ACTH Activity in the Pituitary and Brain of the Least Brook Lamprey, *Lampetra aepyptera*

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A rat adrenal bioassay detected low levels of ACTH activity in the pituitary and brain of larval and adult *Lampetra aepyptera*, a nonparasitic lamprey. This is the first substantial evidence for the existence of this hormone in any cyclostome.

Research on the lamprey pituitary has not revealed whether these cyclostomes possess the same pituitary cell types and trophic hormones as other vertebrates (Percy et al., 1975). For example, Weisbarte et al. (1980) indicate that there is no substantive evidence for the presence of ACTH in cyclostomes. The report of ACTH in *Myxine* and *Lampetra* (Strahan, 1959) is unreliable in view of the insensitivity of the bioassay and the absence of experimental details and quantitative data. As evidenced by a positive lead hematoxylin staining reaction, there is histological evidence for ACTH-secreting cells in the mesoadenohypophysis of lampreys (Larsen and Rothwell, 1972). However, the evidence for hypothyalal control of the interrenal is inconclusive (Hardisty, 1979) considering the small amount of interrenal tissue in lampreys (Hardisty, 1972) and the extremely low levels of plasma corticosteroids (Idler and Truscott, 1980). Plasma corticosteroid levels in hagfish are elevated by injections of mammalian ACTH (Weisbarte et al., 1980), but corticosterone levels in *Lampetra fluviatilis* are unaffected by this treatment (Buus and Larsen, 1975). In both larval and adult sea lamprey, *Petromyzon marinus*, however, administration of mammalian ACTH produces ultrastructural changes in the interrenal cells similar to those seen in the adrenocortical cells of other vertebrates after ACTH stimulation (Youson, 1973).

Based on the results of a sensitive rat adrenal bioassay, we present evidence for the existence of ACTH activity in the pituitary and brain of both larval and adult *Lampetra aepyptera*, the least brook lamprey, a nonparasitic species inhabiting small creeks in the east central United States.

MATERIALS AND METHODS

Lampreys were collected in two small creeks within 5 miles of Athens, Ohio. A rectangular frame dip net with a bag of plankton netting was used to capture both ammocoetes and adults. Adults were taken during the 10-day spawning period in early April 1981 (water temp., 9–16°C). Within 1 hr of capture, lampreys were anesthetized with ethyl-m-aminobenzoate (Sigma), weighed, measured, and assessed for reproductive state. We used 38 adults (weight range 4.1–10.8 g; \( \bar{X} = 6.1 \)) and 6 ammocoetes (0.4–8.5 g; \( \bar{X} = 3.3 \)).

Extracts of pituitaries, brains, or pituitaries and brains were prepared by homogenization in 0.1 N HCl containing 0.5% bovine serum albumin (0.1 ml/pituitary or brain). We extracted the whole brain or, in the case of the pituitary, the dense connective tissue floor of the neurocranium surrounding the hypophysial fenestra. The extracts were heated (90–100°C for 7 min), cooled on ice, and stored frozen until bioassay 1–3 days later.

We bioassayed the extracts using the rat isolated adrenal cortex cell technique of Sayers et al. (1971a, b). This procedure involved dispersion of adrenal cells from the glands by mechanical agitation in Krebs–Ringer bicarbonate (KRB) buffer (pH 7.4) containing 0.2% glucose and 0.25% trypsin. After dispersion, cells were collected by centrifugation and re-
suspended in KRB buffer containing 0.2% glucose and 0.5% bovine serum albumin (KRBGA), plus 0.1% lima bean trypsin inhibitor. Aliquots (0.9 ml) of cell suspension were incubated at 37°C for 60 min with aliquots (0.01–0.1 ml) of lamprey extract or synthetic ACTH 1–24 (Cortrosyn, Organon) as a standard. Fluorometry was used to measure production of steroid by adrenal cells.

This is one of the most sensitive assays for mammalian ACTH. It has the capability of detecting a minimum dose of 0.1 μU (1.0 pg) of ACTH and therefore has the same order of sensitivity as radioimmunoassay. The isolated adrenal cell technique is also highly specific for ACTH. Doses of 10⁷ pg of insulin, oxytocin, vasopressin, angiotensin II, and glucagon do not stimulate steroidogenesis, and the response to 10⁷ pg of α-MSH is less than or equal to the response to 10 pg of ACTH (Sayers et al., 1971b).

RESULTS AND DISCUSSION

ACTH activity is present in both the pituitary and brain of adult and larval lampreys. The data in Table 1 were generated by comparing the steroidogenic response of acidic extracts of lamprey tissue to that of synthetic mammalian ACTH 1–24. Expressed in terms of picograms of synthetic mammalian hormone, the ACTH activity of the lamprey tissue extracts is low. When normalized to body weight, the goldfish pituitary contains approximately 10³ times as much ACTH as the lamprey (Portanova and Eastman, unpublished observation). Alternatively, the lamprey extracts might contain somewhat greater amounts of an ACTH moiety less potent, in terms of affinity and/or intrinsic activity (Ariens and Simonis, 1964), than the synthetic ACTH 1–24 utilized in the rat adrenal bioassay. The relatively small amounts of material available for bioassay, coupled with the low biologic activity of the extracts, did not permit the generation of complete dose-response relationships required to resolve such questions. With these limitations, we believe it is premature to interpret the quantitative data or to formulate hypotheses relating ACTH levels to various phases of the life cycle. Further study of the significance of the apparent postspawning reduction in ACTH levels (Table 1) and the possible role of ACTH in metamorphosis (Sterba, 1955) must await the availability of more specimens. A recent review (Youson, 1980) indicates that there is little information on changes in the levels of any circulating or stored hormones during lamprey metamorphosis.

The presence of ACTH activity in the pituitary and brain of *L. aepyptera* is significant for several reasons. This is the first documentation for the existence of this hormone in any cyclostome, the oldest extant vertebrate group with an evolutionary history of at least 280 million years (Hardisty, 1979). Based on the fact that it stimulates steroidogenesis in rat adrenals, lamprey ACTH is probably structurally similar to mammalian ACTH. Among nonmammalian vertebrates, the amino acid sequence of ACTH is known for only the spiny dogfish shark, *Squalus acanthias*. The remarkable structural similarity between mammalian and chondrichthyan ACTH suggests that ACTH is a phylogenetically conservative molecule (Baker, 1979).

*Lampetra aepyptera* is unique among North American lampreys. Unlike other nonparasitic species, *L. aepyptera* has no ancestral parasitic counterpart within its area of distribution (Hardisty, 1979) and therefore cannot be assigned to a sister species. Consequently, the taxonomic status and proper phylogenetic placement of this species are uncertain (Potter, 1980). *Lampetra aepyptera* is probably an ancient species and possibly the most degenerate (and specialized) lamprey (Bailey, 1980).

As a nonparasitic species, *L. aepyptera* exhibits evolutionary reduction in several morphological features (dentition, alimentary canal, body size) as well as in aspects of the life cycle (no parasitic feeding, no growth after metamorphosis). Furthermore, as the entire life cycle of this species is confined to several hundred meters of a creek, it is not subjected to the stresses associated with extensive migrations for feeding and reproductive purposes. In ad-
<table>
<thead>
<tr>
<th>Sampling date</th>
<th>No. of specimens</th>
<th>Total length (mm)</th>
<th>Estimated age (yr)</th>
<th>ACTH content*</th>
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<td>Pituitary</td>
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<td>pg/gl</td>
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<td>Ammocoetes</td>
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<td>Jun 7</td>
<td>1</td>
<td>57</td>
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<td>Apr 26–Jun 15</td>
<td>3</td>
<td>70–95</td>
<td>2–4</td>
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<tr>
<td>May 24</td>
<td>2</td>
<td>127–170</td>
<td>5</td>
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<tr>
<td>Apr 2c</td>
<td>4</td>
<td>144–180</td>
<td>5–6</td>
<td>10.0</td>
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<td>Apr 12e</td>
<td>7</td>
<td>125–160</td>
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</table>

* Single estimates or, if multiple, ± SE; pituitary and brain totals for adults (right column) are the summed results of individual bioassays of pituitaries and brains; pg/gl/g, picograms/gland and/or brain/gram of body weight.

Based on data in Rohde et al. (1976).

Ripe; spawning.

Spent; postspawning.

Near death.
dition, it is not confronted with the metabolic demands of differential osmoregulation as are the anadromous lampreys. In spite of the elimination of these stressful aspects of the life cycle, ACTH activity is still evident in _I. aepyptera_. This finding suggests that ACTH may also be present in other less specialized lampreys that have retained all components of the life cycle.

ACTH is synthesized and widely distributed in the mammalian brain, where it may function as a neurotransmitter or neuromodulator capable of eliciting behavioral responses (Krieger and Liotta, 1979). Recently, Boer et al. (1979) found ACTH-like immunoreactivity in giant neurons of a snail, a possible indication that this molecule has a long evolutionary history as a biologically active peptide. Little is known about the distribution of ACTH in the central nervous system of nonmammalian vertebrates. However, the presence of this compound in the central nervous system of lampreys, a phylogenetically primitive vertebrate group, is consistent with the hypothesis that ACTH first appeared in peptidergic neurons of the central nervous system where it functioned as a neurotransmitter rather than a hormone.

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**REFERENCES**


