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Gonadotropin-releasing Hormones (GnRH) in Fishes: Evolutionary Data on Their Structure, Localization, Regulation, and Function

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ABSTRACT

Maite Montero and Sylvie Dufour (1996) Gonadotropin-releasing hormones (GnRH) in fishes: evolutionary data on their structure, localization, regulation, and function. *Zoological Studies* **35**(3): 149-160. Since the discovery of GnRH in mammals (mGnRH), a family of homologous decapeptides has been characterized in lower vertebrates. One form (cGnRH-II) is present in all classes of gnathostomes, and coexists with another form exhibiting species-specific molecular variations. The highest diversity has been observed in fishes, where cGnRH-II coexists with dfGnRH in chondrichthyes, with mGnRH in primitive osteichthyes (including some primitive teleosts, the eels), with sGnRH in most teleosts, and with cfGnRH in catfishes; in addition, a 3rd form (sbGnRH) coexists with cGnRH-II and sGnRH in recent teleosts, the perciforms. Immunocytochemistry using highly specific antibodies or in situ hybridization demonstrates a differential distribution of GnRH peptides in teleosts as in other vertebrates: cGnRH-II is located in neurons of the midbrain tegmentum while the other GnRH forms are located in neurons of the anterior brain (olfactory bulbs, telence-

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phalon, and diencephalon). The coexistence of 2 GnRH systems raises the question of their respective physiological roles, particularly in regard to hypophysiotropic function. In teleosts, the adenohypophysis is directly innervated by hypophysiotropic neurons; GnRH from the anterior system (mGnRH, sGnRH, cfGnRH or sbGnRH) is the major molecular form innervating the pituitary. Also, depending on the species of teleost, cGnRH-II fibers contributing to the innervation of the pituitary may be absent, few or numerous, suggesting important differences between species in the hypophysiotropic role of cGnRH-II. Apart from their hypophysiotropic roles, GnRHs may also act as brain neuromediators in teleosts, as in all other vertebrates. Clues to the respective roles of the GnRH forms can also be obtained by comparing how they are regulated. The 2 GnRH forms showed opposite responses in eels subjected to experimental maturation or steroid treatments, with a large stimulation of mGnRH (as well as pituitary gonadotropin) levels, but a decrease in cGnRH-II levels. All these results suggest a major role for the anterior brain GnRH system in the hypophysiotropic control of gonadotropin.

Key words: Evolution, Neuropeptide, Teleost.

INTRODUCTION

In vertebrates, the production of gonadotropins by the pituitary is regulated by gonadotropin-releasing hormone (GnRH), a brain neuropeptide. The discovery of the coexistence of several molecular forms of GnRH in the same species of most vertebrates raises the question of their respective physiological roles.

MOLECULAR DIVERSITY OF GONADOTROPIN-RELEASING HORMONES (GnRHs)

The hypothalamic factor responsible for the control of luteinizing hormone (LH) secretion was first isolated and characterized in mammals (in pig by Matsuo et al. 1971, and in sheep by Amoss et al. 1971). A decapeptide, with the same primary structure was also characterized in several other mammalian species such as rat, mouse, and human (Adelman et al. 1986, Seeburg et al. 1987). This decapeptide, mGnRH (mammalian GnRH) was considered unique until the discovery of molecular variants in non-mammalian vertebrates (King and Millar 1979), and especially in fishes (Sherwood et al. 1983).

Molecular structure of GnRH peptides

Nine molecular forms of GnRH have been characterized (Fig. 1). The mammalian form (mGnRH), discovered in mammals, has also been found in

mGnRH (mammal)	pGlu-His-Trp-Ser-	Tyr-Gly-Leu-Arg-	Pro-Gly-NH2
cGnRH I (chicken)	pGlu-His-Trp-Ser-	Tyr-Gly-Leu-Gln-	Pro-Gly-NH2
cfGnRH (catfish)	pGlu-His-Trp-Ser-	His-Gly-Leu-Asn-	Pro-Gly-NH2
sbGnRH (sea bream)	pGlu-His-Trp-Ser-	Tyr-Gly-Leu-Ser-	Pro-Gly-NH2
sGnRH (salmon)	pGlu-His-Trp-Ser-	Tyr-Gly-Trp-Leu-	Pro-Gly-NH2
dfGnRH (dogfish)	pGlu-His-Trp-Ser-	His-Gly-Trp-Leu-	Pro-Gly-NH2
cGnRH-II (chicken)	pGlu-His-Trp-Ser-	His-Gly-Trp-Tyr-	Pro-Gly-NH2
lGnRH III (lamprey)	pGlu-His-Trp-Ser-	His-Asp-Trp-Lys-	Pro-Gly-NH2
lGnRH I (lamprey)	pGlu-His-	Tyr-	Ser-Leu-Glu-Trp-Lys-Pro-Gly-NH2

Fig. 1. Primary structure of vertebrate GnRHs, identified with the species in which they were first discovered (for review see: King and Millar 1992, Sower et al. 1993, Powell et al. 1994).

other vertebrates such as amphibians and some fish species. Two GnRH forms have been discovered in the chicken hypothalamus: chicken I GnRH (cGnRH-I) (King and Millar 1982a, b) and chicken II GnRH (cGnRH-II) (Miyamoto et al. 1984). Both avian forms are also present in reptiles.

The highest molecular diversity is exhibited by fish species, in which 4 molecular forms have been discovered: salmon GnRH (sGnRH) in *Oncorhynchus keta* (Sherwood et al. 1983), catfish GnRH (cfGnRH) in *Clarias macrocephalus* (Ngamvongchon et al. 1992), dogfish GnRH (dfGnRH) in *Squalus acanthias* (Lovejoy et al. 1992b), and most recently sea bream GnRH (sbGnRH) in *Sparus aurata* (Powell et al. 1994). In agnatha, 2 forms have been characterized: lamprey I GnRH (lGnRH-I) and lamprey III GnRH (lGnRH-III) in *Petromyzon marinus* (Sherwood et al. 1986, Sower et al. 1993).

These nine GnRHs are decapeptides with conserved residues in positions 1, 2, 4, 9, and 10. The amino acid in the 8th position is the most variable. The evolutionary conservation of the N- and C- termini indicates an important functional role for these regions. Indeed, use of numerous synthetic GnRH variants indicated that both ends are involved in interaction with the receptor (for review see: Karten and Rivier 1986, Millar and King 1994, Millar et al. 1994).

Phylogenetic distribution of GnRH forms and coexistence of several forms

Since GnRH is present in very low amounts in the brain, its purification and direct characterization by peptide sequencing requires much starting material, such as several kilograms of brain tissue, for instance, in the first studies in mammals (Amoss et al. 1971, Matsuo et al. 1971) and recent work in fish (Powell et al. 1994). Indirect approaches, based on the chromatographic behavior and antigenic properties of GnRH forms, are therefore often used to identify which forms are present in a given species, for instance in *Anguilla anguilla* (King et al. 1990a). These studies have led to the conclusion that there are examples of species from all vertebrate classes with at least two GnRH forms, and also suggest that in addition to the nine forms presently known, other GnRH forms are likely to exist.

The GnRH peptide family is present in the most ancient vertebrates, the agnatha (which first appeared about 500 million years ago), with two forms, lGnRH-I and lGnRH-III, characterized in lampreys (Sherwood et al. 1986, Millar and King 1994).

In all classes of gnathostomes, and in most species, cGnRH-II is present and coexists with another form exhibiting molecular variations which depend on the species. Among cartilaginous fishes, cGnRH-II alone has been found in the holocephalan (*Hydrolagus colliei* by Lovejoy et al. 1991), while in elasmobranchs, cGnRH-II and dfGnRH are present, together with other still uncharacterized forms (Lovejoy et al. 1992a, b, for review see: Millar and King 1994). Among bony fishes, cGnRH-II coexists with at least one other form: mGnRH, sGnRH, or cfGnRH (for review see: Sherwood and Lovejoy 1989, King and Millar 1992, Millar and King 1994). Recently, Powell et al. (1994) discovered that three forms (cGnRH-II, sGnRH, and sbGnRH in *Sparus aurata*) are present in some teleost species (perciforms).

Amphibians possess both cGnRH-II and mGnRH, with a possible unidentified additional form (King et al. 1994). In reptiles and birds, both cGnRH-I and cGnRH-II coexist, with the possible exception of snakes in which those studied to date have only been found to possess cGnRH-I (for review see: Millar and King 1994).

The coexistence of at least two GnRH forms in a single species of non-mammalian vertebrates triggered the search for various GnRH forms in mammals, which, at that time, were presumed to possess only one form, mGnRH. As a result, cGnRH-II was found, in addition to mGnRH, in several metatherian mammals (marsupials) as well as in some eutherian mammals (insectivores, an order often considered primitive among mammals) (King et al. 1989 1990b, Dellovade et al. 1993, Millar and King 1994). This suggests that the disappearance of cGnRH-II, if it had ever occurred, would have been a recent event, subsequent to the separation of marsupials and eutherian mammals.

Molecular evolution of GnRHs

In gnathostomes, two GnRH molecular lineages are present, probably deriving from gene duplication: on the one hand is the cGnRH-II lineage, which exhibits a total molecular conservation among vertebrates; on the other hand is the variable lineage (dfGnRH, mGnRH, sGnRH, cfGnRH, sbGnRH, cGnRH-I).

In the variable GnRH lineage, mGnRH is the form most highly represented in different vertebrate classes: this form is present in mammals, amphi-

bians, and some groups of fishes. Mammalian GnRH has indeed been detected in the evolutionarily old fish groups, near the tetrapod origin: in the lungfish (*Neoceratodus forsteri* by Joss et al. 1994), in the reedfish (*Calamoichthys calabaricus* by Sherwood et al. 1991), in chondrosteans (*Acipenser* spp. by Sherwood et al. 1991, Leprêtre et al. 1993), in the alligator gar (*Lepisosteus spatula* by Sherwood et al. 1991), in the bowfin (*Amia calva* by Millar and King 1994) and in more primitive teleosts (*Anguilla* spp. by Le Belle et al. 1988, King et al. 1990a). This suggests that mGnRH is the ancestral form of this lineage, at least since the evolution of bony fishes, and that molecular variants such as sGnRH and cGnRH-I may have been derived from the mGnRH gene by a few mutations. The origin of the mGnRH lineage could have been even earlier, as mGnRH has been detected in a cartilaginous fish (*Scyliorhinus canicula* by D'Antonio et al. 1995).

The salmon GnRH form likely appeared during the evolution of teleosts (probably with clupeocephalids): indeed, sGnRH is present in euteleosts and in a clupeid, but not in elopomorphs. The origin of catfish GnRH is still unknown: it has been suggested that this form arose from mGnRH after mutation of two amino acids (King and Millar 1992), but the phylogenetic position of silurids in relation to other groups of teleosts suggests that cfGnRH likely arose from sGnRH. The discovery of a 3rd GnRH form in perciforms, sbGnRH, which coexists with sGnRH and cGnRH-II, suggests the occurrence of a new duplication (of cGnRH-II or sGnRH gene) in this most recent group of teleosts.

Structure of GnRH precursors and genes

The sequences of complementary DNA (cDNA) were initially determined for mGnRH prohormone in various mammalian species (Seeburg and Adelman 1984, Adelman et al. 1986, Mason et al. 1986). The precursor is comprised of 3 domains: the signal sequence, the GnRH peptide, the proteolytic processing site and the GnRH-associated peptide (GAP). In other tetrapods, the cDNAs coding for cGnRH-I and mGnRH precursors were respectively characterized in the chicken (Dunn et al. 1993) and in the clawed toad (Hayes et al. 1994).

In teleosts, the cDNA coding for the sGnRH precursor was determined in salmonids, *Salmo salar* (Klungland et al. 1992) and *Oncorhynchus masou* (Suzuki et al. 1992), and also in a cichlid fish, *Haplochromis burtoni* (Bond et al. 1991); the cDNA coding for the cfGnRH precursor was sequenced in a silurid, *Clarias gariepinus* (Bogerd et al. 1994).

The comparison of GAP sequences shows a very low conservation between tetrapod GAPs and the GAP associated with cfGnRH, and no common amino acids with the GAP associated with sGnRH. There is some sequence identity between the GAP associated with sGnRH in cichlids and salmonids. These data indicate that GAP sequences are considerably less conserved across the phylogenetic spectrum compared to their GnRH partners. This suggests that the conservation of the biological role of GAP may be under fewer constraints than that of GnRH. Sherwood et al. (1993) suggested that full-length transcripts are required for effective processing of GnRH peptides. The evolutionary data confirm the hypothesis of a role of GAP in the structure and stability of the GnRH precursor rather than a function as a biologically active peptide (Andersen and Klungland 1993).

Recently, in addition to the sGnRH precursor, a 2nd cDNA corresponding to the cGnRH-II form was sequenced in a cichlid fish, *Haplochromis burtoni* (White et al. 1994). This precursor is comprised of the signal sequence, the cGnRH-II peptide, the processing site, and the GAP sequence in which the authors suspect the existence of a 2nd processing site leading to the formation of 2 GAPs. However, the determination of the cGnRH-II precursor in another teleost, a silurid fish, *Clarias gariepinus* (Bogerd et al. 1994), did not confirm this hypothesis of the existence of 2 GAPs; the 2nd potential processing site not being present. These studies in teleosts established that the 2 coexisting GnRH forms (sGnRH/cfGnRH and cGnRH-II) were encoded by 2 different genes, and not by a unique gene. These data corroborate the hypothesis that these 2 molecular lineages originated by gene duplication.

The mGnRH gene has been characterized in some mammals (Seeburg and Adelman 1984, Adelman et al. 1986, Mason et al. 1986). In non-mammalian species, the sGnRH gene has been cloned in a salmonid, *Salmo salar* (Klungland et al. 1992), and cGnRH-I in the chicken (Dunn et al. 1993, for review see: Andersen and Klungland 1993). All genes contain 4 exons. The comparison of fish, bird, and mammal genes shows that even if the global structure of GnRH gene is relatively complex and old, it is well conserved: the sequence signal, the GnRH peptide and the GAP are always encoded by the same exons, only an increase in the intron length being observed during the evolution from fishes to mammals (for review see: Andersen and Klungland 1993). The

cGnRH-II gene has not yet been characterized in any species.

LOCALIZATION OF GnRH CELLS IN BRAIN

The coexistence of cGnRH-II with 1 or several of the other GnRH molecular forms appears to be a general, well-conserved feature among chondrichthyes, osteichthyes, and all groups of tetrapods. This raises the question of the respective neuronal distribution of these forms. Several immunocytochemical and radioimmunological studies, using specific antibodies for each form, have been carried out to address this question.

Differential distribution

Immunocytochemical studies of the comparative distribution of distinct GnRH forms, using specific antibodies, were performed in some species, including primitive eutherian mammals (Dellovade et al. 1993, Millar and King 1994), birds (Mikami et al. 1988, Millam et al. 1989), reptiles (Masucci et al. 1992, Tsai and Licht 1993), amphibians (D'Aniello et al. 1991, Conlon et al. 1993, Muske and Moore 1994), and bony fishes (for review see: Kah et al. 1993).

In fish, the immunocytochemical studies of the distribution of distinct GnRH forms were concerned with the distribution of mGnRH and cGnRH-II in a chondrostean, *Acipenser baeri* (Leprêtre et al. 1993), and in a primitive teleost, *Anguilla anguilla* (Montero et al. 1994), and the distribution of sGnRH and cGnRH-II in a salmonid teleost, *Oncorhynchus masou* (Amano et al. 1991). Radioimmunological studies have been used to define the distribution of sGnRH and cGnRH-II in a cyprinid, *Carassius auratus* (Yu et al. 1988), and in a salmonid, *Oncorhynchus mykiss* (Okuzawa et al. 1990), and the distribution of mGnRH and cGnRH-II in *Anguilla anguilla* (Dufour et al. 1993).

Recently, in situ hybridization was applied to brains of several teleosts: studies investigated the localization of sGnRH and cGnRH-II mRNAs in a cichlid fish, *Haplochromis burtoni* (White et al. 1994), that of cfGnRH and cGnRH-II mRNAs in a silurid fish, *Clarias gariepinus* (Bogerd et al. 1994), and that of sGnRH mRNA in 2 salmonids, *Oncorhynchus mykiss* and *Salmo salar* (Bailhache et al. 1994).

Most of the specific studies performed in various vertebrates demonstrated that the various GnRH forms coexisting in the same species are expressed in different neurons (for review see: Muske 1993). Some immunocytochemical studies showed a co-localization of the various GnRH forms in the same neurons (for example, the coexistence of cfGnRH and cGnRH-II in *Clarias gariepinus* by Schulz et al. 1993). However, such co-localizations are possibly due to cross reactions leading to artifacts of localization of immunoreactive structures, as demonstrated by various studies in urodele amphibians (Muske 1993), and by in situ hybridization in *Clarias gariepinus* (Bogerd et al. 1994).

"Anterior" and "posterior" GnRH systems

The cGnRH-II form seems to be strictly localized in cell bodies of the anterior mesencephalon or posterior diencephalon, representing the "posterior" GnRH system according to Muske (1993): in fishes (for review see: Kah et al. 1993, Montero et al. 1994), urodele amphibians (Muske and Moore 1994), birds (Mikami et al. 1988), and mammals (Dellovade et al. 1993). The projections of these cGnRH-II neurons are mainly localized in extrahypophysiotropic areas (particularly in the posterior brain and in the medulla), but may also innervate the hypophysiotropic area in some species (see below).

The other GnRH forms, corresponding to the lineage of GnRH variants in gnathostomes, have been detected in neurons from the olfactory bulbs, telencephalon and diencephalon, forming the "anterior" GnRH system (Muske 1993): this system corresponds to the septo-preoptico-infundibular system in mammals. The major part of GnRH projections to the pituitary (in teleosts) or to the median eminence (in tetrapods) comes from the neurons of this anterior system, but these neurons also send projections to various extrahypophysiotropic areas.

Ontogenesis

Ontogenic data in mammals demonstrate that mGnRH neurons originate from olfactory placodes and migrate into the brain during embryogenesis (Schwanzel-Fukuda and Silverman 1980, Schwanzel-Fukuda and Pfaff 1991). Some data from other tetrapods (in the chicken by Murakami et al. 1991, in the axolotl by Northcutt and Muske 1994), and in fish (in the salmon by Chiba et al. 1994) suggest a similar origin (from olfactory placodes) for GnRH neurons of the anterior system.

Concerning the posterior system, Northcutt

and Muske (1994) recently demonstrated that the ablation of olfactory placodes of an amphibian embryo (the axolotl, *Ambystoma mexicanum*) prevented the appearance of mGnRH neurons, but not that of cGnRH-II neurons. These authors hypothesized that cGnRH-II neurons, which do not come from olfactory placodes, could have a periventricular origin. Thus, the first data on comparative ontogenesis confirm the difference between the anterior and the posterior GnRH systems.

DUAL ROLES OF GnRHs; HYPOPHYSIOTROPIC NEUROHORMONES AND BRAIN NEUROMEDIATORS

The coexistence of 2 GnRH systems raises the question of their respective physiological roles, particularly in regard to hypophysiotropic function.

As pituitary GnRH receptors in lower vertebrates generally have a low specificity towards different GnRH molecular forms (for review see: Millar et al. 1994), clues to the respective roles of GnRHs are provided by their differential projections towards the hypophysiotropic area, combined with data on their action on gonadotropic function.

Pituitary and brain projections of GnRH anterior and posterior systems

In teleosts, the adenohypophysis is directly innervated by hypophysiotropic neurons (for review see: Ball 1981, Peter et al. 1990), corresponding to the median eminence in tetrapods.

The anterior system, expressing various molecular forms (mGnRH, sGnRH, cfGnRH, sbGnRH, cGnRH-I), is always found to innervate the median eminence in tetrapods or its pituitary equivalent in teleosts. Thus, the anterior system plays a major role in the control of the pituitary. In addition to these pituitary projections, the anterior system also projects widely to various areas of the brain, indicating an important role of these molecular GnRH forms as brain neuromediators.

As to the posterior system and its potential hypophysiotropic role, differences are found among vertebrates: in birds and mammals, cGnRH-II neurons do not innervate the median eminence and are therefore not involved in the direct control of the pituitary (Mikami et al. 1988, Dellovade et al. 1993); while in teleosts, various situations are observed. In the goldfish, cGnRH-II may be as abundant as sGnRH in the pituitary (Yu et al. 1988); in the catfish, as in the eel, cGnRH-II is present in the pituitary but much less abundant than the other GnRH form (Dufour et al. 1993, Schulz et al. 1993); in contrast, no cGnRH-II fibers at all are detected in the pituitary of salmonids, where only sGnRH is present (in the masu salmon by Amano et al. 1991, in the rainbow trout by Anglade 1994). These observations suggest important variations among teleost species in the potential role of cGnRH-II as a hypophysiotropic neurohormone. Regarding its role as a brain neuromediator, cGnRH-II projections to different areas of the brain (particularly, the posterior area) have been observed in all cases.

Hypophysiotropic action on gonadotropin (GTH) secretion in teleosts

To address the role of the various GnRH forms on gonadotropic function, parallel studies of their pituitary levels and of their respective abilities to stimulate GTH secretion have been used.

In the goldfish, sGnRH and cGnRH-II are equally effective in stimulating in vitro GTH release (Chang et al. 1990) and they have similar pituitary levels (Yu et al. 1988). This indicates that these two forms possess the same potential role in regulating gonadotropic function. In contrast, in salmonids, while sGnRH and cGnRH-II are also both active on in vitro GTH release (Blaise et al. 1995), the absence of cGnRH-II in the pituitary suggests that this form is not directly involved in the control of gonadotropic function (Amano et al. 1991, Anglade 1994).

An intermediate situation is observed in the eel: mGnRH and cGnRH-II possess the same activity on in vitro GTH release (Montero 1995), but mGnRH is more abundant than cGnRH-II in the pituitary (Dufour et al. 1993). We may therefore assign to mGnRH a major role in the control of the gonadotropic function in this species. However, as both neuropeptides are present in the pituitary, a potential role of cGnRH-II can not be excluded.

In the catfish, a different situation is observed since cGnRH-II is much more active (> 100 times) than cfGnRH in stimulating in vitro GTH release. Binding studies showed that this difference in biological potency results from different affinities for the pituitary receptor (Schulz et al. 1993). It is interesting to note that such a high specificity of the pituitary GnRH receptor was not found in other non-mammalian vertebrates. As catfish GnRH receptor is able to bind with equal affinity not only

cGnRH-II, but also molecular forms such as sGnRH and mGnRH (Schulz et al. 1993), we propose that the apparent selective specificity of catfish receptor towards cGnRH-II could be due to a low recognition of the novel form cfGnRH rather than to a special high specificity. Considering their hypophysiotropic roles, although cfGnRH is more abundant than cGnRH-II in the pituitary, the 2 GnRH forms are still potentially able to play a role in the direct control of gonadotropic function (Schulz et al. 1993).

Studies of pituitary levels and relative abilities to stimulate GTH release by different GnRH forms coexisting in the same species indicate variations in their respective roles in the control of gonadotropic function among teleosts. However, in most teleosts, the results of these studies may be insufficient, and clues to the respective roles of GnRH forms may also be provided by comparing how they are regulated (see below).

Hypophysiotropic action on growth hormone (GH) secretion in teleosts

In teleosts, in addition to the control of pituitary gonadotrophs, GnRHs may also act on somatotrophs. In the goldfish, the 2 GnRH forms (sGnRH and cGnRH-II) are not only able to stimulate GH release in vivo (after GnRH administration), but also in vitro (on primary cultures of pituitary cells) (Marchant et al. 1989, Chang et al. 1990 1993, Wong et al. 1993). In this fish species, GnRH was shown, with the aid of electron microscopy, to bind not only to gonadotrophs but also to somatotrophs (Cook et al. 1991), suggesting the presence of GnRH receptors on somatotrophs. The stimulation of GH release by GnRH was also observed in another cyprinid fish, *Cyprinus carpio* (Lin et al. 1993) as well as in a cichlid fish, *Oreochromis* sp. (Melamed et al. 1995). In contrast, GnRHs were not able to stimulate GH release in a salmonid fish, *Oncorhynchus mykiss* (Blaise et al. 1995), except under the potentiating effect of IGF (insulin-like growth factor) (Blaise 1995). These data show that the importance of the role of GnRH in the control of GH release may vary among teleosts.

A direct effect of GnRH on GH secretion has not been seen in other vertebrate classes, including mammals, and this role may be limited to some teleost species. However, the occurrence of such a phenomenon may be found in some pathological situations in human (Faglia et al. 1973, Dickerman et al. 1981).

DIFFERENTIAL REGULATION OF GnRHs

Until now, very few experimental studies on the differential regulation of various GnRH forms have been available, and they have been concerned with the regulation by sex steroids of cGnRH-I and cGnRH-II in bird species (Sharp et al. 1990, Wilson et al. 1990) and of mGnRH and cGnRH-II in a teleost (the eel, *Anguilla anguilla* L. by Dufour et al. 1993, Montero et al. 1995), using highly specific radioimmunoassays (RIA).

Effects of experimental sexual maturation

In the eel, experimental sexual maturation can be induced by gonadotropic treatment (for instance, injection of carp pituitary extract, in the female), which stimulates both gametogenesis and steroidogenesis. Under the effect of endogenous steroids, both pituitary gonadotropin levels and brain and pituitary mGnRH levels increase (Dufour et al. 1989 1993). In contrast, cGnRH-II levels decrease (Dufour et al. 1993). This indicates a differential regulation of mGnRH and cGnRH-II during sexual maturation in the eel. A differential regulation of the 2 GnRH forms was also observed in the goldfish and the salmon, with an increase in sGnRH but not cGnRH-II in the anterior brain and in the pituitary during natural sexual maturation (Amano et al. 1992, Rosenblum et al. 1994).

These results suggest a principal role for the anterior brain GnRH form (mGnRH in the eel, sGnRH in cyprinids or salmonids) in the hypophysiotropic control of gonadotropin function.

Effects of steroid treatments

In the eel, treatments with various steroids indicate a positive and estrogen-dependent control of mGnRH (as well as of pituitary GTH), and a negative and androgen-dependent control of cGnRH-II (Montero et al. 1995). These results, which agree with data on experimental sexual maturation, suggest the existence of a mGnRH-GTH axis under positive feedback of steroids (estrogen-dependent) during sexual maturation (Montero et al. 1995).

Such a positive feedback of sex steroids has also been demonstrated in juvenile salmonids, suggesting the existence of a sGnRH-GTH axis: a positive effect on GTH synthesis has been found by RIA in the rainbow trout, *Oncorhynchus mykiss* (Crim and Evans 1979, Goos et al. 1986), in the Atlantic salmon, *Salmo salar* (Crim and Peter 1978),

and by northern blot analysis in the salmon *Oncorhynchus tschawytscha* (Xiong et al. 1994). A positive effect on GnRH synthesis has been found by biological assay in *Oncorhynchus mykiss* (Goos et al. 1986) and by in situ hybridization (sGnRH specific) in *Oncorhynchus masou* (Amano et al. 1994).

This important positive feedback of sex steroids on the mGnRH-GTH axis in the eel or the sGnRH-GTH axis in juvenile salmonids could play a major role as an amplifier in the pubertal stimulation of gonadotropic function in these species.

Data on regulation of cGnRH-II by sex steroids have only been obtained in the eel. The negative control of cGnRH-II by sex steroids allows us to exclude the involvement of this form in the stimulation of gonadotropic function during sexual maturation (Montero et al. 1995).

CONCLUSIONS

In teleosts, as in most gnathostomes, 2 distinct GnRH neuronal systems are present, exhibiting differences in the molecular forms expressed, in the localization of their cell bodies and projections, and in their regulation. The anterior system corresponds to cell bodies located in the olfactory bulbs, telencephalon and diencephalon, and expresses various GnRHs among teleosts (mGnRH, sGnRH, cfGnRH, sbGnRH), but this system always plays a major role in the direct control of pituitary gonadotropic function. The posterior system corresponds to cell bodies located in the mesencephalon. This system always expresses the same molecular form, cGnRH-II, but it exhibits large differences in its potential role in the control of gonadotropic function, even among teleosts. Complementary approaches including characterization of the different molecular forms expressed, studies of their localizations, biological activities, and regulations are required to understand their respective physiological roles in a given species.

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魚類性釋素：結構，分佈，調節及功能之進化

Maite Montero[1] and Sylvie Dufour[1]

自哺乳類性釋素(mGnRH)被發現以來，其他非哺乳類之性釋素，也紛紛在其他低等脊椎動物中被發現與確認。而魚類性釋素種類與型態變異最大，有頷上綱（軟骨魚綱及硬骨魚綱）都有雞二型性釋素(cGnRH-II)；另外還共存其他型類之性釋素：在軟骨魚是和沙魚型性釋素(dfGnRH)；原始硬骨魚，（例如：鰻魚），則是與哺乳類型性釋素(mGnRH)；但大部分的硬骨魚卻是和鮭型性釋素(sGnRH)；鯰魚則爲鯰型性釋素(cfGnRH)；另外，在鱸形目，除雞二型性釋素，鮭型性釋素之外，尚有第三型，即鯛型性釋素(sbGnRH)。使用細胞免疫化學或原位雜交技術，已闡明性釋素在腦部之分佈位置。在硬骨魚及其他脊椎動物：雞二型性釋素，分佈於中腦蓋(Tegmentum)，而其他類型性釋素則分佈於前腦（嗅球，終腦及間腦）。此兩大類型性釋素分佈系統共存於腦部，似乎應有其不同生理功能，尤其對腦垂腺之調節。在硬骨魚類之腦垂體中，有控制神經分佈；前腦系統之性釋素(mGnRH, sGnRH, cfGnRH 或 sbGnRH)；是腦垂體中之主要性釋素。另外，視硬骨魚種別而定：腦垂體沒有或含有少數雞二型性釋素神經纖維，因此提示該型性釋素，在不同種類，具有不同的重要性，除調控腦垂體激素分泌角色之外，在硬骨魚類及脊椎動物，GnRHs也擔任神經傳遞之功能。不同類型性釋素之角色，可由其表現之調控而定。從鰻魚的研究中，發現調控兩型性釋素的生成剛好相反；以類固醇激素處理或經由誘導成熟，發現哺乳類型性釋素（以及腦垂體性腺促素）生成較多，而雞二型性釋素生成量則較少。由這些試驗結果提示調控腦垂體性腺促素，主要由腦前端性釋素系統所控制。

關鍵詞：進化，神經胜肽，硬骨魚。

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