

Minireview

Evolution of the role of GnRH in animal (Metazoan) biology

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1. Introduction to gonadotropin releasing-hormone

Gonadotropin releasing-hormone (GnRH) (also called luteinizing hormone-releasing-hormone (LHRH)) is the key hypothalamic neurohormone that is important in the control of reproduction for all vertebrates. Released from the hypothalamus, the GnRH decapeptide travels via the median eminence (neurohemal portal system) in most vertebrates to the pituitary where it binds to specific receptors on the exterior of pituitary gonadotropes. This binding triggers production and release of gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH) in mammals. These gonadotropins are released into the systemic bloodstream and travel to the gonads where they regulate steroidogenesis and gametogenesis.

Research during the past several years has established that there is considerable species diversity in the molecular structure of GnRHs among protochordates and vertebrates (Sower et al., 2004). In vertebrates, the neuroendocrine axis plays a central role in the control of reproduction by integrating internal and external signals during key developmental and life stages. Since GnRH is well established in its role of linking the vertebrate nervous system to the endocrine control of reproduction, it is of interest to establish the presence and function(s) of GnRH in invertebrate species for possible insights in the pre-vertebrate evolution of the GnRH/reproduction relationship. A system that will assure the increase (or at least maintenance) in numbers of individuals of a species despite changes or cycles in the environment, or in availability of new environments, is a key to successful evolution of a species. In evolution, reproduction is the central focus for selective agents. Thus, it is the premise of this review, based on the broad

presence in vertebrate species of the GnRH molecule, that its control of reproduction may have evolved in the invertebrates for the same role as a link between the nervous system and the reproductive system. At this time emerging information has focused on the demonstration of the general phyletic distribution of GnRH in invertebrates. What is needed is a better understanding of the possible function(s) of invertebrate GnRH and how these functions relate to the established patterns of reproductive control in the vertebrates.

2. The GnRH molecule in vertebrates and invertebrates

GnRH was first isolated in 1971 from the brains of pigs and sheep (Amoss et al., 1971; Matsuo et al., 1971). Since then, the GnRH family has expanded to include at least 24 molecular isoforms, 14 from various vertebrate species and 10 from invertebrates. Each GnRH isoform has been named after the organism from which it was first identified (Fig. 1). Except for the octoGnRH from the octopus, in all GnRH peptides in vertebrates and protochordates, certain regions of the molecule have been highly conserved including pGlu¹- and Ser⁴, and the COOH-terminus. These regions and the length of the molecule have remained unchanged during the 500 million years of chordate evolution. The conservation of the NH₂-terminus (pGlu), Ser⁴ and COOH-termini suggests that these regions are significant for the bioactivity and conformation of the peptide, particularly as they relate to effective receptor binding, resistance to enzymatic degradation, and receptor-mediated events required for gonadotropin release. This significance has been supported by numerous activity studies of GnRH analogs in mammalian and other vertebrate systems (Sealfon et al., 1997).

Multiple forms of this hormone have been identified in representative species of all classes of vertebrates.

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GnRH Family of Peptides												
	1			2	3	4	5	6	7	8	9	10
<i>Vertebrate</i>												
Mammal	pGlu			His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	GlyNH ₂
Guinea Pig	pGlu			Tyr	Trp	Ser	Tyr	Gly	Val	Arg	Pro	GlyNH ₂
Chicken - I	pGlu			His	Trp	Ser	Tyr	Gly	Leu	Gln	Pro	GlyNH ₂
Rana	pGlu			His	Trp	Ser	Tyr	Gly	Leu	Trp	Pro	GlyNH ₂
Seabream	pGlu			His	Trp	Ser	Tyr	Gly	Leu	Ser	Pro	GlyNH ₂
Salmon	pGlu			His	Trp	Ser	Tyr	Gly	Trp	Leu	Pro	GlyNH ₂
Whitefish	pGlu			His	Trp	Ser	Tyr	Gly	Met	Asn	Pro	GlyNH ₂
Medaka	pGlu			His	Trp	Ser	Phe	Gly	Leu	Ser	Pro	GlyNH ₂
Catfish	pGlu			His	Trp	Ser	His	Gly	Leu	Asn	Pro	GlyNH ₂
Herring	pGlu			His	Trp	Ser	His	Gly	Leu	Ser	Pro	GlyNH ₂
Chicken -II	pGlu			His	Trp	Ser	His	Gly	Trp	Tyr	Pro	GlyNH ₂
Dogfish	pGlu			His	Trp	Ser	His	Gly	Trp	Leu	Pro	GlyNH ₂
Lamprey - III	pGlu			His	Trp	Ser	His	Asp	Trp	Lys	Pro	GlyNH ₂
Lamprey - I	pGlu			His	Tyr	Ser	Leu	Glu	Trp	Lys	Pro	GlyNH ₂
<i>Invertebrate</i>												
Octopus	pGlu	Asn	Tyr	His	Phe	Ser	Asn	Gly	Trp	His	Pro	GlyNH ₂
Tunicate - I	pGlu			His	Trp	Ser	Asp	Tyr	Phe	Lys	Pro	GlyNH ₂
Tunicate - II	pGlu			His	Trp	Ser	Leu	Cys	His	Ala	Pro	GlyNH ₂
Tunicate-III	pGlu			His	Trp	Ser	Tyr	Glu	Phe	Met	Pro	GlyNH ₂
Tunicate-IV	pGlu			His	Trp	Ser	Asn	Gln	Leu	Thr	Pro	GlyNH ₂
Tunicate-V	pGlu			His	Trp	Ser	Tyr	Glu	Tyr	Met	Pro	GlyNH ₂
Tunicate-VI	pGlu			His	Trp	Ser	Lys	Gly	Tyr	Ser	Pro	GlyNH ₂
Tunicate-VII	pGlu			His	Trp	Ser	Tyr	Ala	Leu	Ser	Pro	GlyNH ₂
Tunicate-VIII	pGlu			His	Trp	Ser	Leu	Ala	Leu	Ser	Pro	GlyNH ₂
Tunicate-IX	pGlu			His	Trp	Ser	Asn	Lys	Leu	Ala	Pro	GlyNH ₂

Fig. 1. Primary structures of GnRHs in vertebrates and invertebrates. There are currently 24 known forms of GnRH: 14 in vertebrates and 10 in invertebrates.

The number of GnRH molecular variants is surprising, particularly in view of the relative constancy of their physiological role in vertebrates (Sower et al., 2004). Growing evidence reveals that almost all vertebrates synthesize at least two isoforms of GnRH. One form, the neuroendocrine form referred to as GnRH-I, is present in the hypothalamus and acts on the pituitary. The second form, referred to as GnRH-II, is extra-hypothalamic and may have no direct involvement in gonadotropin regulation. Because of the confusing nature of the nomenclature of GnRHs, Fernald and White (1999) proposed a nomenclature based on the

anatomical location of GnRH in the brain as well as on the phylogenetic analysis of the precursors of GnRH. Since that time, still more GnRH forms and precursors have been determined; thus Silver and Sower have proposed a modified version of this scheme which includes all known vertebrate forms, and an extensive phylogenetic analysis of most known GnRH precursors (Silver and Sower, 2002) (see Table 1). Based on this scheme, GnRH in vertebrates is divided into four types based on a combination of function, location of expression and molecular phylogenetic analysis.

Table 1

The lineages of vertebrate GnRH: GnRH is divided into four types based on a combination of function, location of expression and molecular phylogenetic analysis

Type GnRH	Brain distribution/origin	Primary GnRH structures identified in vertebrates
GnRH-I	Hypothalamus, diencephalon/olfactory origin	Mammal GnRH in <i>mouse, primate, human, sheep, pig, eel, newt, frog</i> ; chicken GnRH-I in <i>chicken, lizard</i> ; salmon GnRH in <i>goldfish, salmon</i> ; catfish GnRH in <i>catfish</i> ; dogfish GnRH in <i>dogfish</i>
GnRH-II	Midbrain/ventricular ependyma origin	Chicken GnRH-II in <i>mouse, primate, human, chicken, lizard, frog, newt, eel, goldfish, catfish, salmon, medaka red seabream, tilapia, ratfish</i>
GnRH-III	Telencephalon/olfactory origin	Salmon GnRH in <i>medaka, red seabream, tilapia</i>
GnRH-IV	Hypothalamus, diencephalon/ventricular origin	Lamprey GnRH-I and lamprey GnRH-III in <i>lamprey</i>

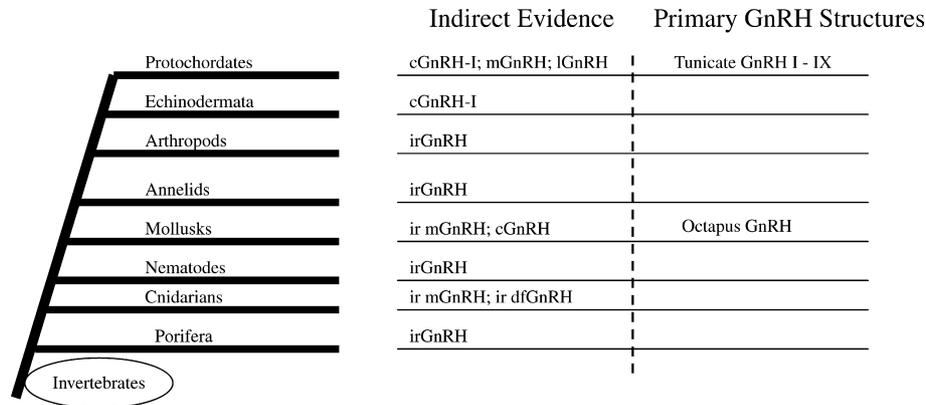


Fig. 2. Taxonomic distribution of GnRH-like peptides and primary GnRH structures in invertebrates. (Adams et al., 2003; Al-Yousuf, 1990; Anctil, 2000; Andries and Tramu, 1984; Cameron et al., 1999; Chang et al., 1983; Craig et al., 1997; Di Cosmo and Di Cristo, 1998; Di Cristo et al., 1995; Di Fiore et al., 2000; Hansen et al., 1982; Iwakoshi et al., 2002; Kelsall et al., 1990; Pazos and Mathieu, 1999; Powell et al., 1996; Schriebman et al., 1986; Tsutsui et al., 1998; Verhaert et al., 1990; Young et al., 1999.) Mammalian GnRH (mGnRH); chicken GnRH-I (cGnRH-I); lamprey GnRH (lGnRH); immunoreactive GnRH (irGnRH); dogfish GnRH (dfGnRH).

The primary structures of ten invertebrate GnRH forms have been identified, nine in two urochordate species, *Chelyosoma productum* and *Ciona intestinalis*, (referred to as tunicate 1 through tunicate 8) (Adams et al., 2003; Powell et al., 1996) and a GnRH of 12 amino acids has been identified in octopus (Iwakoshi et al., 2002) (Fig. 1). Using immunocytochemical, immunological, and chromatography techniques, immunoreactive GnRH or GnRH-like peptide have been demonstrated in Porifera, Coelenterata, Nematoda, Annelida, Mollusca, Arthropoda, Echinodermata, hemichordates, cephalochordates, and protochordates (Rastogi et al., 2002) (Fig. 2). However, caution should be exerted when identifying the ir-GnRH isoform(s) in the invertebrates using indirect means such as HPLC and RIA with various antisera. As an example, it had originally been reported that *C. intestinalis* had two immunoreactive (ir) GnRH peptides, ir-chicken GnRH-I and ir-mammalian GnRH (Di Fiore et al., 2000) using HPLC, RIA, and mass spectrometry; however, six primary structures have been determined by molecular cloning in *C. intestinalis* but none of these isoforms are chicken GnRH-I or mammalian GnRH (Adams et al., 2003). Given that the primary structures have only been determined in tunicates and octopus, it is difficult to propose a phylogenetic scheme. Interestingly, the octopus has retained the crucial Gly residue in the sixth position, which influences the tertiary structure of most of the vertebrate GnRHs. Based on the known primary sequences, a phylogenetic tree is presented in Fig. 3. The GnRH forms essentially form three clades. We would speculate based on this tree that the salmon, chicken GnRH-II, and dogfish are ancient forms that would be likely found in invertebrates. However, until more GnRH forms and their cDNAs and genes as well as function have been identified across invertebrates, it is difficult to predict the phylogeny of GnRH.

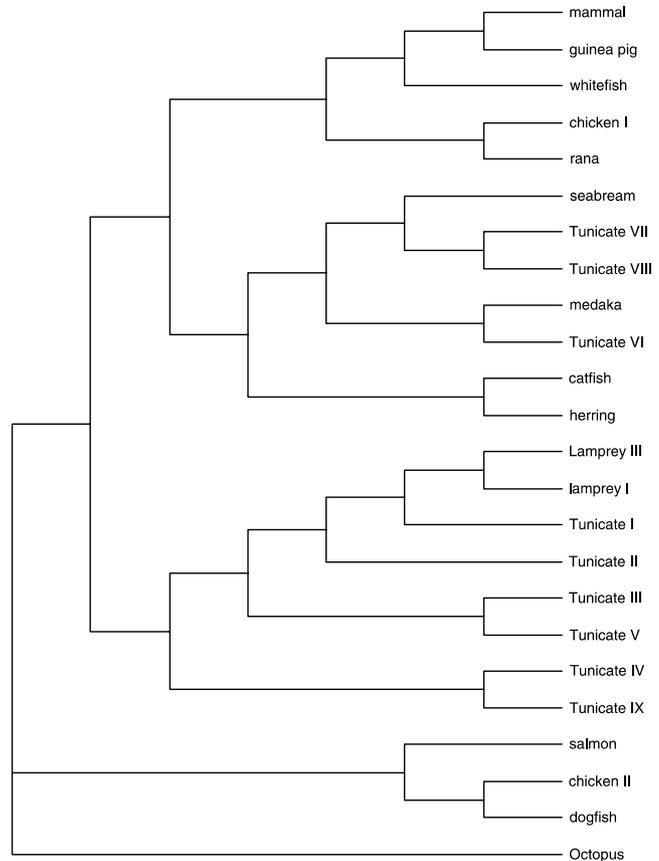


Fig. 3. Phylogenetic analysis of 24 GnRH primary amino acid structures. A tree was constructed using the neighbor joining method rooted with octopus GnRH-like peptide using PAUP (Phylogenetic Analysis Using Parsimony) 4.0 beta10 (Swofford, 2000). Octopus GnRH was considered the out-group in this analysis. (Adams et al., 2003; Adams et al., 2002; Burgus et al., 1972; Carolsfeld et al., 2000; Iwakoshi et al., 2002; Jimenez-Linan et al., 1997; King and Millar, 1982a; King and Millar, 1982b; Lovejoy et al., 1991; Matsuo et al., 1971; Miyamoto et al., 1982; Ngamvongchon et al., 1992; Okubo et al., 2000; Powell et al., 1996; Powell et al., 1994; Sherwood et al., 1983; Sherwood et al., 1986; Sower et al., 1993; Yoo et al., 2000).

3. GnRH associated with reproductive function in invertebrates

The diversity of anatomical distribution patterns observed for irGnRH or irGnRH-like material in invertebrates suggests that GnRH may have multiple functions, not unlike the case for vertebrates. However, consistent with the notion of a role of GnRH in invertebrate reproduction, a few physiological studies in a handful of invertebrate species have indicated that GnRH and its variants and analogs are active in stimulating some aspect of reproduction. In *C. intestinalis*, mammalian GnRH and cGnRH-I, tested in in vitro incubations of gonads, were shown to induce the release of sex steroids (Di Fiore et al., 2000). Injections of various GnRH forms, including the testing of tunicate GnRH-I and tunicate GnRH-II into *C. intestinalis*, induced gamete release (Terakado, 2001). Synthetic forms of the six newly identified *C. intestinalis* GnRHs were injected into adult tunicates and all of these tunicate GnRHs induced release of gametes (Adams et al., 2003). Chang et al. (1983) demonstrated that in amphioxus, *Branchiostoma belcheri* Gray, injection of a mammalian GnRH agonist into the body cavity resulted in an increase in estradiol and testosterone production. In mollusks, Young et al. (1997) observed that synthetic mammalian GnRH can induce an increase in egg-laying in the snail, *Helisoma trivolvis*. In later studies by these same authors, immunocytochemical studies showed that irGnRH cells were located in ganglia innervating the reproductive system indicating that reproduction may be regulated by GnRH in this mollusk (Young et al., 1999).

4. GnRH as a pheromone

It has been difficult to generalize about the function(s) of GnRH in invertebrates due to lack of sequence data for GnRH-like ligands and their putative receptors, and paucity of functional studies. One possibility of the nature of an invertebrate function of GnRH was first suggested by Cameron et al. (1999). In the Hemichordate *Saccoglossus bromophenolosus* immunoreactive (ir)-GnRH was found in conspicuous modified nerve cells in the skin ('mulberry' cells) (Cameron et al., 1999). These authors speculated that because of the orientation of the integumentary mulberry cells in *Saccoglossus*, that GnRH may act as a pheromone. Recently Gorbman et al. (2003) showed in the hemichordate *Saccoglossus* sp. and in the mollusk *Mopalia* sp. (a chiton) that GnRHs, added in low concentrations to the environmental sea water, can rapidly provoke shedding of ripe gametes into the sea water, in what is clearly a pheromonal action. Specifically, two of the peptides, lamprey GnRH-1 and tunicate GnRH-II, stimulated the release of ripe gametes at a concentration of 1.0 mg GnRH/liter.

Three other GnRHs—lamprey GnRH-III, tunicate GnRH-1, and a modified chicken GnRH-II—had no such action under the same test conditions. This finding has led to some exciting speculation concerning the possible or probable role of GnRH's among invertebrates. Since the spawning response could be produced by some GnRH's and not by others, it would appear that some kind of molecular recognition may occur, possibly involving some receptor-signal transduction pathway. Thus it is suggested that GnRH's in at least some invertebrates may function as pheromones serving to stimulate simultaneous spawning of individuals in a population of invertebrates, and in this way assure more successful fertilization in species that must release their gametes into the water in which they live.¹

The breadth of the phyletic distribution of GnRH-like peptides suggests that these molecules have been retained during evolution because they serve an adaptively useful function, possibly the same or similar function throughout the invertebrates. The ubiquity of GnRH's from the coelenterates, a lineage of radially symmetrical metazoans, to the most highly evolved vertebrates indicates that this peptide has served an important adaptive use throughout animal evolution.

Most if not all of the GnRH forms that have been isolated and sequenced in animals are from the nervous system. Thus GnRH release becomes an important feature of the animal reproductive process since it makes possible the use of the peptide when particular seasonal or other sense organ—detected conditions (appropriate physical environment, behavioral signals, etc.) prevail. In this sense we can understand the value of GnRH as a factor regulating the reproductive processes of all animal species, invertebrate and vertebrate (Gorbman et al., 2003). This scenario is a broad one and it remains for future research to fill in the details and to either confirm or deny it.

5. GnRH receptor

Since the first successful cloning of a GnRH receptor transcript from the mouse (Tsutsumi et al., 1992), a total of 32 GnRH receptor cDNAs have been published in 22 organisms: 11 mammals and 11 earlier evolved vertebrates (Sower et al., 2004). In the invertebrates, only one GnRH-like receptor has been identified which is found in *Drosophila melanogaster* (Hauser et al., 1998). The ligand that binds to this receptor has yet to be identified.

¹ The probability that GnRH may act as a pheromone raises an important point. Many of the small invertebrate species are immobilized on or fixed to their substrate so that mating contact for reproductive purposes is not possible. Accordingly, simultaneous gamete release by whole populations of organisms is a most important means of assuring fertilization and successful reproduction.

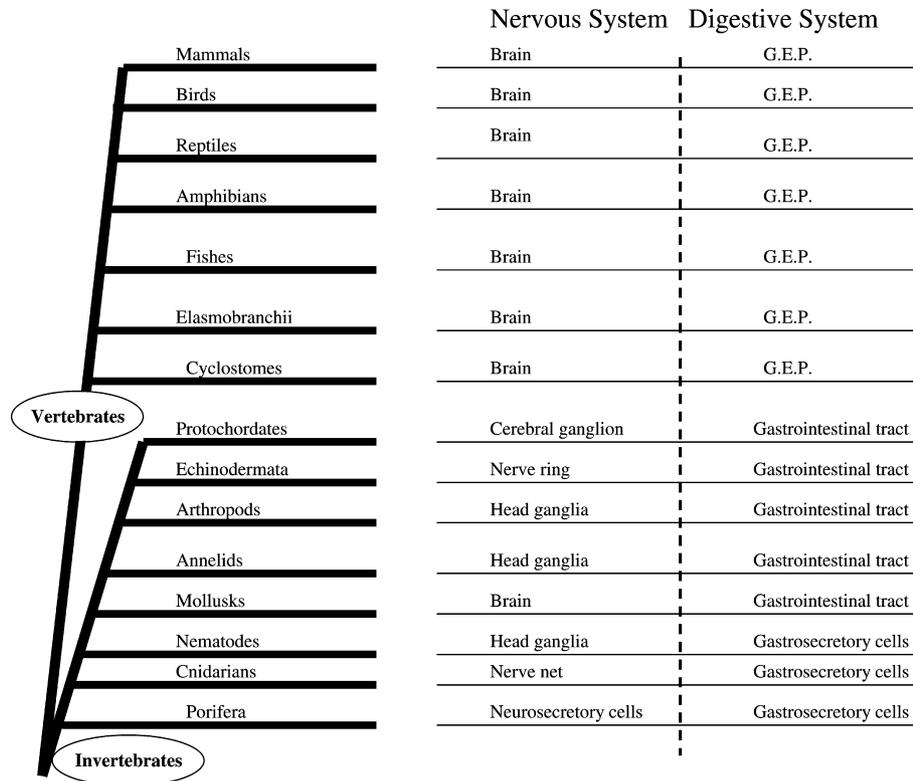


Fig. 4. Schematic evolutionary diagram illustrating the presence/absence and the relative complexity of the nervous and digestive systems of representative classes and phyla of invertebrates and vertebrates.

The authors suggest based on the gene structure that the *Drosophila* and mammalian GnRH receptor genes are evolutionarily related. Once the ligand is identified, it can be determined if indeed this is a functional GnRH receptor involved in some aspect of reproduction in an invertebrate.

6. Summary

The vast differences in levels of complexity of the nervous system between species within a single phylum of invertebrates, between phyla or between agnathans, fishes and mammals, appear to involve more than simple consequences of increased numbers of neurons (Bullock, 1993). Newly recognized variables, such as neurochemicals, anatomical structures and physiological mechanisms, have been added during the long period of evolution. The peptides that have known functions in one species may have been co-opted during evolution by other cells or organs and may have different functions. This is likely the case with GnRH since across vertebrates the expression of GnRH receptors has been reported in many different tissues, although the functional values of differences are unknown at this time. Yet at least one form of GnRH has been highly conserved in structure and function across all vertebrates in terms of regulating reproduction. There are gross variations in

the nervous systems of the invertebrates as shown in Fig. 4. Cephalopod mollusks have a highly developed brain compared to varying degrees of complexity of brain/nerve cells in the invertebrates. In Porifera, there are sensory and neurosecretory cells which occur in clusters. The earliest appearance of neurons is noted in the primitive cnidarian and other coelenterate invertebrates, and these neurons secrete both peptide and nonpeptide regulators (Norris, 1997). Thus it is conceivable that GnRH-like molecules are present even in animal groups that evolved earlier than the coelenterates, such as the Protozoa. These animal forms have not yet been tested for presence of GnRH's. If GnRH-like immunoreactivity occurs in coelenterates, it would appear possible that GnRH-like peptides had an even earlier presence in animal forms that preceded the coelenterates. This interesting possibility deserves appropriate study.

Acknowledgments

This manuscript is dedicated to Professor Aubrey Gorbman as his last published paper. Aubrey and I have worked on this manuscript during the last few months of his life. He was the founder and editor of *General and Comparative Endocrinology*. The first issue was published in April, 1961 and had in the Editorial Statement

the following: “. . .keys to the understanding of complex systems must often lie in the simpler ones from these have evolved. . .” Thus, this manuscript is in dedication and honor to Professor Aubrey Gorbman who not only laid the groundwork for agnathan comparative endocrinology but had significant impact on many areas of comparative endocrinology. This manuscript reflects his first editorial statement of gaining an understanding of the simpler systems in order to understand the more complex systems. I considered him an incredible mentor, good friend, colleague, and collaborator. Aubrey touched so many people: students in the classroom, as a world class researcher, as a founder and editor of a major scientific journal in our field, as a visiting scientist and lecturer to a number of Universities around the world, as a senior author of textbooks on Comparative Endocrinology and as editor for GCE for over 30 years. My career in agnathan endocrinology is dedicated to him.

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