Molecular structure of the human gonadotropin-releasing hormone receptor gene

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Abstract
GnRH receptors belong to the family of G protein-coupled receptor proteins and have been localized to the anterior pituitary, brain and reproductive organs as well as many steroid-dependent tumor tissues. Recently, cDNAs for the GnRH receptors of several species including the human have been cloned. To determine the structure of the gene encoding the human GnRH receptor, we isolated the receptor gene clones from the human genomic libraries. Comparison of the genomic and cDNA sequences revealed that the human GnRH receptor gene is composed of three exons and two introns and spans over 20 kb in size. Exon 1 encodes the 5′ untranslated sequence and nucleotide +1 to +522 in the open reading frame, exon 2 encodes nucleotide +523 to +742 and exon 3 encodes nucleotide +743 to +987 in the open reading frame as well as the 3′ untranslated sequence. Southern blot analysis of genomic DNA and localization of the GnRH receptor gene to a single site on human chromosome 4 (4q13) indicate the presence of a single copy of the gene in the human genome. Several regulatory sequences for various hormones and other regulatory factors were identified, including PEA-3, AP-1, AP-2, and Pit-1 sites. In addition, glucocorticoid/progesterone response element, thyroid hormone response element, and cAMP response element sequences were identified. Reverse transcriptase-primer extension and 5′ RACE analysis of the human pituitary RNA demonstrated the presence of multiple transcriptional start sites upstream of the translational start site. Analysis of the 5′ flanking region of the gene also revealed the presence of multiple TATA and CAAT sequences. The finding of multiple transcriptional start sites raises the possibility of tissue-specific regulation and the existence of variable size transcripts. Chimeras containing 1.26 kb (−534 to 728) of the 5′ flanking region of the receptor gene and the luciferase (Luc) gene expressed a significant luciferase activity when transfected into a human endometrial tumor cell line (HEC-1A) and a breast tumor cell line (MCF-7) but not in a mouse pituitary gonadotrope cell line (αT3–1), suggesting the existence of multiple promoter elements in the gene. These findings indicate a multiplicity of regulation of expression of the GnRH receptor and provide the substrate for detailed investigation in the reproductive system.

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Introduction
Hypothalamic gonadotropin-releasing hormone (GnRH) plays a key role in the reproductive cascade in vertebrates through its regulation of gonadotropin secretion (1). This action of GnRH is mediated through high-affinity receptors present in the cell membranes of gonadotropes. In addition to the role of GnRH in the regulation of gonadotropin secretion it has been reported to have extrapituitary effects in influencing tumor cell growth in a number of human tumors and tumor cell lines (2–10). Inhibition of cell growth and [3H]thymidine incorporation in tumor cells in culture by GnRH agonists and antagonists and the presence of GnRH receptors in these tumors and tumor cell lines (5, 6, 8–12) suggest a direct effect of GnRH agonists and antagonists on cell growth and proliferation. However, the mechanisms by which GnRH and GnRH/GnRH receptor interaction regulate tumor cell growth remain unclear.

Recently, isolation of cDNAs encoding the high-affinity GnRH receptors from mouse (13), human (14, 15), bovine (16), rat (17–20) and sheep (21, 22) pituitary glands have been reported. More recently, Kakar et al. (23) isolated and sequenced GnRH receptors from breast tumor cell line (MCF-7) and an ovarian tumor which were identical in sequence to the human pituitary receptor. In their studies, Kakar et al. (23) also showed that, in addition to the human pituitary, GnRH receptor is also expressed in human breast, breast tumor, ovary, ovarian tumor, prostate, prostate tumor, breast tumor cell lines (MCF-7 and MDA-MB 468) and prostate tumor cell lines (PC-3 and LNCaP). Using PCR and in situ hybridization techniques, the GnRH receptor gene has been localized to chromosome 4q13 (24–26).

GnRH receptor expression is highly regulated in exhibiting both up- and down-regulation by its cognate ligand, gonadal steroids, and peptides (16, 17, 27–31). The presence of response elements for these factors in

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the promoter region of the GnRH receptor gene is therefore anticipated. Recently, Fan et al. (32) reported the partial sequence of the human GnRH receptor gene. However, the complete genomic organization and the promoter region of the human GnRH receptor gene, and the mechanisms involved in altering the quantitative expression of the GnRH receptor number have not yet been determined. As a step toward elucidation of complete genomic organization and transcriptional regulation, we cloned and characterized the human GnRH receptor gene.

Materials and methods

Screening of genomic libraries

A human genomic library prepared from human lung fibroblast cell line (W 138) partially digested with Sau3A and ligated into the XhoI site of XFixII vector was obtained from Stratagene (La Jolla, CA, USA). A second library constructed from the Caucasian male leucocyte by partial MboI digestion and ligated into EMBL3 vector was purchased from Clontech Laboratories (Palo Alto, CA, USA). Both the libraries were screened by hybridizing with a 32P-labeled 1.5 kb human GnRH receptor cDNA as a probe (14). Approximately 5 × 107 plaques were transferred from each library to nitrocellulose membranes and hybridized at 65°C for 16–18 h in hybridizing buffer (0.5 M NaHPO4, pH 7.2, 1 mM EDTA, 7% SDS and 0.1% BSA). The filters were washed three times (20 min each) in 2×SSC/0.1% SDS (1×SSC = 150 mM NaCl and 15 mM sodium citrate, pH 7.0) at room temperature and once at 45°C in 0.2×SSC/0.1% SDS for 45 min (14). Positive clones were plaque-purified by additional rounds of screening. Lambda DNA from the isolated clones was prepared by using Lambda FaZe II purification system from Biotecx (Houston, TX, USA). DNA was digested with various restriction enzymes, separated on a 0.8% agarose gel and analyzed by Southern blot hybridization using the GnRH receptor cDNA as well as synthetic oligonucleotide primers as probes selected from the different regions of the GnRH receptor cDNA sequence. The restricted fragments hybridized to cDNA as well as to oligonucleotide primers were subcloned into plasmid vectors BlueScript (SK+) or pcDNA3 (Invitrogen, San Diego, CA, USA). DNA sequencing was performed with Sequenase (US Biochemical) by the dideoxy chain termination method of Sanger et al. (33). DNA sequence analysis was carried out using the Genetics Computer Group, Inc. (Madison, WI, USA) program on a VAX computer.

Determination of size of the introns and amplification of the 3' end gene sequence

To determine the size of the introns we carried out PCR using a set of oligonucleotide primers containing the sequences of the adjacent exons. The PCR was performed in a final volume of 100 μl, containing 0.5 μg GnRH receptor gene DNA, 50 mM KCl, 10 mM Tris–HCl (pH8.8), 1.5 mM MgCl2, 0.1% Triton X-100, 0.2 mM each of dNTPs, 2.5 units Taq DNA polymerase (Perkin Elmer, Norwalk, CT, USA), and 400 ng of each primer. PCR conditions were 94°C for 1 min, 54°C for 1 min and 72°C for 3 min for 30 cycles in a Perkin Elmer thermal cycler. PCR-amplified DNA was analyzed on an agarose gel after staining with ethidium bromide (34). To amplify the 3' end of the GnRH receptor gene, we used human leucocyte genomic DNA (0.4 μg) as a template. The primers used were sense 5'-GACTCCC TACTATGTCTTAG-3' (gene position, 11 916–11 935, Fig. 2) and antisense 5'-TGTGCATAAGCTGCAAGTTA-3' (gene position, 13 111–13 130, Fig. 2).

Northern blot analysis

Twenty micrograms of the human pituitary total RNA were separated on 1.0% agarose gel containing 1.1% formaldehyde. Northern blot analysis was performed under high stringency conditions using random hexamer primed 32P-labeled human GnRH receptor cDNA as a probe as described previously (17).

RNA fractionation and PCR analysis of the reverse-transcribed mRNA

Total RNA from human pituitary was fractionated through high resolution gel electrophoresis (34). The bands corresponding to 5.0 kb, 2.5 kb and 1.5 kb were excised from the gel. RNA was eluted from each band and was reverse transcribed and used as a template in PCR. The oligonucleotide primers used in PCR were sense 5'-GCTTGAAGCTTTCTGCTGGGA-3' and anti-sense 5'-TGTGCATAACAGTTCAGAG-3' selected from the 5' and 3' ends of the open reading frame of the GnRH receptor cDNA sequence (14). The PCR conditions were 94°C for 1 min, 54°C for 1 min and 72°C for 2 min for 30 cycles. The PCR-generated DNA was analyzed on agarose gel (34).

Southern blot analysis

Human genomic DNA was obtained from Clontech Laboratories and was completely digested with either EcoRI, BamHI, HindIII, PstI, BglII or SacI restriction enzymes. Digested DNA was separated on a 0.8% agarose gel and subjected to Southern blot analysis using 32P-labeled full-length human GnRH receptor cDNA as a probe (34).

Primer extension

Total RNA from the human pituitary was isolated using guanidinium/CsCl gradient as described previously (14). Oligonucleotide primer complementary to the
sense strand 5'–GGGATGCTGTTGTTGATGGCTG–3' (gene position, 1792–1813, Fig. 2) was used for primer extension. The primer was labeled at the 5' end with 32P using T4 polynucleotide kinase (Promega, Madison, WI, USA). Total RNA from human pituitary (10 µg) was hybridized to the primer (10^5 to 10^6 c.p.m.) in the presence of 80% formamide, 400 mM NaCl, 40 mM Pipes (pH 6.4) and 1 mM EDTA by incubation at 85°C for 5 min, and then at 42°C for 16 h. Following precipitation of mRNA templates (35), the hybrids were extended using 30 units avian myeloblastosis virus reverse transcriptase (Promega) in the presence of 10 mM Tris–HCl, pH 8.8, 50 mM KCl, 5 mM MgCl2, 1 mM dNTPs and 1 unit/µl RNase at 42°C for 60 min. The reaction mixture was treated with RNase A, phenol and chloroform-extracted, ethanol-precipitated and resuspended in TE (10 mM Tris/1 mM EDTA, pH 8.0) buffer (35). The primer extension reaction products were analyzed on 6% polyacrylamide gel.

5' RACE analysis

5' RACE analysis kit was obtained from Life Technologies (Gaithersburg, MD, USA). First strand cDNA was prepared from human pituitary total RNA using 5'-GGGATGCTGTTGTTGATGGCTG–3' (gene position, 1792–1813, Fig. 2) as a primer according to the instructions supplied with the 5' RACE system. The cDNA was dC-tailed and subjected to PCR. The primers used for PCR were anchor primer 5'-CUACUAUACUAGGCCACGGTC GACTAADTTACGGGGGIIIGG-3' (sense primer) and antisense primer 5'-AGTGTTCTGTCCAACAT-3' (gene position 1335 to 1351, Fig. 2). The PCR conditions were denaturation at 94°C for 30 s, annealing at 52°C for 30 s and extension at 72°C for 1 min for 35 cycles and then final extension at 72°C for 7 min after the last cycle of amplification. The amplified products were then subcloned in the pCRII vector (Invitrogen). Colonies were screened by using 32P-labeled 5' end gene sequence (1534 to 1351, Fig. 2). The positive clones were isolated and purified using a mini prep system from Promega. Sequencing was performed manually by the dideoxy chain termination method, and also automatically using an Applied Biosystems automated DNA sequencer model 373 (ABI).

Construction of promoter-Luc chimeric plasmids, transfection and luciferase/β-galactosidase assays

The gene sequence from −534 to 1351 (Fig. 2) was amplified by using the sense primer 5'-GCA TAAAATCGGCTA3' (−534 to −517) and anti-sense primer 5'-AGTGTTCTGTCCAACAT-3' (1335 to 1351) in PCR using genomic clone DNA as a template. PCR-generated DNA was subcloned directionally into pGL-3 Basic (Promega) vector at XhoI and HindIII sites. Recombinant plasmid was designated pHGnRHR (−534 to 1351)Luc. A 5' deletion mutation was constructed from pHGnRHR (−534 to 1351)Luc by utilizing unique endonucleases KpnI and PstI sites (generating recombinant plasmid pHGnRHR (729 to 1351)Luc). A 3' deletion mutation was constructed from pHGnRHR (−534 to 1351)Luc by utilizing PstI and HindIII restriction endonucleases (generating recombinant plasmid pHGnRHR (−534 to 728)Luc). The authenticity of these constructs was confirmed by sequencing. Plasmids of all constructs were prepared by using the midi prep system from Promega. The luciferase plasmid constructs (3 µg) were cotransfected with the pSV-β-gal plasmid (1 µg) (Promega) into a mouse pituitary gonadotrope cell line (αT3–1), a human endometrial tumor cell line (HEC-1A) and a human breast tumor cell line (MCF-7) by using lipofectamine.

Figure 1 Organization of the human GnRH receptor gene. (A) The box indicates the gene sequence analyzed. The solid boxes indicate the exons. The relevant restriction enzyme sites are shown. (B) The structure of the human GnRH receptor cDNA. Open boxes indicate the protein coding region and solid boxes the putative transmembrane domains (TM). The location and sizes of the introns are shown.
(10 μl) (Life Biotechnologies) in 60 mm Petri dishes for 16 h (14). After 24 to 30 h of transfection, the cells were washed with PBS and harvested. Luciferase activities were determined by standard procedures utilizing an Optocomp I luminometer (MGM Instruments Inc., Hamden, CT, USA) using the assay kit from Promega. β-Galactosidase activity was used to normalize for variations in transfection efficiency and was determined using standard assays. Each luciferase construct was transfected at least three times and each transfection was performed in duplicate.

Results and discussion

Isolation of genomic clones and sequencing

Using GnRH receptor cDNA as a probe to screen the human genomic library constructed from human lung fibroblast cell line into pFixII vector, we obtained three positive clones. Restriction map analysis of these clones by Southern blot using the full-length GnRH receptor cDNA as well as the primers derived from the 5' and 3' ends of the open reading frame of GnRH receptor
The composed nucleotide sequence of the GnRH receptor gene and its organization are shown in Figs 1 and 2. The overall structure of the gene was determined by comparing the genomic sequence with the cDNA sequence. The length of the introns was determined by PCR analysis using primers derived from the adjacent exons. As shown in Figs 1 and 2, the GnRH receptor gene is composed of three exons and two introns and spans over 22 kb in size. The nucleotide sequences of all the exons were in agreement with that of GnRH receptor cDNA reported previously (14, 15). Exon 1 contains 1099 to 2270 nucleotides. It encodes the 5'-untranslated region which stretches between 576 and 1747 bp, depending on which initiation site is used and nucleotide +1 to +522 in the open reading frame which includes transmembrane domains I–III and a portion of transmembrane domain IV (14). Exon 1 is followed by about 4.2 kb intron. Exon 2 encodes 220 nucleotides in the open reading frame which includes the remainder of the IVth transmembrane domain, transmembrane domain V, and part of intracellular loop 3. Exon 2 is followed by about 5.0 kb intron. Exon 3 encodes the remaining sequence of the open reading frame (+ 743 to + 987) which includes transmembrane domains VI and VII and 3'-untranslated region (Figs 1 and 2). The exact size of exon 3 is not known. The consensus sequences for splicing junction, 5'-gt and 3'-ag (36), were found at the boundaries of both the introns (Figs 1 and 2). Zhou & Sealfon (37) reported the isolation of the mouse GnRH receptor gene and showed that, like the human, mouse GnRH receptor gene is also composed of three exons and two introns and spans over 22 kb. The protein coding sequence is interrupted by two introns and location of the introns in the coding sequence was reported to be at approximately the same positions as the human GnRH receptor (37).

**Figure 3** Primer extension analysis of human GnRH receptor RNA. A synthetic oligonucleotide 22 bp was end-labeled and hybridized to 10 μg total RNA from the human pituitary and analyzed on a 6% polyacrylamide gel. DNA markers are indicated on the left.

To determine the transcriptional start site of the GnRH receptor gene, reverse transcriptase-primer extension analysis was carried out using human pituitary RNA as a template. Initially, we used a 22 nucleotide oligonucleotide primer representing the reverse complement of nucleotides 1792 to 1813 (Fig. 2) selected to be 43 bp downstream of the translational start codon (ATG). A number of dense bands of variable sizes were detected on the autoradiograph (Fig. 3), indicating the existence of multiple transcriptional start sites in the 5' flanking region of the gene. To find the exact transcriptional start site, we used the 5' RACE technique. As shown in Fig. 2, a total of 18 transcriptional start sites were identified in the 5' flanking region of the gene; seven were found in one region (1 to 404) and the remainder were found in another region (931 to 1172). Analysis of the 5' flanking region of the gene also revealed the presence of several TATA boxes (TATAAA) and CAAT boxes residing in close proximity to one another in a cluster-like arrangement. The significance of the existence of these multiple transcriptional start sites and TATA boxes in the human GnRH receptor gene is not known. However, the existence of multiple TATA boxes and transcriptional start sites is not uncommon (38, 39). The finding of multiple transcriptional start sites and TATA boxes raises the possibility of tissue-specific regulation and the existence of variable size transcripts. Consistent with our results, Albarracin et al. (40) recently reported the existence of four transcriptional start sites in the 5' flanking region of the mouse GnRH receptor gene. These investigators identified the major transcriptional start site at 62 nucleotides upstream of the translational start site. As shown in Fig. 2, the human GnRH receptor gene contains 18 transcriptional start sites and the
closest transcriptional start site was identified at 577 nucleotides upstream of the translational start site. While our work was in progress, Fan et al. (32) reported the partial sequence of the human GnRH receptor gene and our study confirms their work. However, our study also contributes additional sequence information and has identified multiple transcriptional start sites in the pituitary. Fan et al. (32) using human brain RNA reported the existence of five transcriptional start sites in the 5′ flanking region of the gene. Transcriptional start sites reported by these investigators were found to be different from those identified in the pituitary (Fig. 2). The existence of multiple and different transcriptional start sites in the pituitary and brain raises the possibility that alternative transcriptional start sites are utilized in pituitary and extrapituitary tissues. Which site(s) of transcriptional initiation is predominantly used in human pituitary and extrapituitary tissues is presently unknown.

Analysis of the 5′ flanking region of the GnRH receptor gene revealed the existence of a number of regulatory sequences for various hormones and regulatory factors. These regulatory sequences include a putative cyclic AMP (cAMP) response element (CRE) (Fig. 2) and a putative glucocorticoid/progesterone response element (GRE/PRE) (Fig. 2) with one base pair deviation from a functional GRE/PRE (41). The presence of CRE and GRE/PRE-like sequences suggest the transcriptional regulation of GnRH receptor gene by cAMP and glucocorticoid or progesterone. Several PEA-3 binding sites (42) which have been reported to function as phorbol ester response elements in some genes were also identified. Additionally, AP-1 recognition site (43) and AP-2 recognition site (44) that would confirm protein kinase C responsiveness were also identified within the 5′ end sequence. Most interesting, Pit-1, an anterior pituitary specific transcriptional factor (44) which is reported to be an important factor in the regulation of anterior pituitary hormones, such as activation of growth hormone (45) and prolactin (46) gene transcription, was also identified in the 5′ flanking region. Pit-1 may play an important role in the regulation of GnRH receptor gene expression. We also identified a number of thyroid hormone response elements which have been reported to regulate growth hormone gene transcription by thyroid hormone (47) in the 5′ flanking region (Fig. 2). However, it remains unknown whether the regulatory elements in the upstream regions of the GnRH receptor gene are active in the transcriptional regulation of the receptor gene.

**Northern blot analysis**

The expression and size of the human GnRH receptor mRNA in human pituitary was determined by Northern blot analysis. As shown in Fig. 4, Northern blot analysis revealed three hybridizing mRNAs with sizes of 5.0 kb, 2.5 kb and 1.5 kb. The 5.0 kb transcript was most abundant. The significance of these additional mRNA species (2.5 kb and 1.5 kb) is not understood but it is noteworthy that multiple species of GnRH receptor mRNA also exist in bovine (16), rat (17), sheep (22, 48, 49) and mouse (13, 50). These multiple transcripts could be the result of initiation at different transcriptional start sites or the product of alternate splicing of the gene. Recently, Zhou & Sealfon (37) reported the isolation of the mouse GnRH receptor gene and demonstrated the existence of multiple transcripts for the receptor in the mouse pituitary gonadotrope tumor cell line (αT31). They reported that some of these transcripts in the αT31 cell line may not contain...
full-length coding sequence and also indicated splice variants. To examine whether a similar situation exists in the human we fractionated the human pituitary RNA on high resolution agarose gel electrophoresis (34). The bands corresponding to 5.0 kb, 2.5 kb and 1.5 kb were excised from the gel. RNA from each band was eluted and reverse transcribed and then used as a template in PCR along with the human pituitary total RNA. The oligonucleotide primers used in PCR were sense 5′-GCTTGAAGCTCTGTCCTGGGA-3′ and antisense 5′-GTAGTCTATCAATCACAGA-3′ derived from the 5′ and 3′ ends of the open reading frame of the human GnRH receptor (14). As shown in Fig. 5, PCR products of size 1024 bp were clearly visibly from human pituitary total RNA (used as a control), 5.0 kb transcript, 2.5 kb transcript and 1.5 kb transcript, suggesting that all the three transcripts for the GnRH receptor present in human pituitary contain full-length coding sequences and are fully spliced. Northern blot analysis of rat anterior pituitary RNA has shown the existence of four transcripts with sizes of 5.0 kb, 4.5 kb, 2.5 kb and 1.3 kb (17). Using this technique to amplify the coding sequence from the multiple transcripts from the rat anterior pituitary, we found that all the transcripts contained full-length coding sequences for the GnRH receptor (results not shown). It therefore seems that, unlike the mouse pituitary gonadotrope cell line, the multiple transcripts of GnRH receptors present in human and rat pituitaries contain full-length coding sequences.

Genomic blot analysis

To determine the number of GnRH receptor genes in the human genome, we carried out Southern blot analysis of the genomic DNA. Human genomic DNA was digested with various restriction enzymes, separated on agrose gel and subjected to Southern blot analysis. 32P-Labeled GnRH receptor cDNA representing its open reading frame was used for hybridization under high stringency conditions (34). As shown in Fig. 6, each digest resulted in multiple fragments which hybridized to the GnRH receptor cDNA. These results are consistent with the expected restriction map analysis of the gene sequence (Fig. 1) as well as with the Southern blot analysis of the genomic clone after digesting with the same enzymes (results not shown). These results, in combination with previous studies from Kakar & Neill (24) showing that the human GnRH receptor gene is localized to a single site on chromosome 4 (4q13), indicate the existence of a single copy for the GnRH receptor gene in the human genome. These results are consistent with those of Zhou & Sealfon (37) who also reported the presence of a single gene for GnRH receptors in rat and mouse. The three major mRNA species seen by Northern blot analysis (Fig. 4) appear to be encoded by a single GnRH receptor gene and are the result of initiation at different transcription start sites or of alternate splicing of the gene due to the presence of multiple polyadenylation signals at the 3′ end of the gene (32).

Transcriptional regulation of the GnRH receptor gene

To characterize the promoter region upstream of the transcriptional start sites, gene sequence −534 to
1351 (Fig. 2) was subcloned immediately upstream to a promoterless and enhancerless luciferase gene (pGL3) basic vector. This construct and 3′- and 5′-deleted constructs (pHGnRHR (−534 to 728)Luc and pHGnRHR (729 to 1351)Luc were transfected into αT3–1, MCF-7 and HEC-1A cells. We selected MCF-7 (breast tumor cell line) and HEC-1A (endometrial tumor cell line) for our studies, these cell lines are well-characterized, estrogen-dependent, and express GnRH receptors. αT3–1 is the mouse pituitary gonadotrope cell line which, although lacking expression of follicle-stimulating hormone-α subunit promoter (51). It has been used to study many aspects of gonadotrope physiology, including GnRH receptor regulation (52–54). Our results clearly demonstrated (Fig. 7) a significant increase in luciferase activity in HEC-1A (20.9-fold) and in MCF-7 cells (16.9-fold) when transfected only with pHGnRHR (−534 to 728) compared with control basic vector, suggesting the existence of a promoter sequence in the 5′ flanking region. No increase in luciferase activity was observed in any of the cell lines when transfected with pHGnRHR (−534 to 1351)Luc or pHGnRHR (729 to 1351)Luc. This indicates that a negative regulatory element exists between 729 to 1351, and a promoter sequence exists between −534 and 728. Recently, Albarracin et al. (40) reported the isolation of 1.2 kb of the 5′ flanking region of the mouse GnRH receptor gene and showed an increase of luciferase activity by 246-fold in αT3–1 cells. 41-fold in the rat pituitary tumor cell line (GH3), 12-fold in the human choriocarcinoma cell line (JEG-3), and 18-fold in the monkey kidney cell line (CV-1) when transfected with chimeric luciferase gene construct containing 1.2 kb of the 5′ flanking region of the mouse GnRH receptor gene compared with the promoterless vector control. Based on these results these investigators suggested the existence of a promoter sequence in the 5′ flanking region of the gene and its preferential expression in cells of gonadotrope origin. In our studies (Fig. 7), we failed to observe any significant increase in luciferase activity in αT3–1 cells. However, an increase in luciferase activity in non-pituitary cells (HEC-1A and MCF-7) was observed to be similar to that reported by Albarracín et al. (40) in non-pituitary cell lines (JEG-3 and CV-1). These results strongly suggest the existence of multiple promoters in the human GnRH receptor gene which may be tissue-specific and that the pituitary-specific promoter resides in the upstream region of the sequence analyzed. We are currently analyzing the upstream sequence of the gene and performing mobility gel shift and in vitro DNase I footprinting assays to determine the exact sequence(s) which promotes the expression of human GnRH receptor gene in human pituitary and tumors.

In conclusion, we have described the genomic organization of the human GnRH receptor gene. This study represents a continuation of our efforts to establish a molecular basis for understanding the normal structure, function and regulation of the GnRH receptor. The determined gene structure and isolated genomic clones would provide a fundamental framework for the identification of specific factors regulating the expression of the GnRH receptor in the pituitary, extrapituitary tissues and tumors.

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