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Biochemistry

Expression cloning of a cDNA encoding the bovine histamine H_1 receptor

(adrenal medulla/*Xenopus* oocyte/[^{3}H]mepyramine/doxepin)

MASAKATSU YAMASHITA*, HIROYUKI FUKUI*†, KAZUSHIGE SUGAMA‡, YOSHIYUKI HORIO*, SEIJI ITO‡, HIROYUKI MIZUGUCHI*, AND HIROSHI WADA*

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

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ABSTRACT **A functional cDNA clone for the histamine H_1 receptor was isolated from a cDNA library of bovine adrenal medulla by a combination of molecular cloning in an expression vector and electrophysiological assay in *Xenopus* oocytes. The H_1 receptor cDNA encodes a protein of 491 amino acids (M_r 55,954) with seven putative transmembrane domains, illustrating the similarity to other receptors that couple with guanine nucleotide-binding regulatory proteins (G protein-coupled receptors). The sequence homology between the H_1 and H_2 receptors is not higher than that between the histamine H_1 and m_1-muscarinic receptors. The cloned receptor protein expressed in COS-7 cells bound specifically to [^{3}H]mepyramine, an H_1 receptor antagonist, and this binding was displaced by H_1 receptor antagonists and histamine with affinities comparable with those in membranes of bovine adrenal medulla. H_1 receptor mRNA was shown to be expressed in brain and in peripheral tissues, including lung, small intestine, and adrenal medulla. This investigation discloses the molecular nature of the H_1 receptor—a receptor that mediates diverse neuronal and peripheral actions of histamine and that may be of therapeutic importance in allergy.**

Since Dale and Laidlaw (1) first reported the contraction of smooth muscle by histamine, the pharmacological significance of this phenomenon has been extensively investigated. Three subtypes of histamine receptor (H_1, H_2, and H_3) are known. The H_1 receptor was identified by Ash and Schild (2) and H_1 receptor antagonists have been used in the therapy of many allergic diseases, including urticaria, allergic rhinitis, pollenosis, and bronchial asthma. In peripheral tissues, the histamine H_1 receptor mediates the contraction of smooth muscles, increase in capillary permeability due to contraction of terminal venules, and catecholamine release from adrenal medulla (3), as well as mediating neurotransmission in the central nervous system (4). Although signal transduction of the H_1 receptor through Ca^{2+} mobilization via an increase in the intracellular inositol 1,4,5-trisphosphate level has been extensively investigated (5, 6), little is known about the molecular structure of the histamine H_1 receptor. Recently, another method for cDNA cloning of Ca^{2+}-mobilizing receptors through their expression in *Xenopus* oocytes has been developed (7). Meyerhof *et al.* (8) and Sugama *et al.* (9) have reported that the injection of poly(A)$^+$ RNA prepared from bovine adrenal medulla into *Xenopus* oocytes resulted in functional expression of the histamine H_1 receptor in oocytes. The present study describes the cloning and sequencing of a cDNA encoding histamine H_1 receptor§ from a cDNA library of bovine adrenal medulla using *in vitro* RNA transcription and electrophysiological assay with *Xenopus* oocytes.

MATERIALS AND METHODS

Materials. [^{3}H]Mepyramine (1073 GBq/mmol) and [α-^{32}P]-dCTP (≈111 TBq/mmol) were purchased from DuPont/NEN). Histamine and (+)-chlorpheniramine were purchased from Wako Pure Chemical (Osaka) and Tokyo Kasei (Tokyo), respectively. Mepyramine and doxepin were purchased from Sigma. (−)-Chlorpheniramine and famotidine were gifts from Smith Kline & French and Yamanouchi Pharmaceutical (Tokyo), respectively. A mammalian expression vector pEF-BOS (10) was donated by S. Nagata of the Osaka Bioscience Institute.

Isolation of Poly(A)$^+$ RNA. Total RNA was extracted by the acid guanidinium isothiocyanate/phenol/chloroform method (11). Poly(A)$^+$ RNA was isolated by chromatography on oligo(dT)-cellulose (12).

Expression Cloning of Histamine H_1 Receptor cDNA. Bovine adrenal medullary poly(A)$^+$ RNA (≈180 μg) was size-fractionated on a 5–25% (wt/vol) sucrose-density gradient. An aliquot (1 μl) of each poly(A)$^+$ RNA fraction (20 μl) was injected into *Xenopus* oocytes, and electrophysiological assay by measuring Ca^{2+}-dependent inward Cl^- currents was done as described (9). The fraction that showed the highest histamine-induced inward Cl^- currents was used for oligo(dT)-primed cDNA synthesis. Double-stranded cDNAs of >2-kilobase (kb) pairs were size-selected by agarose gel electrophoresis followed by elution with Geneclean II (Bio 101, La Jolla, CA) and were ligated into λZAPII (Stratagene) at the *Eco*RI site. The library was divided and amplified in 65 pools of ≈20,000 independent clones each. *In vitro* transcription was done essentially according to the procedure of Julius *et al.* (13). RNA transcripts (≈5 ng) from each pool were individually injected into *Xenopus* oocytes. After incubation for 1–2 days, the oocytes were tested for inward Cl^- currents induced by 100 μM histamine under a voltage clamp at −60 mV. The single positive pool of 20,000 clones was progressively subdivided into smaller pools of 8000, 4000, 400, and 15 clones until finally a single clone was obtained. cDNA encoding the histamine H_1 receptor was sequenced by the M13 chain-termination method (14) using a DNA sequencer (model 370A, Applied Biosystems). The sequence homology search was done by using DNASIS (Hitachi Software Engineering, Yokohama, Japan).

Expression of Histamine H_1 Receptor in COS-7 Cells and Its Determination by [^{3}H]Mepyramine-Binding Assay. An *Eco*RI

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

§The sequence reported in this paper has been deposited in the GenBank data base (accession no. D90430).

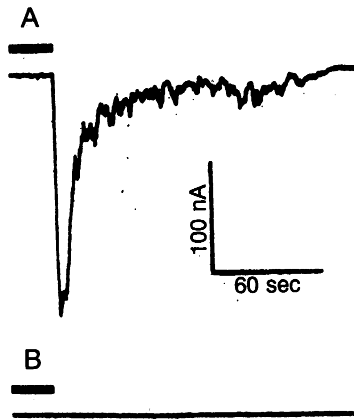

FIG. 1. (*A*) Current trace recorded from a *Xenopus* oocyte injected with *in vitro* synthesized histamine H_1 receptor mRNA. (*B*) Mepyramine (10 μM) was administered 30 sec before histamine application. Recordings were obtained at a voltage-clamped membrane potential of -60 mV. Concentration of histamine applied was 100 μM; horizontal bar indicates duration of application. Data were reproducible ($n = 5$), and representative tracings are shown.

fragment (2.7 kb) of the H_1 receptor cDNA was subcloned into the mammalian expression vector pEF-BOS at the *Bst*XI site. COS-7 cells were transfected by the DEAE-dextran method and were harvested after 60 hr (15). Preparation of membranes from COS-7 cells and [^{3}H]mepyramine-binding assay were done by a described method (16). Nonspecific bindings of [^{3}H]mepyramine to both transfected and nontransfected cells at 2.6 nM radioligand were <10% of total binding to nontransfected cells. Specific binding of [^{3}H]mepyramine to the nontransfected cells was observed (basal control), but that from the transfected cells assayed with 2.6 nM [^{3}H]mepyramine (3.4 pmol/mg of protein) was ≈30 times the basal control (0.1 pmol/mg of protein). Specific binding of [^{3}H]mepyramine to the expressed binding site was calculated by subtracting specific [^{3}H]mepyramine binding to the nontransfected cells from that to the transfected cells.

RNA Blot Analysis. Poly(A)$^+$ RNA prepared from various bovine tissues was separated (7 μg per lane) by formaldehyde/1% agarose gel electrophoresis (17) and transferred to a nylon membrane (Schleicher & Schuell). A 2.7-kb *Eco*RI fragment of the histamine H_1 receptor cDNA was labeled with [α-^{32}P]dCTP by the random-priming method and was

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-107 CCATTGTCCTCTTGAGGGACGTTTCTGCTGTTTCTATTCCTGGCTATTGAATAAGACTGCTGTGAACTCTTGAGAGTGAGCCGTAGCTGGAGGCTACACTTGTGCCA

   1 ATG ACC TGT CCC AAC TCC TCC TGC GTC TTC GAA GAC AAG ATG TGT CAG GGG AAT AAG ACT GCC CCT GCC AAC GAT GCC CAG CTG ACG CCC CTG GTG
   1 Met Thr Cys Pro Asn Ser Ser Cys Val Phe Glu Asp Lys Met Cys Gln Gly Asn Lys Thr Ala Pro Ala Asn Asp Ala Gln Leu Thr Pro Leu Val
                     Δ                                                   Δ

  97 GTG GTC CTG AGC ACC ATC TCC TTG GTC ACA GTG GGA CTC AAC CTG CTG GTC CTG TAT GCT GTG CGC AGC GAG CGG AAA CTA CAC ACC GTG GGG AAC
  33 Val Val Leu Ser Thr Ile Ser Leu Val Thr Val Gly Leu Asn Leu Leu Val Leu Tyr Ala Val Arg Ser Glu Arg Lys Leu His Thr Val Gly Asn
                                 I

 193 CTC TAC ATC GTC AGC CTC TCA GTG GCC GAC CTG ATC GTG GGG GTG GTC GTC ATG CCC ATG AAC ATC CTC TAC CTC CTC ATG TCT AGG TGG TCC CTG
  65 Leu Tyr Ile Val Ser Leu Ser Val Ala Asp Leu Ile Val Gly Val Val Val Met Pro Met Asn Ile Leu Tyr Leu Leu Met Ser Arg Trp Ser Leu
                                         II

 289 GGG CGT CCT CTC TGC CTC TTC TGG CTT TCC ATG GAC TAT GTG GCC AGC ACG GCA TCC ATT TTC AGC GTC TTC ATC TTG TGC ATT GAC CGC TAC CGT
  97 Gly Arg Pro Leu Cys Leu Phe 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
                                                             III

 385 TCT GTG CAG CAG CCC CTC AAG TAC CTG CGG TAT CGT AAC AAG ACC CGA GCA TCC ATC ACC ATC CTA GCC GCC TGG TTT CTC TCC TTC CTG TGG ATT
 129 Ser Val Gln Gln Pro Leu Lys Tyr Leu Arg Tyr Arg Thr Lys Thr Arg Ala Ser Ile Thr Ile Leu Ala Ala Trp Phe Leu Ser Phe Leu Trp Ile
                                                                                                                              IV

 481 ATC CCC ATT CTG GGC TGG CGT CAC TTC CAG CCA AAG ACC CCA GAG CCC CGG GAG GAC AAG TGT GAG ACG GAC TTC TAC AAC GTC ACG TGG TTC AAA
 161 Ile Pro Ile Leu Gly Trp Arg His Phe Gln Pro Lys Thr Pro Glu Pro Arg Glu Asp Lys Cys Glu Thr Asp Phe Tyr Asn Val Thr Trp Phe Lys
                                                                                                          Δ

 577 GTC ATG ACC GCC ATC ATC AAC TTC TAC TTG CCC ACC TTG CTC ATG CTC TGG TTC TAT GCC AAG ATC TAC AAG GCT GTG CGG CAG CAC TGC CAG CAC
 193 Val Met Thr Ala Ile Ile Asn Phe Tyr Leu Pro Thr Leu Leu Met Leu Trp Phe Tyr Ala Lys Ile Tyr Lys Ala Val Arg Gln His Cys Gln His
                                 V

 673 CGG GAG CTC ATC AAC GGG TCC TTC CCC TCC TTC TCT GAC ATG AAG ATG AAG CCG GAG AAC CTC CAG GTG GGC GCT AAG AAA CCG GGG AAG GAG TCC
 225 Arg Glu Leu Ile Asn Gly Ser Phe Pro Ser Phe Ser Asp Met Lys Met Lys Pro Glu Asn Leu Gln Val Gly Ala Lys Lys Pro Gly Lys Glu Ser

 769 CCC TGG GAG GTT CTG AAA AGG AAG CCA AAA GAC ACC GGG GGT GGA CCT GTC TTG AAG CCA CCA TCT CAA GAG CCA AAA GAG GTG ACA TCT CCA GGT
 257 Pro Trp Glu Val Leu Lys Arg Lys Pro Lys Asp Thr Gly Gly Gly Pro Val Leu Lys Pro Pro Ser Gln Glu Pro Lys Glu Val Thr Ser Pro Gly

 865 GTC TTC AGC CAA GAG AAG GAA GAG AAG GAT GGA GAA CTG GGC AAA TTC TAC TGC TTC CCG CTT GAC ACT GTG CAG GCG CAG CCG GAG GCA GAG GGG
 289 Val Phe Ser Gln Glu Lys Glu Glu Lys Asp Gly Glu Leu Gly Lys Phe Tyr Cys Phe Pro Leu Asp Thr Val Gln Ala Gln Pro Glu Ala Glu Gly

 961 AGT GGC AGG GGC TAC GCA ACC ATC AAC CAG AGC CAG AAC CAG CTT GAG ATG GGT GAG CAG GGC CTG AGC ATG CCT GGG GCT AAG GAG GCC TTA GAG
 321 Ser Gly Arg Gly Tyr Ala Thr Ile Asn Gln Ser Gln Asn Gln Leu Glu Met Gly Glu Gln Gly Leu Ser Met Pro Gly Ala Lys Glu Ala Leu Glu

1057 GAT CAG ATC CTA GGT GAC AGC CAG TCC TTC TCC CGG ACA GAC TCG GAC ACC CCC GCA GAG CCG GCA CCA GCG AAA GGC AAG TCG CGA AGC GAG TCT
 353 Asp Gln Ile Leu Gly Asp Ser Gln Ser Phe Ser Arg Thr Asp Ser Asp Thr Pro Ala Glu Pro Ala Pro Ala Lys Gly Lys Ser Arg Ser Glu Ser

1153 AGC ACA GGC CTG GAG TAC ATC AAG TTC ACT TGG AAG AGG CTC CGC TCG CAT TCG AGA CAG TAC GTG TCT GGC TTG CAC ATG AAC CGA GAG CGG AAG
 385 Ser Thr Gly Leu Glu Tyr Ile Lys Phe Thr Trp Lys Arg Leu Arg Ser His Ser Arg Gln Tyr Val Ser Gly Leu His Met Asn Arg Glu Arg Lys

1249 GCC GCC AAG CAG TTG GGT TTT ATC ATG GCG GCC TTC ATC ATC TGC TGG ATT CCT TAC TTC ATC TTC TTC ATG GTC ATT GCC TTC TGT GAG AGC TGC
 417 Ala Ala Lys Gln Leu Gly Phe Ile Met Ala Ala Phe Ile Ile Cys Trp Ile Pro Tyr Phe Ile Phe Phe Met Val Ile Ala Phe Cys Glu Ser Cys
                                                               VI

1345 TGC AAC CAG CAT GTG CAC ATG TTC ACC ATC TGG CTG GGC TAC ATC AAC TCC ACG CTG AAC CCC CTC ATC TAC CCC TTG TGC AAT GAG AAC TTC AAG
 449 Cys Asn Gln His Val 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
                                                               VII

1441 AAG ACC TTC AAG AAA ATT CTG CAC ATT CGT TCC TAG GAGAGACTCCCAGGGGATGCAGCCAAGTGACGCTTACTGATGTCCCTGAAGGAAGTGAAGGAGGAAGCCTGTTGCGTTG
 481 Lys Thr Phe Lys Lys Ile Leu His Ile Arg Ser ***

1556 CCAGGCACCTGGGCCTTCTGGAGTCGGAAGAATGGTCTTAGGGCCTGGGGAGTTTGGAAGGTTCGTAGGCGCAGTGGGAAGAACAGCAGATGGTGGTGGTCAGCAGAGAGATTGTACTCTGAGAGCC
1683 GAATGTTTGCAAGAGACTCAGATCTGTCCAAGCTCTCCTGTTTCTCGGGAATGTGGGGGCCTCAGGCTCACACTCTAATTCCAGCTTTCACACTCAAATTATTGGGCGACTCAAGGGACCTGTGGGT
1810 AGAGTTCCAGTGGACTCTGGAGCCTTCTTCAAATGGAGCTCCACGTCTGTGGAGCAGAGACCTTAGACATGCAGATAGATGCTGTCCCTGACCGGGGTCACCTTGAAAGGCAGGACAGCTACTCCAC
1937 TGTGACTGCCACATCTCAGAACACCTCTCTTCTGAGCCTCTTTTGCAGCTTTCTCCAGATCCAGTGCCTGAACCACCGTGGCTGATTCCACTGTATTATTTCTCACTCATGCACATCTTAAGAGTTG
2064 ATGGGAAATGATGCAGTCTGCACATCCATCATTTTCAAACCCAAATTCCATTCTCCTATTAAAGAAGAGGTTAAATGCCTCCCTCAAAGAGAAAAGGAATATTTTTTAGATGGTTGTATATTAGAAA
2191 CGAAAAAAGAGGAGTGGGGAGAAGAAAGCCAATTGCTTGAGGGCTGTGCCAGGTTGATGTCATTTAAGCCCCATGACACCCCACAACAGGAGAATGGTGTTATGAGAAAAGCAAAGGAGGAACAGCG
2318 AGGTGCAAGAAGGGACCTGAGATCACACAACTAGTTATAAGAAAGCTGGAGCAAACATCCTGTGGCTTCAACTCACTGTAACACATTTTCTCCAAAAGGCAAAAATTTGTTCTATTCAGTATACACA
2445 CACACACACACACACACACACACACATTCATAATGCCTGACAGTGGTGGCAGTTCACAGGAGTATATTGAGAGGAAGAACAGCTGACATAGAATGTGGCCACAGAGGTGATACTTGAAGAAGGAACA
2572 GTATACTTTTTCATCTGGGAATTCTGCTGTGTTTATCCAAGAAACATCATCATGTACTTTTATGGTCATACTTTTTAAGTCAAAACTTACAAAGGAATGGGAACAGGCAGTTTTAGCTGGTGTTTAT
2699 GTTGCAATCTGGTTGTGATTTATATGCTAAAACTGGATGTTAAACTGTAATACATGTAGCCAGTGGGAGTGTCTGTACAAGCTGGTGTTTTATGTCTCGTGTTCCTGTTTGCATGATCTGTTAAAAT
2826 GAGAGATTTTTACCTACTAAAATATGAT 2853
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FIG. 2. Nucleotide and deduced amino acid sequences of the histamine H_1 receptor cDNA clone. Sequences of both strands of cDNA were determined. Positions of the putative transmembrane segments I–VII of the H_1 receptor are indicated below amino acid sequence; the terminal of each segment is tentatively assigned from a hydropathy profile. Triangles indicate potential N-glycosylation sites.

used as a probe (18). Hybridization was done at 42°C in 5× standard saline citrate/20 mM sodium phosphate, pH 7.0/1× Denhardt's solution/50% (vol/vol) formamide/0.1% SDS/ 10% (wt/vol) dextran sulfate/salmon sperm DNA at 100 μg/ml. The membrane was washed with 0.1× standard saline citrate and 0.1% SDS at 42°C.

RESULTS

Isolation of a Histamine H_1 Receptor cDNA. Poly(A)$^+$ RNA isolated from bovine adrenal medulla was size-fractionated in a sucrose-density gradient. Two peaks giving histamine-evoked inward currents in oocytes were observed in the size range of 2.5- to 3.5-kb nucleotides and above 5-kb nucleotides (data not shown). A cDNA library was constructed from poly(A)$^+$ RNA in the fraction of 2.5- to 3.5-kb nucleotides giving the highest response. Of 65 pools tested only one pool gave small inward currents in response to 100 μM histamine. After several subdivisions of the positive pool, a single clone encoding for a functional histamine H_1 receptor was isolated; histamine induced inward Cl^- currents in oocytes injected with *in vitro*-transcribed mRNA from the cloned histamine H_1 receptor cDNA (Fig. 1), and mepyramine, an H_1 receptor antagonist, at 10^{-6} M completely blocked the histamine-induced response in oocytes.

Primary Structure of the Histamine H_1 Receptor. The nucleotide and deduced amino acid sequences of the bovine histamine H_1 receptor are shown in Fig. 2. The clone (2960 nucleotides long) consisted of 107 nucleotides of the 5′ untranslated region, 1473 nucleotides of the coding region, and 1380 nucleotides of the 3′-untranslated region. The histamine H_1 receptor cDNA encodes a protein of 491 amino acids with a M_r of 55,954.

Pharmacological Characterization of [^{3}H]Mepyramine-Binding to the Histamine H_1 Receptor Expressed in COS-7 Cells. For determination of pharmacological characters of the receptor, the *Eco*RI fragment (2.7 kb) of the H_1 receptor cDNA was subcloned into the mammalian expression vector pEF-BOS, and the vector was introduced into monkey kidney COS-7 cells. After 60-hr incubation, the binding of [^{3}H]mepyramine to the membranes from the cells was measured. Specific binding of [^{3}H]mepyramine to the expressed binding site was saturable, and Scatchard plot analysis indicated the presence of a single binding site with a K_d value of 3.2 nM and a B_{max} value of 6.6 pmol/mg of protein (Fig. 3*A*). K_i values of mepyramine, and (+)- and (−)-chlorpheniramines were determined to be 2.6×10^{-9} M, 8.0×10^{-9} M, and 7.6×10^{-7} M, respectively (Fig. 3*B*). These K_d and K_i values and the stereoselectivity of (+)- and (−)-chlorpheniramines for the binding site expressed in COS-7 cells were comparable with those for adrenal medullary membranes. The K_d value was 1.5×10^{-9} M; K_i values were 1.8×10^{-9} M (mepyramine), 4.3×10^{-9} M [(+)-chlorpheniramine], and 4.6×10^{-7} M [(−)-chlorpheniramine], as described (19).

Tissue Distribution of Histamine H_1 Receptor mRNA. Tissue distribution of receptor mRNA was determined by RNA blot analysis (Fig. 4). A band of 3.0-kb nucleotides corresponding to a histamine H_1 receptor mRNA was detected in various bovine tissues. The level of H_1 receptor mRNA was high in the lung and small intestine, moderate in the adrenal medulla and uterus, and lower in the cerebral cortex and spleen. No H_1 receptor mRNA was detectable in the cardiac atrium or liver.

DISCUSSION

In the present study, we isolated and sequenced a cDNA clone for the bovine histamine H_1 receptor by using an oocyte expression system and also examined the pharmacological properties of this receptor and the tissue distribution of its mRNA.

The cloned cDNA had no poly(A)$^+$, but its size [2960 base pairs (bp)] was comparable with that of histamine H_1 receptor mRNA determined by RNA blot analysis. The M_r of encoded H_1 receptor (55,954) was also consistent with the values estimated by photoaffinity labeling of bovine adrenal medulla (M_r 53,000–58,000) (19) and in guinea pig tissues (M_r 56,000–57,000) (20). Hydropathy-profile analysis (21) of the histamine H_1 receptor revealed the existence of seven putative transmembrane domains, indicating a similar topology to those proposed for other G protein-coupled receptors. The histamine H_1 receptor also possesses a characteristic large third cytoplasmic loop and short carboxyl terminus (22), as do the m_1-muscarinic (23) and dopamine-D_2 (24) receptors. We observed another ATG codon 39 bp downstream from the presumed initiation codon. Comparison with Kozak consensus sequence (25) indicated that neither of the two ATG codons had any advantage as an initiation codon. However, as receptors for biogenic amines and acetylcholine possess conservative aspartate residues at position 108 as putative binding sites for their monoamine and tertiary-amine residues (26), we presume that the upstream ATG codon is the

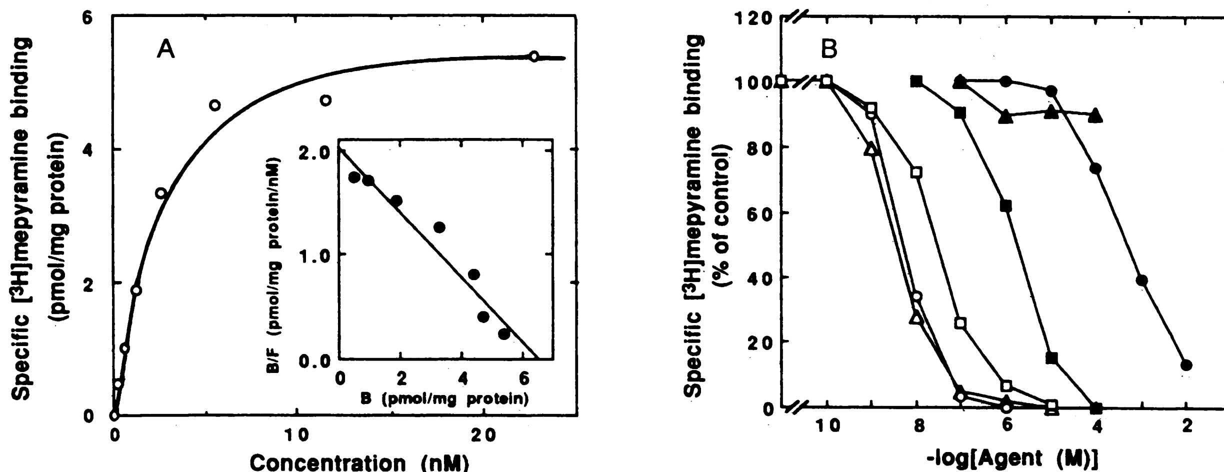

FIG. 3. Binding of [^{3}H]mepyramine to transfected COS-7 cell membranes. (*A*) Saturation isotherm of specific binding of [^{3}H]mepyramine to membranes from COS-7 cells transfected with the receptor cDNA (○). (*Inset*) Scatchard plot of this data. B/F, bound/free. (*B*) Inhibition of [^{3}H]mepyramine-binding to transfected COS-7 cell membranes by various drugs. Membranes were incubated with 4 nM [^{3}H]mepyramine and various concentrations of doxepin (△), mepyramine (○), (+)-chlorpheniramine (□), (−)-chlorpheniramine (■), famotidine (▲), or histamine (●). Data points are means of triplicate experiments.

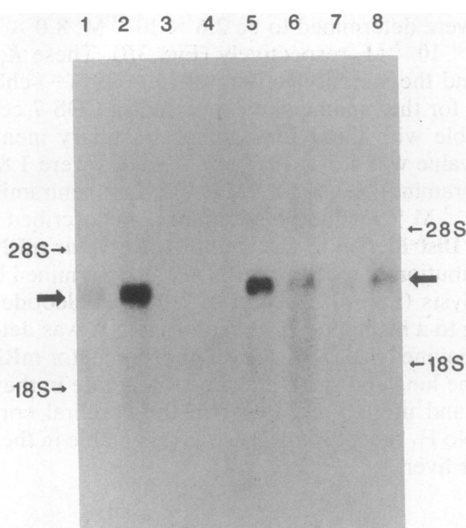

FIG. 4. RNA blot analysis of mRNA isolated from various bovine tissues. Lanes contain 7-μg samples of poly(A)$^+$ RNA from cerebral cortex (lane 1), lung (lane 2), liver (lane 3), cardiac atrium (lane 4), small intestine (lane 5), adrenal medulla (lane 6), spleen (lane 7), and uterus (lane 8). Arrow indicates H_1 receptor mRNA.

initiation codon because it would give a histamine H_1 receptor with the conservative aspartate residue at position 108. The histamine H_1 receptor is highly similar to other G protein-coupled receptors. The sequence of the histamine H_1 receptor is compared with those of some other G protein-coupled receptors in Fig. 5. Sequence homology of transmembrane domains between H_1 and H_2 receptors (40.7%) (27) is not higher than that between H_1 and m_1-muscarinic receptors (44.3%) (23).

There are two potential N-glycosylation sites (Asn-5, Asn-18) in the amino-terminal region with a consensus sequence Asn-Xaa-Ser/Thr (Fig. 2) (29). Mitsuhashi and Payan (30) reported regulation of the affinity of the histamine H_1 receptor by its glycosylation. An additional N-glycosylation site (Asn-187) was observed in the second extracellular loop of the cloned receptor.

The third cytoplasmic loop of the histamine H_1 receptor, which, by analogy, is thought to interact with a G protein, has many serine and threonine residues that may serve as sites for phosphorylation by protein kinases (Fig. 2). Signal transduction through the histamine H_1 receptor is depressed by activation of protein kinase C in various cells (31–33). Thus, the potential sites of phosphorylation in the third cytoplasmic loop may play an important role in regulating signal transduction through the receptor molecule.

Amino acid residues that are conserved in G protein-coupled receptors were also seen in the H_1 receptor: (*i*) Two cysteines (Cys-101 and Cys-181) that have been proposed to form a disulfide bond appear in the first and the second extracellular loops (34). (*ii*) An aspartate residue (Asp-74) is present in the second transmembrane domain. (*iii*) An anionic and cationic amino acid pair (Asp-125 and Arg-126) occurs at the cytoplasmic border of the third transmembrane domain. (*iv*) A conservative sequence of 10 amino acids (Leu-460–Pro-469) is observed in the seventh transmembrane domain.

The H_1 receptor mRNA was visualized by RNA blot analysis in various bovine tissues in which the existence of H_1 receptors was reported (3). The presence of the H_1 receptor mRNA in bovine uterus was clearly demonstrated, whereas only H_2 receptors (35) and both H_1 and H_2 receptors (36) were reported present in the uterus from pharmacological studies. The band of H_1 receptor mRNA from brain was unexpectedly faint (Fig. 4); this observation was surprising because the [^{3}H]mepyramine-binding capacities of brain membranes from various species are reported comparable to those of membranes from peripheral tissues (6). Doxepin is a potent displacer of [^{3}H]mepyramine bound to the histamine H_1 receptor from bovine adrenal medulla (Fig. 3). A doxepin-insensitive subtype of histamine H_1 receptor has been proposed to be present in brain because the binding capacity of [^{3}H]doxepin to rat brain membranes is ≈10% that of [^{3}H]mepyramine (37).

Cardiac atrium and liver did not give detectable bands of H_1 receptor mRNA (Fig. 4). Pharmacological studies indicate the presence of H_1 receptors in heart (3). However, biochemical results (20) show that the M_r of the histamine H_1 receptor in guinea pig heart is 68,000, which is larger than the sizes (M_r 56,000–57,000) of these receptors in lung, intestine, and cerebellum, suggesting a subtype of H_1 receptors in heart in which the H_1 receptor mRNA does not hybridize with the cloned cDNA. A relatively large amount of [^{3}H]mepyramine-binding protein is present in liver and was recently suggested

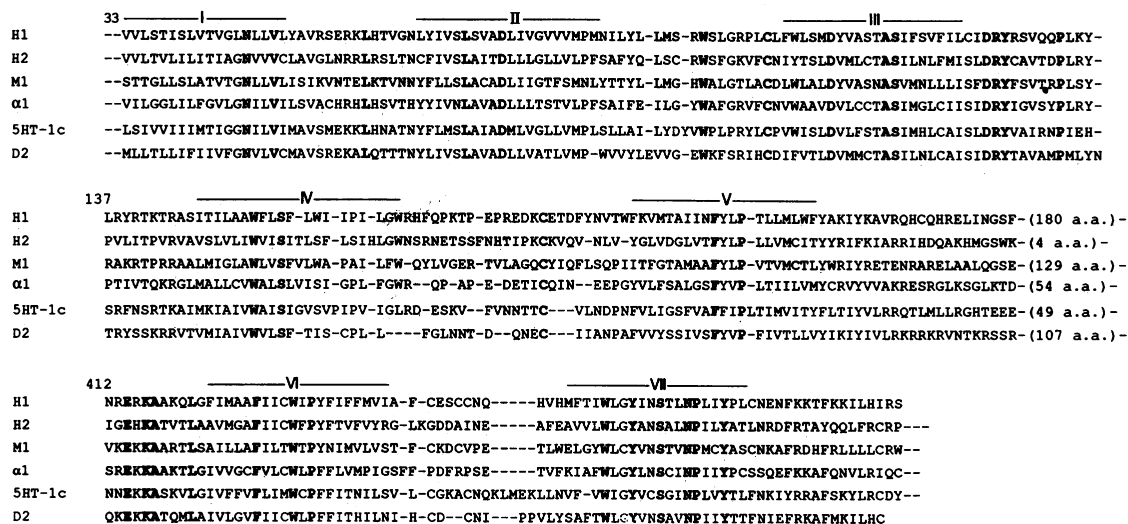

FIG. 5. Alignment of amino acid sequences of bovine histamine H_1 receptor (H1) and some representative G protein-coupled receptors. H2, canine histamine H_2 receptor (27); M1, mouse m_1-muscarinic receptor (23); α1, bovine α_1c-adrenergic receptor (28); 5HT-1c, rat serotonin 1c receptor (13); and D2, rat dopamine D_2 receptor (24). Amino acid residues shown by boldfaced type in sequences are identical; residues nonhomologous with H_1 receptor sequence in the loop between transmembrane segments V–VI are summed in parentheses. Positions of putative transmembrane segments I–VII of H_1 receptor are indicated.

to be a member of the family of debrisoquine-type cytochrome P450s (38).

The receptor cDNA clone for the classical histamine receptor (3), the H_1 receptor, isolated in this study, will be useful for molecular studies of function and regulation of activities mediated through the H_1 receptor molecule and for molecular analysis of possible H_1 receptor subclasses. *In situ* and immunocytochemical studies on localization of the H_1 receptor will also be helpful in analyzing physiological functions of histamine in the central nervous system and in peripheral tissues.

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