Human histamine H2 receptor gene: multiple transcription initiation and tissue-specific expression[1]

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Abstract We have characterized the genomic structure of 12.3 kb of the 5′-flanking region of the human histamine H2 receptor gene. The multiple transcription initiation sites of the human histamine H2 receptor gene were mapped utilizing the 5′-end cap structure of mRNA. We found that a 85 bp segment (−610–−525 bp) immediately upstream of the initiation site exhibits a strong promoter activity in the gastric adenocarcinoma, MNK45, expressing human histamine H2 receptor. A 4.8 kb transcript of the human histamine H2 receptor gene was found in the placenta, spinal cord, lymph node and bone marrow in addition to the previously reported tissues including the heart, brain and stomach, whereas a 1.8 kb transcript was observed in almost all tissues examined. 3′-rapid amplification of cDNA ends revealed the corresponding length of the 3′-untranslated region. These results suggest that the 3′-untranslated region may be involved in the differential expression.

Key words: Histamine receptor; Transcription; Tissue-specific expression; TATA-less promoter

1. Introduction

Histamine, a ubiquitous chemical mediator, has a wide range of biological actions including inflammation, contraction of smooth muscles, dilation of capillaries and stimulation of gastric acid secretion. These effects are mediated by one or combinations of the three pharmacologically defined receptor subtypes, named H1, H2 and H3 [1]. Studies with antagonists have demonstrated the importance of the H2 receptor (H2R) in the regulation of gastric acid secretion, contraction of the heart, gastrointestinal motility, cell differentiation and cell proliferation [2]. The histamine H2R gene has been cloned from man [3], dog [4], rat [5], mouse [6] and guinea pig [7] and the transcripts were detected in the brain and stomach. Detailed histochemical studies revealed that H2R is localized in gastric parietal cells and enteric neurons [8].

To understand the transcriptional regulation of H2R, we have analyzed the genomic structure of the 12.3 kb 5′-flanking region of the human H2R gene. By using the oligo-capping method, that traps the cap structure on the 5′-end of full length mRNA [9–11], we found that H2R contains multiple transcription initiation sites. Corresponding mRNAs were detected in various tissues. An 85 bp segment upstream of the transcription initiation sites showed a strong TATA-less promoter activity and was responsible for transcripts starting from −409 or −367 bp.

2. Materials and methods

2.1. Cell culture and characterization of a human H2R genomic clone

A poorly differentiated human gastric adenocarcinoma cell line, MKN45 (from the Japanese Cancer Research Resources Bank) [13], was cultured at 37°C in RPMI 1640 medium containing 10% fetal calf serum.

A human histamine H2R clone [12] was subcloned into the pBluescript plasmid. Sequencing was performed with either pUC/M13 forward and reverse primers or sequence-specific oligonucleotide primers using a Perkin Elmer-Applied Biosystems 377 Automated Sequencer.

2.2. Oligo-capping analysis

Poly $(A)^+$ RNA was purified using oligo-dT latex beads (Oligotex-dT30 Super, TaKaRa) from RNA isolated with a QuickPrep Total RNA Extraction kit (Amersham Pharmacia Biotech). Oligo-capping was performed as described by Maruyama and Sugano [9–11] with some modifications. The poly $(A)^+$ RNA was treated with bacterial alkaline phosphatase (TaKaRa) and tobacco acid pyrophosphatase (Epicentre Technologies) and then ligated with an RNA oligonucleotide (R-OLIGO, Table 1) using RNA ligase (TaKaRa). After each step, the reaction mixture was subjected to phenol/chloroform extraction and then, the RNA was precipitated with ethanol. The oligo-capped RNA was finally converted to cDNA with Superscript II reverse-transcriptase (Gibco) and a *Not*I poly dT primer adapter (Clontech).

cDNA was amplified by PCR with the D-OLIGO30 and H2REC-GSP1 primers (Table 1) and the products were subjected to second-round PCR with D-OLIGO30 and H2REC-GSP2 (Table 1). The products separated by polyacrylamide gel electrophoresis were sequenced with the nested primer, H2REC-GSP2.

2.3. Construction of promoter/reporter fusion plasmids and transient transfection

Reporter plasmid pGVH2L2D carries the −610–−278 bp region of the H2R gene in front of the luciferase gene of the PGV-B vector (Toyo Ink) [12]. Deletion derivatives were constructed using appropriate restriction enzymes.

45 h after transfection of the plasmids by electroporation, the cells were rinsed twice with phosphate-buffered saline and then lysed in cell lysis reagent PGC-50 (Toyo Ink). Extracts were mixed with a substrate solution (PGL-1500, Toyo Ink) and then, the light intensity was measured with a luminometer, Lumat LB 9501 (Berthod). The pGV-B vector lacking the eukaryotic promoter and enhancer and pGV-C carrying the SV40 promoter and enhancer were used as controls. Cells transfected with a given construct were assayed in triplicate and expression of the co-transfected pact-β-gal plasmid (NipponGene) was used to correct the transfection efficiency.

2.4. 3′-RACE analysis and Northern blot analysis

Full length cDNA was obtained from the poly $(A)^+$ RNA with Superscript II reverse-transcriptase (Gibco) and a *Not*I poly dT

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[1] The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and GenBank nucleotide sequence databases with accession numbers AB023486 and AB023487.

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Table 1
Primers used in this study

Name	Sequence (5′ to 3′)
R-OLIGO	AGC AUG GAG UCG GCC UUG UUG GCC UAC UGG
*Not*I poly dT primer adapter	AAT TCG CGG CCG CTT TTT TTT TTT TTT T
D-OLIGO30	AGC ATC GAG TCG GCC TTG TTG GCC TAC TGG
H2REC-GSP1	GTG GGG AAG GCT TCA TAG ACT GAC
H2REC-GSP2	AGT CCT CAG ACG GTC CCC TGA A
H2REC-GSP12	GTC CAA CGC CTC TCA GCT GTC CA
H2REC-GSP13	CCC TAG CCA TTG GTG CAC AGG ATG

primer adapter. H2REC-GSP12 (Table 1) and a *Not*I poly dT primer adapter were used for the first-round PCR and a part of the product was used for the second-round PCR with a nested primer, H2REC-GSP13 (Table 1) and a *Not*I poly dT primer adapter. The second PCR product was subjected to polyacrylamide gel electrophoresis and then blotted to a Hybond-N membrane (Amersham Pharmacia Biotech). The blot was hybridized with a fluorescein-labelled probe ('probe 3', Fig. 1B) corresponding to the 3′-flanking region of the H2R gene. The PCR products hybridized with the H2R gene-specific probe were sequenced using H2REC-GSP13.

A 1.1 kb *Nco*I fragment of pHISH2R containing the entire open reading frame of H2R was used as a probe ('probe 1', Fig. 1B). Human β-actin cDNA was obtained from Clontech. Hybridization with human multiple tissue Northern blot I and III (Clontech) was carried out as recommended by the manufacturer. Radioactive bands were visualized with a Fuji Film BAS 1000.

3. Results and discussion

3.1. The 12.3 kb sequence of the human histamine H2R gene

A λgt11 clone containing the 12.3 kb 5′-upstream region, 1080 bp coding region and 245 bp 3′-downstream sequence of H2R was isolated from a human genomic library [12] (Fig. 1A). We determined the entire upstream sequence (accession number AB023486), but found no homology with those deposited in the GenBank and EST databases.

3.2. Multiple transcription initiation sites of the human histamine H2 receptor gene

No information on transcription initiation sites is available for H2R genes, although they have been cloned from various species [3–7]. Because the proximal promoter region lacks an apparent TATA box, it is possible that the H2R gene has multiple transcription initiation sites. To unambiguously identify the transcription start sites, we applied the oligo-capping method, that specifically replaces the 5′-cap structure of mRNA with a synthetic RNA oligonucleotide (R-OLIGO) [9–11]. cDNA with an oligo sequence at the 5′-end was amplified with D-OLIGO30 and H2R gene-specific primers. As shown in Fig. 2A, seven major PCR products (numbers 1–7) were obtained. Southern analysis confirmed that six of them (numbers 2–7) were H2R gene-specific (data not shown). The 60 bp fragment (number 1) was a primer-dimer. The six bands (numbers 2–7) were eluted and sequenced directly. Comparison of the genomic and PCR product sequences revealed that band numbers 2, 4, 5 and 7 contained the same initiation site starting at −367 bp (Fig. 2B). The differences in electrophoretic mobility were due to the tandem repeat of the R-OLIGO ligated at the 5′-end or secondary structural differences of single-stranded PCR products. The band number 3 started from −409 bp (Fig. 2B). The H2R gene expressed in either MKN45 (Fig. 2A) or HL60 cells (data not shown) exhibited the same initiation sites. These two initiation sites were located within the *Bgl*II–*Bst*XI region (Fig. 3).

A short 58 bp sequence (accession number AB023487) was found between the R-OLIGO and the sequence of the H2R transcript starting from −525 bp (number 6, Fig. 2A). The

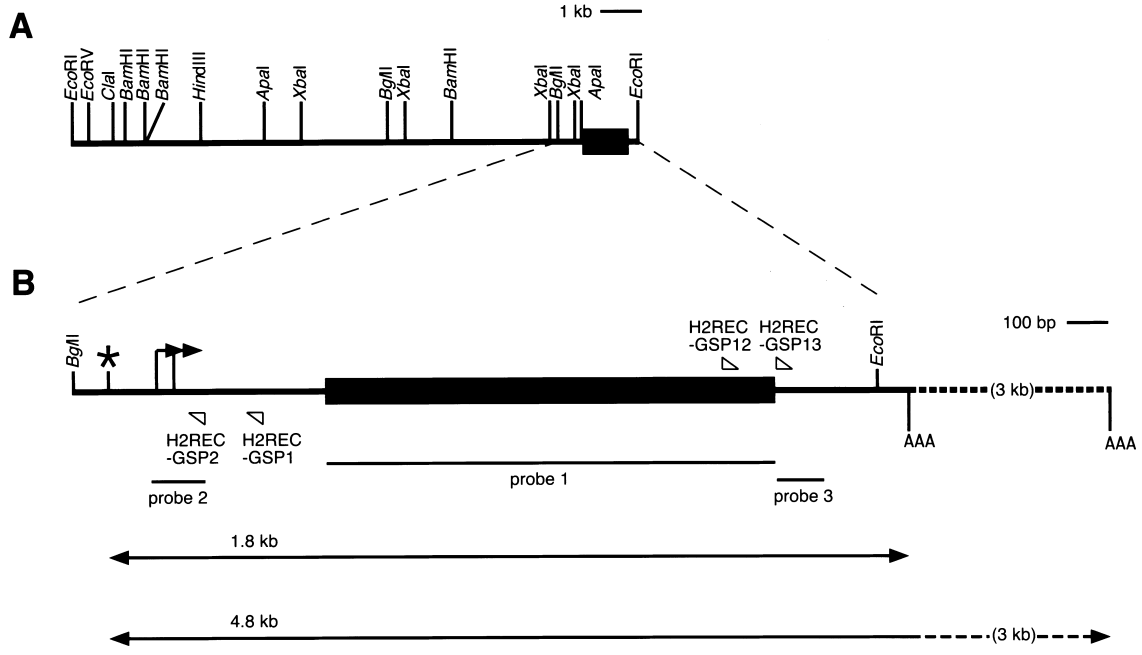

Fig. 1. H2R gene structure and transcription initiation. A: Restriction enzyme map of the H2R gene. The filled box indicates the encoding region. B: The 2.0 kb *Bgl*II–*Eco*RI fragment. The positions of transcription initiation site and splicing acceptor site are shown by arrows and an asterisk, respectively. 'AAA' indicates the putative polyadenylation site. The primers used for oligo-capping or 3′-RACE analysis (also see Table 1) and the probes used for Northern (probe 1, +1–+1080 bp) or Southern blot (probe 2, −421–−292 bp; probe 3, +1084–+1230 bp) analysis are indicated. The predicted sizes of H2R transcripts (1.8 kb and 4.8 kb) are also indicated.

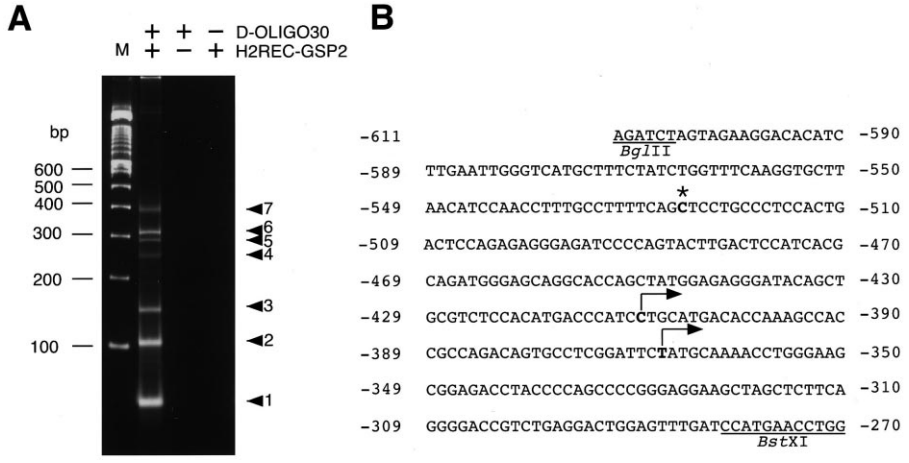

Fig. 2. Determination of the transcription start sites of the H2R gene. A: PCR was performed with the D-OLIGO30 and H2REC-GSP2 primers. M: DNA marker (100 bp ladder, Gibco). No specific PCR product was detected when the PCR reaction was performed without either primer. Southern analysis with probe 2 (Fig. 1B) showed that product numbers 2–7 were H2R-specific. B: The initiation sites were determined by direct sequence analysis of the PCR products eluted from a polyacrylamide gel and the transcription initiation sites and splicing acceptor site are indicated by arrows and an asterisk, respectively.

inserted sequence showed no homology with the 12.3 kb 5′-upstream or other known sequences registered in the DNA databases. The 58 bp sequence inserted at the same position in the H2R mRNA was also found with the 5′-RACE method (data not shown). It is possible that the 58 bp sequence is an exon of the H2R gene existing upstream the 12.5 kb 5′-flanking region. Actually, 'AG', a splice consensus sequence, was found immediately upstream at −525 bp. In order to investigate the origin of the 58 bp sequence, we performed long and accurate PCR using human genomic DNA as template, with primers corresponding to the 58 bp and the 5′-region of the isolated H2R gene, respectively. This result strongly suggested that the 58 bp sequence is an exon of the H2R gene, existing upstream of an approximately 19 kb intron. Further study on the 58 bp sequence and its promoter region is currently in progress.

It has recently been reported that the human histamine H1 receptor contains an intron of approximately 5.8 kb and a short 150 bp exon encoding 5′-untranslated region (UTR), suggesting that the histamine receptor subfamily of G-protein-coupled receptors may contain an intron [14].

3.3. Essential promoter region required for transcription within the BglII–BstXI region

We have reported that a 5′-flanking region (*Bgl*II–*Bst*XI) exhibited promoter activity in MKN45 cells [12]. To identify the essential promoter, we constructed a series of deletions and subjected them to a reporter gene assay. The *Bgl*II–*Bst*XI

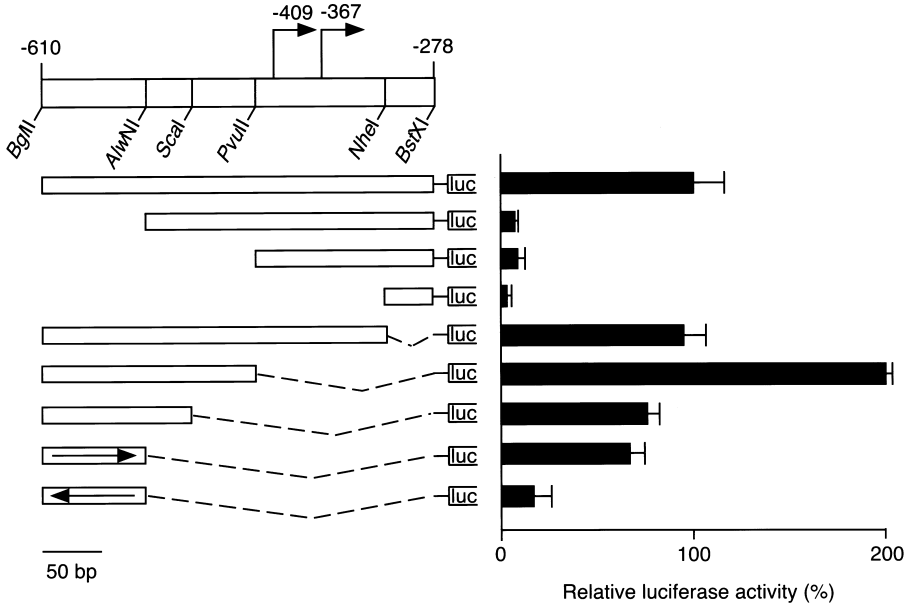

Fig. 3. Essential region for the promoter activity of the H2R gene. A series of deletions of the *Bgl*II–*Bst*XI region of the H2R gene was ligated in front of the luciferase gene (luc), followed by transfection into MKN45 cells. The positions of transcription initiation site are indicated. The direction of the *Bgl*II–*Alw*NI fragment in the reporter constructs is shown by arrows. The normalized luciferase activities with the S.D.s are relative to the activity of the *Bgl*II–*Bst*XI region.

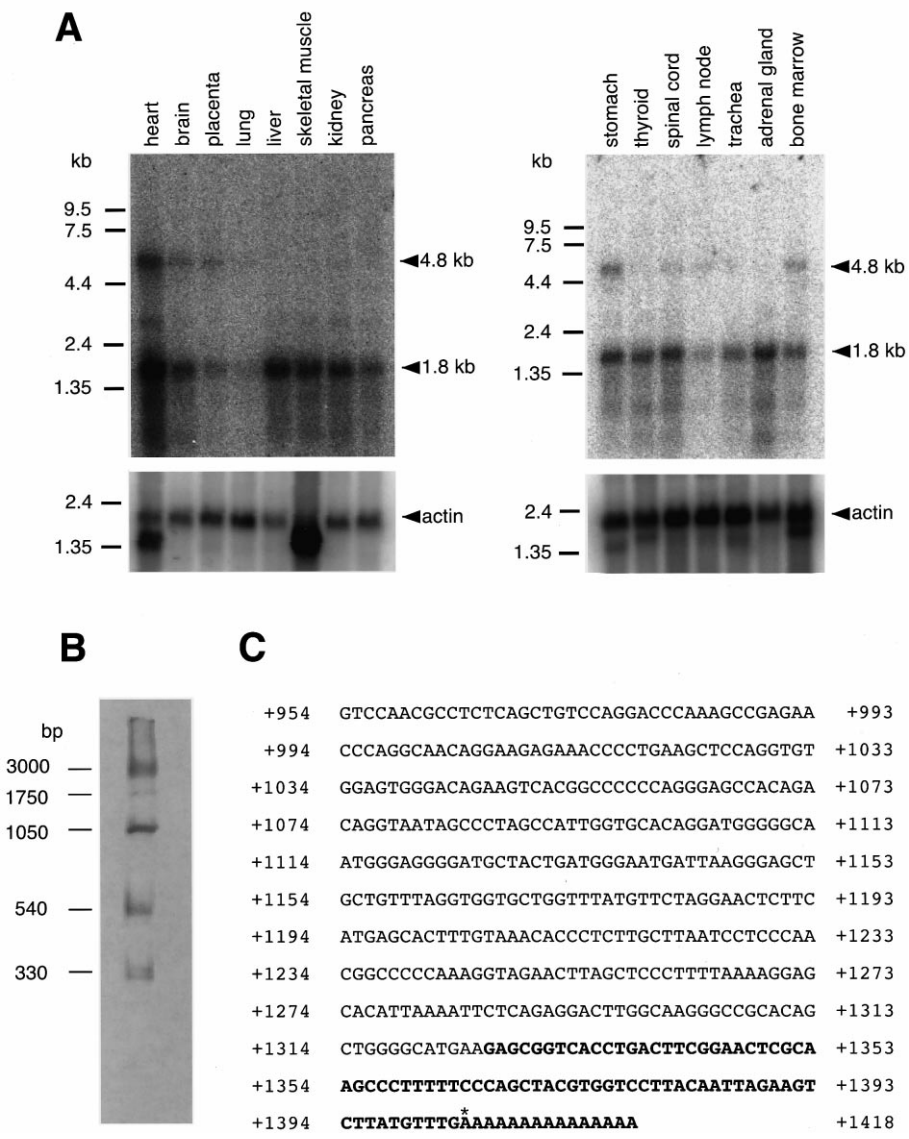

Fig. 4. Transcripts of the H2R gene. A: H2R mRNA in various tissues. Poly (A)$^+$ RNAs (2 μg/lane) from human adult tissues were probed with the encoding region of the human H2R (probe 1, Fig. 1B) or a human β-actin cDNA. The positions of markers and hybridized transcripts (arrowheads) are indicated. B: Southern analysis of the 3′-RACE products. Size of the products hybridized with the probe 3 (Fig. 1B), covering the 3′-flanking region, are indicated. C: The sequence of the 0.3 kb 3′-UTR. The 3′-downstream sequence not found in the genomic clone is shown in bold letters and the polyadenylation site is indicated by an asterisk.

fragment showed a significantly high transcription activity (Fig. 3), confirming previous results [12]. However, transcription was essentially lost when the *Bgl*II–*Alw*NI segment (85 bp), upstream of the initiation sites, was deleted. The 85 bp segment could support transcription, with a similar activity to that of the *Bgl*II–*Bst*XI region, but its activity was completely lost when it was ligated in the reverse direction (Fig. 3, bottom). Essentially the same results were obtained with other human cell lines, such as HeLa, HL60 and 293 cells (data not shown), suggesting that the 85 bp segment exhibits an ubiquitous promoter activity.

3.4. Human H2R transcripts exhibit a wide tissue distribution

Northern analysis using probe 1 (Fig. 1B) revealed a 4.8 kb transcript in the heart, brain and stomach, as previously reported in dog and rodents [4–7] (Fig. 4A, arrowheads). In addition, the transcript was detected in the placenta, spinal cord, lymph node and bone marrow, although the level of expression was variable. It would be interesting to know the physiological functions of H2R in these tissues.

Furthermore, a 1.8 kb transcript was found in almost all tissues examined, with a relatively high expression level. Since the length of 3′-UTR of H2R from man or other species is not clear, we performed 3′-RACE and analyzed the PCR products. Southern analysis with a probe corresponding to the 3′-flanking region ('probe 3', Fig. 1B) gave four major bands (0.3, 0.5, 1.0 and 3.0 kb, Fig. 4B). We have determined the sequence of the 0.3 kb fragment (Fig. 4C) and found that the position of polyadenylation was at +1404 bp. The 0.3 kb or 0.5 kb 3′-UTR, combined with the transcription initiation sites described above, predicts a total mRNA length of approximately 1.8 kb, which corresponds nicely with the size of the smaller transcript identified on Northern blot analysis. In the same way, the 3.0 kb 3′-UTR accounts for the 4.8 kb

transcript. The transcript corresponding to the 1.0 kb 3′-UTR was also detected weakly in several tissues (Fig. 4A). These results suggest that the difference in 3′-UTR of H2R may be involved in its differential expression. The possible roles of H2R in the regulation of cell growth and differentiation have been suggested recently [15–17]. The relationship between the broad expression range of the 1.8 kb transcript and the potential role of H2R in cell proliferation is an interesting possibility to be studied.

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