

**THE PHYSIOLOGY OF REPRODUCTION AND MOLECULAR
EVOLUTION OF GnRH AND GnRH RECEPTORS IN LAMPREYS**

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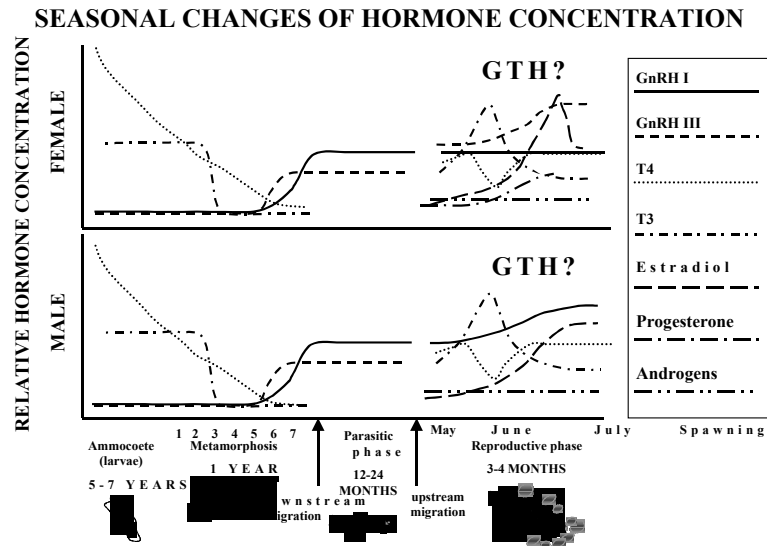
Lamprey Reproductive Physiology in Sea Lamprey

A key neuroendocrine function of the hypothalamus in the control of reproduction is the timed release of the decapeptide, GnRH, which acts on the pituitary to regulate the pituitary-gonadal axis for all vertebrates. Gonadotropins, secreted in response to GnRH, are released from the pituitary gland and are the major hormones influencing steroidogenesis and gametogenesis.

Until about 18 years ago, there had been little evidence for brain control of reproduction in lampreys. However, we have made substantial progress in this area (See Reviews: Sower, 1998, 2002; Sower and Kawauchi, 2001). We now have established a well-defined neuroendocrine axis in lampreys. We identified and sequenced two molecular forms of GnRH in the sea lamprey: lamprey GnRH-I and lamprey GnRH-III. In addition, we have identified the cDNA of lamprey GnRH-I (Suzuki et al., 2000) and lamprey GnRH-III (Silver et al. 2001). Lampreys are the most primitive vertebrates for which there are demonstrated functional roles for multiple GnRH neurohormones involved in pituitary-reproductive activity. Both lamprey GnRH-I and -III have been shown to induce steroidogenesis and spermiation/ovulation in adult sea lampreys. In lampreys undergoing metamorphosis, there is an increase of brain lamprey GnRH-I and -III that coincides with the acceleration of gonadal maturation (Fig 1). In immunocytochemical studies, both ir-lamprey GnRH-I and -III can be found in the cell bodies of the rostral hypothalamus and preoptic area in larval and adult sea lamprey. Most of the ir-GnRH in the brain of larval stage

lampreys has been shown to be lamprey GnRH-III. Thus, lamprey GnRH-III may be the more active form during gonadal maturation. Such information suggests that the structure and function of the GnRHs in vertebrates are highly conserved throughout vertebrate evolution.

Fig 1 - Schematic diagram of the relative circulating hormone concentration during the life cycle of the female sea lamprey. T4, thyroxine; T3, triiodothyronine; GnRH-I, lamprey gonadotropin-releasing hormone-I; lamprey GnRH-III, lamprey gonadotropin-releasing hormone-III; E2, estradiol; and prog, progesterone.



Molecular Cloning of the GnRH Receptor cDNA in the Sea Lamprey

Previous studies from our laboratory have shown two distinct GnRH binding sites in the pituitary of the sea lamprey. Concentration of these binding sites were shown to increase in correlation with increased gonadal maturation and brain GnRH concentration to peak near and at ovulation (Sower, 2002). GnRH's action is modulated through a 7-transmembrane G-protein coupled receptor. The question that arises is whether there are two binding sites or two different

receptors. The cDNA of one GnRH receptor has been cloned in the sea lamprey (Nucci et al., 2002). This receptor has significant identity to several other GnRH receptor cDNAs including 61.2% with the aquatic caecilian *Typhlonectes natans*, 60.7% and 59.8% with the bullfrog receptors 1 and 3, respectively, and 59% with the striped bass *Morone saxatilis*.

Molecular Phylogenetic Analysis Within the Petromyzontiforme Lineage Using The cDNA For Lamprey Gonadotropin Releasing Hormone-III

In addition to our research on the reproductive hormones in the sea lampreys, we have extended these studies to isolate and sequence the cDNA precursor of lamprey GnRH-III in representative species of each of the three families of lamprey in order to assess their phylogenetic relationship. The lamprey lineage is generally considered to be divided into three families, the Petromyzonidae, or holarctic family, found in the northern hemisphere, and the two southern hemisphere families, the Geotriidae and Mordaciidae. The phylogeny of these families has been primarily based on size, shape, and distribution of dentition. The cDNA encoding prepro-lamprey GnRH-III was isolated from two holarctic species, *Petromyzon marinus* and *Lampetra tridentatus*, and one from each southern hemisphere species, *Geotria australis* and *Mordacia mordax* (Silver et al., 2001). Initial segments of the transcript were isolated using PCR on cDNA constructed from total RNA that were extracted from each respective species hypothalami. 5'RACE was used to amplify the rest of the template using the Marathon cDNA Amplification Kit (CLONTECH) and full-length transcripts were isolated. Using PAUP v4.0, the molecular phylogenetic relationship of lamprey GnRH-III between the three families of lamprey was investigated. These data suggest that the lamprey GnRH-III cDNAs of the two southern hemisphere species are highly divergent from the lamprey GnRH-III cDNA of the holarctic species.

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