

GREAT LAKES FISHERY COMMISSION

2000 Project Completion Report¹

**Collection of Lamprey Brains and Pituitaries for Purification of
Hormones**

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GREAT LAKES FISHERIES PROJECT FINAL REPORT

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PROJECT TITLE: Collection of Lamprey Brains and Pituitaries for Purification of Hormones

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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.

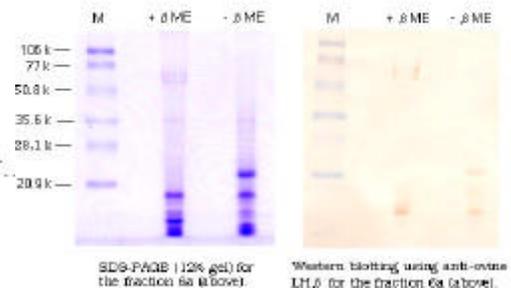
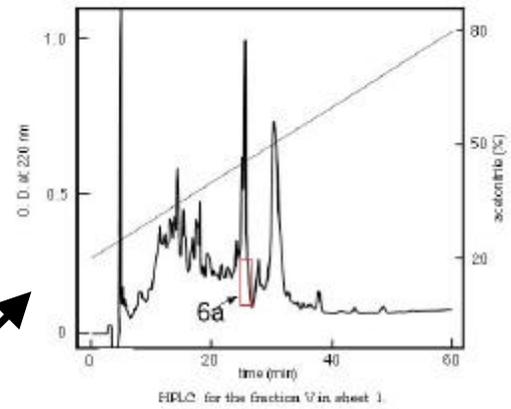
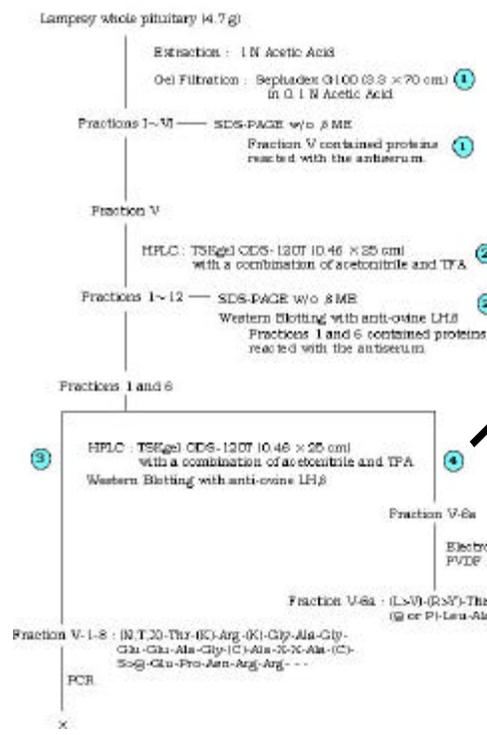
SUMMARY

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 -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
for identification of Lamprey GTH-like protein



LIST OF PEER-REVIEWED PUBLICATIONS IN 2000 SUPPORTED OR PARTIALLY SUPPORTED BY GLFC (the previous and the current grant)

- 2000-** Gazourian, L., E.L. Evans, L. Hanson, C. Chase and S.A. Sower. 2000. The effects of lamprey GnRH-I, -III and analogs on steroidogenesis in the sea lamprey (*Petromyzon marinus*). *Aquaculture*. 188: 147-165.
- Suzuki, K., R.L. Gamble, and S.A. Sower. 2000. Multiple transcripts encoding lamprey gonadotropin-releasing hormone-I precursors. *J. Molec Endocrinol*. 24:365-376.
- Nozaki, M., A. Gorbman and S.A. Sower. 2000. The distribution of lamprey GnRH-III in brains of adult sea lampreys (*Petromyzon marinus*). *Gen. Comp. Endocrinol*. 118: 57-67.
- MacIntyre, J.K, C.Chase, S. Done and S.A. Sower . The interrelationship of PMY and GnRH in the sea lamprey. *Gen. Comp. Endocrinol*. Accepted with revisions
- 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
- Sower, S.A. and E.L. Evans. Steroid feedback in the male sea lamprey, *Petromyzon marinus*. *Comp. Biochem Physiol*. Accepted with revisions.
- Sower, S.A., A. J. McGregor, O. Materne, C. Chase, I. Potter, and J. Joss. 2000. Evidence for lamprey GnRH-I and -III like molecules in the brains of the Southern Hemisphere lampreys. *Geotria australis and Mordacia mordax*. *Gen. Comp. Endocrinol*. In Press.
- 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)
- Bird, D.J., I.C. Potter, S.A. Sower and B.I. Baker. The distribution of melanin-concentrating hormone (MCH) in the lamprey brain. *Comp. Biochem Physiol*. Submitted.
- Takahashi, A., Y. Amemiya, M. Nozaki, S.A. Sower and H. Kawauchi. Evolutionary significance of proopiomelanocortin in agnathan and chondrichthyes. *Comp. Biochem. Physiol*. Submitted.
- Nozaki, M., K. Ominato, A. Takahashi, H. Kawauchi, and S.A. Sower. Adenohypophysial cell types in the lamprey pituitary. *Comp. Biochem. Physiol*. Submitted.
- *Sower, S.A. and H. Kawauchi. UPDATE: Brain and Pituitary Hormones of Lampreys. *Comp. Biochem. Physiol*. Submitted.
- *Sower, S.A. The Physiology of Reproduction in Lampreys and Applications for Male Lamprey Sterilization. *Can. J. Fish. Aquat. Sci*. Submitted.

*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

- Hardisty MW, and Baker BI. 1982.** Endocrinology of lampreys. In: Hardisty MW and Potter IC, eds. *The Biology of Lampreys*. London: Academic Press. Chapt. 33.
- Kawauchi H, Itoh H, and Koide Y. 1991.** Additional evidence for duality of fish gonadotropins. In Scott and al e, eds. Fourth International Symposium of the Reproductive Physiology of Fish. Norwich, U. K. A. P., 19-21.
- Knox CJ, Boyd SK, and Sower SA. 1994.** Characterization and localization of gonadotropin-releasing hormone receptors in the adult female sea lamprey, *Petromyzon marinus*. *Endocrinology* **134**: 492-8.
- Larsen LO. 1980.** Physiology of adult lampreys, with special regard to natural starvation, reproduction, and death after spawning. *Can. J. Fish. Aquat. Sci.* **37**: 1762-1779.
- Larsen LO, and Rothwell B. 1972.** Adenohypophysis. In: Hardisty MW and Potter IC, eds. *The Biology of Lampreys*. New York/London: Academic Press. 1-67.
- Lin YW, Rupnow BA, Price DA, Greenberg RM, and Wallace RA. 1992.** Fundulus heteroclitus gonadotropins. 3. Cloning and sequencing of gonadotropic hormone (GTH) I and II beta-subunits using the polymerase chain reaction. *Mol. Cell. Endocrinol.* **85**: 127-39.
- Materne OL, Gazourian L, Chase C, Pati D, Habibi HR, and Sower SA. 1997.** Characterization of the gonadotropin-releasing hormone binding sites in the pituitary and gonads of the sexually maturing adult lamprey. *XIII Int'l Congress of Comp. Endocrinol.* **1**: 743-746.
- Nozaki M, Ominato K, Takahashi A, Kawauchi H, and Sower SA. 1999.** Possible gonadotropin cells in the lamprey pituitary: colocalization of mammalian LH-like immunoreactivity and glycoconjugate in adult sea lampreys (*Petromyzon marinus*). *Gen. Comp. Endocrinol.* **113**: 23-31.
- Sower SA. 1990.** Gonadotropin-releasing hormone in primitive fishes. *Prog Clin Biol Res* **342**: 73-8.
- Sower SA. 1997.** Evolution of GnRH in fish of ancient origins. In: Parhar IS and Sakuma Y, eds. *GnRH Neurons: Gene to Behavior*. Tokyo: Brain Shuppan Publishers. 486.
- Sower SA. 1998.** Brain and pituitary hormones of lampreys, recent findings and their evolutionary significance. *Amer. Zool.* **38**: 15-38.
- Sower SA, Dickhoff WW, Gorbman A, Rivier JE, and Vale WW. 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.
- Suzuki K, Kawauchi H, and Nagahama Y. 1988.** Isolation and characterization of two distinct gonadotropins from chum salmon pituitary glands. *Gen. Comp. Endocrinol.* **71**: 292-301.
- Swanson P, Suzuki K, Kawauchi H, and Dickhoff WW. 1991.** Isolation and characterization of two coho salmon gonadotropins, GTH I and GTH II. *Biol. Reprod.* **44**: 29-38.
- Van der Kraak G, Suzuki K, Peter RE, Itoh H, and Kawauchi H. 1992.** Properties of common carp gonadotropin I and gonadotropin II. *Gen. Comp. Endocrinol.* **85**: 217-29.
- Wright GM. 1983.** Immunocytochemical study of luteinizing hormone in the pituitary of the sea lamprey, *Petromyzon marinus* L., during its upstream migration. *Cell Tissue Res* **230**: 225-8.