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Evolutionary development of three gonadotropin-releasing hormone (GnRH) systems in vertebrates

E. A. Dubois, M. A. Zandbergen,* J. Peute and H. J. Th. Goos

Research Group of Comparative Endocrinology, Graduate School for Developmental Biology, Faculty of Biology, Utrecht, The Netherlands

ABSTRACT: Gonadotropin-releasing hormone (GnRH) is the neuropeptide that links the brain to the reproductive system. Most vertebrate species express two forms of GnRH, which differ in amino acid sequence, localization, distribution, and embryological origin. The GnRH system in the ventral forebrain produces a species-specific GnRH form and projects toward the gonadotropic cell in the pituitary. The GnRH neurons of this system originate from the olfactory placode and migrate into the brain during early development. The other GnRH system is localized in a nucleus in the midbrain, where large cells express chicken-GnRH-II, of which the function is still unclear. In modern teleosts, a third GnRH system is present in the terminal nerve, which contains salmon GnRH. The three GnRH systems appear at different times during fish evolution. Besides the two accepted lineages in GnRH evolution (of conserved chicken GnRH-II in the midbrain and of mammalian GnRH or species-specific GnRH in the hypophysiotropic system), we propose a third lineage: of salmon GnRH in the terminal nerve. © 2002 Elsevier Science Inc.

KEY WORDS: Gonadotropin-releasing hormone, Evolution, Reproduction, Neuroendocrinology, Brain.

INTRODUCTION

The decapeptide gonadotropin-releasing hormone (GnRH) forms an essential link in the integration of the external and internal stimuli in the control of reproduction in all vertebrates. The brain integrates the available information and transfers the signal to the GnRH neurons: when to release their GnRH content to allow an adequate response by the reproductive axis. GnRH reaches the gonadotropic cells either indirectly via the portal system (in tetrapods) or directly via axon endings (in most teleosts). In the pituitary, GnRH stimulates the synthesis and release of the gonadotropic hormones FSH (follicle-stimulating hormone) and LH (luteinizing hormone). Besides this endocrine function, GnRH probably fulfills a neuromodulatory role in sexual behavior and in transferring olfactory signals. Because it plays these pivotal roles in all vertebrates, it is well known that GnRH is highly conserved throughout evolution.

GNRH: FORMS, GENES, AND PHYLOGENY

The first reports about the existence of a GnRH-like substance date from 1971 [1,2,29]. Since this neuropeptide was first identified in mammals (pig and sheep), it is now known as mammalian GnRH (mGnRH). The original name was luteinizing hormone releasing hormone (LHRH), referring to its stimulatory effect on LH release. We now know that also FSH release is stimulated by the peptide, hence its more general name, GnRH. Until now, 15 forms of GnRH have been identified in various vertebrates and in a protochordate (Table 1). Generally, the GnRHs are named after the species in which they were first discovered. To date, six GnRHs are isolated from fish species (Table 1): salmon GnRH (sGnRH) [52], catfish GnRH (cfGnRH) [3], dogfish GnRH (dfGnRH) [25], seabream GnRH (sbGnRH) [46], herring GnRH (hGnRH) [5], and medaka GnRH (mdGnRH) [36]. Primitive species such as the lamprey and the protochordate *Ciona intestinalis* have their own forms of GnRH: lamprey GnRH I and III [55,57] and tunicate GnRH I and II, respectively [9,44]. Chicken GnRH-I (cGnRH-I) [19] and chicken GnRH-II (cGnRH-II) [31] were both first characterized in chicken. Apart from the common mGnRH, recently the guinea pig GnRH (gpGnRH) was shown as a novel and alternative form of mammalian GnRH [16]. The most recent finding of a novel GnRH was made in an amphibian, the frog *Rana dybowskii*: ranid GnRH (rGnRH) [66].

The GnRH gene consists of three introns and four exons. The second, third, and part of the fourth exon encode for the pre-prohormone, which contains a signal peptide (21–23 amino acids), the GnRH (10 amino acids), a cleavage site (Gly-Lys-Arg), and the GnRH-associated peptide (GAP, 40–60 amino acids) [20,21]. The sequence of the second exon is the most conserved, whereas the other exons show high variability. As a consequence, the signal peptides and the GnRHs are well conserved, but the GAPs show less homology among species.

In all classes of vertebrates and in a few invertebrates, the presence of given GnRH forms was established (Table 2). GnRH is a functionally old and well-conserved peptide, because it already controls reproductive functions in molluscs [67] and protochordates [12,20].

In general, all investigated species to date possess two or three different forms of GnRH (Table 2). The most conserved form of GnRH is cGnRH-II, and it coexists in all classes of vertebrates from the *Chondrichtyes* species onward, together with a species-specific GnRH and a possible third form. The two or three forms of GnRH coexisting in one species are transcribed from different genes. The species-specific forms vary, for example, cGnRH-I in birds, hGnRH in herring, dfGnRH in sharks, sGnRH in salmonids, and mGnRH in primates. But, if a third form is present, as shown for "modern" fishes, it is always the sGnRH form.

* Address for correspondence: Dr. M. A. Zandbergen, Research Group of Comparative Endocrinology, Graduate School for Developmental Biology, Faculty of Biology, Padualaan 8, 3584 CH Utrecht, The Netherlands. Fax: +31 30 2532837; E-mail: M.A.Zandbergen@bio.uu.nl

TABLE 1
AMINO ACID SEQUENCES OF 15 GNRH FORMS

GnRH	1	2	3	4	5	6	7	8	9	10	
Chicken II	pGlu	His	Trp	Ser	His	Gly	Trp	Tyr	Pro	Gly-NH_2	[31]
Mammalian	pGlu	His	Trp	Ser	**Tyr**	Gly	**Leu**	**Arg**	Pro	Gly-NH_2	[2,29]
Rana	pGlu	His	Trp	Ser	**Tyr**	Gly	**Leu**	**Trp**	Pro	Gly-NH_2	[66]
Catfish	pGlu	His	Trp	Ser	His	Gly	Leu	Asn	Pro	Gly-NH_2	[3]
Salmon	pGlu	His	Trp	Ser	**Tyr**	Gly	Trp	**Leu**	Pro	Gly-NH_2	[52]
Herring	pGlu	His	Trp	Ser	His	Gly	**Leu**	**Ser**	Pro	Gly-NH_2	[5]
Medaka	pGlu	His	Trp	Ser	**Phe**	Gly	**Leu**	**Ser**	Pro	Gly-NH_2	[36]
Seabream	pGlu	His	Trp	Ser	**Tyr**	Gly	**Leu**	**Ser**	Pro	Gly-NH_2	[46]
Dogfish	pGlu	His	Trp	Ser	His	Gly	Trp	**Leu**	Pro	Gly-NH_2	[25]
Chicken I	pGlu	His	Trp	Ser	**Tyr**	Gly	**Leu**	**Gln**	Pro	Gly-NH_2	[19]
Guinea pig	pGlu	**Tyr**	Trp	Ser	**Tyr**	Gly	**Val**	**Arg**	Pro	Gly-NH_2	[16]
Lamprey I	pGlu	His	**Tyr**	Ser	**Leu**	**Glu**	Trp	**Lys**	Pro	Gly-NH_2	[57]
Lamprey III	pGlu	His	Trp	Ser	His	**Asp**	Trp	**Lys**	Pro	Gly-NH_2	[57]
Tunicate I	pGlu	His	Trp	Ser	**Asp**	Tyr	**Phe**	**Lys**	Pro	Gly-NH_2	[9]
Tunicate II	pGlu	His	Trp	Ser	**Leu**	**Cys**	**His**	**Ala**	Pro	Gly-NH_2	[9]

Note: The amino acids printed in bold differ from the cGnRH-II form.

GNRH: LOCALIZATION AND ORIGIN

In general, all vertebrate species have at least two forms of GnRH (cGnRH-II and the species-specific GnRH), or even three forms, as discovered in modern fish. The two or three GnRH forms are differentially localized in the brain (Fig. 1) and, moreover, have different embryonic origins as well.

The large cGnRH-II cells are clustered in a distinct nucleus at the fusion site of the anterior midbrain and the posterior diencephalon (the synencephalon), rostral to the oculomotor nucleus. The

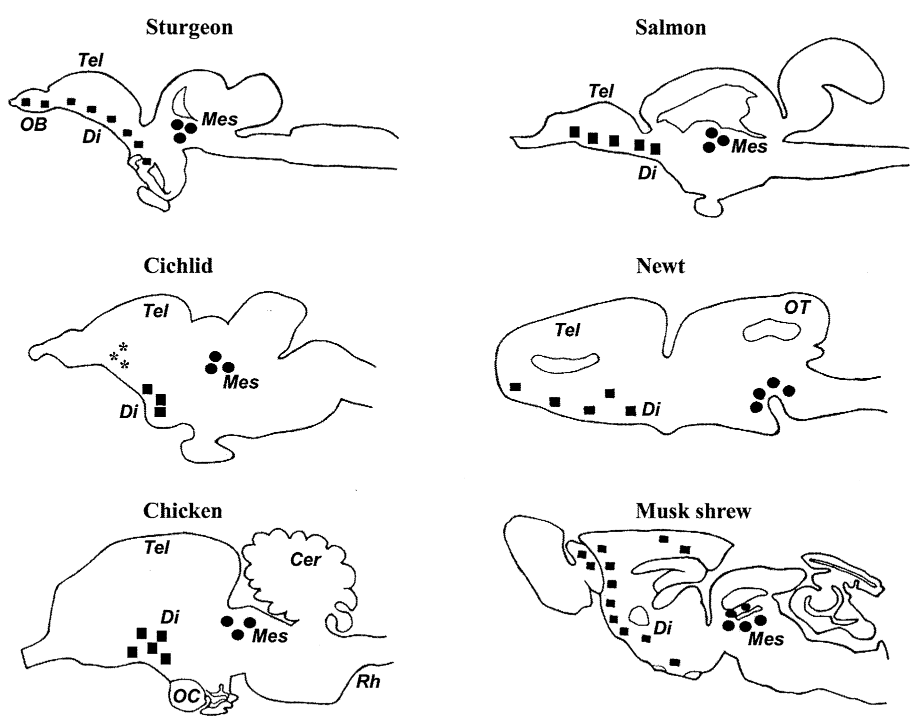

FIG. 1. Sagittal brain sections show gonadotropin-releasing hormone (GnRH) localization in sturgeon, salmon, cichlid, newt, chicken, and musk shrew. The GnRH system in the ventral forebrain is represented by 36, the cGnRH-II system in the midbrain is shown by ∀, and the TN GnRH system in the cichlid is indicated by an asterisk. Images are adapted from Sherwood et al. [54]. Abbreviations: Cer, cerebellum; Di, diencephalons; Mes, mesencephalon; OB, olfactory bulb; OC, optic chiasm; OT, optic tectum; Tel, telencephalon.

TABLE 2

PHYLOGENETIC DISTRIBUTION OF GNRH FORMS ORDERED BY THEIR AREA OF LOCALIZATION IN THE BRAIN: MIDBRAIN (MB), VENTRAL FOREBRAIN (VF) OR TERMINAL NERVE (TN)

	Reference	MB	VF	TN
Invertebrata				
Mollusk *Aplysia californica*	[67]		m?tI?	
Protochordata				
Tunicate *Ciona intestinalis*	[9]		m?cI? tI+II?	
Agnatha				
Lamprey *Petromyzon marinus*	[57]		LI+III	
Hagfish *Myxine glutinosa*	[58]		LIII	
Holocephali				
Ratfish *Hydrolagus colliei*	[27]	cII		
Chondrichthyes				
Atlantic stingray *Dasyatis sabina*	[13]	cII	df LIII?	df
Dogfish *Squalus acanthias*	[25]	cII		df
Osteichthyes				
Reedfish *Calamoichthys calabaricus*	[51]	cII	m	m
Sturgeon *Acipenser transmontanus*	[51]	cII	m	m
Eel *Anguilla anguilla*	[18]	cII	m	m
Herring *Clupea harengus pallasi*	[5]	cII	h	s
Sockeye salmon *Oncorhynchus nerka*	[42]	cII	s	s
Goldfish *Carrasius auratus*	[24]	cII	s	s
African catfish *Clarias gariepinus*	[3]	cII	cf	cf
Pacu *Piaractus mesopotamicus*	[45]	cII	sb	s
Medaka *Oryzias latipes*	[36]	cII	md	s
Pejerry *Odentesthes bonariensis*	[32]	cII	md	s
Red seabream *Pagrus major*	[38]	cII	sb	s
Cichlid *Haplochromis burtoni*	[63]	cII	sb	s
Tilapia *Oreochromis mossambicus*	[40]	cII	sb	s
Lungfish *Protopterus annectens*	[22]	cII	m	
Amphibia				
Frog *Rana ridibunda*	[6]	cII	m	
Newt *Taricha granulosa*	[35]	cII	m	
Frog *Rana dybowskii*	[66]	cII	m r	
Reptilia				
Turtle *Gallus domesticus*	[56]	cII	cI	
American alligator *Alligator mississippiensis*	[26]	cII	cI	
Lizard *Podarcis s. sicula*	[7]	cII	cI	
Aves				
Chicken *Gallus domesticus*	[11]	cII	cI	
Turkey *Gallus meleagris*	[30]	cII	cI	
Mammalia				
Tree shrew *Tupaia glis belangeri*	[17]	cII	m	
Guinea pig	[16]	cII	gp	
Macaque *Macaca mulatta*	[23]	cII	m	
Human	[62]	cII	m	

In invertebrates, protochordates, and Agnathans, the localization of GnRH is unclear, which is indicated by the dashed lines between the different brain regions. Abbreviations: cf, catfish GnRH; cI, chicken GnRH-I; cII; chicken GnRH-II, df; dogfish GnRH; gp; guinea pig GnRH; h; herring GnRH; LI, lamprey GnRH-I; LIII, lamprey GnRH-III; m, mammalian GnRH; md, medaka GnRH; r; rana GnRH; s; salmon GnRH; sb, seabream GnRH; tI, tunicate GnRH-I; tII, tunicate GnRH-II.

function of this GnRH is still under debate. *In vitro* cGnRH-II can be a potent stimulator of gonadotropin release from pituitary gonadotropic cells [4], but *in vivo* levels of cGnRH-II in the pituitary are low. The most commonly accepted function is that of neuromodulator or a role in sexual behavior [28,60]. The ventricular zone is the putative embryonic origin of these cells [41,63]. In chick embryos, this locus has been related to expression of the gene Nkx6.1 [47].

The species-specific GnRH is localized in the ventral forebrain and mostly restricted to the ventral telencephalon, the pre-optic area (POA), the basal hypothalamus, and the pituitary gland. Because during early development neurons of this system were first observed in the olfactory bulb (OB), it has been suggested that they originate from the olfactory region. Moreover, a rare disease, Kallmann's syndrome, which is characterized by hypogonadotropic hypogonadism combined with the disability to smell, suggested an ontogenetic liaison between the olfactory system and reproductive brain areas [39,50]. It was hypothetized that the GnRH neurons of this system originate from the olfactory placode and migrate during development into the brain, colonizing the hypothalamus and the pituitary. Many studies monitored the migration of the GnRH neurons during early development in all classes of vertebrates: salmon [42], platyfish (*Xiphophorus maculatus*) [14], frog [8], lizard [7], chicken [59], rat [15], and rhesus monkey [49]. Firm evidence for this hypothesis was shown by the absence of GnRH neurons in the brain after ablation of the olfactory placode [34].

One GnRH subpopulation, the terminal nerve (TN) ganglion, is localized at the junction of the olfactory nerve and the telencephalon and is present in most vertebrates. This population has no hypophysiotropic function, but it is probably involved in reproductive behavior in relation to olfaction [65]. Its localization in close vicinity of olfactory areas suggests a neuromodulatory function in integrating olfactory signals, which may be evoked by, e.g., pheromones into the brain-pituitary axis. It is unclear whether both the TN and the POA/hypothalamus neurons are derived from the olfactory placode. Parhar et al. [40,43] hypothesized that the GnRH neurons in the terminal nerve originate from the olfactory placode, whereas GnRH neurons in the POA originate in the basal telencephalon. However, we propose that both the TN GnRH neurons and the ventral forebrain neurons originate from the olfactory placode and that they are differentially programmed concerning the timing and distance of migration.

NOMENCLATURE

With the appearance of a third form in the brain, coexisting with cGnRH-II and the species-specific form, the terminology of the forms and systems has become complex. Therefore, Fernald and White [12] proposed a new nomenclature. GnRH1 is the species-specific form and regulates pituitary LH release; GnRH2 is the conserved cGnRH-II in the midbrain. If sGnRH is present in the terminal nerve, it is referred to as GnRH3. This nomenclature is still not widely accepted in the literature and is confusing when the GnRH form in TN and ventral forebrain is similar. Therefore, we propose an alternative nomenclature, in which both the localization and the function of the GnRH forms are combined. MB-GnRH is cGnRH-II in the midbrain; VF-GnRH is the species-specific hypophysiotropic GnRH form in the ventral forebrain; and TN-GnRH is the neuromodulatory salmon GnRH in the terminal nerve.

EVOLUTIONARY ASPECTS OF GNRH

The presence of several molecular forms of GnRH within one species, their phylogenetic distribution, and the recent discovery of new forms [5,16,36,66] tempt one to propose a new evolutionary GnRH tree. Its construction starts with a study of the phylogenetic

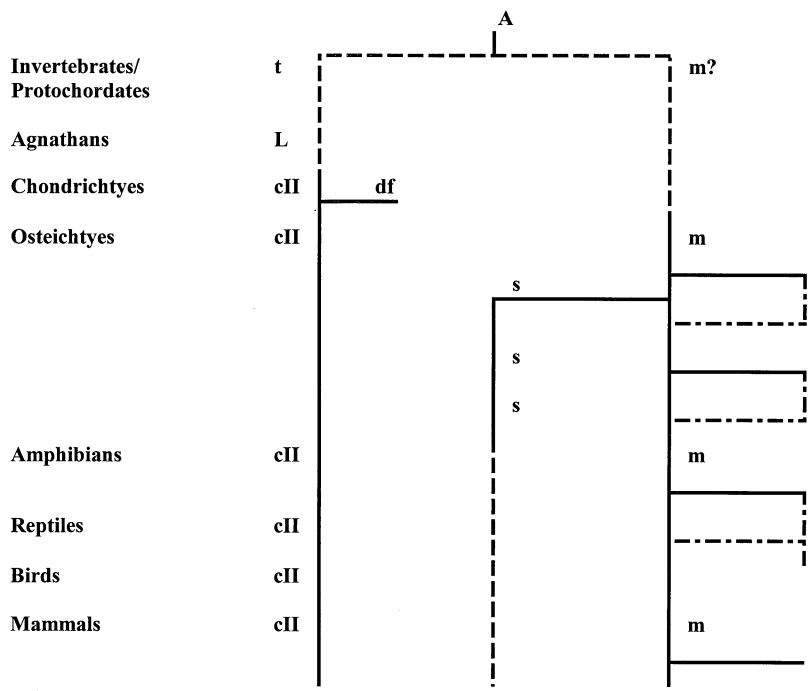

FIG. 2. Hypothetical evolutionary tree of GnRH. Three main lineages are discerned: the cGnRH-II lineage, the species-specific mammalian lineage, and the salmon lineage. Dashed lines indicate uncertain origin. Abbreviations: A, ancestral form; cf, catfish GnRH; cI, chicken GnRH-I; cII, chicken GnRH-II; df, dogfish GnRH; gp, guinea pig GnRH; h, herring GnRH; L, lamprey GnRH; m, mammalian GnRH; md, medaka GnRH; r, rana GnRH; s, salmon GnRH; sb, seabream GnRH; t, tunicate GnRH.

distribution of GnRHs (Table 2) and by comparing GnRH precursors. As in a former model [20], two main lineages are discerned in the evolutionary GnRH tree: the cGnRH-II lineage and the mammalian lineage. Extending that model with more recently obtained data, we propose the following hypothetical tree (Fig. 2). Chicken GnRH-II is supposed to have appeared early in vertebrate evolution, and hence, it is still thought to represent the most conservative GnRH lineage. In addition, it is hypothetized that a second, variable lineage exists with mGnRH, cGnRH-I, gpGnRH, rGnRH, and the "fish" GnRHs. The presence of two or more forms of GnRH within one species suggests that both lineages are probably derived from gene duplication in early vertebrate evolution [20,33,53]. Moreover, the similar architecture of the GnRH genes is important evidence for the gene duplication hypothesis [41]. The place of GnRHs from lampreys and protochordates in this model is unclear; their genes await cloning in order to obtain more information about their possible homology with cGnRH-II or mGnRH. ChickenGnRH-II is probably preceded by the tunicate and lamprey GnRHs, of which lGnRH-III is the putative ancestor of cGnRH-II. DogfishGnRH is probably derived from the cGnRH-II lineage, because these two only differ in one amino acid. Similarly, the seabream and medaka GnRHs and those of higher vertebrates (Rana, chicken-I, and guinea pig) are closely related to mGnRH [37,61] and thus probably originate from the mGnRH lineage. Moreover, when regarding localization and function of the different GnRH forms within the Osteichtyes, it appears that herring, catfish, salmon, seabream, and medaka GnRH are localized in the ventral forebrain and have a hypophysiotropic function similar to the other GnRHs in the mGnRH lineage. This also favors their place in the mammalian GnRH line. When precursors of GnRH genes are compared [37,61], salmon GnRH genes are placed in a separate group. Also because of their special localization in the TN and a different function, we propose a separate lineage for salmon GnRH, which originates during teleost evolution. Until now, only in modern teleosts the presence of this salmon GnRH in the TN has been established. Evolutionary older teleost species, like the African catfish (*Clarias gariepinus*), have a distinct population of GnRH neurons in the TN, but these cells do not project to the pituitary and they express the same species-specific form as the hypophysiotropic GnRH neurons in the ventral forebrain [10]. Apparently, this situation anticipates the origin of a new evolutionary GnRH line, i.e., the salmon GnRH line in the TN of modern teleosts. Whether a special TN-GnRH form is also present in higher vertebrates is still under debate, but new findings indicate the existence of a second GnRH population in the mammalian ventral forebrain brain (Parhar, personal communication)

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