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REVIEW

Gonadotropin-Releasing Hormone: Present Concepts, Future Directions*

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ABSTRACT—There is an overwhelming amount of evidence to indicate that gonadotropin-releasing hormone (GnRH), a decapeptide, is found in multiple molecular forms, and is vital for the functional integration of brain-pituitary-gonadal axis in vertebrates. In simple terms, there is an overall agreement that GnRH acts as a neuroendocrine regulator of pituitary gonadotropin secretion, gonadal steroid secretion, sexual behavior, and reproduction. GnRHs are distributed widely within the vertebrate body, particularly in the brain. The brain GnRH neuronal system(s) varies in its morphology, ontogenesis and function across vertebrates. It is a highly dynamic structure which does not function at the same level throughout life. A large framework of studies completed to date attests to the emerging concept that GnRH neuronal system is regulated by a complex neural circuitry, comprised of diverse neurochemical signals, which may provide excitatory or inhibitory input to GnRH neurons. While general considerations on GnRH systems may be similar among vertebrates, it must not seduce us to generalize the more specific details. In fact, there may occur ontogenesis and reproductive status-related changes and a timetable of complex neuroendocrine events that are probably (certainly) species-specific.

INTRODUCTION

Gonadotropin-releasing hormone (GnRH=LHRH), a 10-amino acid bioregulatory neuropeptide, has a widespread occurrence within the living kingdom, and is not restricted just to vertebrates. In fact, from the evolutionary viewpoint one can go as far back as the yeast cells in which Loumaye *et al.* [55] demonstrated that a mating factor, called α-factor, necessary for sexual reproduction, has a strong similarity to GnRH, as evaluated by its ability to influence pituitary gonadotropin (GtH) secretion.

Brain is certainly the most complex organ of the vertebrate body. Within this highly specialized tissue there are cells characterized by their ability to synthesize GnRH. Originally isolated as a brain peptide, GnRH is well known for its regulatory role in the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary (pars distalis, adenohypophysis, ventral lobe). Since the isolation and structural elucidation of the hypothalamic GnRH in the early-1970s there have been numerous investigations to unveil the distribution pattern of GnRH-like peptide(s) in the brain of all vertebrate classes, from mammals down to cyclostomes [4, 6, 10, 22, 23, 44, 64, 77, 87]. During evolution this neuropeptide has undergone gene duplication with consequent structural diversification which is evident in the presence of multiple molecular forms across vertebrate species [see 45]. So far eight GnRH molecular forms have been described in the vertebrate brain, characterized and named for the vertebrate species in which they were first identified: mammalian (mGnRH), chicken I (cGnRH-I), chicken II (cGnRH-II), salmon (sGnRH), lamprey I and III (lGnRH), catfish (cfGnRH) and dogfish (dfGnRH) [10, 44–46, 64, 87, 86]. From a phylogenetic viewpoint cGnRH-II is considered to be the most conserved GnRH across vertebrates and residues 5, 7 and 8 vary among different forms. So far, only cGnRH-II has been described in all jawed vertebrates. Lamprey GnRH, however, varies from cGnRH-II in positions 3, 5, 6 and 8, and its presence has been demonstrated in cyclostomes only. An endogenous posttransational product of the GnRH precursor has been characterized in mammalian and frog hypothalamus: (Hydroxyproline^9)GnRH [29]. More recently, in *Xenopus laevis*, this form was shown to be distributed in the forebrain, midbrain and hypothalamus [46]. This peptide, as well as the C-terminal fragments of GnRH, are supposed to enhance sexual behavior as GnRH itself [30]. Novel GnRH forms described in oviparous mammals, reptiles, amphibians, bony and cartilaginous fishes and cyclostomes are yet to be characterized and still other GnRH forms are expected to be discovered [45–47, 64, 75]. Furthermore, over a couple of thousand analogs of GnRH ($GnRH_A$) have been synthesized whose availability becomes a powerful tool in understanding the regulatory roles and mechanisms of action of GnRH [42, 83]. The molecular heterogeneity is apparently the basis for a variety of regulatory functions of GnRH: as the stimulator of the reproductive system and sexual behavior, as a neuromodulator and/or neurotransmitter in the central and sympathetic nervous system, and as a paracrine/autocrine regulator in the pituitary, gonads, placenta, and in tumor cells.

This article is intended to give a "bird's eye view" of the morpho-functional features of GnRH neuronal systems

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* This paper is dedicated to our mentor Professor Giovanni Chieffi.

across vertebrates, and to spotlight the new knowledge that has emerged from latest studies.

DISTRIBUTION

Evidences based upon radioimmunoassay (RIA), chromatography (HPLC), *in situ* hybridization histochemistry (ISHH) and immunohistochemistry (ICC) techniques indicate the presence of GnRH-like peptide(s) in several brain as well as outside brain areas of a variety of vertebrate species. Localization of GnRH-producing neurons and their projections has been studied in the brain of mammals, birds, reptiles, amphibians, bony and cartilaginous fishes and cyclostomes [4, 6, 10, 19, 21–23, 40, 50, 56, 57, 64, 68, 77, 78, 87]. In several vertebrates HPLC/RIA analyses have confirmed ICC data as far as the distribution of GnRH-like material in the brain is concerned. However, in some species of birds, reptiles and amphibians ICC and HPLC/RIA data have shown discrepancy regarding the presence and distribution of different molecular forms of GnRH [19, 24, 28, 57, 61, 67, 75, 78, 101, unpublished data].

Modern ICC techniques are considered highly efficient, and yet we can not exclude the possibility that there may be GnRH neurons which fall below the level of detectability of the ICC procedures. Taking into account this reservation, at the present time GnRH-containing neurons and their projections have been described in the rhinencephalon, telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon [6, 10, 19, 21, 23, 57, 64, 87]. Although there are remarkable interspecies differences, a large hypothalamic population constitutes the biggest group of GnRH neurons in the brain of most vertebrates. In the median eminence (ME) no GnRH cell bodies are found. Among mammals, only musk shrew brain contains a cluster of GnRH neurons in the mesencephalon [21], and it is not known whether this feature is common to other primitive mammals. No GnRH neurons have been described posterior to the midbrain in any mammalian species. GnRH neurons in the forebrain, however, send fiber projections to the midbrain in all mammals. In nonmammalian vertebrates, except urodele amphibians [19] and cyclostomes [48], a mesencephalic group of GnRH neurons has been described [50, 57, 61, 64, 65, 101, 106, unpublished data]. Projections of midbrain GnRH neurons in bony fishes may innervate the caudal neurosecretory system (urophysis) [23]. Conspicuous differences in the distribution pattern have been described within each vertebrate subgroup. For example, among anuran amphibians, *Rana esculenta* brain shows perhaps the most extensive network of GnRH neurons and fibers [24, 78], whereas *Pachymedusa dacnicolor* brain contains only a small number of GnRH neurons all located exclusively in the anterior preoptic area (POA) [40] from where axonal projections reach the ME. In cyclostomes, the POA-located GnRH neurons project to the neurohypophysis (PN; pars nervosa) [48]. In most vertebrates GnRH fiber endings in the ME are derived from the forebrain-located neurons, mainly in the hypothalamus and septum, and only in bony fishes do the POA-located GnRH neuron projections directly contact pituitary gonadotropes [23]. Several studies have established that the populations of GnRH cells in the forebrain that project to the pituitary, PN and/or ME are often completely separate from those GnRH neurons that give rise to brain stem and/or spinal cord projections [65]. In fact, a growing body of evidence indicates that there are subpopulations of anatomically distinct GnRH-neuronal systems in the vertebrate brain, and that GnRH neurons may be unipolar, bipolar or multipolar. At a subcellular level GnRH immunoreactivity is distributed around the outside of the nuclear envelope, associated particularly with the rough endoplasmic reticulum, secretory vesicles and Golgi apparatus, and in the neuronal projections [54, 59, 110]. Sex-related differences in subcellular organelles have been described in mares and stallions [59]. To the best of our knowledge, no ultrastructural study of GnRH neurons is available in nonmammalian vertebrates.

Numerous studies have demonstrated that more than one GnRH molecular form may be present in the brain, and there are several reports on a differential distribution of GnRH variants in distinct brain areas [10, 13, 44, 46, 61, 65, 96, 109]. Moreover, in the lizard, *Podarcis sicula*, more than one GnRH form were colocalized in the same neuron [57]. In the musk shrew, mGnRH is distributed all through the forebrain and diencephalon, whereas midbrain-located neurons contain only cGnRH-II [21]. In the midbrain of adult chicken only cGnRH-II is present, while cGnRH-I is present chiefly in the forebrain, diencephalon and ME [43]. However, in a later study, van Gils *et al.* [101] detected cGnRH-II in the ME of chicken as well as Japanese quail. Similarly, Millam *et al.* [61] did not find cGnRH-II in the ME of turkey by ICC, while in a later study RIA analysis showed the presence of cGnRH-II in the posterior pituitary [El Halawani, unpublished, see 60]. Further, in a turtle species, cGnRH-I and II are differentially distributed, the former being most concentrated in the ME in a ratio of 8 : 1 against the latter which is more abundant in the caudal regions [96]. Likewise, in *X. laevis* mGnRH is distributed throughout the brain, whereas cGnRH-II is more concentrated in the midbrain and hindbrain [46]. In the frog, *R. ridibunda*, cGnRH-II neurons, but not mGnRH neurons innervate the neurointermediate lobe of the pituitary; similarly, in *R. esculenta* mGnRH neurons project to ME and PN [78], and using a highly specific cGnRH-II antiserum GnRH neurons were revealed in the midbrain tegmentum and fiber endings in the pars intermedia [unpublished]. Differential distribution of GnRHs has been described in the brain of bony fishes as well [see 45, 47]. Recently, ring dove brain has been shown to contain GnRH-like material-containing nonneuronal cells [88]. Characterized as mast cells, component of the immune system, they are distributed mainly in the medial habenula. This interesting finding could be leading to investigate other vertebrate groups as well.

How does brain GnRH reach the target organs? In

tetrapods GnRH is conveyed to the pituitary via the hypothalamo-hypophyseal portal vessels; however, there are evidences to indicate that GnRH can also be secreted into the cerebral ventricles as well as in brain vessels. In bony fishes, which lack the ME, GnRH neurons are found to directly innervate pituitary gonadotropes. In elasmobranchs, the ventral lobe of the pituitary does not receive a portal supply, and GnRH is evidently released into the general circulation, where, in fact, GnRH and GnRH-binding protein molecules have been determined [83]. In cyclostomes, King *et al.* [48] have suggested that GnRH can be released into the third ventricle and transported by tanycytes to the pituitary; GnRH may also reach pituitary by simple diffusion from neuronal projections in the PN. In elasmobranchs, GnRH may reach pituitary and gonads via general circulation, and its presence has been ascertained in the cerebrospinal fluid [86, 107]. In tetrapods and bony fishes, GnRH can act on the gonads as a paracrine regulatory factor, and the idea is reinforced by the presence of GnRH-like molecules as well as of GnRH-binding sites in the gonads [27, 32, 35].

GnRH neurons have been described in the terminal nerve of mammals, birds, amphibians, and bony and cartilaginous fishes [19, 23, 64, 67, 103]. Fibre projections from these neurons may reach areas as far ahead as nasal capsule and as far behind as rhombencephalon, except the pituitary [69, 103]. In the dwarf gourami, a bony fish, using whole brain *in vitro*, it was shown that terminal nerve GnRH neurons may be the most extensively projecting GnRH cells in the brain [69]. Reptiles and cyclostomes remain the only vertebrate taxa in which GnRH neurons have not been described yet in the terminal nerve. In cyclostomes, however, the presence of a terminal nerve-like structure is still a question of debate.

Besides the brain and terminal nerve, GnRH-like material has been detected in a variety of other structures: gonads, mammary gland, tumor cells, human placenta and pancreas, follicular fluid, milk, olfactory epithelium, sympathetic ganglia, adrenal gland, liver, intestine and retina [10, 12, 35, 44]. GnRH-like material outside the brain and terminal nerve may be similar to or different than one of the GnRH variants characterized in the brain.

ONTOGENESIS

The embryonic origin(s) of GnRH neurons has received considerable interest in recent years. Several reports have described that these neurons, unique among brain cells, originate not in the brain but in the olfactory placode and migrate into the forebrain/diencephalon along the olfactory/terminal nerve. The extracranial origin, time course and route of GnRH neuronal migration has been clarified in some species of mammals, birds and amphibians [19, 64, 65, 84, 92]. In birds and urodele amphibians, this line of evidence has been confirmed by surgical removal of the olfactory placode which results in the elimination of GnRH neurons in the forebrain and diencephalon [1, 63, 64]. In anuran amphibians, however, the midbrain-located group of GnRH neurons is supposed to have an intracranial origin [18, 65, unpublished data]. Among tetrapods, the reptilian GnRH neuronal system is morphologically "atypical" in that GnRH neurons are mainly located in the midbrain and infundibulum [57], and they are not considered to have an extracranial origin [unpublished data]. Interestingly, the midbrain cluster of GnRH neurons is the first to appear during lizard ontogenesis, followed by their appearance in the infundibulum; we argue that these neurons may take origin in the nearby neuroepithelium [unpublished data]. However, the ontogenesis of GnRH neuronal system in reptiles need be investigated in more species to unequivocally clarify the intracranial origin of GnRH neurons in this group. In a bony fish, *Pterophyllus scalare*, the first ontogenetic appearance of GnRH-immunoreactive cells in the pituitary precedes that in the POA and this may be indicative of still another site of origin for GnRH-producing cells [16]. No information on the extracranial origin of GnRH neurons in the brain is available for cyclostomes; in this group Muske [64] suggests another line of origin of the POA-located GnRH neurons, and that is from the ventricular ependyma because of the periventricular localization of GnRH cell bodies. Needless to say, the phylogenetic picture of the embryonic origin of GnRH neuronal systems in the vertebrate brain is far from complete.

Biochemical and ultrastructural differentiation of GnRH neurons, during their olfactory placode-forebrain migration, has been analyzed in the mouse [54], in which GnRH gene is expressed early in ontogenesis [107] and the differentiation of GnRH neurons continues and is coordinated during migration. In this mammal, however, axonal projections are formed only upon entering the forebrain. In contrast, in the rhesus monkey axonal projections are elaborated while GnRH neurons are still in the nasal septum [82]. Similarly, in the chick [92] and newt [19] GnRH axons are seen in the terminal/olfactory nerve during early migratory stage. Prior to migration GnRH neurons are scattered as individual cell bodies in the placode, while during migration they come to lie in close apposition, as confirmed by electron microscopy [92, 110]. Cell-to-cell contact appears to be established when these neurons are located at the adult site. The nature of these contacts must be investigated at the ultrastructural level.

During ontogenesis, besides the morphological and biochemical changes, a remarkable change may also occur in the number of GnRH neurons. In the mouse, in fact, GnRH cell number decreases drastically in the last stages of migration [110], and it is suggested that programmed cell death may be one reason, or that after migration some neurons stop synthesizing GnRH and become undetectable by ICC. There is the need for further investigation as well as the necessity to draw more vertebrate groups in this repertoire.

CORRELATES OF BIOSYNTHETIC AND SECRETORY ACTIVITY, AND FUNCTIONS

Morphological and biochemical correlates

Indeed, the basic theme of investigations related to the morphological, histochemical and biochemical correlates of GnRH secretion and functions has almost always been around the age, sex and reproductive status-related changes in the hypothalamo-hypophyseal-gonadal system. The various steps are, in sequence, comprised of the identification of GnRH-like material in the brain, morphology, distribution and ontogenesis of the GnRH neuronal system, anatomical connections with the pituitary, and an analysis of the GnRH content in the brain, ME, portal blood and systemic circulation under different conditions. Among the central regulatory mechanisms involved in the control of the development of the hypothalamo-hypophyseal-gonadal axis until sexual maturity, the importance of GnRH has been emphasized in all vertebrates [see 31, 98, 104]. In the adult, besides the pulsatile pattern of GnRH secretion, abundantly referred to in mammals, insight into the functional relationships of the hypothalamo-hypophyseal-gonadal system is also gained through evaluating seasonal pattern of GnRH secretion [see 3, 26, 28, 80]. Among tetrapods GnRH content has been evaluated in tissue extract as well as in portal blood, and only in bony and cartilaginous fishes has the radioimmunoassayble GnRH been determined in general circulation [47, 73].

It has hitherto been demonstrated that GnRH neurons undergo morphological, distributional and numerical changes associated with the developmental stage and reproductive status of the animal. Prominent seasonal cycles in GnRH cell morphology are observed in a variety of vertebrates [see 78, 98, 108]. It is evident that a particular morphological feature of the GnRH neuron may be correlated with a particular aspect of reproduction. In hibernating mammals, degranulation of GnRH cells occurs during hibernation, and increased storage after arousal. In the male Djungarian hamster, there occurs an increase in the number of unipolar neurons correlated with the onset of puberty, whereas bipolar GnRH neurons increase only in postpubertal period [108]. An ultrastructural analysis of GnRH neurons in the pony brain has, furthermore, indicated that irregularly-contoured cells in the POA/organum vasculosum lamina terminalis may have higher synthetic activity as compared to most other neurons, and it is suggested that GnRH neurons may utilize ultrashort feedback [59]. In the Syrian hamster, it was ascertained that the inhibitory effects of short days on the reproductive axis are mediated through a suppression of GnRH neurons which, in turn, is reflected as an increase in the net content of GnRH within the brain [97]. In the cow, a quantitative light microscopical study describing morphological changes in GnRH neurons supports the hypothesis of reduced activity of GnRH neurons during early to middle stages of the puerperium [51] which is in line with the concept of postpartum infertility due to the suckling stimulus-mediated suppression of GnRH, and consequently of LH, secretion.

Functions

In all major vertebrate groups, the effects of exogenous GnRH/$GnRH_A$ have been investigated *in vivo* and *in vitro*. These comprise stimulation of pituitary GtH and, in some cases, growth hormone secretion, stimulation of pituitary-thyroid axis, gonadal steroidogenesis, gametogenesis, spermiation, ovulation, and sexual behavior. However, extremely varied experimental protocols, as well as different GnRH forms and/or $GnRH_A$ have been used, and thus it becomes rather arduous to unify these data in order to draw generalized considerations. Nevertheless, all native GnRH variants appear to have at least one characteristic in common, and that is the stimulation of GtH release from the pituitary. A series of studies have also provided evidences that naturally occurring GnRH variants may exhibit different biological potencies in different species in terms of their ability to enhance pituitary GtH secretion [see 10, 53, 73].

In a variety of mammals, including man, and in the domestic fowl, reproductive function can be inhibited by a prolonged treatment with GnRH/$GnRH_A$ [93, 94]. In the adult, initially, GnRH administration enhances pituitary GtH secretion but a continuous exposure to GnRH eventually leads to desensitization of the pituitary to GnRH with the consequent suppression of gonadal function [102]. It is also known that GnRH/$GnRH_A$ exert a differential control over FSH and LH. This may be credited to the fact that GnRH is released in pulses, inducing a pulsatile pattern of LH release, but not of FSH. Nonetheless, chronic GnRH treatment suppresses both LH and FSH. In contrast, in amphibians, it was demonstrated that pituitary is relatively resistant to desensitization due to chronic *in vivo* GnRH exposure which enhances GtH biosynthesis and secretion [53, 89]. However, in the goldfish GnRH desensitization has been reported [34]. Further, in some mammals and birds evidences are that, during ontogenesis, GnRH plays a role in the development of pituitary gonadotropes, and that GnRH, at least in the rat, is important for maintaining FSH synthesis [see 9, 104].

Differential distribution, distinct roles

Do naturally occurring GnRH variants play distinct roles? The answer is yet far from clear. However, it is becoming increasingly evident that native GnRHs are differentially distributed within the brain of jawed vertebrates [13, 21, 43, 46, 61, 96]. In the musk shrew, and some species of birds, reptiles, amphibians and bony fishes a quantitative predominance of cGnRH-II is found not in the forebrain, but in the midbrain and/or hindbrain, and this has led to the assertion that cGnRH-II may have an extrapituitary role. However, a specific role for this form has not yet been established. In birds, in which cGnRH-I seems to predominate in the forebrain/hypothalamus, it is argued that the potential function of cGnRH-I and II may diverge early in development [43, 60]. Among amphibians, based upon the

predominance of mGnRH in the hypothalamus in *X. laevis* it was suggested that this variant may be the prime regulator of GtH release, whereas cGnRH-II, abundant in the hindbrain, may have an extrapituitary role [45]. In *R. esculenta*, however, it was suggested that cGnRH-II may have a hypophysiotropic activity [28]. Further, in this species it was also seen that cGnRH-I and II and mGnRH all enhanced androgen production in intact males, whereas in hypophysectomized males only cGnRH-II enhanced testicular androgen production, suggesting that a cGnRH-II-like molecule, produced in the testis, may be the local paracrine regulator of testicular activity [20, 79]. None of the tetrapod species studied is responsive to lGnRH [see 53], in terms of pituitary GtH secretion. However, lGnRH enhances plasma androgen levels in intact male frog [20]. In the forebrain/hypothalamus of fishes the predominant form varies from mGnRH, sGnRH to catfish or dogfish GnRH, making it evident that any of these variants may have a hypophysiotropic role. However, cGnRH-II neuronal endings may terminate in the bony fish pituitary [see 45]. Perhaps, in lower vertebrates the specificity of GnRH variants is very low. Lovejoy *et al.* [56] have proposed the division of known native GnRHs in two groups: mGnRH, cGnRH-I and catfish GnRH with hydrophilic residues, and cGnRH-II, sGnRH and dogfish GnRH with hydrophobic residues. They suppose that this may lead to clarify, on a structural basis, their distinct functional roles. Naturally, lGnRH is not included in this classification, being this variant present exclusively in cyclostomes.

Interaction with sex steroids

Among vertebrates, the repertoire of GnRH functions appears to be remarkably complex and involves interactions with several hormonal and other factors. GnRH acts upon the pituitary gonadotropes through interaction with membrane-associated high affinity receptors [14]. GnRH-modulated pituitary GtH secretion regulates gonadal activity and reproduction. Sex steroids in turn influence GnRH-regulated GtH biosynthesis and secretion. For this the sex steroid signals from the gonads must be correctly interpreted within the brain. Further, it is necessary to mention that GnRH regulation of pituitary GtH secretion can be modulated by sex steroids at the level of the pituitary as well, positively or negatively [see 71]. Although GnRH neurons do not appear to have sex steroid receptors, their distribution pattern has a remarkable overlapping with that of sex steroid-concentrating neurons, and the latter may even project to other brain areas in order to transmit steroid-influenced signals [10, 22, 25, 62, 74]. Most likely, the GnRH neurons which send their axon terminal in the ME are affected by estrogen-sensitive afferent neuronal systems. Indeed, GnRH neurons in such areas may represent targets for the feedback of steroids, and this is substantiated by a recent study in the rat, in which the rostral medial POA contains estrogen receptors and the GnRH neurons situated in this area are sensitive to estrogen treatment. Changes in GnRH neuronal mRNA levels in this area of estrogen-supplemented ovariectomized rats are taken as the cellular correlates of the positive feedback effects of estrogen on GnRH neurons [74]. This experimental model may be used to examine temporal changes in GnRH gene transcription, GnRH mRNA stability and GnRH translation all contributing to the understanding as to how estrogen triggers the preovulatory hypersecretion of GnRH which leads to LH surge followed by ovulation. However, in the meanwhile pituitary sensitivity to GnRH is enhanced by preovulatory progesterone surge. Similarly, in *R. esculenta*, in which sex steroid-concentrating neurons abound in the anterior POA, gonadectomy in both sexes caused a severe quantitative depletion of GnRH neurons in this area, as evaluated by ICC [41]. Sex steroid-replacement therapy enhanced somal accumulation of immunoreactive material, indicative probably of an increased synthesis and storage. In *R. catesbeiana*, it is suggested that GnRH can enhance pituitary GtH secretion in juveniles, but biosynthesis of GtH is enhanced only at a later time, coincident with gonadal steroid production [90]. These authors suggest that androgens may augment GnRH receptor molecules in the pituitary as well as endogenous secretion of GnRH in the hypothalamus. It thus appears that GnRH-stimulated GtH release is influenced not only by age, sex, season or morpho-functional heterogeneity of pituitary gonadotropes, but also by sex steroids [see 89, 90, 96].

External factors

Of the behavioral cues, courtship enhances GnRH concentration in the terminal nerve in a urodele amphibian [76], whereas in the ring dove, it determines the appearance of nonneuronal GnRH-containing cells in the habenula [88], thus making it obvious that GnRH-producing brain areas may be differentially correlated with diverse reproductive aspects in different species.

An upsurge in interest in the implication of odours in animal reproduction dates long-long back, and it is widely contended that reproductive activity is dependent upon a fully functional olfactory system, and that pheromone signals can be transduced by terminal nerve GnRH system to influence reproductive activity through the modulation of GnRH secretion in the hypothalamus [22, 23, 91]. This concept is strengthened by the fact that limbic (hypothalamus, amygdala, hippocampus, POA) and olfactory systems indeed control reproductive behavior, and extensive GnRH projections between the terminal nerve and limbic systems are well placed to create a chain through the olfactory mucosa, brain, pituitary, and on to the gonad. In a recent study on the electrical activity and morphology of terminal nerve-GnRH neurons in the dwarf gourami, it was shown that these neurons display an endogenous rhythmic discharge pattern, a feature common to all peptidergic and monoaminergic modulator neurons, but are not projected to the pituitary, and thus it was assumed that they do not function as a hypophysiotropic GnRH system, but rather as neuromodulator [69].

Neural modulation of GnRH release and function

Besides the fact that GnRH neurons display neuroendocrine as well as neuromodulator function, their own secretory activity is influenced by multiple neurotransmitters and/or neuromodulators. Only recently is the pivotal importance of such relationhips becoming fully appreciated. Indeed, there are evidences to indicate that several neural circuits are involved in controlling the GnRH neuronal systems. Transmitters and modulators of importance include GABA (γ-amino butyric acid), neuropeptide-Y (NPY), neurotensin, catecholamines, FMRFamide, endogenous opioid peptides (EOP), and others. All these circuits may provide important inputs to the GnRH system and some of them might contain excitatory as well as inhibitory input to GnRH system. It is also conceivable that one or the other component may, in turn, be influenced pre-synaptically by other neural circuits or factors, like sex steroids, cytokines etc. In mammals, GnRH-induced LH release *in vivo* is potentiated by NPY, either probably as a paracrine regulator (NPY neurons may synapse on GnRH neurons in the POA), or through enhancing the binding of GnRH to pituitary GnRH receptors [8, 70]; NPY secretion is enhanced by the preovulatory surge of sex steroids, and in turn it stimulates GnRH secretion. GnRH enhances intracellular calcium levels through openining voltage-sensitive calcium channels and this post-receptor GnRH action is also potentiated by NPY [15]. A number of investigations has suggested that EOPs can act as powerful inhibitors of GtH release acting by primarily decreasing the amplitude of GnRH pulses, and are in turn influenced by gonadal steroids [5, 8]. EOP-containing neurons may communicate with GnRH neural circuitry by adjusting locally the influx of excitatory adrenergic signals along the hypothalamo-hypophyseal axis. There is evidence that corticotropin-releasing hormone (CRF) may deplete GnRH neuronal activity through central opioidergic pathways, although direct effects are also likely to occur [2]. In the rat, dopamine can inhibit calcium ionophore-induced GnRH release [49]. In the goldfish, GnRH release from the POA is inhibited by dopamine and enhnaced by noradrenaline [73]. Noradrenaline, however, may be excitatory to GnRH in the presence of estrogens and inhibitory in their absence [33]. GnRH release and GnRH gene expression can be markedly inhibited by cytokines and the effects may be direct or mediated by opioids and prostaglandins [81]. GABAergic fibers are reported to directly innervate GnRH neuronal cells and until recently this provided morphological basis for a role of the GABAergic system in the regulation of GnRH secretion. Recently, however, Li and Pelletier [52] have shown that the use of $GABA_A$ receptor agonists inhibit not only the release of GnRH but also GnRH gene expression evaluated by ISHH. Simultaneous localization of multiple neuropeptides in the same brain section may yield useful information in relation to the modulation of GnRH neuronal activity on part of the complex neuronal circuitry in vertebrates. Neurons and fiber projections containing GnRH, FMRFamide and EOPs are interspersed in same brain areas and this may be the morphological substrate of physiological interactions between these systems [17, 38, 99, 100]. The coexistence of FMRFamide-like peptide and GnRH within the same neuron in a fish has provided morphological basis to suggest that FMRFamide may have an autocrine action on GnRH secretion [7]. The neural mechanisms involved in the stimulation or inhibition of GnRH release need further study.

Secretory mechanism

One exciting line of investigations is related to the study of neurosecretory mechanisms of GnRH neurons. Based on the conservation of ultrastructural features of differentiated neuroendocrine cells, the pulsatile manner of GnRH secretion and the presence of functional gap junctions in the GnRH neuronal cell line GT1-7 it has recently been suggested that gap junctional coupling between GnRH neurons may be the signalling machinery underlying periodic (pulsatile/circadian/seasonal) secretion of hypothalamic GnRH neuronal system [57]. GnRH-GnRH cell contacts, like synaptic organizations, have been described in mammals [58, 105], and are supposed to be involved in the synchronous activity of an entire population of GnRH neurons. Consequently, innervation of only a few GnRH neurons on part of another category of neuron may account for a cascade excitatory or inhibitory paracrine role of a neurotransmitter/neuromodulator/neuropeptide. At the same time, GnRH-GnRH contact will suggest an autocrine regulation within a GnRH-neuronal population. Taken together, the current data would suggest that a full understanding of the anatomical substrates of GnRH/other neuropeptides interactions may provide the key to the correct interpretation of functional studies.

Binding sites, receptors and mechanism of action

High affinity binding sites for GnRH/$GnRH_A$ have been demonstrated in the pituitary and/or gonads of several vertebrate groups, as well as in the mammalian placenta, adrenal gland and mammary carcinoma [see 10, 11, 27, 44, 45]. GnRH receptor in the mammalian pituitary cells has been characterized and cloned [72]. It is composed of seven transmembrane segments, a feature of G-protein-coupled receptors. Studies are needed to understand the role of G-proteins in mediating the effects of GnRH as well as the amino acid sequence of the GnRH receptor of nonmammalian vertebrates.

In studying the mechanism of action of GnRH on its targets, extracellular and intracellular Ca^{2+}, protein kinase C, inositol phosphates, calmodulin, leukotrienes and arachidonic acid have been implicated in the mediation of GnRH-induced GtH secretion [see 66]. GnRH acts upon the pituitary gonadotropes through specific high affinity membrane-bound receptors [14], by modifying the frequency and amplitude of action potentials generated spontaneously in gonadotropes [37]. In mammals, chronic GnRH/$GnRH_A$ treatment causes desensitization of the pituitary by down-

regulating pituitary GnRH receptors and simultaneously by uncoupling GnRH signal transduction system and inactivating voltage-sensitive Ca^{2+} channels [11, 36, 37, 64]. Among bony fishes, there are two lines of evidences, one supporting the participation of protein kinase C pathway in the mediation of GnRH-stimulated GtH release, while the other supports the involvement of cAMP pathway [see 41].

FINAL COMMENTS

1. The most logical inference that can be drawn from the rather bulky and somewhat bizzare repertoire of data is that a uniform methodological approach should be used throughout vertebrates. However, because of the existence of amazingly diverse situations related to reproduction, the morpho-functional charatecristics of the GnRH neuronal system of a vertebrate group can not necessarily be generalized and extrapolated to other vertebrates. The discrepancies over the identification and distribution of GnRH-like material in the brain and other tissues may depend upon methodological variations, and despite the recognition of inherent difficulties, isolation and sequence analysis of GnRH-like material from more vertebrate species are needed.

2. A relevant amount of studies has provided the basis for additional investigations to fully resolve the questions of how GnRH regulates pituitary-gonadal activity, and reproduction as whole, involving direct GnRH effects on these organs as well as factors (hormonal and not) which in turn influence GnRH system. In relation to the last point it is imperative to clarify whether the stimulatory/inhibitory effects are exerted both on GnRH secretion and biosynthesis, and if there exists a direct relationship between alteration in GnRH release and biosynthesis. Moreover, in ICC analyses of reproductive status-related changes, corresponding ultrastructural studies are required to define whether diminished immunoreactivity is due to decreased biosynthesis or enhanced secretion and, *vice versa*, increased immunoreactivity is owing to an increased biosynthesis or a decline in release.

3. Although there have been some investigations, the potential importance of GnRH on the ontogenesis and postnatal development of pituitary gonadotropes is far from understood. Nevertheless, it is clear that an analysis of the number and distribution pattern of GnRH neuronal subtypes may turn out to be useful in understanding the ontogenetic mechanisms and the potential (differential) role of regional subpopulations of GnRH neurons in the process of sexual maturation. It is also conceivable that embryonic migration of GnRH neurons into the forebrain from the nasal area continues in the postnatal/posthatching/pro and postmetamorphosis period, and it is possible that precocious migration includes undifferentiated cells from the olfactory placode that are capable of division and differentiation at the time of sexual maturation/puberty. Future studies may involve more vertebrate species and, hopefully, be aimed to provide details not only about the extra- and intracranial migratory course of GnRH neurons and the developmental stage at which GnRH neurons reach their adult position, but also on the differential localization of GnRH neurons and processes showing immunoreactivity for different GnRH forms.

4. Morphological, distributional and molecular heterogeneity of GnRH render the variety of roles played by this neuropeptide plausible. There are GnRH receptor sybtypes in the pituitary which has made it possible to ascertain that C-terminal fragments of GnRH, or GAP, or $GnRH_A$ may have similar effects as GnRH itself. Much remains to be done before more concrete inferences may be drawn.

5. Besides electrophysiological methods to study the activity of individual neurons, it is argued that changes in mRNA levels reflect changes in neuronal activity. The availability of quantitative ISHH technique allows for measuring changes in mRNA levels in individual neurons. However, the current methods used to measure GnRH content and GnRH mRNA may not be sensitive enough to detect small changes in GnRH secretion. Hopefully, in the forthcoming years more sophisticated technologies will emerge which will allow us to reveal, record and interpret the electro-chemical and molecular signals released by individual GnRH neurons. There is a total dearth of information on the role of the genome in modulating GnRH neuronal activity. To date only one putative gene has been cloned in all species examined, and it is of comfort that ISHH unveils GnRH gene transcription and expression in neurons where GnRH itself is revealed by ICC.

6. It is hoped that latest studies will stimulate collaborative research among physiologists, biochemists, molecular biologists, genetists, neurobiologists and comparative endocrinologists to unveil the yet "secret" world of GnRH.

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REFERENCES

1 Akutsu S, Takada M, Ohki-Hamazaki H, Murakami S, Arai Y (1992) Origin of luteinizing hormone-releasing hormone neurons in the chick embryo: effect of the olfactory placode ablation. Neurosci Lett 142: 241–244

2 Almeida OFX, Nikolarakis KE, Sirinathsinghji DJS, Hertz A (1989) Opioid-mediated inhibition of sexual behavior and luteinizing hormone secretion by corticotropin-releasing hormone. In "Brain Opioid Systems in Reproduction" Ed by RG Dyer, RJ Bicknell, Oxford University Press, Oxford, pp 149–164

3 Amano M, Aida K, Okumoto N, Hasegawa Y (1992) Changes in salmon GnRH and chicken GnRH-II contents in the brain and pituitary, and GtH contents in the pituitary in female Masu salmon, *Oncorhynchus masou*, from hatching

through ovulation. Zool Sci 9: 375–386

4 Anderson AC, Tonon M-C, Pelletier G, Conlon JM, Fasolo A, Vaudry H (1992) Neuropeptides in the amphibian brain. Int Rev Cytol 138: 89–210

5 Angioni S, Nappi RE, Genazzani AD, Carunchio P, Criscuolo M, Guo A-L, Bidzinska B, Petraglia F (1993) Brain opioids and reproductive function. In "Cellular Communication in Reproduction" Ed by F Facchinetti, IW Henderson, R Pierantoni, A Polzonetti-Magni, J Endocrinol Ltd, Bristol, pp 33–40

6 Barry J, Hoffman GE, Wray S (1985) LHRH-containing systems. In "Handbook of Chemical Neuroanatomy Vol 4" Ed by A Bjorklund, T Hokfelt, Elsevier, Amsterdam, pp 166–215

7 Batten TFC, Cambre ML, Moons L, Vandesande F (1990) Comparative distribution of neuropeptide-immunoreactive systems in the brain of the green molly, *Poecilia latipinna*. J Comp Neurol 302: 893–919

8 Bauer-Dantoin AC, Knox KL, Schwartz NB, Levine JE (1993) Estrous cycle stage-dependent effects of neuropeptide Y on luteinizing hormone (LH)-releasing hormone-stimulated LH and follicle-stimulating hormone secretion from anterior pituitary fragments in vitro. Endocrinology 133: 2413–2417

9 Blake CA, Gary TC, Mascagni F, Culler MD, Negro-Vilar A (1993) Effects of injection of anti-luteinizing hormone (LH)-releasing hormone serum and anti-gonadotropin-releasing hormone-associated peptide serum into neonatal rats on LH and follicle-stimulating hormone cells. Biol Reprod 49: 965–971

10 Chieffi G, Pierantoni R, Fasano S (1991) Immunoreactive GnRH in hypothalamic and extrahypothalamic areas. Int Rev Cytol 127: 1–55

11 Clayton RN (1989) Gonadotropin-releasing hormone: its actions and receptors. J Endocrinol 120: 11–19

12 Clayton RN, Eccleston L, Gossard F, Thalbard J-C, Morel G (1992) Rat granulosa cell expression of the gonadotropin-releasing hormone gene: evidence from *in situ* hybridization histochemistry. J Mol Endocrinol 9: 189–195

13 Conlon JM, Collin F, Chiang YC, Sower SA, Vaudry H (1993) Two molecular forms of gonadotropin-releasing hormone from the brain of the frog, *Rana ridibunda*: purification, characterization, and distribution. Endocrinology 132: 2117–2123

14 Conn PM, Huckle WR, Andrews WV, McArdle GA (1987) The molecular mechanisms of action of gonadotropin-releasing hormone (GnRH) in the pituitary. Rec Prog Horm Res 43: 29–61

15 Crowley WR, Shah GV, Carroll BL, Kennedy D, Dokher ME, Kalra SP (1990) Neuropeptide Y enhances luteinizing hormone (LH)-releasing hormone-induced LH release and elevation in cytosolic Ca^{2+} in rat anterior pituitary cells: evidence for involvement of extracelular Ca^{2+} influx through voltage-sensitive channels. Endocrinology 127: 1487–1494

16 D'Aniello B (1994) Il GnRH nell'ontogenesi dei vertebrati: analisi immunoistochimica. Doctorate Thesis, University of Naples "Federico II"

17 D'Aniello B, Imperatore C, Fiorentino M, Vallarino M, Rastogi RK (1994) Immunocytochemical localization of POMC-derived peptides (adrenocorticotropic hormone, α-melanocyte stimulating hormone and β-endorphin) in the pituitary, brain and olfactory epithelium of the frog, *Rana esculenta*, during development. Cell Tissue Res, (in press)

18 D'Aniello B, Masucci M, di Meglio M, Ciarcia G, Rastogi RK (1991) Distribution of gonadotropin-releasing hormone-like peptides in the brain during development of juvenile male *Rana esculenta*. Cell Tissue Res 265: 51–55

19 D'Aniello B, Masucci M, di Meglio M, Iela L, Rastogi RK (1994) Immunohistochemical localization of GnRH in the crested newt (*Triturus carnifex*) brain and terminal nerve: a developmental study. J Neuroendocrinol 6: 167–172

20 D'Antonio M, Fasano S, de Leeuw R, Pierantoni R (1992) Effects of gonadotropin-releasing hormone variants on plasma and testicular androgen levels in intact and hypophysectomized male frogs, *Rana esculenta*. J Exp Zool 261: 34–39

21 Dellovade TL, King JA, Millar RP (1993) Presence and differential distribution of distinct forms of immunoreactive gonadotropin-releasing hormone in the musk shrew brain. Neuroendocrinology 58: 166–177

22 Demski LS (1984) The evolution of neuroanatomical substrates of reproductive behavior: sex steroid and LHRH-specific pathways including the terminal nerve. Am Zool 24: 809–830

23 Demski LS (1987) Phylogeny of luteinizing hormone-releasing hormone system in protochordates and vertebrates. In "The Terminal Nerve (Nervus Terminalis) Structure, Function, and Evolution Vol 519" Ed by LS Demski, M Schwanzel-Fukuda, Ann NY Acad Sci, pp 1–14.

24 di Meglio M, Masucci M, D'Anillo B, Iela L, Rastogi RK (1991) Immunohistochemical localization of multiple forms of gonadotropin-releasing hormone in the brain of the adult frog. J Neuroendocrinol 3: 363–368

25 di Meglio M, Morrell JI, Pfaff DW (1987) Localization of steroid-concentrating cells in the central nervous system of the frog, *Rana esculenta*. Gen Comp Endocrinol 67: 149–154

26 Fahien CM, Sower SA (1990) Relationship between brain gonadotropin-releasing hormone and final reproductive period of the adult male sea lamprey, *Petromyzon marinus*. Gen Comp Endocrinol 80: 427–437

27 Fasano S, de Leeuw R, Pierantoni R, Chieffi G, van Oordt PGWJ (1990) Characterization of gonadotropin-releasing hormone (GnRH) binding sites in the pituitary and testis of the frog, *Rana esculenta*. Biochem Biophys Res Commun 168: 923–932

28 Fasano S, Goos HJTh, Janssen C, Pierantoni R (1993) Two GnRHs fluctuate in correlation with androgen levels in the male frog *Rana esculenta*. J Exp Zool 266: 277–283

29 Gautron JP, Patton E, Bauer K, Kordon C (1991) (Hydroxyproline^9) luteinizing hormone-releasing hormone: a novel peptide in mammalian and frog hypothalamus. Neurochem Inter 18: 221–235

30 Gautron JP, Patton E, Leblanc P, L'HSitier A, Kordon C (1993) Preferential distribution of C-terminal fragments of (Hydroxyproline^9)LHRH in the rat hippocampus and olfactory bulb. Neuroendocrinology 58: 240–250

31 Goos HJTh (1993) Pubertal development: big questions, small answers. In "Cellular Communication in Reproduction" Ed by F Facchinetti, IW Henderson, R Pierantoni, A Polzonetti-Magni, J Endocrinol Ltd, Bristol, pp 11–20

32 Goubau S, Bond CT, Adelman JP, Misra V, Haynes MF, Murohy BD (1992) Partial characterization of the gonadotropin-releasing hormone (GnRH) gene transcript in the rat ovary. Endocrinology 130: 3098–4000

33 Grossmann R, Dyer RG (1989) Opioid inhibition of brainstem projections to the medial preoptic area in female rats. In "Brain Opioid Systems in Reproduction" Ed by RG Dyer, RJ Bicknell, Oxford University Press, Oxford, pp 112–124

34 Habibi HR (1991) Homologous desensitization of gonadotropin-releasing hormone (GnRH) receptors in the goldfish pituitary: effects of native GnRH peptides and a synthetic GnRH antagonist. Biol Reprod 44: 275–283

35 Habibi HR, Prati D (1993) Endocrine and paracrine control of ovarian function: role of compounds with GnRH-like activity in goldfish. In "Cellular Communication in Reproduction" Ed

by F Facchinetti, IW Hendersen, R Pierantoni, A Polzoneti-Magni, J Endocrinol Ltd, Bristol, pp 59–70

36 Hazum E, Conn PM (1988) Molecular mechanisms of gonadotropin-releasing hormone (GnRH) action. I. The GnRH receptor. Endoc Rev 9: 379–386

37 Heyward PM, Chen C, Clarke IJ (1993) Gonadotropin-releasing hormone modifies action potential generation in sheep pars distalis gonadotropes. Neuroendocrinology 58: 646–654

38 Hoffman GE, Fitzsimmons MD, Watson Jr RE (1989) Relationship of endogenous opioid peptide axons to GnRH neurones in the rat. In "Brain Opioid Systems in Reproduction" Ed by RG Dyer, RJ Bicknell, Oxford University Press, Oxford, pp 125–134

39 Iela L, D'Aniello B, di Meglio M, Rastogi RK (1994) Influence of gonadectomy and steroid hormone-replacement therapy on the gonadotropin-releasing hormone neuronal system in the anterior preoptic area of the frog (*Rana esculenta*) brain. Gen Comp Endocrinol (in press)

40 Iela L, Rastogi RK, Bagnara JT (1991) Reproduction in the Mexican leaf frog, *Pachymedusa dacnicolor*. V. Immunohistochemical localization of gonadotropin-releasing hormone in the brain. Gen Comp Endocrinol 84: 129–134

41 Jobin RM, Ginsberg J, Matowe WC, Chang JP (1993) Down-regulation of protein kinase C levels leads to inhibition of GnRH-stimulated gonadortopin secretion from dispersed pituitary cells of goldfish. Neuroendocrinology 58: 2–10

42 Karten MI, Rivier JE (1986) Gonadotropin-releasing hormone analog design. Structure function studies toward the development of agonists and antagonists. Rationale and perspective. Endoc Rev 7: 44–66

43 Katz IA, Millar RP, King JA (1990) Differential regional distribution and release of two forms of gonadotropin-releasing hormone in the chicken brain. Peptides 11: 443–450.

44 King JA, Millar RP (1991) Gonadotropin-releasing hormones. In "Vertebrate Endocrinology: Fundamentals and Biomedical Applications Vol IV" Ed by PKT Pang, MP Schreibman, Academic Press, New York, pp 1–31

45 King JA, Millar RP (1992) Evolution of gonadotropin-releasing hormones. Trends Endocrinol Metab 3: 339–346

46 King JA, Steneveld AA, Millar RP (1993) Differential distribution of gonadotropin-releasing hormones in amphibian (clawed toad, *Xenopus laevis*) brain. Regulatory Peptides 50: 277–289

47 King JA, Steneveld AA, Millar RP, Fasano S, Romano G, Spagnuolo A, Zanetti L, Pierantoni R (1992) Gonadotropin-releasing hormone in elasmobranch (electric ray, *Torpedo marmorata*) brain and plasma: chromatographic and immunological evidence for chicken GnRH II and novel molecular forms. Peptides 13: 27–35

48 King JC, Sower SA, Anthony ELP (1988) Neuronal systems immunoreactive with antiserum to lamprey gonadotropin-releasing hormone in the brain of *Petromyzon marinus*. Cell Tissue Res 253: 1–8

49 Koike K, Kadowaki K, Hirota K, Ohmichi M, Ikegami H, Sawada T, Miyake A, Tanizawa O (1993) Prolactin stimulates (^{3}H) dopamine release from dispersed rat tubero-infundibular dopaminergic neurons and dopamine decreases gonadotropin-releasing hormone release induced by calcium ionophore. Acta Endocrinol 129: 548–553

50 Kuenzel WJ, Blahser S (1991) The distribution of gonadotropin-releasing hormone neurons and fibers throughout the chick brain (*Gallus domesticus*). Cell Tissue Res 264: 481–495

51 Leshin LS, Rund LA, Kraeling RR, Crim JW, Kiser TE (1992) Morphological differences among luteinizing hormone-releasing hormone neurons from postpartum and estrous cyclic cows. Neuroendocrinology 55: 380–389

52 Li S, Pelletier G (1993) Chronic administration of muscimol and pentobarbital decreases gonadotropin-releasing hormone mRNA levels in the male rat hypothalamus determined by quantitative in situ hybridization. Neuroendocrinology 58: 136–139

53 Licht P, Porter DA (1987) Role of gonadotropin-releasing hormone in regulation of gonadotropin secretion from amphibian and reptilian pituitaries. In "Hormones and Reproduction in Fishes, Amphibians, and Reptiles", Ed by DO Norris, RE Jones, Plenum, New York, pp 61–85

54 Livne I, Gibson MJ, Silverman A-J (1993) Biochemical differentiation and intercellular interactions of migratory gonadotropin-releasing hormone (GnRH) cells in the mouse. Dev Biol 159: 43–656

55 Loumaye E, Thorner J, Catt KJ (1982) Yeast mating pheromone activates mammalian gonadotrophs: evoltionary conservation of a reproductive hormone? Science 218: 1323–1325

56 Lovejoy DA, Fischer WH, Ngamvongchon S, Craig AG, Nahorniak CS, Peter RE, Rivier JE, Sherwood NM (1992) Distinct sequence of gonadotropin-releasing hormone (GnRH) in dogfish brain provides insight into GnRH evolution. Proc Natl Acad Sci USA 89: 6373–6377

57 Masucci M, D'Aniello B, Iela L, Ciarcia G, Rastogi RK (1992) Immunohistochemical demonstration of the presence and localization of diverse molecular forms of gonadotropin-releasing hormone in the lizard (*Podarcis s. sicula*) brain. Gen Comp Endocrinol 86: 81–89

58 Matesic DF, Germak JA, Dupont E, Madhukar BV (1993) Immortalized hypothalamic luteinizing hormone-releasing hormone neurons express a connexin 26-like protein and display functional gap junction coupling assayed by fluorescence recovery after photobleaching. Neuroendocrinology 58: 485–492

59 Melrose PA, Littlefiedl-Chabaud MA, Pickel C, French DD (1993) Regional differences in the sunbcellular characteristics and synaptic connections with gonadotropin-releasing hormone (GnRH) neurons in the basal forebrain of adult ponies: sex-dependent differences found at the beginning of the anovulatory season. Proc 13th Int Symp Equine Physiol Nutrition, Gainsville, Fl, pp 344–345

60 Millam JR, Craig-Veit CB, Petitte JN (1993) Brain content of cGnRH I and II during embryonic development in chickens. Gen Comp Endocrinol 92: 311–317

61 Millam JR, Faris PL, Yougren OM, El Halawani ME, Hartman BK (1993) Immunohistochemical localization of chicken gonadotropin-releasing hormones I and II (cGnRH I and II) in turkey brain. J Comp Neurol 333: 63–82.

62 Morrell JI, Pfaff DW (1981) Characterization of estrogen-concentrating hypothalamic neurons by their axonal projections. Science 217: 1273–1276

63 Murakami S, Kikuyama S, Arai Y (1992) The origin of the luteinizing hormone-releasing hormone (LHRH) neurons in newt (*Cynops pyrrhogaster*): the effect of olfactory placode ablation. Cell Tissue Res 269: 21–27

64 Muske LE (1993) Evolution of gonadotropin-releasing hormone (GnRH) neuronal systems. Brain Behav Evol 42: 215–230

65 Muske LE, Moore FL (1990) Ontogeny of immunoreactive gonadotropin-releasing hormone neuronal system in amphibians. Brain Res 534: 177–187

66 Naor Z (1990) Signal transduction mechanisms of Ca^{2+} mobilizing hormones: the case of gonadotropin-releasing hormone. Endocr Rev 11: 326–353

67 Norgren RB, Lippert J, Lehman MN (1992) Luteinizing

hormone-releasing hormone in the pigeon terminal nerve and olfactory bulb. Neurosci Lett 135: 201–204

68 Nozaki M, Tsukahara T, Kobayashi H (1984) Neuronal systems producing LHRH in vertebrates. In "Endocrine Correlates of Reproduction" Ed by K Ochiai, Y Arai, T Ohioda, M Takahashi, Springer, Berlin, pp 3–27

69 Oka Y, Matsushima T (1993) Gonadotropin-releasing hormone (GnRH)-immunoreactive terminal nerve cells have intrinsic rhythmicity and project widely in the brain. J Neurosci 13: 2161–2176

70 Parker SL, Kalra SP, Crowley WR (1991) Neuropeptide Y modulates the binding of a gonadotropin-releasing hormone (GnRH) analog to anterior pituitary GnRH receptor sites. Endocrinology 128: 2309–2316

71 Pavgi S, Licht P (1993) Inhibition of in vitro pituitary gonadotropin secretion by 17β-estradiol in the frog *Rana pipiens*. Gen Comp Endocrinol 89: 132–137

72 Perrin MH, Bilezikjian LM, Hoeger C, Donaldson CJ, Rivier J, Haas Y, Vale WW (1993) Molecular and functional characterization of GnRH receptors cloned from rat pituitary and mouse pituitary tumor cell line. Biochem Biophys Res Commun 191: 1139–1144

73 Peter RE, Habibi HR, Chang JP, Nahorniak CS, Yu KL, Huang YP, Marchant TA (1990) Actions of gonadotropin-releasing hormmone (GnRH) in goldfish. In "Progress in Clinical and Biological Researc. Progress in Comparative Endocrinology" Ed by A Epple, CG Scanes, MH Stetson, Wiley-Liss, New York, pp 393–398

74 Petersen SL, McCrone S, Shores S (1993) Localized changes in LHRH mRNA levels as cellular correlates of the positive feedback effects of estrogen on LHRH neurons. Am Zool 33: 255–265

75 Powell RC, Ciarcia G, Lance V, Millar RP, King JA (1986) Identification of diverse molecular forms of GnRH in reptile brain. Peptides 7: 1101–1108

76 Propper CR, Moore FL (1991) Effects of courtship on brain gonadotropin hormone-releasing hormone and plasma steroid concentrations in a female amphibian (*Taricha granulosa*). Gen Comp Endocrinol 81: 304–312

77 Rance NE, Young WS, McMullen NT (1994) Topography of neurons expressing luteinizing hormone-releasing hormone gene transcripts in the human hypothalamus and basal forebrain. J Comp Neurol 339: 573–586

78 Rastogi RK, di Meglio M, Iela L (1990) Immunoreactive luteinizing hormone-releasing hormone in the frog (*Rana esculenta*) brain: distribution pattern in the adult, seasonal changes, castration effects, and developmental aspects. Gen Comp Endocrinol 78: 444–458

79 Rastogi RK, Iela L (1992) Spermatogenesis in amphibia: dynamics and regulation. In "Sex Origin and Evolution" Ed by R Dallai, Mucchi Editore, Modena, Italy, pp. 231–249

80 Rhim T-J, Kuehl D, Jackson GL (1993) Seasonal changes in the relationships between secretion of gonadotropin-releasing hormone, luteinizing hormone, and testosterone in the ram. Biol. Reprod. 48: 197–204

81 Rivest S, Rivier C (1993) Interleukin-1β inhibits the endogenous expression of the early gene c-fos located within the nucleus of LHRH neurons and interferes with hypothalamus LHRH release during proestrous in the rat. Brain Res 613: 132–142

82 Ronnekleiv OK, Resko JA (1990) Ontogeny of gonadotropin-releasing hormone-containing neurons in early fetal development of rhesus macaques. Endocrinology 126: 498–511

83 Schally AV, Bajusz S, Redding TW, Zalatnai A, Comaru-Schally AM (1989) Analogs of LHRH: the present and the future. In "GnRH Analogues in Cancer and in Human Reproduction: Basic Aspects Vol 1" Ed by BH Vickery, V Lunenfeld, Kluwer, Dordrecht, pp 5–31

84 Schwanzel-Fukuda M, Pfaff DW (1989) Origin of luteinizing hormone-releasing hormone neurons. Nature 338: 161–164

85 Sherwood NM (1986) Evolution of a neuropeptide family: gonadotropin-releasing hormone. Am Zool 26: 1041–1054

86 Sherwood NM, Lovejoy DA (1993) Gonadotropin-releasing hormone in cartilaginous fishes: structure, location, and transport. Env Biol Fish 38: 197–208

87 Silverman A-J (1988) The gonadotropin-releasing hormone (GnRH) neuronal systems: immunocytochemistry. In "The Physiology of Reproduction Vol 2" Ed by E Knobil, J Neil, Raven Press, New York, pp 1283–1304

88 Silverman A-J, Millar RP, King JA, Zhuang X, Silver R (1994) Mast cells with gonadotropin-releasing hormone-like immunoreactivity in the brain of doves. Proc Natl Acad Sci USA 91 (in press)

89 Stamper DL, Licht P (1993) Further studies on the influence of GnRH on the biosynthesis of gonadotropins in female frogs (*Rana pipiens*) Gen Comp Endocrinol 92: 104–112

90 Stamper DL, Licht P (1994) Influence of androgen on the GnRH-stimulated secretion and biosynthesis of gonadotropins in the pituitary of juvenile female bullfrog, *Rana catesbeiana*. Gen Comp Endocrinol 93: 93–102

91 Stoddart DM (1990) The Scented Ape: The Biology and Culture of Human Odour. Cambridge University Press, pp 79–119

92 Sullivan KA, Silverman A-J (1993) The ontogeny of gonadotropin-releasing hormone neurons in the chick. Neuroendocrinology 58: 597–608

93 Tilbrook AJ, Galloway DB, Williams AH, Clarke IJ (1993) Treatment of young rams with an agonist of GnRH delays reproductive development. Horm Behav 27: 5–28

94 Tilbrook AJ, Johnson RJ, Eason PJ, Walsh DJ, Trigg TE, Clarke IJ (1992) Short-term reduction in egg production in laying hens treated with an agonist of GnRH. Br Poult Sc 33: 611–628

95 Trudau VL, Murthy CK, Habibi HR, Sloley BD, Peter RE (1993) Effects of sex steroid treatments on gonadotropin-releasing hormone-stimulated gonadotropin secretion from the goldfish pituitary. Biol Reprod 48: 300–307

96 Tsai PS, Licht P (1993) Differential distribution of chicken-I and chicken-II in the turtle brain. Peptides 14: 221–226

97 Urbanski HF, Doan A, Pierce M (1991) Immunocytochemical investigation of luteinizing hormone-releasing hormone neurons in Syrian hamster maintained under long or short days. Biol Reprod 44: 687–692

98 Urbanski HF, Doan A, Pierce M, Fahrenbach H, Collins PM (1992) Maturation of the hypothalamo-pituitary-gonadal axis of the male Syrian hamster. Biol Reprod 46: 991–996

99 Vallarino M, Feuilloley M, D'Aniello B, Rastogi RK, Vaudry H (1994) Distribution of FMRFamide-like immunoreactivity in the brain of the lizard *Podarcis sicula*. Peptides (in press)

100 Vallarino M, Salsotto-Cattaneo M, Vaudry H (1993) Immunohistochemical localization of FMRFamide-like peptides in the brain of the frog, *Rana esculenta*. In "Cellular Communication in Reproduction" Ed by F Facchinetti, IW Henderson, R Pierantoni, A Polzonetti-Magni, J Endocrinol Ltd, Bristol, pp 45–48

101 Van Gils J, Absil P, Grauwels L, Moons L, Vandesande F, Balthazart J (1994) Distribution of luteinizing hormone-releasing hormone (LHRH) (LHRH-I and -II) in the quail and chicken brain as demosntrated with antibodies directed against synthetic peptides. J Comp Neurol (in press)

102 Vickery BH (1986) Comparisons of the potential for therapeutic utilies with gonadotropin-releasing hormone agonists and antagonists. Endoc Rev 7: 115–124

103 Wirsig-Wiechmann CR (1993) Pripheral projections of nervus terminalis LHRH-containing neurons in the tiger salamander, *Ambystoma tigrinum*. Cell Tissue Res 273: 31–40

104 Woods JG, Honan MP, Thomas RC (1989) Hypothalamic regulation of the adenohypophyseal-testicular axis in the male chick embryo. Gen Comp Endocrinol 74: 167–172

105 Wray S, Grant P, Gainer H (1989) Evidence that cells expresing luteinizing hormone-releasing hormone mRNA in the mouse are derived from progenitor cells in the olfactory placode. Proc Natl Acad Sci USA 86: 8132–8136

106 Wright DE, Demski LS (1991) Gonadotropin hormone-releasing hormone immunoreactivity in the mesencephalon of sharks and rays. J Comp Neurol 307: 49–56

107 Wright DE, Demski LS (1993) Gonadotropin-releasing hormone (GnRH) pathways and reproductive control in elasmobranchs. Env Biol Fish 38: 209–218

108 Yellon SM, Newman SW (1991) A developmental study of the gonadotropin-releasing hormone neuronal system during sexual maturation in the male Djungarian hamster. Biol Reprod 45: 440–446

109 Yu KL, Sherwood NM, Peter RE (1988) Differential distribution of two moelcular forms of gonadotropin-releasing hormone in discrete brain areas of goldfish (*Carassius auratus*). Peptides 9: 625–630

110 Zheng L-H, Pfaff DW, Scwanzek-Fukuda M (1992) Electron microscopic identification of luteinizing hormone-releasing hormone-immunoreactive neurons in the medial olfactory placode and basal forebrain of embryonic mice. Neuroscience 46: 407–418

Note added in proof

In a redent study, Licht *et al.* [111] identified only cGnRH-II and mGnRH in the brain of three species of *Rana* (*pipiens, ridibunda* and *esculenta*). While both GnRHs were distributed in the telencephalon and diencephalon, only cGnRH-II was found in the cerebellum and medulla. In the platyfish, lGnRH, together with mGnRH and sGnRH was revealed by ICC in some cells of the pituitary gland of animals of all ages [112].

111 Licht P, Tsai P-S, Sotowska-Brochocka J (1994) The nature and distribution of gonadotropin-releasing hormones in brains and plasma of ranid frogs. Gen Comp Endocrinol 94: 186–198

112 Magliulo-Cepriano L, Schreibman MP, Blum V (1994) Distribution of variant forms of immunoreactive gonadotropin-releasing hormone and β-gonadotropins I and II in the platyfish, *Xiphophorus maculatus*, from birth to sexual maturity. Gen Comp Endocrinol 94: 135–150.