

Genomic Cloning of the Rat Histamine H₁ Receptor

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A rat histamine H₁ receptor gene which lacked introns was isolated from a rat genomic library using recently cloned bovine histamine H₁ receptor cDNA [Yamashita *et al.*, Proc. Natl. Acad. Sci. USA, **88**, 11515-11519 (1991)]. The receptor protein deduced from this isolated gene was composed of 486 amino acids and showed characteristic properties of G protein-coupled receptors. At the 5'-flanking region of the receptor gene, we have located potential TATA box sequences and consensus sequences for the glucocorticoid response element and AP-2 element. After being subcloned into a mammalian expression vector, the isolated gene was transfected to C6 glioma cells. These cells showed significant binding toward [³H]mepyramine. The binding was inhibited by H₁ antagonists or histamine. The mode of this binding was comparable to the binding of membranes derived from rat tissues toward [³H]mepyramine. Northern blot analysis detected a 3.0 kb nucleotide band for histamine H₁ receptor mRNAs from rat brain and small intestine when these mRNAs were hybridized with the isolated rat H₁ gene. The present results demonstrate the isolation of the rat histamine H₁ receptor gene. © 1993 Academic Press, Inc.

The histamine H₁ receptor is one of Ca²⁺ mobilizing receptors (1), and mediates immune hypersensitivity in peripheral tissues and neurotransmission in the central nervous system (2). In rat, the H₁ receptor-mediated response was studied extensively in the central nervous system (2), but it was seldom studied in peripheral tissues because of its low sensitivity. We have recently isolated the bovine histamine H₁ receptor cDNA clone and demonstrated this receptor shared a common molecular structure with G protein-coupled receptors (3). In the present study, we report the isolation of the rat histamine H₁ receptor gene ¶ which lacks introns by using the bovine H₁ receptor cDNA as a probe. The expressed rat H₁ receptors were pharmacologically characterized and the tissue distribution of H₁ receptor mRNA was examined.

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¶ Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession No. D12800.

MATERIALS AND METHODS

Materials

[³H]Mepyramine (1.073 GBq/mmol) (Du Pont-New England Nuclear) was purchased from Dai-ichi Chemical Co. (Japan). Histamine and *d*-chlorpheniramine were purchased from Wako Pure Chemical Industries (Japan) and Tokyo Kasei (Japan), respectively. Mepyramine and triprolidine were purchased from Sigma (USA). *l*-Chlorpheniramine and cimetidine were generous gifts from Smith Klein and French (UK).

Screening of Genomic Library

Rat genomic library (Clontech, USA) was screened using the *Eco*RI and *Eco*RI-*Sma*I fragments (2.7 kb and 0.6 kb, respectively) of the bovine H₁ receptor cDNA (3). Duplicate filters were hybridized in 5 X SSC (1 X SSC = 0.15 M NaCl/0.015 M sodium citrate), 20% formamide, 5 X Denhardt's solution, 100 µg/ml denatured E. coli DNA and 10⁶ cpm/ml of [³²P]labeled probes at 42 °C for 16 h. Filters were then washed in 0.2 X SSC and 0.1% SDS at 42 °C. Candidate clones were isolated and re-purified. The isolated clone was subcloned in pUC18. DNA sequencing was performed by dideoxy chain termination method (4) after subcloning in M13 mp18 and mp19 phages using a DNA sequencer (Model 370A, Applied Biosystems, USA).

Expression and Binding Studies

A *Spe*I-*Dra*I fragment (2.3 kb) of H₁ receptor gene was subcloned in the mammalian expression vector pEF-BOS (5) at the *Bst*XI site. The vector was introduced into rat C6 glioma cells by the calcium phosphate method (6) using CellPect Transfection kit (Pharmacia) and the cells were harvested after the 72 h-incubation. Membrane preparation and [³H]mepyramine binding assay were performed as described previously (7).

Northern Blot Analysis

The total cellular RNA was extracted from Wistar rat tissues by the guanidium isothiocyanate/cesium trifluoride method as described by Chirgwin *et al.* with slight modifications (8). Poly (A)⁺ RNA was isolated by oligo(dT)-Latex (Oligotex-dT30, Dai-ichi Chemical Co., Japan), separated on formaldehyde/1% agarose gel electrophoresis (9), and blotted to a nylon membrane (Gene Clean Plus, New England Nuclear, USA). A [³²P]labeled 1.9 kb *Spe*I-*Sph*I fragment of H₁ receptor gene was used as a probe. Hybridization was performed at 42 °C in 5 X SSC, 5 X Denhardt's, 50% formamide, 10% dextran sulfate, 1% SDS and 100 µg/ml salmon sperm DNA. The membrane was washed with 0.1 X SSC and 0.1% SDS at 42 °C.

RESULTS AND DISCUSSION

Isolation of Rat Histamine H₁ Receptor Gene

Two highly co-hybridized clones were isolated from 1.2 x 10⁶ recombinants by screening a rat genomic library with two restriction fragments of the bovine histamine H₁ receptor cDNA. Each of the clones had a 10 kb insert and the same restriction map.

The *Eco*RI fragment (3.5kb) obtained from the clone which contained 990 bp of 5' noncoding region, 1458 bp of the histamine H₁ receptor coding region, and 1061 bp of 3' noncoding region. The nucleotide and deduced amino acid sequences of the fragment are presented in Fig. 1. The rat H₁ receptor gene lacked introns and encoded a protein of 486 amino acids with putative seven transmembrane domains, which is the characteristic for G protein-coupled receptors. Calculated molecular weight of the rat H₁ receptor was 55,690 and was consistent with the values obtained by photoaffinity labeling of rat brain (54,000 - 56,000) (10) and of bovine adrenal medulla (53,000 - 58,000) (11). Similar to the bovine H₁ receptor (3), the rat H₁ receptor possessed a large third

-945 ATCCAGCAGCCCTTCTCAGGCCAGTCAGTTTACTGAGCTCCCTGTGTGGCCATTCTTTCTGGCTGCACAGCCGGTGCATCTGTTCCCAAGGCTTTTCCACTTCCATGGAAATGCAAGCATTCCTCTGGCC -946
 -810 TCACCTGTGGAGCTGGGCACAGGGCTTTCTTTCCAGTCTCTCCAAAGCCCCACCCCTACGGCTCCCTCCCAATTGCTTCTCTCCAAAGCTGCCCTTCTCCAAACAGATTCTTGGACAGTGGCTTAAGCAAA -947
 -675 GAACACATTTCCTACTATAGGAGCAAGATTGACAGATTCTCTCAGCAGCTCTGTGTCAACAAGCTCTAAGCTGACGCTCTGTGGTTTCTTGACATTCTCAATACGCTCAAACTACCATCTGACTCCAAAC -948
 -540 ACCCTTCTCATCTCCCTTTTCAGGAGTACGGTAGAGGGAAGGGTGATTTCATAGCTTGGCATTTTGCACAGGGGAAGATGAGTCTTTTGAACCCACAGCCCTTTTCTGGCCACAGTTTGTGCCAGAA -949
 -405 CCATCAAGAGGCTCCAGATATAGCATTTCTCAGAGTGTTCAGACATCTTCCGCAATAGACTGTGATAACCTACCCCTGTGTGTGTTAACTCTGCTATTAACCTCCACAGATCAGAGGTTTCTTCTCCCTC -950
 -270 TGGTTTATCAACAGTCGATCTCTGATATTCATAATAATCCAGGAATCCAGCAATAGTCTGGCAGCAATGTAGCAGCAGGCAAGCCATGAGTGAATACATGCTCTGATGTGTGTGATGGGTGCCACCAC -951
 -135 CCCAGCAGCTTCTACTCCAGCTGCAGCAGATGGCTGTCTAGGTGACCTCTGATCATCTTGATCTTGACCTTGATCTTGTGTCTTTTCTCCCAAGGAGGAGCATCACTGGAGGCTCCCTCTGTGCAA -952
 1 ATG AGC TTT GCC AAT ACC TCC TCT ACC TTC GAA GAC AAG ATG TGT GAG GGG AAC AGG ACA GCC ATG GCC AGC CCT CAG CTG CTG CCC CTG GTG GTG GTT CTC 192
 1 Met Ser Phe Ala Asn Thr Ser Ser Thr Phe Glu Asp Lys Met Cys Glu Gly Asn Arg Thr Ala Met Ala Ser Pro Gln Leu Leu Pro Leu Val Val Val Leu 34
 103 AGT ACT ATC TCC CTG GTC ACA GTG GGC CTC AAC CTG CTG GTG CTG TAC TCT GTG CAC AGT GAA CCG AAC CTA CAC ACC GTG GGC AAC CTA TAC ATT GTC AGC 204
 35 Ser Ser Ile Ser Leu Val Thr Val Gly Leu Asn Leu Leu Val Leu Tyr Ala Val His Ser Glu Arg Lys Leu His Thr Val Gly Asn Leu Tyr Ile Val Ser 68
 205 CTG TCT GTG GCA GAC CTT ATT GTA GGG GCA GTT GTC ATC CCC ATG AAC ATC CTC TAT CTC ATC ATG ACT AAG TGG TCC TCG GGC CGC CCC CTC TGC CTC TTT 306
 69 Leu Ser Val Ala Asp Leu Ile Val Gly Ala Val Val Met Pro Met Asn Ile Leu Tyr Leu Ile Met Thr Lys Trp Ser Leu Gly Arg Pro Leu Cys Leu Phe 102
 307 TGG CTT TCT ATG GAT TAT GTG GGC AGC ACA GCA TCC ATC TTT AGC GTC TTC ATC CTC TGT ATT GAT CGC TAC CGC TCC GTC CAG CAA CCC CTC CGG TAC CTG 408
 91 Trp Leu Ser Met Asp Tyr Val Ala Ser Thr Ala Ser Ile Phe Ser Val Phe Ile Leu Cys Ile Asp Arg Tyr Arg Ser Val Gln Gln Pro Leu Arg Tyr Leu 136
 409 AGG TAC CGA ACC AAG ACC GGC GCT TCC GCT ACC ATC CTG GGG GCC TGG TGC TTC TCC TTC CTG GTT ATA CCC ATA CTT GGC TGG CAT CAC TTC ATG CCC 510
 137 Arg Tyr Arg Thr Lys Thr Arg Ala Ser Ala Thr Ile Leu Gly Ala Trp Phe Phe Ser Phe Leu Trp Val Ile Pro Ile Leu Gly Trp His His Phe Met Pro 170
 511 CCA GCC CCA CAG CTT CGG GAA CAG AAG TGT GAG ACA GAC TTC TAC AAT GTC ACT TGG TTC AAG ATG ATG ACT CCT ATT ATT AAC TTC TAC CTC CCC ACT TTG 612
 171 Pro Ala Pro Glu Leu Arg Glu Asp Lys Cys Glu Thr Asp Phe Tyr Asn Val Thr Trp Phe Lys Ile Met Thr Ala Ile Ile Asn Phe Tyr Leu Pro Thr Leu 204
 613 CTT ATG CTG TGG TTC TAT GTG AAG ATC TAC AAG GCT GTG GCG CGA CAC TGT GAG CAC CGC CAG CTC ACC AAC GGG TCC CTC CCT TCT TTT TCA GAA CTC AAG 714
 205 Leu Met Leu Trp Phe Tyr Val Lys Ile Tyr Lys Ala Val Arg Arg His Cys Gln His Arg Gln Leu Thr Asn Gly Ser Leu Pro Ser Phe Ser Glu Leu Lys 238
 715 CTG AGG TCA CAC GAT ACC AAG GAA GGT CCC AAG AAA CCT GGG AGA GAG TCT CCC TGG GGG GTT CTA AAA AGG CCA TCA AGA CAC CCC AGT GTA GGA CTG GAT 816
 239 Leu Arg Ser Asp Asp Thr Lys Glu Gly Ala Lys Lys Pro Gly Arg Glu Ser Pro Trp Gly Val Leu Lys Arg Pro Ser Arg Asp Pro Ser Val Gly Leu Asp 272
 817 CAG AAG TCA ACA TCT GAA CAC CCC AAG ATG ACC TCT CCA ACT CTC TTC AGC CAA GAG GGG GAA AGG GAA ACA CCT CCC TGT TTC CTT CTC GAC ATC ATG CAG 918
 273 Gln Lys Ser Thr Ser Glu Asp Pro Lys Met Thr Ser Pro Thr Val Phe Ser Gln Glu Gly Glu Arg Glu Thr Arg Pro Cys Phe Arg Leu Asp Ile Met Gln 306
 919 AAA CAG TCT GTG GCT GAG GGA GAT GTC AGG GGC TCA AAG GGC AAT GAT CAG GGC TTA AGC CAG CCC AAA ATG GAT CAG CAG AGC CTA ATG ACT TGT CGG CGG 1020
 307 Lys Gln Ser Val Ala Glu Gly Asp Val Arg Gly Ser Lys Ala Asn Asp Gln Ala Leu Ser Gln Pro Lys Met Asp Glu Gln Ser Leu Asn Thr Cys Arg Arg 340
 1021 ATC AGT GAG ACA TCA GAG GAT CAC ACC TTG CTG GAT CAA CAG TCC TTC TCC CGG ACC ACA GAC TCA CAC ACA AGC ATA GAG CCA GGG CCG GGC AGA GTC AAA 1122
 341 Ile Ser Glu Thr Ser Glu Asp Gln Thr Leu Val Asp Gln Gln Ser Phe Ser Arg Thr Thr Asp Ser Asp Thr Ser Ile Glu Pro Gly Pro Gly Arg Val Lys 374
 1123 TCG ACA AGC GGG TCT AAG ACT GGC CTG GAT TAC ATC AAA ATC ACC TGG AAG AGC CTC CGC TCA CAG TCC AGA CAG TAT GTG TCC GGG GAG CAG TTG AAG CGA 1224
 375 Ser Arg Ser Gly Ser Asn Ser Gly Leu Asp Tyr Ile Lys Ile Thr Trp Lys Arg Leu Arg Ser His Ser Arg Gln Tyr Val Ser Gly Leu His Leu Asn Arg 408
 1225 GAG CGG AAG CCA AAG CAG TTG GGT TTT ATG CAG TTG ATT CTC TGC TGG ATT CCC TAT TTC ATC TAT CTC TTC ATG GTC ATT GGC TTC TGC AAG ACC 1326
 409 Glu Arg Lys Ala Ala Lys Gln Leu Gly Phe Ile Met Ala Ala Phe Ile Leu Cys Trp Ile Asn Ser Thr Leu Asn Pro Leu Ile Tyr Pro Leu Cys Asn Glu Asn Phe Lys Lys 442
 1327 TGC TGC AGT GAA CCC ATG CAT ATG TCC ACC ATT TGG CTG GGC TAC ATC AAC TCC ACC CTC AAC CCC CTC ATC TAC CCC CTG TGC AAG CAG AAC TTC AAG AAC 1428
 443 Cys Cys Ser Glu Pro Met His Met Phe Thr Ile Trp Leu Gly Tyr Ile Asn Ser Thr Leu Asn Pro Leu Ile Tyr Pro Leu Cys Asn Glu Asn Phe Lys Lys 476
 1429 ACA TTC AAA AAG ATT CTG CAC ATT GGT TCC TAA GGG CAT ACC AAA AGG ATG CCA CAT AGT GGA TAG ACA AGC TCT GAT GTC CAA GAG GGA ACC ACA GGA AGA 1530
 477 Thr Phe Lys Lys Ile Leu His Ile Arg Ser *** 486
 1531 AAGCATGGCTGTCACTAGACCCCTGAGCATTAGGAAGTGAATCTAAGCTCTGGCTGACAGCTCTGGAGGCAATCCCAAGGAGCTGTGACAGGAAGAGGAGCAGAGCGATTGTCTCTGGCTAGAAATCA 1665
 1666 GAGCATCTGAAGAGACCCAGGACAGCATCTCGCTGCCCTCAGGAACTGTGGAGTCTCAGATACCTCTGGAAGTCAAACTCTGACTTAAGTTTCTTGGTAAGTCAAGTGGGAATGCTGTGCTATAGTTCT 1800
 1801 CTGAGGGTTAGCTGTGAGCTCAGACTTCCGACAGAAAGACTGTGTGACTGCAGAGACATGCACCGGACTGCTTCCCTTCCACGGGGTAACTTGGAGAGGCTCGAGAGCTTTTCACTGCAGACCATTTTCAA 1935
 1936 AATGCACTGCTTCGAGCCGAGCTCTCTTACAGACTTGTCCAGCACTATGTGCCCAACCGTGAACCTTGCCTTACCCGTCTCTCAGAGTTTTCAGAAATTAGGACGCTTGTGACTGCACTATTTCACCTCT 2070
 2071 AGTTCTTTTGGGCTTTTAAATGGCGCAGCAACACACACAGTACCAAAATCTCTCTCAATGTCAAGGAGCAGCAGCAACTTTTGAATGGTTTATTTTAAGCAAAAGCAAGCAGACAGCTT 2205
 2206 ATTCTTTAAGACTCCACAGCGGGCTCTCAGCTAGGCGCCCTGGCTGCACAAAGAAAGAGGCTTCTGGTAATCATTTGATCACTCAGCTCAGAAATCGGGATGACAACCTTGTATGGCTCAGTTTCATCACT 2340
 2341 CTCCAAAGGCAAGAAATGTCCCTTCCCATCTACTCAGCATCTCTCCCAAGAGAGTGACAGCTTCCGAGTAACGGGATCGAGCAGCACTTGTCCAGAGGCTGACCTCGGAAGGCAAAATGTCTTTCTT 2475
 2476 CTAAGAGCTGAGTTTCTTGGTGCACATCTGCAAGCATCC

Fig. 1. Nucleotide and deduced amino acid sequences of the rat histamine H₁ receptor gene. Sequences of both strands of DNA were determined. Potential TATA boxes and consensus sequences (AP-2 and GRE) are underlined. Putative transmembrane domains are overlined.

intracellular loop and a short carboxyl terminal, which are common characteristics among Ca^{2+} mobilizing receptors and receptors inhibiting adenylyl cyclase such as m₁-muscarinic (12) and dopamine D₂ receptors (13), respectively. Two ATG codons, 39 bp apart, were located in the isolated rat histamine H₁ receptor gene for a possible initiation codon. Kozak's consensus sequence analysis (14) on the two codons could not determine the one that should be the preferred initiation site; thus, we have assigned the most upstream ATG codon as the presumed initiation site. Similar ATG codons were reported for the bovine H₁ receptor cDNA (3). We located several putative transcriptional regulatory sites: a 5'-noncoding segment of 990 nucleotides containing two potential TATA box sequences (21), one (TAATAA) at position from -238 to -233 and the other (TATAA) at position from -339 to -335; a consensus sequence for glucocorticoid response element (GRE: AGATCAGA) (22) at the position from -293 to -286, and a consensus sequence for AP-2 element (CCCCACCC) at the position from -760 to -753.

When the amino acid sequence of the rat H₁ receptor was compared with that of the bovine H₁ receptor, the amino acid sequence homology between these two receptors was 73% in total and 94% in the presumed transmembrane domain region (Fig. 2). The homology was relatively low in amino-terminal region (62%) and the third extracellular loop region (62%), where the rat H₁ receptor lacked one and four amino acid residues, respectively, as compared with the bovine H₁ receptor. The existence of three potential N-glycosylation sites, Asn-5 and Asn-18 in the amino-terminal region and

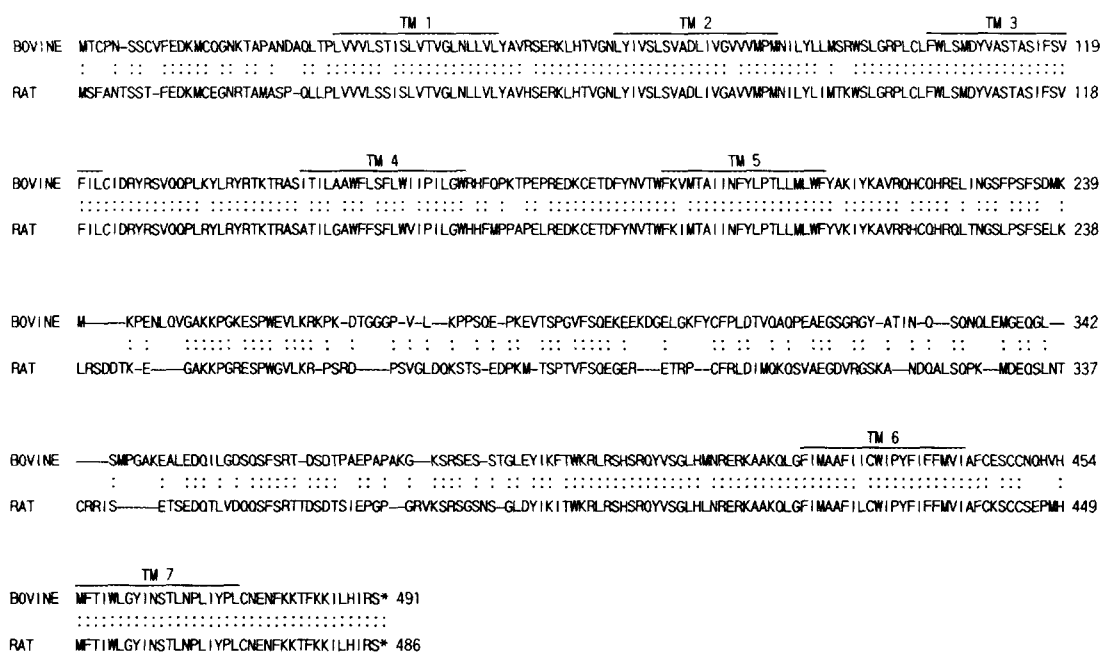


Fig. 2. Comparison of the bovine and rat H₁ receptor amino acid sequences. Putative transmembrane domains are overlined. Two dots (:) indicate amino acid identity. Gaps in the bovine and rat H₁ receptor sequences are indicated by dashes.

Asn-186 in the second extracellular loop, were predicted as the bovine H₁ receptor had a consensus sequence Asn-X-Ser/Thr (X; a less essential amino acid residue) (15). Two cysteins, Cys-100 and Cys-180, similar to those forming disulfide bond in rhodopsin family receptors (16), were found in the first and the second extracellular loops. A number of serine and threonine residues were present in the third intracellular loop. These residues were potential sites for coupling with G protein, since such coupling was regulated by the phosphorylation at these residues. Two potential phosphorylation sites (17) for multifunctional calmodulin-dependent protein kinase II and two sites for cGMP-dependent protein kinase were located in the third intracellular loop in the rat H₁ receptor. Another site for multifunctional calmodulin kinase was found in the second intracellular loop. All multifunctional calmodulin kinase and cGMP-dependent protein kinase sites are conserved in both rat and bovine H₁ receptors. PMA (4 β -phorbol 12-myristate 13-acetate), a protein kinase C activator, inhibited the signal transduction through the H₁ receptor (18); thus, a site for protein kinase C is suggested. There was no consensus sequence found for a typical phosphorylation site designated for protein kinase C (X-R-X-X-R-X-R-X, where X is a less essential amino acid residue) (17); however, we found a similar sequence (K-R-L-R-S-H-S-R-Q), which might act as the site. Asp-107 in the third transmembrane domain, and Thr-194 and Asn-198 both in the fifth transmembrane domain were putative binding sites for histamine amino- and imidazole-moieties. These sites were assigned by the comparison of amino acid sequences of histamine H₂ and other biogenic amine receptors (19, 20) and they were also found in bovine H₁ receptor. Other sites which were conserved among biogenic amine receptors were also present in H₁ receptor; such sites are Asp-73 in the second transmembrane domain, the anionic and cationic amino acid pair (Asp-124 and Arg-125) at the cytoplasmic border of the third transmembrane domain, and the ten amino acid residues (Leu-455 - Pro-464) in the seventh transmembrane domain.

Expression of Rat Histamine H₁ Receptor Gene

Binding study shown in Fig. 3A demonstrated the specific binding of [³H]mepyramine to membranes from the C6 rat glioma cells which were transfected with H₁ receptor gene. The binding was saturable with high concentrations of ligand. The *K_d* and *B_{max}* values were 1.6 nM and 620 fmol/mg protein, respectively. The [³H]mepyramine binding to the membranes was displaced by various H₁ antagonists and histamine in a dose-dependent manner (Fig. 3B). Chlorpheniramine isomers showed a stereoselective inhibition of [³H]mepyramine binding. In contrast, cimetidine, a histamine H₂ receptor antagonist, at 10 μ M had no effect on the [³H]mepyramine binding. The analysis of the data from the competition curve revealed the following *K_i* values: Mepyramine, 1.7 nM; triprolidine, 2.0 nM; *d*-chlorphenyramine, 7.5 nM; *l*-chlorphenyramine, 540 nM; and histamine, 110 μ M. The *K_d* values for [³H]mepyramine binding to membranes from rat tissues had been reported to range from 1.9 to 10.4 nM (2) and the *K_d* value obtained in this study was comparable. These *K_i* values were also comparable to those reported previously using membranes from rat tissues (24, 25). The membranes from nontransfected C6 glioma cells exhibited no specific binding to [³H]mepyramine (data not shown).

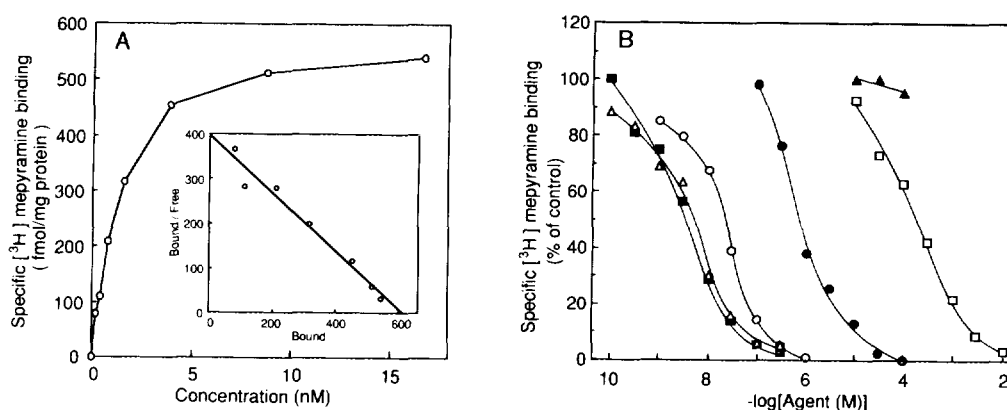


Fig. 3. Binding of [^3H]mepyramine to transfected C6 glioma cell membranes. (A) Saturation isotherms of specific binding of [^3H]mepyramine to membranes from C6 glioma cells with the receptor DNA (\circ). (Inset) Scatchard plot of this data. (B) Inhibition of [^3H]mepyramine binding to transfected C6 glioma cell membranes by various drugs. Membranes were incubated with 4 nM [^3H]mepyramine and various concentrations of *d*-chlorpheniramine (\circ), *l*-chlorpheniramine (\bullet), mepyramine (\blacksquare), triprolidine (\triangle), histamine (\square), or cimetidine (\blacktriangle). Data points are means of triplicate experiments.

Tissue Distribution of Histamine H₁ receptor mRNA

The tissue distribution of rat H₁ receptor mRNA was determined by Northern blot analysis (Fig. 4). A clear band of 3.0 kb which corresponded to the histamine H₁ receptor mRNA was detected in the brain tissue. In contrast, only a faint band was detected in the small intestinal tissue and no positive hybridization signal was observed in the lung and heart tissues. These findings agreed with the report by Chang *et al.* (25) that [^3H]mepyramine binding to membranes from rat brain was several times higher than those from rat peripheral tissues such as lung, ileum and adrenal. It is of interest to point out that the tissue distribution of rat H₁ receptor mRNA is quite different from that

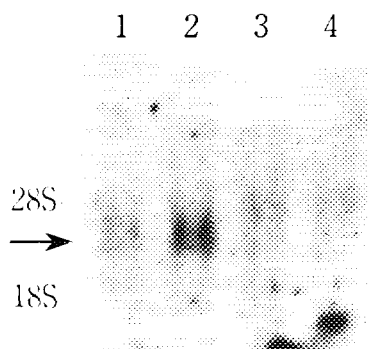


Fig. 4. Northern blot analysis of mRNA isolated from various rat tissues. Lanes contain 7- μg samples of poly(A)⁺RNA from small intestine (lane 1), brain (lane 2), lung (lane 3), and heart (lane 4). Arrow indicates H₁ receptor mRNA.

of bovine H₁ receptor mRNA which is high in the lung and intestine and low in the brain (3). It has been reported that higher affinity towards [³H]mepyramine (lower *K_d* values) was observed in peripheral tissues of the animals which had high peripheral tissue histamine response, *ie*, human and guinea pig, than those had low peripheral tissue histamine response, *ie*, rat, mouse and rabbit (26, 27). These reports suggested that receptors had different affinity towards mepyramine as well as histamine. However, our results in which the low level of H₁ receptor mRNA was found in rat peripheral tissues suggest the amount of H₁ receptors can determine the degree of response towards histamine.

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