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HISTAMINE H_1-RECEPTOR IN THE RETINA: SPECIES DIFFERENCES

Sadako Sawai, *Nai Ping Wang, *Hiroyuki Fukui, Masakatsu Fukuda, Reizo Manabe and *Hiroshi Wada

Department of Ophthalmology and *Department of Pharmacology II, Osaka University School of Medicine, 3-57, Nakanoshima 4, Kitaku, Osaka 530, Japan

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SUMMARY: Histamine H_1-receptors in membranes of the various mammalian retinas were studied by [^{3}H]mepyramine binding assay. Specific [^{3}H]mepyramine bindings to bovine, pig, dog and human retinas were observed with the dissociation constants (K_D), 3.8±1.2 nM, 1.8±0.6 nM, 2.6±0.6 nM and 3.0±0.9 nM, respectively, which were similar to those found in brains. But there was no detectable specific binding in the guinea-pig and rabbit retinas. The number of binding sites (Bmax) ranged from negligible value to 290.7±51.7 fmole/ mg protein(human retina). Some H_1-antagonists acted as potent agents in competing with [^{3}H]mepyramine binding to bovine and pig retinas. These results indicated that histamine H_1-receptors exist in some mammalian retina and have similar characteristics to those in brain membranes, but they distributes in the wide difference of the binding capacities among the species, while in brain variations were smaller.

Mammalian brain contains histamine in at least two different cellular compartments that are neurons and mast cells(1). The neuronal histamine is supposed to play a role as a neurotransmitter or a neuromodulator while non neuronal histamine in the mast cells surrounding the microvessels is suggested to control the vascular tones and permeability under particular pathophysiological conditions(1,2,3). Histaminergic neurons are also suggested to participate in the vascular effects(4). Whichever histamine in brain is considered to act through at least two types of receptors, H_1 and H_2(1), and H_1-receptors have been labeled by [^{3}H]mepyramine(5-9).

Recently histamine was reported to be present in the various vertebrate retinas(10,11), stored in different types of cells from the mast cells in rabbit and bovine(12,13,14), and histamine H_1 receptors were also suggested to be present in the bovine retina by using the [^{3}H]mepyramine binding assay(14,15). Moreover, histaminergic neurons in horizontal cells of the guinea-pig retina

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were demonstrated by an immunohistochemical method(16). In the present study, we demonstrated the existence and the distribution of the histamine H_1-receptors in the various mammalian retinas using the [^{3}H] mepyramine binding assay, and discussed in terms of the significance of the histamine receptors in the retina.

MATERIALS AND METHODS

Materials: [^{3}H]mepyramine (28 Ci/mmol) was obtained from New England Nuclear Corp.. Triprolidine and diphenhydramine were offered by from Wellcome Japan and Tanabe Pharmaceutical Company, respectively, and mepyramine, histamine and other chemicals were purchased from commercial suppliers.

Membrane Preparation and [^{3}H]Mepyramine Binding Assay: Experiments were performed on the eyes of Hartley guinea-pigs, albino New Zealand rabbits, and Beagle dogs. The eyes of bovines and pigs which were obtained from a local slaughter house and human eyes which were enucleated due to some non-inflammatory diseases and seemed to be intact macroscopically were also used. Preparation of the membranes and the assay of [^{3}H]mepyramine binding were performed as described previously(6,10,14,15). Briefly, the sensory retinas of all these species were isolated and homogenized by a Polytron homogenizer for five 20 sec periods in 10-20 volumes of 50mM Na-K-Phosphate buffer(pH 7.4). The homogenates were centrifuged for 20 min at 50,000 g, and the pellets were suspended in the same buffer to give a protein concentration of about 2-10 mg /ml. Samples containing 0.2-1.5mg protein were incubated with [^{3}H]mepyramine (0.3-10nM) in the presence (nonspecific) or absence (total binding) of 10μM triprolidine in a total volume of 0.5 ml at 25℃ for 45 minutes. After incubation, samples were filtered through glass fiber filters (Whatman GF/B), and the radioactivity trapped on the filters after washing was counted in 10 ml of Aquasol-2 (New England Nuclear, Boston, MA). Specific binding was defined as the bound radioactivity calculated by subtracting the nonspecific binding from the total binding. Assays were performed in duplicate and were replicated at least three times. Protein was assayed by the method of Lowry et al.(17) with bovine serum albumin as a standard.

Analysis of Experimental Data: The saturation isotherms of [^{3}H]mepyramine binding were analyzed by nonlinear regression using the equation:

$$B = \Sigma (Bmax_i \cdot F/(K_{Di} + F)) \qquad [1]$$

where B is the specific binding, F is the concentration of [^{3}H]mepyramine, i is the number of binding sites, Bmax is the binding capacity, and K_D is the dissociation constant. A partial "extra sum-of-squares" F-test was used to check whether there was any improvement in goodness-of-fit for the model with additional parameters as compared with that with the original model(18,19). The binding parameters (K_D and Bmax) of [^{3}H]mepyramine binding to one specie's retinal membrane were compared with those of the retinas of other species' by Student's t-test. For this test and the F-test, the level for statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

The specific bindings to bovine, pig, dog and human retinal membranes at various concentrations of [^{3}H]mepyramine were saturable and analyzed by non-

TABLE 1. [^{3}H]MEPYRAMINE BINDING TO H_1-RECEPTORS IN RETINAS OF VARIOUS SPECIES

	K_D(nM)	B_{max}(fmole/mg protein)
Guinea-pig	n.d.	n.d.
Rabbit	n.d.	n.d.
Bovine	3.8±1.2	75.6±15.1
Pig	1.8±0.6	158.8±28.0
Dog	2.6±0.6	114.2±17.0
Human	3.0±0.9	290.7±51.7

Data were analyzed by nonlinear regression using equation [1]. Values were mean S.E.M. (n=4)
n.d.: not detectable.

linear regression using equation [1]. The results were listed in Table 1 and the saturation curves and Scatchard plots for [^{3}H]mepyramine binding to human and bovine membranes were shown in Fig.1 and Fig.2, respectively. There was no improvement in goodness-of-fit using a model with two binding sites (equation [1],i=2) rather than a model with one binding site (equation [1],i=1)(P>0.05) for any species examined.

Bovine, pig, dog and human retinas showed high mepyramine binding affinities with the K_D values of 3.8±1.2 nM, 1.8±0.6 nM, 2.6±0.6 nM and 3.0±0.9 nM, respectively. The K_D value for bovine (3.8±1.2 nM) was statistically differed

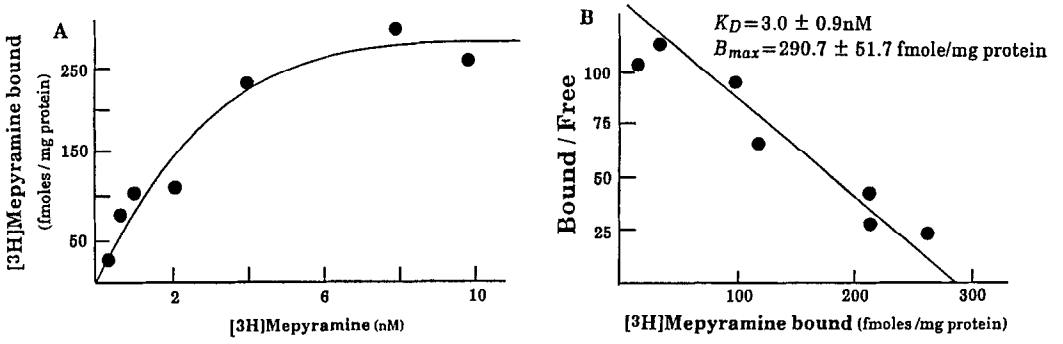

Figure 1. (A) Saturation analysis of [^{3}H]mepyramine binding to H_1-receptors in human retina. Specific [^{3}H]mepyramine bindings, total binding minus non-specific binding, were plotted as a function of increasing concentration. Points were means of three separate experiments, each conducted in duplicate. Equation [1] described in the text was fitted to the data at in (A). (B) Scatchard analysis of the same data.

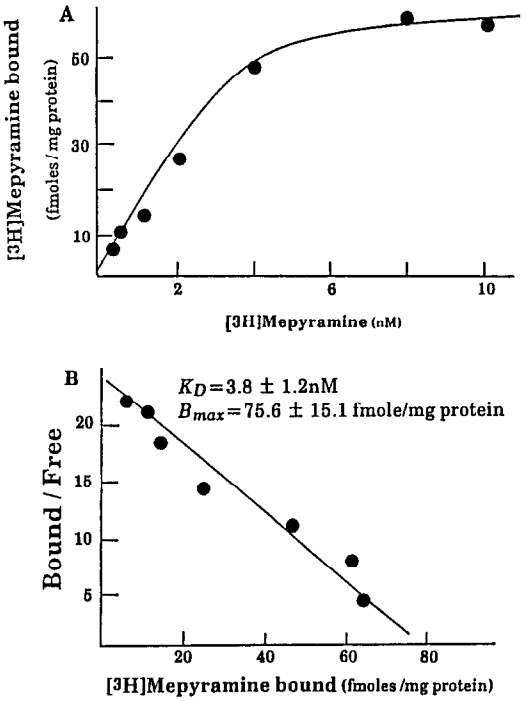

Figure 2. (A) Saturation analysis of [^{3}H]mepyramine binding to H_1-receptors in bovine retina. Specific [^{3}H]mepyramine bindings, total binding minus non-specific binding, were plotted as a function of increasing concentration. Points were means of three separate experiments, each conducted in duplicate. Equation [1] described in the text was fitted to the data in (A). (B) Scatchard analysis of the same data.

from that for pig retinas (1.8± 0.6 nM)($P<0.05$), but the mathematical difference was only 2.0 nM with a standard error difference of 0.6 nM, and so we considered that these two values were similar. Moreover these high affinities of [^{3}H]mepyramine for bovine, pig, dog, and human retinas were in the same order of those reported for brains of other species (6,8,9).

The binding capacities of the retinas of all species examined ranged from negligible value to 290.7± 51.7 fmole/mg protein. Human retinas contained the largest amount of the H_1-receptors with a Bmax =290.7± 51.7 fmole/mg protein which was about 4 times that of the bovine's and 2 times that of the pig's. So they are, approximately the same values with that of the guinea-pig brain (8) and human brain (6), expected relatively high level in various tissues of any species(5). Bmax values of bovine, pig and dog retina were 75.6, 158.8 and 114.2 f mole/mg protein, respectively. However, negligible specific [^{3}H]mepyramine binding was observed in the guinea-pig and the rabbit retinas

even though samples containing 1.5 mg protein for each tube were used.

The result of saturation analysis of the bovine retinas, a K_D of 3.8±1.2 nM and a Bmax of 75.6±15.1 fmole/mg protein, was very similar to that of the previous report by Arbones et al.(14). but different from that by Nowak et al.(15) in which two binding sites (K_{D1}=0.76nM, K_{D2}=7.3nM) were found.

To study the specificity of the [^{3}H]mepyramine binding sites in retinas, some drugs were examined in competition experiments. Drug influences on [^{3}H]mepyramine binding to the retinas of bovine and pig were shown in Table 2. Mepyramine and triprolidine were potent inhibitors of [^{3}H]mepyramine binding in both bovine and pig retinas, which is in good agreement with the binding studies in brain and other tissues(5,6). Diphenhydramine appeared to be less potent in both compared with mepyramine and triprolidine, and histamine to be a weak inhibitor, although having almost the same potencies in the brain according to the previous reported(5,6,9).

These results indicate that the existence of histamine H_1-receptor labeled by [^{3}H]mepyramine was identified in human, dog, pig and bovine retinas and they had very similar characteristics with H_1-receptors in brain previously reported. However, there was no binding activity in rabbit and guinea-pig retinas. The reasons why H_1-receptors are present in some species and absent in others are unknown.

TABLE 2. DRUG INFLUENCES ON [^{3}H]MEPYRAMINE BINDING TO RETINAS OF BOVINE AND PIG (Ki)

DRUG	BOVINE	PIG
Mepyramine	0.7±0.2 nM	2.3±0.7 nM
Triprolidine	5.1±1.5 nM	4.3±1.3 nM
Diphenhydramine	63.9±19.0 nM	27.6±8.2 nM
Histamine	45.8±13.6 μM	34.0±10.0 μM

The inhibition of specific binding of [^{3}H]mepyramine (5nM) was determined with five concentrations of competing drugs assayed in replicate. The mean inhibitory concentration (Ic_{50}) values were determined from the log-probit analysis and Ki values were calculated from the equation $Ki=Ic_{50}/(1+[^3H]\text{mepyramine}/K_D)$. Values were mean ±S.E.M. (n=4)

Recently, histamine content, l-histidine decarboxylase (HDC: a synthesizing enzyme) and histamine-N-methyltransferase (HMT: a catabolizing enzyme) activities in retinas of some animals have been determined(10-13,20,21), but these values showed relatively large variations by species. For example chicken and rat have no histamine content while guinea-pig and cow have 4.26 and 0.189 nmol/g wet weight, respectively(11). Also histamine in human retinas varied from about 1 to 5 nmol/g wet weight depending upon the disease which the eye ball suffered. There was no HDC activity in hen while rabbit and carp had some. And, we couldn't detect the H_1-receptor in the guinea-pig retina which proved to contain HDC immunoreactive cells(16). This may be caused by the incompatibility of the receptor assay and the immunohistochemical method. Moreover, histamine receptors are classified into H_1,H_2 and possibly H_3-receptors, so H_1-receptors labeled by mepyramine binding may not be the only histamine receptors in retina which elicits some physiological actions.

Thus, based on the preliminary results and our results, it was hypothesized that histamine in the retina may concern with more than one function or play a part in specialized or advanced functions, which may reflect the variation of the amounts of histamine H_1-receptors in retinas of the various mammalian species.

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