

REVIEW PAPER

GnRH and GnRH receptors: distribution, function and evolution

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Gonadotropin-releasing hormone (GnRH) was originally identified because of its essential role in regulating reproduction in all vertebrates. Since then, three phylogenetically related GnRH decapeptides have been characterized in vertebrates and invertebrates. Almost all tetrapods investigated have at least two GnRH forms (GnRH1 and GnRH2) in the central nervous system. From distributional and functional studies in vertebrates, GnRH1 in the hypothalamus projects predominantly to the pituitary and regulates reproduction *via* gonadotropin release. GnRH2, which is located in the midbrain, projects to the whole brain and is thought to be involved in sexual behaviour and food intake. GnRH3, located in the forebrain, has only been found in teleost fish and appears to be involved in sexual behaviour, as well as, in some fish species, gonadotropin release. Multiple GnRH receptors (GnRH-Rs), G-protein-coupled receptors regulate endocrine functions and neural transmissions in vertebrates. Phylogenetic and structural analyses of coding sequences show that all vertebrate GnRH-Rs cluster into two main receptor types comprised of four subfamilies. This suggests that at least two rounds of GnRH receptor gene duplications may have occurred in different groups within each lineage. Functional studies suggest that two particular subfamilies of GnRH receptors have independently evolved to act as species-specific endocrine modulators in the pituitary, and these show the greatest variety in regulating neuron networks in the brain. Given the long evolutionary history of the GnRH system, it seems likely that much more remains to be understood about its roles in behaviour and function of vertebrates.

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Key words: GnRH; GnRH receptors; vertebrate.

INTRODUCTION

Gonadotropin-releasing hormone (GnRH), a decapeptide, was originally isolated from the hypothalamus of mammals and is the brain signal that regulates reproductive function *via* the hypothalamic–pituitary–gonadal (HPG) axis. GnRH stimulates the synthesis and release of pituitary gonadotropins, follicle-stimulating hormone and luteinizing hormone to control gametogenesis and sex steroid production. Originally considered and named for its important

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reproductive function, two related GnRH gene-encoding decapeptides (GnRHs), which are not directly involved in HPG axis, have been identified in vertebrates. Within this family of three genes, more than 10 GnRH peptide variants have been identified (Ngamvongchon *et al.*, 1992; Sower *et al.*, 1993; Powell *et al.*, 1994, 1996; Jimenez-Linan *et al.*, 1997; White & Fernald, 1998a; Carolsfeld *et al.*, 2000; Okubo *et al.*, 2000a; Yoo *et al.*, 2000; Montaner *et al.*, 2001; Adams *et al.*, 2002). All GnRH forms are decapeptides with protein residues 1, 4, 9 and 10 conserved, and all have modified amino and carboxyl termini. However, it is still unclear why vertebrates evolved multiple GnRHs or what role these GnRHs play. The first part of this review briefly summarizes the phylogenetic relationships, anatomical distributions and functions of these GnRH ligands. At least two GnRH different forms are in all tetrapods, and teleosts have evolved a unique GnRH form expressed in the forebrain. Although, these GnRH ligands probably have specific functions, they appear to interact and their roles in endocrine and neuromodulation of reproduction will be discussed.

To understand the roles of multiple GnRH systems, it is essential to understand how GnRH ligands interact with their cognate receptors, which are discussed in the second part of this review. The non-systematic nomenclature of GnRH receptors has complicated our understanding of the ligand–receptor relationships in the evolution of GnRH pathways. To resolve this complication, GnRH receptors will be classified according to a particular extracellular domain sequence that identifies functionality. Following this classification, the four subfamilies of GnRH receptors that have evolved independently in each vertebrate lineage demonstrate a diversity of binding affinities and downstream signalling pathways. This review systematizes the nomenclature of various GnRH receptors and applies it to gene sequences, receptor structure, localization information and physiological data across vertebrates. GnRH receptors are promiscuous with respect to GnRH ligands, widely distributed in overlapping regions of the brain and show no evidence of subfunctionalizations. The GnRH receptors seem to have evolved separately and apparently independent from their ligands.

Comparisons of phylogenetic, anatomical and functional data across vertebrates in GnRH systems suggest a wide array of GnRH effects on sensory-motor, cognitive, energy control and other physiological system. However, due to the promiscuity of the GnRH ligands, it is difficult to untangle the effects of particular GnRH–GnRH receptor combinations using conventional methods. It is possible that development of specific receptor blockers or other genetic methods may provide new insights into the evolution and roles of GnRH family members.

THREE TYPES OF GnRH

Molecular phylogeny of GnRH ligands shows that there are three distinct forms, GnRH1, GnRH2 and GnRH3 that arose from a common origin (Fig. 1; Fernald & White, 1999). Most vertebrate classes have only GnRH1 and GnRH2, including some teleosts (King *et al.*, 1990; Lovejoy *et al.*, 1992; Schulz *et al.*, 1993; Okubo *et al.*, 1999), amphibians (Conlon *et al.*, 1993;

Yoo *et al.*, 2000) and mammals (Rissman *et al.*, 1995; Kasten *et al.*, 1996; Mongiat *et al.*, 2006). To date, GnRH3 has only been found in teleosts (White *et al.*, 1995; Yamamoto *et al.*, 1995; Gothilf *et al.*, 1996; Parhar *et al.*, 1998; Okubo *et al.*, 2000a; Amano *et al.*, 2002a; Vickers *et al.*, 2004; Kuo *et al.*, 2005; Mohamed *et al.*, 2005; Pandolfi *et al.*, 2005; Soga *et al.*, 2005; Mohamed & Khan, 2006). Interestingly, the decapeptide sequences of GnRH2 and GnRH3 are completely conserved across vertebrate species, whereas the GnRH1 sequence has diverged in the vertebrate lineage (Fig. 2; Fernald & White, 1999; Millar *et al.*, 2004; Morgan & Millar, 2004).

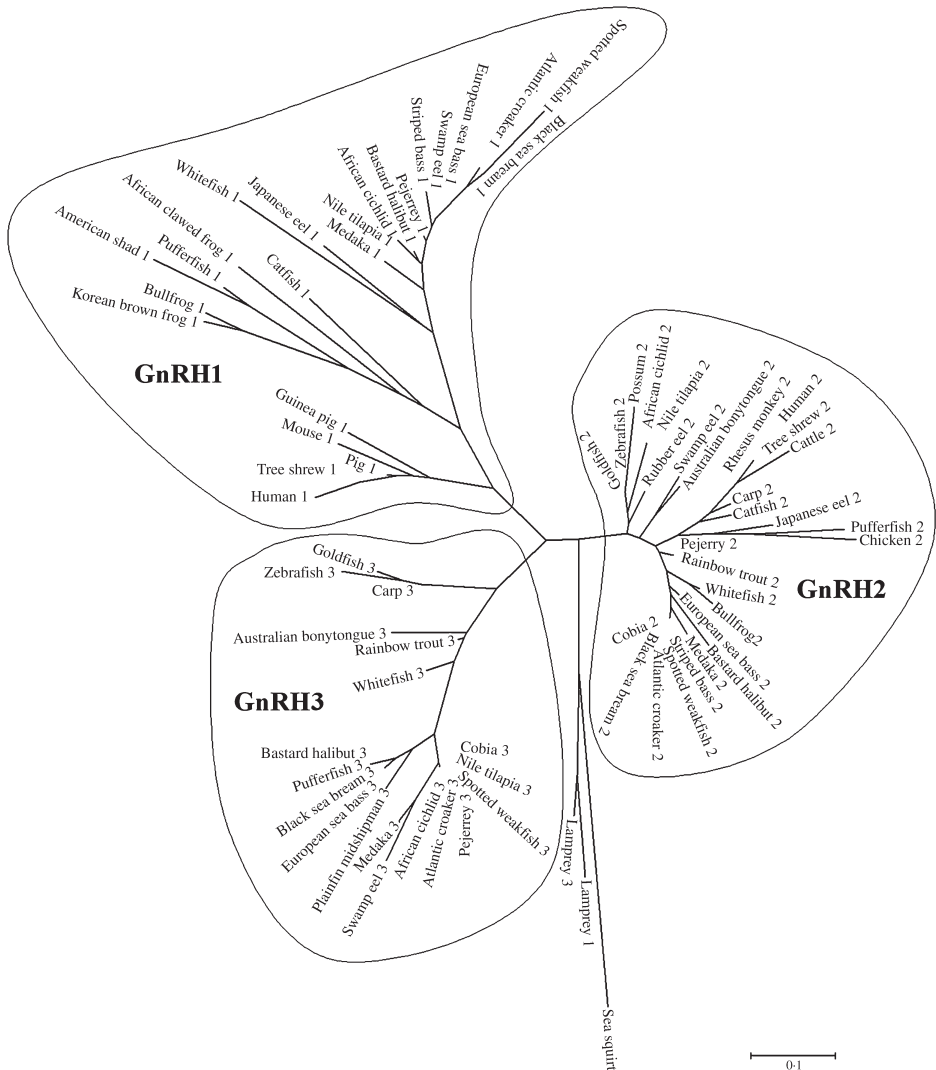
GnRH forms not only regulate reproduction but also clearly have other physiological roles based on their expression patterns, despite relatively limited functional testing. In general, GnRH1 neurons are located in the preoptic area of hypothalamus and project predominantly into the pituitary, where they regulate reproduction *via* gonadotropin release (Davis & Fernald, 1990; Schulz *et al.*, 1993; White & Fernald, 1993; Grober *et al.*, 1994; Muske *et al.*, 1994; Powell *et al.*, 1994; Montero *et al.*, 1995; 1996; Maney *et al.*, 1997; Carolsfeld *et al.*, 2000; White *et al.*, 2002; Amano *et al.*, 2004). In addition to its primary role in regulating reproduction, GnRH1 has also been shown to cause release of growth hormone from the pituitary (Marchant *et al.*, 1989) as well as regulating prolactin (Weber *et al.*, 1997) and somatolactin (Kakizawa *et al.*, 1997). GnRH2 is produced in the midbrain tegmentum near the third ventricle, and GnRH2 neurons project throughout the brain, especially midbrain and hindbrain, as well as having terminals in the third ventricle directly (Montero *et al.*, 1994; Muske *et al.*, 1994; Collin *et al.*, 1995; Rissman *et al.*, 1995; Yamamoto *et al.*, 1995; Di Matteo *et al.*, 1996; Amano *et al.*, 1997; Yoo *et al.*, 2000; Yuanyou & Haoran, 2000; Gonzalez-Martinez *et al.*, 2002a; Steven *et al.*, 2003). In some cases, both GnRH1 and GnRH2 peptides have been detected in the pituitary, but GnRH1 alone is thought to control gonadotropin release (Kim *et al.*, 1995; Mongiat *et al.*, 2006). GnRH3 neurons, which are found only in teleosts, are located in the terminal nerve ganglion near the olfactory bulb and project primarily to the telencephalon but also widely into the whole brain, including the retina and olfactory epithelium (Kudo *et al.*, 1994; Parhar & Iwata, 1994; Chiba *et al.*, 1996; Wirsig-Wiechmann & Oka, 2002; Grens *et al.*, 2005).

The role of the GnRH forms in regulating behaviour has been tested in various species. For example, GnRH1 has been shown to influence reproductive behaviour in musk shrew *Suncus murinus* (Schiml & Rissman, 2000), and GnRH2 has also been implicated in the regulation of female reproductive behaviour in mammals (Kauffman & Rissman, 2004; Barnett *et al.*, 2006), birds (Maney *et al.*, 1997) and teleosts (Volkoff & Peter, 1999). In several studies, Rissman *et al.* have shown that GnRH2 regulates food intake and energy balance (Temple *et al.*, 2003; Kauffman & Rissman, 2004), suggesting that GnRH2 may mediate a balance between survival and reproduction. In teleosts, GnRH3 can influence sexual behaviour, such as nest-building behaviour (Yamamoto *et al.*, 1997; Ogawa *et al.*, 2006), aggressive behaviour (Ogawa *et al.*, 2006) and spawning behaviour (Volkoff & Peter, 1999). Additionally, GnRH3 can influence the signal processing in sensory systems (Behrens *et al.*, 1993; Kinoshita *et al.*, 2007; Maruska & Tricas, 2007), suggesting that

it may play a role in regulating sensory input relative to reproductive state (White *et al.*, 1995). Taken together, we can conclude that GnRH1 has as its main role the regulation of reproduction, while studies of GnRH2 and GnRH3 suggest that these may be neuromodulators of a variety of circuits (Fernald & White, 1999; Soga *et al.*, 2005).

GnRH1 AND GnRH3 SHARE AN ANCESTOR

A distinct anatomical distribution of the three forms of GnRH-expressing neurons has been found in many teleosts, including dwarf gourami *Colisa lalia* (Hamilton) (Yamamoto *et al.*, 1995), African cichlid *Astatotilapia burtoni* (Günther) (White *et al.*, 1995), gilthead sea bream *Sparus aurata* L. (Gothilf



et al., 1996), Nile tilapia *Oreochromis niloticus* (L.) (Parhar *et al.*, 1998) and barfin flounder *Verasper moseri* Jordan & Gilbert (Amano *et al.*, 2002a). However, not all teleosts with three types of GnRH have this distinction between GnRH forms. Some species have overlapping distributions of GnRH1- and GnRH3-producing neurons in the hypothalamus and olfactory bulb (Gonzalez-Martinez *et al.*, 2002a; Vickers *et al.*, 2004; Mohamed *et al.*, 2005; Pandolfi *et al.*, 2005; Mohamed & Khan, 2006). Interestingly, both GnRH1 and GnRH3 neurons arise in the olfactory placode and migrate to their final positions during development (Sullivan & Silverman, 1993; Yoshida *et al.*, 1995; White & Fernald, 1998b; Amano *et al.*, 2002b; Gonzalez-Martinez *et al.*, 2002b; Whitlock *et al.*, 2003; Okubo *et al.*, 2006; Palevitch *et al.*, 2007), suggesting that GnRH1 and GnRH3 may be derived from a gene duplication; this notion also supported by phylogenetic evidence. Additionally, some teleosts have lost the GnRH1 gene, and only have GnRH2 and GnRH3: zebrafish *Danio rerio* (Hamilton) (Steven

FIG. 1. A neighbour-joining phylogenetic tree based on GnRH precursor amino acid sequences of 75 taxa. The sea squirt branch has been modified to half the distance. The other branches are drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 (Tamura *et al.*, 2007). Three cycles represent the separate phylogenetic relationship of three GnRHs in early vertebrate lineage. The GenBank numbers of each GnRH type in different species are below: African cichlid, cichlid, *Astatotilapia burtoni* (GnRH1 AF076961, GnRH2 AF076962 and GnRH3 AF076963); African clawed frog, *Xenopus*, *Xenopus laevis* (GnRH1 L28040); American shad, *Alosa sapidissima* (GnRH AF536381); Atlantic croaker, *Micropogonias undulatus* (GnRH1 AY324668, GnRH2 AY324669 and GnRH3 AY324670); Australian bonytongue, *Scleropages jardinii* (GnRH2 AB047326 and GnRH3 AB047325); bastard halibut, *Paralichthys olivaceus* (GnRH1 DQ074693, GnRH2 DQ008580 and GnRH3 DQ444281); black sea bream, *Acanthopagrus schlegelii* (GnRH1 EU099997, GnRH2 EU099996 and GnRH3 EU117212); bullfrog, *Rana catesbeiana* (GnRH1 AF188754 and GnRH2 AF186096); carp, *Cyprinus carpio* (GnRH2 AY189961 and GnRH3 AY189960); North African catfish, *Clarias gariepinus* (GnRH1 X78049 and GnRH2 X78047); cattle, *Bos taurus* (GnRH2 DQ359716); chicken, *Gallus gallus* (GnRH1 GnRH1 NM_001080877 and GnRH2 AB194408); cobia, *Rachycentron canadum* (GnRH2 AY677174 and GnRH3 AY677173); European sea bass, *Dicentrarchus labrax* (GnRH1 AF224279, GnRH2 AF224281 and GnRH3 AF224280); goldfish, *Carassius auratus* (GnRH2 U30386 and GnRH3 AB017272); guinea pig, *Cavia porcellus* (GnRH1 AF033346); human, *Homo sapiens* (GnRH1 NM_000825 and GnRH2 NM_001501); Japanese eel, *Anguilla japonica* (GnRH1 AB026989 and GnRH2 AB026990); Korean brown frog, *Rana dybowskii* (GnRH1 AF139911); lamprey, *Petromyzon marinus* (GnRH1 AF144481 and GnRH3 AY052628); medaka, *Oryzias latipes* (GnRH1 NM_001104699, GnRH2 NM_001104671 and GnRH3 NM_001104672); mouse, *Mus musculus* (GnRH1 NM_008145); Nile tilapia *Oreochromis niloticus* (GnRH1 AB104861, GnRH2 AB104862 and GnRH3 AB104863); pejerrey, *Odontesthes bonariensis* (GnRH1 AY744689, GnRH2 AY744687 and GnRH3 AY744688); pig, *Sus scrofa* (GnRH1 NM_214274); plainfin midshipman, *Porichthys notatus* (GnRH3 U41669); possum, *Trichosurus vulpecula* (GnRH2 AF193516); pufferfish, *Tetraodon nigroviridis* (GnRH1 AB212811, GnRH2 AB212813 and GnRH3 AB212815); rainbow trout, *Oncorhynchus mykiss* (GnRH2 AF125973 and GnRH3 AY486076); rhesus monkey, *Mucaca mulatta* (GnRH2 AF097356); rubber eel (an amphibian) *Typhlonectes natans* (GnRH2 AF167558); sea squirt, *Ciona intestinalis* (GnRH NM_001039882); spotted weakfish, *Cynoscion nebulosus* (GnRH1 AY796308, GnRH2 AY796309 and GnRH3 AY796310); striped bass, *Morone saxatilis* (GnRH1 AF056314 and GnRH2 AF056313); swamp eel, *Monopterus albus* (GnRH1 AY858056, GnRH2 AY858054 and GnRH3 AY858055); tree shrew, *Tupaia belangeri* (GnRH1 U63326 and GnRH2 U63327); whitefish, *Coregonus clupeaformis* (GnRH1 AY245104, GnRH2 AY245102 and GnRH3 AY245103); zebrafish, *Danio rerio* (GnRH2 NM_181439 and GnRH3 NM_182887).

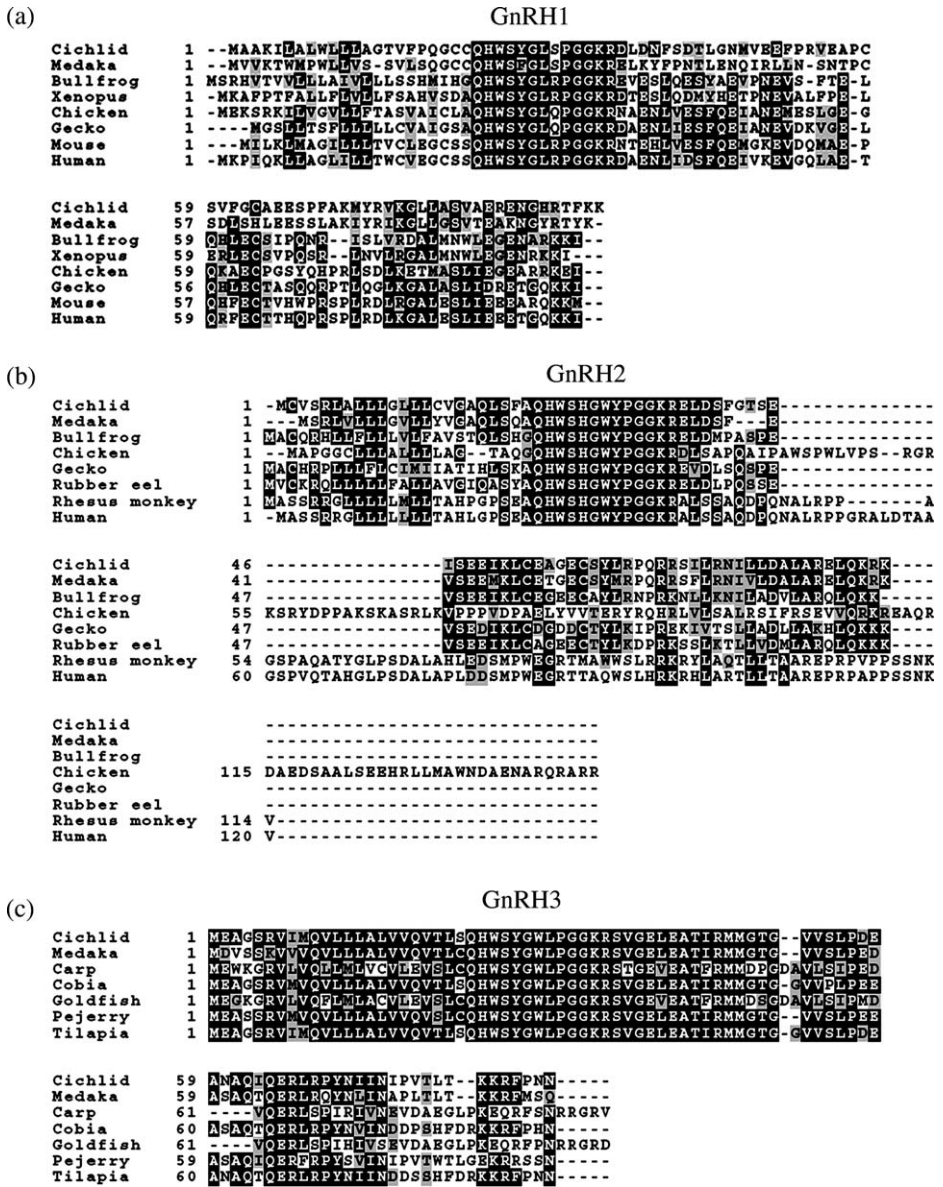


FIG. 2. The predicted amino acid sequence alignments of GnRH1 (a), GnRH2 (b) and GnRH3 (c) and their GnRH-associated peptides (GAP) for eight different species. These examples show that the GnRH1 sequences are more diverse in the difference species. The lines identify the GnRH decapeptides and GAP, respectively. Identical residues are shaded black and similar residues (e.g. same charge) are shaded grey. Hyphens indicate gaps in the sequences among the species. Gecko, *Eublepharis macularius* (GnRH1 DQ269480, GnRH2 AB104485). The GenBank identification is given in the caption of Fig. 1: bullfrog, *Rana catesbeiana*; carp, *Cyprinus carpio*; chicken, *Gallus gallus*; cichlid, *Astatotilapia burtoni*; cobia, *Rachycentron canadum*; goldfish, *Carassius auratus*; human, *Homo sapiens*; medaka, *Oryzias latipes*; mouse, *Mus musculus*; pejerrey, *Odontesthes bonariensis*; rhesus monkey, *Mucaca mulatta*; rubber eel, *Typhlonectes natans*; tilapia, *Oreochromis niloticus*; African clawed toad, *Xenopus laevis*.

et al., 2003; Palevitch *et al.*, 2007), salmonid (Okuzawa *et al.*, 1990; Amano *et al.*, 1991; Ashihara *et al.*, 1995; Ferriere *et al.*, 2001) and goldfish *Carassius auratus* (L.) (Yu *et al.*, 1988). In those species, GnRH3 neurons located in the ventral hypothalamus replace GnRH1 to provide innervation to the pituitary to regulate reproduction (Amano *et al.*, 1995a, b, 2007; Yamada *et al.*, 2002; Onuma *et al.*, 2005) further supporting the idea that GnRH1 and GnRH3 arose from a gene duplication (Fig. 1; O'Neill *et al.*, 1998; Dubois *et al.*, 2002; Guilgur *et al.*, 2006).

GnRH RECEPTORS

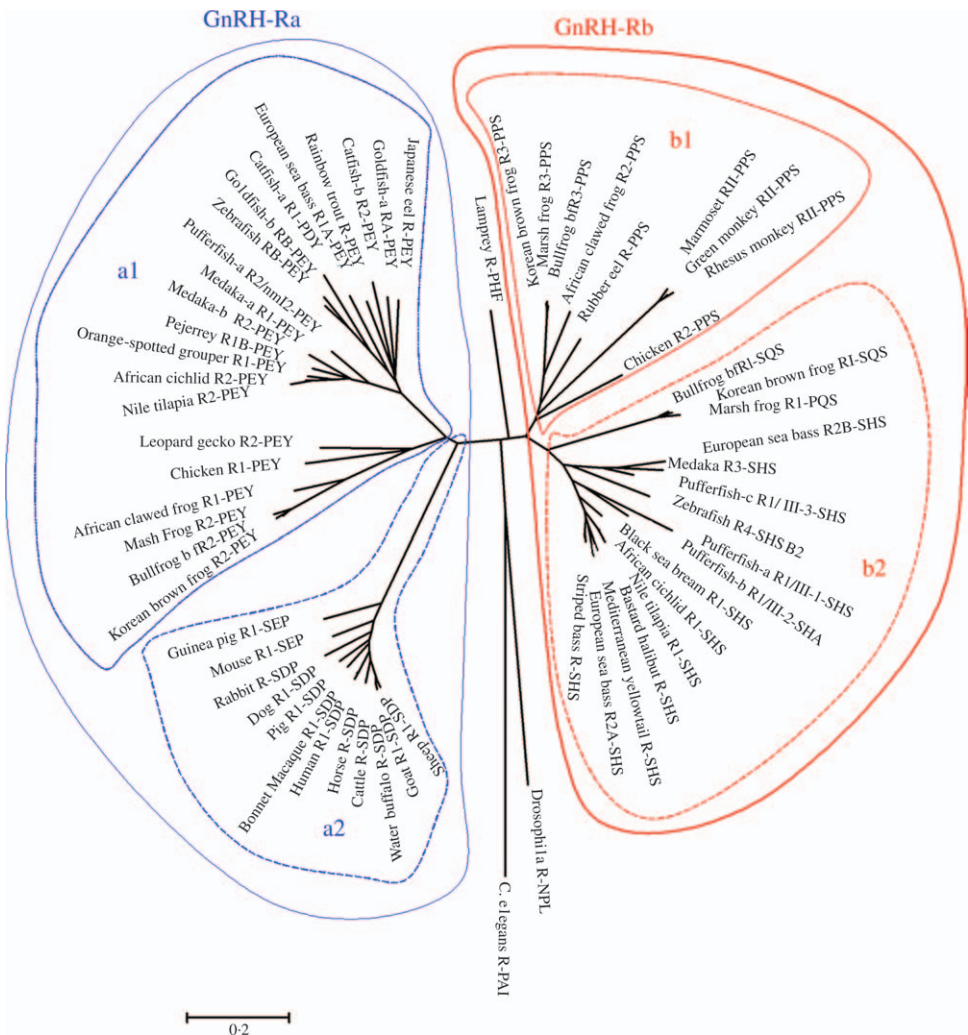
GnRH ACTS VIA COGNATE RECEPTORS

GnRH receptors belong to the G-protein-coupled receptor family, characterized by seven hydrophobic alpha helix transmembrane (TM) domains linked by hydrophilic extra- and intracellular loops (Stojilkovic *et al.*, 1994; Ruf *et al.*, 2003; Millar *et al.*, 2004). The extracellular domains and superficial regions of the TM domains are typically responsible for binding events, especially the third extracellular loop (EC3) (Troskie *et al.*, 1998; Fromme *et al.*, 2004; Millar *et al.*, 2004). These receptors also have an N-terminal extracellular domain and a C-terminal cytoplasmic domain. The intracellular loops and C-terminals mediate the functions of signalling transductions, such as G-protein-binding, propagation of signalling events, desensitization and internalization of GnRH receptors (McArdle *et al.*, 2002; Caunt *et al.*, 2004; Millar *et al.*, 2004; Levavi-Sivan & Avitan, 2005). Several different forms of GnRH receptors have been cloned and characterized in vertebrates (Illing *et al.*, 1999; Okubo *et al.*, 2000b, 2001, 2003; Troskie *et al.*, 2000; Wang *et al.*, 2001; Bogerd *et al.*, 2002; Ikemoto & Park, 2005; Moncaut *et al.*, 2005; Parhar *et al.*, 2005; Flanagan *et al.*, 2007). Like the multiple GnRH forms, multiple GnRH receptors (GnRH-Rs) have been found in an individual species.

The phylogenetic relationships among GnRH receptors are not straightforward, and the nomenclature is confused. In contrast to the clear phylogenetic relationship among the three GnRH ligands, GnRH receptors appear to have had a more complex evolutionary past. Previously, some authors sorted all the vertebrate GnRH receptors into three classes that were postulated to be linked to particular ligands by gene organization and structure of a C-terminal (Millar *et al.*, 2004). However, subsequent studies showed that GnRH receptors are promiscuous, and the link to specific ligands was not uniformly supported. In another scheme, two main groups of GnRH receptors were identified, based on the size of the C-terminal, suggesting that the GnRH receptors may show different transduction cascades and a tendency for the progressive shortening of the C-terminal for modulating the desensitization or internalization of GnRH receptors (Moncaut *et al.*, 2005; Guilgur *et al.*, 2006). In contrast, other research teams suggest that two main groups exist based on the amino acids in one extracellular loop, and emphasizing a role in GnRH-binding affinity (Troskie *et al.*, 1998).

PHYLOGENETIC AND STRUCTURE ANALYSES SUGGEST GnRH RECEPTORS CAN BE CLASSIFIED INTO FOUR SUBFAMILIES

Flanagan *et al.* (2007) proposed two main groups of GnRH receptors (GnRH-Ra and Rb) based on phylogenetic relationships of GnRH-Rs-coding region sequences. This phylogenetic analysis proposes four GnRH-R subfamilies (a1, a2, b1 and b2) corresponding to a set of three conserved amino acids (PEY, SDP, PPS and SHS) in EC3 (Troskie *et al.*, 2000; Flanagan *et al.*, 2007). The phylogenetic patterns of GnRH-Rbs, including some teleosts and all tetrapods, are divergent, whereas GnRH-Ras separate into an a1 non-mammalian group and an a2 mammalian group (Fig. 3). Lamprey *Petromyzon marinus* L. has a single GnRH receptor with a strong binding capacity for all three primary GnRH ligands (Silver & Sower, 2006) but does not have the EC3 feature



of the four subfamilies, suggesting that it could be an ancestral form. One hypothesis is that the original GnRH-R gene may have undergone two rounds of duplication in evolution producing the four extant subfamilies (2R hypothesis; Hughes, 1999). This would suggest that various GnRH-R genes have been lost independently. Thus, in GnRH-Ra, the a1 subtype is found in non-mammals, but the a2 subtype is only found in mammals. In the b group, GnRH-Rb1 is found only in tetrapods and the b2 group is found only in non-mammals (Fig. 3; Flanagan *et al.*, 2007).

Systematic comparison of the structure of GnRH receptors showed not only the conserved feature of EC3 domain in four subfamilies but also the different size range of C-terminals. Generally, GnRH-Ra has a shorter C-terminal tail than GnRH-Rb. One exception is the teleost, Nile tilapia *O. niloticus*, with the C-terminal tail of GnRH-Rb2 (as known as GnRH-R1 of tilapia) being half the size of the C-terminal tail of GnRH-Ra1 (as known as GnRH-R2 of tilapia).

FIG. 3. A neighbour-joining phylogenetic tree based on the multiple-sequence alignment of GnRH-R amino acid sequences of 61 taxa. The sea squirt branch has been modified to half the distance. The other branches are drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Phylogenetic analyses were conducted in MEGA4. Phylogenetic relationships of four subfamilies GnRH-Rs are circled. On the left side, GnRH-Ra includes GnRH-Ra1 and GnRH-Ra2, which has the highly conserved three amino acids PEY and S(D/E)P in the EC3 motif of GnRH-R sequences, respectively. GnRH-Ra2 (right) includes GnRH-Rb1 and GnRH-Rb2, which has the conserved PPS and (S/P)(H/Q)(S/A) in the EC3 domain. To clarify the nomenclature, the original names of GnRH receptors are labelled after the common name and followed by the conserved amino acids in the EC3 motif. The GenBank numbers of each GnRH-R type in different species are below: African cichlid, *Astatotilapia burtoni* (R1-SHS AY705931 and R2-PEY AY028476); African clawed frog, *Xenopus laevis* (R1-PEY AF172330 and R2-PPS AF257320); black sea bream, *Acanthopagrus schlegelii* (R1-SHS AY820276); bonnet macaque, *Macaca radiata* (R1-SDP AF156930); bullfrog, *Rana catesbeiana* (R1-SQS AF144063, R2-PEY AF153913 and R3-PPS AF224277); nematode, *Caenorhabditis elegans* (R-PAI AF039712); catfish, *Clarias gariepinus* (a R1-PDY X97497 and b R2-PEY AF329894); cattle, *Bos taurus* (R-SDP NM_177514); chicken, *Gallus gallus* (R1-PEY AJ304414 and R2-PPS NM001012609); dog, *Canis familiaris* (R1-SDP NM_001003121); drosophila, *Drosophila melanogaster* (R-NPL AF077299); European sea bass, *Dicentrarchus labrax* (R1A-PEY AJ606683, R2A-SHS AJ419594 and R2B-SHS AJ606686); goat, *Capra hircus* (R-SDP EF150356); goldfish, *Carassius auratus* (RA-PEY AF121845 and RB-PEY AF121846); green monkey, *Cercopithecus aethiops* (RII-PPS AF353988); guinea pig, *Cavia porcellus* (R1-SEP AF426176); bastard halibut, *Paralichthys olivaceus* (Temminck & Schlegel) (R-SHS DQ011872); horse, *Equus caballus* (R-SDP AF018072); human, *Homo sapiens* (R1-SDP NM_000406); Japanese eel, *Anguilla japonica* (R-PEY AB041327); Korean brown frog, *Rana dybowskii* (R1-SQS AF236879, R2-PEY AF236877 and R3-PPS AF236878); lamprey, *Petromyzon marinus* (R-PHF AF439802); leopard gecko, *Eublepharis macularius* (R1-PEY AB109032); marmoset, *Callithrix jacchus* (RII-PPS AF368286); marsh frog, *Rana ridibunda* (R1-PQS AY260153, R2-PEY AY260154 and R3-PPS AY260155); medaka, *Oryzias latipes* (a R1-PEY AB057677, b R2-PEY AB057676 and R3-SHS AB083363); Mediterranean yellowtail, *Seriola dumerilii* (R-SHS AJ130876); mouse, *Mus musculus* (R1-SEP NM_010323); Nile tilapia, *Oreochromis niloticus* (R1-SHS AB111356 and R2-PEY AB111357); orange-spotted grouper, *Epinephelus coioides* (R1-PEY DQ536435); pejerrey, *Odontesthes bonariensis* (R1B-PEY DQ875596); pig, *Sus scrofa* (R1-SDP L29342); pufferfish, *Tetraodon nigroviridis* (c R1/III-3-SHS AB212821, b R1/III-1-SHS AB212816, a R1/III-2-SHA AB212818 and a R2/nmI-2-PEY AB212825); rabbit, *Oryctolagus cuniculus* (R-SDP AY781779); rainbow trout, *Oncorhynchus mykiss* (R-PEY AJ272116); rhesus monkey, *Mucaca mulatta* (RII-PPS AF353987); rubber eel (an amphibian), *Typhlonectes natans* (R-PPS AF174481); sheep, *Ovis aries* (R1-SDP NM_001009397); striped bass, *Morone saxatilis* (R-SHS AF218841); water buffalo, *Bubalus bubalis* (R-SDP DQ821403); zebrafish, *Danio rerio* (RB-PEY XM_692308 and R4-SHS NM_001098193).

As the C-terminal can influence the mechanisms of signal transduction, such as the efficiency of coupling to effectors and downstream signalling pathway (McArdle *et al.*, 1999; Castro-Fernandez & Conn, 2002; Levavi-Sivan & Avitan, 2005), receptor desensitization and internalization (Heding *et al.*, 1998; McArdle *et al.*, 2002), it seems likely that this is also a good sorting characteristic for transduction type. The extreme situation is GnRH-Ra2, the mammalian GnRH receptor group, which entirely lacks the C-terminus, and these receptors are not rapidly desensitized or internalized compared with other GnRH-R groups (Lin *et al.*, 1998; Pawson *et al.*, 1998; Hislop *et al.*, 2000). The C-terminal of GnRH-Ra1 comprises between 36 and 61 amino acids. The tail of GnRH-Rb1 includes two groups: shorter C-tails consisting of *c.* 50 amino acids in the primate group, and longer C-tails consisting of *c.* 75 amino acids in the non-primate group. GnRH-Rb2 has the longest C-terminal comprising between 67 and 91 amino acids. Also, in an ancient fish, lamprey *P. marinus*, the GnRH receptor has a longer C-terminal than the GnRH-Rb group, suggesting that the evolutionary tendency could be progressive loss of C-terminal length (Guilgur *et al.*, 2006). Additionally, these receptor subgroups show a different preference of the G-protein-linked intracellular signalling pathway. The GnRH-Ra2 group has been shown to strongly exhibit a preference for the G_{q/11}-linked protein kinase C signalling, but GnRH-Rb does not (Levavi-Sivan & Avitan, 2005).

G_NRH RECEPTORS CAN BIND TO MULTIPLE FORMS OF G_NRH

Generally, all four subfamilies of GnRH receptors can bind with all three GnRH ligands, and all show especially high binding affinity to GnRH2. Slight ligand selectivity is evident between forebrain and midbrain GnRH types (Neill *et al.*, 2001; Wang *et al.*, 2001; Millar, 2003; Okubo *et al.*, 2003; Millar *et al.*, 2004; Flanagan *et al.*, 2007). For example, GnRH-Ra1 has higher binding affinity and higher ligand selectivity to GnRH2. GnRH-Rb2 has higher binding affinity to GnRH2 and GnRH3, but lower ligand selectivity. Although GnRH-Rb1 has been identified in tetrapods with high affinity and selectivity of GnRH2, especially in the primates, this subtype has been lost in rodents and become non-functional in humans (Millar, 2003; Pawson *et al.*, 2003). GnRH-Ra2 only has been identified in mammals and specifically exhibits high affinity and selectivity for GnRH1 (Stojilkovic *et al.*, 1994; Millar *et al.*, 2004). Recently, Caunt *et al.* (2004) showed that the active conformation of GnRH receptors, which is mediated by their C-terminal structure, can influence receptor-binding affinity. This means that discussions of GnRH-R activity must consider both ligand-binding affinity in extracellular domains as well as the influence of the intracellular domain. One caveat is that the studies are all performed in cell culture systems, so that the affinity and specificity may differ *in vivo*.

GnRH-R expression is related to GnRH innervation. For example, the distribution of GnRH-Ra1 and Rb2 is widespread throughout the brain in teleosts (Illing *et al.*, 1999; Madigou *et al.*, 2000; Okubo *et al.*, 2000b, 2001, 2003; Bogerd *et al.*, 2002; Peter *et al.*, 2003; Gonzalez-Martinez *et al.*, 2004; Moncaut *et al.*, 2005; Parhar *et al.*, 2005; Soga *et al.*, 2005; Chen & Fernald, 2006) and in amphibians (Troskie *et al.*, 2000; Wang *et al.*, 2001). However,

GnRH-Rb1 and GnRHb2 are less widely expressed in reptiles (Ikemoto *et al.*, 2004) and birds (Sun *et al.*, 2001; Lovell *et al.*, 2005), while in mammals, GnRH-Ra2 and Rb1 exhibit wider distribution in the whole brain (Millar, 2003; Millar *et al.*, 2004). Given that the GnRH-Rs do not show high fidelity, both GnRH-Rs are likely to be involved in the neural networks controlling reproduction. All vertebrates have been shown to have both GnRH-Ra and Rb expressed in the pituitary and the peripheral tissue, including retina, olfactory epithelium and gonad (Kogo *et al.*, 1995; Alok *et al.*, 2000; Madigou *et al.*, 2000; Okubo *et al.*, 2000b; Sun *et al.*, 2001; Wang *et al.*, 2001; Bogerd *et al.*, 2002; Millar, 2003; Gonzalez-Martinez *et al.*, 2004; Ikemoto *et al.*, 2004; Grens *et al.*, 2005; Moncaut *et al.*, 2005; Parhar *et al.*, 2005; Flanagan *et al.*, 2007). As expected, the distribution of GnRH receptor expression broadly corresponds to the projections of GnRH ligands. The receptor subtypes and their distributions do not change in species that only have two or three types of GnRH (Table I). However, there is not a one-to-one relationship between a specific GnRH ligand and GnRH receptor subtype in brain or peripheral tissues. For example, GnRH1 projecting into the pituitary acts on GnRH-Ra1 and GnRH-Rb2 in an African cichlid *A. burtoni* (Chen & Fernald, 2006), while both GnRH1 and GnRH2 project to the pituitary and act through only GnRH-Rb2 in the bullfrog *Rana catesbeiana* (Wang *et al.*, 2001). It is a general rule that only one GnRH receptor subtype in each species regulates gonadotropin release in the pituitary, such as GnRH-Rb2 in European sea bass *Dicentrarchus labrax* (L.) and African cichlid *A. burtoni* (Au *et al.*, 2006; Moles *et al.*, 2007), GnRH-Ra1 in goldfish *C. auratus* and Nile tilapia *O. niloticus* (Habibi, 1991; Parhar *et al.*, 2005), GnRH-Rb1 in birds (Lovell *et al.*, 2005) and GnRH-Ra2 in mammals (Karges *et al.*, 2003; Ulloa-Aguirre *et al.*, 2004). These studies suggest that GnRH receptors may be selected by their ability to detect synchronous release of GnRH in the pituitary. This difference between the conserved role of GnRH ligand and non-conserved role of GnRH receptor suggests that very different selective forces were at work during evolution. The conservation in the EC3-domain peptide sequence and the size of C-terminal tails suggest that they may have important functional roles for the action of GnRH ligands.

CONCLUSIONS AND FUTURE DIRECTIONS

Clearly, GnRH receptors are promiscuous. Distributions of GnRH-Rs do not correspond either to the fibre distributions of forebrain GnRH (GnRH1 and GnRH3) or to the midbrain (GnRH2). There is no firm correlation between GnRH-R subtype and a particular physiological role. The limited numbers of GnRH-R studies in other taxa make it difficult to identify putative principles of receptor function as related to evolution. It seems that the nomenclature introduced by Flanagan *et al.* (2007), which relates phylogenetic data about structure to actual function, will be useful for future GnRH receptor analysis. Clearly, the two groups of GnRH receptors, which have exhibited the greatest flexibility in regulating neuron networks in the brain, have separately evolved species-specific endocrine modulation in the pituitary.

GnRH, originally named and known for its central role in reproduction must now be considered instead as two or three distinct but related peptides that

TABLE I. Summary of the GnRH ligand expression and GnRH-R distribution in different tissues

GnRH system	Species	GnRH-R classification			Brain							Peripheral tissues							References
		Original name	Name by EC3		FB	POA	MB	HB	Pit	Retina	OE	Gonad	Other						
1, 2	Japanese eel <i>Anguilla japonica</i>	R	a1	++	++	++	++	++	+++	++	++	++	++	++	++	++	++	Okubo <i>et al.</i> , 1999, 2000b	
1, 2	Silver eel <i>A. anguilla</i>	R	a1*	+	+	+	+	+	+++	+	+	+	+	+	+	+	Montero <i>et al.</i> , 1996		
1, 2	Catfish <i>Clarias gariepinus</i>	R1	a1 a	+++	+	+	+	+	+	+	+	+	+	+	+	+	Bogerd <i>et al.</i> , 2002		
1, 2, 3	European sea bass	R2	a1 b	+++	+	+	+	+	+	+	+	+	+	+	+	+	Gonzalez-Martinez <i>et al.</i> , 2004; Moncaut <i>et al.</i> , 2005; Moles <i>et al.</i> , 2007		
1, 2, 3	<i>Dicentrarchus labrax</i>	dIR1A	a1 a	+++	+	+	+	+	+	+	+	+	+	+	+	+			
		dIR1B	b	+	+	+	+	+	+	+	+	+	+	+	+	+			
		dIR2A	b2 a*	++	+	+	+	+	+	+	+	+	+	+	+	+			
		dIR2B	b	++	+	+	+	+	+	+	+	+	+	+	+	+			
		dIR2C	c	+++	+	+	+	+	+	+	+	+	+	+	+	+			
1, 2, 3	African cichlid <i>Astatotilapia burtoni</i>	R2	a1	++	++	++	++	++	++	++	++	++	++	++	++	++	Au <i>et al.</i> , 2006; Chen & Fernald, 2006;		
		R1	b2*	++	++	++	++	++	++	++	++	++	++	++	++	++	Flanagan <i>et al.</i> , 2007		
1, 2, 3	Nile tilapia <i>Oreochromis niloticus</i>	R2 [RA]	a1*	-	-	-	-	-	+	+	+	+	+	+	+	+	Parhar <i>et al.</i> , 2002, 2005;		
		[RB]		+	-	-	-	-	+	+	+	+	+	+	+	+	Soga <i>et al.</i> , 2005†		
		[RII]		++	++	++	++	++	+	+	+	+	+	+	+	+			
2, 3	Goldfish <i>Carassius auratus</i>	R1	b2	+	+	+	+	+	+	+	+	+	+	+	+	+	Illing <i>et al.</i> , 1999;		
		RA	a1 a*	+	+	+	+	+	+	+	+	+	+	+	+	+	Peter <i>et al.</i> , 2003†		
		RB	b*	+	+	+	+	+	+	+	+	+	+	+	+	+	Madigou <i>et al.</i> , 2000		
2, 3	Rainbow trout <i>Oncorhynchus mykiss</i>	R	a1	++	+	+	+	+	+	+	+	+	+	+	+	+			
1, 2	African clawed frog <i>Xenopus laevis</i>	R	a1	+	+	+	+	+	+	+	+	+	+	+	+	+	Troskie <i>et al.</i> , 2000		

TABLE I. Continued

GnRH system	Species	GnRH-R classification		Brain			Peripheral tissues				References	
		Original name	Name by EC3	FB	POA	MB	HB	Pit	Retina	OE		Gonad
1, 2	Bullfrog <i>Rana catesbeiana</i>	bfR2	a1	+			+	-				Wang <i>et al.</i> , 2001
		bfR3	b1	+			+	-				
		bfR1	b2	-			-	+++				
1, 2	Leopard gecko <i>Eublepharis macularius</i>	R	a1	+++++	++	++	++	++	++	+		Ikemoto <i>et al.</i> , 2004
		R1	a1	+	+	+	+	++				
1, 2	Chicken <i>Gallus gallus</i>	R2	b1*	+	+	+	+	++				Sun <i>et al.</i> , 2001; Lovell <i>et al.</i> , 2005
		R1	a2*	+	+	+	+	+				
1	Mouse <i>Mus musculus</i>			+	+	+	+	+				Tsutsumi <i>et al.</i> , 1992; Millar, 2003; Millar <i>et al.</i> , 2004
1, 2	Marmoset <i>Callithrix jacchus</i>	R1	a2	+	+	+	+	+			+	
		R2	b1	+	+	+	+	+			+	
1, 2	Human <i>Homo sapiens</i>	R1	a2	+	+	+	+	+			+	
		R2	b1	+	+	+	+	+			+	

The number of '+' represents the related amount or the existence of GnRH-R, while '-' represents no GnRH-R expression. FB, forebrain; HB, hindbrain; MB, midbrain; OE, olfactory epithelium; Pit, pituitary gland; POA, preoptic area; [RA], data from the staining with antisera to EC3 of *Carassius auratus* GnRH-R; [RB], data from the staining with antisera to EC3 of *C. auratus* GnRH-R; [RIII], data from the staining with antisera to EC3 of amberjack *Seriola sp.* and striped bass *Morone saxatilis*.

*The GnRH-R is related to reproductive cycles, regulation of gonadotropin release or expression on gonadotropes.

†Immunocytochemistry data with antibodies generated against epitopes in EC3.

play multiple roles in organisms. The highly conserved GnRH ligand types act *via* the receptors, of which there are typically two in vertebrate organisms. In contrast to GnRH ligands, GnRH receptors have evolved into a variety of forms and the organizing principles are not yet entirely clear. The receptors have evolved in much more variable forms and the best method for functionally sorting these receptors is in the three peptides in the extracellular loop. It seems likely that one major selective force may have been the transduction cascade utilized by a particular receptor family, which suggests a direction for future research.

What lies ahead? First, an exploration of the role(s) of non-GnRH1 forms, given that our current understanding is extremely limited. For example, GnRH1 itself may play a role in modulating behaviour and other hypothalamic systems in addition to its important regulation of reproductive competence. Beyond this, we have only fragmented knowledge or hints about the possible roles of GnRH2 and GnRH3. As noted, both these forms are strictly conserved through evolution, suggesting their importance for them in all vertebrates. Second, mapping the distribution of GnRH receptors in a variety of organisms could lead to new insights and testable hypotheses about their possible functions. Third, direct assessment of function needs to be conducted for the variety of GnRH forms and receptors. However, functional testing is made difficult because of the lack of fidelity of GnRH forms for particular ligands. Development of specific blockers for GnRH receptor types would greatly facilitate this work. Finally, discovering how the modulation of intracellular-binding affinity affects the receptor–ligand selectivity could be another way of discovering the physiological role(s) of GnRH receptors. The GnRH receptor studies using pharmacological manipulation, such as kinase inhibitors, structural manipulations, chimerical receptors or genetic modification of receptors, should facilitate this work. Given the long evolutionary history of GnRH, it seems likely that much more remains to be understood about its role in behaviour and function of vertebrates.

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