Newly recognized GnRH receptors: function and relative role

Jimmy D. Neill, Lois C. Musgrove and L. Wayne Duck

University of Alabama School of Medicine, Department of Physiology and Biophysics, MCLM 816, 1918 University Boulevard, Birmingham, AL 35294-0005, USA

Hypothalamic gonadotropin releasing hormone (GnRH I) and its pituitary receptor are responsible for the CNS regulation of reproduction. However, a second GnRH (GnRH II) is also expressed in humans and a gene that resembles the GnRH II receptor in fish has been identified in humans and monkeys. The amino-acid sequence of this newly identified, seven-transmembrane, G-protein-coupled receptor in monkeys differs from the human GnRH I receptor by having a C-terminal, cytoplasmic tail. GnRH II is ~400-fold more potent at GnRH II receptors than GnRH I receptors. GnRH I directly inhibits proliferation of human tumor cells, and GnRH II and its receptor might have a similar role. Limited progress has been made, however, because of difficulty translating the mRNA that encodes the human GnRH II receptor. Nevertheless, such receptors are likely to exist in humans because GnRH II is more inhibitory to tumor cell replication than GnRH I, and GnRH I and GnRH II have reciprocal effects on human decidual stromal cells in culture. The focus of this review is the identity of a possible translatable, functional GnRH II receptor in humans. The two possibilities considered are either that GnRH II receptor mRNA is expressed that encodes either 5 or 7 transmembrane domains or that a GnRH II-responsive complex is formed by the GnRH I receptor and fragments derived from the GnRH II receptor.

Despite the apparent sufficiency of GnRH I in regulating reproduction, a second GnRH (GnRH II) is reported to be expressed in humans [3]. GnRH II is 70% identical to GnRH I (Figure 1), and is expressed ubiquitously in human tissues, with the highest levels in kidney, prostate and bone marrow [3]. Until recently, its function in mammals was unknown.

GnRH II receptors have been cloned and sequenced from several species, the first of which was the African catfish [4]. These receptors are in the family of 7TM, GPCR receptors but, unlike the human GnRH I receptor (Figure 2), they have a C-terminal, cytoplasmic tail [4]. Like the GnRH I receptor, the GnRH II receptor couples to the Gq, protein and, therefore, mediates the intracellular production of inositol trisphosphate [Ins(1,4,5)P3] [4].

The existence of GnRH II in humans, coupled with the expression of GnRH II receptors in non-mammals, prompted us to begin a search in 1997 for a human GnRH II receptor. Here, we present progress to date in identifying a gene that encodes the GnRH II receptor in humans, and its cloning and sequencing in monkeys and pigs.

GnRH II receptor gene in humans

The human genome has been searched using the cDNA of either the fish GnRH II receptor [4] or the human GnRH I receptor [2]. The GnRH I receptor sequence was identified on chromosome 4, as reported previously using mapping techniques [5]. A second GnRH-receptor-like sequence, comprised of three exons (Figure 3) was found on chromosome 1, and showed 40% identity with the human GnRH I receptor. However, this sequence has a higher identity (~58%) with the fish GnRH II receptor than with the human GnRH I receptor, which indicates that it is the human GnRH II receptor gene [6–8].

Cloning and characterization of the monkey GnRH II receptor

PCR using primers derived from the presumed human GnRH II receptor gene was used to facilitate cloning and sequencing the cognate receptor from cDNA derived from either the pituitary gland of a rhesus monkey or a green monkey kidney cell line (Figure 3) [6]. The amino-acid sequence derived (Figure 2 and Figure 3) revealed a typical 7TM, GPCR of 379 amino acids. As reported for the fish GnRH II receptor [4], but unlike the human GnRH I

Corresponding author: Jimmy D. Neill (neill@uab.edu).

Figure 1. Amino-acid sequences of gonadotropin releasing hormones (GnRHs), GnRH I, formerly called mammalian GnRH, and GnRH II, previously called chicken GnRH. Residues in GnRH II that differ from those in GnRH I are in red.

Mammalian GnRH (GnRH I)
[pGlu1 His2 Trp3 Ser4 Tyr5 Gly6 Leu7 Arg8 Pro9 Gly10

Chicken GnRH II (GnRH II)
[pGlu1 His2 Trp3 Ser4 His5 Gly6 Trp7 Tyr8 Pro9 Gly10

Vol.15 No.8 October 2004
receptor, the monkey receptor has a C-terminal cytoplasmic tail (Figure 2). The amino-acid sequences of the monkey GnRH receptors showed higher identities with the type II GnRH receptors from non-mammals (55%) than with the human GnRH I receptor (38%) [6]. Confirmatory results in a marmoset monkey were reported later from Millar’s laboratory [9].

Functional analysis of the monkey GnRH II receptor was performed using cDNA transfection of heterologous cells and measuring the production of Ins(1,4,5)P$_3$ after treatment with either GnRH I or GnRH II (Figure 4) [6–8]. GnRH II was active in the nanomolar range, which demonstrates that its receptor is functional. By contrast, GnRH I was ~400-fold less active (Figure 4), which indicates the specificity of the interaction between GnRH II and the GnRH II receptor. Therefore, assignment of the GnRH II receptor to 7TM, GPCR family of receptors is justified and, like the GnRH I receptor, it couples with the $G_q$ family of transduction proteins.

Tissue localization of GnRH II mRNA in humans has been measured with an antisense riboprobe representing mostly the 7TM regions III–VII of the human GnRH II receptor cDNA [6] directed against a region of the human GnRH II receptor cDNA that encodes most of transmembrane regions III–VII. GnRH II receptor RNA was measured using multiple expression arrays of 21 different human tissues; although all tissues expressed significant levels of the RNA, the absolute amount varied eightfold [6]. Therefore, expression of GnRH II receptor mRNA appears to be ubiquitous and parallels the distribution of
the mRNA encoding its ligand, GnRH II [3]. These findings indicate a wide-spread paracrine or autocrine regulatory system in humans.

A more restricted distribution of GnRH II mRNA in human tissues has been reported by Millar’s group [9–11]. This probably results from their use of cDNA probes to localize mRNA, which is far less sensitive than the riboprobe method [6].

**GnRH II receptor genes in non-primate mammals**

Complete genome sequencing has been accomplished only in mice and humans, but the rat genome is nearing completion. Although the genes for GnRH II and its receptor are not present in the mouse genome (Table 1), as expected, genes representing GnRH I and its receptor have been identified easily (Table 1). In pigs, we have isolated and sequenced a cDNA that encodes a protein that belongs to the 7TM, GPCR family and is a functional GnRH II receptor (Figure 5) (GenBank accession no AY542893) [12]. The sequences of the pig and monkey GnRH II receptors are 91% identical (Figure 2) and their functional properties are identical (Figure 4) in terms of GnRH II and I stimulation of Ins(1,4,5)P₃ production [12]. Sheep [13] and cattle [11] occupy a molecular niche between primates and rodents in and express GnRH II-receptor-like transcripts; however, these do not appear to encode functional 7TM GPCRs [13].

In addition to the 7TM GnRH II receptor in pigs, we also identified a 5TM receptor (GenBank accession no AY542893) that is formed by alternative splicing in exon 1 (Figure 5). Thus, TM domains I and II are missing, and the extracellular N-terminus is coupled directly to TM domain III. This receptor is similar to a 5TM GnRH II receptor we reported in humans [7] (GenBank accession no AY081843). Although the functions of human and pig 5TM receptors have not been tested, it should be noted that a 5TM chemokine receptor is functional [14]. The relationship of 5TM receptors to a full-length, appropriately processed GnRH II receptor transcript in humans is discussed later.

**Potential functions of GnRH II and its receptor**

Well-established physiological roles for GnRH II and its receptor are lacking. Nevertheless, because GnRH II has ~10% the activity of GnRH I at the GnRH I receptor [7], it seems reasonable to reassess whether functions that have been ascribed to GnRH II are mislabeled, which might be caused by GnRH II. These functions fall into three categories: (i) gonadotropin secretion; (ii) sexual behavior; and (iii) regulation of peripheral reproductive tissues.

**Gonadotropin secretion**

Although GnRH I seems to be sufficient to account for the hypothalamic control of gonadotropin secretion, there are reports that GnRH II might also be involved. Urbanski et al. [15] reported that GnRH II is expressed in the hypothalamic median eminence, a strategic location for the regulation of gonadotropin secretion. Indeed, administration of GnRH II to monkeys potently stimulates gonadotropin secretion [16]. However, recent findings in monkeys demonstrate that GnRH II increases gonadotropin secretion both in vivo [17] and in vitro [18] by stimulating the GnRH I receptor. In both studies, the investigators used the GnRH I receptor-specific antagonist Antide, which is inactive at the GnRH II receptor [7], to block GnRH II-induced gonadotropin secretion. We have presented similar findings for pig pituitary cells in vitro [19] using Cetrorelix, another GnRH I receptor-specific antagonist [7]. Therefore, in these studies, the effects of GnRH II are exerted via the GnRH I receptor rather than the GnRH II receptor. Of course, such findings do not preclude the possibility that the effect GnRH II at the GnRH I receptor might be physiological. Moreover, continued study of the effect of GnRH II on gonadotropin secretion in species such as the monkey and pig (but not rodents, sheep and cattle) might lead to examples of GnRH II-stimulated gonadotropin secretion that are not inhibited by GnRH I receptor-specific antagonists [20].

**Sexual behavior**

Originally, we postulated [6] that the induction of estrus in rodents with GnRH I [23,24] might involve GnRH II. However, the findings that rodents probably do not express GnRH II and GnRH II receptors [11,12] make
this unlikely. In the meantime, it was reported that GnRH II but not GnRH I induces mating behavior in musk shrews that are nutritionally deficient [25]. This effect is rapid and potent. Similarly, GnRH II but not GnRH I stimulates eating in shrews that are food-restricted [26]. It will be interesting to examine the GnRH II receptors expressed in shrews and to test these effects of GnRH II in other mammals, including primates.

Regulation of peripheral reproductive tissues

There is an extensive literature demonstrating that GnRH I and its agonistic analogs suppress cell proliferation in human tumor cell lines and non-tumor cells [27–30]. These effects are exerted primarily on cells derived from reproductive tissues such as prostate, ovary, endometrium and placenta, but effects on non-reproductive tissues have also been reported [29]. The nature of the GnRH I receptor that mediates these effects is controversial. Some investigators have shown high-affinity, low-capacity binding, which is typical of the pituitary receptor [29,31], and we have shown that the nucleotide sequence of the GnRH I receptor cloned from breast and ovarian tumors is identical to that in the pituitary [32]. In humans, such receptor mRNA also occurs in several non-reproductive tissues such as liver, heart, skeletal muscle, kidney and placenta [33]. The tissue distribution of the GnRH I receptor resembles the ubiquity of tissue expression that is reported for the human GnRH II receptor [7]. However, many investigators report the existence only of low-affinity, high-capacity binding sites for GnRH I [29]. It is not clear why high affinity binding of GnRH I is difficult to detect, but might be caused by low abundance of the GnRH I receptor.

GnRH I mRNA has been detected in most, if not all, of the tissues that are reported to express the GnRH I receptor [28–33]. These findings indicate the existence of a local control system for GnRH I and its receptor. Strong support for an inhibitory regulatory system is provided by Emons et al. [34], who showed that an antibody that neutralizes GnRH I increases the rate of proliferation of ovarian tumor cells. Therefore, it seems that a local function of GnRH I to inhibit cell proliferation in peripheral tissues can be added to the list of established effects for this nominal neurohormone.

A potential role for GnRH II and its receptor, either instead of or in addition to the GnRH I–GnRH I receptor system, has received much interest and experimental attention. This is a result of several factors: the apparent ubiquity of expression of GnRH II [28–36] and its receptor [6–8]; the difficulty in detecting high affinity receptors for GnRH I in peripheral tissues [29]; and the high concentrations of GnRH I and its agonists that are required to suppress tumor-cell proliferation [27]. However, the last observation has been blunted by the recent report [37] that sub-nanomolar concentrations of a GnRH I agonist inhibits tumor-cell proliferation if colony formation rather than cell counts are measured, if cell numbers used in culture are controlled, and if the endogenous release of
stimulatory and inhibitory factors into the culture medium are considered [37].

Although several recent reports identify GnRH II-induced inhibition of tumor-cell growth and regulation of other functions of non-tumor cells [28–30,37–46], few have been able to discriminate between the effects of GnRH II on the GnRH I receptor versus the GnRH II receptor. One of the first to do this was Emons’ laboratory [38], who demonstrated that GnRH II had more potent antiproliferative effects than GnRH I on human ovarian and endometrial tumor cells that express mRNA that encodes the GnRH II receptor. Moreover, GnRH II has antiproliferative effects on tumor cells that do not contain GnRH I receptor mRNA [38]. A more recent publication from the same laboratory [39] reports that experimental ‘knock-down’ of the GnRH I receptor abrogates the antiproliferative effects of GnRH I but not GnRH II. Similar results have come from the laboratory of Leung [45] using human primary cell cultures. In these studies, GnRH II was shown to be a more potent regulator of the steroidogenic activity of granulosa lutein cells [45] and Cetrorelix, a specific GnRH I-receptor antagonist [7,8], inhibited GnRH I but not the GnRH II-induced stimulation of human trophoblasts [43]. Last, GnRH I and GnRH II have reciprocal effects on the same human decidual stromal cell cultures [42]; that is, GnRH I increases and GnRH II decreases the expression of plasminogen activator inhibitor (PAI-1). These findings [38,39,43,45] support the existence of a local regulatory GnRH II/GnRH II receptor system that is functionally relevant in human tissues and cells. Perhaps of more importance is the seemingly inescapable conclusion that humans express a functional GnRH II receptor.

An unusual observation in these studies is that Cetrorelix used alone suppresses tumor proliferation [39,46–48]. The effect of this antagonist in mimicking rather than antagonizing the agonist might involve the GnRH II receptor, as it is observed in cells in which GnRH I receptors have been eliminated [39]. Therefore, in tumor cell lines, at least, the human GnRH II receptor might differ from the receptor in monkeys (Figure 7), which is neither inhibited nor stimulated by Cetrorelix [7,8]. However, Leung’s group has reported that Cetrorelix inhibited the regulatory effects of GnRH I but not GnRH II in human primary cell cultures [42,43]. These findings indicate the possibility that the GnRH II receptor might differ in tumor and non-tumor cells. Further work is needed to distinguish between primary inhibitory effects of GnRH II and secondary effects that involve desensitization by prolonged exposure to GnRH II; such biphasic effects are well-established for some actions of GnRH I.

The human GnRH II receptor

A pseudogene?

The nature of the human GnRH II receptor remains elusive because of the failure to find a mRNA in human cells that encodes a translatable protein, at least according to the standard rules of translation [7,8,10,11,49]. The two potential flaws that might prevent translation are (i) a shift in the reading frame that disrupts the receptor near its N-terminus (between amino acids 9 and 10 in the monkey cognate sequence (Figure 6) and (ii) an in-frame stop codon at amino acid 181 in the second extracellular loop (sequence 4 of Figure 6). Before the recent evidence of a functional receptor became available [38,39,42,43,45], we and others considered the possibility that the human GnRH II receptor gene might be a pseudogene [7,8,10,11,49]. Pseudogenes are common in the human genome, which is estimated to contain almost as many pseudogenes as translatable genes [50]. Many pseudogenes that encode nominal 7TM GPCRs related to odor detection have been reported [51].

Recapitulation of evidence for functional receptor

The recent strong evidence supporting the existence of a functional human GnRH II receptor protein can be recapitulated as follows: (i) GnRH II is more potent than GnRH I at suppressing proliferation of human tumor cells that express mRNA encoding GnRH II receptors but not GnRH I receptors [38]; (ii) the anti-proliferative effects of GnRH II are not abrogated in cells in which GnRH I receptor mRNA is suppressed experimentally and; (iii) reciprocal effects on the same cells are exerted by GnRH I and GnRH II (i.e. GnRH I increased whereas GnRH II decreased PAI-1 expression in human primary cell cultures) [42]. These findings support the conclusion that a functional GnRH II receptor is expressed in humans, the identity of which either resides within or is related to the various GnRH II receptors and receptor fragments in Figure 6 and Figure 7.

Potential corrective shifts in the reading frame

Translating the human GnRH II receptor mRNA into a protein such as that shown for the monkey in Figure 6 and Figure 7 (sequence 3) requires mechanisms to overcome reading-frame shifts in the nucleotide sequence, and to read the stop codon as an amino acid. Examples of both mechanisms have been reported in eukaryotes [52–54]. However, programmed frame-shifting in eukaryotes is rare and the only known example in mammals occurs in a gene that appears to be a relic of retroviral origin [52]. Therefore, we have considered whether an alternative, in-frame non-AUG initiation codon is used, which is downstream of the frame shift. This codon is the nominal valine at position 23 (Figure 6, sequences 4 and 5). If used, this would result in a 7TM receptor with an N-terminal tail that is shortened by 22 amino acids. This nominal valine codon (GUG) meets the two criteria of Kozak [3ggaGUGg] [6], which is translated (Figure 4). This monkey cDNA has a threonine codon (ACG) in place of the surrounding nucleotides are appropriate (Figure 7) that is located immediately downstream of the GUG codon [56]. The probability that this non-AUG codon is used for translation is increased by a similar situation (except that it is not out-of-frame) in the cDNA that encodes the GnRH II receptor in green monkeys (GenBank accession no AF353988) [6], which is translated (Figure 4). This monkey cDNA has a threonine codon (ACG) in place of the AUG initiator codon in rhesus monkeys (Figure 6, sequence 3). The ACG codon is unlikely to support initiation [55,56], and the GUG codon described above for the human sequence is the only alternative site for
## Figure 6: Sequence alignment of gonadotropin releasing hormone (GnRH II) receptors and their fragments.

The seven transmembrane domains are labeled TM I–TM VII, the three extracellular loops as EC1–EC3 and the three intracellular loops as IC1–IC3. Initiating methionine residues for either receptors or receptor fragments are designated as M. 5TM and 7TM indicate five and seven transmembrane-domain receptors and receptor fragment precursors. Sequences used in this and in Figure 7 are redrawn from GenBank. Pig receptors: 5TM, Accession no AY081843; 7TM, Accession no AY542892. Monkey 7TM receptor, Accession no AF353988. Human 7TM receptor and 7TM fragment, Accession no AY077708; 5TM receptor and 5TM fragment, Accession no AY081843.

### Table: Sequence Alignment of Gonadotropin Releasing Hormone (GnRH II) Receptors and Their Fragments

<table>
<thead>
<tr>
<th>Sequence</th>
<th>TM I</th>
<th>EC 1</th>
<th>TM II</th>
<th>IC 1</th>
<th>TM III</th>
<th>EC 2</th>
<th>TM IV</th>
<th>EC 3</th>
<th>TM V</th>
<th>IC 2</th>
<th>TM VI</th>
<th>EC 3</th>
<th>TM VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig (5TM)</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pig (7TM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monkey (7TM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human (7TM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human (5TM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human (5TM) Fragment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Revised by:**

[www.sciencedirect.com](http://www.sciencedirect.com)
initiation of translation \[55,56\] in green monkeys. It is worth noting that methionine is still incorporated into protein when non-AUG codons are used for initiation\[57\].

Recoding of stop codons
Recoding of stop codons to translatable codons (‘stop-codon readthrough’) is relatively common in eukaryotes, including mammals \[52\]. Stop-codon readthrough might result in incorporation of selenocysteine, the 21st amino acid, into proteins when the stop codon is UGA (e.g. in the human GnRH II receptor mRNA) \[7,10,11,49\]. There is evidence from Drosophila that some instances of UGA stop-codon readthrough do not involve selenocysteine, but the signals that promote readthrough are uncertain and the identity of the amino acid(s) incorporated has not been determined \[54\].

Alternative gene splicing
We have reported that alternative splicing of exon 1 in the human GnRH II receptor gene results in the loss of the region that encodes TM regions I and II (GenBank accession no AY081843; sequence 6 in Figure 6 and Figure 7) \[7\]. This splicing event results in an apparent 5TM receptor, and splices out the potential reading-frame disruption near the nominal N-terminus of the receptor (Figure 6 and Figure 7). A 5TM receptor that encodes a functional chemokine receptor has been described \[14\], and we have described a 5TM GnRH II receptor mRNA in pigs that possesses all the characteristics of a translatable protein \[12,19\] (see sequence 1 in Figure 6 and Figure 7). The function of this porcine receptor remains to be tested. Nevertheless, it is consistent with the existence of a 5TM receptor that is encoded by alternative splicing of the human GnRH II receptor gene. Although the 5TM receptor avoids the frame-shift disruption inherent in the full-length 7TM version of the human receptor, it retains the in-frame stop codon. Readthrough of a stop codon is much more common than programmed frame shifting \[52\], so that we hypothesize that a functional human GnRH II receptor might be a 5TM domain receptor that lacks TM regions I and II (sequence 6, Figure 6 and Figure 7).

Alternative translations of peptide fragments
Additional, perhaps alternative, translations of the 7TM and 5TM versions of the human GnRH II receptor mRNAs result in peptide fragments of the two receptor forms (sequences 5 and 7, Figure 6 and Figure 7). The production of these fragments would avoid both of the flaws of translation present in the GnRH II receptor mRNA. As already described for the 7TM receptor, initiation would occur at amino acid 23 and extend through to amino acid 181, resulting in a fragment containing TM domains I–IV (first fragment in sequence 5, Figure 6 and Figure 7). The production of a second peptide fragment requires re-initiation of translation along the same mRNA, for which there is ample precedence in mammals \[58\]. Such a peptide fragment would contain TM regions VI and VII and the nominal C-terminal tail of the receptor (sequence 5, Figure 6 and Figure 7). Similarly, the 5TM receptor (sequence 7, Figure 6 and Figure 7) would produce a
second peptide fragment containing TM domains VI and VII and the C-terminal tail. However, the first peptide (sequence 7) differs from its counterpart in the 7TM receptor (sequence 5) only by lacking TM regions I and II through initiating translation at the cognate start codon used by functional 7TM receptors in the monkey (sequence 3, Figure 6 and Figure 7).

Potential functions for these receptor fragments in the absence of functional GnRH I and GnRH II receptors are problematic. One possibility is that the two fragments of the 7TM receptor might associate non-covalently, thereby constituting a 6TM receptor. There is a precedent for receptor fragments to reassociate into functional units when 7TM receptors are ‘split’, and then co-expressed in heterologous systems [59,60]. However, whether a complex containing six TM domains is functional is unknown.

**Association of GnRH II receptor fragments with the GnRH I receptor**

A final possibility that might result in a functional GnRH II receptor in humans is non-covalent association of the peptide fragments of the receptor (sequences 5 and 7, Figure 6 and Figure 7) with either the type I GnRH receptor or with the 7TM and 5TM domain GnRH II receptor. Precedents for this possibility have been reported: (i) two abbreviated transcripts of the human GnRH I receptor have been published [61,62]. One transcript, which lacks TM regions VI and VII inhibits activity of intact GnRH I receptor when co-expressed [62]; (ii) heterodimerization of different types of opioid receptors [63] and of an opioid receptor with an adrenoceptor [64], is consistent with association between either GnRH II and GnRH I receptors or, perhaps more likely, peptide fragments of the GnRH II receptor and the GnRH I receptor; and (iii) functional rescue a mutant vasopressin receptor has been reported when it is co-expressed with a polypeptide that contains TM domains VI and VII and the C-terminal tail of the receptor [65].

**Conclusions**

A functional GnRH II receptor is present in monkeys and pigs but not in mice, sheep and cattle. GnRH II stimulates gonadotropin secretion in monkeys and pigs by activating the GnRH I receptor rather than the GnRH II receptor. Despite the expression of GnRH II receptor transcripts in human tissues, we and others have been unable to identify a full-length, appropriately processed transcript. Nevertheless, there is strong evidence that a functional GnRH II receptor does exist. For example, GnRH II is more potent than GnRH I in tumor and primary human cells. In addition, abrogation of GnRH I receptor mRNA does not suppress the effects of GnRH II in human cells, and GnRH I and GnRH II exert reciprocal effects on the same cells.

There are two flaws in the GnRH II receptor gene in humans. One is a reading-frame shift that eliminates a translation initiation codon near the N-terminus of the protein. The second is an in-frame stop codon that might terminate translation in the second extracellular loop. We propose that the most likely GnRH II receptor in humans has either 7TM domains (resulting from translation initiation downstream of the frame-shift) or 5TM domains (resulting from alternative splicing that eliminates the frame-shift). Moreover, it is assumed that there is read-through of the stop codon, so that either selenocysteine or another amino acid is substituted for the stop codon. Another possibility is a GnRH II-responsive complex is formed by association between the GnRH I receptor and peptide fragments from the GnRH II receptor. These hypotheses about the nature of the GnRH II receptor are readily testable and clinically relevant because of the potent antiproliferative effects of GnRH II on ovarian, endometrial and prostate-tumor cells.

The identity of the human GnRH II receptor can be tested using either gene ‘knockdown’ by RNA interference or co-transfection of cell lines with cDNAs that represent potential forms of the receptor. In both cases, cells should be used that have established, specific responses to GnRH II and culture conditions that control for the release of stimulatory and inhibitory factors into the culture medium [37]. Last, measured end-points should be refined beyond simple cell counts; possibilities include colony formation [37] and measurement of second messengers and other responses that are related to cell multiplication and growth.

**Acknowledgements**

J.D.N. is supported by NIH Research Grant HD-37121.

**References**

14 Ling, K. et al. (1999) Five-transmembrane domains appear sufficient...
18 Okada, Y. et al. (2003) Evidence that gonadotropin-releasing hormone (GnRH) II stimulates luteinizing hormone and follicle-stimulating hormone secretion from monkey pituitary cultures by activating the GnRH I receptor. Biol. Reprod. 69, 1536–1541
54 Robinson, D.N. and Cooley, L. (1997) Examination of the function of two kelch proteins generated by stop codon suppression. EMBO J. 16, 2492–2492


---

**Have you contributed to an Elsevier publication?**

Did you know that you are entitled to a 30% discount on books?

A 30% discount is available to ALL Elsevier book and journal contributors when ordering books or stand-alone CD-ROMs directly from us.

To take advantage of your discount:

1. Choose your book(s) from www.elsevier.com or www.books.elsevier.com

2. Place your order
   
   **Americas:**
   
   TEL: +1 800 782 4927 for US customers
   
   TEL: +1 800 460 3110 for Canada, South & Central America customers
   
   FAX: +1 314 453 4898
   
   E-MAIL: author.contributor@elsevier.com

   **All other countries:**
   
   TEL: +44 1865 474 010
   
   FAX: +44 1865 474 011
   
   E-MAIL: directorders@elsevier.com

   You’ll need to provide the name of the Elsevier book or journal to which you have contributed. Shipping is FREE on pre-paid orders within the US, Canada, and the UK.

   If you are faxing your order, please enclose a copy of this page.

3. Make your payment

   This discount is only available on prepaid orders. Please note that this offer does not apply to multi-volume reference works or Elsevier Health Sciences products.

   [www.books.elsevier.com](http://www.books.elsevier.com)