Pituitary and extrapituitary actions of gonadotrophin-releasing hormone and its analogues

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The hypothalamic decapetide gonadotrophin-releasing hormone (GnRH) binds to high affinity receptors on pituitary gonadotrophs. These receptors mediate the effects of GnRH on secretion and synthesis of gonadotrophins. The GnRH receptor is coupled to Gq/G11, which activates phospholipase C. This enzyme leads to the generation of several second messenger molecules. Among these, diacylglycerol (DG) and inositol 1,4,5-tris-phosphate (IP3) are critically important. DG leads to activation of protein kinase C and IP3 releases Ca2+ from intracellular pools. Both events result in secretion and synthesis of luteinizing hormone (LH) and follicle stimulating hormone (FSH). In addition, other components of the GnRH signal transduction pathway are involved in cellular responses to GnRH. GnRH receptors and their functions are regulated by GnRH itself or other hormones such as ovarian steroids. The prolonged exposure of pituitary gonadotrophs to GnRH leads to desensitization and consequently to suppressed LH and FSH secretion. This mechanism is employed for the clinical use of GnRH agonists. GnRH antagonists act by competitive binding to the pituitary GnRH receptors. Apart from the well-established pituitary actions of GnRH, receptors for the decapetide have been demonstrated in a variety of extrapituitary tissues. Here we report on the ovarian actions of GnRH which are predominantly inhibitory in the rat ovary. In the human ovary the existence of GnRH receptors is controversial. Recent reports have demonstrated the mRNA for the GnRH receptor in the human ovary. However, to date there is no consensus on the ovarian actions of GnRH or its analogues.

Key words: gonadotrophin-releasing hormone/gonadotrophin-releasing hormone analogues/ovary/pituitary

Introduction

The hypothalamic decapetide gonadotrophin-releasing hormone (GnRH) plays a central role in the control of female reproductive functions. GnRH is released...
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from hypothalamic neurons in a pulsatile manner, binds to specific receptors on pituitary gonadotrophs, and leads to intermittent secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH). Both gonadotrophins are secreted into the systemic circulation and regulate gametogenesis and steroidogenesis in the ovary. The cyclic pattern of gonadotrophin secretion is largely dependent on feedback actions of ovarian steroids on the hypothalamus and the pituitary. The latter seems to be more important in the human since application of pulsatile GnRH to patients with hypothalamic amenorrhoea is able to reinitiate regular menstrual cycles with patterns of gonadotrophin secretion that do not differ from those of normal women (Knobil, 1980).

Chronic agonist stimulation of pituitary gonadotrophs leads to GnRH receptor (GnRH-R) down-regulation and desensitization. This suppression of gonadotrophin secretion and consequently of gonadal function is the basis for the clinical use of GnRH agonists (GnRHag). Their prolonged application leads to suppression of ovarian function. GnRH antagonists (GnRHant), which are not yet available for routine clinical use, suppress gonadotrophin secretion by competing with native GnRH for its receptor (Conn and Crowley, 1991; Sealfon et al., 1997).

In addition to the actions of GnRH on pituitary gonadotrophs, the peptide has been shown to bind to a variety of cell types in extrapituitary tissues. These include cells in several male and female reproductive organs, as well as a number of malignant cells such as endometrial, ovarian and mammary cancer cells (Hsueh and Jones, 1981; Emons et al., 1997). Here, we describe the basic mechanisms of action of GnRH and its analogues on pituitary gonadotrophs which are important for the understanding of the physiology of gonadotrophin secretion and the clinical use of agonistic and antagonistic GnRH analogues. Therefore, we focus on the most important cellular mechanisms of GnRH action. Since GnRH analogues are widely used in reproductive medicine, it might be of concern that they interact with ovarian GnRH receptors that have been described in the human (for review, see Brus et al., 1997). In this brief review we describe the current knowledge concerning this possibility.

**Pituitary actions of GnRH and GnRH analogues**

GnRH binds to specific receptors on pituitary gonadotrophs. GnRH receptors can be regulated by GnRH itself or other hormones. Such regulation may determine the biological activity of these cells. Activation of these receptors leads to generation of several signal transduction molecules that initiate exocytosis of gonadotrophins and are involved in the expression of LH and FSH mRNAs. GnRHag have been synthesized by amino acid substitutions in the native GnRH molecule (Conn and Crowley, 1991). Their application leads to prolonged agonist action on the GnRH receptor, with an initial release of gonadotrophins, clinically known as the flare-up effect, and a prolonged suppression of LH and FSH secretion, which is usually the aim of treatment with GnRHag. The secretion of gonadotrophins that follows GnRHag application results from activation of
mechanisms that are identical to those observed after GnRH administration. Also, the prolonged presence of GnRH at its receptor can be compared to the action of GnRHag. The suppression of gonadotrophin secretion results mainly from homologous desensitization. In contrast, the inhibition of LH and FSH release observed after GnRHant administration is due to competitive blockade of GnRH action at the receptor level.

**GnRH receptor**

The cDNA sequence of the mouse GnRH-R was cloned using the murine gonadotroph cell line αT3-1. The GnRH-R cDNA encodes a 327–328 amino acid protein with seven putative membrane-spanning domains, characteristic of the family of G protein-coupled receptors. The GnRH-R lacks the typical intracellular carboxyl terminus (Stojilkovic et al., 1994; Sealfon et al., 1997). Northern blot analysis revealed the presence of at least two hybridizing mRNAs approximately 4.3 and 2.3 kb in size in αT3-1 cells. This has also been shown in other species. The presence of multiple transcripts raises the possibility that alternative functional forms of the GnRH-R may exist. Specific binding sites have been characterized by using radiolabelled GnRHag. The analogue binds to the GnRH-R with a dissociation constant of 0.20 nM in rat and 0.32 and 4.8 nM in human pituitary tissue (Wormald et al., 1985; Horn et al., 1991). The total number of binding sites is 0.31 pM/mg (Horn et al., 1991).

**GnRH-R regulation**

GnRH-R number is regulated by GnRH in a time- and dose-dependent manner. Moderate doses of GnRH or GnRHag lead to an initial decrease of GnRH-R followed by a prolonged increase. Continuous exposure of gonadotrophs to high concentrations of GnRH induced a decrease of binding sites (Table I). Some authors have reported independent regulation of GnRH-R number and GnRH-R mRNA levels, suggesting post-transcriptional homologous receptor regulation. However, others have demonstrated parallel time- and dose-dependent actions of GnRH and GnRHag on receptor number and mRNA levels (Hazum and Conn, 1988; Clayton, 1989; Kaiser et al., 1997).

Receptor-bound GnRH or GnRHag is internalized via receptor-mediated endocytosis. The internalized complex subsequently undergoes dissociation, followed by degradation of the ligand and partial recycling of the receptors. In contrast, GnRHant analogues remain bound at the cell surface for a long period. Endocytosis of the receptor–ligand complex is not required for GnRH-induced secretion. Also, desensitization of gonadotrophs by agonistic GnRH analogues can be dissociated from internalization (Kaiser et al., 1997).

Heterologous regulation of the GnRH-R has been shown to occur after steroid treatment of intact animals or isolated anterior pituitary cells (Table I). In primary cultures of rat pituitary cells, oestradiol increased GnRH-R number after long-term treatment and decreased GnRH binding after short-term treatment. Such
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effects might be responsible for oestradiol effects on GnRH-induced gonadotrophin secretion. Progesterone had exclusively stimulatory actions on GnRH-R, although the steroid exerts inhibitory actions on agonist-stimulated LH release. In αT3–1 cells, oestradiol reduced GnRH-R numbers, indicating differences between physiological cellular responses of αT3–1 cells and primary pituitary cells (Stojilkovic and Catt, 1995; Kaiser et al., 1997).

The gonadal peptides activin A and follistatin have been shown to be regulators of GnRH-R. Activin A may modulate the responsiveness of gonadotrophs to GnRH by increasing the expression of the GnRH-R, an action which is antagonized by follistatin (Kaiser et al., 1997).

Several second messenger molecules of GnRH signal transduction pathways (see below) seem to be involved in GnRH-R regulation. Protein kinase C (PKC) may mediate GnRH-R up-regulation by GnRH. In contrast, cAMP down-regulates GnRH-R mRNA in αT3–1 cells.

GnRH signal transduction

Research into cellular mechanisms of GnRH action in the past 10 years has resulted in rapid advances in our basic understanding of GnRH signal transduction. The majority of the experiments were performed in rat pituitary cells and cells of the murine gonadotroph cell line αT3–1. Activation of GnRH-R by GnRH leads to G protein coupling, generation of inositol phosphates, increase in cytoplasmic Ca²⁺ concentrations, translocation of PKC, production of cAMP and activation of mitogen-activated protein kinases (Hazum and Conn, 1988; Huckle and Conn, 1988; Clayton, 1989; Naor, 1990; Stojilkovic et al., 1994; Hille et al., 1995; Stojilkovic and Catt, 1995; Kaiser et al., 1997; Naor et al., 1998).

G protein coupling/phospholipase C

GnRH-induced gonadotrophin secretion is not altered by pertussis toxin, which excludes the participation of the Gi and Go subfamilies in GnRH action. Recently, it has been demonstrated that members of the newly defined pertussis toxin-insensitive Gq/G₁₁ subfamily of G proteins mediate the control of phospholipase C (PLC) activity by GnRH in pituitary gonadotrophs (Hsieh and Martin, 1992; Shah and Milligan, 1994). Down-regulation of Gq and G₁₁, which occurs after sustained exposure of gonadotrophs to GnRH, may be a component of the desensitization of the cellular response (see below). Activation of the G proteins leads to increased PLC activity, which results in phosphoinositide breakdown with generation of inositol phosphates (IP) and diacylglycerol (DAG) (Naor et al., 1998). In αT3–1 cells the concentration of inositol 1,4,5-tris-phosphate [Ins(1,4,5)P₃] increases rapidly (within 10 s) and reaches a maximum after 30 s. Ovarian steroids have been shown to enhance or inhibit GnRH-induced IP production. In αT3–1 cells we have demonstrated modulation of IP production by oestradiol and progesterone that is consistent with the steroid actions on GnRH-R numbers in primary pituitary cells (Emons et al., 1992; Ortmann et al., 1997).
Ins(1,4,5)P₃ binds to specific intracellular receptor channels and releases Ca²⁺ from the endoplasmic reticulum (Naor, 1990; Stojilkovic and Catt, 1995; Kaiser et al., 1997).

Calcium signalling

GnRH induces dose-dependent and biphasic (spike/plateau) increases of intracellular Ca²⁺ concentrations ([Ca²⁺]ᵢ) in primary rat pituitary cell cultures and αT3-1 cells. These changes of [Ca²⁺]ᵢ match the kinetics of Ins(1,4,5)P₃ production. The initial spike phase of the [Ca²⁺]ᵢ response is independent of extracellular Ca²⁺, while the sustained plateau phase is dependent on Ca²⁺ influx through voltage-sensitive Ca²⁺ channels. The extracellular Ca²⁺ independent phase of the Ca²⁺ signal is primarily an Ins(1,4,5)P₃-mediated rise in [Ca²⁺]ᵢ due to release from intracellular stores. The voltage-sensitive Ca²⁺ entry pathway can be influenced by PKC (Stojilkovic and Catt, 1995).

Measurements of [Ca²⁺]ᵢ in single gonadotrophs loaded with indo-1 AM, the Ca²⁺ sensitive dye, by dual-wavelength fluorescence microscopy have revealed a complex pattern of dose-dependent GnRH-induced Ca²⁺ signals. These range from subthreshold to oscillatory and biphasic responses. Increasing concentrations of GnRH lead to increased frequency of Ca²⁺ oscillations while their amplitude is not significantly changed. We have shown that agonist-induced Ca²⁺ signals are influenced by oestradiol and progesterone. Such steroid actions may be responsible for their modulatory effects on GnRH-stimulated gonadotrophin secretion (Ortmann et al., 1992a, 1994; Hille et al., 1995; Stojilkovic and Catt, 1995). The mechanism that mediates the actions of ovarian steroids on Ca²⁺ signals is thought to be located distal from IP formation, since steroid effects on agonist-induced IP production and increases of [Ca²⁺]ᵢ are not necessarily parallel (Emons et al., 1992; Ortmann et al., 1995).

Protein kinase C

GnRH induces phosphoinositide turnover which results in generation of IP and DAG. DAG activates PKC. Rat pituitary cells express the α, βII, δ, ε and γ subspecies of PKC. Activators of PKC (phorbol ester) induce LH secretion from pituitary gonadotrophs, while inhibitors of PKC lead to reduced secretory responses to GnRH. In permeabilized pituitary cells, the α and β isoenzymes mediate the exocytotic response. Furthermore, PKC activates LHB and FSHβ gene expression. Different PKC subspecies might be responsible for the mediation of various GnRH-induced cellular responses. It has been suggested that PKCα and PKCβ are potential candidates for the mediation of exocytosis (Naor et al., 1998). Also, steroids influence PKC activity and phorbol ester-induced gonadotrophin release, indicating that this signal transduction pathway is involved in steroid modulation of GnRH-induced exocytosis from gonadotrophs (Naor, 1990; Ortmann et al., 1992b).

Cyclic nucleotides

Increases of cAMP and cGMP in response to GnRH have been described. These observations are controversial; cAMP seems to play a role in the sensitization
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of the gonadotroph to GnRH. The production of cAMP can be enhanced by oestradiol. This effect could be involved in the stimulatory action of oestradiol on GnRH-induced LH secretion (Naor, 1990; Kaiser et al., 1997).

Mitogen-activated protein kinase

Mitogen-activated protein kinases (MAPK) are involved in transmitting extracellular growth and differentiation signals into the cell nucleus. Recently, it has been shown that some G protein-coupled receptors can activate MAPK and that MAPK may also be involved in nonproliferative signalling. Stimulation of MAPK activity was increased by GnRH in αT3–1 cells and primary rat pituitary gonadotrophs. This action is receptor-mediated and might involve a signalling pathway that is coupled to the pertussis toxin-sensitive G\textsubscript{i} or G\textsubscript{o} pathway. Furthermore, MAPK can be activated by PKC since GnRH-induced activation of MAPK is blocked in PKC-depleted cells (Kaiser et al., 1997).

Arachidonic acid

Arachidonic acid (AA) is liberated from cultured rat pituitary cells in response to GnRH application. AA itself and its lipoxygenase metabolites induce LH release from these cells. Thus AA or leukotrienes may serve as second messengers in gonadotrophs. Progesterone exerted stimulatory actions after short-term and inhibitory actions after long-term treatments on GnRH-induced AA liberation from perifused pituitary cells which were consistent with the steroid actions on Ca\textsuperscript{2+} signalling (Ortmann et al., 1996; Naor et al., 1998).

Desensitization

Pulsatile GnRH release from the hypothalamus is necessary for the maintenance of physiological LH and FSH secretion and biosynthesis. Sustained exposure of the pituitary gonadotroph to high concentrations of GnRH reduces the response of the cells to subsequent stimulation with GnRH. This process, termed homologous desensitization, leads to suppression of gonadotrophin secretion and is the primary mechanism of action of GnRHag. The exact mechanism of homologous desensitization is not known. Rapid desensitization is characteristic for a number of G protein-coupled receptors (Figure 1). The mechanism involves uncoupling of the receptor from its regulatory G protein, and thus subsequent signal transduction. The third intracellular loop and the C-terminal tail of these receptors seem to be critical for the desensitization process. However, the GnRH-R lacks both components (Kaiser et al., 1997; Sealfon et al., 1997).

Long-term homologous desensitization seems to involve the PKC pathway. In contrast to IP production, the Ca\textsuperscript{2+} response can be rapidly desensitized. High concentrations of GnRH lead to reduction of GnRH-R numbers without effects on affinity. Thus, one mechanism of action of long-term desensitization may be receptor loss (Figure 1). On the other hand, it is well known that changes in GnRH-R number can be uncoupled from those in gonadotroph responsiveness,
Homologous desensitization of GnRH receptors

- rapid or early desensitization - not present
- intermediate desensitization
  - receptor loss
  - reduced efficiency Ins (1,4,5)P₃
  - induced Ca²⁺ mobilization
  - inactivation of Ca²⁺ channels
- long-term desensitization
  - receptor loss
  - gonadotrophin pool depletion
  - desensitization of the PKC-pathway

Figure 1. Mechanisms of rapid, intermediate and long-term desensitization. GnRH = gonadotrophin-releasing hormone; Ins(1,4,5)P₃ = inositol 1,4,5-tris-phosphate; PKC = protein kinase C.

demonstrating the involvement of additional mechanisms (Conn and Crowley, 1991; Stojilkovic et al., 1994; Kaiser et al., 1997).

GnRH antagonists

Antagonistic analogues of GnRH result from multiple amino acid substitutions at positions 1, 2, 3, 6, 8 and 10 in the native GnRH molecule. The first GnRHant were synthesized more than 20 years ago (Karten and Rivier, 1986). However, their clinical application was hampered by oedematogenic and anaphylactoid reactions. New generations of GnRHant have been developed that are free of such side-effects. Two of these compounds, Cetrorelix and Ganirelix, are currently being tested in clinical studies (Rabinovici et al., 1992; Nestor et al., 1992; Diedrich et al., 1994; Nelson et al., 1995; Rivier et al., 1996; Fujimoto et al., 1997). These studies have shown the ability of these compounds to suppress gonadotrophin secretion immediately and thus gonadal steroid production. Therefore, GnRHant are likely to replace the GnRHag since they do not induce gonadotrophin release, which is not desired in most of the indications for GnRHag. Currently, GnRHant have been mainly tested in ovarian stimulation protocols, but may be used for other purposes, such as treatment of endometriosis, uterine fibroids, polycystic ovary syndrome and sex steroid-dependent cancer.

As mentioned earlier, the administration of GnRHant leads to immediate suppression of LH and FSH secretion (Rabinovici et al., 1992; Diedrich et al., 1994; Felberbaum et al., 1995; Albano et al., 1997; Fujimoto et al., 1997). The principal mechanism of action of these compounds is the competitive blockade of GnRH-R. Therefore, GnRHant inhibit GnRH-induced signal transduction. Since antagonistic analogues are free of any agonist activity, they do not induce the initial burst of gonadotrophin secretion that is characteristic of GnRHag. This is of importance for certain clinical applications of GnRHant, such as their use...
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Table I. Examples for homologous and heterologous regulation of gonadotrophin-releasing hormone receptor (GnRH-R) numbers. Arrows indicate time- and dose-dependent up(↑)- or down(↓)-regulation of GnRH-R numbers by various hormones.

<table>
<thead>
<tr>
<th>Homologous regulation</th>
<th>Heterologous regulation</th>
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<tbody>
<tr>
<td>GnRH ↑↓</td>
<td>Oestradiol ↑↓</td>
</tr>
<tr>
<td>GnRH agonist ↑↓</td>
<td>Progesterone ↑</td>
</tr>
<tr>
<td>GnRH antagonist ↓</td>
<td>Activin ↑</td>
</tr>
<tr>
<td></td>
<td>Follistatin ↓</td>
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in ovarian stimulation. Apart from their action to compete with GnRH for receptors on gonadotroph cell membranes, recent data indicate that prolonged treatment with GnRHa leads to down-regulation of GnRH receptors. Both occupied and unoccupied receptors were decreased after in-vivo treatment of rats with the GnRHa Cetrorelix, with maximal suppression of receptor numbers after 3–6 h. This action of the GnRHa might contribute to the mechanism by which the peptide inhibits gonadotrophin secretion (Table I; Halmos et al., 1996).

Furthermore, the GnRHa have the advantage that the reversal of their inhibitory action appears to be faster and, due to the nature of competitive blockade of GnRH action at the receptor level, the degree of gonadal suppression can be controlled by the dose of GnRHa (Albano et al., 1997; Fujimoto et al., 1997). For a number of indications, long-acting preparations are necessary, since the GnRHa suffer from the fact that they should be present at all times in the bloodstream to prevent the action of endogenous GnRH effectively.

Ovarian actions of GnRH and GnRH analogues

The expression of GnRH and its receptors has been described in a number of benign and malignant extrapituitary tissues. In female reproductive organs, the actions of GnRH have been shown to be predominantly inhibitory. These include inhibition of ovarian steroidogenesis, ovulation, ovum transport, ovum implantation, pregnancy and uterine growth (Hsueh and Jones, 1981). Some of these effects are due to direct actions of GnRH in the respective tissues. Our current knowledge on the ovarian actions of GnRH stems mainly from experiments in the rat. In this species, GnRH has inhibitory effects on granulosa and luteal cells. Here it inhibits gonadotrophin-induced steroidogenesis (Hsueh and Jones, 1981). Since hypothalamic GnRH does not reach the ovary in concentrations that could be responsible for these effects, it has been claimed that a paracrine mechanism may be the basis for a physiological role of GnRH in the ovary. The inhibitory actions of GnRH on steroidogenesis involve suppression of agonist-induced cAMP production as well as reduced activity of 3β-hydroxysteroid dehydrogenase (3β-HSD), which metabolizes the precursor pregnenolone to progesterone. Furthermore, FSH-induced increase of 20α-hydroxysteroid
dehydrogenase activity (20α-HSD), which leads to conversion of progesterone to its inactive metabolite 20α-hydroxyprogren-4-en-3-one, is enhanced by GnRH (Hsueh and Jones, 1981). The actions of GnRH in the rat ovary are mediated via high affinity binding sites which have been demonstrated in granulosa and luteal cells (Clayton et al., 1979; Jones et al., 1980; Pieper et al., 1981). In non-human primates, GnRH inhibited steroidogenesis, an effect that was not due to interaction with high affinity binding sites (Wickings et al., 1990).

In contrast to the considerable body of information on the functions of GnRH in the rat ovary, relatively little is known about the role of the decapeptide in the human ovary. Data on the presence of GnRH binding sites in the human ovary are controversial. High affinity binding has been demonstrated in granulosa cells of the dominant follicle by in-situ autoradiography (Latouche et al., 1989). Low affinity binding sites have been detected in the corpus luteum (Bramley et al., 1985). In contrast, Clayton and Huhtaniemi (1982) were not able to show GnRH binding in human corpora lutea. It has been suggested that degradation of GnRH tracers prevented the measurement of specific GnRH binding sites in some cases (Bramley and Menzies, 1996).

Recent studies analysed GnRH binding sites in preovulatory follicles and granulosa lutein cells obtained at oocyte retrieval for in-vitro fertilization in spontaneous unstimulated cycles. It has been demonstrated that specific GnRH agonistic and antagonistic binding is present in human luteinized granulosa cells. The $K_a$ values indicate high affinity binding sites, comparable with the binding that was measured in rat pituitary, ovary and granulosa cells. In contrast, no binding was observed in homogenates of preovulatory follicles (Brus et al., 1997).

Recently, the human GnRH-R has been cloned and characterized (Kakar et al., 1992). Since then, several studies have investigated the expression of GnRH-R mRNA. Receptor transcripts were demonstrable by Northern blots of rat ovary RNA (Whitelaw et al., 1995). In contrast, Northern blots did not detect receptor mRNA in the human ovary. However, GnRH-R mRNA was detected in the human ovary by reverse transcriptase–polymerase chain amplification (Kakar et al., 1992; Peng et al., 1994). GnRH-R mRNA is expressed in granulosa lutein cells and the human ovary across different functional stages, implying that multiple ovarian compartments may express GnRH-R (Minaretzis et al., 1995a). The level of GnRH-R mRNA shows a wide range of individual differences and is ~200-fold lower in the ovary than in the pituitary. Furthermore, the expression of GnRH-R mRNA as assessed by in-situ hybridization has been shown to be very low in the human corpus luteum (Fraser et al., 1996). Since the concentration of hypothalamic GnRH in the systemic circulation is considered to be too low to interact with the ovarian receptors, it has been proposed that GnRH or GnRH-like peptides produced in the ovary activate GnRH-R (Aten et al., 1986).

GnRH analogues are being used increasingly in reproductive medicine. Their systemic application leads to plasma concentrations of the peptide analogues that might be able to activate ovarian GnRH-R. Therefore, it is of interest whether the introduction of agonistic and antagonistic analogues of GnRH has adverse or beneficial effects on ovarian functions. However, information on these
potential GnRH analogue actions is sparse. GnRHag exerted discrepant effects on steroidogenesis in granulosa lutein cells in vitro (Casper et al., 1982, 1984; Tureck et al., 1982; Parinaud et al., 1988; Bussenot et al., 1993; Furger et al., 1996). The GnRH antagonist Cetrorelix was only recently tested for its potential actions on steroidogenesis (Minaretzis et al., 1995b). It was shown that GnRHant is able to inhibit aromatase activity. We have performed a number of in-vivo and in-vitro treatment paradigms to investigate this possibility. Cultured granulosa lutein cells from patients who had been treated with Cetrorelix or Ganirelix during ovarian stimulation showed basal and human chorionic gonadotrophin-stimulated oestradiol and progesterone production that was not different from that of cells that were obtained from patients who received a GnRHag (Ortmann et al., 1998, 1999). Furthermore, we exposed granulosa lutein cells to 1 nmol/l Cetrorelix or Ganirelix in vitro and did not detect significant effects on steroid production. Others (Minaretzis et al., 1995b) have reported similar results in a smaller group of patients.

**Summary and conclusions**

High affinity GnRH-R on pituitary gonadotrophs can be activated by native GnRH or GnRHag. Both of them regulate the number of GnRH-R, which may be associated with the secretory response of these cells. Activation of GnRH-R initiates a series of steps that lead to a cascade of intracellular responses. The main intracellular signal that leads to exocytosis of gonadotrophins is the increase of [Ca\(^{2+}\)]. Other signal transduction molecules exert positive and negative controls that are important for the amplification, maintenance and termination of cellular activation pathways. Their exact role in various cellular responses is still unclear.

The ability of GnRHag to suppress gonadotrophin secretion is caused by long-term desensitization of gonadotrophs, which involves receptor loss and uncoupling from signal transduction mechanisms. In contrast, GnRHant act mainly through competition with native GnRH for the specific membrane receptor. Thus their application does not lead to an initial release of gonadotrophins.

The human ovary has been shown to express GnRH receptors, although this is still a matter of debate. Also, GnRHag induced inhibitory actions on steroidogenesis in some but not all reports. The sparse information on the effects of GnRHant on steroidogenesis of human granulosa lutein cells did not reveal any significant effect, and presumably their application does not directly influence this ovarian function during in-vivo application of the compound.

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