Clinical and molecular genetics of the human GnRH receptor

Beate Karges1,4,*, Wolfram Karges2,* and Nicolas de Roux3

1Pediatric Endocrinology, University Children’s Hospital, University of Ulm, 89075 Ulm, 2Division of Endocrinology, Department of Internal Medicine 1, University of Ulm, 89081 Ulm, Germany and 3INSERM U584, Hôpital Necker, 75006 Paris, France

4To whom correspondence should be addressed at: University Children’s Hospital, University of Ulm, Prittwitzstrasse 43, D 89075 Ulm, Germany. E-mail beate.karges@medizin.uni-ulm.de

A functional GnRH receptor (GnRH-R) in the anterior pituitary is critical for normal LH/FSH secretion, pubertal development and reproduction. Inactivating mutations of the GnRH-R have been identified in patients with idiopathic hypogonadotrophic hypogonadism. In this article we summarize phenotypic characteristics of these patients and focus on specific functional alterations of the human GnRH-R. In-vitro studies using recombinant receptor constructs demonstrate that GnRH-R missense mutations result in impaired ligand binding and reduced signal transduction, causing gonadotrophin deficiency. A detailed molecular understanding of receptor inactivation may help to design new GnRH agonists to therapeutically modulate GnRH-R function.

Key words: gene mutation/GnRH receptor/G protein-coupled receptor/hypogonadotrophic hypogonadism/infertility

Introduction

Idiopathic hypogonadotrophic hypogonadism (IHH) is characterized by absent or incomplete pubertal development and infertility. Substantial progress has been made in the understanding of this syndrome since the identification of inactivating mutations of the GnRH receptor (GnRH-R) gene in patients with IHH (de-Roux et al., 1997).

Loss of GnRH-R function equally affects male and female patients with isolated hypogonadotrophic hypogonadism. Impaired GnRH action needs to be distinguished from other causes of gonadotrophin deficiency (Achermann et al., 2002), including Kallmann syndrome characterized by impaired olfactory function. In patients with IHH and normal sense of smell, the prevalence of GnRH-R mutations has been estimated at 40% in familial cases and 16% in sporadic cases (Bananova et al., 2001).

In this review we summarize currently available clinical and functional data concerning GnRH mutations in humans. We focus on recent progress in understanding molecular aspects of altered GnRH-R function, and we translate this evidence into clinical patient management.

GnRH receptor: a G protein-coupled heptahelical receptor

The GnRH-R belongs to the family of rhodopsin-like G protein-coupled receptors (GPCR) containing seven transmembrane domains. These transmembrane helices (TMH) are connected by six alternating intracellular and extracellular loops, with the amino-terminus located on the extracellular side and the carboxy-terminus on the intracellular side (Stojilkovic et al., 1994). The seven transmembrane domains are oriented counterclockwise when viewed from the extracellular surface, forming a hydrophilic core. Physical proximity between TMH 1, 2 and 7 was suggested by experimental analyses of mutant GnRH-R (Zhou et al., 1994; Ballesteros et al., 1998) as well as by analogy with the three-dimensional crystal structure of rhodopsin, a prototype heptahelical receptor (Palczewski et al., 2000).

The gene encoding the human GnRH-R is located on chromosome 4q13.2-13.3, spanning 18.9 kb. The GnRH-R coding sequence comprises three exons, coding for a 328 amino acid protein with >85% homology within mammalian species, with near identity inside the transmembrane domains. However, compared with other heptahelical hormone receptors, the GnRH-R has several unique features: the absence of a cytoplasmic C-terminal tail, replacement of Tyr by Ser in the conserved DRY motif (Asp-Arg-Tyr) located at the intracellular part of TMH3, and the reciprocal exchange of two highly conserved amino acids, Asp in TMH2 and Asn in TMH7. A single glycosylation site (N18) improves GnRH-R expression (Davidson et al., 1995). Extracellular disulphide bonds between C14–C200 and C114–C196 (Cook and Eidne, 1997; Davidson et al., 1997) stabilize the structure of the functional GnRH-R protein.

The natural ligand of the GnRH-R is GnRH (also called LH-releasing hormone, LHRH), a decapeptide synthesized by hypothalamic neurons and secreted in a pulsatile fashion into the
pituitary portal circulation. GnRH interacts with high affinity receptors in cell membranes of gonadotrophs in the anterior pituitary, leading to biosynthesis and release of the gonadotrophins LH and FSH (Figure 1). The pulse-timing and concentration levels of GnRH are crucial for gonadal steroidogenesis and normal reproductive function (Seminara et al., 1998). GnRH binds to its receptor in a hairpin structure with the NH2- and COOH-terminal domains contributing to receptor binding and activation, whereas the central region is mostly involved in conferring flexibility to the molecule (Karten and Rivier, 1986).

**Physical interaction of GnRH ligand and its receptor**

The ligand-binding pocket of the GnRH-R is formed by residues in the extracellular loops (ECL) and in the interior of the TMH, predicting that GnRH partially enters into the transmembrane core (Hoffmann et al., 2000). Similar binding mechanisms have been suggested for the structurally related thyrotrophin-releasing hormone (TRH) and vasopressin (VP) (Gershengorn and Osman, 2001). In addition, the extracellular domain of the GnRH-R seems to play a role for ligand binding (Davidson et al., 1997). Based on computational and experimental data, it has been proposed that the binding pocket is formed by residues in TMH2 (Asp98), ECL1 (Trp101, Asn102), TMH3 (Lys121), TMH5 (Asn212) and ECL3 (Asp302) (Zhou et al., 1995; Davidson et al., 1997; Flanagan et al., 2000; Hoffmann et al., 2000; Hovelmann et al., 2002).

Specific interaction between agonist and its G protein-coupled receptor requires sequential conformational changes until the active receptor state is reached (Gether, 2000). Rotation of TMH3 and proximity of TMH 2, 3 and 7 are essential for effective receptor activation. During ligand binding, His2 of GnRH interacts with Lys121 in TMH 3 and Asp98 in TMH 2, turning the orientation of the Lys121 side chain from interactions with Asp98 and Glu90 in TMH2 towards TMH7, placing His2 in proximity of the C-terminus of GnRH (Flanagan et al., 2000; Blomenrohr et al., 2001; Hovelmann et al., 2002). In contrast, this movement of the Lys121 side-chain from TMH2 towards TMH7 is absent in the absence of ligand and during antagonist binding (Hovelmann et al., 2002). The C-terminal glycaminamide of GnRH is supposed to interact with Asn102 and Trp3 with Trp279 in TMH6 (Chauvin et al., 2000). Arg8 of GnRH and Asp302 side-chains interact to induce a high-affinity ligand conformation of GnRH (Fromme et al., 2001).

**GnRH receptor activation and signal transduction**

Ligand-induced activation of GPCR such as GnRH-R requires the release of constraining intramolecular bonds maintaining the receptor in an inactive conformation (Schoneberg et al., 1999). Ionic interaction, including protonation of the conserved Arg139 at the intracellular transition of TMH3 and the interaction of side-chains of residues Arg139 and Asp318 located in TMH7 are involved in stabilizing the active GnRH-R state (Ballesteros et al., 1998). With the counterclockwise rotation of transmembrane helices during sequential ligand binding, receptor activation is now considered a dynamic process.

Ligand-induced signal transduction of the GnRH-R is illustrated in Figure 1. The movements of the TMH are thought to cause changes in the intracellular loops (ICL) leading to increased coupling to heterotrimeric G proteins containing α, β and γ subunits. Specificity of signal transduction is transmitted by residues in ICL3. G protein activation is further regulated by critical amino acid residues in TMH2, ICL2 and TMH7 (Flanagan et al., 1999).

The GnRH-R activates both the Gα and Gβγ signalling pathway in LβT2 pituitary gonadotroph cells (Liu et al., 2002b). The predominant activation of Gα11-proteins in αT3-1 cells, CHO-K1 cells and COS-7 cells (Grosse et al., 2000) induces phospholipase C (PLC) activity, catalysing the generation of intracellular inositol phosphates (IP3) and diacylglycerol (DAG) from phosphatidyl inositol phosphate (PIP2). IP3 mobilizes calcium ions (Ca++) from the endoplasmatic reticulum (ER), activating protein kinase C (PKC) and mitogen-activated protein kinase (ERK) (Liu et al., 2002a), involving RAF-1 and MEK (Naor et al., 1998). Phosphorylated ERK activates transcription factors via phospholipase A2 (PLA2), resulting in gene transcription of gonadotrophin subunits and synthesis and release of LH and FSH (Naor et al., 1998) (Figure 1). The FSHβ gene promoter is more sensitive to GnRH stimulation than the LHβ or gonadotrophin α-subunit gene promoters in GH3 cells (Bedecarrats et al., 2003). Similarly, the molar quantity of GnRH required to induce FSH secretion in vivo is less than that for LH (Brown and McNeilly, 1999).

**Identification of inactivating human GnRH-R mutations**

IHH has long been considered to result from deficient hypothalamic GnRH or impaired GnRH action, as exogenous GnRH may initiate gonadotrophin secretion (Waldstreicher et al., 1996). Therefore, GnRH represented a promising candidate gene for clinical mutation analysis, in particular as hypogonadal hpg mice carrying a GnRH gene deletion have been identified (Mason et al., 1986). To some surprise, however, no mutations of the human GnRH gene have so far been detected in patients with IHH (Weiss et al., 1991; Layman et al., 1996).
Clinical and molecular genetics of GnRH-R

Table I. In-vitro characteristics of human GnRH receptor mutants causing hypogonadotrophic hypogonadism

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Signal transduction</th>
<th>Ligand binding</th>
<th>Cell surface expression</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>N10K</td>
<td>+</td>
<td>(+)</td>
<td>+</td>
<td>Costa et al. (2001)</td>
</tr>
<tr>
<td>T32I</td>
<td>–</td>
<td>NT</td>
<td>+</td>
<td>Beranova et al. (2001)</td>
</tr>
<tr>
<td>E90K</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Maya-Nunez et al. (2002)</td>
</tr>
<tr>
<td>Q106R</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
<td>Bedecarrats et al. (2003), Nunez et al. (2001), de-Roux (1999)</td>
</tr>
<tr>
<td>A129D</td>
<td>–</td>
<td>–</td>
<td>NT</td>
<td>Caron et al. (1999)</td>
</tr>
<tr>
<td>R139H</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Costa et al. (2001)</td>
</tr>
<tr>
<td>S168R</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Prolong et al. (1999)</td>
</tr>
<tr>
<td>A171T</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Karges et al. (2003)</td>
</tr>
<tr>
<td>C200Y</td>
<td>–</td>
<td>NT</td>
<td>+</td>
<td>Beranova et al. (2001)</td>
</tr>
<tr>
<td>S217R</td>
<td>–</td>
<td>–</td>
<td>NT</td>
<td>Beranova et al. (2001), de Roux et al. (1999), Layman et al. (1998)</td>
</tr>
<tr>
<td>*177T</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>Silveira et al. (2002b)</td>
</tr>
</tbody>
</table>

With two exceptions (L314X, *177T), all mutations are missense, leading to a single amino acid substitution.

*Intron 1 mutation, leading to protein truncation at amino acid position 177. + normal; (+) partial; – absent; NT = not tested.

The GnRH-R was initially not considered an ideal candidate gene in IHH, as exogenous GnRH administration (e.g. in the context of clinical LH-RH testing) results in significant increases of LH/FSH secretion. After the demonstration of partial loss-of-function mutations in other GPCR such as thyrotrophin receptor (TSH-R) (Sunthornthepvarakui et al., 1995; de Roux et al., 1996) and LH receptor (LH-R) (Latronico et al., 1996; Laue et al., 1996) in other human diseases, the discovery of inactivating GnRH-R mutations causing IHH was made in patients with normal response to exogenous GnRH (de-Roux et al., 1997).

It is not well understood why mutations of the GnRH ligand have not been observed in humans with IHH (Bo-Abbas et al., 2003). In contrast, mutations of both receptors for LH (Themmen and Huhtaniemi, 2000) and TSH (Szkudlinski et al., 2002), as well as of their corresponding glycoprotein ligands (Doeker et al., 1998; Achermann et al., 2001; Szkudlinski et al., 2002), have been identified. Naturally occurring LH receptor (Themmen and Huhtaniemi, 2000) or TSH receptor (Wonerow et al., 2001) mutations may result in receptor inactivation, or in constitutive receptor activation. However, no activating mutations of the GnRH-R gene have been described (Chanson et al., 1998).

GnRH-R germline mutations in hypogonadotrophic hypogonadism

Since 1997 (de Roux et al., 1997), a variety of inactivating mutations of the GnRH-R in patients with IHH have been described (Table I). With two exceptions (Kottler et al., 2000; Silveira et al., 2002b), all currently known GnRH-R mutations are missense mutations, leading to single amino acid substitutions. In the majority of patients, compound heterozygous mutations, i.e. the combination of two different GnRH mutations each located on a single allele, have been identified (Table II).

The most common GnRH-R mutations are located in the first extracellular loop (Q106R) and in the third intracellular loop (R262Q). Other natural mutations of the GnRH-R are distributed throughout the entire coding sequence (Figure 2). According to functional in-vitro criteria, GnRH-R mutations can be classified in partial or complete loss-of-function mutations (Table I). A silent polymorphism at nucleotide 453 (C to T transition) encoding for serine at codon 151 located in ICL2 segregated with the Q106R mutation in patients of different populations (Kottler et al., 2000; Costa et al., 2001; Karges et al., 2003).

Partially inactivating substitutions of the GnRH-R frequently found in familial hypogonadotropic hypogonadism are Q106R and R262Q. Q106R, located in the first extracellular loop (Figure 2), markedly reduces ligand affinity (de Roux et al., 1997), suggesting involvement of this residue in hormone binding. Since specificity of signal transduction is transmitted by residues in the third intracellular loop, R262Q showed normal agonist binding but reduced signal transduction (de Roux et al., 1997).

Despite a comparable reduction in GnRH-stimulated inositol phosphate (IP) production, the R262Q and Q106R GnRH-R variants have differential effects on other signal transduction pathways, as ERK activity is decreased to a larger extent in mutant R262Q than in Q106R (Bedecarrats et al., 2003). Similarly, the promoter activity of the α gonadotrophin subunit after GnRH stimulation is more severely affected by the R262Q than the Q106R receptor variant (Bedecarrats et al., 2003). Another substitution located in ICL3 (L266R) completely abolishes signal transduction and ligand binding in the GnRH-R (Beranova et al., 2001; Chabbert-Buffet et al., 2001). Loss of hormone binding and signalling may be due to disturbed cell membrane expression as observed in the GnRH-R variant E90K (TMH2), indicating specific importance of E90 for cell membrane targeting (Maya-Nunez et al., 2002). Normal receptor function may be further
affected by mutations changing electrostatic, ionic or hydrogen bond interactions with adjacent helices, e.g. A129D in TMH3 (Caron et al., 1999), S168R in TMH4 (Pralong et al., 1999), and S217R in TMH5 (de Roux et al., 1999). The complete lack of ligand binding and signal transduction in GnRH-R variants normally expressed at the cell surface is poorly understood. Using a molecular modelling approach, we recently demonstrated that an additional hydrogen bond between TMH4 and TMH3 impedes conformational mobility in GnRH-R mutation A171T, thus explaining loss of sequential binding and receptor activation despite normal surface expression (Karges et al., 2003).

Clinical phenotype of patients with GnRH-R mutations

Patients with genomic GnRH-R mutations present with a wide spectrum of clinical symptoms, reflecting the variable phenotypic expression of disease and the age at diagnostic evaluation. Clinical features in male patients include microphallus and descended testes in childhood and failure of pubertal development in adolescence (Caron et al., 1999; Pralong et al., 1999; Beranova et al., 2001; Costa et al., 2001; Söderlund et al., 2001; Karges et al., 2003) as well as infertility in adults (de Roux et al., 1997, 1999; Chabbert-Buffet et al., 2001; Pitteloud et al., 2001). Women with GnRH-R mutations typically have delayed puberty and primary amenorrhea (Kottler et al., 2000; Beranova et al., 2001; Layman et al., 2001; Silveira et al., 2002b). The variable onset and disease expression explains why patients with GnRH-R mutations may consult different medical specialties, including paediatrics, endocrinology, urology and gynaecology. Overall, hypogonadism due to GnRH-R mutations is considered part of the phenotypically variable and genetically heterogeneous IHH syndrome. GnRH-R mutations account for up to 40% of familial cases of IHH (Beranova et al., 2001). Recently, loss-of-function mutations of the G protein-coupled receptor 54 gene (GPR54 gene) have been identified as another cause of normosmic autosomal-recessive IHH (de Roux et al., 2003).

All male patients described with GnRH-R mutations have microphallus and reduced testicular volumes, with one exception (Pitteloud et al., 2001), reflecting fetal onset of GnRH deficiency. Using clinical and biochemical markers in patients with idiopathic hypogonadism, the severity of GnRH deficiency and time of onset may be stratified (Pitteloud et al., 2002a). It is likely that most patients with inactivating GnRH-R mutations have hypogonadism as early as at birth, although diagnosis has not been reported in early infancy so far. Isolated micropenis and cryptorchidism has also been reported in patients with mutations in LH-R (Themmen et al., 2000) and androgen receptor (Sultan et al., 2001), but these defects predominantly cause male pseudohermaphroditism. Male patients with inactivating GnRH-R mutations complain of a lack of virilization and reduced sexual function, and gynaecomastia was noted in ~50% of male patients (de Roux et al., 1997, 1999; Caron et al., 1999; Pralong et al., 1999; Chabbert-Buffet et al., 2001; Pitteloud et al., 2001; Söderlund et al., 2001; Karges et al., 2003).

Females with GnRH-R mutations typically present with incomplete or absent pubertal development, including incomplete thelarche and primary amenorrhea due to low estrogen production (de Roux et al., 1999; Kottler et al., 2000; Seminara et al., 2000; Beranova et al., 2001; Layman et al., 2001; Silveira et al., 2002b). The uterus is pre-pubertal sized and ovaries are small because of absent follicular stimulation. In contrast to constitutional delay of puberty, there is no developmental progress to full sexual maturity in such patients. Adrenarche occurs normally, and final height is not reduced in males and females with GnRH-R mutations. For further diagnostic differentiation between constitutional delay of growth and IHH due to GnRH-R mutations, determination of free α subunit (FAS) before and after GnRH administration may be useful, showing a failure of exaggerated FAS response in patients with GnRH-R mutations (Seminara et al., 2000; Mainieri and Elnekeave, 2003).

Reproductive function

Spontaneous pulsatility of the gonadotrophins is abnormal in individuals with GnRH-R mutations, showing a reduced amplitude but normal frequency (de Roux et al., 1997; Beranova et al., 2001) or an absent pulsatility of LH and FSH secretion (de Roux et al., 1997; Caron et al., 1999; de Roux et al., 1999; Seminara et al., 2000; Beranova et al., 2001; Pitteloud et al., 2001). Infertility in male patients is caused by absent spermatogenesis and impaired sexual function. There is no report about spontaneous fertility, although spermatogenesis was preserved in one individual carrying the Q106R/R262Q receptor variant (de Roux et al., 1997). Erectile and ejaculatory disorder was the main complaint of another individual homozygous for GnRH-R Q106R with normal testicular size of 17 ml (Pitteloud et al., 2001). Therapy with hCG was initiated because of desired fertility, resulting in a normal sperm count. After 4 months of hCG treatment, successful conception and subsequent delivery of a healthy child was reported (Pitteloud et al., 2001).

In female patients with GnRH-R mutations, reproductive function is severely compromised, but ovulation, conception and normal pregnancy may be achieved with gonadotrophin treatment (Seminara et al., 2000). In one individual homozygous for Q106 GnRH-R, spontaneous pregnancy was reported after pulsatile GnRH therapy, followed by loss of early pregnancy. However, pregnancy was successfully maintained to term under repeated GnRH therapy (Dewailly et al., 2002). Pulsatile GnRH has also induced conceptions in an individual carrying the Q106R/R262Q GnRH-R variant, but recurrent loss of pregnancy was reported (Seminara et al., 2000). Because of the presence of GnRH and GnRH-R in human endometrium and preimplantation embryos, a putative role for GnRH in the support of early pregnancy has been suggested (Raga et al., 1999; Casan et al., 2000; Seminara et al., 2000).

Diagnostic administration of exogenous GnRH results in a secretion of LH and FSH in a subgroup of patients with GnRH-R mutations with residual receptor function (Table I). High doses of GnRH, as typically used in GnRH stimulation testing (100 μg), are required to achieve this effect. LH and FSH secretion in such individuals is dose-dependent, and gonadotrophin release is augmented by repeated GnRH administration (‘receptor priming’) (Seminara et al., 2000).

Genotype–phenotype correlations

For a variety of human GnRH mutants, the extent of functional impairment has been comprehensively studied in vitro, using...
Male patients had a penile length <3rd percentile and low serum testosterone, breast stage Tanner 1 in females, testicular volume <3 ml in males) or partial clinical decision-making during infertility therapy, as pulsatile studies using mutant GnRH-R constructs may be helpful for mostly respond to exogenous GnRH. Patients with completely (Table II), and affected individuals carrying mutation Q106R function.

determined by the GnRH-R variant with the less severe loss of mutations, with two GnRH-R variants, one on each allele. As a mind that most patients have compound heterozygous GnRH-R experimental in-vitro data to a clinical setting, one has to keep in recombinant GnRH-R constructs (Table I). When transferring experimental in-vitro data to a clinical setting, one has to keep in mind that most patients have compound heterozygous GnRH-R mutations, with two GnRH-R variants, one on each allele. As a whole, comparison of compound heterozygous with homozygous patients suggests that their phenotype and the response to GnRH is determined by the GnRH-R variant with the less severe loss of function.

Overall, patients homozygous for Q106R present with the mildest phenotype (Pitteloud et al., 2001; Dewailly et al., 2002) (Table II), and affected individuals carrying mutation Q106R mostly respond to exogenous GnRH. Patients with completely inactive GnRH-R variants are characterized by complete hypogonadism without response to exogenous GnRH. Functional in-vitro studies using mutant GnRH-R constructs may be helpful for clinical decision-making during infertility therapy, as pulsatile GnRH treatment will not restore gonadotrophin release in individuals with refractory receptor function (Pralong et al., 1999; Layman et al., 2002).

Comparative studies in siblings carrying the same compound heterozygous GnRH-R variants showed inter-individual variation of GnRH response. Female patients with the Q106R/S217R/ R262Q GnRH-R mutant showed an increase of LH and FSH after exogenous GnRH administration, while their brother had no response to GnRH (de Roux et al., 1999). Clinically, both sisters presented partial hypogonadism with spontaneous thelarche and primary amenorrhea, while the male propositus had complete hypogonadism with microphallus and testes volume of 3 ml (de-Roux et al., 1999). Similar phenotypic heterogeneity has been described in a family with three siblings compound heterozygous for the R262Q/A129D GnRH-R variant (Caron et al., 1999). These observations suggest that phenotypic differences in patients with identical GnRH-R mutation may be due to modifier genes involved in gonadotroph function.

### Therapeutic strategies for patients with IHH and GnRH-R mutations

With minor exceptions, the treatment of patients with GnRH-R mutations is similar to HRT in other forms of central hypogonadism, following established clinical practice (Zitzmann and Nieschlag, 2000; Silveira et al., 2002a). In male individuals, androgen replacement is commonly achieved by i.m. injection of testosterone enanthate, or by transdermal testosterone administration using adhesive patches or gel (Steidler et al., 2003). Specific aspects of hormone therapy need to be considered in childhood, including dose titration and timing. If started at pubertal age, testosterone therapy leads to an adult size penis within 2 SD of the mean (Bin-Abbas et al., 1999). As spermatogenesis cannot be initiated by testosterone replacement, gonadotrophin therapy is required if induction and maintenance of reproductive function is desired (Buchter et al., 1998; Liu and Handelsman, 2003). Male patients with complete gonadotrophin deficiency may benefit from stimulation of Sertoli cell development using recombinant FSH or long-acting FSH analogues before the induction of puberty (Bouloux et al., 2001; Pitteloud et al., 2002b).

In female patients, to restore a normal menstrual cycle, cyclic estrogen/progesterin replacement is the treatment of choice (Silveira et al., 2002a; Timmreck and Reindollar, 2003). As in other patients with hypogonadism, general treatment goals include the induction of normal sexual development and function, improved fertility, and the acquisition and preservation of normal bone mineral density to prevent osteoporosis.

In patients with inactivating GnRH-R mutations, fertility treatment was successfully performed with hCG or hCG/hMG (Layman et al., 2001; Pitteloud et al., 2001). In contrast, pulsatile GnRH is not successful in the majority of such patients. There are several reports that GnRH therapy failed to induce spermatogenesis (Pralong et al., 1999) or ovulation (Layman et al., 2001) because of partial or complete resistance to pulsatile GnRH treatment. Rarely, high doses of pulsatile GnRH were able to increase levels of gonadotrophins and steroids sufficient for the induction of pregnancy (Seminara et al., 2000; Dewailly et al., 2002). With one exception (Dewailly et al., 2002), successful paternity and motherhood in patients with GnRH-R mutations was

### Table II. Correlation of GnRH-R genotype and clinical phenotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Clinical hypogonadism&lt;sup&gt;a&lt;/sup&gt;</th>
<th>In-vivo response to GnRH&lt;sup&gt;b&lt;/sup&gt;</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele 1</td>
<td>Allele 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q106R</td>
<td>Q106R</td>
<td>Partial</td>
<td>Dewailly et al. (2002), Pitteloud et al. (2001)</td>
</tr>
<tr>
<td>L266R</td>
<td>Partial</td>
<td>Normal</td>
<td>Beranova et al. (2001)</td>
</tr>
<tr>
<td>N10K</td>
<td>Partial</td>
<td>Normal</td>
<td>Costa et al. (2001)</td>
</tr>
<tr>
<td>N10K</td>
<td>Complete</td>
<td>Normal</td>
<td>Costa et al. (2001)</td>
</tr>
<tr>
<td>R262Q</td>
<td>Partial</td>
<td>Normal</td>
<td>Beranova et al. (2001), de Roux et al. (1997), Layman et al. (1998)</td>
</tr>
<tr>
<td>R262Q</td>
<td>Complete</td>
<td>Partial</td>
<td>Seminara et al. (2000)</td>
</tr>
<tr>
<td>A171T</td>
<td>Complete</td>
<td>Normal</td>
<td>Karges et al. (2003)</td>
</tr>
<tr>
<td>L314X</td>
<td>Complete</td>
<td>Normal</td>
<td>Kottler et al. (2000)</td>
</tr>
<tr>
<td>R262Q</td>
<td>A129D</td>
<td>Partial</td>
<td>Caron et al. (1999), Layman et al. (2002)</td>
</tr>
<tr>
<td>Y284C</td>
<td>Complete</td>
<td>Normal</td>
<td>Layman et al. (2001)</td>
</tr>
<tr>
<td>Y284C</td>
<td>Complete</td>
<td>partial</td>
<td>Layman et al. (2001)</td>
</tr>
<tr>
<td>Q106R/</td>
<td>S217R&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Normal</td>
<td>de Roux et al. (1999)</td>
</tr>
<tr>
<td>Q106R/</td>
<td>S217R&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Complete</td>
<td>de Roux et al. (1999)</td>
</tr>
<tr>
<td>T32I</td>
<td>C200Y</td>
<td>Complete</td>
<td>Beranova et al. (2001)</td>
</tr>
<tr>
<td>E90K</td>
<td>E90K</td>
<td>Complete</td>
<td>Soderlund et al. (2001)</td>
</tr>
<tr>
<td>C279Y</td>
<td>C279Y</td>
<td>Absent</td>
<td>Beranova et al. (2001)</td>
</tr>
<tr>
<td>S168R</td>
<td>S168R</td>
<td>Complete</td>
<td>Pralong et al. (1999)</td>
</tr>
<tr>
<td>L266R</td>
<td>L266R</td>
<td>Complete</td>
<td>Chabbert-Buffet et al. (2001)</td>
</tr>
<tr>
<td>Intron1</td>
<td>Intron1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Absent</td>
<td>Silveira et al. (2002b)</td>
</tr>
</tbody>
</table>

The majority of patients carry compound heterozygous GnRH-R mutations, characterized by different GnRH-R variants on each allele.

<sup>a</sup> The extent of clinical hypogonadism was subclassified as complete (breast stage Tanner 1 in females, testicular volume <3 ml in males) or partial (breast stage Tanner >2 in females, testicular volumes >3 ml in males). All male patients had a penile length <3rd percentile and low serum testosterone, and all female patients had primary amenorrhea and low serum estradiol levels.

<sup>b</sup> LH/FSH increase after intravenous administration of exogenous GnRH ("GnRH testing").

<sup>c</sup> Double mutant Q106R/S217R on one allele.

<sup>d</sup> Intron 1 mutation, causing deletion of exon 2, frameshift and premature termination.
only achieved with gonadotrophin treatment (de Roux et al., 1997; Seminara et al., 2000; Pitteloud et al., 2001). In a single case, successful pregnancy was reported after clomiphene treatment in such a patient (de Roux et al., 1997). After fertility therapy, individuals homozygous for the Q106R GnRH-R variant may exhibit a spontaneous reversal of hypogonadotrophic hypogonadism (Pitteloud et al., 2001; Dewailly et al., 2002).

The pharmacological rescue of GnR-R mutants with altered cell-surface expression was recently demonstrated in vitro (Janovick et al., 2002; Leanos-Miranda et al., 2002). IN3, a GnRH-R antagonist considered to function as a folding template, has been shown to restore ligand binding and inositol phosphate production in a variety of mutant GnRH receptors, (Leanos-Miranda et al., 2002), suggesting that defective intracellular transport due to protein misfolding significantly contributes to the pathogenesis of GnR-R dysfunction.

Based on the ability of IN3 to restore receptor function, an in-vitro classification system for GnR-R mutants has been proposed (Leanos-Miranda et al., 2002). The potential clinical utility of this system still remains to be determined. Very recently, other GnRH peptidomimetic antagonists were evaluated as potential rescue molecules (‘pharmacoperones’) for human GnR-R variants (Janovick et al., 2003). An identical approach was successfully studied in misfolded vasopressin receptor mutants (Morello et al., 2000). Taken together, the development of pharmacological chaperones to overcome receptor-misrouting may offer new therapeutic approaches in GnR-R-related hypogonadism and other diseases involving GPCR.

Molecular modelling of the GnR-R has successfully been used to study functional effects of specific agonists and antagonists on GnR-R activation (Hoffmann et al., 2000). Therefore, computational data of ligand–receptor interaction in functionally altered human GnR-R substitutions may further facilitate the design of new agonists to therapeutically modulate GnR-R function.

Clinical utility of GnR-R gene analysis

The identification and functional characterization of GnR-R mutations in patients with IHH has considerably extended our understanding of gonadotrophin deficiency, and recent molecular studies have provided the perspective of a rational drug design to treat gonadotrophin-sensitive human diseases (Hoffmann et al., 2000; Janovick et al., 2003). However, the clinical benefit of GnR-R mutational screening for the individual patient presenting with overt hypogonadism is currently limited. If induction of fertility is not an immediate treatment goal, patients with GnR-R mutations will typically receive HRT with testosterone or estrogen/progestin, as outlined above (Silveira et al., 2002a; Liu and Handelsman, 2003). Identification (and eventual in-vitro characterization) of disturbed GnR-R function may, however, guide fertility treatment, as pulsatile GnR therapy is less likely to restore reproductive function than gonadotrophin therapy in affected male and female patients (Pralong et al., 1999; Layman et al., 2001). Other advantages associated with mutation analysis are the identification and early treatment of (oligosymptomatic) siblings with hypogonadism, and the option of genetic family counselling.

GnR-R gene analysis should be considered in patients with IHH and preserved olfactory function, in particular if other family members are affected by hypogonadism. Notably, as in other autosomal-recessive genetic disorders, a negative family history does not exclude inheritance. Before initiating GnR-R analysis, other causes of central hypogonadism, including Kallmann’s syndrome, combined pituitary insufficiency, brain tumours, as well as constitutional delay of puberty should be ruled out (Achermann et al., 2002; Kalantaridou and Chrousos, 2002). Following established guidelines, informed patient consent and counselling should be integral parts of GnR-R genetic analyses.

Conclusion

The GnR-R is involved in genetically determined changes of gonadotrophin function in the anterior pituitary, because GnR-R amino acid substitutions result in decreased responsiveness to GnR-R. The pathophysiology of altered hormone–receptor interaction determines the phenotype of hypogonadism and the efficiency of treatment in individual patients. The design of new GnR-R agonists and antagonists is facilitated by in-vitro mutagenesis studies and molecular receptor modelling of variant GnR-R. Such efforts will eventually contribute to novel therapeutic approaches to modulate disturbed GnR-R function in patients with hypogonadotropic hypogonadism.

Acknowledgements

This work was supported in part by a Fellowship Award of the German Society of Pediatric Endocrinology (APE) (to B.K.).

References


Bouloux, P.M., Handelsman, D.J., Jochenhovel, F., Nieschlag, E., Rabinovici,
Clinical and molecular genetics of GnRH-R

receptor contribute differentially to ligand interaction. Biochemistry, 39, 8133–8141.


B. Karges, W. Karges and N. de Roux


