Expression and Function of the Gonadotropin-releasing Hormone Receptor Are Dependent on a Conserved Apolar Amino Acid in the Third Intracellular Loop*

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The coupling of agonist-activated heptahelical receptors to their cognate G proteins is often dependent on the amino-terminal region of the third intracellular loop. Like many G protein-coupled receptors, the gonadotropin-releasing hormone (GnRH) receptor contains an apolar amino acid in this region at a constant distance from conserved Pro and Tyr/Asn residues in the fifth transmembrane domain (TM V). An analysis of the role of this conserved residue (Leu237) in GnRH receptor function revealed that the binding affinities of the L237I and L237V mutant receptors were unchanged, but their abilities to mediate GnRH-induced inositol phosphate signaling, G protein coupling, and agonist-induced internalization were significantly impaired. Receptor expression at the cell surface was reduced by replacement of Leu237 with Val, and abolished by replacement with Ala, Arg, or Asp residues. These results are consistent with molecular modeling of the TM V and VI regions of the GnRH receptor, which predicts that Leu237 is caged by several apolar amino acids (Ile233, Ile234, and Val240 in TM V, and Leu237, Leu263, and Val269 in TM VI) to form a tight hydrophobic cluster. These findings indicate that the conserved apolar residue (Leu237) in the third intracellular loop is an important determinant of GnRH receptor expression and activation, and possibly that of other G protein-coupled receptors.

The hypothalamic decapeptide, gonadotropin-releasing hormone (GnRH), controls the activity of the reproductive system by regulating the synthesis and release of luteinizing hormone and follicle-stimulating hormone from the anterior pituitary gland (1, 2). In pituitary gonadotrophs, the actions of GnRH are mediated by specific high affinity receptors that promote G protein-dependent stimulation of phosphoinositide turnover and calcium mobilization (3). The cloned GnRH receptors of mouse, rat, human, sheep, cow, and pig exhibit more than 85% amino acid identity among species (4). The hydrophathy analysis of the GnRH receptor coding region is consistent with the seven transmembrane domain structure that is characteristic of the G protein-coupled receptor (GPCR) superfamily. Mammalian GnRH receptors exhibit several unique structural features, including the absence of an intracellular carboxyl-terminal tail, reciprocal exchange of the conserved Asp and Asn residues in transmembrane domains (TM) II and VII, and replacement of Tyr with Ser in the Asp-Arg-Tyr motif located at the junction of TM III and the second intracellular loop (IL2) (3, 4).

In several GPCRs, mutational analysis has shown that regions of the third intracellular loop (IL3), in particular its amino- and carboxyl-terminal portions, and sometimes the cytoplasmic tail of the receptor, are important determinants of receptor-G protein coupling (5–10). In some GPCRs, the first and second intracellular loops have also been shown to be important in interaction with G proteins and activation of signal transduction (10). For example, we have recently demonstrated the dependence of cAMP-induced signaling from the GnRH receptor on specific residues in IL1 that are not essential for activation of the phosphoinositide signaling pathway (11). The role of IL3 in GnRH receptor function has not been examined in detail, but recent evidence has indicated its ability to couple the receptor to Gα2 and Gαq11-mediated signal transduction pathways. However, this study did not identify specific amino acid residues within the loop that are functionally important for receptor-G protein coupling and selectivity (12). Although the GnRH receptor has the unusual structural features mentioned above, it also contains several conserved residues and sequences in its TM helices and loops that are typical of other members of the GPCR superfamily. One of these is a hydrophobic amino acid (Leu237) located at the NH2-terminal region of IL3. This residue is positioned at a constant distance from the conserved Pro and Tyr residues in TM V (Tyr is replaced by Asn in the GnRH receptor), and is present in most of the GPCRs that are coupled to Gαq11. This suggests that Leu237 is a critical structural determinant of the specificity of receptor-G protein coupling.

The location of this apolar residue in the GnRH receptor (see Fig. 1) indicates that it might play an important role in the receptor activation process. In fact, the importance of a corresponding apolar residue in the agonist-induced activation of the AT1a angiotensin receptor has been demonstrated (13). Additionally, secondary structure predictions suggest that this region is an amphiphilic α-helical extension of the fifth transmembrane helix. Synthetic peptides corresponding to the NH2-terminal region of the IL3 of certain GPCRs were shown to activate the corresponding G proteins in vitro (7, 8), confirming the proposed importance of this region in receptor-G protein activation. However, few of the residues that are required for receptor activation have been identified (8–10), and the structural elements determining the coupling specificity of GPCRs are not well defined. Identification of specific amino acids that...
Thermo Sequenase radiolabeled terminator cycle sequencing kits were marked with analytical grade quality. The role of amino acid Leu237, shown as bold in IL3, was examined in the present study. The conformation of the conserved third loop hydrophobic amino acid (Leu237) in GnRH receptor function. Leucine was replaced with residues of different characteristics, to maintain the apolarity but with different side-chain lengths, or by basic or acidic residues. The mutant GnRH receptors were transiently expressed in COS-1 cells and analyzed for ligand binding, agonist-stimulated inositol phosphate production, and agonist-induced internalization of the hormone-receptor complex. Our findings indicate that the presence of the conserved apolar amino acid (Leu) in IL3 of the GnRH receptor is critical for its expression, receptor-G-protein activation, and signal transduction. These results are consistent with the prediction of molecular modeling that Leu237 of the GnRH receptor is surrounded by several apolar residues forming a tightly packed hydrophobic cluster.

EXPERIMENTAL PROCEDURES

Materials—GnRH and its superagonist analog (des-Gly⁴-lys⁴-Ala⁵)GnRH N-ethylamide, GnRH-Ag) were obtained from Peninsula Laboratories, Inc. (Belmont, CA). Fugene™6 transfection reagent was purchased from Roche Molecular Biochemicals, cell-culture related in M199, was examined in the present study. The role of amino acid Leu237, shown as bold in IL3, was examined in the present study. The role of amino acid Leu237, shown as bold in IL3, was examined in the present study. The role of amino acid Leu237, shown as bold in IL3, was examined in the present study. The role of amino acid Leu237, shown as bold in IL3, was examined in the present study. The role of amino acid Leu237, shown as bold in IL3, was examined in the present study. The role of amino acid Leu237, shown as bold in IL3, was examined in the present study. The role of amino acid Leu237, shown as bold in IL3, was examined in the present study.

The binding affinity and abundance of the mutant receptors were determined in transfected COS-1 cells incubated with 2 mM 125I-GnRH-Ag in binding medium (M199 containing 25 mM HEPES and 0.1% bovine serum albumin) in the absence or presence of increasing concentrations of unlabeled peptide for 3–4 h at 4 °C. The cells were then rapidly washed twice with ice-cold phosphate-buffered saline (PBS) (pH 7.4) and solubilized in 0.5 M NaOH, 1% SDS solution for measurement of cell-associated radioactivity by γ-spectrometry. All time studies were performed in duplicate on at least three occasions, and displacement curves were analyzed for binding affinity and capacity by the LIGAND program using a one-site model (16). The nonspecific binding of 125I-GnRH-Ag to wild-type or mutant receptors, determined in the presence of excess unlabeled agonist (1 μM), was always less than 5% of the respective total binding.

For internalization studies, transfected COS-1 cells were washed once with binding medium before the addition of 2 nM 125I-labeled GnRH-Ag. After incubation at 37 °C for the indicated times, the cells were washed twice with ice-cold PBS (pH 7.4) and incubated with 1 ml of 50 mM acetic acid, 150 mM NaCl (pH 2.8) for 12 min to remove surface-bound tracer. The acid-released radioactivity was collected to determine the receptor-bound radioactivity, and the internalized (acid-resistant) radioactivity was quantitated after solubilizing the cells in NaOH/SDS solution. Radioactivities were measured by γ-spectrometry, and the internalized radioligand at each time point was expressed as a percentage of the total (acid-resistant + acid-released) binding.

Receptor Expression—In addition to radioligand binding assays, an indirect ELISA protocol was used to quantitate the expression of epitope-tagged wild-type or mutant GnRH receptors in the plasma membrane. COS-1 cells were seeded at a density of 90,000 cells/well in 48-well plates and transfected after 24 h with wild-type or mutant receptor cDNAs. After 48 h, the cells were fixed with 4% paraformaldehyde in PBS for 30 min at room temperature. After washing with PBS three times, the cells were incubated with 10% FBS and 0.25% trypsin in M199 medium containing 10% fetal bovine serum to block nonspecific binding sites, the cells were incubated at 37 °C for 2 h with a monoclonal antibody directed against the HA epitope tag (Babco, at a dilution of 1:500 in DMEM). Plates were then washed in DMEM and incubated with a 1:2000 dilution (in DMEM) of peroxidase-conjugated goat-anti-mouse IgG antibody (Sigma) for 1 h at room temperature. Hydrogen peroxide (0.03%) and o-phenylenediamine (5 mM) in 0.1 M phosphate-citrate buffer (pH 5.0), serving as substrate and chromagen, respectively, were then added, and the plates were kept in the dark for 30 min. The enzymatic reaction was terminated with 1 M H₂SO₄ containing 0.05 M Na₂SO₃, and the color development was measured at 495 nm using a Titertek Multiskan plate reader.

Inositol Phosphate Production—COS-1 cells were labeled 24 h after transfection by incubation in inositol-free DMEM containing 20 μM [³²P]inositol as described previously (17). After 24 h, cells were washed with inositol-free M199 medium and preincubated in the same medium containing 10 mM LiCl for 30 min at 37 °C, then stimulated with 10⁻¹⁰ to 10⁻⁶ M GnRH for 20 min. Incubations were terminated by the addition of ice-cold perchloric acid (5% (v/v) final concentration). The inositol phosphates were extracted and separated by anion exchange chromatography as described previously (17), and their radioactivities were measured by liquid scintillation β-spectrometry.

Binding to COS-1 Cell Membranes—For membrane binding experiments, cells were cultured and transfected in 100-mm cell culture dishes. After 48 h, radioligand binding was performed on the crude membrane fraction in the absence or presence of 10 μM GTPγS as described previously (17), and the bound radioactivity was measured by γ-spectrometry.

Molecular Modeling of the Mouse GnRH Receptor—Homology modeling was used to construct a model of the mouse GnRH receptor protein, employing the most recent model of the seven transmembrane helix bundle of bovine rhodopsin (18) as the template. The calculations were performed on an Indigo2 (IMPACT 10000) Silicon Graphics workstation equipped with program CHARMM 23.2, modeling software.
Binding characteristics of wild-type and Leu<sup>237</sup> mutant mouse GnRH receptors expressed in COS-1 cells

| Receptor  | $K_d$ (nM) | Binding sites | Receptor expression by ELISA |
|-----------|------------|---------------|-----------------------------|
| Wild type | 2.3 ± 0.07 | 2.0 ± 0.31    | 100 ± 5.0                   |
| L237I     | 2.4 ± 0.03 | 2.7 ± 0.46    | 106 ± 7.4                   |
| L237V     | 1.4 ± 0.13 | 0.9 ± 0.13    | 67 ± 6.3                    |
| L237A     | --         | --            | 3.5 ± 1.6                   |

The results are means ± S.E. of three independent experiments, each performed in duplicate.

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**Role of IL3 Apolar Residue in GnRH Receptor Function**

- **Expression of Wild Type and Mutant GnRH Receptors**—To assess the role of the conserved aliphatic leucine residue at position 237 in IL3 in GnRH receptor function, five substitution mutations were created. The conserved apolar amino acid was changed to aliphatic residues (Ile, Val) to maintain the hydrophobic environment, to the apolar short side-chain residue Ala, or to charged amino acids (Arg and Asp). COS-1 cells were transfected with cDNA encoding either the wild-type or mutant GnRH receptors to compare their binding, signaling, and internalization properties. The agonist binding displacement curves for these receptors are shown in Fig. 2, and the binding parameters calculated from these data using the LIGAND program are summarized in Table I. The wild-type receptor and both of the detectably expressed mutant receptors (L237I and L237V) bound the radioligand with high affinity, and Scatchard analysis of the binding data yielded linear plots consistent with a single class of GnRH-binding sites. The L237I receptor showed a normal binding affinity, and that of the L237V mutant was slightly but consistently increased over that of the wild-type receptor. The $B_{max}$ values for L237I was similar to that of the wild-type receptor, and that of the L237V mutant was reduced by about 50% (Table I). The $B_{max}$ values determined by radioligand binding were in general agreement with the measurements of cell-surface expression by ELISA (Fig. 3 and Table I).

In contrast, the L237A, L237R, and L237D mutants showed no radioligand binding (Fig. 2), consistent with their lack of expression at the cell surface as indicated by ELISA measurements (Fig. 3). Immunoblot analysis of lysates of cells transfected with these mutant receptors, using antibody to the HA tag, revealed the presence of GnRH receptor protein with the same size and abundance as the wild-type protein (data not shown). These findings indicate that the lack of cell-surface expression of the L237A, L237R, and L237D receptors may lie at the level of receptor folding, processing, and/or targeting to the plasma membrane.

**Effect of Leu<sup>237</sup> Mutations on GnRH-mediated Inositol Phosphate Signaling**—The ability of the mutant receptors to couple to phospholipase C via $G_{i/1}$ proteins was determined by measuring the inositol phosphate responses of transfected COS-1 cells stimulated with $10^{-10}$ to $10^{-6}$ M GnRH in the presence of 10 mM LiCl. Under these experimental conditions, the major accumulated products of phosphoinositide hydrolysis in GnRH receptor-transfected cells are InsP<sub>2</sub> and InsP<sub>3</sub> (24).

The GnRH-induced InsP<sub>3</sub> dose-response curves mediated by L237I and L237V receptors were similar to those of the wild-type receptor, but the EC<sub>50</sub> values for agonist stimulation were increased by 10–20-fold (Fig. 4). EC<sub>50</sub> values were 0.9 ± 0.22 nM for WT, 11.8 ± 0.95 nM for L237I, and 20.5 ± 6.3 nM for L237V receptors ($n = 3$). The L237A, L237R, and L237D mutant receptors showed no detectable inositol phosphate responses, consistent with their lack of cell-surface expression.

**Effect of Guanosine Thiotriphosphate (GTP<sub>γ</sub>S) on $^{125}$I-GnRH Agonist Binding to Wild-type and Mutant GnRH Receptors**—The ability of L237I and L237V mutant receptors to interact with G proteins was further evaluated by measuring the effect of GTP<sub>γ</sub>S on $^{125}$I-GnRH agonist binding to cell membranes of COS-1 cells expressing wild-type and mutant receptors. Treatment with GTP<sub>γ</sub>S reduced agonist binding to the wild-type receptor by about 30%. This reduction in agonist binding was due to a decrease in the affinity of the receptor for GnRH and reflects the normal coupling of the activated receptor to G protein(s) (24). However, the inhibitory effect of the GTP ana-
log on agonist binding to the L237I and L237V receptors was relatively small (8.0 ± 1.1%, n = 3) (Fig. 5), consistent with the impaired ability of these mutants to mediate inositol phosphate production in response to GnRH stimulation (Fig. 4).

Internalization by Wild-type and Mutant GnRH Receptors—The effects of mutations on agonist-induced internalization of the receptor-hormone complex were evaluated in cells expressing wild-type or mutant receptors by measuring the kinetics of 125I-GnRH-Ag uptake over a period of 60 min at 37 °C (Fig. 6). A direct comparison between the wild-type and mutant receptors, made by plotting the percentage of bound tracer internalized with increasing time of incubation, showed that the cells expressing L237I and L237V receptors internalized the labeled agonist at slower rates (Fig. 6A). The amount of tracer sequestered after 60 min was 60% of that of the wild-type receptor (Fig. 6B). These results suggested that Leu237 is not a major determinant of internalization of the GnRH receptor.

DISCUSSION

The functional role of the conserved Leu237 in the amino-terminal region of the third intracellular loop of the GnRH receptor was analyzed in mutant receptors in terms of its cell-surface expression, ligand binding, agonist-induced signal transduction, and internalization. Our findings indicate that signal generation efficiency, measured by the stimulation of inositol phosphate production by GnRH, was significantly impaired in the L237I and L237V mutant receptors. In addition, the agonist-induced internalization for these receptors was reduced by 40%. The binding properties of these receptors for the GnRH agonist were largely unchanged from those of the wild-type receptor, indicating that these substitutions did not alter the integrity of the receptor. Thus, it appears that Leu237 is critical for G protein coupling of the GnRH receptor and subsequent phospholipase C activation.

Although substantial reductions in signal transduction efficiency were observed for the Ile237 and Val237 mutants, the partial retention of inositol phosphate signaling indicates that other regions or residues in the intracellular loops are involved in G protein activation. This was also the case for receptor internalization, which was significantly decreased but not abol-
lished. A large body of literature on various GPCRs, recently reviewed by Wess (10), indicates that the IL3 loop is of critical importance for proper G protein recognition, but is not usually the sole determinant of the coupling properties of a given receptor. Rather, the IL3 appears to act in a cooperative manner with other receptor domains to permit optimum coupling and selectivity. Furthermore, the heterogeneity in amino acid sequence and size of IL3 for various GPCRs suggests that the secondary structure, rather than the primary sequence and/or the length of the loop, is important in determining G protein coupling and activation. For example, in muscarinic and catecholamine receptors, both the amino and carboxyl termini of IL3, as well as some regions within IL2, have been shown to be important in G protein binding and activation (25–31); for the rhodopsin receptor, both IL2 and IL3 appear to interact with the G protein transducin, G

Of the conserved hydrophobic residue (Leu147, in the middle of the transmembrane domain) impaired GnRH-induced inositol phosphate production (17). Impairment in signaling was also observed following replacement of the conserved hydrophobic residue (Leu147, in the middle of IL2) with Asp or Ala (24). Similarly, mutation of Arg146 to Pro in IL2 of the human GnRH receptor led to defective coupling (4). In another study, co-expression of the wild-type human GnRH receptor and a splice variant lacking one-third of the carboxyl-terminal region, including IL3, significantly reduced the signaling ability of the wild-type receptor, presumably due to direct physical interactions between the intracellular loops of the wild-type and truncated forms of the receptor (35). These results suggest that a specific conformation of IL2 is necessary for productive coupling to G protein(s). Recently, the third intracellular loop of the rat GnRH receptor was implicated in coupling the receptor to both G

and G

mediated signal transduction pathways (12), but no regions in the loop were identified. In another study, deletion of the carboxyl portion (Ala260–Leu265) of IL3 of the rat GnRH receptor was found to abolish receptor binding and signaling, probably due to lack of expression of the mutant receptor (36). Additionally, mutation of Ala261 (corresponding to Ala260 in the mouse or rat receptor) in the carboxyl-terminal region of IL3 in the human GnRH receptor led to impaired G protein-coupled signaling (37). In some GPCRs, including the 5α-adrenergic receptor, mutation of the corresponding residue leads to constitutive activation of the receptor (38). Thus, variable effects on receptor function have been observed following mutation of the conserved residue in different receptors.

It has been shown that the second and third intracellular loops of several GPCRs, particularly their NH

and COOH-terminal regions, and sometimes the membrane proximal region in the carboxyl terminus of the receptor, are important sites for G protein coupling and specificity (10). In particular, the amino-terminal region of IL3 is generally considered to be an important determinant of G protein coupling and specificity. In many GPCRs, the amino acids in this region form an amphiphilic helix with nonpolar and positively charged surfaces. Mutational studies targeting the charged surface have not shown major perturbations in the functional characteristics of the mutant receptors (39–41). In the AT1a receptor, replacement of all positively charged residues in this region did not influence the ability of the receptor to activate G proteins (40). However, the presence of a conserved apolar amino acid in this region, corresponding to the Leu237 residue analyzed in the present study, was shown to be critical in the agonist-induced activation of the AT1a receptor (13). Replacements of the conserved leucine with charged amino acids or the short apolar amino acid, alanine, did not impair AT1a receptor expression but interfered with its internalization and signal transduction functions. In the present study, these replacements caused impairment of both expression and function of the GnRH receptor. In the yeast pheromone receptor (Ste2), replacement of a similarly located Leu residue in IL3 with Ala (42), His, or Arg (43) interfered with α-factor-induced responses. Thus, the role of this residue in receptor activation is conserved in distantly related GPCRs.

Mammalian GnRH receptors, like other GPCRs, undergo endocytosis following agonist binding, but their internalization proceeds relatively slowly. Although receptor internalization and signaling both require the active conformation of the receptor, the structural determinants of the two processes are not identical. Some of these motifs may be common or overlapping, and other might be distinct. For example, sequences in the cytoplasmic tail of the angiotensin AT1a receptor were found to be critical determinants of receptor internalization, but had no significant role in angiotensin II-induced signal transduction (44). Conversely, mutant receptors with impaired signaling ability were shown to undergo rapid endocytosis (45). On the other hand, mutation of the highly conserved Tyr215 in the fifth transmembrane domain impaired agonist-induced internalization of the mutant AT1a receptor and also abolished its ability to mediate inositol phosphate response (46). In the GnRH receptor, several mutations associated with impairment of signaling were found to concomitantly decrease receptor internalization (17, 24). In this context, it is noteworthy that in the present study the extent of internalization was reduced but still readily demonstrable, and the EC

values for GnRH-induced inositol phosphate production were significantly increased. Analysis of the Leu237 mutant receptors revealed that receptor internalization and inositol phosphate signaling have a common amino acid requirement at this position, suggesting that mutations of this residue interfere with an event that affects both processes. Despite the parallel impairment of the signaling properties and internalization of the L237I and L237V receptors, replacement of this single amino acid did not cause a major perturbation of receptor structure, as indicated by the retention of high binding affinity for the GnRH agonist.

We attempted to delineate the molecular details of the interhelical microdomains surrounding Leu237 by a computational model, derived as described under “Experimental Procedures.” The NH

and COOH-terminal membrane proximal portions of IL3 loop are depicted as α-helical extensions of TM V and TM VI, respectively (18). Molecular modeling of the GnRH receptor predicted that Leu237, a highly conserved residue in GPCRs, is part of a hydrophobic cluster composed of aliphatic residues in TM V and TM VI (see Fig. 7). The neighboring residues that may interact with or restrict the position of the leucine side chain by forming a hydrophobic cage are Ile233, Ile234, and Val240 in TM V, and Leu262, Leu265, and Val269 in TM VI. The roles of the individual amino acids surrounding the predicted hydrophobic cluster will be examined in future studies. Such
molecular models can assist in defining the structural basis for the receptor phenotypes observed in mutagenesis experiments. It has been proposed that conserved amino acid residues serve to maintain the general topological structure of the GPCRs, and certain of their functions. To maintain such a well packed apolar cluster (Fig. 7), not only the polarity but also the size of the residue in Leu237 position is important. Mutations may cause rearrangement of the apolar cluster and consequently induce structural change(s) in the receptor. For the Leu to Ile or Val GnRH receptor mutants, which retain binding and possess agonist affinities similar to that of the wild-type receptor, it is assumed that the overall structure of the receptor is not perturbed and the ligand binding site is maintained. However, the decreased InsP production and internalization of these mutants may reflect the structural change induced by the mutations. Conversely, the mutant receptors (Leu to Ala, Arg, or Asp) exhibit no detectable agonist binding, suggesting that these substitutions disrupt the apolar microenvironment and induce significant structural change that impairs surface expression of the receptor protein. These defects may be at the levels of protein folding and/or the intracellular processing of the mutant receptors. In the structural context, these results suggest that mutations of the conserved Leu237 to Ile or Val are tolerated for maintaining the hydrophobic environment, as these receptors were well expressed. However, the mutant receptors are less efficient in signaling than the wild-type receptor and give submaximal InsP responses, suggesting that these substitutions disrupt the apolar microenvironment and induce significant structural change that impairs surface expression of the receptor protein.

A comparison of the sequences of the amino-terminal region of IL3 of a subfamily of GPCRs (13) has revealed that many such receptors have a nonpolar residue in the position corresponding to Leu237 of the GnRH receptor, identified by its distance from the conserved Pro and Tyr residues in the fifth transmembrane domain (Tyr is replaced by Asn in the GnRH receptor). The identity of this apolar amino acid in GPCRs shows some correlation with the receptor-G protein coupling specificity, but this is not strictly conserved (13). When this comparison was extended, it was noted that the fourth residue located upstream of the conserved apolar amino acid corresponding to Leu237 is almost invariably an aliphatic (usually Ile) residue in GPCRs. Thus, at the NH2-terminal region of IL3 a structural determinant associated with G protein coupling can be depicted as XXXXXX. In this sequence, Tyr may be a frequent determinant of receptor function such as G protein recognition/activation and coupling, and Leu may act in cooperation with other regions of the receptor to provide the structural basis of coupling specificity. The notion that hydrophobic or noncharged residues located within the amino-terminal region of IL3 are of critical importance for G protein recognition is also supported by several loss of function mutagenesis studies performed in GPCRs, including the β2-adrenergic, thyrotropin, parathyroid hormone/parathyroid hormone-related peptide, thromboxane A2, glucagon-like peptide, and rhodopsin receptors (10, 39, 47–51).

In summary, the data presented in this study provide evidence for the importance of a highly conserved apolar residue (Leu237) located in the amino-terminal region of the third intracellular loop, in the expression, agonist-mediated signaling, and internalization of the GnRH receptor. These findings are in accordance with the predicted role of this residue, based on its location in a hydrophobic cluster predicted by molecular modeling of the GnRH receptor. The concomitant impairment of expression, internalization, signaling, and G protein coupling of the mutant GnRH receptor suggest that this hydrophobic residue is an important determinant of multiple aspects of its activation mechanism. It is probable that the corresponding residue of other GPCRs, as exemplified by the angiotensin AT1a receptor (13), is likewise a significant factor in receptor expression and/or agonist activation and G protein coupling.

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Fig. 7. Molecular modeling of the GnRH receptor. Three-dimensional computational model of transmembrane domains V and VI of the mouse GnRH receptor, illustrating the hydrophobic cluster surrounding the conserved residue Leu237 (shown in blue).
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