Dose–response relationship of 15α-hydroxylated sex steroids to gonadotropin-releasing hormones and pituitary extract in male sea lampreys (Petromyzon marinus)

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Abstract

The sea lamprey (Petromyzon marinus) is one of the earliest extant vertebrates for which the hypothalamic-pituitary-gonadal (HPG) axis has been shown to control and regulate reproduction in a similar fashion to gnathostome vertebrates. While the two forms of gonadotropin-releasing hormones in the sea lamprey (GnRH I and GnRH III) have been studied extensively, their in vivo effect on synthesis of 15α-hydroxytestosterone (15α-T) and 15α-hydroxyprogesterone (15α-P) have only been partially characterized. In the present study, plasma concentrations of 15α-T and 15α-P were measured in prespermiating sea lampreys that were given a single injection of either GnRH I or GnRH III in doses ranging from 5 to 100 μg/kg, or of pituitary extract (as a source of gonadotropin). Plasma was sampled at 1–6 h and 6–48 h post-injection, in separate experiments, in order to characterize the peak and duration of responses. 15α-T plasma concentrations increased slightly in response to all three treatments, but not in a dose-dependent manner, and the timing of peak concentrations varied between doses. However, 15α-P plasma concentrations showed a greater range of response (between 1 and 100 ng/ml) and were clearly correlated with the injection dose. Plasma concentrations of 15α-P also responded to far lower doses of GnRH I and GnRH III than any other steroid previously investigated in lampreys. The plasma concentrations of 15α-P peaked at 6 h after injection for all three treatments, and levels reached a mean of 53.1 ng/ml. In female lampreys that were injected twice with 50 μg/ml GnRH I or III, 15α-T concentrations did not exceed 0.5 ng/ml and 15α-P concentrations did not exceed 1 ng/ml. These results lend further support to the hypothesis that 15α-P plays an important role in the reproductive endocrinology of male lampreys.

Keywords: Lamprey; Steroid; GnRH; 15α-hydroxyprogesterone; Progesterone

1. Introduction

The sea lamprey (Petromyzon marinus) is one of the earliest extant vertebrates for which the hypothalamus has been found to coordinate reproductive physiology. Investigations of the hypothalamic hormones have been extensive (Sower and Kawauchi, 2001; Sower, 1998, 2003), and have shown that there are two distinct forms of gonadotropin-releasing hormone (GnRH) native to lamprey, GnRH I (Sherwood et al., 1986) and GnRH III (Sower et al., 1993). Injections of either type of GnRH accelerate final maturation in both male and female lampreys (Sower et al., 1993; Gazourian et al., 1997, 2000). It has not yet been demonstrated whether the GnRHs act via release of gonadotropin (GTH) from the pituitary gland (the major pathway in other vertebrates) or directly on the gonads. It was only very recently (Sower et al., 2006) that the β-subunit of GTH was identified. It is likely that GTH mediates the response
between GnRH and gonadal hormones, with the gonadal hormones being the proximate regulators of physiological changes associated with reproduction, as has been shown in all other vertebrates. However, this remains to be proven.

The steroids produced by lamprey gonads have received occasional attention over many years. It has been established that, unlike other vertebrates, male sea lampreys synthesize 15α-hydroxysteroids in the testes, namely 15α-hydroxytestosterone (15α-T; Kime and Callard, 1982; Bryan et al., 2003; Lowartz et al., 2003) and 15α-hydroxyprogesterone (15α-P; Kime and Rafter, 1981; Bryan et al., 2004; Lowartz et al., 2003, 2004). The 15α-hydroxysteroids are synthesized by the testes, circulate in the plasma, and the plasma concentrations of these steroids in males are responsive to injections of either type of GnRH at relatively large doses (Bryan et al., 2003, 2004; Young et al., 2004a). It has been found that injections of GnRH have a much greater effect on the plasma concentrations of the 15α-hydroxysteroids than on their classical equivalents, testosterone (T) and progesterone (P) (Sower et al., 1985; Sower, 1989; Deragon and Sower, 1994; Gazourian et al., 2000). Plasma concentrations of 15α-T increased 2- to 5-fold in response to injections of either type of GnRH (Young et al., 2004a), while concentrations of T did not change significantly in response to heterologous gonadotropin (Sower et al., 1985). Plasma concentrations of 15α-P increased from <1 ng/ml to 36 ng/ml after injection with 50–200 μg/kg of GnRH (Bryan et al., 2004), while plasma concentrations of P, starting from approximately the same baseline, only increased to a maximum of 3 ng/ml in lampreys injected with GnRH (5–200 μg/kg GnRH I, Sower, 1989; 100–200 μg/kg GnRH III, Deragon and Sower, 1994; 50–100 μg/kg GnRH I, III, and analogues, Gazourian et al., 2000).

The aim of the present study was to characterize the magnitude, sensitivity, and timing of the response of 15α-T and 15α-P in male lampreys to a range of doses of GnRH I, GnRH III and pituitary extract (as a proxy for GTHβ). Some data on females has also been included to show that differing from previous experiments where two serial injections were used (Sower, 1989; Deragon and Sower, 1994; Gazourian et al., 2000; Bryan et al., 2004; Young et al., 2004a,b). Blood samples (0.8–1.0 ml) were collected from the caudal vein using heparinized syringes. After centrifugation of blood samples at 1000g for 20 min, plasma was collected and stored at –80°C until analyzed by RIA for 15α-T (Bryan et al., 2003) and 15α-P (Bryan et al., 2004).

In order to characterize both the immediate and long-term changes in sex steroid levels, while limiting sampling to quantities that would not significantly reduce blood volume and therefore have confounding effects, two separate experiments were designed. A short-term, 6-h experiment was designed to examine the immediate changes in sex steroid concentrations, and a long-term 48-h experiment was designed to examine the duration of the changes in sex steroid concentrations. For the 6-h experiment, lampreys were given 100 μg/kg doses of either type of GnRH or saline as a control (3 treatments total, 10 lampreys per treatment). Blood was sampled at 0, 1, 2, 4, and 6 h after the injection. For the 48-h dose–response experiment, five doses of each type of GnRH were given (5, 10, 20, 40, and 80 μg/kg, plus saline control; 11 treatments total, 10 lampreys per treatment). Blood was sampled at 0, 6, 12, 24, and 48 h after the injection.

To obtain pituitary extract, sea lamprey pituitary glands from 400 adults were collected in June 2000, at Hammond Bay Biological Station. The frozen pituitary glands were homogenized in 20 ml of 20 mM Tris-buffer, pH 7, containing protease inhibitor cocktails (Roche). This mixture was centrifuged at 1000g for 20 min allowing recovery of the supernatant. The protein concentration was determined using DCA protein analysis kit (Pierce, Rockford, IL). The protein concentration for the extract was 6.7 mg/ml. One millilitre of the extract was equivalent to 20 lamprey pituitary glands. Lampreys were given a single intraperitoneal injection equivalent to 1, 5, or 10 pituitaries or a 0.9% saline as a control (4 treatments total, 10 lampreys per treatment). Blood was sampled at 0, 6, 12, 24, and 48 h after the injection.

To confirm that neither type of GnRH resulted in the production and circulation of additional steroids that might cross-react with the antibodies used in the RIAs, small amounts of plasma from several individuals from the 80 μg/kg treatment group sampled at 6 h post-injection were pooled for both GnRH I and III. The plasma pools were extracted using solid phase extraction cartridges (Sep-pak C18, Waters, Milford, MA), that were washed with 5 ml dH2O, and eluted with 5 ml methanol. The methanol extracts were evaporated under nitrogen, redissolved, and fractionated by reverse-phase High Performance Liquid Chromatography (HPLC) as described by Bryan et al. (2002, 2004). Then 20 μl each of fractions 21–75 were assayed for 15α-T and 15α-P, and the peaks of reactivity were compared to the known elution points of the steroids.

In order to investigate the circulating concentrations of 15α-hydroxysteroids in female lampreys, an experiment using one dosage of each type of GnRH was performed. Preovulating female sea lampreys were given two serial injections, 24 h apart, of 0.9% saline (control), or GnRH I or III (50 μg/kg). Blood was sampled 6 h after the second injection. This is the same experimental design as done previously to investigate both 15α-hydroxysteroids in prespermiating males (Bryan et al., 2004; Young et al., 2004a) and classical steroids (Sower, 1989; Deragon and Sower, 1994; Gazourian et al., 2000).

Statistical analyses of the 6-h GnRH experiment were done per time interval using Analysis of Variance (ANOVA) in which type of injected hormone was the factor. The concentrations of 15α-T and 15α-P were analyzed separately. Analysis of the 48-h GnRH experiment was performed only at the peak response time because responses merged at the beginning and end of the experiment. A two-way ANOVA was used in which type of injected hormone and dosage were the factors. Least Squares Difference (LSD) test was used for multiple comparisons among dosages. Analysis of the pituitary extract experiment was done with a one-way ANOVA in which pituitary equivalent dosage at the peak response time was the factor. Multiple comparisons between doses were done using Fisher’s LSD tests. For the preovulating female experiment, for each steroid a two-way ANOVA was used in which type of injected hormone and time were the factors, and multiple comparisons between treatments and between sampling times were done using Fisher’s LSD tests.
3. Results

3.1. Animals

The lampreys from the 6-h GnRH injection experiment had lengths of (mean ± SEM) 497 ± 3.3 mm and weights of 265 ± 4.9 g. The lampreys from the 48-h GnRH injection experiment had lengths of 497 ± 5.9 mm and weights of 260 ± 9.4 g. The lampreys from the pituitary extract injection experiment had lengths of 477 ± 6.0 mm and weights of 227 ± 8.9 g. Of the 180 PSM lampreys used in all three experiments, two misidentified females were found and removed from analysis. The female sea lamprey had lengths of 479 ± 7.3 mm and weights of 232 ± 10.3 g.

3.2. Six-hour experiment

In the 6-h GnRH injection experiment, both 15α-T and 15α-P were evaluated for their responses to injections of GnRH I and GnRH III across a short time scale. The plasma levels of 15α-T showed few differences between the control, GnRH I, or GnRH III treatment groups (Fig. 1a).

Fig. 1. Concentrations of 15α-hydroxytestosterone (15α-T, Graph a) and 15α-hydroxyprogesterone (15α-P, Graph b) in response to GnRH I and III injections (100 μg/kg) across a 6-hour time course. Each point represents a mean for immunoreactive steroid concentrations measured in plasma from 10 sea lampreys with bars representing the SEM.

Measured responses in all treatments had a range from 0.25 ng/ml to 0.72 ng/ml. Although 15α-T plasma levels after injection with GnRH I was higher than in the other two treatments, they started with a higher concentration at 0 h. Both treatments and control groups exhibited nearly equal, gradual rates of increase from 0 h to 6 h. In contrast, plasma concentrations of 15α-P in response to either type of GnRH injection showed immediate and highly significant increases compared to those in control animals (Fig. 1b). While the control group had 15α-P plasma concentrations ranging from 1.2 ng/ml to 2.0 ng/ml across all intervals, the plasma concentrations of the two GnRH-injected groups rose at each consecutive interval from 2.1 ng/ml for GnRH I and 2.2 ng/ml for GnRH III at 0 h to 48.2 ng/ml for GnRH I and 55.8 ng/ml for GnRH III at 6 h. No statistical difference in 15α-P concentrations between the GnRH I and GnRH III treatments was found at any of the time intervals.

3.3. Forty-eight hour experiment

In the 48-h GnRH injection experiment, plasma concentrations of both 15α-T and 15α-P were evaluated for their responses to injections of GnRH I and GnRH III across a range of incremental doses. The time intervals of peak responses for 15α-T were not consistent between doses, ranging from 6 h to 24 h (Figs. 2a and 3a), so 12 h was chosen as a common point of comparison between doses (Figs. 2c and 3c). GnRH III (mean 0.65 ng/ml) elicited higher (p = 0.025) 15α-T plasma concentrations than GnRH I (0.55 ng/ml) at 6 h, GnRH III (0.76 ng/ml) elicited higher (p = 0.008) 15α-T plasma concentrations than GnRH I (0.61 ng/ml) at 12 h, and GnRH III (0.70 ng/ml) elicited higher (p = 0.058) 15α-T plasma concentrations than GnRH I (0.61 ng/ml) at 24 h. When comparing the effects of dosage for either GnRH I or III, significant differences were only found between treatments and the control (Table 1). Although 15α-T plasma concentrations increased in response to both GnRH treatments and a semblance of a dose effect appears in both Figs. 2c and 3c, the inherent variation seen at time 0 h (prior to injection) and the lack of pronounced differences in 15α-T plasma levels between treatments failed to reveal any effects of dosage for 15α-T.

The time interval of peak response for 15α-P occurred at 6 h and was consistent among all doses (Figs. 2b and 3b). GnRH III (53.1 ng/ml) elicited higher (p < 0.0001) 15α-P plasma concentrations than GnRH I (21.0 ng/ml) at 6 h (Figs. 2d and 3d). In contrast to 15α-T, 15α-P plasma concentrations showed significantly different responses to dosage for both GnRH I and GnRH III (Table 2). The trend of dose responses seen for 15α-P at the peak response interval of 6 h appears similar to the trends seen for 15α-T at its peak response interval (Figs. 2c and 3c). However, the magnitude of the changes in plasma concentrations of 15α-P is much greater and the errors around the means are relatively smaller (Figs. 2d and 3d) thereby allowing greater discrimination among doses.
3.4. Pituitary extract experiment

The pituitary extract injection experiment evaluated the changes in plasma concentrations of 15α-T and 15α-P to different doses of pituitary extract (Fig. 4). The peak response interval was again used as the comparison point for the treatments and was found to occur at 6 h for both 15α-T and 15α-P. Pituitary-injected lampreys showed increases in 15α-T plasma concentrations in response to the injections \((p < 0.0001)\), but at 6 h, the variation in responses among treatments masked any detectable differences among the treatments or the control. The plasma concentrations of 15α-T measured at all intervals and for all doses fell in the same range of 0.2–0.6 ng/ml as seen in the GnRH injection experiments.

The changes in 15α-P plasma levels were more pronounced and showed statistical differences between treatments. All pituitary-treated lampreys showed an increase in 15α-P plasma levels at 6 h. Among the treatments and control at 6 h, the 10 pituitary equivalent treatment group (24.6 ng/ml) had plasma concentrations of 15α-P significantly greater than the other two treatments and control \((p < 0.05)\). The 5 pituitary equivalent treatment group (12.7 ng/ml) had 15α-P plasma levels significantly greater than the control \((p < 0.05)\), but not the 1 pituitary equivalent treatment. The 1 pituitary equivalent treatment group (9.1 ng/ml) and the control group (2.1 ng/ml) were not found to have statistically different 15α-P plasma levels. The magnitude of response of 15α-P to the pituitary dosages was similar to the responses seen to the range of administered GnRH I dosages, but less than half the magnitude of the responses to GnRH III injections.

3.5. HPLC-fractionated plasma

The profiles of immunoreactivity (ir) for 15α-T and 15α-P in HPLC-fractionated plasma revealed that no new cross-reacting steroids were circulated in response to
Fig. 3. Concentrations of 15α-hydroxytestosterone (15α-T) and 15α-hydroxyprogesterone (15α-P, Graph b) in response to five doses of GnRH III across a 48-hour time course. Each point represents a mean for 10 sea lampreys with bars representing the SEM. The GnRH III dosage response profiles at the peak interval of 15α-T (Graph c) and 15α-P (Graph d) are shown adjacently.

Table 1
Summary of p-values for comparisons of 15α-hydroxytestosterone (15α-T) plasma concentrations in response to different doses of GnRH at 12 h after injection during the 48-h dose–response experiment

<table>
<thead>
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<th>10</th>
<th>20</th>
<th>40</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH I (μg/kg)</td>
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<td>0.2035</td>
<td>0.0078</td>
<td>0.0001</td>
<td>&lt;0.0001</td>
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<tr>
<td></td>
<td>5</td>
<td>0.7766</td>
<td>0.1078</td>
<td>0.0529</td>
<td>0.1704</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.7752</td>
<td>0.5898</td>
<td>0.8796</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.9996</td>
<td>0.9999</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.9952</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GnRH III (μg/kg)</td>
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<td>0.1419</td>
<td>0.0007</td>
<td>0.1506</td>
<td>0.0007</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.4242</td>
<td>1.0</td>
<td>0.4036</td>
<td>0.1249</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.4071</td>
<td>1.0</td>
<td>0.9835</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.3869</td>
<td>0.1174</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>40</td>
<td>0.9869</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Underlined values are significant at p < 0.05.

Table 2
Summary of p-values for comparisons of 15α-hydroxyprogesterone (15α-P) plasma concentrations in response to different doses of GnRH at the peak response interval (6 h) during the 48-h dose–response experiment

<table>
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<tr>
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<th>10</th>
<th>20</th>
<th>40</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH I (μg/kg)</td>
<td>0</td>
<td>0.1568</td>
<td>0.0006</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
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<tr>
<td></td>
<td>5</td>
<td>0.3460</td>
<td>0.1744</td>
<td>&lt;0.0001</td>
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<tr>
<td></td>
<td>10</td>
<td>0.9989</td>
<td>0.0092</td>
<td>0.9223</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.0269</td>
<td>0.9908</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.1156</td>
<td></td>
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<tr>
<td>GnRH III (μg/kg)</td>
<td>0</td>
<td>0.0189</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.0434</td>
<td>0.3235</td>
<td>0.0042</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.9310</td>
<td>0.9605</td>
<td>0.0136</td>
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<td>20</td>
<td>0.4860</td>
<td>0.0007</td>
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</tr>
<tr>
<td></td>
<td>40</td>
<td>0.1121</td>
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Underlined values are significant at p < 0.05.
injections of GnRH or pituitary extract (Fig. 5). The profiles of ir-15α-T in HPLC-fractionated plasma revealed one large peak for lampreys treated with either GnRH I or GnRH III. This peak corresponded to the known elution position of 15α-T. The ir-15α-P profile revealed two peaks for lampreys treated with either GnRH I or GnRH III. The larger of the two peaks corresponded to the elution position of 15α-P standard, as seen previously (Bryan et al., 2004).

3.6. Female sea lampreys

Concentrations of circulating 15α-hydroxylated steroids increased significantly, but only marginally, in response to two injections of GnRH (Table 3). In preovulating females, concentrations of 15α-T were significantly higher at 6 h after the second injection in fish injected with both GnRH I (p = 0.0059) and GnRH III (p = 0.0004). Concentrations of 15α-T in each treatment group did not change significantly between 6 h and 24 h after the second injection, but only lampreys injected with GnRH III had 15α-T plasma concentrations significantly higher than controls at 24 h (p = 0.0007). Concentrations of 15α-P were also significantly higher at 6 h after the second injection in fish injected with both GnRH I (p = 0.0001) and GnRH III (p < 0.0001). Plasma concentrations of 15α-P in both treatment groups decreased after 24 h (GnRH I, p = 0.0097; GnRH III, p = 0.0005), but remained higher than controls (GnRH I, p = 0.0228; GnRH III, p = 0.0003).

Table 3

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Time (h)</th>
<th>Treatment</th>
<th>Control</th>
<th>GnRH I</th>
<th>GnRH III</th>
</tr>
</thead>
<tbody>
<tr>
<td>15α-T</td>
<td>6</td>
<td>0.15 ± 0.02</td>
<td>0.26 ± 0.02</td>
<td>0.30 ± 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.15 ± 0.01</td>
<td>0.20 ± 0.02</td>
<td>0.25 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>15α-P</td>
<td>6</td>
<td>0.12 ± 0.02</td>
<td>0.44 ± 0.06</td>
<td>0.39 ± 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.12 ± 0.01</td>
<td>0.19 ± 0.04</td>
<td>0.24 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Concentrations are in ng/ml, and are expressed as the mean ± SEM. Underlined values are significantly higher (p < 0.05) than control plasma concentrations.
4. Discussion

In male lampreys, 15α-P plasma concentrations exhibit a clear dose–response relationship with both forms of GnRH and with pituitary extract. In all experiments 15α-P plasma levels peaked at 6 h after stimulation. Plasma 15α-P concentrations respond to much lower doses of GnRH and show a much greater response than any other steroid previously investigated in sea lampreys. 15α-P levels also responded in a similar fashion to injections of pituitary extract, implying, but by no means proving, that the GnRHs act primarily, as in other vertebrates, through release of GTH from the pituitary, and not directly on the gonads.

The progressive increase in sex steroid response to each increasing dose represents the first time that the range of GnRH dosages that differentially affect the production of a sex steroid has been demonstrated in male sea lampreys. This identification of the correct dosage range and knowledge of differential responses will be useful for future investigations on lamprey reproductive endocrinology.

Previous studies have shown that both types of GnRH are effective in increasing steroidogenesis and hastening maturity in male sea lampreys (Deragon and Sower, 1994). While the physiological effects of the two forms of GnRH are similar, comparisons of GnRH levels in male sea lamprey brains combined with immunocytochemical evidence has led to the hypothesis that GnRH III is the major form of GnRH regulating gonadal maturation (Sower, 1998; Sower and Kawauchi, 2001). The present study supports this hypothesis by demonstrating that GnRH III increases plasma concentrations of steroids significantly more than GnRH I.

While showing significant increases in response to GnRH injections (see also Young et al., 2004a,b), 15α-T plasma concentrations do not rise as steeply as those of 15α-P and also do not exhibit a clear dose–response relationship with GnRH. The pattern of the changes in 15α-T is also quite different from the pattern observed for 15α-P in that 15α-T takes more time to reach its peak and remains elevated for longer periods of time. The relatively poor response of 15α-T may be because it plays no role in the physiology of the lamprey. On the other hand, it could be because androgens are not important during the final stages of testis development (when spermatogenesis is complete). It is well known in teleosts that there is a ‘switch’ from C19 to C21 steroid synthesis in the final stages of gamete maturation in both males and females (Scott et al., 1983; Baynes and Scott, 1985; Barry et al., 1990). In male North Sea plaice (Pleuronectes platessa; Vermeirssen et al., 1998) implanted with GnRH, concentrations of C19 steroids initially rise significantly in response to GnRH stimulation but drop off as soon as C21 concentrations start to rise. In male dab (Limanda limanda) injected with gonadotropin (Canario and Scott, 1991), C19 steroids stay stable for a short time and then fall—at the same time as C21 steroids rise. In Atlantic halibut (Hippoglossus hippoglossoides) treated with GnRH (Vermeirssen et al., 2004), C19 steroid concentrations fall immediately—again at the same time as C21 steroid concentrations rise. These examples show that the relatively poor response of 15α-T to GnRH in the present study is not unusual or unexpected and most probably reflects the advanced stage of testis maturation of the males. Experiments probably need to be carried out on males at earlier stages of gonadal development (when androgens would be expected to have a key role) in order to investigate the role of 15α-T.

Compared to the classical steroids measured previously in lampreys, 15α-P circulates at much greater concentrations in response to injections with GnRH in prespermatogenic male lampreys. Basal levels of P are usually less than 1 ng/ml, and rise to 2–3 ng/ml (Sower, 1989; Deragon and Sower, 1994; Gazourian et al., 2000). Levels of T are very low and have not been shown to change in response to injections with heterologous gonadotropin (Sower et al., 1985). Concentrations of 17β-estradiol (normally considered to be a female hormone) are as high as 2 ng/ml or less in untreated male sea lampreys and can increase to as high as 8 ng/ml after GnRH or heterologous gonadotropin injections (Sower et al., 1985; Sower, 1989; Deragon and Sower, 1994; Gazourian et al., 2000; Young et al., 2004b). However, the effect of GnRH on 17β-estradiol and P concentrations has a poor dose-dependency and also requires relatively high doses of GnRH (100–200 μg/kg) in order to demonstrate a significant increase in steroid concentrations (Sower, 1998). With 15α-P, however, a response can be detected to a single dose as low as 5 μg/kg of either GnRH I or GnRH III (and the results indicate that even lower doses are likely to be effective), plasma concentrations have a wide range (from <1 ng/ml to ca. 100 ng/ml) and the response is highly dose-dependent. The concentration of 15α-P in plasma undoubtedly represents the ‘best measure’ yet for the effects of GnRH stimulation in male sea lampreys.

Presently, it is difficult to interpret the effect of the pituitary extract on steroid secretion. It seems probable that it was due to the extract containing GTHβ. However, changes in GTHβ concentrations in plasma in response to GnRH I and GnRH III and changes in plasma steroid concentrations in response to GTHβ (as opposed to crude pituitary extract) have yet to be quantified. Expression of the GTHβ gene has been shown to increase in response to 100 μg/kg injections of GnRH, but not to 50 μg/kg injections (Sower et al., 2006). If GTHβ is stored within pituitary cells and released when GnRH binds to GnRH receptors, changes in GTHβ gene expression may not reflect the amount of GnRH necessary to cause a short-term increase in circulating GTHβ. However, it is also possible that GnRH is affecting steroid levels not only through GTHβ, but also by direct action on the testes. This is supported by research showing that receptors for GnRH have been detected in lamprey gonads (Silver et al., 2005) and that GnRH can directly stimulate steroidogenesis in the gonads (Gazourian et al., 1997, 2000).
Two alternative hypotheses for the ability of the pituitary extract to increase 15α-P concentrations in plasma are that: (1) the extract contained GnRH that either acted via the intact pituitary glands of the injected males or directly on the gonads (as described above); or (2) 15α-P (and/or immunoreactive 15α-P-like steroids) are actually stress steroids that are made by the presumptive interrenal tissue (present in the kidneys) and are responsive to corticotrophin in the pituitary extracts. However, this second possibility is very unlikely. If 15α-P were a stress steroid, then there should have been a handling response in the saline-injected males and also in GnRH-injected females. However, there was no such response (Fig. 4; Table 3). The data, instead, point strongly to the fact that not only is 15α-P primarily a reproductive steroid, but that it is also a male steroid.

The sensitivity and magnitude of the response of 15α-P reinforces the hypothesis that this steroid plays a major hormonal role in male lampreys. This hypothesis may be further supported by experiments demonstrating the presence of a receptor for this steroid and determining its function.

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