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ON THE PITUITARY GLAND OF THE SAFFRON COD, *ELEGINUS GRACILIS*, AND THEIR COLD ADAPTATION

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Abstract: The seasonal variation of the pituitary gland of the saffron cod, *Eleginus gracilis*, was investigated by light microscopy and immunohistochemistry.

Growth hormone(GH)-secreting cells were found in the proximal pars distalis. Prolactin(PRL)-secreting cells were located in most part of the rostral pars distalis. In the fish collected during the winter, the immunoreactions with both GH and PRL were very weak. On the contrary, in the fish collected during the summer, the positive immunoreactions with both GH and PRL were remarkable. However, there were no seasonal differences in the sizes of nuclei of GH- and PRL-secreting cells between the winter and the summer fish.

GH action for annual antifreeze glycoprotein cycle and PRL role for the kidney function are discussed. The results may suggest the possibility of PRL clearing of antifreeze glycoprotein of the serum by increasing glomerular filtration during the summer.

1. Introduction

Many species of Antarctic teleosts have been reported to have aglomerular kidneys (DOBBS *et al.*, 1974; DOBBS and DEVRIES, 1975a). It has also been reported that most Antarctic fishes have freezing-point-depressing glycoproteins (antifreeze glycoproteins) in their blood sera (DEVRIES *et al.*, 1970). Aglomerularism in these fishes may be related to the conservation of serum glycoproteins which have "antifreeze" properties (DOBBS *et al.*, 1974; DOBBS and DEVRIES, 1975b).

Antifreeze glycoproteins have been recently reported not only in Antarctic fishes but also in high-latitude cold-water fishes; further it has been noted that blood levels of these substances in the cold-water fishes show seasonal changes (DUMAN and DEVRIES, 1974a, b; FLETCHER, 1977; HEW *et al.*, 1981).

Winter flounder, *Pseudopleuronectes americanus*, survives the ice-laden coastal water of Newfoundland during the winter by synthesizing and accumulating antifreeze glycoproteins in the serum (DUMAN and DEVRIES, 1976; HEW and YIP, 1976; FLETCHER *et al.*, 1985). These substances appear in flounder serum during November, reach peak values by January and disappear during May (FLETCHER, 1977). Evidence suggests that this annual cycle is primarily endogenous, but can be affected by photoperiod

and temperature (FLETCHER and SMITH, 1980; FLETCHER, 1981). Pituitary has been shown to be necessary for control of the antifreeze glycoprotein cycle (FLETCHER *et al.*, 1978; HEW and FLETCHER, 1979; FOURNEY *et al.*, 1984). Recently the growth hormone (GH) has been shown as a major pituitary factor regulating the annual antifreeze glycoprotein cycle in the winter flounder, whereas prolactin (PRL) has no effect for them (IDLER *et al.*, 1989). Further investigations of the endocrine control on the antifreeze glycoprotein level and of the cold adaptation of these fishes are interesting.

Saffron cod, *Eleginus gracilis*, also survives the ice-laden coastal water of Hokkaido during the winter by possessing higher concentration of antifreeze glycoprotein in the serum than during the summer (BURCHMAN *et al.*, 1984).

In the present study, therefore, an attempt has been made to observe the pituitary of the saffron cod, especially GH- and PRL-secreting cells, by light-microscopy and immunohistochemistry and to compare them between winter and summer.

2. Materials and Methods

Saffron cod, *Eleginus gracilis*, were collected in February and June 1991 from Notsuke Bay located on the Okhotsk Sea side of Hokkaido, Japan. It is known that the saffron cod migrate into shallow beaches during intense cold winter (January–March) and spawn under the platelet ice. During the summer, they migrate to some deep place. The numbers and mean standard length of saffron cod used in this study, and the water temperature and salinity at collecting points are shown in Table 1. No attempt was made to separate the sexes.

The brain including the pituitary gland was fixed in acetic acid-free Bouin's or Bouin-Holland solution, embedded in paraffin and sectioned at 6 μm using routine procedures. Sections were stained with hematoxylin and eosin. Immunohistochemical staining was carried out according to the avidin-biotin-peroxidase complex (ABC)

Table 1. Number and standard length (SL) of saffron cod used in this study, and the water temperature and salinity at collecting points.

	Winter (February 26, 1991)			Summer (June 27, 1991)		
Number of fish	6			8		
Mean SL (mm)	309			216		
(Range) (mm)	(290–330)			(170–280)		
	Depth (m)	Temperature (°C)	Salinity (‰Cl)	Depth (m)	Temperature (°C)	Salinity (‰Cl)
	0.0*	–1.62	24.60	0.0	11.55	32.00
	0.1	–1.10	30.26	1	11.53	32.07
	0.4	–1.10	30.27	2	11.52	32.06
	0.6	–1.07	30.27	3	11.51	32.06
	0.8	–1.06	30.30	4	11.37	31.92
	1.0	–1.04	30.42	5	10.21	32.02
	1.2	–1.03	30.31	6	9.86	32.24
	1.4	–1.01	30.34	7	9.82	32.26
				8	9.79	32.27

*Just under the platelet ice.

method. Alternate sagittal sections of the pituitary were stained with either anti-chum salmon GH antiserum (KAWAUCHI *et al.*, 1986) or anti-chum salmon PRL antiserum (NAITO *et al.*, 1983).

The measurements of the cross-sectional area of GH and PRL cell nuclei were made on 20 cells from each fish. The observable nuclei were selected for outlining their boundaries from the photograph, and then their cross-sectional area were determined. The statistical analyses of data from the two groups (the winter fish and summer fish), were performed by Student T test.

3. Results and Discussion

Pituitary of the saffron cod is nearly ovoid in shape and partly embedded in the basal part of the hypothalamus. In the rear of pituitary, a well-developed vascular sac is located.

GH- and PRL-secreting cells in the pituitary were identified by immunohistochemical staining with antisera raised against the chum salmon hormones. GH cells are found in the proximal pars distalis (Fig. 1). These cells are irregular in shape with an irregular nucleus situated frequently in the center of the cell. PRL cells are located in most part of the rostral pars distalis (Fig. 2). These cells are irregular in outline with a round nucleus situated in the center of the cell.

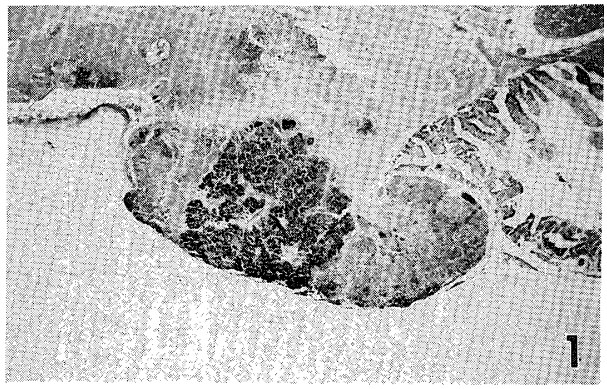
In the fish collected during winter (winter fish), the immunoreactions with both GH and PRL are very weak (Figs. 3W and 4W). In the winter fish, there are few immunopositive cells for GH antiserum, scattered in the proximal pars distalis (Fig. 3W). On the contrary, in the fish collected during summer (summer fish), the positive immunoreactions with GH and PRL are both remarkable (Figs. 3S and 4S). However, there are no seasonal differences on the cross-sectional area of GH- and PRL-secreting cell nuclei between the winter fish and the summer fish (Table 2).

In the winter flounder, hypophysectomy results in the biosynthesis of antifreeze glycoprotein and maintenance of high plasma levels of it throughout the year (FLETCHER *et al.*, 1978; HEW and FLETCHER, 1979; FOURNEY *et al.*, 1984). Pituitary transplants or injections of winter flounder pituitary homogenates inhibit the antifreeze glycoprotein biosynthesis (FLETCHER, 1979; FLETCHER *et al.*, 1984). Recently the result indicated that GH blocks the antifreeze glycoprotein mRNA transcription in the liver (FLETCHER

Table 2. Seasonal differences in the cross-sectional areas (μm^2) of the GH- and PRL-secreting cell nuclei in the pituitary of saffron cod, Eleginus gracilis.

	No. of Fish (SL, mm)	No. of Cells	Area of Nucleus (μm^2)
GH-Secreting Cells			
Winter Fish	4 (295 – 330)	80	11.76 ± 0.29
Summer Fish	7 (190 – 280)	140	10.25 ± 0.15
PRL-Secreting Cells			
Winter Fish	4 (295 – 330)	80	9.30 ± 0.20
Summer Fish	7 (190 – 280)	140	9.15 ± 0.14

Values are means and standard error.
SL: Standard length.



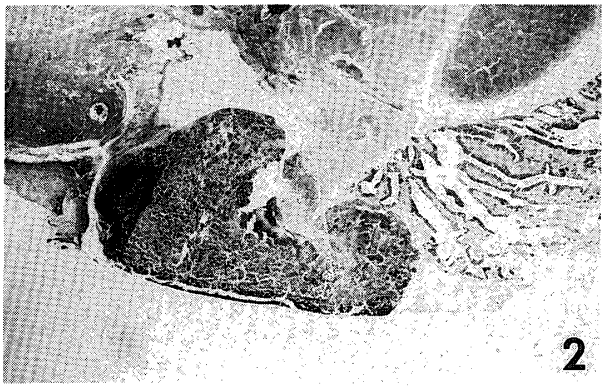


Fig. 1. Growth hormone-secreting cells located in the proximal pars distalis of the pituitary in saffron cod (summer fish). × 25.

Fig. 2. Prolactin-secreting cells located in the rostral pars distalis of the pituitary in saffron cod (summer fish). × 25.

Fig. 3W and 3S. Immunoreaction with salmon growth hormone of the winter fish (W) was weaker than that of the summer fish (S). × 260.

Fig. 4W and 4S. Immunoreaction with salmon prolactin of the winter fish (W) was weaker than that of the summer fish (S). × 260.

et al., 1989). More recently it has been shown that GH is a major pituitary factor regulating the annual serum antifreeze glycoprotein cycle in the winter flounder and PRL has no effect for them (IDLER *et al.*, 1989).

However, PRL is the most versatile of all hormones in its effect. In addition to the many other biological systems, PRL has direct renal effects in teleosts. PRL in stickleback may increase the ion reabsorption and/or the glomerular filtration rate and excrete highly hypo-osmotic urine (LAM and HOAR, 1967). In kidney of stickleback, the increase in size of glomerular tufts was more marked in spring fish than in winter fish after transfer into freshwater (OGAWA, 1968). Such seasonal difference in size of glomerular tufts would be closely associated with the seasonal variation in PRL secretion from the pituitary. In the stickleback the administration of PRL increased both urine flow and glomerular filtration rate (LAM and LEATHERLAND, 1969). These results appeared to be a specific glomerular effect as the glomerular capillary tuft was enlarged after treatment with PRL.

Even if GH is a major pituitary hormone regulating the synthesis of antifreeze gly-

coprotein in the liver, it is needed to take account of the effect of PRL on the secretion of them from the kidney.

Molecular weights of antifreeze glycoproteins range from 2600 to 33700 (DEVRIES, 1982). Molecules of this size are readily filtered through glomerular membranes. In our previous report on the saffron cod kidney (KITAGAWA *et al.*, 1990), the observations showed shrunk glomerular tufts during the winter in which the glomeruli would be non-functional and similar to aglomerular kidneys. This may be related to the conservation of antifreeze glycoproteins for low water temperature.

In the present study, there were no changes in the sizes of nuclei of both GH- and PRL-secreting cells between winter and summer respectively. However, the immunoreactivities of these cells during summer were remarkable. Further studies are certainly required to examine not only the nucleus size but also the cell size for these cells.

The results of this study suggest the possibility of PRL clearing the antifreeze glycoprotein by the increasing glomerular filtration during the summer.

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