence of various concentrations of unlabeled mepyramine (○) or quinine (●). In (B), inhibition of [^{3}H]mepyramine binding with unlabeled mepyramine was carried out in the absence (○) or presence (△) of 10^{-5} M quinine. Kd values of [^{3}H]mepyramine binding to membranes from C6 cells, bovine cerebral cortex and liver were 1, 6.8 and 32.6 nM,respectively. Ki values shown in "RESULTS AND DISCUSSION" were calculated as described previous (6).

those reported previously (15), but in the presence of 10^{-5} M quinine the bindings were suppressed to various extents. The inhibitions by quinine of [^{3}H]mepyramine binding to membranes were 60.7 % in the hypophysis, 57.3 % in the pons, 18.8 % in the thalamus, 18.7 % in the hypothalamus, 17.3 % in the cerebellum, 12.9 % in the striatum, and only 9.8 % in the hipocampus, 9 % in the cerebral cortex, and 8.1% in the medulla oblongata. Palacios *et al.* (16) studied the distribution of

Table 1. [^{3}H]Mepyramine binding in various areas of bovine brain in the presence and absence of 10^{-5} M quinine

	Specific Binding (fmole/mg protein)		MBP
	quinine (-)	quinine (+)	(%)
Cerebral cortex	23.03 ± 0.92	20.94 ± 1.91	8.99
Cerebellum	34.93 ± 1.54	28.89 ± 3.92	17.25
Pons	12.61 ± 3.02	5.45 ± 2.30	57.28
Thalamus	9.52 ± 1.58	7.66 ± 0.95	18.76
Hypothalamus	11.04 ± 1.06	8.87 ± 1.10	18.67
Hippocampus	10.18 ± 1.20	9.02 ± 0.71	9.80
Striatum	16.62 ± 2.10	14.29 ± 1.07	12.86
Medulla oblongata	8.05 ± 2.05	7.03 ± 0.75	8.12
Medulla spinalis	5.64 ± 1.38	5.49 ± 2.06	2.70
Hypophysis	98.70 ± 15.29	38.41 ± 6.15	60.72

Values are means ± S.D. (n=3).

H_1 receptors in rat brain by autoradiographic examination with [^{3}H]mepyramine, whereas Watanabe *et al.* (17) demonstrated histaminergic neurons and fibers in rat brain by fluorescent immunohistochemical analysis with histidine decarboxylase (HDC) as a marker. Comparison of results by these two methods, indicated a difference in the distribution of mepyramine binding sites from that of HDC-like immunoreactivity in certain areas, especially the pons, hippocampus, medulla oblongata and thalamus (18, 19). For example, in the pons [^{3}H]mepyramine binding was high, but few histaminergic nerve fibers were observed. We found that more than 50 % of the [^{3}H]mepyramine binding in the pons was due to MBP (Table 1). These results suggest that differences in the distributions of [^{3}H]mepyramine binding sites and histaminergic fibers reflect the presence of MBP.

Doxepin binds to the histamine H_1 receptor with high affinity. The Kd value for the high affinity binding site of [^{3}H]doxepin in rats is 0.02 nM (20). However, the binding capacity of the H_1 receptor labeled with [^{3}H]doxepin is about 10 % of that with [^{3}H]mepyramine in rat brain (20, 21). Fewer high-affinity [^{3}H]doxepin binding sites than [^{3}H]mepyramine binding sites have been detected in the pons and cerebellum (22), suggesting that there are subclasses of histamine H_1 receptors. But our experiments showed that the pons and cerebellum contain significant amounts of MBP, so MBP may not been recognized in the [^{3}H]doxepin binding study.

Cytochrome P450 is mainly located in the liver, but is also present in the brain (23, 24) and other tissues, such as the intestine, lung, kidney, pancreas and testis (25, 26). As MBP could also be present in these tissues, re-examination of the amounts of histamine H_1 receptor and MBP in various tissues is necessary. In addition, it should be noted that many drugs bind not only to functional proteins, but also to cytochrome P450 (14). Recently, [^{3}H]GBR-12935 binding protein, which was believed to be the dopamine transporter in both rat and human brain, has been identified as debrisoquine 4-hydroxylase (24). Our study also indicates the necessity for caution in radio-ligand binding studies.

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