A putative corticosteroid hormone in Pacific lamprey, *Entosphenus tridentatus*

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1. Introduction

The stress response is found across all vertebrate groups (Tort and Teles, 2011) and is mediated by glucocorticoids acting on glucocorticoid receptors and ultimately causing changes in the expression of stress-response genes. The ability to perceive and respond to stressful situations is considered an adaptive response (Wendelaar Bonga, 1997). Stress in fish is known to reduce concentrations of gonadal steroids and is associated with higher ovarian atresia rates, reduced egg size in females and lower sperm counts in males, which can lead to direct influences on reproductive success (Iwama et al., 1997). Stress in fish is also known to reduce the amount of available energy. However, exposure to stressors of increased duration or frequency can cause the physiological responses to become maladaptive to the organism (Barton, 2002) by dramatically reducing the amount of energy available for reproduction and immunity (Wendelaar Bonga, 1997). Stress in fish is also known to reduce the amount of available energy. However, exposure to stressors of increased duration or frequency can cause the physiological responses to become maladaptive to the organism (Barton, 2002) by dramatically reducing the amount of energy available for reproduction and immunity (Wendelaar Bonga, 1997).

Great efforts have been put forth to elucidate the mechanisms of the stress response in vertebrates and demonstrate the conserved response across different vertebrate groups, ranging from similarities in the activation of the hypothalamic–pituitary–adrenal axis to the release and role of corticosteroids. There is however, still very little known about stress physiology in the Pacific lamprey (*Entosphenus tridentatus*), descendants of the earliest vertebrate lineage, the agnathans. In this paper we demonstrate that 11-deoxycortisol, a steroid precursor to cortisol in the steroidogenic pathway, may be a functional corticosteroid in Pacific lamprey. We identified the putative hormone in Pacific lamprey plasma by employing an array of methods such as RIA, HPLC and mass spectrometry analysis. We demonstrated that plasma levels of 11-deoxycortisol significantly increased in Pacific lamprey 0.5 and 1 h after stress exposure and that lamprey corticotropin releasing hormone injections increased circulating levels of 11-deoxycortisol, suggesting that the stress response is under the control of the HPA/I axis as it is in higher vertebrates. A comprehensive understanding of vertebrate stress physiology may help shed light on the evolution of the corticosteroid signaling system within the vertebrate lineage.

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Steroid receptors are referred to as ligand-activated transcriptional factors (Bolander, 2004) and the advent of steroid receptor signaling and the responses mediated by these receptors are thought to have had an important role in the early evolution of vertebrates as well as their survival (Baker, 1997). Tetrapods have two corticosteroid receptors which have divergent functions and ligands, the mineralocorticoid receptor whose ligand is aldosterone and the glucocorticoid receptor whose ligand is either corticosterone or cortisol depending on the group (Bury and Sturm, 2007).

It is hypothesized that a large scale genome duplication event early in the gnathostome lineage is responsible for creating the six steroid receptor orthologs present in the sarcopterygians: the estrogen receptor, progesterone or cortisol depending on the group (Bury and Sturm, 2007).

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Three steroid receptors have been identified in sea lamprey: the estrogen receptor, progestin receptor, androgen receptor, glucocorticoid receptor, and mineralocorticoid receptor (Baker, 1997; Thornton, 2001). Teleosts have two distinct glucocorticoid receptors present (GR1 and GR2) and a mineralocorticoid receptor likely from a gene duplication event that happened early in the teleost lineage (Bury et al., 2003). Three steroid receptors have been identified in sea lamprey: the estrogen receptor, progestin receptor, and corticosteroid receptor (Thornton, 2001).

Corticosteroids regulate metabolism by directly increasing gluconeogenesis, protein turnover, and lipolysis (Mommsen et al., 1999). In addition, they have also been shown to affect the immune system by increasing susceptibility to pathogen and reducing lymphocyte levels in fish (Wendelaar Bonga, 1997). The active glucocorticoid is taxa dependent and includes cortisol in most mammals and ray finned fish (Sangalang et al., 1971; Barton et al., 1987; Barton, 2002), corticosterone in most birds, amphibians and reptiles (Tyrrell and Cree, 1998; Moore and Jessop, 2003), and possibly 1x-hydroxycorticosterone in elasmobranchs (Idler and Truscott, 1969; Anderson, 2012). Recently, a putative corticosteroid in one of the early vertebrates, sea lamprey (Petromyzon marinus), was shown to be 11-deoxycortisol, a precursor molecule to cortisol (Close et al., 2010), offering a clue about the evolution of steroid signaling in the early vertebrates. However, it is premature to draw a conclusion that 11-deoxycortisol is a widespread corticosteroid hormone adopted by other jawless vertebrates. This warrants further characterization of corticosteroids in additional agnathan species, for example, Pacific lamprey.

We therefore investigated whether a more recently derived species of agnathan, Pacific lamprey, also uses 11-deoxycortisol as a functional corticosteroid. We examined if Pacific lamprey produce 11-deoxycortisol by identifying the steroid in the plasma using HPLC and mass spectrometry. Further, we determined if 11-deoxycortisol would respond to sea lamprey CRH and ACTH, which may indicate regulatory mechanisms by the HPI in this fish. Finally we examined if 11-deoxycortisol concentrations were elevated after acute stress.

2. Materials and methods

2.1. Animals

Adult Pacific lampreys were collected in Stamp Falls, British Columbia in June 2010 and August 2012, and in the Skeena River, British Columbia in August 2013. Fish were kept in covered, flow through, insulated tanks at 10–12 °C filled with dechlorinated tap water and were acclimated for at least 1 week before experiments. All fish were maintained in accordance with the Canadian Council on Animal Care and research experiments performed were approved by the UBC Animal Care Committee.

2.2. Chemicals

11-Deoxycortisol antibody was purchased from American Research Products (Waltham, MA, USA), antibodies for 11-deoxycorticosterone and corticosterone were purchased from Novus Biologicals (Littleton, CO, USA) and cortisol antibody was purchased from Millipore (Temecula, CA, USA). Standard 11-deoxycortisol, 11-deoxycorticosterone, cortisol and corticosterone were purchased from Sigma (Sigma Aldrich Chemical Co., St. Louis, MO, USA). 11-Deoxycorticosterone, 11-deoxycorticosterone, cortisol and corticosterone radioligands were purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). The putative sea lamprey CRH was custom synthesized by New England Peptide (Gardner, MA, USA) based on the deduced amino acid sequence identified from the Sea lamprey genome database (Roberts et al., 2014). ACTH was custom synthesized by Bachem Inc. (Torrance, California, USA). Acetonitrile (HPLC grade) was purchased from Fisher Scientific (Fair Lawn, NY, USA), formic acid (puriss. p.a. for mass spectroscopy) from Fluka (Steinheim, Germany), and ultra pure water was prepared in our laboratory using a Milli-Q Synthesis system (Millipore, Billerica, MA, USA). All other chemicals were purchased from Sigma Aldrich.

2.3. Plasma samples

Blood (1–2 ml) was collected from anesthetized Pacific lamprey via caudal vein using heparinised vacutainer tubes and spun at 1000 × g at 4 °C for 12 min. Plasma was stored at −80 °C until use.

2.4. Radioimmunoassay

Prior to radioimmunoassay (RIA), all plasma samples were subjected to ether extraction using a protocol outlined in Brenner et al. (1973). Briefly, each plasma sample was ether extracted in duplicate using 20 μl of plasma per replicate. 200 μl of diethyl ether was added to each tube, then tubes were vortexed for 30 s, the phases were separated by centrifugation (1000 × g for 2 min at 4 °C), the bottom of the tubes were dipped into a bucket containing dry ice and methanol to freeze the aqueous layer and the organic phase was transferred to a clean test tube. This process was carried out three times to extract the steroids from the plasma. The organic phase was dried under a stream of N2 gas. The dried tubes received 20 μl of RIA buffer and then were used for RIA.

RIA was performed as in Scott et al. (1980). Briefly, RIA was conducted in glass culture tubes (10 mm × 75 mm, Fisher Scientific, Ottawa, Canada) in duplicate. Assay buffer was made of 50 mM sodium phosphate, pH 7.4, 0.2% BSA, 137 mM NaCl, 0.40 mM EDTA, and 0.77 mM sodium azide. Nine standard tubes were made ranging from 1.95 to 500 pg ml-1 of standard steroid (11-deoxycortisol, 11-deoxycorticosterone, cortisol or corticosterone) in 100 μl assay buffer. Blank, total and maximum tubes received 100 μl of assay buffer. Sample tubes received 20 μl of either ether extracted plasma or HPLC fractionated plasma and 80 μl assay buffer for a total of 100 μl per tube. Radiolabel-buffer solution was made such that 100 μl of this solution would contain 5000 DPM. 100 μl of this solution was added to blank tubes. Antibody was added to the label-buffer solution such that 50% of the radiolabel was bound to the antibody in the absence of any standard steroid. All tubes (except blank tubes) received 100 μl of this antibody-label-buffer solution. Tubes were incubated overnight at 4 °C. The next day tubes were placed on ice and 500 μl of 0 °C charcoal solution (50 mM sodium phosphate, pH 7.4, 0.1% gelatin, 1.0% dextrancoated charcoal) was added to all tubes (except total tubes which received 500 μl assay buffer). After 15 min, the tubes were centrifuged at 1000 × g, 4 °C for 12 min, decanted into 7 ml scintillation vials (Fisher Scientific, Ottawa, Canada), and mixed with 5 ml scintillation cocktail (RPI Corp.). DPM were counted with an LS-6500 scintillation counter (Beckman Coulter, Mississauga, Ontario, Canada).

2.5. Screening for 11-deoxycortisol, 11-deoxycorticosterone, cortisol and corticosterone using high performance liquid chromatography (HPLC) and RIA

Steroids were extracted from 5 mL of adult Pacific lamprey plasma by filtering with a 0.45 μm filter (Millipore, Billerica, MA, USA) and the filtered plasma was passed through an activated Sep-Pak (Waters, Mississauga, Canada) and eluted with 10 mL of methanol. The methanol steroid extract was then dried down using a CentriVap Concentrator (Labconco, Kansas City, MO, USA) and Benchtop Freeze Dryer (VirTis, Gardiner, NY, USA). The dried sample was redissolved in 700 μL of solvent A (0.01% formic acid in deionized water) and 300 μL of solvent B (70% acetonitrile and 0.01% formic acid in deionized water). The sample was centrifuged at 12,000 × g for 10 min and then loaded onto a C18 reverse-phase HPLC column (Alltima, 4.6 mm × 250 mm, Alltech, Deerfield, IL, USA) fitted with a guard module. Oven temperature was kept at 40 °C. Solvents A and B were used to create the column gradient and the development pattern was as follows: 0–10 min: 28% B; 10–60 min: 28–100% B; 60–90 min 100% B. Total development time was 90 min (flow rate: 0.5 ml/min) and fractions were collected every min between 20 and 80 min into scintillation vials (Fisher Scientific, Ottawa, Canada). Fractions 21–80 were screened using RIA (described above) to determine the concentration of 11-deoxycortisol, 11-deoxycorticosterone, cortisol and corticosterone. Position of standard 11-deoxycortisol, 11-deoxycorticosterone, cortisol and corticosterone was determined by loading 6 μg dissolved in 700 μL solvent A and 300 μL solvent B) onto the C18 reverse phase HPLC column, in separate runs, and the eluate was monitored for UV absorption with a photodiode array detector (Analytical Technologies Group, Gorton, CT, USA).

2.6. Confirmation of RIA measurements of 11-deoxycortisol: pre- and post-acute stress experiments

To confirm that the measured 11-deoxycortisol concentrations in the plasma by RIA represent the actual concentrations of the steroid, a study was designed and conducted as described below. Following acclimation, Pacific lamprey (n = 4) were netted from holding tanks, placed into a bucket and immediately bled to get a pre-acute stress plasma sample. Following the bleed, fish were placed into a dry bucket for 5 min to induce a stress response, fish were then placed back into holding tanks. After 1 h, fish were netted, placed into a bucket and quickly bled once again to get a post-acute stress plasma sample. 1 ml of each pre- and post-acute stress plasma for each individual was used for steroid extraction and HPLC fractionation as explained in Section 2.6. Fractions 41–70 were used in RIA’s to quantify the amount of 11-deoxycortisol and 11-deoxycorticosterone as explained in Section 2.5 except 100 μL of the fractions were used instead of 20 μL.

2.7. Liquid chromatography–mass spectrometry

The HPLC fraction with 11-deoxycortisol immunoreactivity and synthetic 11-deoxycortisol were further analyzed by ultra high performance liquid chromatography–tandem mass spectrometer (UHPLC/MS/MS) in separate runs. The UHPLC/MS/MS system consisted of an Agilent 1290 Infinity Binary Pump, a 1290 Infinity Sampler, a 1290 Infinity Thermostat, and a 1290 Infinity Thermostatted Column Compartment (Agilent, Mississauga, Ontario, Canada) connected to an AB Sciex QTrap®5500 hybrid linear ion-trap triple quadrupole mass spectrometer equipped with a Turbo Spray source (AB Sciex, Concord, Ontario, Canada). The mass spectrometer was operated in positive ionization mode and data were acquired using the Analyst 1.5.2. software, on a Microsoft Windows XP Professional operating platform. Chromatographic separation was achieved using a Waters Acquity UPLC BEH C18, 1.7 μm, 2.1 × 100 mm column maintained at 30 °C. The mobile phase consisted of water with 0.1% formic acid (A), and acetonitrile with 0.1% formic acid (B) was applied at a flow rate of 200 μl/min, with a linear gradient of 0–100% B over 8 min. The analyze of interest was detected in multiple reaction monitoring (MRM) using the transition at m/z 347.2 → 109.2. The declustering potential, collision energy were 80 units, and -40 eV, respectively, and nitrogen was used as a desolvation and collision gas.

2.8. CRH dose response

Following acclimation, adult Pacific lamprey were placed in a bucket containing a buffered solution of MS-222 (0.06 g/l) and NaHCO3 (0.15 g/l). Once anesthetized, fish were injected intraperitoneally with either 0.90% saline as a control (female n = 6–13; male n = 1–3), or a specific dose of sea lamprey CRH peptide (0.1, 50, 100 μg/kg dissolved in 0.90% saline solution). Fish were placed in a recovery bucket and then returned to holding tanks after injections and blood samples were taken 1 h after injections. Plasma 11-deoxycortisol concentrations were determined by performing a RIA on ether extracted plasma samples.

2.9. ACTH injection experiments

Pacific lamprey were removed from holding tanks and placed in a bucket containing buffered MS-222 solution. After anesthetization, Pacific lamprey were injected intraperitoneally with either 0.90% saline (n = 10; 5 males and 5 females) or a mixture of 4 ACTH peptides (n = 10; 5 males and 5 females) (ACTH1–60, ACTH1–59P, ACTH1–59P), each at a concentration of 100 μg per kg body weight dissolved in 0.90% saline. Fish were placed in a recovery bucket and then returned to holding tanks following injections. Blood samples were taken 1 h after injection. Plasma samples were stored at −80 °C until used to determine plasma 11-deoxycortisol levels by performing an RIA on the ether extracted plasma.

2.10. Acute stress response

Following acclimation, adult female Pacific lamprey (n = 7–10) were netted from tanks and placed into a dry bucket for 5 min to induce stress. Fish were placed back into tanks after the dewatering and blood samples were taken at various time intervals after stressor exposure (0.5, 1, 2, 4 h). Control groups (n = 10) were left in the tanks undisturbed and bled immediately after netting and anesthetizing. Plasma 11-deoxycortisol concentrations were determined by performing a RIA on ether extracted plasma samples.

2.11. Statistical analysis

Data are expressed as mean ± SE, and statistical significance is assumed at P < 0.05. Statistical analyses were performed using Prism 5.00 (GraphPad Software Inc, California, USA). For the acute stress and CRH injection experiments, significant differences between means were evaluated using one way ANOVA followed by Dunnett’s comparisons. Differences between the means in the ACTH injection experiments were evaluated using a t-test. Differences between the means of pre and post stressed fractions in the pre- and post-acute stress experiment were evaluated by performing a two way ANOVA followed by a Bonferroni post-test.
3. Results

3.1. RIA of HPLC fractions

The highest immunoreactive peak was at fraction 48 with the 11-deoxycorticosterone antibody (Fig. 1B). The cortisol and 11-deoxycorticosterone antibodies also showed slight immunoreactivity with fraction 48. Authentic standard 11-deoxycorticosterone also eluted at fraction 48, corresponding to the highest immunoreactive peak from the HPLC fractionated plasma. Immunoreactive cortisol did not appear to be above background levels. Two very small immunoreactive peaks for corticosterone and 11-deoxycorticosterone were also evident from the screening (Fig. 1A).

3.2. Confirmation of RIA measurements of 11-deoxycortisol: pre- and post-acute stress experiments

There was no significant difference in concentrations of 11-deoxycorticosterone in fractionated lamprey plasma (Fig. 2A). Fraction 48 showed significantly higher (P < 0.001) concentrations of 11-deoxycorticosterone in the post stress group in comparison to the pre stress group (Fig. 2B). No other fractions showed significantly different immunoreactivity between the pre- and post-stress groups.

3.3. Identification of 11-deoxycortisol by UHPLC/MS/MS

MRM experiments using m/z 347.0 → 109.2 transition on fraction 48 and synthetic 11-deoxycortisol confirmed the presence of 11-deoxycortisol, as evidenced in the peak at 4.75 min (Fig. 3). A peak from fraction 48 coincided with that from the synthetic standard, confirming the identity of 11-deoxycortisol.

3.4. CRH dose responses

Pacific lamprey injected with sea lamprey CRH showed significantly increased plasma concentrations of 11-deoxycortisol in comparison to saline injected controls (female n = 6–13/treatment; male n = 1–3/treatment) (Fig. 4). Saline injected Pacific lamprey had plasma 11-deoxycortisol concentrations of 0.60 ± 0.09 ng/ml (mean ± SEM) for females and 0.55 ± 0.15 ng/ml for males and 0.1 μg/kg CRH dose induced a statistically significant (P < 0.05) increase in females with plasma 11-deoxycortisol rising to 3.39 ± 0.60 ng/ml and an increase, although not significant, in males to 2.07 ± 0.47 ng/ml. Plasma 11-deoxycorticosterone levels were significantly increased in the 50 μg/kg of females and males to 2.91 ± 0.29 ng/ml and 3.27 ± 0.39 ng/ml, respectively and a significant increase in females injected with the 100 μg/kg doses to 3.53 ± 0.29 ng/ml. Due to low sample size, statistical analyses on males in the 100 μg/kg treatment group could not be performed.

3.5. ACTH injection experiments

Pacific lamprey injected with the 4 ACTH peptides used did not increase plasma concentrations of 11-deoxycortisol (Fig. 5)
compared to the saline injected control group. Saline injected Pacific lamprey had plasma 11-deoxycortisol concentrations of 2.48 ± 0.44 ng/ml and 2.90 ± 0.71 ng/ml for females and males respectively whereas Pacific lamprey injected with 100 \( \mu \)g/kg of an ACTH peptide mixture had plasma concentrations of 2.32 ± 0.69 ng/ml and 2.36 ± 0.64 ng/ml for females and males, respectively.

3.6. Acute stress experiment

Acute stress induced by 5 min of dewatering caused circulating concentrations of 11-deoxycortisol to significantly \((P < 0.0001)\) increase by 0.5 h. Basal levels of 11-deoxycortisol were 0.59 ± 0.057 ng/ml (mean ± SEM) and significantly increased to 1.15 ± 0.11 ng/ml by 0.5 h and 0.91 ± 0.06 ng/ml by 1 h (Fig. 6). Although not statistically significant, plasma 11-deoxycortisol concentrations were higher 2 h after the acute stress (0.62 ± 0.09 ng/ml) compared to controls. Plasma concentrations of 11-deoxycortisol returned to basal levels by 4 h (0.57 ± 0.04 ng/ml).

4. Discussion

Results from the present study indicate that 11-deoxycortisol may potentially function as a corticosteroid in Pacific lamprey. 11-Deoxycortisol was identified in the plasma of Pacific lamprey using HPLC fractionation, RIA and UHPLC/MS/MS analysis. Plasma 11-deoxycortisol levels responded to acute stress and sea lamprey-CRH injections, but not sea lamprey ACTH injections, demonstrating the possibility of regulation by an HPI axis. Close et al. (2010) showed that 11-deoxycortisol is present and functions as a corticosteroid in sea lamprey. Our data support the hypothesis...
that 11-deoxycortisol is present in Pacific lamprey plasma and may act as a functional corticosteroid.

We isolated and identified 11-deoxycortisol from adult Pacific lamprey plasma. To reduce the chance of misinterpreting cross reactivity of the 11-deoxycortisol-antibody with other steroids, the pooled plasma was fractionated by HPLC and then each fraction was screened using a RIA developed for 11-deoxycortisol. The highest immunoreactivity was found at fraction 48 with the 11-deoxycortisol antibody and had an elution time identical to that of standard 11-deoxycortisol under the same HPLC conditions. To further confirm the presence of 11-deoxycortisol, fraction 48 was examined with UHPLC/MS/MS and the identification of 11-deoxycortisol in the fraction was verified. The combination of all the aforementioned methods provides direct evidence for the identification of the steroid being measured by our RIA and the presence of 11-deoxycortisol in the circulation of Pacific lamprey.

We demonstrated that the antibody used in the 11-deoxycortisol RIA was highly specific with low cross reactivity with other steroids in the lamprey plasma. In addition, fraction 48 in the post-stress group was the only fraction which had significantly higher concentrations of 11-deoxycortisol compared to the pre-stress group in the RIA developed for 11-deoxycortisol. We were also able to eliminate 11-deoxycorticoctosterone as a candidate corticosteroid since there were no significant differences in any fractions between the pre- and post-stressed groups in the 11-deoxycorticoctosterone RIA. The identification of 11-deoxycortisol in fraction 48 was finally verified using mass spectrometry.

The CRH gene is highly conserved among vertebrates (Lovejoy and Balment, 1999; Chang and Hsu, 2004; Lovejoy and Jahan, 2006) and Roberts et al., (2014) have shown that the sea lamprey CRH peptide sequence is very similar to a wide range of other vertebrates. Human CRH and sea lamprey CRH have high sequence similarity in addition to function similar to that of standard 11-deoxycortisol under the same HPLC conditions. To further confirm the presence of 11-deoxycortisol, fraction 48 was examined with UHPLC/MS/MS and the identification of 11-deoxycortisol in the fraction was verified. The combination of all the aforementioned methods provides direct evidence for the identification of the steroid being measured by our RIA and the presence of 11-deoxycortisol in the circulation of Pacific lamprey.

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In the present study, we used sea lamprey CRH to understand the potential functionality of an HPI hormone; (2) the synthesized ACTH molecules may not have formed the correct structural conformation; (3) the ACTH molecules may have degraded rapidly upon injection; or (4) potentially, 11-deoxycortisol may not be the functional corticosteroid and thus does not respond to ACTH. Further research is required to elucidate the possible cause of such observation.

Acute stress is known to elicit corticosteroid release among vertebrates, however, different species have different latency periods between stressor recognition and significant increases in corticosteroid levels (Barton, 2002). Five min handling stress and confinement in brown trout caused significant increases in cortisol levels and highest plasma levels were detected at 60 min (Sumpter et al., 1985). Cortisol concentrations significantly increased in spardin red porgy after 8 min of net handling and were highest at 2 h following stress exposure (Rottlant and Tort, 2005). Net handling and confinement of gilthead sea bream caused a significant increase in cortisol concentrations by 1 h (Rottlant et al., 2001). Close et al. (2010) found similar results that sea lamprey significantly increased 11-deoxycortisol plasma concentrations 1 h after dewatering stress and that concentrations of 11-deoxycortisol were back to basal levels 24 h post stress. Although the rate of corticosteroid release during HPI axis activation varies, it is commonly accepted that the rise in corticosteroid concentrations generally occurs within minutes rather than hours in teleosts (Pankhurst, 2011). This is similar to our results in Pacific lamprey where a significant increase in plasma 11-deoxycortisol concentrations was seen 0.5 h post stress with a return to basal levels by 2 h.

The present study serves as a starting point in understanding the role of 11-deoxycortisol in the stress response of Pacific lamprey. Future work should use Pacific lamprey-CRH to better understand effect on plasma 11-deoxycortisol and determine the presence of a dose–response relationship between CRH and 11-deoxycortisol. The relationship seen with sea lamprey CRH injections and 11-deoxycortisol concentrations should be supplemented with future work examining the role of the pituitary hormone, ACTH, to understand the potential functionality of an HPI axis in lamprey. The biochemical characterization, localization and function of the lamprey corticosteroid receptor should also be examined. Pacific lamprey are modern representatives of the most basal vertebrate group, and therefore, identification of a functional corticosteroid in a greater number of lamprey species will be pivotal in understanding the role corticosteroids played in early vertebrates.

In conclusion, by identifying 11-deoxycortisol in the plasma using several different methods as well as looking at the biological effects of induced stress and sea lamprey CRH injections on circulating plasma 11-deoxycortisol levels, we have demonstrated that 11-deoxycortisol is a putative corticosteroid hormone in Pacific lamprey, Entosphenus tridentatus. Gen. Comp. Endocrinol. (2014), http://dx.doi.org/10.1016/j.ygeno.2014.06.019.
lamprey. This is the second species of lamprey in which 11-deoxy-
cortisol has been identified and shown to increase plasma levels in
response to stress; this work along with future studies on lamprey
corticosteroid signaling can contribute to the understanding of the
evolution of corticosteroid signaling.

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