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Kinetic Studies of Growth Hormone and Prolactin during Adaptation of Coho Salmon, *Oncorhynchus kisutch*, to Different Salinities

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The kinetics of growth hormone (GH) and prolactin (PRL) in coho salmon (*Oncorhynchus kisutch*) transferred from fresh water (FW) to seawater (SW) and *vice versa* were examined to help clarify the osmoregulatory roles of the two hormones during periods of migration to different salinities. Chum salmon GH or PRL was administered by a single injection intraarterially, and metabolic clearance rate (MCR) and secretion rate (SR) of injected hormones were calculated from the disappearance of the hormones from the plasma. When coho salmon smolts were acclimated to SW, MCR, SR, and plasma level of GH in SW-adapted (2–3 weeks) fish were twice as great as those in fish in FW. On the other hand, there was no difference in the kinetics of GH between the adult coho salmon in SW and those adapted to FW (2–3 weeks). The transfer of the adult coho salmon from SW to FW was followed after 2 days by a rise in plasma level and SR of PRL, which tended to stay at high levels after 2–3 weeks. The MCR of PRL increased significantly after 2–3 weeks in FW. These results support the likelihood of an important role of GH in SW adaptation and of PRL in FW adaptation in coho salmon.

There has been increasing interest in endocrine control of salmonid osmoregulation, with particular emphasis on the juveniles during the period of seawater migration (smolts) and on sexually mature adults during freshwater (FW) entry. Several studies have suggested that cortisol, a putative seawater (SW)-adapting hormone in teleosts, is involved in osmoregulatory processes also in salmonids. Increased plasma level or metabolic clearance rate (MCR) of cortisol has been observed after transfer of several salmonids from FW to SW (Redding *et al.*, 1984; Nichols and Weisbart, 1985; Patiño *et al.*, 1987; Young *et al.*, 1989). Recently, McCormick and Bern (1989) reported *in vitro* stimulation of Na^+–K^+-ATPase activity by cortisol in coho salmon gill, indicating a direct action of cortisol on an osmoregulatory surface.

Although the importance of prolactin (PRL) in FW adaptation in teleosts has been well documented (see Bern, 1983; Hirano, 1986; Hirano *et al.*, 1987), several studies indicate that PRL is not essential for FW survival of hypophysectomized salmonids (see Komourdjian and Idler, 1977; Nishioka *et al.*, 1987). However, an increase in plasma levels of PRL has been commonly observed after transfer of salmonids from SW to FW, whereas decreased levels have been seen during FW to SW transfer (Hirano *et al.*, 1985; Prunet and Boeuf, 1985; Prunet *et al.*, 1985, 1989; Hasegawa *et al.*, 1987; Young *et al.*, 1989; Ogasawara *et al.*, 1989), suggesting a possible "FW-adapting" role. On the other hand, growth hormone (GH), which has a structural homology close to that of PRL, has been implicated in seawater adaptation

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of salmonids, although the distinction between its growth-stimulatory and its osmoregulatory effects is ambiguous (see Hirano *et al.*, 1987). However, Bolton *et al.*, (1987) and Collie *et al.* (1989) demonstrated that the ability of exogenous chum salmon GH to increase SW adaptability in rainbow trout is independent of its growth-promoting effect. Plasma levels of GH increased after transfer of coho and chum salmon into SW (Sweeting *et al.*, 1985; Hasegawa *et al.*, 1987). Increased MCR and secretion rate (SR) of GH were observed 4 days after transfer of "nonmigratory" rainbow trout to 75% SW despite the same plasma levels (Sakamoto *et al.*, 1990).

Coho salmon (*Oncorhynchus kisutch*) undergo morphological and physiological changes referred to as smoltification, which prepares the fish for growth in SW and migration to feeding areas in the ocean (see review by Hoar, 1988). After life in SW, the coho salmon return to FW to undergo final maturation. To date, there is no information concerning the plasma MCR of pituitary hormones in such anadromous species. In the present study, changes in MCR of GH were examined during SW adaptation of coho salmon smolts and changes in MCR of PRL were examined during FW adaptation in adult stage.

MATERIALS AND METHODS

Fish. Yearling smolts of coho salmon (*O. kisutch*), weighing 150–350 g, were obtained from Sasaki Trout Station (Hachimantai, Iwate) and maintained in running FW aquaria (500 liters) at 12–15°. Some fish were acclimated to SW at 13° for 2–3 weeks. Experiments using the smolts were conducted in November 1988, when smolts were transferred to seapens. Artificial diet (Masu, Nihon Nosan Kogyo) was supplied several times daily until the day before cannulation. Adult coho salmon (1.1–2.5 kg, GSI = 0.1–4%) were obtained from seapens of Kesengawa Fisheries Cooperation Hatchery (Kesengawa, Iwate) and maintained in SW at 15° without feed for 2–3 weeks before the experiment. Some fish were acclimated to FW at 14°. The experiments using the adult fish were conducted during June and August 1988. Final maturation of the gonads would have occurred by September of that year. The adults had not been feeding before the transfer. There was no significant difference in body weight between FW and SW groups. Transfer to different salinities was achieved by switching off the FW tap and switching on the SW tap to the aquarium or *vice versa*.

Cannulation. Fish were cannulated via the dorsal aorta, held individually in restraining plastic boxes (20 liters) with circulating water, and allowed to acclimate for 2–6 days before use as described by Ishimatsu *et al.* (1988). The cannula was connected outside the box with a plastic syringe filled with heparinized saline (100 U/ml in 0.9% NaCl). Manipulation of the catheter was accomplished without disturbing the fish. Immediately after blood sampling, a volume of 0.9% saline, equal to that of the blood removed, was returned to the fish.

Hormones. Recombinant chum salmon GH (rsGH lot R-3) supplied by Tokyo Research Laboratory, Kyowa Hakko Kogyo, was dissolved in saline containing 0.2% (w/v) bovine serum albumin (BSA) and the pH was adjusted to 10 with NaOH (50–100 μg rsGH/ml). Natural chum salmon PRL (sPRL) supplied by Professor H. Kawauchi, School of Fisheries Sciences, Kitasato University, was dissolved in distilled water (1 mg/ml) and then diluted to 30–40 μg/ml with saline containing 0.2% BSA. The fish received intraarterial injections of 10 ng/g body wt rsGH or 7.5 ng/g body wt sPRL in a volume of 0.1 ml/kg body wt.

Plasma variables during adaptation to different salinities. In smolts, blood samples were taken at the start of the experiment and plasma concentrations of cortisol, sodium, and endogenous GH were recorded.

Adult fish (two males and two females) were transferred from SW to FW during 15–30 min between 9 and 12 AM, immediately after removal of blood samples (0.2–0.3 ml) in SW. Thereafter, blood samples (0.2–0.3 ml) were taken 15 and 30 min and 1, 2, 3, 6, 12, 24, 48, 72, 120, and 168 hr after entry to FW, and changes in plasma sodium, GH, and PRL levels were recorded.

MCR of GH and PRL. GH MCR was measured in smolts in FW, in fish 2 days after transfer to SW, and in fish after 2–3 weeks in SW. The GH MCR was also measured in the adult fish in SW and in those acclimated to FW for 2–3 weeks. PRL MCR was measured in the adult fish in SW, in fish 2 days after transfer to FW, and in fish after 2–3 weeks in FW. The methods of MCR measurement were essentially the same as previously described (Sakamoto *et al.*, 1990). Initially, a 0.2-ml blood sample was removed from the fish, and rsGH or sPRL solutions diluted with 0.2–0.6 ml salmon blood were injected, followed immediately by 0.6 ml saline. Blood samples (0.05–0.2 ml) were taken through the cannula 2, 5, 10, 30, 60, 120, and 180 min after the injection for determination of the hormone concentration. The total blood volume removed during the MCR determination was less than 10% of the total blood volume of the fish.

Analytical techniques. Plasma concentrations of sodium were determined by atomic absorption spectrophotometry (Hitachi 180-50). PRL, GH, and cortisol concentrations in the plasma were measured by radioimmunoassays as described by Hirano *et al.* (1985), Bolton *et al.* (1986), and Takahashi *et al.* (1985), respectively.

Calculations of MCR. The MCR, defined as the volume of plasma cleared of the hormone per unit time, was calculated by the numerical method of Normand and Fortier (1970) according to the formula,

$$\mathrm{MCR} = \frac{R}{\int_0^\infty x\,dt},$$

where R represents the injected dose of the hormone, x is the plasma concentration of the injected hormone at a given time after the injection, and dt is the change in time. On the assumption that plasma concentration of the endogenous hormone is in a steady-state condition during MCR measurement (180 min), all points on the decay curve were used to calculate the MCR. The secretion rate of the hormone was calculated by multiplying the MCR by the endogenous (initial) concentration of the hormone.

Statistics. Statistical significances were examined by one-way analysis of variance, Duncan's new multiple range test (Duncan, 1955), and Student's t test, where appropriate.

RESULTS

Transfer of Smolts to SW

Coho salmon smolts exposed to SW showed increased plasma Na concentrations after 2 days and maintained concentrations higher than the FW level throughout the experiment. Plasma cortisol levels were highly variable, and there were no significant differences throughout the experiment (Table 1).

TABLE 1
PLASMA SODIUM AND CORTISOL LEVELS IN CANNULATED COHO SALMON SMOLTS

Fish	*n*	Sodium (m*M*)	Cortisol (ng/ml)
Fresh water	5	123 ± 2[a]	369 ± 64
2 days in seawater	4	196 ± 7*	1384 ± 516
2–3 weeks in seawater	6	177 ± 7*	346 ± 65

[a] Mean ± SEM.

* Significantly ($P < 0.001$) different from the fish in fresh water.

Changes in plasma levels, MCR, and SR of GH after SW transfer are presented in Fig. 1. There was no significant change in GH MCR in the fish 2 days after transfer to SW. After 2–3 weeks in SW, the MCR increased significantly ($P < 0.001$). Plasma GH levels were significantly ($P < 0.05$) higher than the FW level in the fish 2 days after SW transfer and also in the SW-acclimated fish. A similar tendency was

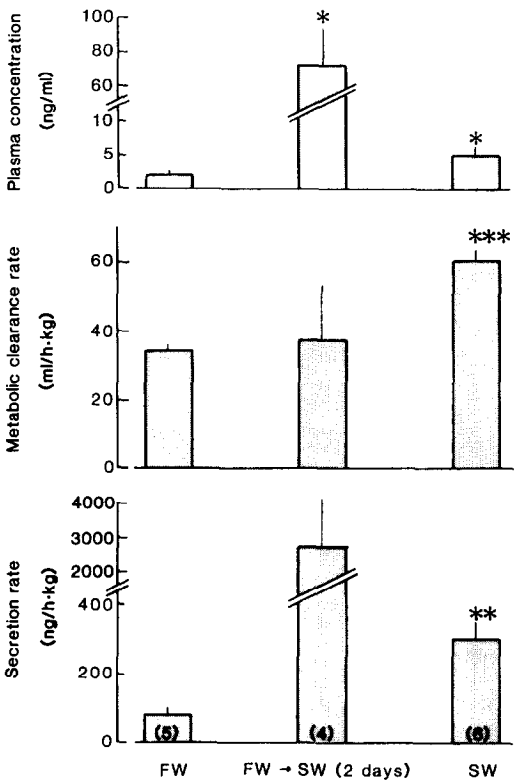

FIG. 1. Growth hormone (GH) kinetics in coho salmon smolts in fresh water (FW), 2 days after exposure to seawater (SW), and after 2–3 weeks in SW. After collection of blood samples for determination of initial levels of sodium and endogenous hormones, fish were injected with 10 ng chum salmon GH/g body wt intraarterially. Results are expressed as means ± SEM. Numbers of the fish used are indicated in parentheses. *, **, ***: Significantly different from the fish in FW at 5, 1, and 0.1% levels, respectively.

seen in the calculated secretion rate, although only SW-acclimated fish showed significant ($P < 0.01$) elevation compared with the fish in FW.

Plasma Variables of Adult Coho Salmon Transferred To FW (Fig. 2)

The adult coho salmon transferred from SW to FW were able to regulate their plasma Na levels within 1 week. No significant change was seen in plasma GH levels during FW adaptation. Plasma PRL levels were low in SW, and there was no significant change during the first 24 hr in FW. A significant ($P < 0.05$) increase was observed after 3 days, and the level remained elevated throughout the experiment.

GH and PRL Kinetics in Adult Fish

No significant change was observed in plasma concentration, SR, and MCR of GH 2–3 weeks after acclimation to FW (Fig. 3).

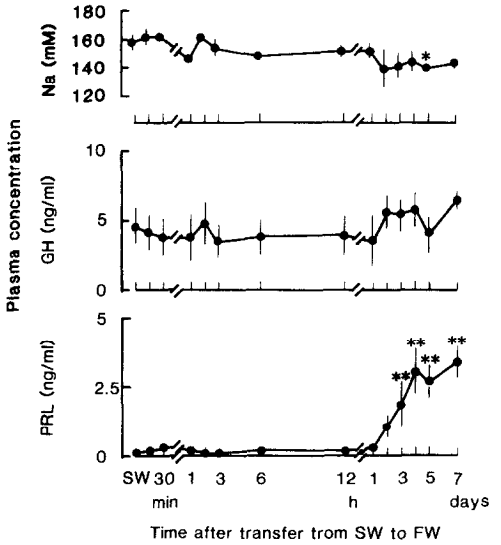

FIG. 2. Time course of changes in plasma sodium, GH, and PRL levels in adult coho salmon during adaptation to fresh water (FW). Repeated blood samples were taken from the fish in seawater (SW) and during 7 days after transfer to FW. Results are expressed as means ± SEM ($n = 4$). *, **: Significantly different from the level in SW at 5 and 1% levels, respectively.

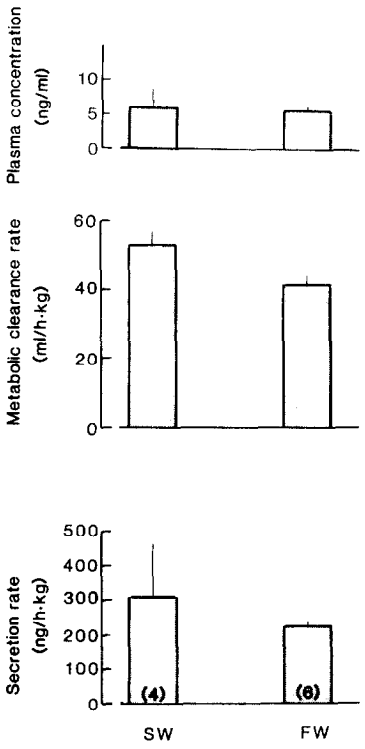

FIG. 3. Growth hormone (GH) kinetics in adult coho salmon in seawater (SW) and after 2–3 weeks in fresh water (FW). After collection of samples for determination of endogenous plasma GH levels, fish were injected with 10 ng chum salmon GH/g body wt intraarterially. Results are expressed as means ± SEM. Numbers of the fish used are indicated in parentheses.

The MCR of PRL showed a tendency to increase progressively in fish transferred to FW, although only the value of the group fully acclimated to FW was significantly ($P < 0.01$) different from that of fish in SW. Plasma PRL concentrations both in the fish 2 days after transfer to FW and in the fish fully acclimated to FW were significantly ($P < 0.05$) higher than those in the fish in SW. A similar tendency was also seen in the calculated secretion rate, with a significant ($P < 0.05$) increase 2 days after the transfer (Fig. 4).

DISCUSSION

This study demonstrates GH and PRL

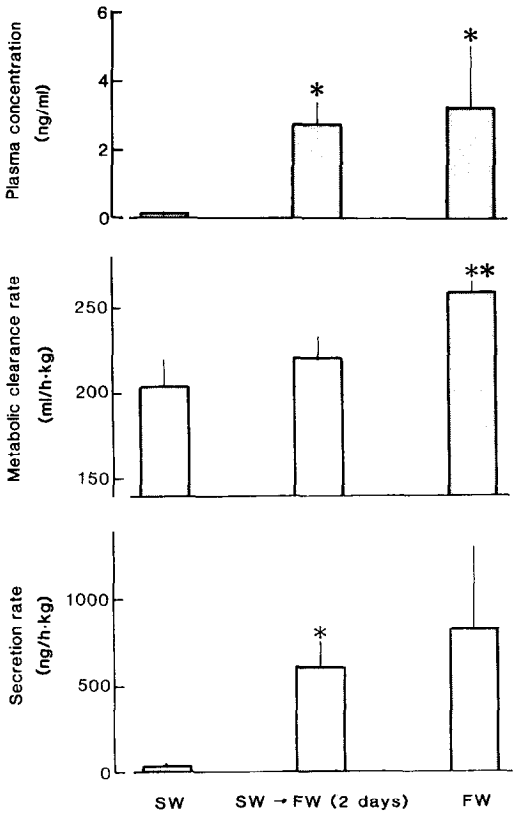

FIG. 4. Prolactin (PRL) kinetics in adult coho salmon in seawater (SW), 2 days after exposure to fresh water (FW), and after 2–3 weeks in FW. After collection of samples for determination of endogenous plasma PRL levels, fish were injected with 7.5 ng chum salmon PRL/g body wt intraarterially. Results are expressed as means ± SEM (n = 7). *, **: Significantly different from the fish in SW at 5 and 1% levels, respectively.

dynamics during adaptation of coho salmon to different salinities. Plasma GH increased markedly 2 days after transfer of coho salmon smolts to SW. The level in the fully adapted smolts was still significantly higher than that in FW fish. Similarly GH levels in SW higher than those in FW have been observed in noncannulated coho salmon smolts by Sweeting *et al.* (1985), whereas Young *et al.* (1989) reported no change in GH levels after SW transfer of another stock of coho salmon smolts. As plasma concentrations of the hormone are the results of an equilibrium between the secretion rate and MCR, a complete assessment of GH dynamics requires kinetic study as in the present study.

MCR and SR of GH significantly higher than those in fish in FW were observed in SW-acclimated (2–3 weeks) smolts, consistent with the role of GH in SW adaptation suggested for salmonids (see Hirano *et al.*, 1987). However, MCR reflects the distribution, utilization, and degradation of the hormone. In the "nonmigratory" rainbow trout, the MCR of GH increased temporarily and returned to the FW levels 3–4 weeks after transfer to 75% SW, suggesting that GH is involved in the development of hypoosmoregulatory mechanisms especially during the early phase of adaptation (Sakamoto *et al.*, 1990). Therefore, the chronic elevation of GH turnover observed in "migratory" coho salmon smolts, which grow in the ocean, might reflect not only the osmoregulatory action of GH but also its growth-stimulatory role in the marine environment.

In cannulated coho salmon smolts, plasma cortisol levels were extremely high throughout the experiment, indicating that the fish were under severe stress: the size of the fish (150–350 g) used may have been too small for cannulation. Cortisol stimulated the release of GH from tilapia proximal pars distalis in culture (Nishioka *et al.*, 1985; Helms *et al.*, 1987). Chronic stress resulted in an elevation of plasma PRL (Avella *et al.*, 1991). Therefore, such stress could also affect the hormone MCRs.

Adult coho salmon adjusted their plasma Na level within 7 days after transfer to FW, which is in agreement with the results of similar transfer experiments using noncannulated fish (Nagahama *et al.*, 1977). There was no significant change in plasma PRL levels within 24 hr after transfer to FW, and a significant increase was seen after 2–3 days, apparently reflecting the increase in calculated SR. These changes were not directly correlated with the changes in

plasma Na level. Similar observations have been made in immature chum salmon (Hasegawa *et al.*, 1987; Ogasawara *et al.*, 1989). Plasma Na level may not directly affect PRL secretion. Hirano *et al.* (1985) found an increase in plasma PRL levels after transfer of mature female chum salmon to FW, whereas no change was detected in males. In the present study, performed about 2 months before the spawning period, the difference was unclear between the PRL dynamics of males and females. Further studies are needed on the sex difference in turnover of PRL in salmonids.

In adult coho salmon, there was no significant difference in GH turnover between the fish in SW and those fully adapted to FW, whereas turnover of PRL increased after transfer from SW to FW. Brewer and McKeown (1980) reported a shorter half-life of injected ^{125}I-labeled ovine PRL in coho salmon transferred to FW than in SW fish. The increased turnover of PRL is consistent with the well-established role of PRL in maintaining hydromineral balance of several euryhaline teleosts in FW (See Nicoll, 1981; Bern, 1983; Hirano, 1986). Fryer (1979) found that tilapia PRL binding to tilapia kidney membranes was greater with FW-acclimated than with SW-acclimated fish. The increased clearance of PRL from blood after transfer of coho salmon to FW may reflect increased PRL binding to receptors.

The mode of action of GH in osmoregulation has also yet to be determined. As discussed above, elevated turnover of GH in SW-adapted coho salmon smolts might reflect both SW-adapting and growth-promoting actions of GH. GH may influence SW survival indirectly through effects on energy metabolism. As in mammals, GH appears to have lipolytic, diabetogenic, and protein-anabolic effects in salmonids (McKeown *et al.*, 1975; Higgs *et al.*, 1976; Markert *et al.*, 1977). Another possible mode of GH action during SW adaptation is through effects on other mediators, such as somatomedins and cortisol, a steroid with SW-adapting actions in coho salmon (Richman *et al.*, 1987; McCormick *et al.*, 1989). In coho salmon, GH seems to facilitate stimulatory actions of ACTH on cortisol secretion (Young, 1988).

Evidence for direct action of GH on ion-transporting epithelia such as the gill, gut, and kidney is scanty: tilapia GH-binding sites are presented in the gill and kidney of coho salmon (Fryer and Bern, 1979) and salmonid GH-binding sites are presented in these tissues of masu salmon and rainbow trout (Ikuta *et al.*, 1989; Le Bail *et al.*, 1991). Thus, the sites and mechanisms of GH and PRL actions during adaptation to different salinities represent a fascinating area for future studies, including receptor dynamics and the role of secondary hormone mediators.

In this study, kinetic parameters of GH and PRL were determined following a single intraarterial injection of unlabeled hormones. Experiments with rainbow trout using the same procedures have shown that the metabolic clearance of GH is independent of the amount of GH injected over a wide range of doses (8–40 ng/g body wt) (Sakamoto *et al.*, 1990). Similar results were reported in the clearance of GH and PRL in mice (Shinha *et al.*, 1979a,b) and of GH in man (Taylor *et al.*, 1969). In rainbow trout, the kinetic parameters of GH were calculated from the data obtained up to 90 min after GH injection. In the present study, the disappearance of GH and PRL was analyzed up to 180 min after injection. The MCR of GH (40–60 ml/hr · kg) in both smolts and adult coho salmon were substantially lower than GH MCR in trout (80–420 ml/hr · kg). The difference between the two species could be due to the difference in the time-course of blood sampling. According to Down *et al.* (1988), the plasma half-life of recombinant chicken GH injected intraperitoneally or intramasucularly in juvenile coho salmon was considerably longer (about 10 hr). The difference may be

due to the use of a heterologous hormone and to the difference in the route of hormone injection.

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