Changes in Brain Gonadotropin-Releasing Hormone, Plasma Estradiol 17-β, and Progesterone During the Final Reproductive Cycle of the Female Sea Lamprey, Petromyzon marinus

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ABSTRACT  Changes in ovarian morphology, brain gonadotropin-releasing hormone (GnRH), plasma estradiol, and progesterone were examined during the 1988 and 1989 spawning migrations of the adult female sea lamprey, Petromyzon marinus. There were significant increases through time in brain GnRH (1989) and plasma estradiol (1988 and 1989), with progesterone levels fluctuating (1988 and 1989) during the freshwater phase of the spawning migrations. In 1989, brain GnRH and plasma estradiol levels gradually increased through time until just prior to spawning when levels decreased. During 1988, there were no significant changes in GnRH, which may reflect lower temperatures in that year. These data provide new information on brain GnRH during the final maturational processes in the female sea lamprey. © 1992 Wiley-Liss, Inc.

The sea lamprey, Petromyzon marinus, is an anadromous fish which spawns once in its lifetime. Following the migration from seawater to freshwater, adult sea lampreys undergo final reproductive maturation and spawning, after which they die. Recent evidence indicates that similar to other vertebrates, hypothalamic gonadotropin-releasing hormone (GnRH) regulates the pituitary-gonadal axis in sea lampreys.

GnRH has been defined as the major regulatory hypothalamic peptide involved in the reproduction of most vertebrates. In lampreys, the primary structure of GnRH has been identified as pGlu-His-Tyr-Ser-Leu-Glu-Trp-Lys-Pro-Gly-NH₂ (Sherwood et al., '86). Using an antibody generated to lamprey GnRH in immunocytochemical studies, King et al. ('88) demonstrated GnRH in the preoptico-neurohypophysial region of the lamprey hypothalamus. Fahien and Sower ('90) have reported significant changes in brain GnRH levels of the male sea lamprey during the period of final reproductive maturation. These studies suggest a relationship between GnRH and gonadal activity.

Physiological studies by Sower ('89) and Sower et al. ('85a, '87) provide further evidence for the biological function of GnRH in sea lamprey. Injection of lamprey GnRH into female sea lamprey resulted in increased plasma levels of estradiol and progesterone, and accelerated the onset of ovulation (Sower et al., '87). These studies indicate that GnRH, as in other vertebrates, is involved in the reproductive processes of the sea lamprey.

Estradiol and progesterone are two steroids that have been associated with the reproductive activity of the sea lamprey and have both been measured as indicators of gonadal activity (Sower et al., '83, '85a,b; Fahien and Sower, '90; Linville et al., '87). Plasma estradiol levels fluctuated during the spawning migration of the lamprey, increasing significantly during spermiation (Fukayama and Takahashi, '85; Sower et al., '85a; Fahien and Sower, '90) and decreasing significantly at ovulation (Fukayama and Takahashi, '85; Sower et al., '85a). Progesterone has been detected in the plasma of the sea lamprey but trends were not as consistent as with estradiol, and its function is unknown (Fahien and Sower, '90; Linville and Sower, '87).

Developmental changes in the unfertilized, in vivo lamprey egg at the light microscope level have been previously described by Larsen ('70) and Lewis and McMillan ('65). Lewis and McMillan ('65) studied the developmental changes in the landlocked sea lamprey, Petromyzon marinus, from the appearance of primordial germ cells to ovulation, while Larsen examined the changes associated with ovu-
loration in the river lamprey (*Lampetra fluviatilis*). Developmental changes in the lamprey egg during the final maturational and ovulation stages at the light microscope level have not been previously described for the anadromous sea lamprey.

The objective of this study was to investigate the seasonal relationships between changes in brain GnRH, plasma estradiol 17-β, and progesterone and activities of the ovary in the female sea lamprey, *Petromyzon marinus*, during the period of final reproductive maturation.

**METHODS AND MATERIALS**

Adult female sea lampreys (*Petromyzon marinus*) were collected and sampled biweekly from May 25 to June 14, 1988 and from May 20 to June 12, 1989. The lampreys, captured during their spawning migration from the ocean, were obtained from the upstream portion of a denile-type fish ladder on the Cocheco River in Dover, New Hampshire. Immediately after capture the lampreys (n = 6–10/sampling day in 1988 and n = 4–10/sampling day in 1989) were sampled for blood, brain, and gonadal tissues. In order to sample lampreys undergoing the final stages of oogenesis and spawning, a group of lampreys was collected at the Cocheco River in early May and transferred and maintained in an artificial stream at the University of New Hampshire Anadromous Fish and Aquatic Invertebrate Research (AFAIR) laboratory. Female sea lampreys (n = 10/sampling day in 1988 and n = 5–10/sampling day in 1989) were sampled weekly at the AFAIR lab from June 2 to July 7, 1988 and from May 31 to July 10, 1989 for blood, brain, and gonadal tissues. The conditions of the artificial stream have been previously described by Fahien and Sower (’90).

**Sampling procedures**

Lampreys were sexed by palpation of the abdomen early in the season (females having a softer abdomen than males), and by palpation and the presence of secondary sex characteristics later in the season, when the males developed a dorsal rope-like thickening and an enlarged branchial region and the females showed percloacal swelling. Lampreys removed from either the fish ladder or the stream were immediately sampled for blood as previously described by Fahien and Sower (’90). After centrifugation, the plasma from individual blood samples was stored at −20°C until assayed for estradiol and progesterone. Following blood collection, the fish were decapitated and the brains removed as previously described by Fahien and Sower (’90). Individual brains were placed on dry ice until transferred to a −80°C freezer, where the tissue remained until extracted and assayed for gonadotropin-releasing hormone (GnRH). Each lamprey was sampled for a section of ovary just posterior to the liver, which was placed in Bouin’s solution and prepared for histological examination as described by Sower et al. (’86). The ovaries were examined for morphological changes occurring within the ovary during final maturation and spawning.

**Radioimmunoassays**

Brains were extracted as previously described (Yu et al., ’87; Fahien and Sower, ’90) and duplicate 10 μl or 25 μl aliquots of brain extract were assayed for GnRH, using a lamprey GnRH antibody (J.A.K., 1467 at a final dilution of 1:50,000), as previously described by Stopa et al. (’88). The lower limit of detection was 39 pg/tube, with binding efficiencies ranging from 53% to 61% (1988) and from 30% to 37% (1989) and with coefficients of interassay variation of 2.6% (1988) and 12% (1989). Plasma estradiol 17-β was measured from duplicate 100 μl plasma aliquots by RIA as described by Sower and Schreck (’82). The lower limit of detection was 15.6 pg/tube, with binding efficiencies of 36–43% (1988) and 32% (1989), and coefficients of interassay variation of 9% (1988) and 14% (1989). Plasma progesterone was measured by RIA, as described by Sower et al. (’83). The lower limit of detection was 7.8 pg/tube, with binding efficiencies of 24%–30% (1988) and 25–30% (1989), and with coefficients of interassay variation of 13% (1988) and 6% (1989).

**Statistics**

Differences in hormone concentrations were analyzed by a Student-Newman-Kuels test after a preliminary analysis of variance.

**RESULTS**

**Reproductive cycle**

Lampreys first reached the upstream portion of the fish ladder in the third week of May in both 1988 and 1989. By this time, the oocytes were in the spawning stage of development, as described by Lewis and McMillan (’65). The germinal vesicle was located peripherally (Fig. 1), the vitelline membrane was double (Fig. 1), and the theca had begun to separate from the vitelline membrane (Fig. 1). By June 23, 1988 and June 21, 1989, the theca had separated from the vitelline membrane along nearly the entire egg surface (Figs. 1, 2). By July 18, 1988 and July 5, 1989, ovulation was observed in some of the fish sampled. At ovulation, the follicle cells of the thecal layer were no longer associated with
Fig. 1. (Top plate): Longitudinal section through a lamprey egg. a: Germinal vesicle; b: pole plasm with channels. (Bottom plate): Animal end of a lamprey egg. a: Germinal vesicle; b: elevated theca; c: amorphous material; d: double vitelline membrane; e: yolk platelets.
Figure 2.
the oocytes. A gelatinous material, similar to that found earlier in the thecal elevation of the animal pole, was present on portions of ovulated eggs (Fig. 2).

As a result of the intense vitellogenesis which occurs during the marine phase, most of the cytoplasm within the oocyte was filled with yolk platelets at the time of entrance into freshwater. The central portion of the oocyte was occupied by large equally sized platelets, while the peripheral margin contained smaller platelets (Fig. 1). At the beginning of the freshwater migration, the vitelline membrane was double, with the inner and outer portions being of about equal thickness (Fig. 1). After ovulation, the majority of oocytes still had a double vitelline membrane. Sometimes only a single-layer vitelline membrane was observed (Fig. 2). The pole plasm, a region within the animal pole of the oocyte located between the nucleus and vitelline membrane, was already present upon entrance into freshwater. This area was filled with small yolk granules and projections or channels, as they have been described by Larsen ('70), appeared to span the distance from the germinal vesicle to the vitelline membrane (Fig. 1).

**Temperature, GnRH, and estradiol**

Temperatures were lower in 1988 (less than 18°C) than in 1989 (greater than 20°C) between June 19 and July 9 at the AFAIR laboratory (Fig. 3). In 1989, brain GnRH and plasma estradiol 17-β levels covaried, increasing gradually through time until just prior to spawning and then decreasing (Figs. 4, 5). In 1988, brain GnRH and plasma estradiol 17-β covaried; however, plasma estradiol increased slightly in lampreys sampled at the AFAIR laboratory. Plasma progesterone levels fluctuated during the sampling periods in both years and at both locations.

**GnRH**

**May-June**

GnRH was detected in 48 of the 59 fish sampled in 1988 and in 41 of the 44 fish sampled in 1989. GnRH levels did not change significantly in fish sampled at the Cocheco River in 1988. In 1989 GnRH increased significantly ($P < 0.05$) from June 9 (34.6 ± 7.3 ng/brain) to June 12 (81.6 ± 17.0 ng/brain) (Fig. 4).

**Estradiol 17-β**

Estradiol 17-β increased through time during the spawning migration in 1989 at the Cocheco River and in both 1988 and 1989 at the AFAIR laboratory.

**June-July**

GnRH was detected in 51 of the 60 fish sampled in 1988 and in 36 of the 45 fish sampled in 1989. Mean GnRH levels did not vary significantly through time in 1988 (Fig. 5). In 1989 GnRH varied significantly through time (Fig. 5). GnRH increased significantly ($P < 0.05$) from May 31 (11.7 ± 1.9 ng/brain) to June 21 (67.7 ± 10.0 ng/brain), and decreased significantly from July 5 (71.7 ± 15.8 ng/brain) to July 10 (35.1 ± 7.4 ng/brain).

**Estradiol 17-β**

Estradiol 17-β increased through time during the spawning migration in 1989 at the Cocheco River and in both 1988 and 1989 at the AFAIR laboratory.

**May-June**

Estradiol was detected in 54 of the 59 fish sampled in 1988 and in 39 of the 44 fish sampled in 1989. Estradiol levels in fish sampled in 1988 did not vary significantly (Fig. 4). Plasma estradiol from fish sampled in 1989 varied significantly ($P < 0.05$), increasing from June 6 (1.01 ± 0.10 ng/ml plasma) to June 12 (1.78 ± 0.19 ng/ml plasma) (Fig. 4).
June-July

Estradiol was detected in 57 of the 60 fish sampled in 1988 and in 40 of the 45 fish sampled in 1989. In 1988 estradiol increased significantly from June 9 (0.32 ± 0.07 ng/ml plasma) to June 30 (0.98 ± 0.10 ng/ml plasma) (Fig. 5). In 1989 estradiol increased significantly (P < 0.05) from May 31 (0.62 ± 0.07 ng/ml plasma) to June 21 (2.03 ± 0.33 ng/ml plasma) and, while not significantly, decreased on July 10 (0.95 ± 0.15 ng/ml plasma) (Fig. 5).

Progesterone

Plasma progesterone levels at both locations in 1988 and 1989 fluctuated significantly (P < 0.05) through time.

May-June

Plasma progesterone was detected in 56 of the 59 fish sampled in 1988 and in 43 of the 44 fish sampled in 1989. In 1988 progesterone decreased significantly (P < 0.05) from May 31 (0.37 ± 0.04 ng/ml plasma) to June 3 (0.07 ± 0.002 ng/ml plasma), then increased significantly (P < 0.05) from June 7 (0.08 ± 0.006 ng/ml plasma) to June 10 (0.41 ± 0.06 ng/ml plasma) (Fig. 4). In 1989 progesterone increased significantly (P < 0.05) from June 2 (0.18 ± 0.01 ng/ml plasma) to June 12 (0.31 ± 0.04 ng/ml plasma) (Fig. 4).

June-July

Progesterone was detected in 56 of the 60 fish sampled in 1988 and in 43 of the 45 fish sampled in 1989. In 1988 progesterone decreased significantly (P < 0.05) from June 2 (0.17 ± 0.02 ng/ml plasma) to June 9 (0.07 ± 0.004 ng/ml plasma) and then increased on June 16 (0.16 ± 0.04 ng/ml plasma) (Fig. 5). In 1989 progesterone increased significantly (P < 0.05) from June 21 (0.14 ± 0.008 ng/ml plasma) to July 5 (0.33 ± 0.05 ng/ml plasma) (Fig. 5).
DISCUSSION

Despite the importance of GnRH in the reproductive processes of the female sea lamprey (see review by Sower, '90), the dynamics of brain GnRH have yet to be examined during sexual maturation and spawning in the female sea lamprey. The results of the present study demonstrated that total brain GnRH, plasma estradiol 17-ß, and progesterone levels changed through time during final maturation in the female sea lamprey. In 1989, brain GnRH and plasma estradiol 17-ß levels gradually increased through time until just prior to spawning, when levels decreased. In 1988 no changes were observed in brain GnRH or plasma estradiol 17-ß, except for a slight increase in plasma estradiol 17-ß in fish sampled in May-June. Plasma progesterone levels fluctuated during the sampling periods in both years and at both locations.

The two noted elevations in GnRH observed in 1989 may be one aspect of an overall gradual increase in brain GnRH that occurs in the female sea lamprey during the spawning migration. Breton et al. ('86) reported a slow prolonged increase in brain GnRH between the end of vitellogenesis and the final stages of ovarian maturation in the brown trout (*Salmo trutta*). Similarly, increased levels of immunoreactive brain GnRH were detected in tilapia (*Oreochromis mossambicus*), and the male sea lamprey (Fahien and Sower, '90) during their respective spawning seasons. In the silver eel (*Anguilla anguilla*) radioimmunoassay data (Dufour et al., '85) suggested that steroids increase GnRH synthesis but do not release, thus leading to an accumulation of the peptide in axonal endings. Therefore, it seems that the increase in brain GnRH observed in the female sea lamprey during the spawning migration in 1989 may represent GnRH that can be released during final maturation. However, synthesis, storage, and release rates of brain GnRH in the lamprey are unknown, and further studies are needed before brain GnRH changes in the lamprey can be accurately interpreted.

The lack of observed changes in GnRH in 1988 is unexplained. One possible explanation may be the lack of elevated temperatures in June-July, 1988 compared to the elevated temperatures noted in 1989. The water temperatures from 1989 are more similar to temperatures noted in previous years than to temperatures in 1988 (Fahien and Sower, '90). There is good evidence for an association between temperature and final maturational processes of adult sea lampreys (see review by Sower, '90). In Fahien and Sower's study ('90), there was a

rise in brain concentrations of GnRH, coincident with an increase in temperature, just prior to spawning in adult male lampreys. In earlier experiments of female sea lampreys, injections of a mammal GnRH analog sufficient to elevate plasma estradiol would not evoke ovulation at lower environmental temperatures of 13°C (Sower et al., '83). Upstream spawning sea lampreys kept at temperatures below 15.5°C will not ovulate or spermatize unless the temperature is elevated closer to their optimal spawning temperatures of 21°C. These data suggest that changes in brain GnRH may be influenced by environmental temperature, which may explain the significant changes that occurred in 1989 but not in 1988.

Yu et al. ('87) observed decreases in brain (hypothalamus, pituitary, olfactory bulbs, and telencephalon) GnRH to be correlated with increases in serum GTH in ovulating goldfish, while Breton et al. ('83) noticed a decrease in pituitary GnRH to coincide with minimum estradiol levels present at ovulation. As a result, Breton et al. ('83) suggested that estradiol could exert a negative feedback on both brain and pituitary GnRH, with GnRH being released in the pituitary only when estradiol levels reach their minimum values. As with Fahien and Sower ('90) in their study on the male sea lamprey, a decrease in brain GnRH was noted in female sea lampreys sampled near the end of the reproductive period in 1989. The increase prior to this final decrease in brain GnRH observed in the female sea lamprey may, among other activities, function in ovulation. The covariance of brain GnRH and plasma estradiol observed in this study suggest a possible feedback mechanism existing between brain GnRH and plasma estradiol in the lamprey.

The major morphological changes which occurred during the final maturation processes of the spawning phase of the sea lamprey life cycle involved the separation of the theca from the oocyte. The separation of the theca from the entire oocyte prior to ovulation appeared to differ from the morphological changes described for the landlocked *P. marinus* and the *L. fluviatilis*. The germinal vesicle of the sea lamprey was located at the periphery of the oocyte upon entrance into freshwater. The pole plasm, a term used by Larsen ('70), was also present at this time. Due to the lack of cortical granules and the presence of a perivitelline space in this region at fertilization, Larsen ('70) suggested that the channels within the pole plasm may have a function in the formation of the fertilization membrane. The oocytes of the lamprey are located within sacs of peritoneal epithelium with their vegetative
poles resting on the epithelium (Busson-Mabillot, '67). At ovulation, the oocyte must therefore pass by both the theca and peritoneal-epithelium in order to enter the body cavity. Larsen ('70) stated that the most important changes during this phase involve the follicle cells of the vegetative pole and the thecal elevation at the animal pole. Both Larsen ('70) and Yorke and McMillan ('79) had described a thickening of the follicle cells of the vegetative pole, in *L. fluviatilis* and landlocked *P. marinus*. This was not observed during this study on *P. marinus*, or by Lewis and McMillan ('65) and Sower et al. ('85) during their studies with landlocked sea lamprey. This may be a result of the extended period spent in freshwater by *L. fluviatilis*, which spends up to 6 months in freshwater before spawning, as compared to the 5 or 6 week freshwater resident period of *P. marinus*.

In the present study, an elevation of the theca from the vitelline membrane at the animal pole, consistent with Larsen's observation, was present in fish upon entrance into freshwater. Larsen ('70) observed this characteristic in January, a few months after entrance into freshwater. Lewis and McMillan ('65) also described a fluid between the vitelline membrane and thecal membrane immediately before spawning, which histologically is quite similar to the structure noted in this study and by Larsen ('70). Both Larsen ('70) and Lewis and McMillan ('65) suggested that this elevation may create the weak spot through which the oocyte can be expelled from the surrounding cell layers.

Not described by either Larsen ('70) or Lewis and McMillan ('65) was the separation of the theca from the vitelline membrane around the entire oocyte. Approximately 10 days before the first sign of ovulated eggs, this separation of the theca and vitelline membranes was noted. It is possible that this separation is a continuation of the separation which was noticed earlier at the animal pole. Larsen ('70) discussed the possibility of ovulation taking place as a result of pressure changes caused by the uptake of water. It seems possible that the separation of the theca and vitelline membrane could result from a gradual increase in osmotic pressure. After ovulation, the eggs were free of follicle cells. A gelatinous substance was noted on a number of ovulated eggs and may create the weak spot through which the oocyte can be expelled from the surrounding cell layers.

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Plasma estradiol 17-β and progesterone were measured as indicators of gonadal activity in the sea lamprey. The levels of these steroids changed significantly during the spawning migration. However, only estradiol demonstrated any consistent pattern. Similar to the studies by Sower et al. ('85b) and Fukayama and Takahashi ('85), the present study showed significant changes in plasma estradiol 17-β in females during final maturation, with increases in plasma estradiol observed in May-June samplings in 1989 and in June-July samplings in 1988 and 1989. Similarly, estradiol levels at the end of the spawning season in 1989 decreased, as in ovulated females observed by Sower et al. ('85b), Linville et al. ('87), and Fukayama and Takahashi ('85).

The role of estradiol during the maturation and ovulation of the sea lamprey is largely unknown. The presence of gonadal steroids has been linked to the formation of secondary sex characteristics in the sea lamprey (Larsen, '74; Evennett and Dodd, '63). Pickering ('76) associated the presence of estradiol with the process of vitellogenesis in the river lamprey and Fukayama and Takahashi ('85) observed an increasing gonadosomatic index (suggesting ongoing vitellogenesis) until just before spawning in the Japanese River lamprey (*Lamproptera japonica*). Likewise, Busson-Mabillot ('67) suggested from histological studies of the ovaries of *Lampetra planeri* that vitellogenesis ends just prior to ovulation. In *P. marinus*, vitellogenesis is completed prior to final maturation (Sower et al., 1985b), which indicates that estradiol has other functions, such as the development of secondary sex characteristics.

Progesterone changed significantly during the spawning migrations, fluctuating through time in 1988 and gradually increasing through time in 1989. Unlike estradiol, reports of progesterone profiles are conflicting. In this study progesterone levels in the female sea lamprey were higher than those reported for the male sea lamprey by Fahien and Sower ('90). However, Linville et al. ('87) reported higher progesterone levels in male than in female sea lamprey. One explanation for the differences may be the different populations sampled. The fish used in this study and by Fahien and Sower ('90) were obtained from the Cocheo River of New Hampshire, while Linville et al. ('87) obtained their
fish from a landlocked population of sea lamprey in the Ocqueoc River of Michigan. Due to the low levels of progesterone in the plasma of the sea lamprey, actions of the hormone have not been proposed. Linville et al. (’87) found no correlation between progesterone levels and reproductive behaviors, and thus suggested that progesterone may function in nonbehavioral aspects of reproduction.

In summary, the results of this study have shown that brain concentrations of GnRH occurring with plasma estradiol 17-β fluctuate during the final reproductive cycle of female sea lamprey (Petromyzon marinus), which supports the concept of a hypothalamic-hypophysial-gonadal axis functioning to mediate reproduction in the sea lamprey. Whether progesterone acts directly or is merely a precursor to active forms needs to be determined.

ACKNOWLEDGMENTS

We thank D. Adams, J. Calvin, M.J. James, J. Pardo, L. Penney, and C. Schmidt for excellent technical assistance. This work was supported by the National Science Foundation (DCB-8602907 and DBC-8904919).

LITERATURE CITED


