Collection of Lamprey Brains and Pituitaries for Purification of Hormones

by:

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FINAL REPORT
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PROBLEM STATEMENT AND OBJECTIVES: Gonadotropin(s) (GTH) have not been isolated from lamprey pituitary glands, so that measurement of plasma GTH has not been possible. Thus, we have been unable to directly assess the response of the GnRH analogs that we have been testing for sterilizing lampreys. Previous studies have indicated that a lamprey GnRH analog (antagonist) may be useful as a method to sterilize male sea lampreys for use in a sterile-male-release program in the Great Lakes. The biochemical investigation of lamprey GTH has been very difficult over the past few years, we have attempted a wide variety of biochemical and molecular methods in identifying the GTH. We have now isolated and purified all lamprey pituitary hormones except GTH. Thus, the objective of this proposal was to have a major collection of pituitaries and other tissues from lampreys at Hammond Bay Biological Station. These pituitaries are now being used for the molecular and biochemical studies for the identification of GTH.

RATIONALE AND RELEVANCE TO COMMISSION OBJECTIVES: One of the goals of the Great Lakes Fishery Commission is to suppress sea lamprey populations to target levels by several methods including the development and use of alternate control techniques to reduce reliance on lampricides to 50% of current levels (1990). For the past few years, my laboratory has been testing GnRH analogs for sterilizing sea lampreys and has provided evidence that a lamprey GnRH analog may present a potential viable alternative to bisazir for use in the sterile male program. The potential for using GnRH analogs is exciting because these compounds are proteins which are easily degraded within the organism, non-toxic to humans and other organisms, easy to administer, low in cost, and relatively easy to synthesize. However, before further work can progress on this research, it is essential and critical that lamprey GTH be identified in order that these analogs can be designed and tested.

PROCEDURES: We decapitated 6,012 lampreys in June, 2000 at Hammond Bay Biological Station. We dissected the brains and pituitaries by use of microscopes and immediately placed the tissues in containers on dry ice. These samples were shipped back to NH and Japan for isolation of GTH.
SUMMARY

Gonadotropins, in response to GnRH, are released from the pituitary gland and are the major hormones regulating steroidogenesis and gametogenesis. Gonadotropins have not been identified from either the lampreys or hagfish. Prior to the late 1980's, it was considered by many researchers that fish only had one gonadotropin, although it had been suggested that there were two. Two gonadotropins, GTH-I and GTH-II, were first identified in chum salmon by Suzuki et al., (Suzuki, Kawauchi & Nagahama, 1988). Subsequently, the duality of the gonadotropins has been shown in a number of other teleost fish; coho salmon (Swanson, Suzuki, Kawauchi & Dickhoff, 1991); carp (Van der Kraak, Suzuki, Peter, Itoh & Kawauchi, 1992); bonito (Kawauchi, Itoh & Koide, 1991); and killifish (Lin, Rupnow, Price, Greenberg & Wallace, 1992). It is now generally accepted that teleosts have two gonadotropins, GTH-I (which is FSH-like) and GTH-II (which is LH-like). It is suggested that GTH-I is involved in regulating gonadal steroidogenesis in the regulation of puberty and early gonadal development and GTH-II is involved in regulating the final stages of reproductive maturation and spawning in salmon ([Swanson, 1991 #56]).

Evidence from physiological and immunocytochemical studies strongly support the presence of a gonadotropin-like molecule in lampreys indicating that a reasonably typical pituitary-gonadal relationship exists in this group (Hardisty & Baker, 1982; Larsen & Rothwell, 1972; Sower, 1990; Sower, 1998). In river lampreys (Lampetra fluviatilis), hypophysectomy and substitution therapy with pituitary extracts or mammalian GTHs indicated pituitary regulation of the gonads (Larsen, 1980). Moreover, injection of salmon gonadotropin preparation into adult spawning sea lamprey advanced ovulation by several weeks and elevated plasma estradiol levels (Sower, Dickhoff, Gorbman, Rivier & Vale, 1983). In addition, previous studies demonstrated that there are two distinct high affinity binding sites in the pituitary for lamprey GnRH-I and GnRH-III and that these hormones differentially regulate lamprey pituitary function(Knox, Boyd & Sower, 1994; Materne, Gazourian, Chase, Pati, Habibi & Sower, 1997; Sower, 1997; Sower, 1998). A mammalian-like immunoreactive luteinizing hormone was shown to be present by immunocytochemistry in the pituitary of the sea lamprey (Wright, 1983).

Based on this evidence of a GTH-like molecule in lampreys, we have been working on the identification of GTH as well as the other pituitary hormones. More recently, using immunocytochemistry, Nozaki et al. (Nozaki, Ominato, Takahashi, Kawauchi & Sower, 1999) detected immunoreactive (ir) GTH in the sea lamprey pituitary using two different cytochemical approaches. Based on these recent results, we have been performing molecular and protein isolation procedures for lamprey GTH using probes to ovine luteinizing hormone (oLH). We now have a partial sequence of a lamprey GTH-like protein and a cDNA of a putative GTH sequence (A. Takahashi, K. Tanimizu, H. Kawauchi, and S.A. Sower, unpublished). This is shown in the following figure. We will continue our purification and sequencing studies with the pituitaries that we collected in the summer of 2000.
Experimental Report

Lamprey whole pituitary (4.7 g)

Extraction: 1 N Acetic Acid

Filtration: Sephacryl 400 (3.9 x 70 cm) in 0.1 M Acetic Acid

Fractions 1-14: SDS-PAGE w/o JME
Fraction 15 contained protein

Fractions 1 and 6: Western Blotting with antisera L150

HPLC: TSKgel CDX-120T (50 x 25 cm) with a combination of acetonitrile and TFA

Fractions 1-12: SDS-PAGE w/o JME
Western Blotting with antisera L150

Fractions 1 and 6: Western Blotting with antisera L150

Fraction V-6a: Lys-Ag-Ala-Arg with Phe-Ala-Ala-Ala-X-Lys-

PCR

SDS-PAGE (12% gel) for the fraction 6a (below)
Western blotting using antisera L150 for the fraction 6a (above)
LIST OF PEER-REVIEWED PUBLICATIONS IN 2000 SUPPORTED OR PARTIALLY SUPPORTED BY GLFC (the previous and the current grant)


Sower, S.A. and E.L. Evans. Controlled release of D-Ala6, Pro9 Net mammalian GnRH in the sea lamprey (Petromyzon marinus). Aquaculture. Accepted with revisions


Reed, K.L., MacIntyre, J.M., M. Nozaki, A. Gorbman, S.A. Sower and S. A. Tobet. The development of γ-aminobutyric acid neurons and their relationship to gonadotropin-releasing hormone neurons in the larval and adult sea lamprey, Petromyzon marinus. (In Revision)


*Note: these last two papers are manuscripts from talks given at the International Fish Endocrinology Symposium and at the Sea Lamprey International Symposium II, respectively.
D. REFERENCES CITED


