J. steroid Biochem. Vol. 23, No. 5B, pp. 757–764, 1985
Printed in Great Britain.

0022-4731/85 $3.00 + 0.00

GONADOTROPIN-RELEASING HORMONE AS A PARACRINE HORMONE AND NEUROTRANSMITTER IN EXTRA-PITUITARY SITES

AARON J. W. HSUEH and JAMES M. SCHAEFFER
Department of Reproductive Medicine, M-025, University of California, San Diego, La Jolla, CA 92093, U.S.A.

Summary—Gonadotropin-releasing hormone (GnRH), in addition to its classical releasing action at the pituitary level, acts on multiple extrapituitary sites to regulate various reproductive functions. In the rat ovary, specific high affinity GnRH receptors have been identified in granulosa and theca cells. These binding sites mediate the inhibitory effects of GnRH and its agonists on gonadotropin-stimulated estrogen, progestin and androgen biosynthesis. At the granulose cell level, GnRH treatment decreases aromatase activity as well as the biosynthesis of pregnenolone and progesterone via inhibition of cholesterol side-chain cleavage and 3β-hydroxysteroid dehydrogenase enzymes. High concentrations of GnRH also stimulate low but significant levels of various steroids. In addition, treatment with high concentrations of GnRH induces ovulation and oocyte maturation in hypophysectomized rats. This is associated with the ability of GnRH to stimulate plasminogen activator activity in cultured granulosa cells. In the rat testis, GnRH receptors have been identified in Leydig but not Sertoli cells. Treatment with GnRH inhibits gonadotropin-stimulated androgen biosynthesis by the cultured Leydig cells. The inhibitory effect of GnRH on testicular androgen production occurs at sites distal to the formation of cyclic AMP and pregnenolone and may be due to decreases in the activity of the enzyme 17α-hydroxylase and 17–20 desmolase. Since hypothalamic GnRH is unlikely to act at the gonadal level, several laboratories have attempted to isolate gonadal GnRH-like peptide which may serve as the ligand for specific gonadal GnRH receptors. Although the presence of ovarian GnRH-like substance still remains elusive, testicular GnRH-like substance has been identified. This gonadal peptide(s) may be an important local paracrine hormone. In addition to its action at the gonadal level, GnRH or GnRH-like peptides may play an important role as a neurotransmitter in the central nervous system. Exogenous administration of GnRH in selected brain areas has been shown to modulate sexual behavior in experimental animals, while neural pathways containing GnRH-like immunoreactive substances have been identified in several brain areas. We have recently synthesized a bioluminescent GnRH analog capable of serving as a specific GnRH ligand for a bioluminescent ligand receptor assay which is more sensitive than classical ^{125}I-ligand assays. We have identified GnRH receptors in small, discrete brain regions. Thus, GnRH and GnRH-like peptides may play important paracrine and neurotransmitter roles in the regulation of various reproductive functions in extra-pituitary sites.

INTRODUCTION

Following the purification and sequencing of GnRH and the synthesis of potent GnRH agonists, many laboratories investigated the effects of these peptides on various reproductive functions. Administration of GnRH or its potent agonists results in sustained increases in serum gonadotropins. Since pituitary gonadotropins are essential for normal gonadal functions and since hypothalamic GnRH was believed to act solely on the pituitary gland, treatment with high doses of GnRH or its agonists had been predicted to be a potential means for enhancing fertility.

In contrast, paradoxical inhibitory actions of GnRH and its agonists have been documented in animal models (Table 1; 1). In female rats, long-term administration of pharmacological doses of GnRH or GnRH agonists inhibit ovarian steroidogenesis, ovulation, ovum transport, ovum implantation, pregnancy, uterine growth, and ovarian-dependent mammary tumorigenesis. Similarly, in male rats, long-term administration of high doses of GnRH or GnRH agonists inhibit testicular steroidogenesis, spermatogenesis and male accessory sex organ growth.

Table 1. Inhibition of reproductive functions by chronic treatment with GnRH or its agonists

Females	Implantation delay and pregnancy termination
	Decreases in ovarian steroidogenesis and gonadotropin receptor content
	Inhibition of follicular maturation and ovulation
	Decreases in uterine and oviductal growth
	Delay of puberty
	Delay of parturition
	Inhibition of ovarian-dependent mammary tumorigenesis
Males	Decreases in testicular androgen production
	Decreases in testicular gonadotropin receptor content
	Decreases in testis weight
	Inhibition of spermatogenesis
	Decreases in ventral prostate and seminal vesicle growth

At least three possible mechanisms can be proposed to explain the paradoxical, inhibitory actions of GnRH and its agonists: (1) chronic stimulation of the anterior pituitary by high doses of GnRH or its agonists may desensitize the gonadotrophs to hypothalamic GnRH, resulting in decreased circulating gonadotropins and subsequent atrophy of reproductive organs; (2) treatment with pharmacological doses of GnRH or its agonists may stimulate the release of high levels of LH which, in turn, results in LH-induced desensitization of gonadal cells to subsequent LH action, and (3) GnRH and its agonists may exert an extrapituitary, direct inhibitory action upon gonadal cells.

We have used primary cultures of ovarian granulosa and testicular Leydig cells to provide conclusive evidence on the direct inhibitory effect of GnRH and its agonists on gonadal functions *in vitro* [2–5].

DIRECT MODULATION OF GONADAL FUNCTIONS BY GnRH

Granulosa cells

Ovarian granulosa cells, obtained from preantral follicles of immature hypophysectomized rats, respond *in vitro* to FSH with the production of estrogens and progestins. Concomitant treatment with GnRH results in the inhibition of FSH-stimulated estrogen and progestin production with ED_{50} values of approximately 10^{-10} M. Also, the inhibition of FSH-stimulated steroidogenesis by GnRH can be blocked by concomitant treatment with a GnRH antagonist [3, 6]. The counteracting effect of GnRH antagonists at the ovarian level suggests that the actions of GnRH and its analogs are mediated through stereospecific recognition sites in the granulosa cells. Comparison between the pituitary and ovarian potencies of ten GnRH agonists and seven GnRH antagonists indicates an overall agreement of responsiveness between ovarian granulosa cells and pituitary gonadotrophs [7], suggesting the binding specificity of GnRH receptors in these tissues are similar.

FSH also stimulates LH and prolactin (PRL) receptor formation by cultured granulosa cells. Concomitant treatment with GnRH or its agonists inhibits the FSH stimulation of LH and PRL receptor formation [3, 8, 9].

In addition to the inhibitory effects of GnRH on FSH-stimulated steroidogenesis and receptor formation, GnRH inhibits FSH-stimulated cAMP formation [10, 11, 12]. In contrast, GnRH enhances FSH-stimulated prostaglandin production [10], as well as the FSH-stimulated increases in granulosa cell protein content. GnRH has a biphasic effect on FSH-stimulated glycolysis, first enhancing and then inhibiting FSH action [13].

In addition to the induction of functional LH and PRL receptors, FSH treatment of cultured granulosa cells *in vitro* also increases the responsiveness of granulosa cells to β_2-adrenergic agents. Treatment of these granulosa-luteal cells with LH increases estrogen and progestin production [14]. In contrast, treatment with PRL or β_2-adrenergic agents stimulates progesterone production without affecting aromatase activity [14, 15]. Concomitant treatment with GnRH inhibits LH stimulation of estrogen production [6]. GnRH treatment also inhibits progesterone production stimulated by LH, PRL or β_2-adrenergic agents [6, 16]. Furthermore, PRL treatment increases LH receptor content, while GnRH treatment inhibits PRL action [6].

The *in vitro* studies were extended to *in vivo* experiments using immature, hypophysectomized rats. GnRH or a GnRH agonist inhibits the FSH-stimulated increases in ovarian weight, ovarian LH receptor content, and granulosa cell aromatase activ-

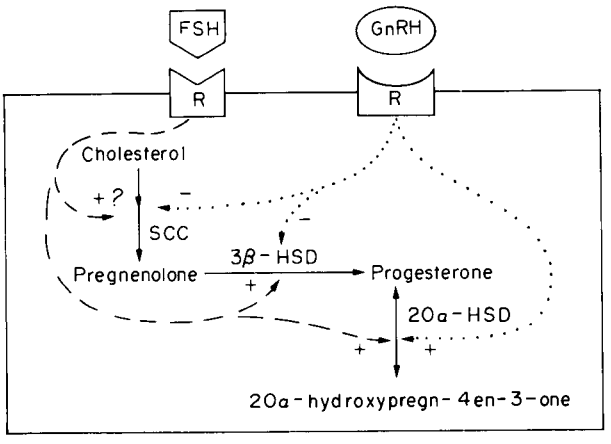

Figure 1. Mechanism of GnRH action in the granulosa cells: modulation of steroidogenic enzymes by FSH and GnRH. SCC: side-chain cleavage enzymes; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 20α-HSD, 20α-hydroxysteroid dehydrogenase; 20α-hydroxypregn-4-en-3-one, 20α-dihydroprogesterone.

ity [1, 8]. The inhibitory effects are blocked by *in vivo* treatment with a GnRH antagonists [6].

The rat granulosa cell culture system was used to examine the mechanism by which GnRH inhibits the FSH stimulation of progesterone production (Fig. 1). Both FSH and GnRH act on their respective receptors in the granulosa cells. FSH treatment *in vitro* markedly stimulates the activity of 3β-hydroxysteroid dehydrogenase (3β-HSD), which converts the precursor pregnenolone to progesterone. FSH treatment also increases the activity of 20α-hydroxysteroid dehydrogenase (20α-HSD), which metabolizes progesterone to its inactive metabolite, 20α-hydroxypregn-4-en-3-one (20α-OH-P). Concomitant treatment with GnRH partially inhibits the stimulatory effect of FSH on 3β-HSD activity but further enhances the FSH stimulation of 20α-HSD activity, thereby decreasing the conversion of pregnenolone to progesterone and increasing the breakdown of progesterone [17, 18]. Furthermore, measurement of pregnenolone biosynthesis in granulosa cells treated with cyanoketone (an inhibitor of 3β-HSD activity) shows that FSH stimulates pregnenolone production and GnRH inhibits FSH action, suggesting that GnRH may affect additional steroidogenic enzymes (such as cholesterol side-chain cleavage enzymes) before pregnenolone production [19]. The major effects of GnRH are probably the decreased pregnenolone production coupled with increased conversion of progesterone to 20α-OH-progesterone.

Leydig cells

Treatment with hCG substantially stimulates androgen production in cultured testicular cells obtained from hypophysectomized immature or adult rats. Concomitant treatment with GnRH or a GnRH agonist results in a dose-dependent inhibition of androgen production [4, 20]. Furthermore, the inhibitory effect of GnRH is blocked by concomitant treatment with a potent GnRH antagonist [5], suggesting that the actions of these peptides are mediated through stereospecific testis recognition sites.

An extrapituitary inhibitory effect of GnRH on testicular functions has also been observed in hypophysectomized rats *in vivo*. Treatment of immature hypophysectomized male rats with high doses of GnRH or a GnRH agonist for 5 days decreases the FSH maintenance of testis weight and Leydig cell androgen biosynthesis [21]. Moreover, treatment with FSH or PRL and growth hormone in hypophysectomized adult rats maintain testis weight, LH receptor content, and steroidogenic responsiveness, whereas concomitant treatment with GnRH or an agonist inhibits the action of the pituitary hormones [22].

Primary cultures of adult testis cells were used to examine the mechanism of the GnRH inhibition of hCG-stimulated testosterone production (Fig. 2). Both LH/hCG and GnRH act on their respective receptors in the plasma membrane. The inhibitory effect of GnRH on Leydig cell androgen production appears to be the result of decreases in the activities of 17α-hydroxylase and 17–20 desmolase [5]. In cells treated with cyanoketone and spironolactone to prevent pregnenolone metabolism, hCG stimulates pregnenolone biosynthesis while concomitant GnRH treatment did not affect hCG action. Likewise, GnRH-induced decreases of androgen production are not accompanied by decreases of hCG-stimulated cAMP production [5]. Furthermore, GnRH inhibits testosterone production stimulated by a cAMP analog, suggesting that GnRH may act at steps distal to cAMP biosynthesis [5, 22].

PRESENCE OF GnRH RECEPTORS AND GnRH-LIKE PEPTIDES IN THE GONADS

Using radiolabelled GnRH agonists, specific high affinity ($K_d = 10^{-10}$ M) binding sites have been

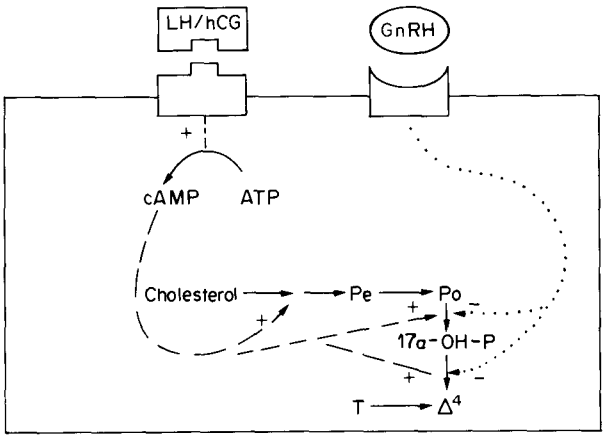

Figure 2. Regulation of testicular Leydig cell androgen biosynthesis by LH/hCG and GnRH. Pe, pregnenolone; Po, progesterone; 17α-OH-P, 17α-hydroxyprogesterone; Δ^4, androstenedione; T, testosterone.

demonstrated in granulosa [24, 25] and luteal [25–28] cells. GnRH receptors are widely distributed in luteal, thecal, and granulosa cells at all stages of cellular differentiation [29]. Furthermore, GnRH binding sites have been characterized in granulosa cells using photoaffinity and fluorescence labeling methods [30]. Likewise, the inhibitory effects of GnRH in the testis are believed to be mediated by the specific, high affinity ($K_d = 10^{-10}$ M) GnRH binding sites which have been demonstrated in Leydig, but not Sertoli, cells [31, 32, 33].

GnRH is different from most classical hormones in that the circulation of this neuropeptide is restricted to the brain portal vessel. While hypothalamic GnRH in the brain portal vessel interacts with pituitary GnRH receptors [34], the peripheral concentration of GnRH is lower than 10^{-11} M [35]. Thus, the hypothalamic neuropeptide is not present in the systemic circulation in sufficient concentration to interact with extrapituitary binding sites. While the extrapituitary actions of GnRH on gonadal functions may represent an evolutionary vestigial phenomenon, recent evidence indicates that GnRH-like peptides are secreted by extra-hypothalamic tissues.

GnRH-like peptides have been found in rat testicular tissue [36–40]. These peptides may play an important paracrine role in the regulation of gonadal functions.

EXTRAHYPOTHALAMIC GnRH IN THE BRAIN AND THE FACILITATION OF SEXUAL BEHAVIOR BY GnRH

In addition to the dense innervation of GnRH-containing neurons to the primary portal plexus and the organum vasculosum of the lamina terminalis (OVLT), GnRH fibers are widely distributed throughout the rat central nervous system [41, 42]. The GnRH-containing cells (of which there are 2000 or less) are diffusely distributed over a region extending from the septal nuclei and diagonal band of Broca through the preoptic area, into the anterior hypothalamus [41, 42]. Whether there are GnRH cells in the rat medial basal hypothalamus has been debated, though King *et al.*[43] have visualized few such cells in the region between the arcuate and ventromedial nuclei. Furthermore, workers in several laboratories have reported the existence of GnRH-containing neurons in cortical region of the rat including the hippocampus, olfactory bulb, perpiriform cortex, cingulate cortex, and amygdala [41, 42, 44, 45]. GnRH neurons in the medial preoptic (MPO) area seem to project to the median eminence, suprachiasmatic and septal nuclei, amygdala and organum vasculosum lamina terminalis. In the guinea pig, cells in the medial septum seem to innervate the central mesencephalon; axons of these cells enter the stria medullaris, go to the medial habenular nucleus and the interpeduncular nucleus via the fasciculus retroflexus.

Although the function of extra-hypothalamic GnRH is unclear, several lines of evidence suggest a neuromodulatory role for GnRH. Using iontophoretic administration of GnRH, responsive cells were found widely distributed throughout the hypothalamus, preoptic area and septum as well as in the cerebral cortex and midbrain central gray [46, 47]. In addition, administration of GnRH to hypophysectomized, castrated, and estrogen-treated female rats increases lordosis [48–51], indicating GnRH has behavioral effects not mediated by the pituitary. Furthermore, GnRH increases copulatory behavior in castrated male rats [52]. Neurotropic effects of GnRH were detected when this peptide was microiontophoresed into the central gray area [53, 54]. Infusion of GnRH into the central gray has an immediate facilitative effect on the lordosis reflex in castrated female rats primed with estrogen [55], while passive immunization against endogenous GnRH by anti-GnRH gamma globulin diminishes the reflex.

GnRH RECEPTORS IN DISCRETE BRAIN REGIONS

The presence of immunoreactive GnRH in brain neurons and the findings that some neurons are responsive to GnRH suggests that GnRH or GnRH-like peptide may serve as a neurotransmitter or neuromodulator in the central nervous system. However, specific GnRH receptors have not been identified in the brain, possibly due to receptors being localized within discrete, small brain regions which cannot be detected with the conventional ^{125}I-ligand binding assay. Recently, we reported the synthesis of luciferin derivatized α-bungarotoxin which was used as a probe to study specific α-bungarotoxin binding sites [56]. Because of the extreme sensitivity of the luciferin–luciferase reaction, the bioluminescent ligand is a more sensitive probe than ^{125}I-α-bungarotoxin for the study of nicotinic cholinergic receptors.

It has been demonstrated that substitution of the 6th position of GnRH with modified D-amino acids containing bulky groups such as biotin and chlorambucil does not significantly impair its bioactivity (5 or 7). We, therefore, used the agonist [des-Gly^{10},D-Glu^{6},Pro^{9}-NHEt]GnRH (GnRH-A) for conjugation to luciferin [57]. The bioactivity of GnRH-A luciferin was assessed by measuring the release of LH from rat anterior pituitary cells maintained in culture. As shown in Figure 3, the dose-response curves of LH release in response to increasing concentrations of GnRH-A and GnRH-A luciferin are parallel. In three experiments, GnRH-A is 5.4 ± 1.7-fold more potent than GnRH, and GnRH-A luciferin is 2.2 ± 0.8-fold more potent than GnRH. These results demonstrate that the conjugation of luciferin to GnRH-A results in a 2.5-fold loss of LH releasing activity. The potency of the conjugate is, however, still higher than that of GnRH.

Specific GnRH-A-luciferin binding was used to quantitate GnRH receptors in pituitary and several

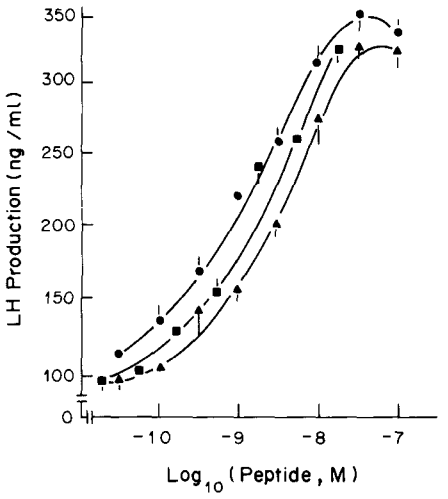

Figure 3. Effect of GnRH agonists on LH release by cultured rat anterior pituitary cells. Anterior pituitary cells were prepared and cultured for 2 days. After washing with 2 ml portions of culture media, cells were treated for 5 h in the presence or absence of various concentrations of GnRH (▲), the GnRH agonist (●), or GnRH A-luciferin (■). Data are expressed as mean ± SE. Three or four culture dishes were used for each datum point with duplicate determinations for each culture. Similar data were obtained in three separate experiments.

extra-pituitary tissues and various regions of the rat brain [57]. The highest concentration of GnRH binding sites are in the anterior pituitary. This is followed by binding in the interpeduncular nucleus, periaqueductal gray, habenula, ovary, and testes. Considerably lower, but significant concentrations of GnRH binding sites were found in the hypothalamus. No binding was detected in the olfactory bulb, cerebral cortex or cerebellar cortex.

High concentrations of GnRH receptors in the interpeduncular nucleus and habenula is consistent with studies demonstrating GnRH-containing axons traveling from the medial septum via the fasiculus retroflexus to the medial habenular nucleus and interpeduncular nucleus [41, 58]. GnRH receptors in the periaqueductal gray correlates with reports showing that GnRH infusion into this area enhances lordosis in castrated female rats primed with estrogen [55]. Thus, GnRH receptors detected in various brain regions may be important in mediating the actions of GnRH in the facilitation of sexual behavior.

EXTRA-PITUITARY ACTIONS OF GnRH: MULTIPLE TARGET ORGANS AND SPECIES VARIATION

In addition to their actions on the gonads and central nervous system, GnRH and its agonists also directly affect the functions of many other organs through extrapituitary actions. Findings of the direct extrapituitary actions of GnRH and its receptors as well as the demonstration of endogenous ligands outside the central nervous system are summarized in Table 2. GnRH treatment affects the functions of various gonadal cells as well as modulates the functions of both female and male accessory sex organs [59–63], the placenta [63], fat cells and frog sympathetic ganglia. An antiandrogenic action of GnRH has also been demonstrated in the mouse kidney [64]. In addition, GnRH-like substances have been identified in the rat testis [40], human placenta [65, 66] and frog sympathetic ganglia [67, 68].

Although the rat model has been used extensively to examine the direct effects of GnRH on gonadal functions, the direct gonadal action of GnRH is not limited to this species (Table 2). *In vitro* treatment with GnRH or its agonists modulates steroidogenesis in ovarian cells from women [69, 70, 71], rabbit [71], pig [11] and chicken [72]. In addition low, but not high, affinity GnRH receptors have been identified in human corpus luteum [73, 74]. Furthermore, GnRH treatment has been shown to stimulate ovulation in perfused rabbit ovaries [71]. In contrast, no GnRH binding sites have been identified in ovine, bovine, procine [75] and monkey [76] ovaries. While there is only limited data on the species specificity of direct GnRH action on testicular steroidogenesis, GnRH treatment *in vitro* modulates steroidogenesis in frog testes [77]. In contrast, the mouse appears to be

Table 2. Species variation of the direct gonadal action of GnRH

Animals	Cells and functions	Reference
Frog	Increase testis androgen production	Segal and Adjuwon, 1979 [77]
Fowl	Enhance progesterone production in granulosa cells	Hertelendy *et al.*, 1982 [72]
Rat	Increase and decrease steroidogenesis by granulosa, theca, luteal and Leydig cells	Multiple reports
Mouse	No effect on Leydig cells	Bex and Corbin, 1981 [78] Hunter *et al.*, 1982 [79]
Rabbit	Increase progesterone and ovulation in perifused ovary	Koos *et al.*, 1982 [71]
Porcine	Inhibit granulosa cell cAMP and progesterone production	Massicotte *et al.*, 1980 [11]
Bovine	No GnRH binding in the ovary	Brown and Reeves, 1983 [75]
Ovine	No GnRH binding in the ovary	Brown and Reeves, 1983 [75]
Monkey	No GnRH binding to corpora lutea and testis	Asch *et al.*, 1981 [76]; Clayton and Huhtaniemi, 1982 [74]
Human	Decrease gonadotropin-stimulated progesterone production in granulosa cells; blocked by antagonist	Tureck *et al.*, 1982 [70]
	No effect on progesterone production by granulosa cells	Casper *et al.*, 1982
	Decrease androgen production by ovarian tumor cells	Lamberts *et al.*, 1980 [69]
	No binding to corpus luteum and testis	Clayton and Huhtaniemi, 1982 [74]
	Low affinity binding to corpus luteum	Popkin *et al.*, 1983 [73]

resistant to the direct modulatory effects of GnRH in both females and males [78–80].

EVOLUTION OF GnRH AND RELATED PEPTIDES: THEIR ROLE AS PARACRINE HORMONES AND NEUROTRANSMITTERS

Based on the above discussion, it is apparent that GnRH and related peptides have diverse actions in various target tissues. In addition to the anterior pituitary, GnRH and related peptides may play important paracrine and neurotransmitter roles in the gonads, central nervous system, sympathetic ganglia and placenta.

Studies on the structural identity of various GnRH-like peptides have revealed the presence of at least four different peptides. The mammalian hypothalamic GnRH has also been found in the amphibian brain and retina. In contrast, Glu^8-GnRH and His^5,Trp^7,Tyr^8-GnRH are present in the avian brain, whereas Trp^7,Leu^8-GnRH has been isolated from teleost brain and may be present in amphibian brain as well as amphibian sympathetic ganglia [81–84]. It is not surprising to find reports suggesting that the mammalian gonadal GnRH-like peptide may be structurally different from the mammalian hypothalamic GnRH [40]. Furthermore, the chicken and salmon hypothalamic GnRH were less effective than mammalian hypothalamic GnRH in LH release by rat pituitary cells. Although the stereospecificity of the rat gonadal GnRH receptor is apparently similar to that of the rat pituitary receptor [7], future studies may reveal interesting evolutional differences of the stereospecificity of GnRH receptors in various target tissues (such as the pituitary and the central nervous system) or in various species (e.g. human gonadal GnRH receptor may be different from pituitary receptors).

Acknowledgements—This work was supported by National Institutes of Health Research Grant HD-14084 and Program Project Grant HD-12303.

REFERENCES

1. Hsueh A. J. W. and Jones P. B. C.: Extrapituitary actions of gonadotropin-releasing hormone. *Endocr. Rev.* **2** (1981) 437–461.
2. Hsueh A. J. W. and Erickson G. F.: Extrapituitary action of gonadotropin-releasing hormone: Direct inhibition of ovarian steroidogenesis. *Science* **204** (1979) 854–855.
3. Hsueh A. J. W. and Ling N. C.: Effect of an antagonistic analog of gonadotropin-releasing hormone upon ovarian granulosa cell function. *Life Sci.* **25** (1979) 1223–1230.
4. Hsueh A. J. W., Schreiber J. R. and Erickson G. F.: Inhibitory effect of gonadotropin-releasing hormone upon cultured testicular cells. *Molec. Cell. Endocr.* **21** (1981) 43–49.
5. Hsueh A. J. W., Bambino T. H., Zhuang L. Z., Welsh Jr. T. H. and Ling N. C.: Mechanism of the direct action of gonadotropin-releasing hormone and its antagonist on androgen biosynthesis by cultured rat testicular cells. *Endocrinology* **112** (1983) 1653–1661.
6. Jones P. B. C. and Hsueh A. J. W.: Direct effects of gonadotropin-releasing hormone and its antagonist upon ovarian functions stimulated by FSH, prolactin and LH. *Biol. Reprod.* **24** (1981b) 747–759.
7. Hsueh A. J. W., Adashi E. Y., Tucker E., Valk C. and Ling N. C.: Relative potencies of GnRH agonists and antagonists on ovarian and pituitary functions. *Endocrinology* **112** (1983) 689–695.
8. Hsueh A. J. W., Wang C. and Erickson G. F.: Direct inhibitory effect of gonadotropin-releasing hormone upon follicle-stimulating hormone induction of luteinizing hormone receptors and aromatase activity in rat granulosa cells. *Endocrinology* **106** (1980) 1697–1705.
9. Navickis R. J., Jones P. B. C. and Hsueh A. J. W.: Modulation of prolactin receptors in cultured rat granulosa cells by FSH, LH and GnRH. *Molec. Cell. Endocr.* **27** (1982) 77–88.
10. Clark M. R., Thibier C., Marsh J. M. and LeMaire W. J.: Stimulation of prostaglandin accumulation by luteinizing hormone-releasing hormone (LHRH) and LHRH analogs in rat granulosa cells *in vitro*. *Endocrinology* **107** (1980) 17–23.
11. Massicotte J., Veilleux R., Lavoie M. and Labrie F.: An LHRH agonist inhibits FSH-induced cyclic AMP accumulation and steroidogenesis in porcine granulosa cells in culture. *Biochem. biophys. Res. Commun.* **94** (1980) 1362–1366.
12. Knecht M., Katz M. S. and Catt K. J.: Gonadotropin-releasing hormone inhibits cycling nucleotide accumulation in cultured rat granulosa cells. *J. biol. Chem.* **256** (1981) 34–36.
13. Billig H., Magnusson C., Ekholm C. and Hillensjö T.: Biphasic effect of a GnRH agonist on glycolysis in cultured rat granulosa cells. *Biol. Reprod.* **26** Suppl. (1982) 152A.
14. Wang C., Hsueh A. J. W. and Erickson G. F.: Induction of functional prolactin receptors by follicle-stimulating hormone in rat granulosa cells *in vivo* and *in vitro*. *J. biol. Chem.* **254** (1979) 11330–11336.
15. Adashi E. Y. and Hsueh A. J. W.: Stimulation of β_2-adrenergic responsiveness by follicle-stimulating hormone in rat granulosa cells *in vitro* and *in vivo*. *Endocrinology* **108** (1981) 2170–2178.
16. Jones P. B. C., Valk C. and Hsueh A. J. W.: Mechanism of the regulation of progesterone biosynthesis by prolactin (PRL) and β_2-adrenergic agents in cultured rat granulosa cells: antagonistic effect of gonadotropin-releasing hormone (GnRH). *Biol. Reprod.* **29** (1983) 572–585.
17. Jones P. B. C. and Hsueh A. J. W.: Direct stimulation of ovarian progesterone metabolizing enzyme by gonadotropin-releasing hormone in cultured granulosa cells. *J. biol. Chem.* **256** (1981) 1248–1254.
18. Jones P. B. C. and Hsueh, A. J. W.: Regulation of ovarian 3β-hydroxysteroid dehydrogenase by GnRH and follicle-stimulating hormone in cultured rat granulosa cells. *Endocrinology* **110** (1982) 1663–1671.
19. Jones P. B. C. and Hsueh A. J. W.: Pregnenolone biosynthesis by cultured rat granulosa cells: modulation by FSH and gonadotropin-releasing hormone. *Endocrinology* **111** (1982) 713–721.
20. Hsueh A. J. W.: Direct effects of gonadotropin-releasing hormone on testicular Leydig cell functions. *Ann. N.Y. Acad. Sci. U.S.A.* **383** (1982) 249–271.
21. Hsueh A. J. W. and Erickson G. F.: Extra-pituitary inhibition of testicular function by luteinising hormone releasing hormone. *Nature* **281** (1979) 66–67.
22. Bambino T. H., Schreiber J. R. and Hsueh A. J. W.: Gonadotropin-releasing hormone and its agonist inhibit testicular luteinizing hormone receptor and steroidogenesis in immature and adult hypophysectomized rats. *Endocrinology* **107** (1980) 908–917.

23. Hsueh, A. J. W.: Direct effects of gonadotropin releasing hormone on testicular Leydig cell functions. In *The Cell Biology of the Testis. Ann. N.Y. Acad. Sci. U.S.A.* **383** (1982) 249–271.
24. Jones P. B. C., Conn P. M., Marian J. and Hsueh A. J. W.: Binding of gonadotropin-releasing hormone agonist to rat ovarian granulosa cells. *Life Sci.* **27** (1980) 2125–2132.
25. Pieper D. R., Richards J. S. and Marshall J. C.: Ovarian gonadotropin-releasing hormone (GnRH) receptors: characterization, distribution and induction by GnRH. *Endocrinology* **108** (1981) 1148–1155.
26. Clayton R. N., Harwood J. P. and Catt K. J.: Gonadotropin-releasing hormone analogue binds to luteal cells and inhibits progesterone production. *Nature* **282** (1979) 90–92.
27. Harwood J. P., Clayton R. N. and Catt K. J.: Ovarian gonadotropin-releasing hormone receptors. I. Properties and inhibition of luteal cell function. *Endocrinology* **107** (1980) 407–413.
28. Harwood J. P., Clayton R. N., Chen T. T., Know G. and Catt K. J.: Ovarian gonadotropin-releasing hormone receptors. II. Regulation and effects on ovarian development. *Endocrinology* **107** (1980) 414–421.
29. Pelletier G., Seguin C., Dubé C. and St Arnaud R.: Distribution of LHRH receptors in the rat ovary. *Biol. Reprod.* **26** Suppl. (1982) 151A.
30. Hazum E. and Nimrod A.: Photoaffinity labeling and fluorescence distribution studies of gonadotropin-releasing hormone receptors in ovarian granulosa cells. *Proc. natn. Acad. Sci. U.S.A.* **79** (1982) 1747–1750.
31. Clayton R. N., Katikeneni M., Chan V., Dufau M. L. and Catt K. J.: Direct inhibition of testicular function by gonadotropin-releasing hormone: mediation by specific gonadotropin-releasing hormone receptors in interstitial cells. *Proc. natn. Acad. Sci. U.S.A.* **77** (1980) 4459–4463.
32. Bourne G. A., Regiani S., Payne A. H. and Marshall J. C.: Testicular GnRH receptors—characterization and localization on interstitial tissue. *J. clin. Endocr. Metab.* **51** (1980) 407–409.
33. Sharpe R. M. and Fraser H. M.: Leydig cell receptors for luteinizing hormone-releasing hormone and its agonists and their modulation by administration or deprivation of the releasing hormone. *Biochem. biophys. Res. Commun.* **95** (1980) 256–262.
34. Eskay R. L., Mical R. S. and Porter J. C.: Relationship between luteinizing hormone-releasing hormone concentration in hypophyseal portal blood and luteinizing hormone relase in intact, castrated, and electrochemically stimulated rats. *Endocrinology* **100** (1977) 263–270.
35. Nett T. M., Akbar A. M. and Niswender G. D.: Serum levels of luteinizing hormone and gonadotropin-releasing hormone in cycling, castrated and anesthetized ewes. *Endocrinology* **94** (1974) 713–718.
36. Sharpe R. M. and Fraser H. M.: HCG stimulation of testicular LHRH-like activity. *Nature* **287** (1980) 642–643.
37. Dutlow C. M. and Millar R. P.: Rat testis immunoreactive LH-RH differs structurally from hypothalamic LH-RH. *Biochem. biophys. Res. Commun.* **101** (1981) 486–494.
38. Paull W. K., Turkelson C. M., Thomas C. R. and Arimura A.: Immunohistochemical demonstration of a testicular substance related to luteinizing hormone-releasing hormone. *Science* **213** (1981) 1263–1264.
39. Nagendranath N., Joso T. M. and Juneja H. S.: Bioassayable gonadotropin-releasing hormone-like activity in the spent nutrient medium of rat Sertoli cells in primary cultures. *Horm. Metab. Res.* **15** (1983) 99–102.
40. Bhasin S., Heber D., Peterson M. and Swerdloff R.: Partial isolation and characterization of testicular GnRH like factors. *Endocrinology* **112** (1983) 1144–1146.
41. Whitkin J. W., Paden C. M. and Silverman R. J.: The LHRH systems in the rat brain. *Neuroendocrinology* **35** (1982) 429–438.
42. Krey L. C. and Silverman A. J.: Luteinizing hormone releasing hormone. In *Brain Peptides* (Edited by D. T. Krieger, M. J. Brownstein and J. B. Martin). John Wiley, New York (1983).
43. King J. C., Tobet S. A., Snavely F. L. and Arimura A.: The LHRH system in normal and neonatally androgenized female rats. *Peptides* **1** (1980) 85–100.
44. Barry J.: Immunohistochemistry of LH-RH producing neurons of vertebrates. *Int. Rev. Cytol* **60** (1979) 179–221.
45. Phillips H. S., Hostetter G., Kerdelhue B. and Kozlowski G. P.: Immunocytochemical localization of LHRH in central olfactory pathways of hamster. *Brain Res.* **193** (1980) 574–579.
46. Samson W. K., McCann S. M., Chud L., Dudley C. A. and Moss R. L.: Intra- and extra-hypothalamic LH-RH distribution in the rat with special reference to mesencephalic sites which contain both LHRH and single neurons responsive to LHRH. *Neuroendocrinology* **31** (1980) 66–72.
47. Renaud L. P., Martin J. B. and Brazeau P.: Hypothalamic releasing factors: physiological evidence for a regulatory action on central neurons and pathways for their distribution in brain. *Pharmac. Biochem. Behav.* **75** (1976) 171–178.
48. Pfaff D. W.: Luteinizing hormone-releasing factor (LRF) potentiates lordosis behavior in hypophysectomized ovariectomized female rats. *Science* **182** (1973) 1148–1149.
49. Moss R. L. and Foreman M. M.: Potentiation of lordosis behavior by intrahypothalamic infusion of synthetic luteinizing hormone-releasing hormone. *Neuroendocrinology* **20** (1976) 176–181.
50. Foreman M. M. and Moss R. L.: Effects of subcutaneous injection and intrahypothalamic infusion of releasing hormones upon lordotic response to repetitive coital stimulation. *Horm. Behav.* **8** (1977) 219–234.
51. Moss, R. L.: Role of hypophysiotropic neurohormones in mediating neural and behavioral events. *Fedn Proc.* **36** (1979) 1978–1983.
52. Moss R. L., Dudley C. A. and Foreman N. N.: Synthetic LRF; a potentiator of sexual behavior in the rat. In *Hypothalamic Hormones* (Edited by M. Motta, P. G. Crosignani and L. Martini). Academic Press, London (1975) p. 269.
53. Kawakami M. and Sakuma U.: Responses of hypothalamic neurons to the microiontophoresis of LHRH, LH and FSH under various levels of circulatory ovarian hormones. *Neuroendocrinology* **15** (1974) 290–307.
54. Renaud L. P., Martin J. B. and Brazeau P.: Depressant action of TRH, LHRH and somatostatin on activity of central neurons. *Nature* **255** (1975) 233–235.
55. Sakuma Y. and Pfaff D. W.: LHRH in the mesencephalic central grey can potentiate lordosis reflex of female rats. *Nature* **283** (1980) 566–567.
56. Schaeffer J. M. and Hsueh A. J. W.: α-Bungarotoxin-luciferin as a bioluminescent probe for characterization of acetylcholine receptors in the CNS. *J. biol. Chem.* **259** (1984) 2055–2058.
57. Schaeffer J. M. and Hsueh A. J. W.: Identification of GnRH binding sites in the central nervous system using a bioluminescent ligand receptor assay. *7th Int. Congr. Endocr.* (1985) Abstr 2780.
58. Conrad L. C. A. and Pfaff D. W.: Efferents from medial basal forebrain and hypothalamus in the rat. *J. Comp. Neurol.* **169** (1976) 185–220.
59. Jones R. C.: The effect of luteinizing releasing hormone (LRH) and two agonist analogues on uterine growth in

hypophysectomized, ovariectomized and intact rats. *Int. J. Fert.* **24** (1979) 188–192.
60. Liu Y. and Li S.: Studies on the mechanism of inhibitory action of a LHRH analogue on decidual formation. II. Experiments in pseudopregnant rats. *Acta Zool. Sin.* **26** (1980) 1–9.
61. Sundaram K., Cao Y.-Q., Wang N.-G., Bardin C. W., Rivier J. and Vale W.: Inhibition of the action of sex steroids by gonadotropin-releasing hormone (GnRH) agonists: a new biological effect. *Life Sci.* **28** (1981) 83–88.
62. Rao I. M. and Reddy P. R. K.: Direct inhibitory effect of gonadotropin releasing hormones in the uterus of rat. *Life Sci.* **34** (1984) 2257–2263.
63. Siler-Khodr T. M. and Khodr G. S.: Dose response analysis of GnRH stimulation of hCG release from human term placenta. *Biol. Reprod.* **25** (1981) 353–358.
64. Lecomte P., Wang N.-G., Sundaram K., Rivier J., Vale W. and Bardin C. W.: The antiandrogenic action of gonadotropin-releasing hormone and its agonists on the mouse kidney. *Endocrinology* **110** (1982) 1–6.
65. Khodr G. S. and Siler-Khodr T. M.: Placental luteinizing hormone-releasing factor and its synthesis. *Science* **207** (1980) 315–317.
66. Lee J. N., Seppälä M. and Chard T.: Characterization of placental luteinizing hormone-releasing factor-like material. *Acta endocr. Copenh* **96** (1981) 394–397.
67. Jan Y. N., Jan L. Y. and Brownfield M. S.: Peptidergic transmitters in synaptic boutons of sympathetic ganglia. *Nature* **288** (1980) 380–382.
68. Adams P. R. and Brown D. A.: Luteinizing hormone-releasing factor and muscarinic agonists act on the same voltage-sensitive K^+-current in bullfrog sympathetic neurons. *Br. J. Pharmac.* **68** (1980) 353–355.
69. Lamberts S. W. J., Timmers J. M., Oosterom R., Verleun T., Rommerts F. G. and DeJong F. H.: Testosterone secretion by cultured arrhenoblastoma cells: suppression by a luteinizing hormone-releasing agonist. *J. clin. Endocr. Metab.* **54** (1982) 450–454.
70. Tureck R. W., Mastroianni L. Jr, Blasco L. and Strauss J. F. III: Inhibition of human granulosa cell progesterone secretion by a gonadotropin-releasing hormone agonist. *J. clin. Endocr. Metab.* **54** (1982) 1078–1080.
71. Koos R. D., Ahren K. E. B., Janson P. O. and LeMaire W. J. A.: Effect of a GnRH agonist on the rabbit ovary perfused *in vitro*. *Endocrinology* **100** (Suppl.) (1982) 178.
72. Hertelendy R., Linter F., Asem E. K. and Raab B.: Synergistic effect of GnRH on LH-stimulated progesterone production in granulosa cells of the domestic fowl (*Gallus domesticus*). *Gen. comp. Endocr.* **48** (1982) 117–122.
73. Popkin R., Bramley T. W., Currie A., Shaw R. W., Baird D. T. and Fraser H. M.: Specific binding of LHRH to human luteal tissue. *Biochem. biophys. Res. Commun.* **114** (1983) 750–756.
74. Clayton R. N. and Huhtaniemi I. T.: Absence of GnRH receptors in human gonadal tissue. *Nature* **299** (1982) 56–59.
75. Brown J. L. and Reeves J. J.: Absence of specific LHRH receptors in ovine, bovine and procine ovaries. *Biol. Reprod.* **29** (1983) 1179–1182.
76. Asch R. H., van Sickle M., Rettor V., Balmaceda J. P., Eddy C. A., Coy D. H. and Schally A. V.: Absence of LH-RH binding sites in corpora lutea from rhesus monkeys. *J. clin. Endocr. Metab.* **53** (1981) 215–217.
77. Segal S. J. and Adejuwon C. A.: Direct effect of LHRH on testicular steroidogenesis in *Rana pipiens*. *Biol. Bull. mar. biol. Lab., Woods Hole* **157** (1979) 393.
78. Bex F. J., Corbin A. and France E.: Resistance of the mouse to the antifertility effects of LHRH agonists. *Life Sci.* **30** (1982) 1263–1269.
79. Hunter M. G., Sullivan M. H. F., Dix C. J., Aldred L. F. and Cooke B. A.: Stimulation and inhibition by LHRH analogues of cultured rat Leydig cell function and lack of effect on mouse Leydig cells. *Molec. cell. Endocr.* **27** (1982) 31–39.
80. Sundaram K. and Wang N.: Mice are insensitive to the antitesticular effects of LHRH agonists. *Endocrinology* **100** (Suppl.) (1982) 90.
81. Sherwood N., Eiden L., Brownstein M., Spiess J., Rivier J. and Vale W.: Characterization of a teleost–gonadotropin-releasing hormone. *Proc. natn. Acad. Sci. U.S.A.* **80** (1983) 2794–2798.
82. King J. A. and Millar R. P.: Structure of chicken hypothalamic LHRH. II. Isolation and characterization. *J. biol. Chem.* **257** (1982) 10729–10732.
83. Eiden L. E., Loumaye E., Sherwood N. and Eskay R. L.: Two chemically and immunologically distinct forms of LHRH are differentially expressed in frog neural tissues. *Peptides* **3** (1982) 323–327.
84. Miyamoto K., Hasegawa Y., Igarashi M., Kangawa K. and Matsuo H.: Structural determination of the second gonadotropin-releasing hormone in chicken hypothalamus. *7th International Congress of Endocrinology*, Abstr. 1776.