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Multiple Gonadotropin-Releasing Hormone Neuronal Systems in Vertebrates

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Gonadotropin-releasing hormone (GnRH) was originally isolated as a hypothalamic peptide that regulates reproduction by stimulating the release of gonadotropins. Using comparative animal models has led to the discovery that GnRH has a more ancient evolutionary origin. During evolution, GnRH peptide underwent gene duplication and structural changes to give rise to multiple molecular forms of GnRHs. Mammalian GnRH initially considered to be the sole molecular form, is now grouped as a family of peptides along with GnRH variants determined from representatives in all classes of vertebrates. Vertebrate species, including primates and humans have more than one GnRH variant in individual brains; a unique GnRH form in the forebrain and chicken II GnRH in the midbrain. Furthermore, several species of bony fish have three molecular variants of GnRH: salmon GnRH, seabream GnRH and chicken II GnRH. Also, it has been shown that in addition to the olfactory placodes and the midbrain, there is a third embryonic source of GnRH neurons from the basal diencephalon in birds and fish, which might be true for other vertebrates. Therefore, comparative animal models like fish with discrete sites of expression of three molecular variants of GnRH in individual brains, could provide insight into novel functions of GnRH variants, conservation of gene regulation, and mechanisms governing reproduction in vertebrates.

During vertebrate evolution, the primary structure of gonadotropin-releasing hormone (GnRH), has been remarkably conserved, suggesting that its functions are of utmost importance. GnRH represents the first step in a cascade of events coordinating the complex physiology of reproduction and reproductive behavior in vertebrates. Variant forms of GnRH have been isolated from the brains of non-mammalian vertebrates, and more than one form exist in individual brains (King and Millar, 1997; Sherwood et al., 1997). Recent research has also demonstrated the existence of three distinct receptors, which have evolved in conjunction with three distinct GnRH ligand classes present in many vertebrates (Troskie et al., 1998), and it has become increasingly clear that GnRH has other functions in addition to stimulating the release of gonadotropins. The present review discusses the multiplicity of GnRH, multiple embryonic sources of GnRH neurons, multiple roles of GnRH molecular variants and the usefulness of comparative animal models to gain insights into the physiology of production, regulation and functions of GnRH molecules.

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GnRH molecular variants

At present, there are ten distinct forms of GnRHs that have been isolated from vertebrates and two from protochordates (Jimenez-Linan et al., 1997; Sherwood et al., 1997; Sower, 1997). All known forms are ten amino acids in length (Fig. 1). The most common structural variation among the different forms of GnRHs occur frequently in amino acid positions 5-8. However, the essential molecular sequences at the ends of the decapeptide, the pyroglutamyl-modified amino-terminus and the amidated carboxy-terminus have remained unchanged during evolution. Evidence suggests that in most species from all the major vertebrate groups, chicken II GnRH is the most highly conserved form, while the second or the third form (seabream GnRH in some bony fish) vary structurally in different species. The fact that more than one GnRH molecule existence in protocodates (tunicates) and jawless fish (lampreys: Sherwood et al., 1997; Sower, 1997), strengthens the evidence that the presence of multiple forms of GnRH in a single species is an ancient pattern in evolution (Fig. 2).

Genes encoding the different GnRH forms have the same architecture of four exons separated by three introns and this has been highly conserved throughout evolution, despite changes in the size and sequences of exons and introns. In each gene, the first exon

GnRH	1	2	3	4	5	6	7	8	9	10	
TUNICATE I	pGLU	HIS	TRP	SER	**ASP**	**TYR**	**PHE**	**LYS**	PRO	GLY	HN2
TUNICATE II	pGLU	HIS	TRP	SER	**LEU**	**CYS**	**HIS**	**ALA**	PRO	GLY	HN2
LAMPREY I	pGLU	HIS	**TYR**	SER	**LEU**	**GLU**	**TRP**	**LYS**	PRO	GLY	HN2
LAMPREY III	pGLU	HIS	TRP	SER	**HIS**	**ASP**	**TRP**	**LYS**	PRO	GLY	HN2
DOGFISH	pGLU	HIS	TRP	SER	**HIS**	GLY	**TRP**	**LEU**	PRO	GLY	HN2
CATFISH I	pGLU	HIS	TRP	SER	**HIS**	GLY	LEU	**ASN**	PRO	GLY	HN2
SALMON	pGLU	HIS	TRP	SER	TYR	GLY	**TRP**	**LEU**	PRO	GLY	HN2
SEABREAM	pGLU	HIS	TRP	SER	TYR	GLY	LEU	**SER**	PRO	GLY	HN2
CHICKEN I	pGLU	HIS	TRP	SER	TYR	GLY	LEU	**GLN**	PRO	GLY	HN2
CHICKEN II	pGLU	HIS	TRP	SER	**HIS**	GLY	**TRP**	**TYR**	PRO	GLY	HN2
GUINEA PIG	pGLU	**TYR**	TRP	SER	TYR	GLY	**VAL**	ARG	PRO	GLY	HN2
MAMMAL	pGLU	HIS	TRP	SER	TYR	GLY	LEU	ARG	PRO	GLY	HN2

Fig. 1. Comparison of twelve primary structures of GnRH peptides isolated from various species. Amino acids in bold letters indicate variation with respect to the mammalian GnRH (see Sherwood et al., 1997; Jimenez-Linan et al., 1997).

encodes the 5′-untranslated region; the second and third exons encode the signal peptide, GnRH decapeptide, a proteolytic cleavage site and the GnRH-associated peptide (GAP); and the fourth exon has the carboxy terminus of GAP and the 3′-untranslated region. Comparisons between the cDNA precursors encoding the distinct members of the GnRH family show complete conservation of the proteolytic cleavage site, high homology of the decapeptide, but high structural divergence in the GAP region (see Sherwood et al., 1997; Yu et al., 1997). The cDNAs encoding precursors for the mammalian GnRH preprohormone have been determined in an amphibian and in mammals (Adelman et al., 1986; Hayes et al., 1994); chicken I GnRH precursor from birds (Dunn et al., 1993); chicken II GnRH precursor from bony fish and mammals including humans (Bogerd et al., 1994; White et al., 1995; Kasten et al., 1996; White et al., 1998); salmon GnRH precursor from several species of bony fish including salmonids (Bond et al., 1991; Klungland et al., 1992; Suzuki et al., 1992); seabream GnRH precursor from bony fish (White et al., 1995; Gothilf et al., 1996) and catfish GnRH precursor from the African catfish (Bogerd et al., 1994). In some vertebrates, more than one cDNA encoding GnRH precursors have been sequenced from the brain of a single species. For example, mammals (tree shrew, humans) express mammalian GnRH and chicken II GnRH (Kasten et al., 1996; Lescheid et al., 1997; White et al., 1998) and some species of bony fish contain three GnRH molecules (salmon GnRH, chicken II GnRH, seabream GnRH: White et al., 1995; Gothilf et al. 1996). Therefore, identification by chemical sequence analysis and molecular cloning confirmed GnRH as a family of structurally related peptides.

SPECIES	GnRH TYPE										
	M	CII	CI	SB	CF	S	DF	LI	LIII	TI	TII
PROTOCHORDATES											
Tunicate										TI	TII
JAWLESS FISH											
Lampery								LI	LIII		
CARTILAGINOUS FISH											
Dogfish		CII					DF				
BONY FISH						S					
Salmon		CII									
Catfish		CII			CF	S					
Seabream/Cichlid		CII		SB							
AMPHIBIANS											
Salamander	M	CII				S					
REPTILES											
Alligator		CII	CI								
BIRDS											
Chicken		CII	CI								
MAMMALS											
Rat/Mouse	M	CII									
Musk shrew	M	CII									
Human	M	CII									

Fig. 2. Phylogenetic distribution of GnRHs. M; mammalian GnRH, CII; chicken II GnRH, CI; chicken I GnRH, SB; seabream GnRH, CF; catfish GnRH, S; salmon GnRH, DF; dogfish GnRH, LI; lamprey I GnRH, LIII; lamprey III GnRH, TI; tunicate I GnRH, TII; tunicate II GnRH (see Sherwood et al., 1997; Sower, 1997; King and Millar 1997; Chen et al., 1998; White et al., 1998).

Comparative studies demonstrate a remarkable similarity, across most vertebrates, in the general distribution pattern of GnRH cell bodies and fiber pathways, suggesting that these systems are phylogenetically conserved (Muske, 1997; Sherwood et al., 1997). Localization studies have demonstrated differential distribution of GnRH molecular variants in distinct brain areas (Fig. 3). Chicken II GnRH neuronal system is the conserved GnRH system present in the midbrain of most bony fish, amphibians, reptiles, birds and mammals (Muske, 1997). A second neuronal group in the basal forebrain of all vertebrate classes express unique GnRH form in each species: mammalian GnRH in mammals, catfish GnRH in catfish, salmon GnRH (terminal nerve) and seabream GnRH (preoptic area) in the bony fish (Parhar, 1997).

Embryonic sources of GnRH subtypes

The embryonic origin of terminal nerve GnRH neurons from the olfactory placode and their subsequent migration into the forebrain is unique among brain cells of some mammalian and non-mammalian species of vertebrates (Schwanzel-Fukuda and Pfaff, 1989; Muske, 1997; Parhar, 1997). During migration, the terminal nerve GnRH neurons are strongly associated with fibers immunoreactive for polysialylated form of neural cell adhesion molecules (PSA-NCAM) in rodents and in birds (Murakami et al., 1991; Schwanzel-Fukuda, 1997). The same set of cells with developmental origins in the olfactory placodes is supported by the loss of basal forebrain- preoptic GnRH neurons, following ablations of the olfactory placodes in amphibians and birds (Murakami et al., 1992; Northcutt and Muske, 1994), and microinjections of NCAM antibodies into the olfactory placodes in mice (Schwanzel-Fukuda, 1997).

The lack of specific markers in most vertebrates do not allow distinction between GnRH neurons associated with the terminal nerve and subpopulations of preoptic GnRH neurons. However, in mammals, there is compelling evidence that there exists more than one population of preoptic GnRH neurons. For example, under

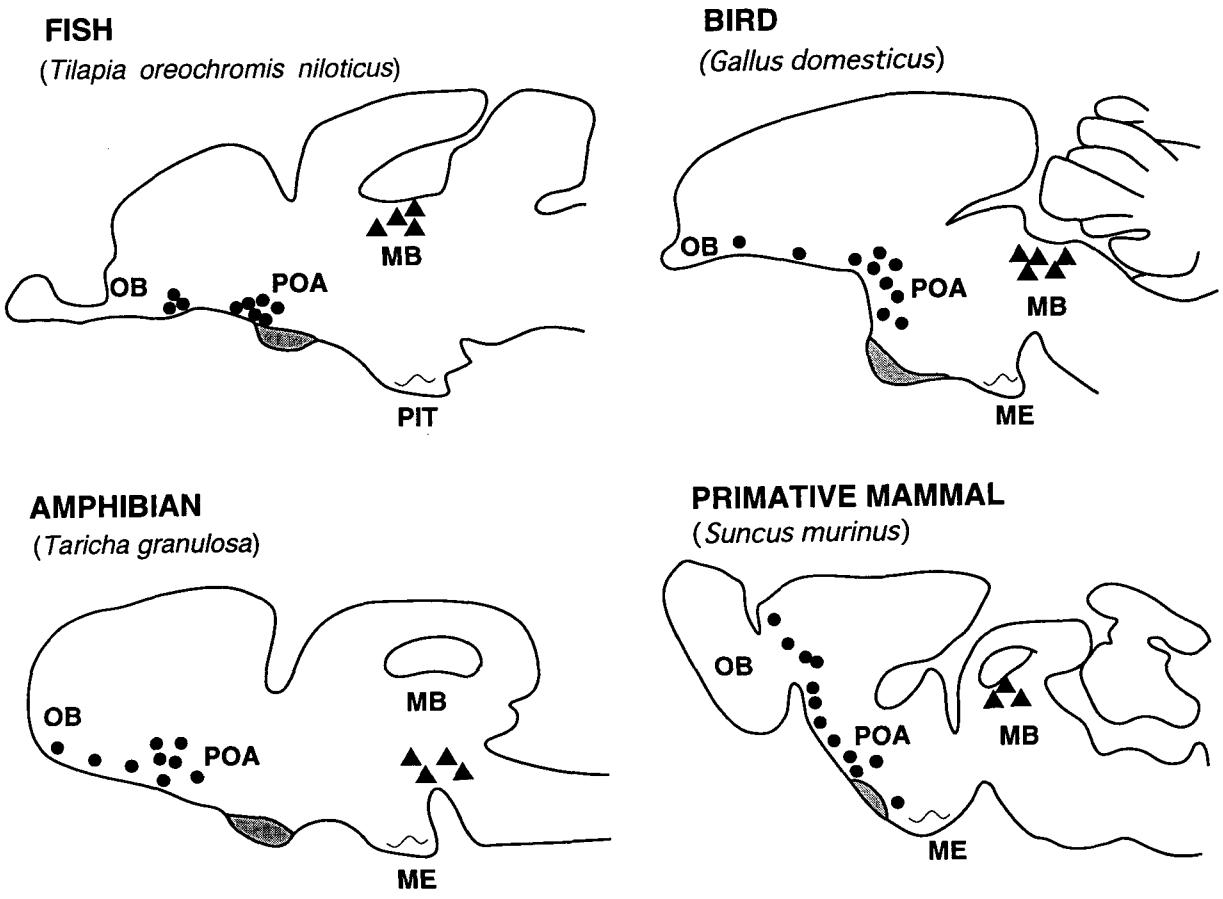

Fig. 3. Diagrammatic representations of sagittal brain sections illustrating the location of GnRH neurons in *Oreochromis niloticus* (Parhar, 1997), *Taricha granulosa* (Muske and Moore, 1994), *Gallus domesticus* (Millam et al., 1993), *Suncus murinus* (Rissman et al., 1995). Unique GnRH forms have been localized in the forebrain and chicken II GnRH in the mid/hindbrain (see Sherwood et al., 1997; Muske, 1997). MB, midbrain; ME, median eminence; OB, olfactory bulb; PIT, pituitary; POA, preoptic area.

certain physiological states only a subpopulation of preoptic GnRH neurons express galanin (Merchenthaler et al., 1991) or c-Fos (Lee et al., 1990). Furthermore, not all GnRH neurons of the septum-preoptic area are under gonadal steroid influence (Herbison, 1998). Therefore, in mammals, it is possible that subpopulations of preoptic GnRH neurons could originate from progenitor cells in the olfactory placodes (Quanbeck et al., 1997). Conversely, subpopulations of preoptic GnRH neurons could have developmental origins from different embryonic sites, the olfactory placodes, and the basal diencephalon. This, however, remains to be tested.

In fish and in birds several lines of evidence suggest that the preoptic GnRH neurons have an independent origin and are different from GnRH neurons of the terminal nerve-nucleus olfactoretinalis: (1) the lampreys lack a terminal nerve and terminal nerve GnRH neurons but not preoptic GnRH neurons (Eisthen and Northcutt, 1996); (2) the lack of preoptic but not the terminal nerve GnRH neurons in the medaka provide convincing evidence that the preoptic and the terminal nerve GnRH neurons do not share a common progenitor cell (Parhar et al., 1998); (3) developmental studies have shown preoptic GnRH neurons originating from the rostrobasal preoptic region and the organum vasculosum of the lamina terminalis (Norgren and Chen, 1994; Parhar and Sakuma, 1995; Tobet et al., 1996; Parhar, 1997); (4) differentiated preoptic GnRH neurons are small, fusiform in shape and scattered, whereas undifferentiated and differentiated GnRH neurons of the terminal nerve-nucleus olfactoretinalis are large, round in shape and present as clusters (ganglia); (5) in many species of bony fish, the terminal nerve GnRH neurons but not the preoptic GnRH neurons co-express molluscan cardioexcitatory tetrapeptide (FMRFamide: Stell et al., 1984) and (6) the newly discovered cDNA sequence of preoptic specific seabream GnRH is different from the terminal nerve-nucleus olfactoretinalis specific salmon GnRH cDNA sequence (White et al., 1995; Gothilf et al., 1996). Furthermore, the GnRH neurons of the terminal nerve-nucleus olfactoretinalis develop during early embryonic life, these cells are developmentally regulated, serve as neuromodulators and might play no role in reproduction (Parhar, 1997). On the other hand, the expression of preoptic GnRH neurons coincides with

various stages of sexual differentiation, maturation and smoltification-metamorphosis (Hayes et al., 1994; Parhar and Iwata, 1996; Parhar, 1997). Preoptic GnRH neurons are regulated by sex steroids, serve as a hypophysiotropic hormone and play an important role in reproduction.

The chicken II GnRH neuronal system is an ancient highly conserved GnRH system reported in the midbrain of bony fish, amphibians, reptiles, birds and mammals (see reviews by Muske, 1997; Parhar, 1997; Sherwood et al., 1997). From early developmental stages, the presence of midbrain GnRH neurons close to the ventricles, suggests that they differentiate from precursor cells in the ventricular ependyma (Parhar, 1997). The expression of chicken II GnRH mRNA and peptide in the midbrain argues that these neurons are different from the terminal nerve salmon GnRH and preoptic seabream GnRH synthesizing neurons. Furthermore, ablations of the olfactory placodes in a urodele amphibian results in bilateral loss of the terminal nerve-anterobasal forebrain GnRH neurons but not the midbrain chicken II GnRH, demonstrating that the chicken II GnRH neurons do not originate from the olfactory placodes (Northcutt and Muske, 1994).

The three different populations of GnRH-containing neurons, in certain species of bony fish, have three separate embryonic origins: salmon GnRH from the olfactory placodes; seabream GnRH from the preoptic area; chicken II GnRH from the ependymal cells of the third ventricle, which might also be true for other vertebrates species (Parhar, 1997; Parhar et al., 1998).

Multiple targets and biological activities of GnRH

In mammals, GnRH from hypothalamic neurons reaches the pituitary gland through a specialized portal system to induce the synthesis and secretion of gonadotropic hormones but in bony fish, morphological evidence support direct pituitary innervation by salmon-, seabream-, chicken II-, and mammalian-GnRH nerve terminals (Parhar and Iwata, 1994; Parhar et al., 1995; Parhar, 1997) which regulate pituitary functions. In mammals, birds, reptiles, amphibians, and bony fish, all known GnRH variants (mammalian-, chicken I and II-, salmon-, catfish-, dogfish-, seabream-, and lamprey-GnRH) can stimulate *gonadotropin* release to some degree across species (King and Millar, 1997; Sherwood et al., 1997). GnRH variants have also been shown to regulate the release of *growth hormone* and *prolactin* in mammals and in some species of fish (Sherwood et al., 1997; Weber et al., 1997; Yu et al., 1997). The isolation of three distinct GnRH receptors which apparently have evolved in conjunction with three distinct GnRH ligands may help to explain the regulation of different pituitary endocrine cells by GnRH variants in the future (Troskie et al., 1998).

GnRH may have originated as a regulator of reproduction, however, during evolution GnRH variants have also acquired non-reproductive and extra-pituitary functions. For example, within the central nervous system, GnRH acts as a neurotransmitter or neuromodulator, and has effects on reproductive behavior. Outside the central nervous system, mammalian GnRH has been shown in the placenta, endometrium, gonads, adrenal glands and breast tumor cells and large quantities of chicken II GnRH in the kidneys (King and Millar, 1997; White et al., 1998).

Multiple roles and regulatory mechanisms

In mammals, preoptic-GnRH neurons can autoregulate and synchronize the activity of other preoptic-GnRH neurons through GnRH-GnRH cell contacts (Leranth et al., 1985). GnRH neuronal activity is also influenced by sex steroids and a number of excitatory as well as inhibitory inputs to the GnRH system which include neurotransmitters and neuromodulators (catecholamines, -amino butyric acid, neurotensin, neuropeptide-Y, endogenous opioid peptides, corticotrophin-releasing hormone, glutamate: Kalra and Kalra, 1997; Kim et al., 1997). Similar studies in non-mammalian vertebrates have shown that sex steroids, gonadal maturation, pheromone signals, social interactions, temperature, photoperiods, developmental age and body mass can change GnRH content of the brain, and GnRH neuronal cell activity (Yu et al., 1997; Soga et al., 1998). It is still unclear whether steroids act directly on GnRH-containing cells or indirectly by activating neurons closely-associated with GnRH neurons.

Extracerebral terminal nerve GnRH neurons seen during juvenile stages of vertebrates decrease in number with increasing age (Schwanzel-Fukuda and Pfaff, 1989; Parhar et al., 1995). Intracerebral terminal nerve-GnRH ganglia is a distinctive feature of most bony fish (Munz and Claas, 1987). The expression of terminal nerve GnRH from early stages of development indicates that GnRH might be involved in the development of the reproductive brain, imprinting of odors and olfactory memory. Terminal nerve GnRH neurons in sexually mature but not immature fish are regulated by gonadal steroids (Parhar, 1997; Soga et al., 1998). However, the role of terminal nerve-olfactory system in reproduction is questionable since in some species of fish the terminal nerve is either absent or regressed during adult stages (Eisthen and Northcutt, 1996).

In fish, the concurrent development of preoptic GnRH neurons, GnRH fibers in the pituitary, gonadal sex differentiation and the appearance of steroid producing cells in the gonads, suggest a role of preoptic GnRH in sex differentiation (Parhar, 1997). Furthermore, an increase in preoptic GnRH cell size and cell numbers is linked to elevated levels of plasma sex steroids and the onset of gonadal maturation, thus emphasizing the role of preoptic neurons in vertebrate reproduction and reproductive behaviors (Grober et al., 1994; Soma et al., 1996). In salmonids, and frogs undergoing meta-

morphosis, there is an increase in GnRH expression in the basal forebrain which coincides with the acceleration of gonadal maturation (Hayes et al., 1994; Parhar et al., 1995; Parhar and Iwata, 1996). Whether metamorphosis initiates basal forebrain GnRH expression or vice versa is not known. However, growth promoting factors and high levels of thyroid hormones secreted at the time of smoltification-metamorphosis might regulate GnRH gene expression and GnRH cell differentiation (Parhar and Iwata, 1996).

In some species of fish midbrain chicken II GnRH expression begins at the same time as the terminal nerve GnRH while in others later, almost at the time of maturation (Parhar et al., 1995; Parhar, 1997). The significance of early or delayed expression of chicken II GnRH among species is unclear. Although no known function has been described for chicken II GnRH, in rodents, musk shrew, newts and birds, midbrain neurons have been implicated in mating and courtship behaviors (Rissman, 1997). In fish, midbrain chicken II GnRH neurons compared to forebrain GnRH neurons, are not the targets of steroid hormones (Soga et al., 1998) and therefore they might be under different regulatory mechanisms and have functions besides reproduction.

Perspectives

Comparative animal models have been very useful for elucidating some aspects of reproduction. Studies in amphibians laid the ground work proposing hypothalamic GnRH neurons arise from precursor cells in the olfactory placodes and migrate into the brain along with the developing terminal nerve fibers (Muske and Moore, 1987, 1990). The migratory route of GnRH neurons was later confirmed by surgically ablating the olfactory placodes of developing bird and amphibian embryos because these species develop outside the uterine environment and therefore fertilized eggs can be obtained for experimental manipulations at earlier developmental stages than mammals (Akutsu et al., 1992; Murakami et al., 1992; Northcutt and Muske, 1994). GnRH neuronal migration from the olfactory placodes into the forebrain has been charted in a wide variety of vertebrate species ranging from fish (Parhar et. al, 1995), rodents and humans (Schwanzel-Fukuda and Pfaff, 1989; Schwanzel-Fukuda, 1997). The clinical importance of this phenomenon has been shown by Schwanzel-Fukuda, Pfaff and coworkers (1989), that in X-linked Kallmann patients GnRH neurons fail to migrate from the olfactory placodes into the basal forebrain.

The molecular diversity of GnRHs was first shown in non-mammalian vertebrates (King and Millar, 1997). The multiplicity of GnRH has recently expanded with the discovery of chicken II GnRH in the brain of humans and other mammals (Jimenez-Linan et al., 1997; Lescheid et al., 1997; White et al., 1998). The discoveries of molecular variants of GnRHs in non-mammalian vertebrates and the cloning of three GnRH genes from the brain of a single species of bony fish are likely to increase our understanding of functions (novel) of GnRH variants and the conservation of mechanisms of gene regulation. Furthermore, GnRH variants could be a potential source to generate synthetic GnRH analogs for therapeutic applications.

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