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Influence of Seawater Adaptation on Prolactin and Growth Hormone Release from Organ-Cultured Pituitary of Rainbow Trout

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ABSTRACT—When immature rainbow trout (*Oncorhynchus mykiss*) were accliomated to 80% seawater for 2 weeks, the plasma osmolality and sodium level were slighlty but significantly higher than the levels in freshwater fish. Plasma prolactin (PRL) level of seawater-adapted fish was significantly lower than that of freshwater fish, whereas plasma growth hormone (GH) was significantly higher in seawater fish. Pituitary PRL and GH contents were significantly lower in seawater fish than in freshwater fish. When pituitaries were cultured in serum-free medium for 48 hr, an acute decrease in PRL release was seen within 12 hr in culture. Thereafter, a basal level of PRL release (0.2–0.5 ng/pituitary·hr) was maintained, and the level was significantly lower in seawater fish than freshwater fish. On the other hand, GH release from the pituitary of both seawater and freshwater fish (200–500 ng/pituitary·hr) was about 100 times greater than the basal PRL release throughtout the experiment, and there was no difference between the fish in seawater and those in fresh water. GH release from the trout pituitary seems to be predominantly under inhibitory control of the hypothalamus, thus resulting in an increased GH release under culture condition, and possibly masking the effect of seawater adaptation. The decreased PRL release in seawater-adapted fish may be related to the reduction in pituitary PRL content.

INTRODUCTION

Osmoregulatory roles of prolactin (PRL) as a freshwater-adapting hormone is well established in many euryhaline teleosts [1–3]. In salmonids, a reduction in plasma level of PRL has been frequently observed after transfer from fresh water to seawater [4–8]. The decrease in plasma PRL in response to seawater is most likely to be releated to its sodium retaining action, which is inhibitory to maintenance of ion balance in seawater. On the other hand, recent studies suggest that growth hormone (GH) plays an important role in seawater adaptation, especially in salmonids, and the seawater-adapting effect seems to be independent of growth promotion [9, 10]. An increase in plasma GH level has been observed during seawater acclimation in several salmonid species [6, 8, 11–13]. An increased metabolic clearance rate of GH has also been seen when rainbow trout and coho salmon are transferred from fresh water to seawater [12, 13]. Transfer of the fish to environment of different salinity generally causes chages in plasma osmolality. Reduction in osmotic pressure of culture medium is known to affect *in vitro* release of PRL in several teleost species [14, 15]. In salmonids, however, changes in osmotic pressure of medium within physiological range did not affect PRL and GH release *in vitro* [16, 17]. According to Kelley *et al.* [18], the basal release of newly synthesized PRL and PRL synthesis in organ-cultured pituitaries of coho salmon smolts were greater in freshwater fish than in the fish acclimated to seawater for 4 weeks; the PRL cells *in vitro* appear to retain at least some of their *in situ* characteristics. The present study was undertaken to examine the influrence of environmental salinity on spontaneous release of PRL and GH from organ-cultured pituitary of rainbow trout.

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MATERIALS AND METHODS

Immature rainbow trout (*Oncorhynchus mykiss*), weighing about 100 g, were obtained from a commercial source in Tokyo. They were reared in recirculating freshwater tank at 12°C for more than a week until use. Twelve fish were transferreed directly to a recirculating tank of 80% seawater (salinity 30 ppt) at 12°C. As a control, the same number of the fish were transferred to a freshwater tank. They were fed commercially prepared pellets (Oriental, Chiba) during the experiment. Two weeks after the transfer, they were anesthetized in 2-phenoxyethanol (0.1 ml/liter), and blood was collected from the caudal vessels with a syringe needle treated with ammonium heparin. After centrifugation at 10,000 rpm for 5 min, the plasma was separated and kept frozen at −80°C until analyses. The pituitaries taken out upon decapitation were cultured in a 96-well multiple plate containing 200 μl Eagle's minimum essential medium with Earle's salts (Gibco, New York), penicillin (100 U/ml), streptomycin (100 U/ml), and Fungizone (0.25 μg/ml, M. A. Bioproducts, Maryland). The pH of medium was adjusted to 7.3 by sodium bicarbonate, and the osmotic pressure was 300 mOsm. Pituitaries were incubated at 12°C under an atmosphere of 95% O_2 and 5% CO_2 for 48 hr. The medium was changed every 12 hr. Each pituitary was sonicated in 1 ml 0.1% Triton X-100 in 10 mM phosphate buffered saline (pH 7.3). The medium and pituitary homogenates were stored at −80°C. As controls, unincubated pituitaries were also collected immediately after decapitation.

Plsma sodium level was measured by an atomic absorption spectrophotometry (Hitachi, Tokyo). Osmotic pressure was measured by a vapor pressure osmometer (Wescor, Utah). The medium and pituitary concentrations of PRL and GH were measured by homologous radioimmunoassays [19, 20].

RESULTS

When rainbow trout was acclimated to 80% seawater for 2 weeks, a significant increase was seen in both plasma osmolality and sodium level (Table 1). Plasma PRL level was significantly lower in seawater fish than in freshwater fish. In

TABLE 1. Plasma osmolality and concentrations of sodium, PRL and GH of rainbow trout acclimated to fresh water or 80% seawater

Environment	No. of fish	Osmolality (mOsm/kg)	Na (mEq/liter)	PRL (ng/ml)	GH (ng/ml)
fresh water	12	297±3	152±1	0.8±0.2	5.8±2.9
80% seawater	12	334±4**	169±2**	0.4±0.1*	14.8±2.0*

Data are expressed as mean±SEM.
*,** Signifiantly different from the level in freshwater fish at $P<0.05$ and $P<0.001$, respectively, by Student's *t*-test.

TABLE 2. Pituitary contents of PRL and GH of rainbow trout acclimated to fresh water or 80% seawater

	Environment	No. of fish	PRL (μg/pituitary)	GH (μg/pituitary)
unincubated pituitery	fresh water	6	2.67±0.30	19.9±1.5
	80% seawater	6	1.71±0.15*	15.9±0.8*
pituitary incubated for 48 hr	fresh water	6	2.73±0.33	14.0±1.6*
	80% seawater	6	2.07±0.24	13.1±1.1*

Data are expressed as mean±SEM.
* Significantly different from the content in unincubated pituitary of freshwater fish at $P<0.05$ by Student's *t*-test.

contrast, plasma GH level of seawater fish was significantly higher than that of freshwater fish.

PRL and GH contents in the unincubated pituitary were significantly smaller in the fish acclimated to 80% seawater than those in freahwater fish. However, there was no significant difference in the residual pituitary contents of both PRL and GH between the fish in fresh water and 80% seawater (Table 2).

As shown in Fig. 1, PRL release from the organ-cultured pituitary was 3–5 ng/pituitary·hr during the first 12 hr of incubation, and there was no difference between the fish acclimated to fresh water and those to 80% seawater. Thereafter, PRL release decreased markedly to a basal level of less than 0.5 ng/pituitary·hr. The basal release of PRL of freshwater fish (0.4–0.5 ng/pituitary·hr) was significantly greater than that of the fish in 80% seawater (about 0.2 ng/pituitary·hr). In contrast, GH release from the cultured pituitary was about 150 ng/pituitary·hr during the first 12 hr, about 30 times greater than the PRL release during the same period, and higher levels (200–450 ng/pituitary·hr) were maintained throughout the experiment (Fig. 2). There was no significant difference in GH release between the fish in fresh water and those in seawater at any period.

DISCUSSION

In the present study, plasma PRL level of rainbow trout acclimated to 80% seawater was significantly lower than in freshwater fish. This is in agreement with previous observations in several salmonid species [4–8]. The reduction in plasma PRL level seems to reflect decreased PRL relesae *in vivo* during seawater adaptation. PRL release from organ-cultured pituitary of seawater adapted rainbow trout was also significantly lower than that of freshwater fish, indicating that the influence of seawater adaptation on PRL release is still existent in the cultured pituitary at least for 48 hr. In coho salmon smolts, Kelley *et al.* [18] reported that the basal release of newly synthesized PRL, as measured by incubating the pituitary with [^{35}S]-methionine for 20 hr, was lower in seawater fish than in freshwater fish. The acute decline of PRL release within the first 12 hr in culture observed in the present study does not seem to be due to the exhaustion of PRL stored in pituitary, since a considerable amount of PRL remained in the pituitary after 48 hr in culture. Gonnet [21] reported that dominant hypothalamic control of PRL release in rainbow trout is stimulatory, based on their studies with perfused pituitaries. The acute decline in PRL release within the first 12 hr in culture may be caused by disappearance of the stimulatory control by hypothalamus.

In teleosts, a considerable number of studies have focused on direct effect of osmotic pressure on PRL release *in vitro* [14, 15]. A reduction in osmotic pressure of cultrue medium directly stimulated PRL release in several euryhaline teleosts such as a goby, tilapia and eel [22–25]. In rainbow trout, however, the response of PRL release needed a large variation in osmotic pressure [17]. In the present study, pituitaries of seawater-adapted fish were incubated in the same culture medium as the pituitary of freshwater fish with osmolality of 300 mOsm/kg. In spite of a reduction in osmotic pressure from 330 mOsm/kg in plasma to 300 mOsm/kg in culture medium, PRL release from seawater fish pituitary was still lower than in freshwater fish. In salmonids in general, changes in osmotic pressure with physiological ranges seem to have no significant influence on PRL release from organ-cultured pituitary [16–18].

As is discussed above, osmolality of the culture medium is not the cause of the decrease in PRL release in seawater-adapted fish. It is also unlikely that the effect of hypothalamic hormones persists for more than 48 hr on PRL cells in the cultured pituitary, although hypothalamic nerve endings in the trout pituitary were found intact electron microscopically after 8 days of culture [unpublished observation]. On the other hand, PRL content in the unincubated pituitary decreased significantly 2 weeks after transfer from fresh water to seawater. In rainbow trout weighing about 200 g, Prunet *et al.* [4] reported that pituitary PRL content increased within 2 days after transfer to seawater, possibly as a result of an acute decrease in PRL release. Thereafter, PRL content decreased gradually to the initial level 3 weeks after transfer. In coho salmon smolts, pituitary content and synth-

esis of PRL decreased 4 weeks after transfer to seawater [18]. Although there was no significant difference in the residual PRL content between the fish in fresh water and in seawater after 48 hr in culture, the reduction in *in vitro* release of PRL in seawater fish as compared with that in freshwater fish seems to be related to the smaller pituitary content before the culture.

In agreement with previous reports in rainbow trout and other salmonids [6, 8, 11, 13], plasma level of GH increased after adaptation of the trout to 80% seawater. However, *in vitro* release of GH in seawater fish was not different from that of freshwater fish. Nagahama *et al.* [26] suggested electron microscopically that GH cells in coho salmon pituitary were more active in seawater-adapted fish than in the fish in fresh water. Estimated from the metabolic clearance rate of exogenously administrated GH, GH released from the pituitary *in vivo* increased during seawater adaptation of rainbow trout and coho salmon [12, 13]. In salmonids, seawater adaptation seems to stimulate both release and sythesis of GH. Smaller GH content in the unincubated pituitary in seawater fish than in freshwater fish observed in the present study may reflect greater secretion rate than the rate of synthesis. In our recent study, activation of GH release and synthesis was observed in serum-free culture of rainbow trout pituitary, indicating that predominant control of GH secretion in rainbow trout is inhibitory [27]. Thus, the influence of seawater adaptation on GH release, if any, seems to be masked by the increased GH cell activity under the culture condition. Further studies are needed with dispersed or isolated pituitary cells without nerve endings of hypothalamic neurons to clarify the influence of seawater adaptation on PRL as well as GH release from the trout pituitary.

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REFERENCES

1 Nicoll, C. S. (1981) Role of prolactin in water and electrolyte balance in vertebrates. In "Prolactin". Ed. by R. B. Jaffe, Elsevier, New York, pp. 127–166.

2 Bern, H. A. (1983) Functional evolution of prolactin and growth hormone in lower vertebrates. Amer. Zool., **23**: 663–671.

3 Hirano, T. (1986) The spectrum of prolactin action in teleosts. In "Comparative Endocrinology: Developments and Directions". Ed. by C. L. Ralph, A. R. Liss, New York, pp. 53–74.

4 Prunet, P., Boeuf, G. and Houdebine, L. M. (1985) Plasma and pituitary prolactin levels in rainbow trout during adaptation to different salinities. J. Exp. Zool., **235**: 187–196.

5 Prunet, P., Boeuf, G., Bolton, J. P. and Young, G. (1989) Smoltification and seawater adaptation in Atlantic salmon (*Salmon salar*): Plasam prolactin, growth hormone, and thyroid hormones. Gen Comp. Endocrionl., **74**: 355–364.

6 Hasegawa, S., Hirano, T., Ogasawara, T., Iwata, M., Akiyama, T. and Arai, S. (1987) Osmoregulatory ability fo chum salmon, *Oncorhynchus keta,* reared in fresh water for prolonged periods. Fish Physiol. Biochem., **4**: 101–110.

7 Young, G., Björnsson, B. Th., Prunet, P., Lin, R. J. and Bern, H. A. (1989) Smoltification and seawater adaptation in coho salmon (*Oncorhynchus kisutch*): Plasma prolactin, growth hormone, thyroid hormones, and cortisol. Gen. Comp. Endocrionl., **74**: 335–345.

8 Yada, T., Takahashi, K. and Hirano T. (1991) Seasonal changes in seawater adaptability and plasma levels of prolactin and growth hormone in landlocked sockeye salmon (*Oncorhynchus nerka*) and amago salmon (*O. rhodurus*). Gen. Comp. Endocrionol., **82**: 33–44.

9 Bolton, J. P., Collie, N. L., Kawauchi, H. and Hirano, T. (1987) Osmoregulatory actions of growth hormone in rainbow trout (*Salmo gairdneri*). J. Endocrinol., **112**: 63–68.

10 Collie, N. L., Bolton, J. P., Kawauchi, H. and Hirano, T. (1989) Survival of salmonids in seawater and the time-frame of growth hormone action. Fish Physiol. Biochem., **7**: 315–321.

11 Sweeting, R. M., Wagner, G. F. and McKeown, B. A. (1985) Changes in plasma glucose, amino acid nitrogen and growth hormone during smoltification and seawater adaptation in coho salmon, *Oncorhynchus kisutch.* Aquaculture, **45**: 185–197.

12 Sakamoto, T., Ogasawara, T. and Hirano, T. (1990) Growth hormone kinetics during adaptation to a hyperosmotc environment in rainbow trout. J. Comp. Physiol. B, **160**: 1–6.

13 Sakamoto, T., Iwata, M. and Hirano, T. (1991) Kinetic studies of growth hormone and prolactin during adaptation of coho salmon, *Oncorhynchus*

kisutch, to different salinities. Gen. Comp. Endocrinol., **82**: 184–191.

14 Ball, J. N. (1981) Hypothalamic control of the pars distalis in fishes, amphibians, and reptiles. Gen. Comp. Endocrino., **44**: 135–170.

15 Nishioka, R. S., Kelley, K. M. and Bern, H. A. (1988) Control of prolactin and growth hormone secretion in teleost fishes. Zool. Sci., **5**: 267–280.

16 Suzuki, R., Kishida, M., Ogasawara, T., Hasegawa, S. and Hirano, T. (1987) Prolactin and growth hormone secretion during long-term incubations of the pituitary pars distalis of mature chum salmon, *Oncorhynchus keta*. Gen. Comp. Endocrinol., **68**: 76–81.

17 Gonnet, F., Prunet, P., Tonon, M. C., Dubourg, P., Kah, O. and Vaudry, H. (1988) Effect of osmotic pressure on prolactin release in rainbow trout: *in vitro* studies. Gen. Comp. Endocrionol., **69**: 252–261.

18 Kelley, K. M., Nishioka, R. S. and Bern, H. A. (1990) *In vitro* effect of osmotic pressure and cortisol on prolactin cell physiology in the coho salmon (*Oncorhynchus kisutcn*) during the parr-smolt transformation. J. Exp. Zool., **254**: 72–82.

19 Hirano, T., Prunet, P., Kawauchi, H., Takahashi, A., Ogasawara, T., Kubota, J., Nishioka, R. S., Bern, H. A., Takada, K. and Ishii, S. (1985) Development and validation of a salmon prolactin radioimmunoassay. Gen. Comp. Endocrinol., **59**: 266–276.

20 Bolton, J. P., Takahashi, A., Kawauchi, H., Kubota, J. and Hirano, T. (1986) Development and validation of a salmon growth hormone radioimmunoassay. Gen. Comp. Endocrionol., **62**: 230–238.

21 Gonnet, F., Barret, A., Grouselle, D. and Prunet, P. (1989) Hypothalamic control of prolactin release in the rainbow trout, *Salmo gairdneri*: *in vitro* studies. Fish Physiol. Biochem., **7**: 301–308.

22 Nagahama, Y., Nishida, R. S., Bern, H. A. and Gunther, R. L. (1975) Control of prolactin secretion in teleosts, with special reference to *Gillichthys mirabilis* and *Tilapia mossambica*. Gen. Comp. Endocrinol., **25**: 166–188.

23 Grau, E. G., Shimoda, S. K., Ford, C. A., Helms, L. M. H., Cooke, I. M. and Pang, P. K. T. (1986) The role of calcium in prolactin release from the pituitary of a teleost fish *in vitro*. Endocrinology, **119**: 2848–2855.

24 Grau, E. G., Ford, C. A., Helms, L. M. H., Shimoda, S. K. and Cooke, I. M. (1987) Somatostatin and altered medium osmotic pressure elicit rapid changes in prolactin release from the rostral pars distalis of the tilapia, *Oreochromis mossambicus, in vitro*. Gen. Comp. Endocrinol., **65**: 12–18.

25 Suzuki, R., Kaneko, T. and Hirano, T. (1991) Effects of osmotic pressure on prolactin and growth hormone secretion from organ-cultured eel pituitary. J. Comp. Physiol. B, **161**: 147–153.

26 Nagahama, Y., Clarke, W. C. and Hoar, W. S. (1977) Influence of salinity on ultrastructure of the secretory cells of the adenohypophyseal pars distalis in yearling coho salmon (*Oncorhynchus kisutch*). Can. J. Zool., **55**: 183–198.

27 Yada, T., Urano, A. and Hirano, T. (1991) Growth hormone and prolactin gene expression and release in the pituitary of rainbow trout in serum-free culture. Endocrinology, **129**: 1183–1192.