Solubilization, Characterization and Partial Purification of 
$[^3H]$Mepyramine-Binding Protein, a Possible Histamine 
H$_1$ Receptor, from Rat Liver Membrane

Hiroyuki FUKUI, Nai Ping WANG, Takehiko WATANABE* and Hiroshi WADA
Department of Pharmacology II, Osaka University School of Medicine, Osaka 530, Japan
*Department of Pharmacology I, Tohoku University School of Medicine, Sendai 980, Japan

Accepted October 21, 1987

Abstract—$[^3H]$Mepyramine binding protein, a possible subtype of histamine H$_1$ receptors, was solubilized from rat liver membrane with 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) and Tween 60 as detergents and glycerol as an enhancer of solubilization. The optimal concentration of CHAPS was 10 mM and that of glycerol was 20% or more (v/v). The molecular weight of the $[^3H]$mepyramine binding protein-detergent complex was determined to be 670K by Sepharose CL-4B gel filtration and 800K by sucrose density gradient sedimentation. By target size analysis, the molecular weights of both the membrane-bound and solubilized $[^3H]$mepyramine binding protein were determined to be 162K. These values are similar to those of other well-characterized H$_1$-receptor proteins, though slightly different. Simultaneous computerized analysis of the data obtained by $[^3H]$mepyramine binding to the solubilized $[^3H]$mepyramine binding protein indicated the presence of a single binding site with a $K_d$ value of 19.0±5.6 nM and a binding capacity ($B_{max}$) of 6.6±2.1 pmole/mg protein. The $K_i$ value of cold mepyramine for $[^3H]$mepyramine binding to the solubilized receptor was 20±4 nM, whereas those of diphenhydramine, d-chlorpheniramine and triprolidine were all 2.9±0.8 µM, or about 150 times that of mepyramine. These data on the molecular and binding characteristics of the solubilized protein reported here suggest that there is a subtype of histamine H$_1$ receptor in rat liver membrane.

The solubilized preparation retained 90% and 75% of its $[^3H]$mepyramine binding activity after storage at -80°C and 4°C, respectively, for 20 days. The solubilized $[^3H]$mepyramine binding protein was purified 30-fold by Sepharose CL-4B gel filtration, Bio Gel HTP hydroxylapatite, Octyl Sepharose 4B and hydroxylapatite HPLC column chromatographies.

Histamine receptors are classified pharmacologically into two subtypes, H$_1$ and H$_2$ receptors (1, 2), although recently Arrang and coworkers (3) suggested the presence of a presynaptic H$_3$ receptor. H$_1$ receptors are present in smooth muscles and in the brains of most animals. Stimulation of histamine H$_1$ receptors appears to increase the concentration of intracellular free calcium ion by opening of calcium channels (4) or increase of phosphatidylinositol turnover (5). However, the mechanism of signal transduction through the H$_1$ receptor at the molecular level is still unknown. Histamine H$_1$ receptor can be determined by assay of $[^3H]$mepyramine binding (6). Solubilized and purified H$_1$ receptors would be useful for studies on the molecular mechanisms regulating their activity. Solubilization of the H$_1$ receptor has been reported (7-11), but purification of the receptor is very difficult because of its very low content in tissues. Recently rat liver was reported to contain the highest $[^3H]$mepyramine binding activity (12), though the physiological function of histamine in the liver is unknown.

In this paper, the $[^3H]$mepyramine binding protein existing in rat liver membrane was
solubilized, characterized, and partially purified, and it was proposed that this protein may represent one of the subtypes of histamine H₁ receptors.

Materials and Methods

Reagents

[^3H]Mepyramine (26 Ci/mole) was purchased from New England Nuclear. 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and Tween 60 were obtained from Dojindo Chemicals and Nakarai Chemicals, respectively. Triprolidine, diphenhydramine, mequitazine, terfenadine and astemizole were generous gifts from Wellcome Japan, Tanabe, Nippon Shoji, Dow Chemical and Jansen Kyowa, respectively. Mepyramine, d-chlorpheniramine, cimetidine, histamine, d-tubocurarine, noradrenaline, adrenaline and serotonin were obtained from commercial sources. Standard molecular weight marker proteins were obtained from Oriental Yeast or Worthington. Sepharose CL-4B and Octyl Sepharose 4B were from Sigma. Bio Gel HTP was from Bio Rad.

Preparation of stock solution of Na/K phosphate buffer (0.5 M, pH 7.4 at 50 mM)

The stock solution of Na/K phosphate buffer (0.5 M, pH 7.4 at 50 mM) was prepared as follows: 270 g of Na₂HPO₄·12H₂O and 33.2 g of KH₂PO₄ were dissolved in ca. 800 ml of distilled water; the pH was adjusted to 7.4 with a solution of 3.5 M NaOH and 1.5 M KOH by monitoring the pH of 10 times diluted buffer solution; and the volume was then adjusted to 1,000 ml.

Preparation of the membrane fraction of rat liver

Rats were killed by cervical dislocation, and their livers were rapidly removed and then homogenized in 10 volumes of ice-cold 10 mM Na/K phosphate buffer with a Polytron (setting 8) for six 30 sec periods at 15 sec intervals. The suspension was centrifuged (50,000×g for 20 min), and the pellet was used as the crude membrane fraction.

Solubilization of [^3H]mepyramine binding protein

The crude membrane fraction from 10 g of liver was resuspended in 50 ml of ice-cold 10 mM Na/K phosphate buffer, mixed with an equal volume of Na/K phosphate buffer (100 mM) containing 20 mM CHAPS, 0.4% (w/v) Tween 60 and 60% (v/v) glycerol, homogenized by 50 strokes of a glass homogenizer and stood on ice for 60 min. The suspension was then centrifuged at 100,000×g for 60 min, and the solubilized binding protein was recovered in the supernatant.

[^3H] Mepyramine binding assay

The sample containing the solubilized [^3H] mepyramine binding protein was incubated with 2 nM[^3H] mepyramine in the absence (for measuring total binding) or the presence (for measuring non-specific binding) of unlabeled 20 μM triprolidine. The reaction mixture (total volume 300 μl) was incubated at 25°C for 60 min, and the mixture was then applied on a gel filtration column (0.75 cm×6 cm) of Cellulofine GC 200 m equilibrated with Na/K phosphate buffer (50 mM) containing 1 mM CHAPS, 0.2% (w/v) Tween 60 and 3% (v/v) glycerol (Buffer A). The column was washed with Buffer A (600 μl).[^3H] Mepyramine bound to the binding protein was recovered with Buffer A (700 μl), while free[^3H] mepyramine was retained on the column. The radioactivity eluted was measured in 10 ml of Aquasol II scintillation cocktail. Specific binding was defined as the radioactivity bound, calculated by subtracting the non-specific binding in the presence of 20 μM triprolidine from the total binding. Assays of [^3H] mepyramine binding were performed in duplicate or triplicate.

Molecular weight determinations

1) Gel filtration: A Sepharose CL-4B column (1.5 cm×17 cm) was equilibrated and eluted with Buffer A and calibrated with molecular weight markers: thyroglobulin (Mr. 670K), ferritin (Mr. 445K) and catalase (Mr. 240K). The flow rate was 3 ml/hr, and fractions of 2 ml were collected. The [^3H] mepyramine binding and absorbance at 278 nm of each fraction were measured.

2) Sucrose density gradient centrifugation: Linear gradients of 5 to 50% (w/v) sucrose in Buffer A (8.5 ml) were prepared. A sample of 1.5 ml of solubilized[^3H] mepyramine binding protein, which had been eluted from Sephadex G-100 with Buffer A to lower the concentration of glycerol, was layered on the
gradient. Aliquots (1.5 ml) of the molecular weight markers, thyroglobulin (Mr. 670K), β-galactosidase (Mr. 560K), catalase (Mr. 240K) and alcohol dehydrogenase (Mr. 148K) were layered on identical gradients in separate centrifuge tubes. The gradients were centrifuged at 100,000 x g for 16 hr in a Hitachi RPS-40T rotor. After centrifugation, a capillary was inserted into the bottom of the centrifuged tubes, the gradients were pumped out and fractions of 1 ml were collected. The [3H]mepyramine binding and absorbance at 278 nm of each fraction were measured.

3) Gamma-ray irradiation with 60Co: [3H]: Mepyramine binding proteins in the membrane fraction and those solubilized with detergents were frozen in dry ice and exposed to 60Co at various distances for 24 hr. Then the remaining [3H]mepyramine binding activity of each sample was determined.

Molecular size was calculated as described by Kempner and Schlegel (13). The logarithm of the percentage of remaining [3H]mepyramine binding activity was plotted against the radiation dose. A single exponential plot of inactivation versus the radiation dosage was obtained. The molecular weight was determined from the dose of radiation for 37% survival [D37 (Mrad)] by the equation:

\[ \text{Molecular weight} = f \times 6.4 \times 10^{-5} D_{37} \]

where \( f \) is a temperature correction factor that is related to the temperature at which the radiation inactivation was performed; 1.9 is a suitable value at 193*K (14).

Determination of the dissociation constant and binding capacity

The specific bindings of different concentrations of [3H]mepyramine to solubilized [3H]mepyramine binding protein were determined, and data were analyzed by nonlinear least squares regression (15). The binding capacity and affinity of the binding site were determined by the equation:

\[ B = B_{\text{max}} \cdot \frac{F}{K_D + F} \]

where \( B \) represents bound mepyramine, \( F \) represents free mepyramine, \( K_D \) is the dissociation constant of [3H]mepyramine binding to each receptor site and \( B_{\text{max}} \) is the total concentration of the receptor site (10).

Determination of \( K_i \) values for [3H]mepyramine binding

The inhibition of specific [3H]mepyramine binding was determined with different concentrations of competing drugs. The IC50 values were determined by logit-probit analysis and \( K_i \) values were calculated from the following equation (16):

\[ K_i = \frac{IC50}{L} \cdot \frac{1}{K_D} \]

where IC50 is the inhibitor concentration which should produce half maximal inhibition of [3H]mepyramine binding, \( L \) is the used concentration of [3H]mepyramine, and \( K_D \) is the dissociation constant of [3H]mepyramine.

Purification of [3H]mepyramine binding protein from rat liver

All procedures were carried out at 4°C unless otherwise stated.

1) Sepharose CL-4B gel filtration: A Sepharose CL-4B column (2.5 cm x 62 cm) was equilibrated with Buffer A. Solubilized [3H]mepyramine binding protein (50 ml) extracted from the membrane fraction of rat liver was applied to the column, and material was eluted with Buffer A at a flow rate of 12 ml/hr. Fractions of 20 ml (#22–29) were collected, and their [3H]mepyramine binding and absorbance at 278 nm were measured. The fractions (#22–29) with [3H]mepyramine binding activity were combined for further purification.

2) Bio Gel HTP column chromatography: A Bio Gel HTP column (1.5 cm x 11 cm) was equilibrated with Buffer A. Solubilized [3H]-mepyramine binding protein from Sepharose CL-4B was applied to the column. The column was washed thoroughly, and their material was eluted with 100 ml of a linear gradient of 50–250 mM Na/K phosphate buffer. The peak fractions (#17–24) of [3H]mepyramine binding activity were combined for further purification.

3) Octyl Sepharose 4B column chromatography: An Octyl Sepharose 4B column (1.5 cm x 11 cm) was equilibrated with 50 mM Na/K phosphate buffer containing 1 mM CHAPS, 0.02% (w/v) Tween 60 and 3% (v/v)
glycerol (Buffer B). The solubilized $[^3$H]$mepyramine binding protein from Bio Gel HTP was dialyzed against Buffer B and applied to the column. The column was washed thoroughly, and then material was eluted with 100 ml of a linear gradient of Tween 60 (0.02–0.5% (w/v)). The peak fractions (21–24) of $[^3$H]$mepyramine binding activity were combined for further purification.

4) Hydroxylapatite HPLC column chromatography: An HCA hydroxylapatite HPLC column (A-7610) (Mitsuitohatsu Chemical Co.) was operated with the HPLC system of Toyo Soda Co. at 20°C. Solubilized $[^3$H]$mepyramine binding protein from Octyl Sepharose 4B was dialyzed against Buffer A, filtered through a Millex-GV 0.22 nm filter (Millipore) and applied to the column equilibrated with Buffer A. Material was eluted with 100 ml of a linear gradient of 50–250 mM Na/K phosphate buffer at a flow rate of 1 ml/min and fractions of 2 ml were collected. The $[^3$H]$mepyramine binding and absorbance at 278 nm of each fraction were measured.

Determination of protein concentration

Protein concentration was determined from the absorbance at 278 nm, assuming that an absorbance of 1.0 corresponded to 1 mg protein/ml, irrespective of the buffer.

Results

Solubilization of $[^3$H]$mepyramine binding protein

When the $[^3$H]$mepyramine binding protein in the crude membrane fraction was solubilized with 50 mM Na/K phosphate buffer containing various concentrations of CHAPS and glycerol and without Tween 60 using a glass homogenizer and the solubilized binding protein was recovered in the supernatant by the centrifugation at 100,000 x g for 60 min, the highest $[^3$H]$mepyramine binding activity was recovered at 10 mM CHAPS and 20% or more (v/v) glycerol (Fig. 1 A,B). However, the centrifugation of the supernatant containing the $[^3$H]$mepyramine binding protein obtained in the absence of Tween 60 at 100,000 x g for more than 1 hr resulted in a decrease of binding activity, and on Sepharose CL-4B gel filtration, the binding protein was eluted at the void volume (data not shown). The $[^3$H]$mepyramine binding protein solubilized in the presence of 10 mM CHAPS, 30% (v/v) glycerol and 0.2% (w/v) Tween 60 was not affected by the centrifugation at 100,000 x g for longer than 1 hr (data not shown) and was ressolved on Sepharose CL-4B filtration (Fig. 2A). Tween 60 did not affect the binding activity of the solubilized $[^3$H]$mepyramine binding protein at concentrations from 0.2 to 5.0% (w/v) (data not shown). However, the optimal concentration of Tween 60 on the solubilization was not determined.

Fig. 1. The effects of (A) CHAPS and (B) glycerol on the solubilization of $[^3$H]$mepyramine binding protein from rat liver. Solubilized protein from liver membrane fraction (375 μg of protein in membrane fraction) was used for each binding assay. $[^3$H]$mepyramine binding activities in assay tubes (fmol) vs. concentrations of CHAPS or glycerol were plotted. The solubilization of the receptor is described in ‘Materials and Methods’.
Molecular weight determinations of the $[^3]H$-mepyramine binding protein-detergent complex by gel filtration and sucrose density gradient sedimentation

The solubilized $[^3]H$-mepyramine binding protein was chromatographed on a Sepharose CL-4B gel filtration column calibrated with molecular weight standards (Fig. 2A), giving rise to a single peak of $[^3]H$-mepyramine binding. Bound $[^3]H$-mepyramine to the eluted receptor was replaced with 2 μM cold mepyramine or 20 μM cold triprolidine, but only partially replaced by 2 μM cold triprolidine. From the standard curve of the Ve/Vo value vs. molecular weight obtained with the marker proteins, the molecular weight of the $[^3]H$-mepyramine binding protein-detergent complex was determined to be 670K (Fig. 2B).

The solubilized $[^3]H$-mepyramine binding protein was layered on a sucrose density gradient and centrifuged as described in "Materials and Methods". The sucrose density gradient was fractionated and the $[^3]H$-mepyramine binding activity of each fraction was determined (Fig. 3A). From the standard curve of Rf values of migration vs. molecular weights obtained with marker proteins, the molecular weight of the $[^3]H$-mepyramine binding protein-detergent com-
plex was determined to be 800K (Fig. 3B).

Target size analysis of membrane-incorporated and solubilized \[^3H\]mepyramine binding proteins

The membrane-incorporated and solubilized \[^3H\]mepyramine binding proteins were subjected to irradiation by \(^{60}Co\). The inactivations of the \[^3H\]mepyramine binding activities of both preparations showed the same linear exponential decrease with increase in the radiation dosage (Fig. 4). The \(K_D\) values of \[^3H\]mepyramine for the remaining \[^3H\]mepyramine binding protein were unchanged (data not shown). The molecular weight of the solubilized \[^3H\]mepyramine binding protein was similar to that of the binding protein in the membrane and was determined to be 162K.

Dissociation constant (\(K_D\)) of solubilized \[^3H\]mepyramine binding protein

The saturation curve of specific binding to solubilized \[^3H\]mepyramine binding protein at different concentrations of \[^3H\]mepyramine is shown in Fig. 5A. Figure 5B shows the Scatchard plot of the same data. The \(K_D\) value and the binding capacity (\(B_{max}\)) were determined with a computer to be 19.0\(\pm\)5.6 nM and 6.6\(\pm\)2.1 pmole/mg of protein, respec-
Fig. 4. Radiation inactivation-target size analysis of membrane incorporated and solubilized [3H]-mepyramine binding protein from rat liver. [3H]-Mepyramine binding protein in the membrane fraction of rat liver and solubilized [3H]mepyramine binding protein from the same membrane fraction were frozen in dry ice and irradiated with 60Co as described in "Materials and Methods". The radiation dose was determined as described in "Materials and Methods". The figure shows a semilogarithmic plot of the relative [3H]mepyramine binding activity vs. the radiation dose. (○) and (●) show values for membrane-bound and solubilized [3H]mepyramine binding protein, respectively. [3H]Mepyramine binding activity of liver membrane used for irradiation was 0.4 pmole/mg protein, and that of solubilized protein was 0.5 pmole/mg protein. These activities were taken as 100%, respectively.

Fig. 5. Saturation curve (A) and Scatchard plot (B) of specific [3H]mepyramine binding to the solubilized [3H]mepyramine binding protein. Each assay used 80 μg solubilized protein from the liver membrane fraction. Values are means ±S.D. (n=4).
Table 1. $K_i$ values of various drugs for $[^3H]$mepyramine binding to solubilized $[^3H]$mepyramine binding protein

| Drug                  | $K_i$ (M) |
|-----------------------|-----------|
| Mepyramine            | 2.0±0.4×10^{-8} |
| Triprolidine          | 2.9±0.8×10^{-6} |
| Diphenhydramine       | 2.9±0.8×10^{-6} |
| $d$-Chlorpheniramine  | 2.9±0.8×10^{-6} |
| Mequitazine           | 4.8±0.3×10^{-6} |
| Terfenadine           | 0.9±0.3×10^{-6} |
| Astemizole            | 2.3±0.7×10^{-6} |
| Cimetidine            | 2.3±0.4×10^{-4} |
| Histamine             | 3.8±0.9×10^{-4} |
| $d$-Tubocurarine      | 3.9±0.3×10^{-3} |
| Noradrenaline         | 0.9±0.3×10^{-2} |
| Adrenaline            | 0.9±0.3×10^{-2} |
| Serotonin             | 0.9±0.3×10^{-2} |

All values are means±S.E.M. (n=6).

Effectively, by nonlinear regression.

$K_i$ values of various ligands for $[^3H]$mepyramine binding to the solubilized $[^3H]$mepyramine binding protein

The $K_i$ values of various ligands for the solubilized $[^3H]$mepyramine binding protein were examined (Table 1). Mepyramine showed very high affinity ($K_i=20±4$ nM), whereas $H_1$ antagonists such as diphenhydramine, $d$-chlorpheniramine and triprolidine showed about 150 times lower affinities ($K_i=2.9±0.8$ AM). The other $H_1$ antagonists, mequitazine, terfenadine and astemizole, which have weaker side-effects on the CNS, also showed lower affinities. Histamine and cimetidine had lower affinities than $H_1$ antagonists. The non-histamine ligands, $d$-tubocurarine, noradrenaline, adrenaline and serotonin, showed the weakest inhibitions of $[^3H]$mepyramine binding.

Stability of solubilized $[^3H]$mepyramine binding protein

Solubilized $[^3H]$mepyramine binding protein was stored at either 4°C or −80°C and the remaining activities of $[^3H]$mepyramine binding were measured after different storage periods. $[^3H]$Mepyramine binding activity was slowly inactivated as a single exponential function of the period of storage at both 4°C and −80°C (Fig. 6). The residual $[^3H]$mepyramine binding activities after storage at 4°C and −80°C for 20 days were 75% and 90%, respectively.

Partial purification of $[^3H]$mepyramine binding protein

Sepharose CL-4B gel filtration (Fig. 7A): The crude solubilized $[^3H]$mepyramine binding protein obtained from rat liver membranes by extraction and centrifugation as described in “Materials and Methods” was applied to a Sepharose CL-4B column. On elution with Buffer A, the peak of $[^3H]$mepyramine binding activity was observed after elution with 540
Fig. 7. Chromatographies for partial purification of the solubilized [3H]mepyramine binding protein. A, B, C and D show the elution profiles on Sepharose CL-4B gel filtration, Bio Gel HTP-hydroxylapatite, Octyl Sepharose 4B and hydroxylapatite HPLC column chromatographies, respectively. The fractions of eluate from each column were assayed for specific [3H]mepyramine binding (cpm) (●—●) and monitored for absorbance at 278 nm (■—■). Aliquots of 30 µl, 20 µl, 15 µl and 30 µl from fractions of Sepharose CL-4B, Bio Gel HTP, Octyl Sepharose 4B and hydroxylapatite HPLC column chromatographies, respectively, were assayed. The concentration of Na/K phosphate buffer for Bio Gel HTP chromatography and that of Tween 60 for Octyl Sepharose 4B column chromatography are indicated by solid lines ( ). In A, fraction collection was initiated after the column was washed with 540 ml of buffer. The conditions for the chromatographies are described in "Materials and Methods".
ml of eluate.

**Table 2. Purification of \[^3H\]mepyramine binding protein from rat liver membrane**

| Step                              | Total activity (pmole) | Total protein (mg) | Specific activity (pmole/mg prot.) | Yield (%) |
|-----------------------------------|------------------------|-------------------|------------------------------------|-----------|
| Crude soluble                     | 373                    | 373               | 1.0                                | 100       |
| Sepharose CL-4B                   | 265                    | 78.0              | 3.4                                | 71        |
| Bio Gel HTP                       | 124                    | 20.7              | 6.0                                | 33        |
| Octyl sepharose                   | 39.7                   | 2.5               | 15.8                               | 10.6      |
| Hydroxylapatite HPLC              | 24.6                   | 0.82              | 30.9                               | 6.6       |

**Big Gel HTP column chromatography (Fig. 7B):** The \[^3H\]mepyramine binding fraction from Sepharose CL-4B was applied to a Bio Gel HTP column. \[^3H\]Mepyramine binding activity was adsorbed to the column. The column was washed with Buffer A, and then material was eluted with a linear gradient of 50 mM to 250 mM Na/K phosphate buffer (pH 7.4). The peak of \[^3H\]mepyramine binding activity was eluted with 200 mM buffer.

**Octyl Sepharose 4B hydrophobic chromatography (Fig. 7C):** The fraction of \[^3H\]mepyramine binding activity from the Bio Gel HTP column was then applied to an Octyl Sepharose 4B column. Some protein was not adsorbed, but the \[^3H\]mepyramine binding protein was adsorbed to the column; and after development with a gradient of 0.02–0.5% (w/v) of Tween 60, the \[^3H\]mepyramine binding protein was eluted with 0.5% (w/v) of the detergent.

The \[^3H\]mepyramine binding protein eluted from Octyl Sepharose 4B was purified further by hydroxylapatite HPLC column chromatography (Fig. 7D). The elution profile was essentially the same as on Bio Gel HTP column chromatography, but resolution of the H₁ receptor from other proteins was better. The peak of \[^3H\]mepyramine binding activity was eluted with 180 mM phosphate buffer. The partial purification of \[^3H\]mepyramine binding protein is summarized in Table 2. Starting from a total activity of 373 pmoles of \[^3H\]mepyramine binding activity from 10 g of rat liver, 6.6% was recovered by the purification steps with 30-fold purification.

**Discussion**

In the present study, the \[^3H\]mepyramine binding protein was solubilized in an active form from rat liver membrane using CHAPS and Tween 60 as detergents and glycerol as an enhancer of solubilization. CHAPS is a synthetic detergent (17, 18) that has recently been used to solubilize various receptors (19–24), and like digitonin and deoxycholate, it may solubilize membrane proteins in the form of subpopulations. Thus, the "solubilized membrane protein" obtained with these detergents may still contain small membrane fragments that are not precipitated by centrifugation at 100,000×g for 1 hr. Toll and Snyder reported solubilization of the H₁ receptor with \[^3H\]mepyramine binding as a marker in an active form from rat brain and guinea pig brain using digitonin as a detergent (10). We tried to solubilize rat hepatic \[^3H\]mepyramine binding protein with digitonin preparations from several commercial sources. Each preparation had a different solubility in water, but we could not solubilize the \[^3H\]mepyramine binding protein with any of them (data not shown). This may be partly because digitonin is obtained from a natural source and/or hepatic \[^3H\]mepyramine binding protein is a subtype of H₁ receptor with a different state in the membrane. CHAPS, which is a synthetic compound, solubilized \[^3H\]mepyramine binding protein reproducibly in combination with Tween 60 and glycerol. When the \[^3H\]mepyramine binding protein was solubilized with CHAPS and glycerol in the absence of Tween 60, \[^3H\]mepyramine binding activity was detected in the supernatant, but it decreased on centrifugation at 100,000×g for more than 1 hr (data not shown). On solubilization in the presence of 0.2% (w/v) Tween 60, the \[^3H\]mepyramine binding activity in the supernatant was not affected by centrifugation at 100,000×g for more than 1 hr. The results of molecular weight determination by gel filtration and...
Sucrose density gradient sedimentation indicates that the binding site was actually solubilized.

The molecular weight of solubilized [3H]-mepyramine binding protein determined by target size analysis was 162K, and this value was the same as that of membrane incorporated [3H]-mepyramine binding protein (Fig. 4). However, the molecular weight of solubilized [3H]-mepyramine binding protein determined by gel filtration (670K) or sucrose density gradient sedimentation (800K) was much larger than that of [3H]-mepyramine binding protein determined by target size analysis (162K) (Figs. 2, 3 and 4). Several explanations of this difference in molecular weight can be considered. One is that the apparent molecular weight of the solubilized [3H]-mepyramine binding protein may be increased by formation of a protein-detergent complex. This idea is supported by the observations of Suarez et al. (25) and Haga (26) that the apparent molecular weight of cytochrome c oxidase and the muscarinic acetylcholine receptor, respectively, were higher when these proteins were solubilized with detergents. Another possibility is that the [3H]-mepyramine binding protein may consist of subunits and that the molecular weight of the [3H]-mepyramine binding subunit may have been determined by target size analysis. A third possibility is that the [3H]-mepyramine binding protein may not be globular and so the apparent molecular weight was overestimated by gel filtration. A fourth possibility is that when the [3H]-mepyramine binding protein is solubilized with detergent, the density of the receptor-detergent complex may have been less, resulting in an apparent increase in molecular weight. Probably the difference in the molecular weight is due to several of these possibilities. Kuno et al. (27) reported a molecular weight of 160K for the H1 receptor of rat brain determined by target size analysis. This value is very similar to that of the [3H]-mepyramine binding protein in rat liver (28).

Uchida (7) prelabeled the histamine H1 receptor from smooth muscle of cat small intestine with [3H]-dibenamine in combination with several blockers of receptors other than the histamine H1 receptor and solubilized it with Triton X-100. He found that the molecular weight of the H1 receptor-detergent complex was about 100K. We determined the molecular weight of [3H]-mepyramine binding protein-detergent complex to be 670K by gel filtration and 800K by sucrose density gradient sedimentation, and by target size analysis, it was 162K. Thus the molecular nature of the solubilized H1 receptor obtained by Uchida is quite different from that of our preparation. Dibenamine may not be specific for H1 receptors. Osband and McAffrey (8) reported solubilization of the H1 receptor from calf thymus with Nonidet P-40, monitoring the receptor by measuring [3H]-histamine binding activity, while Gavish et al. (9) reported its solubilization from rat brain with digitonin, monitoring the receptor by [3H]-mepyramine binding activity. However, they did not determine the molecular size of the receptor-detergent complex by gel filtration or sucrose density gradient sedimentation and so did not demonstrate its solubilization. Toll and Snyder (10) determined the molecular weight of the H1 receptor-digitonin complex from guinea pig brain to be 430K by gel filtration and sucrose density gradient sedimentation. This was less than our values for some unknown reason. Possible reasons are that 1) we used different detergents for solubilization of the [3H]-mepyramine binding protein (H1 receptor), 2) digitonin solubilized a subunit of the [3H]-mepyramine binding protein (H1 receptor) and 3) [3H]-mepyramine binding proteins (H1 receptors) from rat liver and guinea pig brain have different molecular weights.

We observed a single binding site for [3H]-mepyramine binding protein solubilized from rat liver membranes. The dissociation constants (Kd) of [3H]-mepyramine binding for the H1 receptors in membranes (28) and in solubilized preparations were very similar. These data indicated that the [3H]-mepyramine binding protein was solubilized without appreciable change in its nature.

The Kd value of mepyramine for [3H]-mepyramine binding to the receptor was about 150 times less than those of diphenhydramine, d-chlorpheniramine and tripolidine. This is why a higher concentration of displacer (20 μM tripolidine) was used.
However, the solubilized binding protein was eluted as a single peak on gel filtration and showed high affinity to mepyramine and lower affinity to triprolidine (Fig. 2A). These data show that only one $[3\text{H}]$mepyramine receptor seems to exist in rat liver membrane. The $K_i$ values of $\alpha$-chlorpheniramine and triprolidine were reported to be about 30–50 times larger than the $K_i$ value of $[3\text{H}]$mepyramine for rat intestinal membranes (16). Moreover, the $K_i$ values of diphenhydramine, $\alpha$-chlorpheniramine and triprolidine are reported to be very close to that of mepyramine for both membrane bound (29–31) and solubilized (9) $H_1$ receptors from rat brain. It is considered that $[3\text{H}]$mepyramine binding protein in rat liver may be one of the subtypes of histamine $H_1$ receptors with different $K_i$ values for $H_1$ antagonists. Further investigations are still necessary.

For studies on the molecular mechanism of signal transduction through the histamine $H_1$ receptor, it is desirable to purify the receptor. The solubilized $[3\text{H}]$mepyramine binding protein was fairly stable on prolonged storage at 4°C or –80°C, and its stability seemed sufficient to allow its purification. In this work, we purified the $[3\text{H}]$mepyramine binding protein 30 times from rat liver by Sepharose CL-4B gel filtration, Bio Gel HTP hydroxylapatite, Octyl Sepharose 4B and hydroxylapatite HPLC column chromatographic steps. The resulting preparation had the highest purity reported so far (10). The physiological role of histamine in rat liver is unknown; and to our knowledge, there are no reports of effects of histamine on liver function. Although $[3\text{H}]$-mepyramine binding activity is widely used to characterize histamine $H_1$ receptor, we have to note the possibility that the $[3\text{H}]$mepyramine binding site in rat liver is a receptor for some substance other than $H_1$ receptor, because $H_1$ antagonists have other actions such as anticholinergic, local anesthetic, etc. (32). The possible role of $[3\text{H}]$mepyramine binding protein (histamine $H_1$ receptor) of rat liver in glycogenolysis is currently being investigated in our laboratory (33).

Acknowledgments: We are grateful to Dr. H. Hayashi for help with HPLC; to Mr. T. Ikeda, Institute of Scientific and Industrial Research, Osaka University, for help with gamma-irradiation with $^{60}\text{Co}$; to Tanabe Pharmaceutical Co., Nippon Shoji Kaisha, Ltd., Jansen Kyowa Pharmaceutical Co., Dow Chemical Co. and Wellcome Japan Pharmaceutical Co. for generous gifts of drugs; and to Mrs. K. Tsuji for typing. We thank Dr. I. Imamura for valuable suggestions. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, the Tokyo Biochemical Research Foundation, Japan and the Uehara Memorial Foundation, Japan.

References

1. Ash, A.S.F. and Schild, H.O.: Receptors mediating some actions of histamine. Br. J. Pharmacol. Chemother. 27, 427–430 (1966)
2. Black, J.W., Duncan, W.A.M., Durant, C.J., Ganellin, C.R. and Parsons, E.M.: Definition and antagonism of histamine $H_2$-receptors. Nature 236, 385–390 (1972)
3. Arrang, J.-M., Garbarg, M. and Schwartz, J.-C.: Auto-inhibition of brain histamine release mediated by a novel class ($H_3$) of histamine receptor. Nature 320, 832–837 (1983)
4. Karaki, H. and Weiss, G.B.: Calcium channels in smooth muscle. Gastroenterology 87, 960–970 (1984)
5. Jones, L.M., Cockcroft, S. and Michell, R.H.: Stimulation of phosphatidylinositol turnover in various tissues by cholinergic and adrenergic agonists, by histamine and by caerulein. Biochem. J. 182, 869–676 (1979)
6. Hill, S.J., Young, J.M. and Marrian, D.H.: Specific binding of $^3\text{H}$-mepyramine to histamine $H_1$ receptors in intestinal smooth muscle. Nature 270, 361–363 (1977)
7. Uchida, M.: Purification of histamine receptor (VI) an improved double labeling methods with "double protection". Japan. J. Pharmacol. 27, 781–789 (1977)
8. Osband, M. and McCaffrey, R.: Solubilization, separation, and partial characterization of histamine $H_1$ and $H_2$ receptors from calf thymocyte membranes. J. Biol. Chem. 254, 9970–9972 (1979)
9. Gavish, M., Chang, R.S.L. and Snyder, S.H.: Solubilization of histamine $H_1$, GABA and benzodiazepine receptors. Life Sci. 25, 783–790 (1979)
10. Toll, L. and Snyder, S.H.: Solubilization and characterization of histamine $H_1$ receptors in brain. J. Biol. Chem. 257, 13693–13601 (1982)
11. Wang, N.P., Fukui, H., Watanabe, T. and Wada, H.: Solubilization and characterization of histamine $H_1$-receptor from guinea-pig brain. Bioge-
nic Amines 4, 7–17 (1987)

12 Imoto, M., Tsuchie, K., Tanaka, M., Sugiyama, S. and Ozawa, T.: Predominance of histamine H₁ receptors on liver plasma membrane. Biochem. Biophys. Res. Commun. 129, 885–889 (1985)

13 Kempner, E.S. and Schlegel, W.: Size determination of enzymes by radiation inactivation. Anal. Biochem. 92, 2–10 (1979)

14 Kempner, E.S. and Haigler, H.T.: The influence of low temperature on the radiation sensitivity of enzymes. J. Biol. Chem. 257, 13297–13299 (1982)

15 Burgisser, E.: Model testing in radioligand/receptor interaction by Monte Carlo stimulation. J. Recept. Res. 3, 261–281 (1983)

16 Chang, R.S.L., Tran, V.T. and Snyder, S.H.: Characteristics of histamine H₁-receptors in peripheral tissue labeled with [³H]mepyramine. J. Pharmacol. Exp. Ther. 209, 437–442 (1979)

17 Simons, W.F., Koski, G., Streaty, R.A., Hjelmeland, L.M. and Klee, W.A.: Solubilization of active opiate receptors. Proc. Natl. Acad. Sci. U.S.A. 77, 4623–4627 (1980)

18 Hjelmeland, L.M.: A nondenaturing zwitterionic detergent for membrane biochemistry: Design and synthesis. Proc. Natl. Acad. Sci. U.S.A. 77, 6368–6370 (1980)

19 Liscia, D.L. and Vonderhaar, B.K.: Purification of a prolactin receptor. Proc. Natl. Acad. Sci. U.S.A. 79, 5930–5934 (1982)

20 Wouters, W., Dan, J.V., Leysen, J.E. and Laduron, P.M.: Solubilization of rat brain serotonin-S₂ receptors using CPAPS/salt. Eur. J. Pharmacol. 115, 1–9 (1985)

21 Hirose, S., Akiyama, F., Shinjo, M., Ohno, M. and Murakami, K.: Solubilization and molecular weight estimation of atrial natriuretic factor receptor from bovine adrenal cortex. Biochem. Biophys. Res. Commun. 130, 574–579 (1985)

22 Lambert, M., Svoboda, M., Furnelle, J. and Christophe, J.: Solubilization from rat pancreatic plasma membranes of a cholecystokinin (CCK) agonist-receptor complex interacting with guanine nucleotide regulatory proteins coexisting in the same macromolecular system. Eur. J. Biochem. 147, 611–617 (1985)

23 Matsui, H., Asakura, M., Tsukamoto, T., Imafuku, J., Ino, M., Saitoh, N., Miyamura, S. and Hasegawa, K.: Solubilization of α₂-adrenergic receptor. J. Neurochem. 44, 1625–1632 (1985)

24 Takemura, M., Fukui, H., Yamamoto, Y., Hosomi, N. and Wada, H.: Solubilization and characterization of the nitrendipine receptor of rat brain. J. Neurochem. 48, 1185–1190 (1987)

25 Suarez, M.D., Revzin, A., Narlock, R., Kempner, E.S., Thompson, D.A. and Ferguson-Miller, S.: The functional and physical form of mammalian cytochrome C oxidase determined by gel filtration, radiation inactivation, and sedimentation equilibrium analysis. J. Biol. Chem. 259, 13781–13799 (1984)

26 Haga, T.: Molecular size of muscarinic acetylcholine receptors of rat brain. FEBS Lett. 113, 68–72 (1980)

27 Kuno, T., Kubo, N. and Tanaka, C.: Molecular size of histamine H₁ receptor determined by target size analysis. Biochem. Biophys. Res. Commun. 129, 639–644 (1985)

28 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)

29 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 (1979)

30 Chang, R.S.L., Tran, V.T. and Snyder, S.H.: Heterogeneity of histamine H₁-receptors: species variations in [³H]mepyramine binding of brain membranes. J. Neurochem. 32, 1653–1663 (1979)

31 Hill, S.J. and Young, J.M.: Histamine H₁ receptors in the brain of the guinea-pig and the rat: the differences in ligand binding properties and regional distribution. Br. J. Pharmacol. 68, 687–696 (1980)

32 Douglas, W.W.: Histamine antagonists: H₁ and H₂-blocking agents. In The Pharmacological Basis of Therapeutics, Seventh Edition, Edited by Gilman, A.G., Goodman, L.S., Rall, T.W. and Murad, F., p. 618–628, McMillan, New York (1985)

33 Fukui, H., Takemura, M., Wang, N.P., Kuurosawa, K., Furuya, E., Tagawa, K. and Wada, H.: Hepatic histamine H₁ receptor: Purification of the receptor and effect of histamine on glycogenolysis. Japan. J. Pharmacol. 40, Supp. 155P (1986)