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ELEVATION OF PLASMA SOMATOLACTIN CONCENTRATIONS DURING ACIDOSIS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Summary

Somatolactin (SL) is a putative pituitary hormone of the growth hormone (GH)/prolactin (PRL) family in fish; its physiological function has yet to be determined. Acidosis was induced in rainbow trout (*Oncorhynchus mykiss*) by exposure to acidic water (pH 4.5) or by exhaustive exercise, and plasma concentrations of SL, PRL and GH as well as other plasma parameters were examined. A decrease in blood pH was observed in fish from 1 day after water acidification until the end of the experiment at day 7. Plasma SL levels in the acid-exposed fish increased, reached a peak on day 1 and then returned to the initial level by day 4. No change was seen in plasma concentrations of PRL throughout the experiment. Plasma levels of GH, in contrast, decreased in the acid-exposed fish on days 2 and 4. Plasma cortisol levels in the acid-exposed fish were higher than the control level on days 4 and 7, although plasma cortisol levels did not increase above the initial level in response to water acidification. There was no significant change in the expression of SL-, PRL- and GH-mRNA in the pituitary gland. Levels of plasma Na^+ and lactate were reduced 12 h after water acidification and remained low throughout the experiment. Exhaustive exercise in shallow water at neutral pH (7.5) resulted in a transient but pronounced acidosis, associated with increases in plasma SL, cortisol, Ca^{2+}, phosphate and lactate levels. Plasma SL levels returned to the initial level along with the recovery of blood acid–base status. In contrast, plasma cortisol levels stayed elevated even 24 h after exercise. There was no correlation between plasma PRL and GH levels and blood pH. Elevation of plasma SL levels during acidosis suggests the possible involvement of SL in acid–base regulation in rainbow trout.

Key words: somatolactin, acidosis, rainbow trout, acidic water, exercise, *Oncorhynchus mykiss*.

Introduction

Somatolactin (SL), a pituitary hormone in teleosts, was isolated for the first time from the pituitary gland of the Atlantic cod (*Gadus morhua*) (Rand-Weaver *et al.* 1991*b*). The corresponding protein was isolated from the Japanese flounder (*Paralichthys olivaceus*), and the complete structure of the protein was elucidated from its cDNA sequence (Ono *et al.* 1990). Because this protein is structurally related to both growth hormone (GH) and prolactin (PRL), it was named somatolactin (Ono *et al.* 1990; Kawauchi, 1993; Rand-Weaver and Kawauchi, 1993).

Although a definitive physiological function for SL has yet to be determined, previous studies have suggested the involvement of SL in various biological processes (see Kaneko and Hirano, 1993). In coho salmon (*Oncorhynchus kisutch*), plasma SL levels increased during sexual maturation (Rand-Weaver *et al.* 1992; Rand-Weaver and Swanson, 1993), and *in vitro* gonadal steroidogenesis was stimulated by SL (Planas *et al.* 1992). Immunoreactive SL cells were activated in spawning sockeye, chum and chinook salmon (*O. nerka*, *O. keta* and *O. tshawytscha*; Olivereau and Rand-Weaver, 1994*a*,*b*), suggesting that SL may be involved in the regulation of gonadal function in salmonids. However, plasma SL levels in rainbow trout (*O. mykiss*) increased in response to stress (Rand-Weaver *et al.* 1993; Kakizawa *et al.* 1995*a*). Activation of SL cells was observed in rainbow trout transferred from Ca^{2+}-rich water to low-Ca^{2+} water (Kakizawa *et al.* 1993). Furthermore, an involvement of SL in lipid metabolism has also been suggested in a cobalt variant of rainbow trout, which lacks SL-producing cells and accumulates a large amount of abdominal fat (Kaneko *et al.* 1993). We have also suggested an involvement of SL in energy mobilization in chum salmon during the spawning migration and in rainbow trout during acute stress and exhaustive exercise (Kakizawa *et al.* 1995*a*,*b*).

Rand-Weaver *et al.* (1991*a*) demonstrated that SL cells correspond to periodic-acid-Schiff (PAS) -positive cells of the pars intermedia (PIPAS cells) in several teleost species;

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salmonids lack PIPAS cells, possibly because of the absence of N-glycosylation sites in salmon SL (Takayama *et al.* 1991). Even before the discovery of SL, histological studies showed that PIPAS cells are activated under conditions of low environmental osmolality or [Ca^{2+}] in goldfish (*Carassius auratus*) and eel (*Anguilla anguilla* L.) (Olivereau *et al.* 1980, 1981*a,b*) and when tilapia (*Oreochromis mossambicus*) and molly (*Poecillia latipinna*) are exposed to a black background (van Eys, 1980; Ball and Batten, 1981). Activation of PIPAS cells was also observed in goldfish exposed to acidic water (Wendelaar Bonga *et al.* 1986). In our previous report, elevation of plasma SL levels was observed in exhaustively exercised rainbow trout (Kakizawa *et al.* 1995*a*). Since a decrease in blood pH (acidosis) usually accompanies exhaustive exercise in fish (see Cameron, 1989; Heisler, 1993), the elevation of plasma SL levels observed in the exercised fish could be attributed to an effect of acidosis.

The present study was carried out to investigate the possible involvement of SL in acid–base regulation in *Oncorhynchus mykiss*. Acidosis was induced by exposure to acidic water or exhaustive exercise, and changes in blood acid–base and ionic status as well as plasma concentrations of SL, PRL, GH and cortisol were examined.

Materials and methods

Fish

Immature rainbow trout [*Oncorhynchus mykiss* (Walbaum)], weighing about 100 g, were obtained from a commercial source in Tokyo and maintained in a stock tank with recirculating fresh water (pH 7.5, [Ca^{2+}] 0.6 mmol l^{-1}, [Na^{+}] 1.0 mmol l^{-1}, [Cl^{-}] 1.0 mmol l^{-1}) at 15 °C for more than 1 week before use. They were fed on commercial trout pellets (Oriental no. 6, Chiba, Japan) in a ration equivalent to 1 % body mass per day until 24 h before blood sampling.

Blood and pituitary sampling

Fish were removed from experimental tanks and anaesthetized with 0.02 % phenoxyethanol. Each fish was bled only once. Blood was collected from caudal vessels using a syringe, and blood pH was analyzed immediately. Subsequently, blood was centrifuged at 10 000 ***g*** for 5 min at 4 °C to obtain plasma. After measurement of ion concentrations (see below), the plasma was stored at −80 °C until further analyses.

In the experiment examining the effects of exposure to acidic water, the pituitary gland was collected for the analysis of mRNA expression. The pituitary was dissected out soon after blood sampling and stored at −80 °C until analysis.

Experimental procedures

Exposure to acidic water

About 60 fish were reared in a tank (1000 l) with recirculating fresh water (pH 7.5, 15 °C). On the first day of the experiment (day 0), the water pH was reduced from 7.5 to 4.5 by adding 1 mol l^{-1} H_2SO_4. It took about 1 h to adjust the pH to 4.5. Thereafter, the water pH was maintained at 4.5 with 0.025 mol l^{-1} H_2SO_4 using a pH controller (FD-02, Tokyo Glass, Tokyo). On days 0, 0.5 (12 h), 1, 2, 4 and 7, the blood and pituitary gland were collected. The fish were unfed during the experiment.

Exhaustive exercise in shallow water

The experimental protocol was essentially the same as that of our previous study (Kakizawa *et al.* 1995*a*), following the method of Milligan and Wood (1986). The fish were removed individually from the stock tank and forced to swim by being chased constantly for 8 min in 10 l of shallow water to induce severe acidosis. The fish were then transferred to a tank containing recirculating fresh water (200 l). Fish were bled before the exercise (initial), just after exercise (0 h), and 3 and 24 h into the recovery period. The fish did not respond to further stimulus immediately after exercise. However, the exercise was not lethal, as the fish recovered 5–10 min after transfer back to the recovery tank.

Analyses

Blood pH and plasma levels of ions and metabolites

Blood pH was measured using a pH analyzer (AVL 9110, Graz, Austria). Plasma levels of Na^{+} and Ca^{2+} were determined with an electrolyte analyzer (AVL 984-S, Graz, Austria), and Cl^{-} levels with a chloridometer (Buchler 4-2500, USA). Plasma levels of phosphate and lactate were measured using Phospha B-Test Wako (Wako, Osaka) and Lactate Test BMY (Boehringer-Mannheim, Tokyo), respectively.

Plasma hormone levels

Plasma concentrations of SL, PRL, GH and cortisol were measured by specific radioimmunoassays as described by Kakizawa *et al.* (1993), Hirano *et al.* (1985), Bolton *et al.* (1986) and Takahashi *et al.* (1985), respectively. The minimal detectable levels, ED_{50}, and intra- and inter-assay coefficients of variation for each assay were 0.5 ng ml^{-1}, 11.7 ng ml^{-1}, 2.5 % (N=10) and 3.9 % (N=10) for SL, 0.1 ng ml^{-1}, 0.82 ng ml^{-1}, 5.9 % (N=4) and 13.5 % (N=4) for PRL, 0.6 ng ml^{-1}, 18.8 ng ml^{-1}, 3.9 % (N=7) and 4.1 % (N=6) for GH, and 0.16 ng ml^{-1}, 5.2 ng ml^{-1}, 10.4 % (N=4) and 10.8 % (N=4) for cortisol. No cross-reaction was seen among SL, PRL and GH.

Expression of mRNA in the pituitary

The protocol was essentially the same as described by Sakamoto and Hirano (1993). Total RNA was extracted from the pituitary gland using the phenol/chloroform method (Chomczynski and Sacchi, 1987) and quantified spectrophotometrically. Samples (1–10 μg) of total RNA were transferred to a nylon filter (Nytran, Schleicher and Schuell, Dassel, Germany) by vacuum blotting after electrophoresis, and covalently attached to the filter by ultraviolet cross-linking. The mRNA of each hormone was detected by hybridization to the corresponding ^{32}P-labelled probes; salmon SL-cDNA probe (Takayama *et al.* 1991), labelled with

a multi-prime labelling kit (Amersham, Tokyo) and denatured by boiling, and oligonucleotide probes for salmon PRL and GH (Yada *et al.* 1991), labelled with a 3′-end labelling kit (Amersham, Tokyo). After hybridization for 16–20 h at 55 °C in a solution containing 6× standard saline citrate (SSC; 1×SSC is 0.15 $mol\,l^{-1}$ NaCl, 0.015 $mol\,l^{-1}$ sodium citrate), the filters were washed in 2×SSC, containing 0.1 % SDS, at room temperature for 20 min and then twice in 1×SSC, containing 0.1 % SDS, at 60 °C for 1 h. The hybridization signal was detected by autoradiography at −70 °C for 1–3 days, and the intensity of the signal was quantified by densitometry using a chromatoscanner (CS-9000, Shimazu, Kyoto). The intensity of each signal was normalized against total RNA content (Yada *et al.* 1991). The mRNA levels for each hormone for the individual fish were expressed as a percentage of the average mRNA level in fish at the start of the experiment.

Statistical analyses

For statistical analyses, Student's *t*-test or Duncan's multiple-range test was applied after Bartlett's test for variance.

Results

Exposure to acidic water

No mortality was seen throughout the 7 day experimental period. Pronounced acidosis occurred in the fish exposed to acidic water from day 1 to day 7. Plasma concentrations of Na^+, Cl^- and lactate began to decrease 12 h (Na^+ and lactate) or 24 h (Cl^-) after water acidification, and were lower than those in the control fish until the end of the experiment, although not significantly so for plasma [Cl^-] on days 2 and 4. The degree of reduction was greater for plasma Na^+ levels than for plasma Cl^- levels throughout the experiment. Plasma Ca^{2+} level declined 12 h after water acidification, returned to the initial level by day 1, but decreased again on day 7. There was no change in plasma phosphate level (Fig. 1).

Plasma SL levels increased after exposure to acidic water, reached a peak on day 1 and thereafter returned to the initial level by day 4. SL levels were again significantly higher than the initial level on day 7. In contrast, there was no difference in plasma PRL concentrations between the control fish in fresh water and the fish exposed to acidic water. Plasma GH levels in acid-exposed fish were significantly lower than the initial level on days 1, 2 and 4, and significantly lower than the

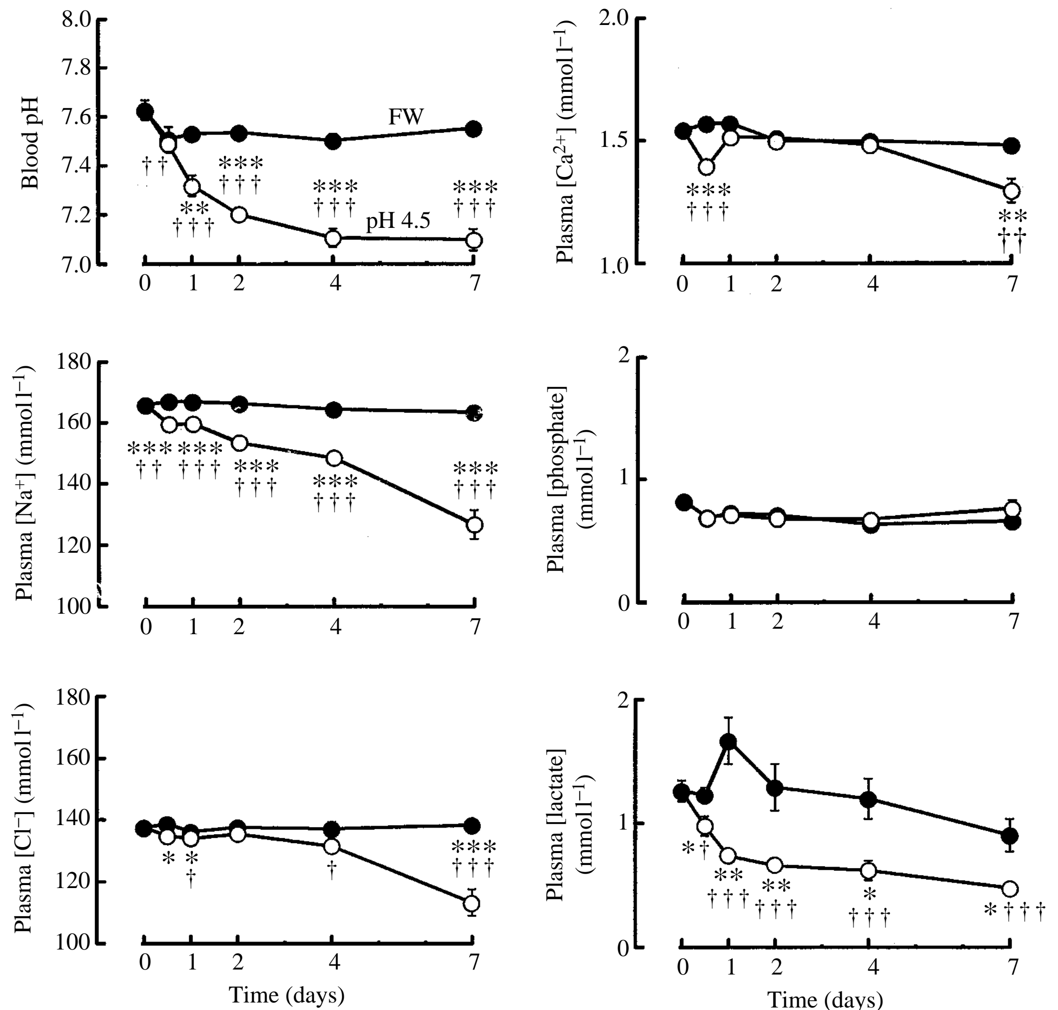

Fig. 1. Blood pH and plasma concentrations of Na^+, Cl^-, Ca^{2+}, phosphate and lactate in rainbow trout after exposure to acidic water (pH 4.5). Each point represents the mean ± S.E.M. (N=10). Error bars are omitted in cases where the values are within the symbols. †$P<0.05$, ††$P<0.01$, †††$P<0.001$, significantly different from the initial value. *$P<0.05$, **$P<0.01$, ***$P<0.001$, significantly different from the value for the control (pH 7.5) fish on the same day. Filled circles, control fish; open circles, acid-exposed fish.

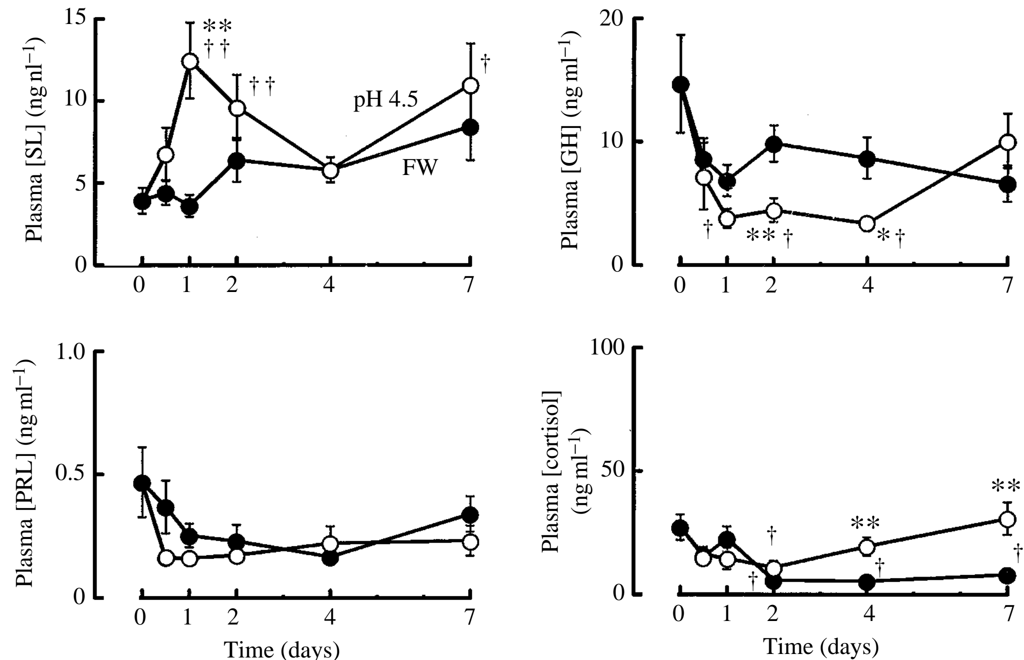

Fig. 2. Plasma concentrations of somatolactin (SL), prolactin (PRL), growth hormone (GH) and cortisol in rainbow trout after exposure to acidic water (pH 4.5). Each point represents the mean ± S.E.M. (N=10). †P<0.05, ††P<0.01, significantly different from the initial value. *P<0.05, **P<0.01, significantly different from the value for the control (pH 7.5) fish on the same day. Filled circles, control fish; open circles, acid-exposed fish.

control level on days 2 and 4. Plasma levels of cortisol in the acid-exposed fish were significantly higher than those in the control fish on days 4 and 7 (Fig. 2).

The levels of SL-, PRL- and GH-mRNA expression in the pituitary of the acid-exposed fish were not significantly different from the initial levels or from the control levels (Fig. 3).

Exhaustive exercise

Exhaustive exercise resulted in a pronounced blood acidosis. Blood pH was maximally depressed immediately after the 8 min of exercise, began to return to the initial level by 3 h after exercise and had returned to the control level after 24 h. There were no changes in plasma levels of Na^+ and Cl^- immediately after the exercise. Plasma [Na^+] was lower than the initial level 24 h after the exercise, and plasma [Cl^-] was lower 3 and 24 h after exercise. Plasma concentrations of Ca^{2+} and phosphate increased and reached maxima immediately after exercise. Plasma [Ca^{2+}] returned to the initial level after 3 h, whereas plasma phosphate level was still above the initial level at 24 h after exercise. Plasma lactate level began to increase immediately after the exercise, reached a peak after 3 h and then returned to the initial level after 24 h (Fig. 4).

Plasma SL levels increased markedly immediately after exercise and returned to the initial level along with the disappearance of acidosis. Plasma cortisol levels also increased immediately after exercise but remained high at the end of the experiment. No change was seen in plasma levels of PRL and GH immediately after exercise. Plasma PRL levels were

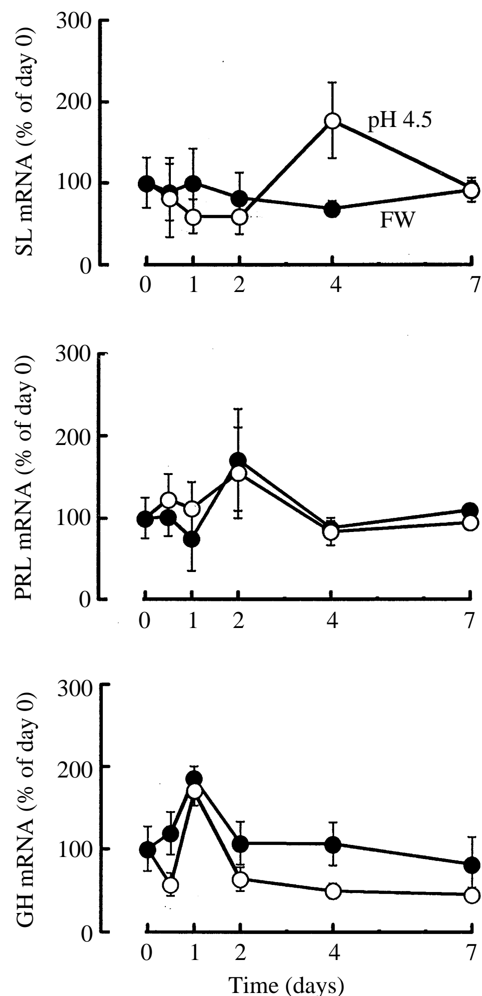

Fig. 3. The levels of somatolactin (SL), prolactin (PRL) and growth hormone (GH) mRNA expression in rainbow trout after exposure to acidic water (pH 4. 5). Each value is expressed as a percentage of the mean initial level. Each point represents the mean ± S.E.M. (N=6). Error bars are omitted in cases where the values are within the symbols. Filled circles, control fish; open circles, acid-exposed fish.

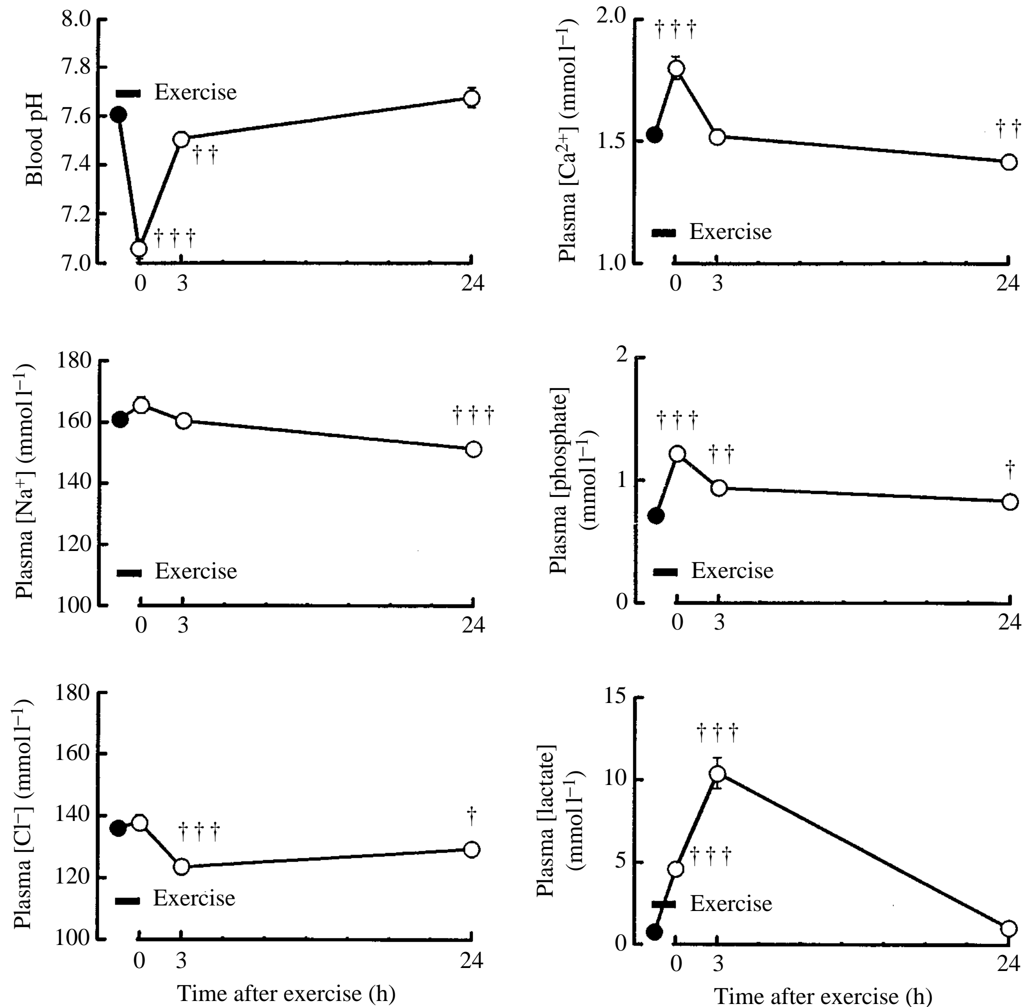

Fig. 4. Blood pH and plasma concentrations of Na^+, Cl^-, Ca^{2+}, phosphate and lactate in rainbow trout before (filled circles) and 0, 3 and 24 h after (open circles) exhaustive exercise (bar). Each point represents the mean ± S.E.M. (N=8). Error bars are omitted in cases where the values are within the symbols. †P<0.05, ††P<0.01, †††P<0.001, significantly different from the value before exercise.

reduced 3 and 24 h after exercise, whereas plasma GH levels increased at 24 h (Fig. 5).

Discussion

Effects of acidosis on plasma SL, PRL, GH and cortisol levels

In the present study, plasma SL levels increased significantly 1 day after water acidification. Although plasma SL levels returned to the initial level on day 4, they were again higher than the initial level on day 7. This may partly reflect an increase in the turnover rate of SL from day 2 to day 4; plasma hormone levels reflect an equilibrium between secretion and consumption. Plasma levels of PRL, in contrast, did not change during acidosis, whereas levels of plasma GH decreased on days 2 and 4 in acid-exposed fish. Plasma cortisol levels in acid-exposed fish were higher than the control levels on days 4 and 7, although plasma cortisol levels did not increase above the initial level in response to water acidification. Plasma SL levels were also elevated immediately after exhaustive exercise, when pronounced acidosis occurred, and the level showed a tendency to decrease along with the disappearance of acidosis. Plasma cortisol levels also increased immediately after the exercise and remained elevated even after the recovery from acidosis. However, plasma PRL and GH levels did not change at the time of acidosis, although plasma PRL levels decreased and plasma GH levels increased after the recovery from exercise. Throughout the two experiments, levels of plasma SL only increased at the time of acidosis. The observation that there was no change in SL-mRNA levels after transfer to acidic water seems to indicate that SL secretion, rather than synthesis, was stimulated in the acid-exposed fish, probably reflecting the nature of the SL cell's response to acidosis induced by water acidification.

Wendelaar Bonga *et al.* (1986) demonstrated an activation of PIPAS cells, possibly SL cells, in goldfish exposed to acidic water. However, no activation of PIPAS cells was seen in tilapia exposed to acidic water, although PRL cells were activated (Wendelaar Bonga *et al.* 1984*a*,*b*). Because blood pH was not measured in these studies, the relationship between the activity of SL cells and the blood acid–base status is not clear. In the present study, an elevation of plasma SL concentration was also seen in exercised trout exposed to water of neutral pH. Thus, blood acidosis, rather than environmental acidification, seems to be the primary stimulus for an increase in plasma SL level.

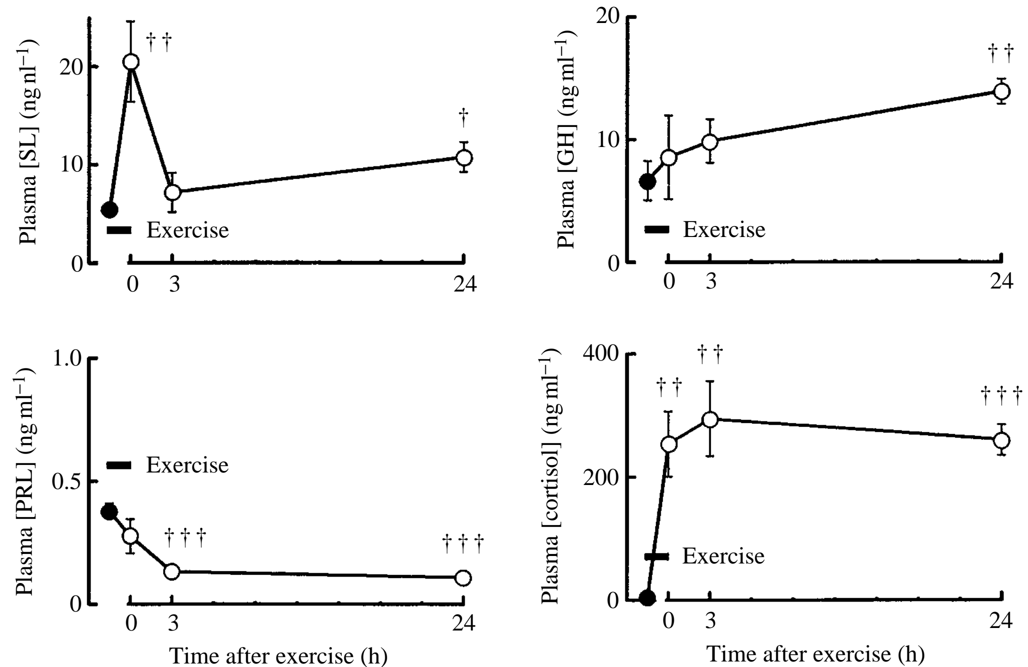

Fig. 5. Plasma concentrations of somatolactin (SL), prolactin (PRL), growth hormone (GH) and cortisol in rainbow trout, before (filled circles) and 0, 3 and 24 h after (open circles) exhaustive exercise. Each point represents the mean ± S.E.M. (N=8). Error bars are omitted in cases where the values are within the symbols. †P<0.05, ††P<0.01, †††P<0.001, significantly different from the value before exercise.

PRL is known to have an important role in adaptation to a hypo-osmotic environment in teleosts (Hirano, 1986; Brown and Brown, 1987). Because acidification of water increases branchial permeability, resulting in a net loss of ions in freshwater fish (Wood, 1989; Perry and Laurent, 1993), an involvement of PRL in the adaptation to an acidic environment has been suggested (Wendelaar Bonga and Balm, 1989; Perry and Laurent, 1993). Activation of PRL cells in response to water acidification was observed in brook trout (Notter *et al.* 1976) and tilapia (Wendelaar Bonga *et al.* 1984*a*,*b*), although no change was seen either in the plasma level or the cellular activity of PRL in rainbow trout exposed to acidic water (Balm *et al.* 1995). The relationship between plasma PRL levels and acidosis is not clear in these previous studies, because blood pH was not measured, and exposure to acidic water does not necessarily result in acidosis. In the present study, there was no elevation of plasma PRL levels or PRL-mRNA expression in rainbow trout during acidosis caused by exposure to acidic water or exhaustive exercise; rather, plasma PRL levels decreased 3 and 24 h after exhaustive exercise. These results indicate that acidosis itself may not be a key stimulus to PRL secretion.

According to Wendelaar Bonga and Balm (1989), an acidic environment had an inhibitory effect on growth and on GH cell activity in tilapia. In the present study, a decrease in plasma GH level was seen in acid-exposed trout, whereas an increase was seen only at 24 h after exhaustive exercise. There were no changes in GH-mRNA levels during acidosis. Thus, GH may not be directly involved in acid–base balance in rainbow trout. The decrease in plasma GH level in acid-exposed fish on days 2 and 4, when a pronounced decrease in plasma [Na^+] was also observed, seems to reflect the involvement of GH in hypo-osmoregulation in salmonids (see Sakamoto *et al.* 1993).

Cortisol is also one of the strong candidates for the hormones involved in adaptation to an acidic environment. Elevation of plasma cortisol levels and/or activated interrenal cells have frequently been observed in teleost species exposed to acidic water (Wendelaar Bonga and Balm, 1989), although Balm and Pottinger (1993) reported that there was no change in plasma cortisol levels after exposure of rainbow trout to acidic water. In the present study, however, plasma levels of cortisol in acid-exposed fish did not increase from the initial level. However, plasma cortisol levels in acid-exposed fish on days 4 and 7 were higher than the control levels, which showed a tendency to decrease even though the constant blood pH and plasma ion levels strongly indicated that the physiological condition of the control fish was stable. One of the reasons for this may be that each fish was bled only once, and the data at each time point were obtained from different fish. In the exhaustively exercised fish, however, a pronounced increase in plasma cortisol levels was observed and the levels stayed high even after the disappearance of acidosis 24 h after exercise. This is in sharp contrast with plasma SL levels, which had fallen to levels only slightly above the initial value by that time. Because cortisol is known to have a role in energy mobilization, mainly through carbohydrate metabolism (McKeown, 1984; Wendelaar Bonga and Balm, 1989), high levels of cortisol in the exercised fish during the recovery period may imply that cortisol is primarily involved in mobilization of the energy consumed during the exhaustive exercise, rather than in acid–base regulation.

Blood acid–base and ionic status

Blood acidosis was observed from 24 h after water acidification until the end of the experiment. Prior to the decrease in blood pH, plasma Na^+ level started to decrease.

Plasma Cl^- levels were less affected by water acidification than were plasma Na^+ levels throughout the experiment. The influence of Ca^{2+} concentration in ambient water on blood acid–base and ionic status has been well studied (see Wood, 1989). In rainbow trout exposed to soft acidic water containing less than $0.2\,mmol\,l^{-1}$ Ca^{2+}, plasma levels of both Na^+ and Cl^- are greatly reduced (McDonald *et al.* 1980) and net losses of Na^+ and Cl^- are almost equal (McDonald, 1983). In contrast, in acidic water containing more than $0.3\,mmol\,l^{-1}$ Ca^{2+}, there was a greater decrease in plasma [Na^+] than in plasma [Cl^-] (McDonald *et al.* 1980; McDonald, 1983). This relationship between the external Ca^{2+} concentration and the extent of plasma Na^+ and Cl^- disturbances is seen over a wide range of environmental Ca^{2+} levels (Wood, 1989). In the present study, the Ca^{2+} concentration of ambient water was about $0.6\,mmol\,l^{-1}$, and our results are in good agreement with those of the previous studies.

A transient decrease in plasma Ca^{2+} concentration was observed 12 h after water acidification. However, the level was restored by 24 h and remained at the same level until day 4. According to Hobe *et al.* (1984), whole-body net loss and efflux of Ca^{2+} are maximal and influx of Ca^{2+} is minimal 12 h after water acidification, and both flux rates return to their initial levels at 24 h. Our data therefore suggest that the effects of an acidic environment on ion permeability and ion fluxes are different for Ca^{2+} and for the monovalent ions Na^+ and Cl^-.

In the present study, a transient but pronounced acidosis was observed following exhaustive exercise. As shown by the marked elevation in plasma lactate level, which was not observed in acid-exposed trout, metabolic acid produced during exercise might be the main cause of acidosis. The acidosis resulted in changes in plasma ionic status, reflecting the compensation of the blood acid–base disturbance.

Plasma Cl^- levels decreased significantly during the period of recovery from exercise. The decreased [Cl^-] could be a result of an increase in electroneutral Cl^-/HCO_3^- exchange, compensating for the acidosis (Wood, 1989; Perry and Laurent, 1993). Na^+ is considered to be electroneutrally exchanged with H^+ (Wood, 1989; Lin and Randall, 1991; Perry and Laurent, 1993). In spite of the pronounced reduction in blood [H^+] (increase in blood pH) during the recovery from acidosis, no significant elevation of plasma [Na^+] was observed in the present study. The acidosis in the exercised trout may be compensated mainly by bicarbonate uptake rather than by H^+ excretion.

Possible physiological function(s) of SL

Previous studies have demonstrated increases in plasma SL levels, activities of SL cells and expression of SL-mRNA under various conditions in salmonids (Rand-Weaver *et al.* 1992, 1993; Rand-Weaver and Swanson, 1993; Kakizawa *et al.* 1993, 1995*a*,*b*; Olivereau and Rand-Weaver, 1994*a*,*b*). These results imply that SL may be a multifunctional hormone. However, it is entirely possible that there is an essential stimulating factor that may be common to the various biological events related to changes in SL levels.

On the basis of the stimulatory effects of low environmental Ca^{2+} levels on the activities of SL cells, a hypercalcaemic action of SL has been suggested in rainbow trout (Olivereau *et al.* 1981*a*,*b*; Kakizawa *et al.* 1993). In the present study, the return of the decreased plasma [Ca^{2+}] or elevation of plasma [Ca^{2+}] and elevation of SL levels coincided with a decrease in blood pH in rainbow trout exposed to acidic water or exercised exhaustively. In trout exposed to acidic water, plasma Ca^{2+} decreased temporarily after 12 h, but returned towards the initial level by day 1, whereas plasma SL levels remained elevated for 2 days. This result suggests a possible involvement of SL in Ca^{2+} metabolism. The simultaneous increase in plasma Ca^{2+} and SL levels in the exercised fish seems to argue against a hypercalcaemic role for SL (Kakizawa *et al.* 1993; Kaneko and Hirano, 1993). The elevation of plasma [Ca^{2+}] in the exercised fish might be partly attributable to dissociation of calcium phosphate in bone and/or scales caused by acidosis (Herrmann-Erlee and Flik, 1989). We conclude that a direct action of SL in Ca^{2+} metabolism is not indicated by the present study.

Elevation of plasma SL levels was observed in coho salmon during gonadal maturation (Rand-Weaver *et al.* 1992; Rand-Weaver and Swanson, 1993) and in chum salmon during the spawning migration (Kakizawa *et al.* 1995*b*). Activation of SL cells was seen in chinook, chum and sockeye salmon during the spawning migration (Olivereau and Rand-Weaver, 1994*a*,*b*). However, information on blood acid–base and ionic status in these experimental fish is very limited, but significant reductions in plasma Na^+ and Ca^{2+} levels in chum salmon after entry to a river have been described (Kakizawa *et al.* 1995*b*). Salmonids need significant energy mobilization during their spawning migration and final maturation (Olivereau and Rand-Weaver, 1994*a*,*b*; Kakizawa *et al.* 1995*b*). According to Tang *et al.* (1989), acidosis was more marked in rainbow trout exercised in fresh water than in sea water. They ascribed the difference to high concentrations of basic equivalents (HCO_3^-) and of other exchangeable ions (Na^+ and Cl^-) in sea water compared with fresh water. It could thus be speculated that SL levels increased during gonadal maturation in response to a metabolic acidosis caused by an increased energy mobilization during the spawning migration and final maturation.

An increase in plasma SL concentration was also observed in rainbow trout during acute stress (Rand-Weaver *et al.* 1993; Kakizawa *et al.* 1995*a*). Although blood acid–base status was not examined in these studies, responses of plasma ionic status and hormone levels in the stressed trout were similar to those in the present study. Because plasma ionic status influences blood acid–base balance, it is probable that an acidosis occurred in the stressed fish.

In summary, plasma SL level was increased during acidosis induced by water acidification or exercise. These results suggest that SL may be involved in acid–base regulation in rainbow trout.

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