Vol. 190, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1993 Pages 294-301

Genomic Cloning of the Rat Histamine H1 Receptor

Katsumi Fujimoto, Yoshiyuki Horio, Kazushige Sugama* †
Seiji Ito*, Ye Qi Liu and Hiroyuki Fukui ‡

Department of Pharmacology II, Faculty of Medicine, Osaka University, Suita 565, Japan
* Department of Cell Biology, Osaka Bioscience Institute, Suita 565, Japan

Received December 7, 1992

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 [^{3}H]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 [^{3}H]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.

The histamine H1 receptor is one of Ca^{2+} 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.

† Present address: Beyer Yakuhin Ltd., Kanda-cho, Nishi-ku, Kobe 651-23, Japan.

‡ To whom reprint requests should be addressed at the Department of Pharmacology II, Faculty of Medicine, Osaka University, 2-2 Yamadaoka, Suita 565, Japan.

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

0006-291X/93 $4.00

MATERIALS AND METHODS

Materials

[^{3}H]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 H$_1$ 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. coil DNA and 10^6 cpm/ml of [^{32}P]labeled probes at 42 ℃ for 16 h. Filters were then washed in 0.2 X SSC and 0.1% SDS at 42℃. 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 H$_1$ receptor gene was subcloned in the mammalian expression vector pEF-BOS (5) at the BstXI site. The vector was introduced into rat C6 glioma cells by the calcium phosphate method (6) using CellPhect Transfection kit (Pharmacia) and the cells were harvested after the 72 h-incubation. Membrane preparation and [^{3}H]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 [^{32}P]labeled 1.9 kb *Spe*I-*Sph*I fragment of H$_1$ receptor gene was used as a probe. Hybridization was performed at 42℃ 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℃.

RESULTS AND DISCUSSION

Isolation of Rat Histamine H$_1$ Receptor Gene

Two highly co-hybridized clones were isolated from 1.2 x 10^6 recombinants by screening a rat genomic library with two restriction fragments of the bovine histamine H$_1$ 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 H$_1$ 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 H$_1$ 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 H$_1$ 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 H$_1$ receptor (3), the rat H$_1$ receptor possessed a large third

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 m_1-muscarinic (12) and dopamine D_2 receptors (13), respectively. Two ATG codons, 39 bp apart, were located in the isolated rat histamine H_1 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 H_1 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 H_1 receptor was compared with that of the bovine H_1 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 H_1 receptor lacked one and four amino acid residues, respectively, as compared with the bovine H_1 receptor. The existence of three potential N-glycosylation sites, Asn-5 and Asn-18 in the amino-terminal region and

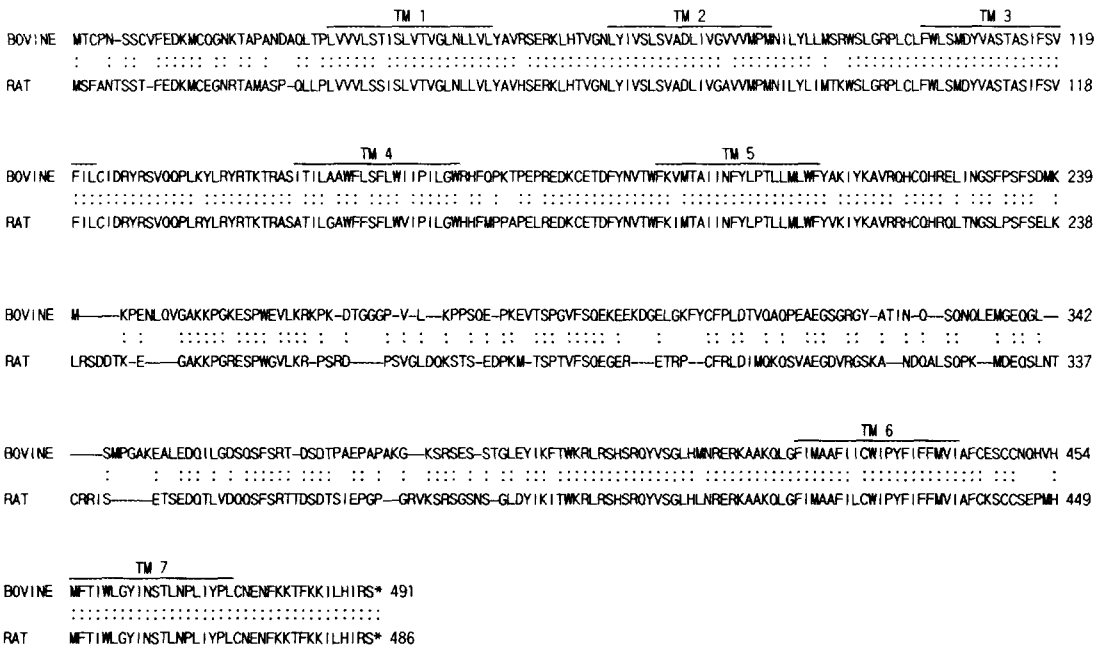

Fig. 2. Comparison of the bovine and rat H_1 receptor amino acid sequences. Putative transmembrane domains are overlined. Two dots (:) indicate amino acid identity. Gaps in the bovine and rat H_1 receptor sequences are indicated by dashes.

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 (4β-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 [^{3}H]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 [^{3}H]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 [^{3}H]mepyramine binding. In contrast, cimetidine, a histamine H2 receptor antagonist, at 10 μM had no effect on the [^{3}H]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 μM. The *K*d values for [^{3}H]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 [^{3}H]mepyramine (data not shown).

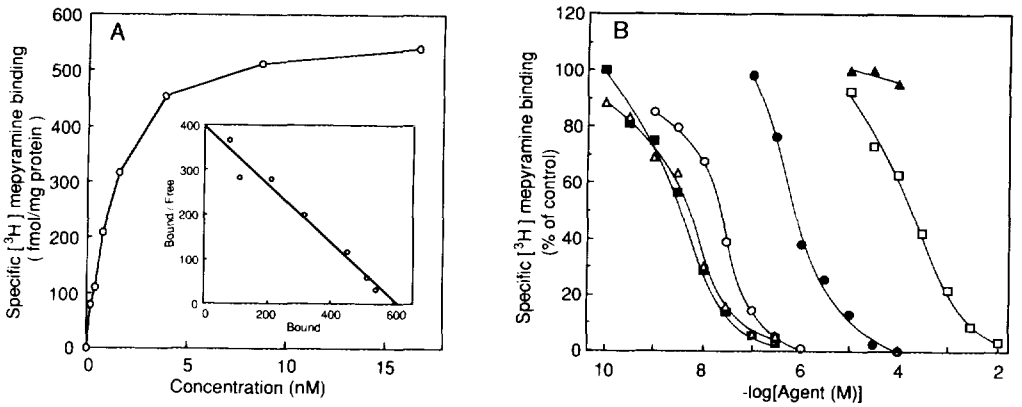

Fig. 3. Binding of [^{3}H]mepyramine to transfected C6 glioma cell membranes. (A) Saturation isotherms of specific binding of [^{3}H]mepyramine to membranes from C6 glioma cells with the receptor DNA (○). (Inset) Scatchard plot of this data. (B) Inhibition of [^{3}H]mepyramine binding to transfected C6 glioma cell membranes by various drugs. Membranes were incubated with 4 nM [^{3}H]mepyramine and various concentrations of *d*-chlorpheniramine (○), *l*-chlorpheniramine (●), mepyramine (▩), triprolidine (△), histamine (□), or cimetidine (▲). 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 [^{3}H]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

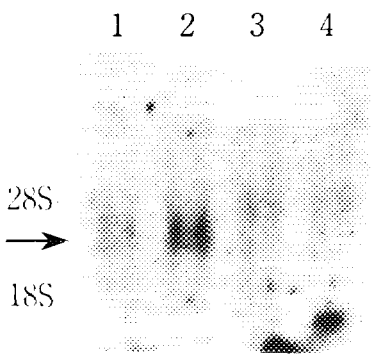

Fig. 4. Northern blot analysis of mRNA isolated from various rat tissues. Lanes contain 7-μg samples of poly(A)$^+$RNA from small intestine (lane 1), brain (lane 2), lung (lane 3), and heart (lane 4). Arrow indicates H1 receptor mRNA.

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 affiniy towards [^{3}H]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.

ACKNOWLEDGMENTS

This investigation was supported in parts by Grant-in Aid 03670102 from the Ministry of Education, Science and Culture of Japan, and by grants from the Mochida Memorial Foundation for Medical and Pharmaceutical Research, Brain Science Foundation and Kowa Life Science Foundation.

REFERENCES

1. Inagaki, N., Fukui, H., Ito, S., Yamatodani, A. and Wada, H. (1991) Proc. Natl. Acad. Sci. USA **88**, 4215-4219.
2. Fukui, H. (1991) in: Histamine receptors: H1- and H2-receptors (Watanabe, T. and Wada, H. eds.) Histamine Neurons: Morphology and Function, pp. 61- 83, CRC Press, Boca Raton.
3. Yamashita, M., Fukui, H., Sugama, K., Horio, Y., Ito, S., Mizuguchi, H. and Wada, H. (1991) Proc. Natl. Acad. Sci. USA **88**, 11515-11519.
4. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA **74**, 5463-5467.
5. Mizushima, S. and Nagata, S. (1990) Nucleic Acid Res. **18**, 5322.
6. Graham, F.L. and van der Eb, A.J. (1973) Virology **52**, 456-467.
7. Inagaki, N., Fukui, H., Taguchi, Y., Wang, N.P., Yamatodani, A. and Wada, H. (1989) Eur. J. Pharmacol. **173**, 43-51.
8. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry **18**, 5294-5299.
9. Lehrach, H., Diamond, D., Wezney, J.M. and Boldtkew, H. (1977) Biochemistry **16**, 4743-4751.
10. Ruat, M. and Schwartz, J.-C. (1989) J. Neurochem. **53**, 335-339.
11. Yamashita, M., Ito, S., Sugama, K., Fukui, H., Smith, B., Nakanishi, K. and Wada, H. (1991) Biochem. Biophys. Res. Commun. **177**, 1233-1239.
12. Shapiro, R.A., Scherer, N.M., Habecker, B.A., Subers, E.M. and Nathanson, N.M. (1988) J. Biol. Chem. **263**, 18397-18403.
13. Bunzow, J.R., Van Tol, H.H.M., Grandy, D.K., Albert, P., Salon, J., Christie, M., Machida,C.A., Neve, K.A. and Civelli, O. (1988) Nature (London) **336**, 783-787.
14. Kozak, M. (1984) Nucleic Acid Res. **12**, 857-872.
15. Kornfeld, R. and Kornfeld, S. (1985) Annu. Rev. Biochem. **54**, 631-664.
16. Strader, C.D., Sigal, I.S. and Dixon, R.A.F. (1989) FASEB J. **3**, 1825-1832.
17. Kemp, B.E. and Pearson, R.B. (1990) Trends Biochem. Sci. **15**, 342-346.
18. Fukui, H., Inagaki, N., Ito, S., Kubo, A., Kondoh, H., Yamatodani, A. and Wada, H. (1991) in: Histamine H1 receptors on astrocytes in primary cultures: a possible target for histaminergic neurones (Timmerman, H. and van der Goot, H. eds.) New Perspectives in Histamine Research, Agents Actions Supplements, Vol. 33, pp. 161-180, Birkhauser Verlag, Basel.
19. Gantz, I., Schaffer, M., DelValle, J., Logsdon, C., Campbell, V., Uhler, M. and Yamada, T. (1991) Proc. Natl. Acad. Sci. USA **88**, 429-433.

20. Strader, C.D., Sigal, I.S., Candelore, M.R., Rands, E., Hill, W.S. and Dixon, R.A.F. (1988) J. Biol. Chem. **263**, 10267-10271.
21. O'Shea-Greenfield, A. and Smale, S.T. (1992) J. Biol. Chem. **267**, 1391-1402.
22. Payvar, F., DeFranco, D., Firestone, G.L., Edgar, B., Wrange, O., Okret, S., Gustafsson, J.-A. and Yamamoto, K.R. (1983) Cell **35**, 381-392.
23. Roesler, W.J., Vandenbark, G.R. and Hanson, R.W. (1988) J. Biol. Chem. **263**, 9063-9066.
24. Tran, V.T., Chang, S.L. and Snyder, S.H. (1978) Proc. Natl. Acad. Sci. USA **75**, 6290-6294.
25. Chang, R.S.L., Tran, V.T. and Snyder, S.H. (1979) J. Pharm. Exp. Ther. **209**, 437-442.
26. Chang, S.L., Tran, V.T. and Snyder, S.H. (1979) J. Neurochem. **32**, 1653-1663.
27. Hill, S.J. and Young, J.M. (1980) Br. J. Pharmacol. **68**, 687-696.