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15α-Hydroxytestosterone induction by GnRH I and GnRH III in Atlantic and Great Lakes sea lamprey (*Petromyzon marinus* L.)

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Abstract

The sea lamprey (*Petromyzon marinus* L.) represents one of the two most ancient classes of vertebrates and possesses a functional hypothalamus–pituitary–gonadal axis. However, the presence and functionality of androgens in the sea lamprey remain elusive. Recently, 15α-hydroxytestosterone (15α-T) has been found in sea lamprey gonads and blood plasma. In this study we examined changes of circulatory concentrations of 15α-T in response to gonadotropin releasing hormone (GnRH) treatments. Plasma concentrations of 15α-T in sea lamprey increased 2–5 times for all GnRH-injected sea lamprey compared to controls ($P < 0.0001$). However, there were no differences among responses: (1) to the two forms of GnRH (lamprey GnRH I or lamprey GnRH III), (2) to the doses delivered (50, 100, or 200 μg/kg), or (3) between post-injection sample intervals (8 or 24 h). Between lampreys from the Atlantic Ocean and Great Lakes sites, two of seven GnRH form and dosage comparisons showed between-site differences, but were not believed to represent an overall between-site difference. These are the first data to show a response of a C19 steroid to GnRH stimulation in sea lamprey.

1. Introduction

The sea lamprey (*Petromyzon marinus* L.) is among the most primitive of extant vertebrates and has a complex life cycle (Hardisty and Potter, 1971). Sea lampreys begin their lives in freshwater as blind, filter-feeding ammocoetes (larvae). After three to seven years, metamorphosis occurs and the ammocoetes become sexually immature, free-swimming juveniles that migrate to the sea (Atlantic Ocean) or lakes (Great Lakes). During an approximate 15-month parasitic phase, gametogenesis progresses and spermatogonia proliferate and develop into primary and secondary spermatocytes in males. After migration to freshwater streams, both sexes undergo final reproductive maturation. The semelparous life cycle of the sea lamprey ends soon after spawning in streams. The sea lamprey is the only jawless fish in which the hypothalamus–pituitary–gonadal (HPG) axis has been established (Sower, 1998; Sower and Kawauchi, 2001). Therefore, further knowledge regarding the reproductive physiology of sea lampreys can aid in understanding the evolution of endocrine controls for reproduction seen in more modern vertebrates.

The neuroendocrine components of the reproductive endocrine system are highly conserved among vertebrates, including lampreys (Sower, 1998; Sower and Kawauchi, 2001). However, the roles of gonadal steroids appear to differ in lampreys. Several groups have detected very low concentrations of immunoreactive testosterone in plasma of male and female sea lampreys, but have been unable to demonstrate that it increases in response to injections of lamprey gonadotropin releasing hormone (GnRH) and heterologous gonadotropin(s), or that its circulatory concentrations and profiles are sexually dimorphic (Katz et al., 1982; Linville et al.,

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1987; Sower et al., 1985a,b). In addition, Ho et al. (1987) demonstrated estrogen binding, but not androgen binding to receptors in lamprey testis. These studies were later supported by a study that identified cDNA that appeared to encode a putative estrogen receptor, but did not identify any cDNA sequences that are homologous to typical vertebrate androgen receptors in sea lampreys (Thornton, 2001; Thornton and DeSalle, 2000). These findings support the hypothesis (Sower, 1990, 1998) that cyclostomes may not use androgens as male reproductive hormones.

One other possible explanation for the absence of experimental evidence for typical androgens and their corresponding receptors is that lamprey androgens are structurally different from those identified in other vertebrates. Kime and Rafter (1981) and Kime and Callard (1982) showed that the testis of *Lampetra fluviatilis* and *P. marinus* have the capacity to hydroxylate testosterone at the C15 position, forming 15α-hydroxytestosterone (15α-T). This has recently been confirmed by two studies. Lowartz et al. (2003) found steroids produced in vivo and in vitro by sea lampreys that have elution times corresponding to 15α-hydroxylated steroids when analyzed with high performance liquid chromatography (HPLC). Bryan et al. (2003) used HPLC, thin layer chromatography (TLC), and microchemical analysis to further show that sea lampreys produce 15α-T in vitro. Bryan et al. (2003) also developed a radioimmunoassay (RIA) for 15α-T and showed this steroid to be present in the blood plasma of male sea lampreys (and to a lesser extent in females). Despite the identification of 15α-T in sea lampreys, the function of this steroid has yet to be characterized.

This study was designed to determine a potential endocrine role of 15α-T in the sea lamprey through induction by GnRH, thus showing this sex steroid to be a possible component of the well-established, and functional hypothalamus–pituitary–gonadal (HPG) axis of the sea lamprey (Sower and Kawauchi, 2001). In particular, the effects of lamprey GnRH I (Sherwood et al., 1986) and GnRH III (Sower et al., 1993) on 15α-T plasma concentrations were measured. Using adult male sea lampreys, an experiment was designed to test: (1) the effects of the administration at different doses and at different times post-treatment of exogenous lamprey GnRH I and GnRH III on 15α-T plasma concentrations and (2) to determine if there was a difference in response between adult pre-spermiating male sea lampreys from the Great Lakes or Atlantic Ocean.

2. Methods

Pre-spermiating, adult male sea lampreys were collected during late-spring (May) spawning migrations. Anadromous lampreys were trapped at the Cocheco River fish ladder in Dover, New Hampshire, USA. Great Lakes lampreys were trapped at the mouths of two Lake Huron tributaries, the AuGres and AuSable Rivers in Michigan, USA. Atlantic Ocean lampreys were held at the University of New Hampshire (Durham, NH) and Great Lakes lampreys were held at the Hammond Bay Biological Station (USGS-BRD, Millersville, MI) in tanks containing approximately 160 L of continuous-flow water for at least 2 days prior to treatment applications. Water at the New Hampshire site came from the Oyster River while the Michigan site used Lake Huron water. Temperature during acclimation and experimental periods was maintained at 16 °C (± 1 °C) for both sites. Lampreys were weighed and measured for use in subsequent dosage calculations.

Lamprey GnRH I and lamprey GnRH III were synthesized by American Peptide Company Inc. (Sunnyvale, CA) and dissolved in 0.6% saline less than 30 min prior to administration. Each site used seven treatments, twelve lampreys per treatment, with two timed injections, and two timed bleedings as done previously (Deragon and Sower, 1994; Gazourian et al., 2000; Sower, 1989). Each group of twelve lampreys were injected intraperitoneally with either 0.6% saline (control), lamprey GnRH I (50, 100, or 200 μg/kg body weight), or lamprey GnRH III (50, 100, or 200 μg/kg body weight). The two injections, 48 h apart, were administered beginning at 8:00 a.m. Blood samples (0.5–1.0 ml) were collected 8 and 24 h after the two sets of injections by cardiac puncture using heparinized syringes. After centrifugation of blood samples, plasma was collected and stored at −80 °C until analyzed for 15α-T by RIA according to Bryan et al. (2003). The RIA for 15α-T was conducted using raw plasma. The antibody raised against 15α-T was used at a dilution of 1:100,000 and radiolabel was dispensed so that there were 7000 dpm per tube. In the absence of standard, the antibody bound approximately 50% of the available radiolabel. The sensitivity of the RIA was 39 pg/ml plasma and had a detectable range from 2 to 500 pg/tube.

The reliability of the 15α-T RIA, when applied to plasma from GnRH-injected lampreys was validated in three ways. First, 5 ml of pooled plasma from GnRH-treated lampreys were extracted using a solid phase extraction cartridge (Sep-Pak C18; Waters, Milford, MA), fractionated using HPLC (Bryan et al., 2003), and 20 μl of each fraction was then assayed for 15α-T using RIA. This was done in order to confirm that immunoreactivity was restricted to the expected elution position of 15α-T, and that production of no new immunoreactive compounds had resulted from GnRH induction. Second, dilutions of pooled plasma from GnRH-treated lampreys were combined with labeled 15α-T in the absence of antibody. This was done to confirm the absence of plasma binding proteins (which if they were present, might interfere with the RIA). Third, standard 15α-T at

a range of dilutions was added to plasma from GnRH-injected lamprey and assayed at volumes of 25, 50, and 100 μl (total volume brought to 100 μl with assay buffer). This was done to establish parallelism within the assay.

Results were analyzed at multiple treatment levels. Violations of normality and variance equality precluded the use of a factorial analysis of variance (ANOVA). For comparisons among several treatment groups, Kruskal–Wallis (K–W) tests were used. *T* tests or Wilcoxon signed-ranked tests (W) were used for simple group comparisons. There were 14 interval comparisons (7 treatments × 2 sites) between the 8 and 24 h samples. If those comparisons proved not significant, then the two intervals were pooled. Second, comparisons of the three dosage levels for GnRH I and GnRH III were made. Third, differences between the two forms of lamprey GnRH were evaluated by dose and site. Finally, between-site differences were assessed by GnRH treatment and dose.

3. Results

During the course of the experiment, 24 of the 168 lampreys died. Mortalities among treatments appeared random with the highest number (50%) seen in a control treatment. Lamprey size differed between the sites where Great Lakes sea lampreys had a mean length of 509 mm and mean weight of 243 g while Atlantic sea lampreys had an approximate mean length of 700 mm and an approximate mean weight of 800 g.

The RIA of HPLC fractions from GnRH-injected male plasma revealed a single peak of immunoreactivity corresponding to the known elution position of 15α-T (Fig. 1). The test for binding proteins in the plasma was negative. The test of parallelism returned a line with a slope equal to the known values and an $r^2 = 0.86$.

Plasma analyzed using RIA yielded 15α-T concentrations ranging from 0.07 to 4.52 ng/ml and one extreme value of 7.97 ng/ml that was excluded from analyses. Average standard reference curve error was 13% at the midpoint and 29% at the low end. The data were not normally distributed (Shapiro-Wilk, $P < 0.0001$) at both sites and variation among the Atlantic lamprey data ($\sigma^2 = 1.20$) was more than double that of the Great Lakes site ($\sigma^2 = 0.55$). Treatment subset groupings of data did favor some parametric comparison tests, but non-parametric tests were necessarily used in most cases.

The 14 interval comparison tests showed three significant differences between the two sample times (8 and 24 h). This proportionally small number of differences was believed to not represent a true interval difference and led to the pooling of these two time intervals for further analyses. There was also no evidence of dose response differences within Great Lakes-GnRH I

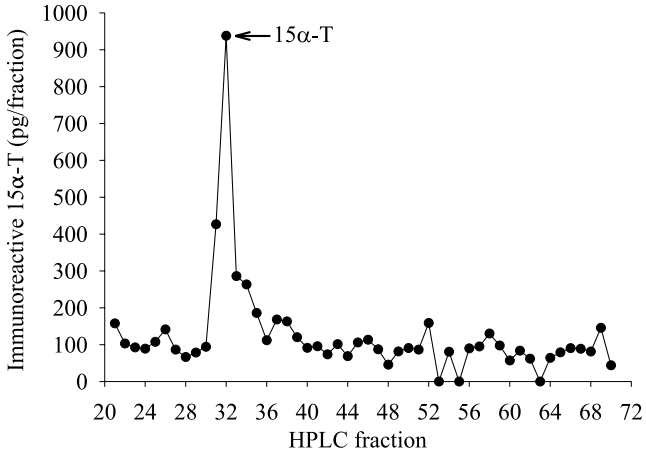

Fig. 1. Radioimmunoassay of HPLC-fractionated plasma from GnRH-injected male sea lampreys. The elution point of 15α-hydoxytestosterone (15α-T) standard is indicated with an arrow.

($P = 0.82$), Great Lakes-GnRH III ($P = 0.58$), Atlantic-GnRH I ($P = 0.11$), and Atlantic-GnRH III ($P = 0.19$) groups. Additionally, no significant difference in 15α-T concentrations was found between lampreys injected

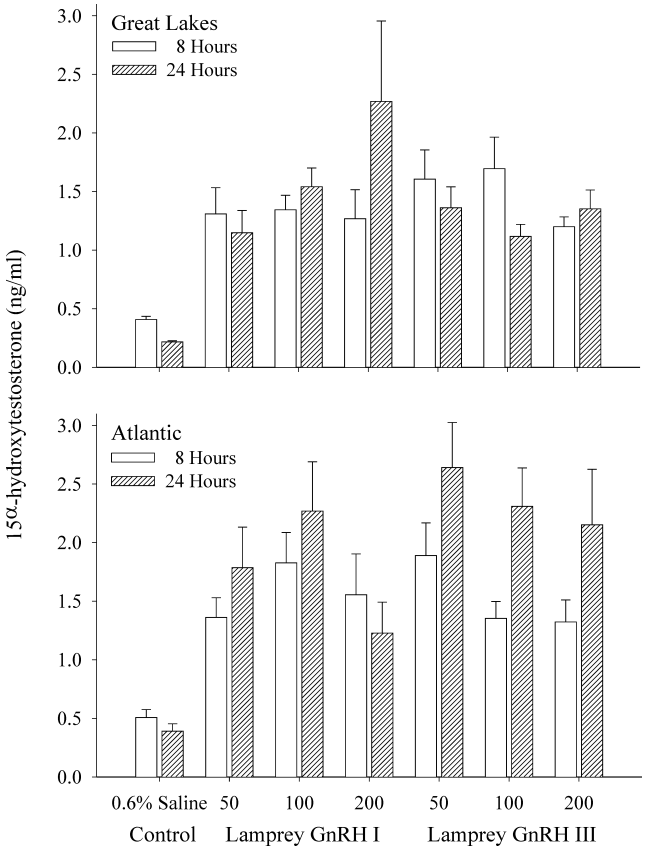

Fig. 2. Differences in 15α-hydroxytestosterone blood plasma concentrations for male sea lampreys per site, GnRH form, dosage, and time interval. The *X*-axis labels indicate the GnRH form and dosage in μg/kg of lamprey body weight. Bars represent mean treatment values and error bars show mean standard error.

with GnRH I or GnRH III at the Great Lakes site ($P = 0.93$), nor at the Atlantic Site ($P = 0.16$).

Only two of seven GnRH form and dosage specific treatment comparisons differed between the two sites. The GnRH III, 50 μg/kg treatment differed between sites (W, $P = 0.018$) where median 15α-T plasma concentrations were 2.04 ng/ml at the Atlantic site and 1.26 ng/ml at the Great Lakes site. The control groups (saline-injected) also differed (W, $P = 0.006$) where median 15α-T plasma concentrations for Atlantic sea lampreys were 0.44 ng/ml and 0.29 ng/ml for Great Lakes sea lampreys. The ratios of 15α-T concentrations in Great Lakes versus Atlantic sea lampreys were similar for both GnRH-injected lampreys (1:1.31) and control group lampreys (1:1.52).

The effect of GnRH injections (regardless of dose or form) was evaluated by comparing 15α-T concentrations between all GnRH-injected lampreys of each site to their corresponding saline-injected control group. Because the Great Lakes control group showed some differences between time intervals, the comparisons were made per interval and with combined interval data. Large differences in sample size from pooling data led to GnRH-specific and dosage-specific comparisons. At both sites and for every level of classification (interval, dose, and GnRH form), GnRH-injected lampreys always had significantly higher (K–W, $P < 0.0001$) plasma concentrations of 15α-T than control groups (Fig. 2).

4. Discussion

The results showed that both GnRH I and GnRH III elicited increases in 15α-T production and release in the blood plasma of adult male sea lampreys. GnRH-injected sea lampreys had 2–5 times greater 15α-T concentrations than their respective controls. The identity and presence of 15α-T in sea lamprey blood plasma was only recently confirmed and shown to be present at higher concentrations than immunoreactive testosterone (Bryan et al., 2003; Lowartz et al., 2003). This study now shows that 15α-T has a physiological response to GnRH and may be part of the HPG axis as induced by GTH.

The response of 15α-T further adds to the growing understanding of the unique endocrine system of the sea lamprey. To date, research has demonstrated the effects of hypothalamus and pituitary hormones on 17β-estrodial and progesterone production in sea lampreys (Sower and Kawauchi, 2001). GnRH I and GnRH III directly stimulate the pituitary and increase steroidogenesis (Gazourian et al., 2000). In vitro receptor localization studies have identified areas of the pituitary with an affinity for GnRH I and GnRH III (Knox et al., 1994) and estrogen receptor sites in the testis (Ho et al., 1987). In male sea lampreys, immunoreactive 17β-estradiol (E_2) has been found in blood plasma (Sower et al., 1983; Sower, 1989; Katz et al., 1982), whose concentrations increase in response to injections of lamprey GnRH I, GnRH III, and GnRH analogs (Deragon and Sower, 1994; Gazourian et al., 1997; Sower, 1989; Sower et al., 1983, 1985b, 1993). Additionally, receptors for E_2 have been found in the testis (Ho et al., 1987). Concentrations of 15α-T showed the same proportional (injected versus control) increased responses to GnRH I and GNRH III as E_2 responses have shown (Young, unpublished data). Expected receptor gene sequences for androgens were not found in the liver of lamprey (Thornton, 2001), but the author indicated that does not exclude the possibility that very different receptor sequences exist for androgens in sea lampreys. Finding this response of 15α-T now requires further research to reveal whether it, like E_2, has receptor binding sites in the testis, thus supporting the hypothesis that C19 steroids may have an androgenic role in the sexual development and maturation processes of sea lampreys.

A possible explanation for the absence of change in 15α-T concentrations between the 8 and 24 h sampling times may be that the duration selected was too short to observe the progression of increase and was not long enough to observe the pending decrease. Previous studies have shown that final maturation was accelerated by one or more injections of GnRH (Sower, 1989). The first injection is believed to activate the system, making it more sensitive to further injections. Sower (1989) shows how a single GnRH injection maintains the concentration of E_2, and progesterone (P) after 24 h, whereas after two successive GnRH injections, concentrations began to decrease after 24 h. The results from this study appear to indicate that for 15α-T responses, the two successive GnRH injections did not push the lampreys past the initial activation stage, thus the samples at the 8 h and 24 h sample times showed no differences in concentration.

The absence of dose-dependent responses indicates that the minimum GnRH doses used in this study were sufficient to induce the maximum 15α-T response in sea lamprey. Deragon and Sower (1994), using four successive injections, found E_2 concentrations to increase within 4 h, yet found no difference in responses between 100 and 200 μg/kg doses. Gazourian et al. (1997), using four successive injections, found that concentrations of both E_2 and P rose after 4 h and began to descend after 24 h. Gazourian et al. (2000), using a single injection and doses of 50 and 100 μg/kg, found similar elevated E_2 and P responses after 4 h, but a less pronounced decline after 24 h, and that the 50 μg/kg dosage elicited lower responses in some instances. All studies found that higher temperatures generally produced higher concentrations of steroids. Based on the experimental design of these previous studies, the injection regime, sampling intervals, and temperature used in this study were expected

to elicit differential 15α-T concentration responses between intervals and among doses. The fact that differences were not seen in these treatments as were seen for E_2 and P in other studies suggests that the production of 15α-T may be regulated differently than the production of estrogens and progestogens.

Within the range of doses used in this study, lamprey GnRH I and III were equipotent in increasing concentrations of 15α-T. Sower et al. (1993) first identified GnRH III and found that it elicited E_2 responses similar to those of GnRH I. Other studies have also shown that GnRH I and GnRH III are equally effective in elevating E_2 and P concentrations in sea lamprey (Sower, 1998). Gazourian et al. (1997) showed that GnRH I and GnRH III were equipotent in stimulating steroidogenesis and inducing ovulation in females. Only Deragon and Sower (1994) found different responses to the two GnRH forms where GnRH III in males was more potent than GnRH I in its ability to induce spermiation and increase the concentrations of E_2 and P. The elevated concentrations of 15α-T seen in response to both GnRH I and GnRH III indicate that at the doses examined, the different GnRH forms appear to have equal effects on this steroid and are similar to the responses of estrogens and progestogens.

The two GnRH form and dose-specific comparisons that differed between sites are not believed to represent an overall between-site difference. There was less than 39% difference in median plasma concentrations between sites for both of these two comparisons. This is low when compared to the over 72% difference in median plasma concentration between GnRH-treated lampreys and their respective controls. Although lamprey size and environmental conditions do differ between sites, the majority of the data support the conclusion that there is minimal if any difference in 15α-T plasma concentrations between the two sites.

The 15α-hydroxylated form of testosterone found in sea lampreys is unconventional and may be indicative of more specialized functions necessary in this ancient vertebrate. There have been 15α-hydroxylated steroids found in other organisms, but not as sex hormones (Brown et al., 1979; Giannopoulos and Solomon, 1970; Levy et al., 1965). A possible explanation for the use of 15α-T by lampreys is the assumption that a hematophagous parasite needs different forms of hormones than its hosts to avoid interaction with exogenous hormones. However, because sea lampreys only have a short parasitic phase while the majority of their life cycle is spent as larvae with immature gonads, the reason for needing hydroxylated testosterone and its specific functions are not yet known. Though the origins and function of 15α-T may be in question its placement in the HPG axis, and response to lamprey GnRH add significant information toward the further understanding of the reproductive endocrinology of this ancient fish.

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References

Brown, K.I., Long, D.W., Bacon, W.L., Braselton, W.E., 1979. Evidence for the presence of 15-hydroxylated estrogens in the peripheral plasma of the laying turkey. Gen. Comp. Endocrinol. 39, 552–560.

Bryan, M.B., Scott, A.P., Černý, I., Yun, S.S., Li, W., 2003. 15α-Hydroxytestosterone produced in vitro and in vivo in the sea lamprey, *Petromyzon marinus*. Gen. Comp. Endocrinol. 132, 418–426.

Deragon, K.L., Sower, S.A., 1994. Effects of lamprey gonadotropin-releasing hormone-III on steroidogenesis and spermiation in male sea lampreys. Gen. Comp. Endocrinol. 95, 363–367.

Gazourian, L., Derragon, K.L., Chase, C.F., Debananda, P., Habibi, H.R., Sower, S.A., 1997. Characteristics of GnRH binding in the gonads and effects of lamprey GnRH-I and -III on reproduction in the adult sea lamprey. Gen. Comp. Endocrinol. 108, 327–339.

Gazourian, L., Evans, E.L., Hanson, L., Chase, C.F., Sower, S.A., 2000. The effects of lamprey GnRH-I, -III, and analogs on steroidogenesis in the sea lamprey (*Petromyzon marinus*). Aquaculture 188, 147–165.

Giannopoulos, G., Solomon, S., 1970. Isolation of 15α-hydroxyandrostenedione and 15α-hydroxytestosterone from human pregnancy urine. Biochemistry 9, 596–602.

Hardisty, M.W., Potter, I.C., 1971. The general biology of adult lampreys. In: Hardisty, M.W., Potter, I.C. (Eds.), The biology of lampreys, vol. 1. Academic Press, New York, pp. 127–206.

Ho, S.M., Press, D., Liang, L.C., Sower, S.A., 1987. Identification of an estrogen-receptor in the testis of the sea lamprey, *Petromyzon marinus*. Gen. Comp. Endocrinol. 67, 119–125.

Katz, Y., Dashow, L., Epple, A., 1982. Circulating steroid hormones of anadromous sea lampreys under various experimental conditions. Gen. Comp. Endocrinol. 48, 261–268.

Knox, C.J., Boyd, S.K., Sower, S.A., 1994. Characterization and localization of gonadotropin-releasing-hormone receptors in the adult female sea lamprey, *Petromyzon marinus*. Endocrinology 134, 492–498.

Kime, D.E., Callard, C.V., 1982. Formation of 15α-hydroxylated androgens by the testis and other tissues of the sea lamprey, *Petromyzon marinus*, in vitro. Gen. Comp. Endocrinol. 4, 267–270.

Kime, D.E., Rafter, J.J., 1981. Biosynthesis of 15-hydroxylated steroids by gonads of the river lamprey, *Lampetra fluviatilis*, in vitro. Gen. Comp. Endocrinol. 44, 69–76.

Levy, H., Hood, B., Cha, C.H., Carlo, J.J., 1965. The monohydroxylation of 17β-estradiol at the 15α-, 16α-, and 16β-positions by bovine adrenal perfusion. Steroids 5, 677–686.

Linville, J.E., Hanson, L.H., Sower, S.A., 1987. Endocrine events associated with spawning behavior in the sea lamprey (*Petromyzon marinus*). Horm. Behav. 21, 105–117.

Lowartz, S., Petkam, R., Renaud, R., Beamish, F.W.H., Kime, D.E., Raeside, J., Leatherland, J.F., 2003. Blood steroid profile and in vitro steroidogenesis by ovarian follicles and testis fragments of adult sea lamprey, *Petromyzon marinus*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 134, 365–376.

Sherwood, N.M., Sower, S.A., Marshak, D.R., Fraser, B.A., Brownstein, M.J., 1986. Primary structure of gonadotropin-releasing hormone from lamprey brain. J. Biol. Chem. 216, 4812–4819.

Sower, S.A., 1989. Effects of lamprey gonadotropin-releasing hormone and analogs on steroidogenesis and spermiation in male sea lampreys. Fish Physiol. Biochem. 7, 101–107.

Sower, S.A., 1990. Neuroendocrine control of reproduction in lampreys. Fish Physiol. Biochem. 8, 365–374.

Sower, S.A., 1998. Mini-review: brain and pituitary hormone of lampreys, recent findings, and their evolutionary significance. Am. Zool. 38, 15–38.

Sower, S.A., Chiang, Y.C., Lovas, S., Conlon, J.M., 1993. Primary structure and biological activity of a third gonadotropin-releasing hormone from lamprey brain. Endocrinol. 132, 1125–1131.

Sower, S.A., Dickhoff, W.W., Gorbman, A., Rivier, J.E., Vale, W.W., 1983. Ovulatory and steroidal responses in the lamprey following administration of salmon gonadotropin and agonistic and antagonistic analogues of gonadotropin-releasing hormone. Can. J. Zool. 61, 2653–2659.

Sower, S.A., Kawauchi, H., 2001. Review: update: brain and pituitary hormones of lamprey. Comp. Biochem. Physiol. B 129, 291–302.

Sower, S.A., Plisetskaya, E., Gorbman, A., 1985a. Changes in plasma steroid and thyroid and insulin during final maturation and spawning of the sea lamprey *Petromyzon marinus*. Gen. Comp. Endocrinol. 58, 259–269.

Sower, S.A., Plisetskaya, E., Gorbman, A., 1985b. Steroid and thyroid hormone profiles following a single injection of partly purified salmon gonadotropin or GnRH analogues in male and female sea lamprey. J. Exp. Zool. 235, 403–408.

Thornton, J.W., DeSalle, R., 2000. A new method to localize and test the significance of incongruence: detecting domain shuffling in the nuclear receptor superfamily. Syst. Biol. 49, 183–201.

Thornton, J.W., 2001. Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. Proc. Natl. Acad. Sci. USA 98, 5671–5676.