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Effects of Lamprey Gonadotropin-Releasing Hormone-III on Steroidogenesis and Spermiation in Male Sea Lampreys

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The biological activities of lamprey gonadotropin-releasing hormone-III (GnRH-III) were determined in the adult male sea lamprey, *Petromyzon marinus*. One injection of lamprey GnRH-III at 0.1 or 0.2 μg/g body wt stimulated plasma estradiol and progesterone levels in adult male sea lampreys undergoing final maturation. Four successive injections of lamprey GnRH-III at 0.1 μg/g or lamprey GnRH-I at 0.2 μg/g induced spermiation in 78 or 30% of the lampreys, respectively, compared to 0% in controls by Day 16. In summary, lamprey GnRH-III is biologically active in stimulating the pituitary–gonadal axis in adult male lampreys.

The primary structure of gonadotropin-releasing hormone-III (lamprey GnRH-III) has recently been determined in sea lampreys, *Petromyzon marinus*, as pGlu–His–Trp–Ser–His–Asp–Trp–Lys–Pro–Gly–NH_2 (Sower *et al.*, 1993). The primary structure of lamprey GnRH-III differs in three amino acids compared with that of lamprey GnRH-I. Lamprey GnRH-III is more closely related to the other members of the GnRH family than lamprey GnRH-I. Lamprey GnRH-III has 80% identity with chicken GnRH-II and dogfish GnRH, 70% identity with catfish GnRH-I, lamprey GnRH-I, and salmon GnRH, and 60% identity with mammal GnRH and chicken GnRH-I (Sower *et al.*, 1993).

The significance of two forms of lamprey GnRH in the brain of the lamprey is currently under investigation. Ovulatory, spermiation, and steroidogenic responses to lamprey GnRH-I have been well documented in adult sea lampreys (Sower *et al.*, 1987; Sower, 1989, 1990). In preliminary studies, lamprey GnRH-III has also been shown to be biologically active in adult female lampreys as determined by increased levels of plasma steroids (Sower *et al.*, 1993). Administration of lamprey GnRH-III significantly stimulated plasma estradiol in a dose-related manner compared to controls. Lamprey GnRH-III was detected in brain samples of lampreys undergoing metamorphosis (Youson and Sower, 1991). In these studies, there was an increase of brain GnRH during metamorphosis which coincided with the acceleration of gonadal maturation. Thus, based on the biological activity of lamprey GnRH-III in adult female lampreys and the occurrence of this peptide during metamorphosis in lampreys, we suggest that both lamprey GnRH-I and -III are neurohormones involved in reproduction in lampreys. However, the biological activity of lamprey GnRH-III has not been determined in adult male sea lampreys. Therefore, the objective of this study was to determine the effects of lamprey GnRH-III on spermiation and plasma levels of estradiol and progesterone in adult male sea lampreys.

MATERIALS AND METHODS

Lampreys. Adult sea-run sea lampreys were collected in a trap located at the top of the salmon ladder

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at the Cocheco River in Dover, New Hampshire in May and June during their upstream spawning migration from the ocean. The lampreys were transported to the freshwater fish hatchery at the University of New Hampshire and maintained in an artificial spawning channel supplied with flow-through of reservoir water at ambient temperature range of 13–20°C under natural photoperiod. A total of 60 lampreys were used in two experiments.

Peptides. Lamprey GnRH-III was generously donated by Dr. Russell Doolittle, University of California at San Diego. Lamprey GnRH-I was obtained from Peninsula Laboratories, Inc., CA.

Experiment 1. In experiment 1, lampreys were tested with a single injection of lamprey GnRH-III on June 21, 1993. Each group of 10 lampreys was tested with saline (control), lamprey GnRH-III (0.1 μg/g body wt), or lamprey GnRH-III (0.2 μg/g body wt). The adult male lampreys were maintained at a water temperature of 19°C. Within 30 min of injections, all peptides were dissolved in 0.6% NaCl in distilled water and were injected intraperitoneally. The lampreys were anesthetized with 0.2 g/liter ethyl m-amino benzoate methanesulfonate (MS222). Blood samples were collected 4 hr after the injections by cardiac puncture with heparinized syringes. After centrifugation of the blood samples, plasma was collected and stored at −20°C until assayed for estradiol and progesterone. Plasma estradiol and progesterone levels were measured by radioimmunoassay as described previously (Sower *et al.*, 1983; Sower and Schreck, 1982).

Experiment 2. In experiment 2, lampreys were tested with four successive injections of saline, lamprey GnRH-I, or lamprey GnRH-III. Three groups of 10 lampreys each were injected with saline, lamprey GnRH-I (0.1 μg/g body wt), or lamprey GnRH-III (0.2 μg/g body wt). Four successive injections were performed on June 8, 11, 15, and 18 with the adult male lampreys maintained at water temperature in the range of 13–20°C. The lampreys were checked approximately every other day for a period of 40 days to determine if they had spermiated. Spermiation occurred when, upon gentle pressure, sperm was expressed for the first time (Sower, 1989).

Statistical analysis. Data for the hormone concentrations were analyzed by a Student–Newman–Keuls test after preliminary analysis of variance. The percentage spermiation was analyzed by a 2 × 2 contingency table followed by the Bonferonni approach. In all tests, the level of significance for differing groups was $P < 0.05$.

RESULTS

Single Injection: Estradiol and Progesterone Response

Plasma estradiol increased significantly ($P < 0.05$) in response to lamprey GnRH-III at 0.1 μg/g (2.611 ng/ml ± 0.197 SE) and 0.2 μg/g (2.329 ng/ml ± 0.135 SE) compared to controls (1.270 ng/ml ± 0.098 SE); however, the response was not dose related (Fig. 1). Progesterone levels also increased significantly ($P < 0.05$) in all lamprey GnRH-III-treated lampreys (1.424 ng/ml ± 0.234 SE and 2.028 ng/ml ± 0.492 SE for 0.1 and 0.2 μg/g treatments, respectively) compared to controls (0.197 ng/ml ± 0.056 SE); however, the response was not dose related (Fig. 1).

Four Successive Injections: Spermiation Response

On Day 16, spermiation had occurred in

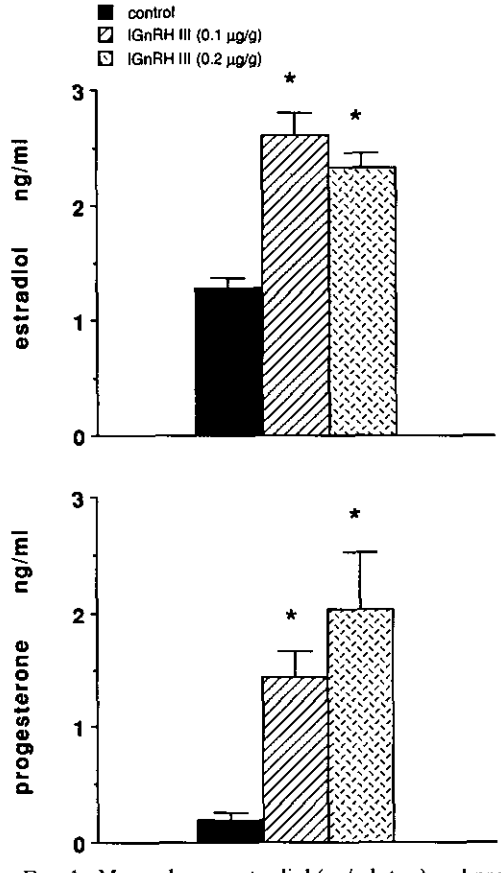

FIG. 1. Mean plasma estradiol (ng/ml; top) and progesterone (ng/ml; bottom) injected with saline (control), lamprey GnRH-III (0.1 μg/g), or lamprey GnRH-III (0.2 μg/g). Plasma samples were taken 4 hr after injection from each lamprey. Bars depict SEM. *$P < 0.05$.

78% of the lampreys injected with lamprey GnRH-III at 0.2 μg/g and 30% of lampreys injected with lamprey GnRH-I at 0.1 μg/g compared to 0% spermiation for controls (Fig. 2). By Day 28, lampreys injected with lamprey GnRH-III had a 100% spermiation response compared to 50% spermiation response of lampreys injected with lamprey GnRH-I and 0% spermiation in the controls (Fig. 2).

DISCUSSION

In the present study, lamprey GnRH-III stimulated steroidogenesis and induced spermiation in adult male sea lampreys during their final reproductive stage. These data provide further evidence to a recent study in which lamprey GnRH-III was shown to increase plasma steroid levels in adult female sea lampreys (Sower *et al.*, 1993), thus suggesting that both lamprey GnRH-I and -III are neurohormones involved in reproduction in the lamprey.

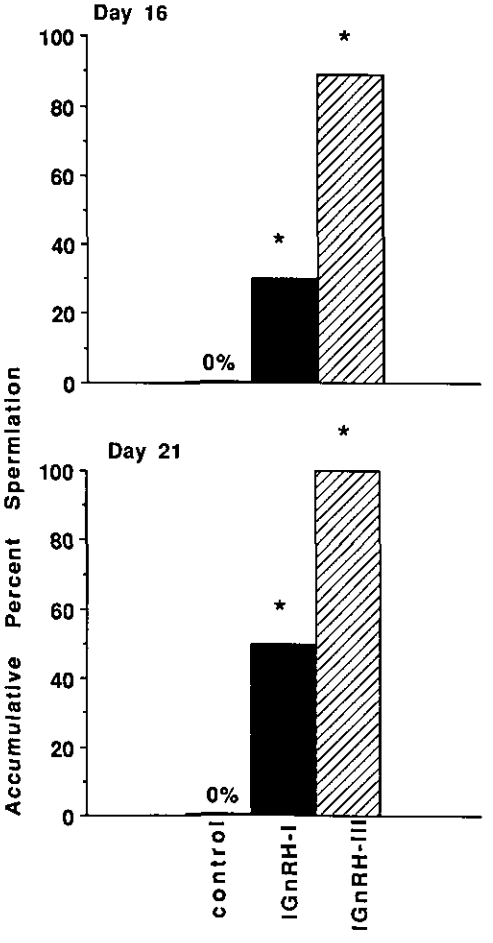

FIG. 2. Percentage spermiation on Day 16 (top) and Day 21 (bottom) after four successive injections of male sea lampreys with saline (control), lamprey GnRH-I (0.1 μg/g), or lamprey GnRH-III (0.2 μg/g). *$P < 0.05$.

Sower (1989) demonstrated that lamprey GnRH-I stimulated levels of progesterone and estradiol in adult male sea lampreys after single and two successive injections of lamprey GnRH-I. In this same study, lamprey GnRH-I was determined to induce spermiation in adult male sea lampreys compared to controls after four successive injections of lamprey GnRH-I. In the present study, lamprey GnRH-III was also shown to stimulate levels of both progesterone and estradiol in the adult male lamprey after a single injection of lamprey GnRH-III and induce spermiation after four successive injections of lamprey GnRH-III. In both studies, neither lamprey GnRH-III nor lamprey GnRH-I appeared to produce a dose-related response in levels of estradiol and progesterone. The percentage spermiation data demonstrate that the injection of adult male sea lampreys with lamprey GnRH-III induced a higher percentage spermiation after Days 16 and 21, indicating that lamprey GnRH-III may be more potent as a neurohormone than lamprey GnRH-I in the adult male sea lamprey. This is supported by the fact that lamprey GnRH-III brain content concentration was determined to be three times greater than that of lamprey GnRH-I (Sower *et al.*, 1993). However, until the release rates of lamprey GnRH-I and lamprey GnRH-III are known and gonadotropins can be directly measured the differences in potency of lamprey GnRH-I and -III can only be inferred.

Plasma levels of estradiol and progesterone were used as indicators of reproductive activity in response to lamprey GnRH injections similar to earlier studies (Sower *et al.*, 1987; Sower, 1989, 1990). Previous

physiological studies in male lampreys (Sower *et al.*, 1985; Sower, 1989; Katz *et al.*, 1982; Fukayama and Takahashi, 1985) and the demonstrated absence of androgen receptors in the lamprey testis (Ho *et al.*, 1987) suggest that testosterone may not have a role during the final spermatogenic phases in adult male lampreys. As reviewed in Sower (1990), estradiol appears to be associated with reproductive activity in male sea lampreys. The role of progesterone in male reproductive activity has yet to be determined, although progesterone levels were demonstrated to be significantly higher in males compared to females during final reproductive stages (Linville *et al.*, 1987).

Currently, eight primary structures of GnRH have been determined in various representatives of vertebrates (Sower *et al.*, 1993). Chromatographic and immunological studies using antibodies to these known forms of vertebrate GnRH have established that there are two or more forms of irGnRH present within the brains of representative vertebrate species (King and Millar, 1991). Chicken GnRH-II appears to be the most highly conserved GnRH molecule within the vertebrate classes, although its function is still unknown. The significance of multiple forms of GnRH within the brain of a species has not been well understood, although the presence of GnRH in extrahypothalamic brain regions suggests that GnRH molecules have multiple functions such as neurotransmitters and/or neuromodulators (Calvin *et al.*, 1993).

Lampreys are the first vertebrates to clearly demonstrate a dual role for GnRH molecules as neurohormones involved in reproductive activity. Both lamprey GnRH-I and -III have been shown to induce steroidogenesis and spermiation and/or ovulation in adult sea lampreys (Sower *et al.*, 1987, 1993; Sower, 1989). Studies using antisera directed toward either lamprey GnRH-I or lamprey GnRH-III have localized both lamprey GnRH-I and lamprey GnRH-III in larval sea lampreys (Tobet *et al.*, in press). GnRH concentrations were the greatest in the rostral hypothalamus and preoptic area, while lower concentrations were localized to areas of the neurohypophysis and mesencephalon. Tobet *et al.* suggested that in the larval stage, the majority of the irGnRH was lamprey GnRH-III indicating that GnRH-III perhaps is the more important form in maturation of GnRH cells and fibers. Additionally, lamprey GnRH-I and -III were detected in brain samples of lampreys undergoing different stages of metamorphosis coinciding with the acceleration of gonad maturation (Youson and Sower, 1991). To date, these data support a dual role of lamprey GnRH-III as neurohormones involved in reproduction in lampreys.

In most vertebrates, there appear to be two principle GnRH pathways which express different molecular forms of GnRH and which project to different targets (see review, Muske, 1993). In the first GnRH system, GnRH from the terminal nerve–septo–preoptic system originates from the olfactory placode and is the principle regulator of gonadotropin release from the pituitary; this terminal nerve has been associated with the olfactory system in vertebrates (Muske, 1993). More recent evidence suggests that the GnRH neurons in the terminal nerve have a neuromodulatory function, regulating neuronal excitability in brain regions (Leprêtre *et al.*, 1993). The second GnRH system is composed of GnRH neurons in the periventricular regions of the posterior diencephalon and/or midbrain (Muske, 1993). Chicken GnRH-II seems to be the predominant form of GnRH in this pathway. With the exception of the frog, *Rana ridibunda* (Conlon *et al.*, 1993), the localization of chicken GnRH-II has been generally in regions not typically associated with pituitary function. Neither of these two GnRH systems have been demonstrated to be present in the lamprey. Although the organization of GnRH neurons

in the lamprey seems similar to that of the terminal nerve–septo–preoptic pathway, GnRH neurons are not localized around the terminal nerve, and it is likely that lampreys do not have a terminal nerve (King *et al.*, 1988). King *et al.* (1988) demonstrated a large number of immunoreactive GnRH neurons in the periventricular preoptic area of the sea lamprey, which are more superficially distributed in other vertebrates.

In summary, lamprey GnRH-III stimulated steroidogenesis and induced spermiation in adult male sea lampreys. These data suggest that lamprey GnRH-III, like lamprey GnRH-I, is also a major hypothalamic neurohormone involved in the reproduction of the sea lamprey.

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