

Advances in understanding gonadotrophin-releasing hormone receptor structure and ligand interactions

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Gonadotrophin-releasing hormone (GnRH) is the central regulator of the reproductive system and its analogues are used widely in the treatment of diverse diseases. The GnRH receptor is a member of the large family of G-protein-coupled receptors (GPCRs) which have seven transmembrane domains. Knowledge of these receptors has assisted the development of molecular models of the GnRH receptor that allow prediction of its three-dimensional configuration and the way GnRH binds and activates its receptor. Comparison with other GPCRs led to the discovery that Lys¹²¹, in the third transmembrane domain, has a role in agonist binding. The history of GnRH structure–activity studies has allowed the identification of an acidic residue in the third extracellular loop of the receptor that is required for binding of mammalian GnRH, while synthetic GnRH analogues have showed that Asn¹⁰², in the second extracellular loop, may interact with the carboxy-terminus of GnRH. These residues can now be incorporated into the receptor models that are being used to design orally active non-peptide GnRH analogues for contraception and treatment of a variety of reproductive disorders.

Gonadotrophin-releasing hormone (GnRH) and its analogues are clinically valuable for the treatment of a variety of reproductive disorders including infertility, precocious puberty, uterine fibroids, endometriosis and polycystic ovarian disease, and have therapeutic value in the treatment of cancers of the prostate, breast, pancreas, ovary and pituitary (Conn and Crowley, 1991). Understanding of the structure of the GnRH receptor will lay the foundation for the design of a new generation of GnRH analogues, for the regulation of reproductive function and treatment of reproductive disorders. Recent cloning of the GnRH receptor makes it possible to examine how GnRH recognizes and activates its receptor at a molecular level, and allows us to re-examine established but previously untested hypotheses.

Structure–activity relationship of GnRH ligands

Gonadotrophin-releasing hormone is a decapeptide with eleven naturally occurring structural variants identified in animals ranging from protochordates through fish, amphibians, reptiles and birds (King and Millar, 1997). The structures vary most at amino acid positions five, six, seven and eight (Fig. 1). Most species contain two or more forms of GnRH: a hypothalamic form, which varies in structure among the different species, and a highly conserved form (chicken II GnRH), which predominates in extra-hypothalamic brain and neural tissue and is proposed to have a neuromodulator role (Jones, 1987).

Gonadotrophin-releasing hormone is flexible in solution, assuming many different conformations. Only a small subset

of these conformations bind to the receptor with high affinity. The biologically active conformation of GnRH is believed to contain a bend in the middle portion of the molecule (Fig. 2; Karten and Rivier, 1986). The blocked amino-terminal pGlu and carboxy-terminal GlyNH₂ residues are required for high GnRH activity and can be substituted only with similar uncharged groups (Coy *et al.*, 1975). Thus, many GnRH analogues contain an ethylamide substitution of the carboxy-terminal GlyNH₂ (Karten and Rivier, 1986). Modification of the conserved amino-terminal residues His² and Trp³ led to the development of GnRH antagonists and implies that these residues have a role in activating the GnRH receptor (Fig. 2). The Arg⁸ residue has an important role in determining high-affinity binding to mammalian GnRH receptors but, as might be expected from the high variability of position eight residues in natural GnRH variants, Arg⁸ is not required by non-mammalian GnRH receptors (Millar *et al.*, 1989). It has been proposed that Arg⁸ has a role in stabilizing the peptide conformation that is preferred by mammalian GnRH receptors (Shinitzky and Fridkin, 1976). GnRH can also be constrained in its active conformation by substituting the achiral Gly⁶ residue with a D-amino acid (Monahan *et al.*, 1973) or incorporating a τ -lactam ring at the Gly-Leu peptide bond (Freidinger *et al.*, 1980). Constraining the peptide conformation enhances binding to the mammalian pituitary GnRH receptor, but is much less effective in increasing interaction with the chicken GnRH receptor (Millar *et al.*, 1986). This indicates that the chicken GnRH receptor and probably other non-mammalian receptors do not bind GnRH in the same conformation as do the mammalian GnRH receptors.

	1	2	3	4	5	6	7	8	9	10	
Mammal	pGlu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly	NH ₂
Chicken 1	pGlu	His	Trp	Ser	Tyr	Gly	Leu	Gln	Pro	Gly	NH ₂
Seabream	pGlu	His	Trp	Ser	Tyr	Gly	Leu	Ser	Pro	Gly	NH ₂
Catfish	pGlu	His	Trp	Ser	His	Gly	Leu	Asn	Pro	Gly	NH ₂
Salmon	pGlu	His	Trp	Ser	Tyr	Gly	Trp	Leu	Pro	Gly	NH ₂
Dogfish	pGlu	His	Trp	Ser	His	Gly	Trp	Leu	Pro	Gly	NH ₂
Chicken II	pGlu	His	Trp	Ser	His	Gly	Trp	Tyr	Pro	Gly	NH ₂
Lamprey III	pGlu	His	Trp	Ser	His	Asp	Trp	Lys	Pro	Gly	NH ₂
Lamprey I	pGlu	His	Tyr	Ser	Leu	Glu	Trp	Lys	Pro	Gly	NH ₂
Tunicate I	pGlu	His	Trp	Ser	Asp	Tyr	Phe	Lys	Pro	Gly	NH ₂
Tunicate II	pGlu	His	Trp	Ser	Leu	Cys	His	Ala	Pro	Gly	NH ₂

Fig. 1. Comparison of the structures of naturally occurring GnRH variants. Conserved features of GnRH structure, indicated in the yellow box, are likely to have important functional roles, while sidechains of nonconserved residues, indicated in blue boxes, are either unimportant or confer specificity for a particular GnRH receptor.

Structure of the GnRH receptor

Deduced amino acid sequence

The amino acid sequence of the GnRH receptor was first elucidated when the mouse GnRH receptor was cloned from cDNA of the α T3 gonadotrope cell line (Fig. 3; Tsutsumi *et al.*, 1992). The sequence was confirmed by Reinhart *et al.* (1992) and Perrin *et al.* (1993) and provided the basis for the cloning of GnRH receptors from human (Kakar *et al.*, 1992; Chi *et al.*, 1993), rat (Eidne *et al.*, 1992; Kaiser *et al.*, 1992; Perrin *et al.*, 1993), sheep (Brooks *et al.*, 1993; Illing *et al.*, 1993), cattle (Kakar *et al.*, 1993) and pig (Weesner and Matteri, 1994) pituitaries. The sequences of these cloned mammalian GnRH receptors exhibit high homology, with greater than 80% amino acid identity between any two sequences, indicating that the cloned GnRH receptors belong to a single subtype that recognizes the mammalian hypothalamic form of GnRH.

The GnRH receptor exhibits the characteristic features of G-protein-coupled receptors (GPCRs) (Fig. 3). It consists of a single amino acid chain with an extracellular amino-terminal domain, with no cleaved terminal signal sequence, and seven hydrophobic segments which probably form a bundle of membrane-spanning α -helices connected by extracellular and intracellular loops. The GPCRs terminate with a cytosolic carboxy-terminal domain. The GnRH receptor contains most of the amino acid sequence patterns that are highly conserved among the rhodopsin family of GPCRs (Baldwin, 1993). The high degree of conservation of particular amino acid residues among the rhodopsin-type GPCRs suggests that members of this family of receptors may share a common structural framework and mechanism of activation. Thus, much of what is known about other GPCRs may also apply to the GnRH receptor, and insights into the structure and mechanism of activation of the

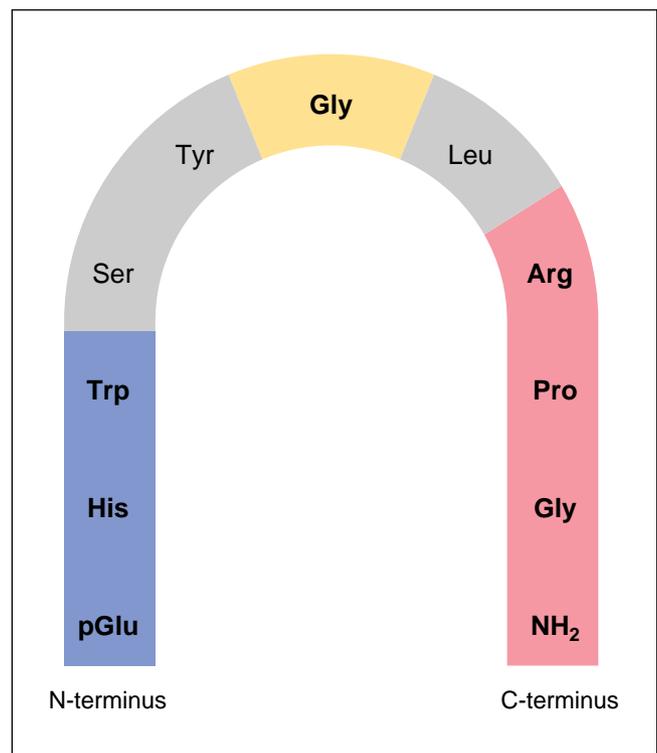


Fig. 2. Structure–activity relationship of GnRH. The amino-terminal residues (blue) have a role in receptor activation, while the achiral Gly residue in position six (yellow) allows GnRH to assume the β -turn conformation required for high-affinity interaction with mammalian receptors. The carboxy-terminal residues (pink) are required for specificity and high-affinity binding to the GnRH receptor.

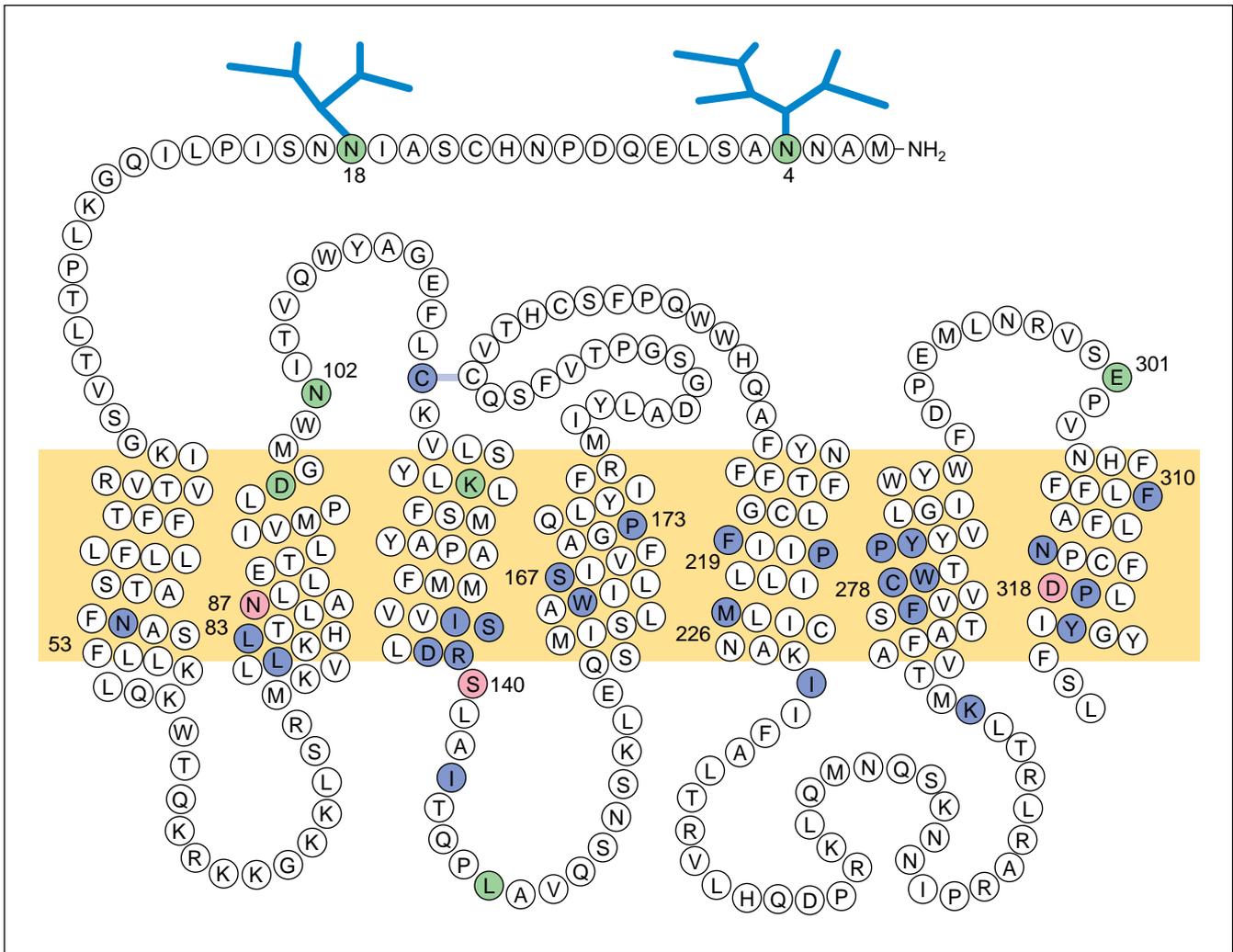


Fig. 3. Two-dimensional representation of the mouse GnRH receptor, a member of the G-protein-coupled family of receptors (GPCRs). Amino acid motifs that are highly conserved throughout the rhodopsin group of GPCRs probably have a role in receptor activation. Indicated by grey circles, they include Asn⁵³ in helix I; Leu⁸⁰ and Leu⁸³ in helix II; Cys¹¹⁴ and Ile¹³⁵-Ser-X-Asp-Arg¹³⁹-X-X-Ile¹⁴³ in helix III; Trp¹⁶⁴, Ser¹⁶⁷ and Pro¹⁷³ in helix IV; Phe²¹⁹-X-X-Pro²²², Met²²⁶ and Ile²³³ in helix V; Lys²⁶⁶ and Phe²⁷⁵-X-X-Cys-Trp-x-Pro-Tyr²⁸² in helix VI; Phe³¹⁰, Asn³¹⁴ and Pro³¹⁹-X-X-Tyr³²² in helix VII. Some of the residues in positions that are highly conserved among GPCRs are different in the GnRH receptor and provide insight into the structure of GPCRs. These residues are indicated by pink circles: Asn⁸⁷, Ser¹⁴⁰, Asp³¹⁸. Other residues that affect receptor function and are marked by green circles: Asn⁴, Asn¹⁸, Asp⁹⁸, Asn¹⁰², Lys¹²¹, Leu¹⁴⁷ and Glu³⁰¹.

GnRH receptor can be expected to have general application among the GPCRs.

Unusual features of the GnRH receptor sequence

In addition to the characteristic features of the rhodopsin-type GPCRs (described in the legend of Fig. 3), the cloned GnRH receptor also has some unique features.

(i) *Lack of carboxy-terminal domain and desensitization.* The GnRH receptor lacks a cytoplasmic carboxy-terminal domain. In other GPCRs this domain is frequently docked to the membrane by palmitoylation of a Cys residue (Strader *et al.*, 1994) and has a role in short-term homologous desensitization resulting from the ligand-stimulated phosphorylation of Ser and Thr residues

(Palczewski and Benovic, 1991). Consistent with the absence of a carboxy-terminal domain, GnRH receptor-mediated inositol phosphate production does not exhibit rapid desensitization (Davidson *et al.*, 1994a; McArdle *et al.*, 1995). However, increase of intracellular calcium concentrations, a signalling event distal to inositol phosphate production, does exhibit GnRH-dependent desensitization after 1 h of exposure to GnRH (McArdle *et al.*, 1995). This may account for the well-described desensitization of physiological responses to GnRH administration (Clayton, 1989). The lack of a carboxy-terminal tail and rapid desensitization may have been selected for in the mammalian GnRH receptor to allow the protracted LH surge necessary for ovulation. The first cytoplasmic loop of the GnRH receptor is unusually long and may substitute for the absent carboxy-terminal domain in its usual role of receptor internalization (Tsutsumi *et al.*, 1992).

(ii) *Modified DRY motif at boundary of helix III and second cytoplasmic loop.* At the cytosolic end of the transmembrane helix III of the GnRH receptor, a Ser residue (Ser¹⁴⁰) replaces the Tyr residue of the 'DRY' (Asp-Arg-Tyr) motif which is highly conserved amongst the GPCRs (Fig. 3; Probst *et al.*, 1992). The Asp/Glu and Arg residues of this motif have been implicated in the coupling of some GPCRs to their G proteins (Baldwin, 1994). Mutation of Ser¹⁴⁰ to Tyr or Ala (Davidson *et al.*, 1994b; Arora *et al.*, 1995) did not affect coupling of the GnRH receptor to cytosolic G proteins. However, the Tyr¹⁴⁰ mutant receptor exhibited an increased rate of receptor internalization and increased agonist binding affinity, suggesting that this mutated residue has subtle effects on receptor conformation (Arora *et al.*, 1995). Conserved residues located in this region of the GnRH receptor (Ile¹³⁵, Ile¹⁴³ and Leu¹⁴⁷) do have roles in G protein coupling, confirming the importance of helix III and the second cytoplasmic loop for interaction with G proteins (Arora *et al.*, 1995; Kitanovic *et al.*, 1996).

Post-translational modification: glycosylation

Inhibition of *N*-glycosylation using tunicamycin or cleavage of terminal sialic acid residues results in a decreased number of GnRH receptors in rat pituitary cells (Schwartz and Hazum, 1985). However, polysaccharide moieties do not affect the affinity of ligand binding and therefore probably do not constitute part of the ligand-binding domain (Schwarz and Hazum, 1985; Hazum, 1987). Mutagenesis of the three *N*-glycosylation consensus sequences (Asn-X-Ser/Thr) in the cloned mouse GnRH receptor shows that two of these sites, the Asn⁴ and Asn¹⁸ residues, are glycosylated during transient expression in COS-1 cells, while Asn¹⁰² is not (Davidson *et al.*, 1995). However, the GnRH receptor in native pituitary membranes migrates with a lower apparent M_r than does the wildtype receptor expressed in COSM6 cells (Perrin *et al.*, 1993). This indicates less polysaccharide modification and suggests that only one residue is glycosylated in the pituitary. Since the Asn⁴ site is not conserved in other species of GnRH receptor, it is possible that only Asn¹⁸ is glycosylated in the pituitary. Mutation of Asn⁴ or Asn¹⁸ decreases receptor expression, indicating that the polysaccharide moieties affect the number of receptors present in the cell (Davidson *et al.*, 1995). However, these mutations do not affect the affinity of ligand binding to the receptor, confirming the earlier indication that polysaccharides do not have a direct function in ligand binding (Davidson *et al.*, 1995).

Models of G-protein-coupled receptors and the tertiary structure of the GnRH receptor

Theoretical models of receptor activation

The transmission of the hormone message by the receptor to the signal transduction pathway within the cell has been presumed to involve a change in receptor conformation (Kenakin, 1993). For GPCRs, the active conformation is related to a ternary complex consisting of hormone, receptor and G protein. This model includes an initial binding step common to both agonists and antagonists, followed by a transition step, exclusive to agonists, which leads to formation of the ternary complex. The model also allows for spontaneous formation of a receptor-G protein complex, which has higher affinity for

agonist ligands and is stabilized by binding of agonists. The receptor returns to the low-affinity conformation when GTP binds to the G protein and the complex dissociates (De Lean *et al.*, 1980). This model has recently been revised to accommodate constitutively active receptors that signal in the absence of agonist and inverse agonists that inhibit basal signal transduction (Samama *et al.*, 1993). The revised model proposes that receptors exist in equilibrium between an inactive R conformation and an active R* conformation. Agonist binding shifts the equilibrium towards R*. The R* conformation has high affinity for agonists, and is the only form that can bind G proteins (Samama *et al.*, 1993).

Models of receptor tertiary structure

It has not been possible to obtain crystals of GPCRs or study their tertiary structure directly by X-ray crystallography. Consequently, their structure can only be predicted through the use of computer based molecular models (Baldwin, 1993; Schwartz, 1994; Ballesteros and Weinstein, 1995).

Different GPCR models involve different, distinct ligand binding arrangements, all of which are compatible with known rank orders of binding affinity. In the absence of direct structural information, this ambiguity makes it necessary to test structural details of receptor models such as helix-helix interactions and the relative location in space of various domains (Ballesteros and Weinstein, 1995).

An unusual feature of the GnRH receptor has allowed identification of interhelical interactions. Two residues that are highly conserved in GPCRs, Asp in helix II and Asn in helix VII, appear to have undergone reciprocal mutation to Asn⁸⁷ and Asp³¹⁸ in the GnRH receptor (Fig. 3), suggesting that the two residues interact with each other. Mutation of Asn⁸⁷ in helix II to Asp abolished receptor function, but a second mutation, recreating the arrangement found in other GPCRs (Asp⁸⁷Asn³¹⁸), regenerated ligand binding. This restoration of binding by reciprocal mutation shows that the sidechains of two residues in helices II and VII have complementary roles in maintaining the structure of the receptor and occupy the same microenvironment within the receptor (Zhou *et al.*, 1994).

The helix II Asp in GPCRs has been widely studied. Mutating it disrupts multiple aspects of receptor-G protein interactions in many GPCRs (Schwartz, 1994). Similarly, mutation of Asp³¹⁸ in helix VII of the GnRH receptor, alone or in the double mutant, decreases the inositol phosphate response to GnRH, suggesting that the functional role of the helix II Asp in other GPCRs may have been transferred to the helix VII Asp in the GnRH receptor (Zhou *et al.*, 1994). Alternatively, both residues may function in helix-helix interactions that relay the conformational changes associated with receptor activation.

The importance of the conserved Cys bridge

The conserved Cys residue at the extracellular end of the third transmembrane helix in the GnRH receptor probably forms a disulfide bridge with one of the Cys residues in the second extracellular loop. The occurrence of this second Cys residue is highly conserved, but its position is variable (Schwartz, 1994). The disulfide bridge probably stabilizes the active conformation of GPCRs (Baldwin, 1994). The importance of a disulfide bridge in the GnRH receptor was shown by an

early experiment in which treatment with the reducing agent dithiothreitol decreased the affinity of GnRH agonist binding (Keinan and Hazum, 1985).

The ligand-binding domain

The receptor conformation that binds agonist ligands with high affinity (R^*) is thought to differ from that which binds antagonists (R) (Samama *et al.*, 1993). This implies that some of the receptor amino acid residues involved in the binding of agonists will differ from those involved in antagonist binding.

The agonist-binding domain

Since receptor activation is initiated by binding of an agonist ligand, the conformational changes associated with receptor activation must have their origin in the ligand binding pocket (Findlay and Eliopoulos, 1990). The ligand binding sites of rhodopsin and the β -adrenergic receptor are located within the transmembrane helical bundle (Strader *et al.*, 1994). Specific residues in the β -adrenergic receptor have been identified as contact points for each of the functional chemical groups in the ligand. The most important of these is Asp¹¹³ in transmembrane helix III, which has been shown to form a salt bridge with the amine group in the ligand (Strader *et al.*, 1994).

(i) *Lys¹²¹ in helix III has a role in agonist binding.* The GnRH receptor has a basic residue, Lys¹²¹, in helix III at the position analogous to the Asp¹¹³ of the β -adrenergic receptor. Substitution of Lys¹²¹ with an uncharged Gln decreases the binding affinity of agonist analogues but does not affect antagonist binding affinity (Zhou *et al.*, 1995). This indicates that Lys¹²¹ may interact with agonist ligands, but not with antagonists. GnRH antagonists differ from agonists chiefly in their amino termini, where the pGlu-His-Trp sequence of GnRH is substituted with aromatic D-amino acids. Thus, an amino-terminal residue of GnRH may interact with Lys¹²¹ of the receptor. The interaction clearly does not involve a salt bridge, since there are no negative charges in GnRH. The electro-positive Lys¹²¹ sidechain may form a hydrogen bond with the electron-dense aromatic rings of the His² or Trp³ residues of GnRH, the imino group of His² (Zhou *et al.*, 1995), or a carbonyl group in the peptide backbone. The importance of a Lys residue was confirmed by treatment of the cloned GnRH receptor with an amino-group modifying reagent, 2,4,6-trinitrobenzenesulfonic acid, which destroyed GnRH receptor function (Zhou *et al.*, 1995).

(ii) *The extracellular domains in ligand binding.* Peptide ligands are more complex than the biogenic amines. Their larger size means that they have many more functional groups, both in the amino acid sidechains and in the peptide backbone, which have the potential to interact with specific receptor residues. The ligand-binding pocket of peptide receptors is likely to be larger than those of the biogenic amines in order to accommodate the larger size of their ligands. Consequently, in addition to residues in the transmembrane domains, the extracellular domain has been implicated in binding of diverse peptide ligands (Fong *et al.*, 1992; DeMartino *et al.*, 1994; Hjorth *et al.*, 1994; Walker *et al.*, 1994).

(iii) *Role of acidic residues in conferring selectivity for mammalian GnRH.* Since the Arg⁸ of GnRH is required for high-affinity binding to mammalian GnRH receptors, it has been postulated that the positively charged Arg interacts with a negatively charged acidic residue in the receptor (Hazum, 1987). Chemical modification of the GnRH receptor in pituitary membranes indicates that at least one, and possibly two, carboxyl groups are involved in GnRH binding (Keinan and Hazum, 1985). The carboxyl groups have been attributed to Glu or Asp residues or to polysaccharide sialic acid residues (Hazum, 1987). Mutagenesis of the glycosylation consensus sequences in the mouse GnRH receptor showed that polysaccharides do not affect ligand-binding affinity (Davidson *et al.*, 1995). However, mutation of acidic amino acids identified two acidic residues, Asp⁹⁸ and Glu³⁰¹, in transmembrane helix II and the third extracellular loop of the mouse GnRH receptor, which affect receptor function. The Glu³⁰¹Gln mutant receptor exhibited decreased affinity for mammalian GnRH, but unchanged affinity for the neutral [Gln⁸]-GnRH, and increased affinity for the negatively charged analogue [Glu⁸]-GnRH. Thus, the Glu³⁰¹ residue of the mouse GnRH receptor plays a role in the recognition of Arg⁸ in the ligand (Flanagan *et al.*, 1994).

(iv) *Glu³⁰¹ may affect ligand conformation.* Mammalian GnRH receptors preferentially bind GnRH peptides in a conformation that is stabilized by Arg⁸ and constrained by incorporation of a D-amino acid in position six. Although the Glu³⁰¹ residue of the mouse GnRH receptor is necessary for high affinity binding of mammalian GnRH, ligands that were conformationally constrained by the incorporation of D-Trp⁶ bound both the wildtype and the mutant Glu³⁰¹Gln GnRH receptor with high affinity. This suggests that the mutant Glu³⁰¹Gln GnRH receptor retains a preference for the conformation constrained by D-amino acids in position six, but it cannot induce this preferred conformation in unconstrained GnRH. Thus, GnRH may be induced to assume a high-affinity conformation by an interaction that involves the Glu³⁰¹ receptor residue and Arg⁸ (Flanagan *et al.*, 1994).

(v) *Asn¹⁰² confers high potency of peptides with carboxy-terminal glycinamide residues.* Mutation of Asn¹⁰² to Gln increases binding affinity for GnRH, while subsequent mutation of Asn¹⁰² to Ala causes a large decrease in the potency of GnRH in stimulating signal transduction (Davidson *et al.*, 1996). Similar large decreases are seen for all GnRH analogues that contain the polar GlyNH₂ carboxy-terminal residue present in native GnRH, but only small losses are seen for analogues containing the apolar N-ethylamide substitution at the carboxy terminus (Davidson *et al.*, 1996). These results are consistent with a hydrogen bond between the sidechain of Asn¹⁰² in the receptor and one of the polar groups of the GlyNH₂ moiety of GnRH (Davidson *et al.*, 1996).

It is concluded that GnRH agonist binding involves residues located in helix III (Lys¹²¹) and at the top of helices II (Asn¹⁰²) and VII (Glu³⁰¹ in the mouse receptor, Asp³⁰² in the human receptor) (Fig. 4). This would imply that these residues are in close proximity in the three-dimensional architecture of the GnRH receptor, consistent with the proposed arrangement of the transmembrane helices of the GPCR family of proteins

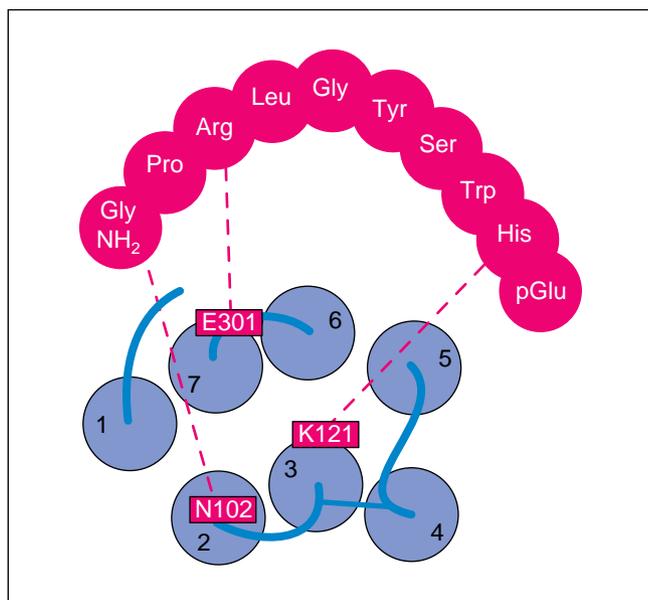


Fig. 4. Diagrammatic representation of GnRH and the mouse GnRH receptor ligand binding pocket. GnRH has been enlarged relative to the receptor, for clarity and to emphasize the relationship of specific residues of GnRH with residues that have been identified in the receptor. The membrane-spanning helices of the receptor are represented by numbered cylinders with connecting extracellular loops shown. The residues that affect ligand binding, Asn¹⁰², Lys¹²¹ and Glu³⁰¹, are identified. The GnRH residues, GlyNH₂, Arg⁸ and an amino-terminal residue, His², which are affected by mutation of the receptor residues, are indicated by dashed lines.

(Baldwin, 1993), which is based on the projection map of rhodopsin (Schertler *et al.*, 1993).

The antagonist-binding domain

Agonist and antagonist ligands do not necessarily occupy the same ligand-binding domain in GPCRs even though antagonists appear to be competitive inhibitors of agonist binding (Strader *et al.*, 1994). It is proposed that the competitive behaviour arises from a volume exclusion effect in which agonist and antagonist binding pockets overlap in space while interacting with different receptor residues (Fong *et al.*, 1992; Huang *et al.*, 1994; Strader *et al.*, 1994). However, it has also been observed that some (non-peptide) antagonists are not simple competitive inhibitors of agonist binding, and it has been proposed that they inhibit agonist access by an allosteric mechanism that stabilizes an inactive receptor conformation (Rosenkulde *et al.*, 1994).

The GnRH receptor also appears to have different binding domains for agonist and antagonist ligands. Mutation of the Lys¹²¹ residue affects binding of agonists, but not of antagonists (Zhou *et al.*, 1995). Thus, Lys¹²¹ may be an agonist contact site, but it is clearly not part of an antagonist binding site. Trypsin treatment of the GnRH receptor that had been covalently coupled to a photoactive agonist or antagonist shows that the agonist and antagonist are attached to different parts of the receptor (Janovick *et al.*, 1993). Thus, two quite different approaches,

mutagenesis of Lys¹²¹ and proteolytic cleavage of the receptor, show that the binding sites for GnRH agonists and antagonists differ.

In other peptide receptor systems, non-peptide antagonists have provided useful pharmacological tools. Because their binding sites are frequently different from the binding sites of both agonists and peptide antagonists, they exhibit different sensitivities to receptor mutagenesis and allow analysis of the ligand-binding properties of mutant receptors which have low affinities for peptide ligands (Fong *et al.*, 1992; Gether *et al.*, 1993). It is anticipated, therefore, that development of non-peptide ligands (whether agonist or antagonist) for the GnRH receptor will facilitate further studies of the receptor, in addition to providing orally active drugs.

Concluding remarks

The cloning of the GnRH receptor has provided information on the primary structure of the receptor and has confirmed the expectation that it belongs to the GPCR family. It provides some explanation for the structure–activity relationships of GnRH in terms of its receptor. Lack of knowledge of the three-dimensional structure of the receptor limits further understanding of its interactions with known GnRH analogues and the development of new analogues. Combined approaches using molecular modelling based on comparison with other GPCRs and experimental molecular biology and pharmacology are being used to develop a three-dimensional model of the GnRH receptor. This evolving model is being used to explore the way in which GnRH interacts with the receptor. Examination of the coordinated changes during the evolution of both the GnRH ligand and its receptor will provide further insight into receptor structure and the ligand-binding site. The emerging understanding of receptor structure will provide a useful model of the ligand binding site on which to design orally active non-peptide GnRH analogues which may form the basis of a new generation of non-steroidal contraceptives with application in both men and women.

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