

In Proceedings of 4th International Symposium in Reproduction Physiology of Fish, ed. A.P. Scott, J.P. Sumpter, D.E. Kime, and M.S. Rolfe. FishSymp 91, Sheffield, England. pp. 11-12.

DEVELOPMENT OF RADIOIMMUNOASSAYS FOR TWO STURGEON GONADOTROPINS

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Summary

Radioimmunoassays for the measurement of two sturgeon gonadotropins, stGTH 1 and stGTH 2, in the pituitary and plasma of sturgeon were developed using rabbit antiserum to purified sturgeon pituitary fractions that exhibited distinctly different gonadotropin activity both *in vitro* and *in vivo*. In females, plasma concentrations of stGTH 1 were elevated during the prematurational stages, dropping prior to spawning. In contrast, stGTH 2 plasma concentrations rose at ovulation. These data suggest that like salmonids, sturgeon have two gonadotropins controlling reproductive development.

Introduction

The white sturgeon (*Acipenser transmontanus*) and other Chondrosteian species are becoming important species in aquaculture. Unfortunately, little is known about the endocrine control of reproduction in these species. To study the neuroendocrine control of reproduction of the white sturgeon, two gonadotropins (GTH) have been isolated from sturgeon pituitary extracts and radioimmunoassays (RIAs) have been developed to measure these GTHs.

Methods and Results

Pituitary glands were collected from prespawning sturgeon from the delta of the Volga River near the Caspian Sea (Farmer et al., 1981). Most likely, the pituitaries were collected from two species, *Acipenser güldenstädti* and *Acipenser stellatus*. Glycoprotein fractions potentially containing GTHs were extracted from these glands by methods employed and described previously for other species (Farmer and Papkoff, 1977, Papkoff et al., 1982). Two purified preparations designated GTH 14 and GTH 20 were identified as potentially distinct gonadotropins by virtue of their fractionation behavior during extraction. These two preparations were tested for their biological activity and RIAs were developed to measure the occurrence of the two GTHs during reproductive development.

To determine gonadotropic activity, both GTHs were assessed by an *in vitro* germinal vesicle breakdown (GVBD) bioassay. Eggs collected by catheterization from wild white sturgeon in the prespawning stage were incubated in Leibovitz culture media with progesterone, GTH 14, GTH 20 or without treatment (control). The germinal vesicle breakdown (GVBD) response was 0% for controls, 100% for progesterone, 38% for GTH 14 and 73% for GTH 20. These data suggest that both GTHs can induce GVBD, but GTH 20 appears to have the greatest effect.

To develop the RIAs, relatively specific polyclonal antibodies to the two GTHs were raised in rabbits. Each antibody showed a minimum cross reactivity with the other sturgeon GTH preparation, a sturgeon growth hormone-prolactin preparation and the GTHs of other species (Table 1). The antibodies were used in a double antibody RIA patterned after the one developed by Niswender et al. (1969). Both antigens were iodinated using the 1,3,4,5-tetrachloro-3 α , 6 α -diphenylglycoluul (IODO-GEN) method described by Moberg et al. (1981). Standards were from the same highly purified preparations used for the assay tracers. The minimum detectable concentration for the GTH 14 assay is 0.84 ng/ml with an inter- and intra-assay variability of 10.2 and 7.3 % respectively. The minimum detectable concentration for the GTH 20 assay is 1.25 ng/ml with an inter- and intra-assay variability of 9.7 and 6.1%. Both RIAs exhibited parallel dilution responses for serum and for pituitary extracts of both male and female sturgeon.

Table 1. Cross reactivity of related pituitary hormones with GTH 14 and GTH 20 assays.

Hormones	Cross Reactivity (%)	
	GTH 14 Assay	GTH 20 Assay
GTH 14	--	2.0
GTH 20	9.3	--
Hake GTH	< 0.0095	< 0.0095
Salmon GTH	< 0.0095	< 0.0095
Tilapia GTH	< 0.0095	< 0.0095
Carp GTH	< 0.0095	0.7
Gillichthes GTH	< 0.0095	< 0.0095
Ovine GTH	< 0.0095	< 0.0095
Sturgeon Growth Hormone-Prolactin	0.5	0.6

Supported by NOAA Sea Grant NA 85 AA-D-SG140 (R/A-73)

(Moberg; R/A-73)

Plasma was collected over a two year period from three cultured females that reached maturity. During vitellogenesis, plasma GTH 14 was elevated, but dropped prior to spawning. In three of the fish that we bled when spawning was induced with carp pituitary extract, there was a significant increase in GTH 20 with only a slight increase in GTH 14. Since carp pituitary extracts do not significantly cross react with our two antibodies (see Table 1), we believed that the carp pituitary extracts induced ovarian steroid synthesis and these steroids in turn induced GTH secretion (Jalabert et al., 1977). These preliminary data suggest that GTH 14 may control early development while GTH 20 is responsible for final maturation.

The gonadotropin releasing hormone (GnRH) analog des-GLY¹⁰-[D-Ala⁶]-LHRH ethylamide (GnRH_a) is similar in structure to sturgeon GnRH (Sherwood and Lovejoy, 1989) and will induce spawning in sturgeon (Doroshov and Lutes, 1984). To determine if this GnRH analog would induce secretion of the two GTHs, 10 µ/kg of GnRH_a was injected IP into mature male sturgeon. Blood samples were taken 6, 24 and 48 hours post injection and assayed for GTH content. At all three samplings following GnRH_a injection, both GTH 14 and GTH 20 were elevated with the peak response occurring at 24 hours after the GnRH_a injection.

Discussion

The two GTHs that we have isolated from sturgeon pituitaries appear to be functional analogs of GTH I and GTH II in salmonids (Kawauchi et al., 1989; Swanson et al., 1989), controlling reproductive development in the sturgeon. We believe that GTH 14, like GTH I in salmonids, is primarily responsible for ovarian development while GTH 20, like salmonid GTH II, induces final maturation. In order to maintain consistency in the fish endocrine literature, we suggest that GTH 14 be named stGTH I and GTH 20 be referred to as stGTH 2.

The RIAs that we have developed to measure these two GTHs, have the sensitivity and specificity to permit further study of the role of gonadotropins in the regulation of reproduction in sturgeon.

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