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Plasma Prolactin and Cortisol Concentrations of Stressed Coho Salmon, *Oncorhynchus kisutch*, in Fresh Water or Salt Water¹

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Juvenile coho salmon, *Oncorhynchus kisutch*, adapted to fresh water or seawater were either acutely handled or continuously stressed by severe confinement. Chronic stress, independent of external salinity, caused a gradual increase in the concentration of circulating prolactin that persisted for 1 to 5 days but lagged behind the cortisol response which peaked much more rapidly and remained elevated. Acutely stressed fish showed a rapid, more transient increase in plasma cortisol titer with no apparent effect on prolactin. Confinement appeared to be more stressful to fish in salt water than to those in fresh water, as judged by their sodium regulatory ability, hormone profiles, and mortality. Stress always elevated plasma prolactin concentrations, regardless of medium or developmental stage.

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It is well known that stress can trigger prolactin (Prl) secretion in mammals: numerous studies have shown that physical (cold shock, handling, and acute restraint) and psychological stress cause transient increases in plasma Prl level (Willoughby, 1980; Drago *et al.*, 1985; Seltzer *et al.*, 1986). However, very limited information concerning the relationship between stress and Prl is available on fish. Only one study using heterologous assay reported a decrease of plasma Prl level in goldfish subjected to serial sampling and restraint (Spieler and Meier, 1976). These differences between mammals and fish led us to further investigate this aspect in fish using a homologous radioimmunoassay for salmon Prl.

Prolactin is believed to act as an osmoregulatory hormone in teleosts and is considered to be of more importance in fresh water (FW) than in seawater (SW) (Hirano and Mayer-Gostan, 1978; Loretz and Bern, 1982; Prunet *et al.*, 1985; Hirano, 1986). Stress is known to cause hydromineral imbalance (Mazeaud *et al.*, 1977; Eddy, 1981) and concomitant elevation in the concentration of the stress hormone cortisol (Mazeaud *et al.*, 1977; Donaldson, 1981; Schreck, 1981).

Our objectives were to evaluate the effects of acute (brief) and chronic (continuous) stress on prolactin in a euryhaline species, the coho salmon (*Oncorhynchus kisutch*), adapted to FW or SW. Plasma cortisol concentration was followed as an indicator of stress. Our data clearly indicate that chronic stress causes an increase in plasma Prl levels.

MATERIALS AND METHODS

Experiment 1. Yearling coho salmon parr from

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Eagle Creek National Fish Hatchery were acclimated to Oregon State University's Smith Farm Experimental Hatchery (Corvallis, OR). On 8 February 1987, fish averaging 28.5 g were randomly sorted into 100-liter circular FW flow-through tanks (11°) to a final density of about 10 g/liter. Fifteen days later fish in one tank were acutely stressed by catching them with a dip net and suspending them in the air for 30 sec before returning them to their tank. Another group of fish was subjected to chronic stress by crowding them to a density of about 400 g/liter in a perforated bucket (25 cm in diameter) suspended in their tank; the fish remained under these conditions throughout the sampling period, which started immediately before the stress and lasted for 9 days. An unstressed group of fish served as controls. Two tanks were used for each group of fish (control, acute stress, chronic stress). The chronic stress experiment was repeated, also with duplicate tanks, on 10 March 1987, with fewer sampling times and fish averaging 39 g.

Experiment 2. Underyearling coho salmon, considered to be in the smolt stage, were obtained from Oregon Aqua-Foods Hatchery (Newport, OR). They were transferred to Oregon State University's Hatfield Marine Science Center (Newport, OR) and randomly distributed into 380-liter circular tanks with either flowing FW (18°) or SW (15°), at a density of about 5–10 g/liter. After 42 days acclimation (on 24 July 1987), tanks of fish were either subjected to chronic stress or left as controls, as described for Experiment 1. At this time, the average weights of fish were 30 g (FW) and 20 g (SW). The water flow rates were greater than 1 liter/min/tank, allowing a maximum $[O_2]$ of 10 mg/liter in each tank. Because fish in SW subjected to continuous stress experienced some mortality (about 20%) starting at 5 hr onset of stress, they were sampled more frequently and only during the first 24 hr.

General procedures and analyses. During acclimation fish were fed twice daily with Oregon Moist Pellet diet at about 2% of their body weight; feeding was discontinued 48 hr before the initiation of stress. Sampled fish were rapidly netted and killed by immersion in 200 mg/liter ethyl *m*-aminobenzoate methane-sulfonate (MS 222) (Strange and Schreck, 1978; Barton *et al.*, 1985). Blood was collected from the severed caudal peduncle and plasma was stored at -20° until analysis. There was insufficient plasma in some samples to allow for all assays.

Concentrations of total plasma electrolyte (total Na^+ and K^+) were measured with a Nova 1 sodium-potassium analyzer (Nova Biomedical, Newton, MA). Plasma cortisol concentration was measured by radioimmunoassay (RIA) as described by Forster and Dunn (1974) and modified by Redding *et al.* (1984). Plasma prolactin was assayed using highly purified chinook salmon (*O. tshawytscha*) prolactin (Prunet and Houdebine, 1984); a homologous RIA for salmon plasma prolactin was performed according to Hirano *et al.* (1985) as slightly modified by Prunet *et al.* (1985).

Statistical analyses of the data followed methods of Winer (1971) and Affi and Azen (1979). Data were subjected to one-way analysis of variance followed by the Fisher's protected least significant difference (PLSD) multiple comparison test after testing for homogeneity of variance with the F_{max} test. Prolactin and cortisol data were transformed into their natural logarithms or square roots to increase homogeneity of variances. Data from replicate groups were not statistically different and were pooled.

RESULTS

Experiment 1. Coho salmon parr subjected to chronic stress, starting on 23 February, showed a threefold increase in the concentration of circulating prolactin (Fig. 1A). Two peaks were observed, one 5 hr and a second 24 hr after the onset of stress. Prolactin returned to resting levels between the two peaks and again by 2 days after the onset of the stress. Plasma prolactin was apparently not affected by the acute stress and also did not vary during the experiment in control animals. Plasma cortisol levels increased more rapidly than prolactin, peaking within 1 hr after the onset of either acute or chronic stress (Fig. 2). Duration of the cortisol response was greater than that of the first peak in prolactin level and never returned to resting values within 12 hr of continuous stress. Cortisol concentration in the acutely stressed fish remained elevated for less than 3 hr. Cortisol concentrations of control fish showed an unexplained transient increase at 24 hr and 2 days of stress, thus no conclusion can be drawn from this period.

Prolactin dynamics of fish subjected to continuous stress on 10 March appeared similar to those observed in February (Fig. 1B). Although the starting level of plasma was higher in control fish at this time than it was in February, it did not change significantly during the course of the study.

Experiment 2. As in Experiment 1, the concentration of circulating prolactin was significantly increased when FW-adapted smolts were subjected to chronic confinement (Fig. 3A). Results differed from those

FIG. 1. (A) Plasma prolactin concentrations in coho salmon parr subjected to acute (handling, ■) or chronic (confinement, ♦) stress in fresh water on 23 February 1987. Controls (□). (B, insert) Plasma prolactin concentrations in coho salmon parr subjected to chronic (confinement, shaded bars) stress in fresh water on 10 March 1987. Controls, striped bars. Statistical comparisons: values significantly different from controls with ** $P < 0.001$ and * $P < 0.05$. Each point represents the mean $\pm$ SEM ($n = 5-25$).

obtained in February (Experiment 1) in that the initial resting concentration was higher and the elevation in titer started later and lasted longer, returning to control levels within 5 days. As before, plasma titers of cortisol peaked earlier than those of prolactin and were still significantly above resting concentrations at 5 days (Fig. 3B). Plasma Na^+ in these fish declined continuously during the first day of stress but recovered within 5 days (Fig. 3C). Although plasma K^+ appeared to be elevated initially during stress, the increase in the resting concentration on Day 5 makes interpretation difficult (Fig. 3D).

Seawater adapted smolts that were chronically stressed experienced some mortality. Their plasma prolactin concentration increased significantly ($P < 0.05$) by 24 hr of confinement (Fig. 4A). Plasma cortisol titers were elevated by 5 hr after onset of stress and remained elevated thereafter (Fig. 4B). Both plasma Na^+ and K^+ initially showed a pattern similar to that of

plasma cortisol in stressed fish (Figs. 4C and 4D), except that the concentration of K^+ fluctuated over the course of the study. Both electrolyte profiles demonstrated a trend toward recovery by 24 hr of stress as K^+ concentration returned to control level and Na^+ concentration was significantly lower than the 9-hr-stress sample ($P < 0.001$).

DISCUSSION

The concentration of circulating prolactin was always increased by severe, continuous stress, regardless of the salinity of the ambient medium or the developmental stage of the fish. The exact dynamics of this stress-related prolactin increase and its duration, however, appeared to vary and could be attributed to rearing history, age and size of the fish, or the experimental environment (water temperature, season, etc.). The stimulation of plasma prolactin by stress is well known in mammals (Willoughby, 1980; Drago *et al.*, 1985; Selt-

FIG. 2. Plasma cortisol concentrations in coho salmon parr subjected to acute (handling, ■) and chronic (confinement, ◆) stress in fresh water on 23 February 1987. Controls (□). Statistical comparisons: values significantly different from controls with $**P < 0.001$ (from Time 24 hr, no statistical comparisons with the controls were considered). Each point represents the mean $\pm$ SEM ($n = 5-23$).

zer *et al.*, 1986). These findings in fish are contrary to those of Spieler and Meier (1976), who used a heterologous radioimmunoassay. They reported that stress depressed serum prolactin levels in goldfish, *Carassius auratus*. Differences may be attributable to differences in physiological tolerances of the study animals (euryhaline versus stenohaline), to the nature of the stressors, or to the assay systems used; heterologous assay systems may yield results that are difficult to interpret (Nicoll, 1975, 1981).

Given the observed increase in plasma prolactin concentration during chronic stress, it is interesting that acute stress did not appear to affect plasma prolactin levels. The acute stressor to which our fish were subjected elicited a cortisol stress response, although not of the same duration as that observed in response to the chronic stressor. Similar dynamics of circulating cortisol consequent to stress in salmonids has been well documented (Mazeaud *et al.*, 1977; Donaldson, 1981; Schreck, 1981; Pickering *et al.*, 1982; Sumpter *et al.*, 1986).

FIG. 3. Concentrations of plasma prolactin (A), cortisol (B), Na^+ (C), and K^+ (D) in coho salmon smolt subjected to chronic (confinement, ◆) stress in fresh water on 24 July 1987. Statistical comparisons: values significantly different from controls at the same time point with $**P < 0.001$ and $*P < 0.05$. The number of fish studied is indicated near each point, which represents the mean $\pm$ SEM.

Levels of prolactin in control fish were relatively stable in each trial but varied somewhat according to the sampling time of year.

Circulating prolactin concentration also varied according to external salinity in both

FIG. 4. Concentrations of plasma prolactin (A), cortisol (B), Na^+ (C), and K^+ (D) in coho salmon smolt subjected to chronic (confinement, $\blacklozenge$) stress in salt water on 24 July 1987. Statistical comparisons: values significantly different from controls with $**P < 0.001$ and $*P < 0.05$. The number of fish studied is indicated near each point, which represents the mean $\pm$ SEM.

unstressed and stressed smolting fish. The finding that resting prolactin appeared higher in FW- than in SW-adapted animals is in agreement with other studies using a homologous RIA in salmonids (Prunet *et al.*

et al., 1985; Hasegawa *et al.*, 1987; Young *et al.*, 1989; Avella *et al.*, 1990). The increase in plasma prolactin during stress in FW could be explained by the purported role of this hormone in restoring the stress-related osmoregulatory dysfunction as evidenced by the hyponatremia found in our fish (Fig. 3C) and hydromineral disturbance reported by others (Lahlou and Giordan, 1970; Pic *et al.*, 1974; Mazeaud *et al.*, 1977; Pic, 1978; Eddy and Bath, 1979; Eddy, 1981). Prolactin is known to help fish maintain homeostasis in FW (Hirano and Mayer-Gostan, 1978; Loretz and Bern, 1982; Hirano, 1986), and lowered plasma osmotic pressure has often been related to increased plasma prolactin level (Prunet *et al.*, 1985; Avella *et al.*, 1990). The potential involvement of prolactin in response to stress in SW is difficult to explain. It is possible, of course, that this hormone is playing some other, nonhydromineral (Clarke and Bern, 1980) or immunologic role after stress in either medium. Similarly, the hyperkalemia following stress in either FW- or SW-adapted fish could reflect compensation for stress-induced acid-base imbalance (Redding and Schreck, 1983; Turner *et al.*, 1983).

Elevation in plasma prolactin consequent to chronic stress always lagged behind that of cortisol. It is tempting to speculate that both hormones are playing some osmoregulatory role following the onset of stress. Cortisol has a mineralocorticoid function (Maetz, 1969) and is important in maintaining homeostasis in SW (Mayer *et al.*, 1967; Hirano and Utida, 1968; Epstein *et al.*, 1971). Interactions between cortisol and stress-related osmotic disturbances have been described in fish (Redding and Schreck, 1983) and in mammals (Oosterom *et al.*, 1985). We were unable to find a correlation between circulating cortisol and prolactin levels in chronically stressed FW parr of smolt. Thus, any relationship between plasma prolactin and cortisol is as yet unclear.

In conclusion, we have shown that stress can affect circulating prolactin concentrations in coho salmon. This phenomenon needs to be considered in further studies involving these parameters in fish. The mechanism by which stress induces stimulation of plasma prolactin remains to be elucidated.

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