Divalent Cations Increase the Binding Capacity of the
$[^{3}H]$Mepyramine Binding Site, a Possible Histamine $H_1$ Receptor,
in Rat Liver Membranes

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Abstract—The $[^{3}H]$mepyramine binding to rat liver membranes was about 2-fold higher in the presence of 2 mM CaCl$_2$ than in the absence. However, the $[^{3}H]$-mepyramine binding to rat brain membranes was not affected by the presence of 2 mM CaCl$_2$. Scatchard analysis showed that 2 mM CaCl$_2$ did not change the affinity ($K_D$) of liver membranes to $[^{3}H]$mepyramine, but increased the binding capacity ($B_{max}$). CaCl$_2$ had a half maximal effect (ED50) at 3 μM and a maximal effect at concentrations of more than 10 μM. Other divalent cations, Mg$^{++}$, Mn$^{++}$, Sr$^{++}$ and Ba$^{++}$, also increased the $[^{3}H]$mepyramine binding, whereas monovalent cations, Na$^+$, K$^+$, Li$^+$ and Rb$^+$, had no effect.

The brain and many peripheral tissues have been demonstrated to contain histamine $H_1$ receptors by the $[^{3}H]$mepyramine binding assay (1-4). Of the membranes from the various tissues examined, those of rat liver were reported to have the highest $[^{3}H]$-mepyramine binding activities (5). It was reported that some receptors were affected by various ions. Baudry and Lynch (6) reported that the binding capacity of rat hippocampal membranes to $[^{3}H]$glutamate was increased by Ca$^{++}$ and other cations. Pasternak et al. (7) reported that divalent cations, Mn$^{++}$, Mg$^{++}$, Ca$^{++}$ and Ni$^{++}$, increased the binding capacity of rat brain membranes to $[^{3}H]$-dihydromorphine. Tsai and Lefkowitz (8) reported that Mg$^{++}$ increased and Na$^+$ decreased the binding affinity of rabbit platelet membrane to $[^{3}H]$-dihydroergocriptine. Thus the effect of ions on $[^{3}H]$mepyramine binding to histamine $H_1$ receptors was of interest. Chang and Snyder (9) did not observe any ionic factor related to the brain histamine $H_1$ receptor by the $[^{3}H]$mepyramine binding assay. However, we found that the $[^{3}H]$-mepyramine binding to rat liver membranes was increased by Ca$^{++}$ and other divalent cations. This paper shows that Ca$^{++}$ increased the binding capacity ($B_{max}$) of rat liver membranes to $[^{3}H]$mepyramine, and this increase was induced at a very low concentration of Ca$^{++}$.

Materials and Methods

Materials: $[^{3}H]$Mepyramine was purchased from Amersham. Triprolidine was a generous gift from Wellcome Japan. Trans 1,2-cyclohexandiaminetetraacetic acid (CyDTA) was obtained from Dojindo Laboratories. Other chemicals used were standard commercial products.

Membrane preparation: Male Wistar rats (150–200 g) were sacrificed by decapitation, and their livers and brains were rapidly isolated. Membranes were prepared by homogenizing the tissues on ice in a Polytron (Kinematica, Model K) for six 30-sec periods at 8/10 of the full speed in 10 volumes of 50 mM Tris-HCl, pH 7.4. The homogenate was centrifuged at 50,000×g for 20 min, and the pellet was suspended in the same buffer at a concentration of 0.2 g tissue/ml (about 15–25 mg of protein/ml).

Assay of $[^{3}H]$mepyramine binding and
analysis of the data: The assay was done in triplicate according to the method of Tran et al. with slight modifications (3). Samples containing 1.5–2.5 mg protein were incubated with various concentrations of $^{[3]H}$mepyramine in the presence (nonspecific binding) and absence (total binding) of 20 μM triprolidine in 50 mM Tris-HCl, pH 7.4, at 25°C for 1 hr (total volume, 0.5 ml). The effect of cations on $^{[3]H}$mepyramine binding to liver membranes was studied by the incubation with each chloride salt at 2 mM. After incubation, samples were filtered on glass fiber filters (Whatman GF/B), and the filters were washed four times with 3 ml of ice-cold 50 mM Tris-HCl, pH 7.4. The radioactivity trapped on the filters was counted in 10 ml of Aquasol-2 (New England Nuclear). Specific binding was defined as the radioactivity bound, calculated by subtracting the nonspecific binding from the total binding. The saturation isotherms of $^{[3]H}$mepyramine binding were analyzed by nonlinear regression as described previously (10). Statistical evaluation of significant differences was performed with the Student’s t-test.

Protein assay: Protein was assayed by the method of Lowry et al. (11) with bovine serum albumin as a standard.

Results

$^{[3]H}$Mepyramine binding to rat liver and brain membranes were determined in the presence and absence of 2 mM CaCl₂ (Fig. 1). $^{[3]H}$Mepyramine binding to rat liver membranes in the presence of Ca²⁺ was 180–240% of that in the absence of Ca²⁺ (P<0.01), whereas $^{[3]H}$mepyramine binding to brain membranes was not affected by the presence of Ca²⁺. The addition of 1 mM CyDTA, a strong chelator of divalent cations, tended to decrease the $^{[3]H}$mepyramine binding to rat liver membranes, but the difference was not statistically significant (P>0.05). The $^{[3]H}$mepyramine binding to rat brain membranes was not changed by the addition of 1 mM CyDTA.

For determination of whether the affinity ($K_D$) or binding capacity ($B_{max}$) was changed by Ca²⁺, liver membranes were incubated with various concentrations of $^{[3]H}$mepyramine in the presence and absence of 2 mM CaCl₂, and Scatchard analyses were made (Fig. 2). There was no improvement in goodness-of-fit either in the presence or the absence of CaCl₂ (P>0.05), when a two-binding-site model was used instead of a one-binding-site model. The $K_D$ values of $^{[3]H}$mepyramine binding in the absence and presence of 2 mM CaCl₂ were 17.5±3.2 nM and 19.2±5.3 nM, respectively. These values did not differ significantly. The binding capacity ($B_{max}$) of $^{[3]H}$mepyramine binding in the presence and the absence of 2 mM CaCl₂ were 3.4±0.7

Fig. 1. $^{[3]H}$Mepyramine binding to rat liver and brain membranes in the presence and absence of 2 mM CaCl₂ or 1 mM CyDTA. Specific $^{[3]H}$mepyramine binding was determined as described in Materials and Methods. The concentration of $^{[3]H}$mepyramine used was 2 nM. The specific $^{[3]H}$mepyramine bindings of brain and liver membranes to test tubes were 1.23±0.06 fmoles and 12.0±0.9 fmoles without additions (NONE) and were taken as 100%, respectively. Values are means±S.E. from five separate experiments, each conducted in triplicate.
pmole/mg protein and 1.5±0.4 pmole/mg protein, respectively. These results showed that Ca++ increased the binding capacity to 230% of that in its absence (P<0.01), but did not affect the affinity.

The effect of Ca++ concentration on [3H]-mepyramine binding to liver membranes was examined (Fig. 3). [3H]Mepyramine binding to the membranes increased with an increase in the concentration of Ca++ to a maximum attained at 10 μM, and the concentration for a half maximal effect (ED50) was 3 μM.

The effect of various divalent and monovalent cations at 2 mM on [3H]mepyramine binding to liver membranes were examined (Fig. 4). The divalent cations Ca++, Mg++, Ba++ and Sr++ increased [3H]mepyramine binding (P<0.01), Ca++ having the most marked effect. The monovalent cations Na+, K+, Li+ and Rb+ had no effect.

**Discussion**

The [3H]mepyramine binding assay is widely used for determination of histamine H₁ receptors (1-4, 12-14). Imoto et al. (5) observed very high [3H]mepyramine binding to rat liver membranes. We confirmed their observation and found that the livers from other animals also possessed higher [3H]-mepyramine binding than their other body tissues (15). We also found that [3H]-mepyramine binding to rat liver membranes determined in the presence of 2 mM CaCl₂ was 180–240% of that determined in the absence of CaCl₂. This increase was due to Ca++, not to Cl⁻, because the binding assay was done in the presence of 50 mM Tris-HCl, pH 7.4, and 2 mM NaCl did not affect the
Fig. 4. Effects of various cations on [3H]mepyramine binding to rat liver membranes. Specific [3H]-mepyramine binding was determined as described in Materials and Methods. The concentration of [3H]-mepyramine used was 2 nM. Specific [3H]mepyramine bindings in the presence of 2 mM cations were expressed as percentage of specific binding in the absence of cations (control). The specific [3H]-mepyramine binding of the control was 10.2±0.7 fmole, which was taken as 100%. Values are means±S.E. from three separate experiments, each conducted in triplicate.

binding activity (Fig. 4). Chang and Snyder (9) reported that neither the affinity nor the binding capacity of guinea pig brain membranes to [3H]mepyramine was affected by the presence of the divalent cations Mn++ and Mg++ or the monovalent cation Na+. We also observed that [3H]mepyramine binding to rat brain membranes was not affected by CaCl₂ (Fig 1). Thus the [3H]mepyramine binding sites in rat liver and brain differ in sensitivity to Ca++. These results suggest that rat liver and brain histamine H₁ receptors are different subtypes. Scatchard analysis showed that Ca++ increased the binding capacity (Bₘₐₓ) without changing the affinity (Kᵦ) of the binding sites on liver membranes (Fig. 2). This means that it induced an increase in the number of the binding sites (receptors).

Baudry and Lynch (6) reported that the glutamate receptor in the hippocampus is regulated by divalent cations, which increase the binding capacity without changing the affinity to glutamate. Their further investigations showed that this increase was inhibited by leupeptin, an inhibitor of thiol proteases (16). We observed that leupeptin inhibited the increase of [3H]mepyramine binding to rat liver membranes induced by Ca++ (H. Fukui et al., unpublished data). We are assuming that there are two forms of the [3H]mepyramine binding sites, the exposed and the hidden binding sites. Proteolysis of the hidden [3H]mepyramine binding site or the neighboring protein by the leupeptin sensitive protease, possibly a Ca⁺⁺ protease, is considered to expose the hidden binding site to the outside of the membrane. The existence of the hidden [3H]mepyramine binding site is supported by the fact that the solubilization of the rat liver membranes increased the binding capacity of the membranes to [3H]mepyramine (data not shown).

The physiological roles of histamine H₁ receptor in rat liver membranes are not elucidated. Recently it was reported by Gracia-Sainz et al. (17) that histamine stimulated glycogenolysis, glyconeogenesis and ureagenesis in isolated rat hepatocytes mainly through histamine H₁ receptors.

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References
1 Hill, S.J., Young, J.M. and Marrian, D.H.: Specific binding of ³H-mepyramine to histamine H₁ receptors in intestinal smooth muscle. Nature 270, 361–363 (1977)
2 Hill, S.J., Emson, P.C. and Young, J.M.: The binding of [³H]mepyramine to histamine H₁ receptors in guinea-pig brain. J. Neurochem. 31, 997–1004 (1978)
3 Tran, V.T., Chang, R.S.L. and Snyder, S.H.: Histamine H₁ receptors identified in mammalian brain membranes with [³H]mepyramine. Proc. Natl. Acad. Sci. U.S.A. 75, 6290–6294 (1978)
4 Chang, R.S.L., Tran, V.T. and Snyder, S.H.: Characteristics of histamine H₁-receptors in peripheral tissues labeled with [³H]mepyramine. J. Pharmacol. Exp. Ther. 204, 437–442 (1979)

5 Imoto, M., Tsuchie, K., Tanaka, M., Sugiyama, S. and Ozawa, T.: Predominance of histamine H₁ receptors on liver plasma membrane. Biochem. Biophys. Res. Commun. 127, 885–889 (1985)

6 Baudry, M. and Lynch, G.: Regulation of glutamate receptors by cations. Nature 282, 748–750 (1979)

7 Pasternak, G.W., Snowman, A.M. and Snyder, S.H.: Selective enhancement of [³H]opiate agonist binding by divalent cations. Mol. Pharmacol. 11, 736–744 (1975)

8 Tsai, B.S. and Lefkowitz, R.J.: Agonist-specific effects of monovalent and divalent cations on adenylate cyclase-coupled alpha adrenergic receptors in rabbit platelets. Mol. Pharmacol. 14, 540–548 (1978)

9 Chang, R.S.L. and Snyder, S.H.: Histamine H₁-receptor binding sites in guinea pig brain membranes: regulation of agonist interactions by guanine nucleotides and cations. J. Neurochem. 34, 916–922 (1980)

10 Fukui, H., Wang, N.P., Watanabe, T. and Wada, H.: Solubilization, characterization and partial purification of [³H]mepyramine-binding protein, a possible histamine H₁ receptor, from rat liver membrane. Japan. J. Pharmacol. 46, 127–139 (1988)

11 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275 (1951)

12 Carswell, H. and Nahorski, S.R.: Distribution and characteristics of histamine H₁-receptors in guinea-pig airways identified by [³H]mepyramine. Eur. J. Pharmacol. 81, 301–307 (1982)

13 Casale, T.B., Rodard, D. and Kaliner, M.: Characterization of histamine H₁ receptors on human peripheral lung. Biochem. Pharmacol. 34, 3285–3292 (1985)

14 Nishimura, J., Kanaide, H., Miwa, N. and Nakamura, M.: Specific binding of [³H]mepyramine to histamine H₁-receptors in the sarclemma from porcine aorta and coronary artery. Biochem. Biophys. Res. Commun. 126, 594–601 (1985)

15 Wang, N.P., Fukui, H., Matsuoka, H. and Wada, H.: Determination of the molecular size of the hepatic H₁-receptor by target size analysis. Biochem. Biophys. Res. Commun. 137, 593–598 (1986)

16 Baudry, M. and Lynch, G.: Regulation of hippocampal glutamate receptors: evidence for the involvement of a calcium-activated protease. Proc. Natl. Acad. Sci. U.S.A. 77, 2298–2302 (1980)

17 Garcia-Sainz, J.A., Carmen de la Garza, M., Contreras-Rodriguez, L.J. and Majera-Alvarado, A.: Effects of histamine on the metabolism of isolated rat hepatocytes: roles of H₁- and H₂-histamine receptors. Mol. Pharmacol. 31, 253–258 (1987)