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# GONADOTROPIN-RELEASING HORMONE IN PRIMITIVE FISHES

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In this report, I will review recent data on the gonadotropin-releasing hormone (GnRH) in relation to reproductive function in the primitive groups of agnathan and chondrichthyan fishes. In most vertebrates, the hypothalamus and pituitary have well-defined roles in the control of reproduction. The hypothalamus in response to external and internal cues releases GnRH which, in turn, stimulates pituitary gonadotropin(s) secretion(s). However, there is very limited evidence on the function of hypothalamic GnRH in agnathan and chondrichthyan fishes. These primitive vertebrates are of particular importance in understanding possible hypothalamic-pituitary relationships since they represent the two oldest lineages of vertebrates. The agnathans are subdivided as myxinoids (hagfish) and petromyzonids (lampreys), and the chondrichthyans are subdivided into elasmobranchs (sharks and rays) and holocephalians (chimaeras and ratfishes). GnRH as a stimulator of the pituitary-gonadal axis appears to be conserved through vertebrate evolution. However, information is fragmentary, particularly in these groups of fish which probably represent the least understood of all vertebrates.

## STRUCTURE OF GNRH AND IDENTIFICATION OF GNRH-LIKE FORMS

Purification and sequence analysis of GnRH has been a sufficiently difficult task that only five GnRH molecules from vertebrates have been identified and structurally characterized. The structure of GnRH has been determined in only one species of primitive fishes, sea lamprey (*Petromyzon marinus*) (Sherwood *et al.*, 1986). The lamprey GnRH differs in five amino acids compared with mammalian GnRH and chicken GnRH I and in four amino acids compared with salmon GnRH add chicken GnRH II. The $NH_2$-terminal, $pGlu^1$-$His^2$ and $Ser^4$ and the COOH-terminal regions, and the length of the molecule have remained highly conserved during 550 million years of evolution.

Chromatographic and immunologic studies using antibodies to the known GnRHs have revealed the nature of GnRHs in other vertebrates. In hagfish, the

TABLE 1. Distribution of GnRHs in Primitive Fishes by Chromatographic and Immunological Studies

| Species | mGnRH | cGnRH I | cGnRH II | sGnRH | lGnRH | UNK |
|---|---|---|---|---|---|---|
| Class: Agnatha | | | | | | |
| Hagfish | | | | | | |
| *Myxine glutinosa* | | | | | | X |
| Lamprey | | | | | | |
| *Petromyzon marinus* | | | | | X | X(2) |
| Class: Chondrichthyes | | | | | | |
| Sharks and Rays | | | | | | |
| *Poroderma africanum* | X | | X | X | | X(4) |
| *Raja erinacea* | X | | X | X | X | |
| Ratfish | | | | | | |
| *Hydroloagus colliei* | | | X | | | |

UNK, unidentified molecular variant of GnRH

presence of irGnRH was reported (King and Millar, 1980), while in other studies GnRHs were not detected in hagfish brain (Sherwood and Sower, 1985; Crim *et al.*, 1979; Nozaki and Kobayashi, 1979). More recently, we demonstrated GnRH-like peptide in hagfish brain using a lamprey GnRH antibody (Sower, unpublished). Hagfish is likely to have a GnRH variant which is different from the known GnRHs, however, further studies are required to verify the existence of a GnRH-like molecule in hagfish brain. In sea lampreys, a second form of GnRH has been identified in brain but only the amino acid composition and not the sequence is known (Sherwood *et al.*, 1986). In addition, a third form of irGnRH has been detected using the lamprey GnRH antibody (Sower, unpublished).

In chondrichthyans, Powell *et al.* (1986) demonstrated seven GnRH molecular forms in the extracts from brains of dogfish (*Poroderma africanum*), three of which co-eluted with synthetic mammal GnRH (mGnRH), chicken GnRH II (cGnRH II) and salmon GnRH (sGnRH). The other four GnRH-like forms are considered novel forms. The dominant form of immunoreactive GnRH-like peptide in brains from dogfish shark (*Squalus acanthias*) and ratfish (*Hydrolagus colliei*) eluted with synthetic chicken GnRH II (Sherwood and Sower, 1985). GnRH-like forms identified in brains from skate (*Raja erinacea*) were similar to mammal GnRH, chicken GnRH II, salmon GnRH and lamprey GnRH as well as several other novel forms (Bolduc *et al.*, 1988).

## DISTRIBUTION OF GNRH

In most vertebrates, GnRH released by the hypothalamus reaches the anterior pituitary by a hypophysial portal system and in teleost fishes by direct innervation. Unlike most vertebrates, there is the absence of a hypothalamo-hypophysial

link of either a hypophysial portal system or nervous connections between pituitary (gonadotropic region) and hypothalamus in agnathans and chondrichthyans. This suggests that GnRH reaches the pituitary either by a systemic route or by simple diffusion (reviewed by Dodd and Dodd, 1985).

TABLE 2. Distribution of GnRH as Determined by Immunocytochemistry

| Species | Hypothalamus | Nervus Terminialis | Extra Hypothalamic |
|---|---|---|---|
| Class: Agnatha | | | |
| Hagfish | 0 | 0 | + |
| Lamprey | +++ | 0 | 0 |
| Class: Chondrichthyes | | | |
| Sharks | + | +++ | |

In hagfish, immunocytochemical studies have failed to indicate the presence of a GnRH-like peptide in the hypothalamus of *Eptatretus stouti* (Crim *et al.*, 1979) and *E. burgeri* (Nozaki and Kobayashi, 1979). A recent immunocytochemical study utilizing antibodies to lamprey GnRH or salmon GnRH also failed to demonstrate any immunoreactivity in the hypothalamus of *Myxine glutinosa*, but did indicate the presence of immunoreaction in extra hypothalmic brain with antibody to salmon GnRH (King, Anthony and Sower, unpublished). In a recent review, Dodd and Dodd (1985) concluded that the hypothalamus plays little or no role in reproductive control in hagfish.

In lampreys unlike the hagfish, there is immunocytochemical evidence for hypothalamic influence on the pituitary by diffusion of GnRH from the neurohypophysis to the pars distalis,of the pituitary (Nozaki *et al.*, 1984; King *et al.*, 1988). GnRH-like neurons identified by immunocytochemistry project their fibers primarily into the neurohypophysis from the preoptic region (Nozaki *et al.*, 1984; Crim *et al.*, 1979; King *et al.*, 1988). In these same studies, GnRH was not demonstrated to be widely distributed in extra hypothalamic regions as noted for other neuropeptides within the lamprey brain. Thus, there is evidence of normal occurrence of GnRH in a part of the lamprey brain homologous with that brain region in higher vertebrates in which GnRH localization forms part of a neuroendocrine mechanism for gonadotropin secretion.

In chondrichthyans, there is a hypophysial portal system between the hypothalamus and pituitary except to the lobe of pituitary that is considered to be the gonadotropic region (reviewed by Dodd and Dodd, 1985); the ventral lobe (in elasmobranchs) and the buccal lobe (in holocephalans) are separated from the rest of the pituitary (Dodd and Dodd, 1985). These authors conclude that GnRH must reach these lobes by peripheral circulation. There has been only one reported immunocytochemical study of GnRH distribution in the brain in chondrichthyans.

Nozaki *et al.* (1984) demonstrated using mammalian antibodies to GnRH that most of the immunoreactivity was in the nervus terminalis system with very few positive perikarya found in the hypothalamus and no positive fibers in the median eminence in *Triakis scyllia.*

## BIOLOGICAL ACTIVITY OF GNRH

With the exception of hagfish, evidence has been presented for the existence of a gonadotropic hormone in the pituitary gland of species of agnathans and chondrichthyans (reviewed by Dodd and Dodd, 1985). However, investigations on the role of GnRH in reproductive processes have been impeded by the lack of a purified gonadotropin that can be used in assays to measure pituitary function. Therefore, to date, biological activity of GnRH has been assessed by steroidogenesis or gametogenesis in *in vivo* studies. Gonadotropin has only been purified in one primitive fish, the dogfish (*Scyliorhinus canicula*) (Sumpter *et al.*, 1978); yet no action of GnRH on possible pituitary GTH release has been documented in dogfish.

TABLE 3. GnRH as Stimulator of Reproductive Functions

| | Hagfish | Lamprey | Ratfishes | Sharks/Skates |
|---|---|---|---|---|
| Biological Activity | 0 | +++ | + | + |

In hagfish, there is no evidence as yet for a role of GnRH in stimulating reproductive activity. Injections of mammal GnRH analogue had no effect on plasma estradiol or progesterone levels (MacKichan, Sower and Gorbman, unpublished). There have been so few studies and many questions yet answered that hypothalamic influence on reproductive processes remains unresolved in hagfish.

In lampreys, the availability of synthetic lamprey GnRH has made it possible to measure plasma steroid levels and gametogenesis as measures of reproductive function. In experiments using lamprey GnRH and analogs, lamprey GnRH stimulated steroidogenesis and ovulation or spermiation in adult sea lampreys during their final reproductive stage indicating a regulatory influence of the hypothalamus on the pituitary-gonadal axis (Sower *et al.*, 1987; Sower, 1989). A mammalian GnRH analog or antagonists of mammalian GnRH and lamprey GnRH stimulated or inhibited, respectively, the reproductive system, suggesting that the pituitary GnRH receptor is capable of binding variant forms of GnRH (Sower *et al.*, 1983, 1987). In *in vitro* studies, lamprey GnRH has been demonstrated to potentiate estradiol release in co-cultures of testis and pituitary (Sower,

unpublished). This information, combined with the immunocytochemical studies, provide evidence for the regulatory influence of the hypothalamus on the pituitary-gonadal axis.

In chondrichthyans, there are very few studies on the biological activity of GnRH. In dogfish, injection of dogfish hypothalamic extracts or mammal GnRH elevated sex steroid levels and induced ovulation (Jenkins and Dodd, 1980). In another study, injection of chicken GnRH II elevated plasma levels of progesterone, testosterone and estradiol in the female skate (*Raja erinacea*) (Fileti and Callard, unpublished). In ratfish, mammal GnRH induced ovulation (Dodd and Dodd, 1985).

In summary, data for the structure and function of GnRH in agnathans and chondrichthyan fishes is very limited, and many questions concerning hypothalamic regulation remain unresolved. Certain aspects of the GnRH molecule and function appear to be conserved through vertebrate evolution; however, the role of GnRH in these primitive fishes is far from understood.

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