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POSSIBLE INVOLVEMENT OF SOMATOLACTIN IN THE REGULATION OF PLASMA BICARBONATE FOR THE COMPENSATION OF ACIDOSIS IN RAINBOW TROUT

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Summary

Somatolactin is a putative pituitary hormone of the growth hormone/prolactin family in fish. Its function is still unknown. The effects of environmental hypercapnia and hypoxia, acid (HCl) infusion and exhaustive exercise on plasma somatolactin levels were examined in the chronically cannulated rainbow trout to study the possible physiological roles of somatolactin. Respiratory acidosis induced by hypercapnia (2 % CO_2) did not affect plasma somatolactin level. In contrast, metabolic acidosis induced by acid infusion and exercise increased plasma somatolactin level. Blood pH was depressed to a similar extent by both types of acidosis, whereas plasma [HCO_3^-] was elevated by respiratory acidosis but reduced by metabolic acidosis. A moderate hypoxia (water P_{O_2} 9.3 kPa) affected neither acid–base status nor plasma somatolactin level. A more severe hypoxia (water P_{O_2} 6.1 kPa) resulted in metabolic acidosis accompanied by an apparent rise in plasma somatolactin level, although the difference in somatolactin level from the control value was not statistically significant. Somatolactin immunoneutralization retarded recovery of plasma [HCO_3^-] following acid infusion. These results indicate that somatolactin is involved in the retention of HCO_3^- during metabolic acidosis but not in the active accumulation of HCO_3^- for acid–base compensation of respiratory acidosis in rainbow trout *Oncorhynchus mykiss*.

Key words: somatolactin, bicarbonate, metabolic acidosis, respiratory acidosis, hypercapnia, acid infusion, exercise, hypoxia, rainbow trout, *Oncorhynchus mykiss*.

Introduction

Somatolactin is a putative pituitary hormone, whose presence has been confirmed only in fish. The name was devised because the protein is structurally related to both growth hormone (somatotropin) and prolactin.

Although a definitive physiological function for somatolactin has yet to be determined, changes in plasma levels of somatolactin and the activity of somatolactin cells during various biological processes have been described in previous studies (see Kaneko and Hirano, 1993; Kaneko, 1996). In salmonids, an involvement of somatolactin in the regulation of gonadal function was suggested by an elevation of plasma somatolactin levels (Rand-Weaver *et al.* 1992; Rand-Weaver and Swanson, 1993) and by activation of immunoreactive somatolactin cells (Olivereau and Rand-Weaver, 1994*a*,*b*) during the spawning season. Plasma somatolactin levels in rainbow trout (*Oncorhynchus mykiss*) increased in response to stress (Rand-Weaver *et al.* 1993; Kakizawa *et al.* 1995). Activation of somatolactin cells was observed in rainbow trout transferred from calcium-rich to calcium-poor water (Kakizawa *et al.* 1993). Furthermore, an involvement of somatolactin in lipid metabolism has also been suggested in a cobalt variant of rainbow trout, which lacks somatolactin-producing cells and accumulates large amounts of abdominal fat (Kaneko *et al.* 1993). In addition, an elevation of plasma somatolactin level during adaptation to a dark background was observed in red drum (*Sciaenops ocellatus*) (Zhu and Thomas, 1995).

We recently found that plasma somatolactin concentration was increased during acidosis induced by water acidification or exhaustive exercise (Kakizawa *et al.* 1996). Water acidification directly raises plasma [H^+] (lowers pH) by affecting transepithelial ion transfer (Wood, 1989), whereas exhaustive exercise entails acidosis caused by dissociation of the lactic acid formed anaerobically. Plasma lactate levels were

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markedly increased after exercise (Kakizawa *et al.* 1996). Water acidification can also accompany a reduction of blood oxygen levels (Packer, 1979). Thus, it is possible that somatolactin was secreted in response to hypoxaemia.

In the present study, we studied the effects of environmental hypercapnia, two levels of hypoxia, acid infusion and exhaustive exercise on plasma somatolactin levels and acid–base status using chronically cannulated rainbow trout. The partial pressure of oxygen (P_{O_2}) and oxygen content were also monitored in the hypoxia experiments to evaluate the importance of blood oxygen level for somatolactin secretion. Furthermore, plasma somatolactin levels were artificially depressed by immunoneutralization, and the effect of acid infusion on acid–base status in these fish was compared with that in control trout.

Materials and methods

Experimental animals

In all experiments except for environmental hypoxia, immature rainbow trout *Oncorhynchus mykiss* (Walbaum), weighing 800–1200 g, were obtained from Shizuoka Prefectural Fisheries Experimental Station, Japan, and reared in a stock tank with recirculating fresh water (pH 7.5; 0.6 mmol l^{-1} Ca^{2+}; 1.0 mmol l^{-1} Na^+; 1.0 mmol l^{-1} Cl^-) at 12 °C for more than 3 weeks before use. Fish were fed commercial trout pellets (Oriental no. 6, Chiba, Japan) at a ration equivalent to 1 % of body mass per day until 2 days before experiments. The hypoxia experiment was conducted at the National Research Institute of Aquaculture, Nikko Branch, where mature rainbow trout of both sexes weighing 700–1400 g were reared in flowing fresh water at 9 °C.

Surgery

Each fish was anaesthetized by immersion in a solution of 0.01 % tricaine methane sulphonate (MS-222, Sigma, St Louis, MO, USA) in tap water buffered with $NaHCO_3$. During surgery, the gills were irrigated to maintain anaesthesia with a solution of 0.005 % MS-222, aerated with pure O_2.

The dorsal aorta was cannulated using a catheter (PE50, Clay Adams, Parsippany, NJ, USA) by application of a modified Seldinger technique, similar to the procedure of Soivio *et al.* (1975). After surgery, the fish was allowed to recover for at least 20 h in a recirculating water system consisting of fish chambers and a 200 l reservoir tank, or in a flow-through water system consisting of fish chambers and a 150 l tank (hypoxia experiment). The water in the tank was vigorously aerated. The fish chambers were covered to prevent visual disturbance of the fish.

Blood sampling

Arterial blood (1.0 ml or 1.5 ml in the hypoxia experiment) was sampled through a catheter into a 1 ml glass syringe whose dead space was filled with heparinized (lithium salt, Sigma, 50 i.u. ml^{-1}) saline. 0.2 ml (or 0.5 ml in the hypoxia experiment) was used for pH (and P_{O_2}) determinations. The remaining blood was centrifuged at 10 000 ***g*** for 5 min at 4 °C to obtain plasma. The plasma was used for measurements of total CO_2 (T_{CO_2}), Na^+, K^+, Ca^{2+}, Cl^- and somatolactin levels. In the hypoxia experiment, the O_2 content of whole blood was also determined. Plasma samples for measurement of ion and somatolactin levels were stored at –80 °C until analysis. Blood used for P_{O_2} and pH measurements was reinfused through the cannula after analysis. The red blood cells obtained after centrifugation were suspended in heparinized (sodium salt, Sigma, 50 i.u. ml^{-1}) saline containing 1.2 mmol l^{-1} Ca^{2+} (Ca^{2+}-saline) and also reinfused into the fish.

Experimental protocols

Environmental hypercapnia

After two control samples had been taken 2 h apart, the system water was equilibrated with 2 % CO_2, and arterial blood samples were taken subsequently 1, 3, 6, 24 and 48 h after the onset of hypercapnia and 1, 3 and 24 h post-hypercapnia.

Acid infusion

The protocol was essentially the same as described by Tang *et al.* (1988). After two control samples had been taken 2 h apart, the fish was infused with 0.25 ml per 100 g body mass of 0.1 mmol l^{-1} HCl in Ca^{2+}-saline, followed by a Ca^{2+}-saline wash (0.1 ml per 100 g body mass). The infusion rate was 0.5 ml min^{-1} (infusion took 4–6 min). The time when the acid infusion finished was defined as time zero. Arterial blood samples were taken 5, 30 and 120 min post-infusion.

Exhaustive exercise

The protocol was essentially the same as described by Kakizawa *et al.* (1995). After two control samples had been taken, the fish was forced to swim by chasing it for approximately 20 min in a 1000 l water tank until it failed to respond to further stimulation. An arterial blood sample was taken immediately after the exercise. All fish recovered 5–10 min after chasing.

Environmental hypoxia

After two control samples had been taken (2 h apart), water in the flow-through system was bubbled with N_2 to achieve a final water P_{O_2} (Pw_{O_2}) of 9.3 kPa (first experiment) or 6.1 kPa (second experiment). Subsequently, arterial blood samples were taken 1 and 2 h after the onset of hypoxia, and 1 and 15 h post-hypoxia.

Acid infusion into immunoneutralized fish

To decrease plasma somatolactin levels, the fish was infused with 0.1 ml per 100 g body mass of specific rabbit anti-salmon somatolactin serum, diluted with Ca^{2+}-saline (1:10), after two control samples had been taken. The infusion rate was 0.5 ml min^{-1} (infusion took 100–140 s). The first sample (1.0 ml) was taken 3 min after the infusion ended. 17 min after the first sample had been taken (20 min after the antiserum infusion), the fish was infused with acid, and blood samples were taken as described above for the acid infusion.

Analytical methods

Arterial blood pH and P_{O_2} were determined using pH (E301/E351, Cameron Instruments, Port Aransas, TX, USA) and P_{O_2} (E101, Cameron Instruments) electrodes, adjusted to the water temperature. T_{CO_2} was determined using a Capni-con V (Cameron Instruments). Plasma P_{CO_2} was calculated from the measured pH and T_{CO_2} values, using the Henderson–Hasselbalch equation with appropriate constants (Boutilier *et al.* 1985). Plasma [HCO_3^-] was calculated by subtracting the fraction of molecular CO_2 from T_{CO_2}. Plasma O_2 content was monitored using an Oxycon (Cameron Instruments).

Plasma levels of Na^+, K^+ and Ca^{2+} were determined using an electrolyte analyzer (AVL 984-S, Graz, Austria) and Cl^- levels using a chloridometer (Buchler 4-2500, USA).

Plasma concentrations of somatolactin were measured by the specific radioimmunoassay described by Kakizawa *et al.* (1993). The immunoglobulin G (IgG) in the plasma obtained from the immunoneutralized fish was completely removed using Protein A (IgG-sorb, The Enzyme Center, Malden, MA, USA), which did not affect the plasma somatolactin assay. The minimal detected level was 0.5 ng ml^{-1}, the ED_{50} was 11.7 ng ml^{-1}, the intra-assay coefficient of variation was 2.5 % (N=10) and the inter-assay coefficient of variation was 3.9 % (N=10).

Statistical analysis

Dunnett's test was used to determine statistically significant differences between the mean control values and other values in the same experimental series. Student's t-tests or paired t-tests (effects of exhaustive exercise) were applied after Bartlett's test for variance to detect statistically significance differences between control and experimental groups at the same time point in the experiments for acid infusion and immunoneutralization. Data are presented as means ± S.E.M. A level of P<0.05 was considered significant.

Results

Environmental hypercapnia

The pH decreased by approximately 0.5 units from the control level of 7.96±0.09 to 7.47±0.05 at 1 h, and then gradually increased to 7.70±0.05 at 48 h, although the latter value was still significantly lower than the control value. The pH compensation resulted from an increase in plasma [HCO_3^-] from 14.3±1.3 mmol l^{-1} at 1 h to 33.1±4.4 at 48 h. Blood P_{CO_2} remained at approximately 1.7 kPa throughout the hypercapnic period. Upon recovery, blood P_{CO_2} declined rapidly, while plasma [HCO_3^-] slowly returned to the control level. The discrepancy in the time course of changes in P_{CO_2} and [HCO_3^-] resulted in a transient alkalosis at 3 h post-hypercapnia (Fig. 1).

There was no significant change in the plasma concentration of somatolactin, which remained within a narrow range of values (4.2–6.7 ng ml^{-1}) throughout the experiment. Plasma [Na^+] increased transiently at 1 h, returned to the control level by 3 h, and decreased at 72 h. Plasma [Cl^-] was significantly reduced (115±6 mmol l^{-1}) below the control level (127±5 mmol l^{-1}) at 24 h, remained low until hypercapnia ended, and returned to the control level at 72 h (24 h after hypercapnia). Plasma [Ca^{2+}] showed a similar time course of change. Plasma [K^+] showed a transient but significant decrease at 1 h and increase at 49 h (1 h after hypercapnia) (Fig. 2).

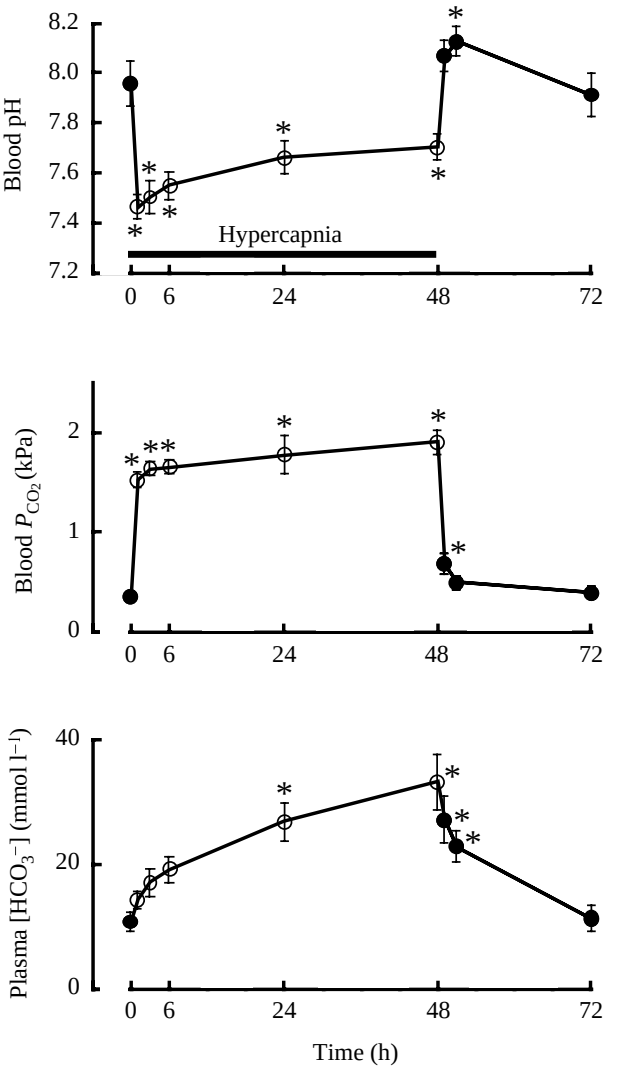

Fig. 1. Effects of environmental hypercapnia on blood pH, P_{CO_2} and plasma [HCO_3^-] in rainbow trout. Each point represents the mean ± S.E.M. (N=6). Error bars are omitted in cases where the values are within the symbols. *Significantly different from control values (time 0), P<0.05.

Acid infusion

Blood pH in acid-infused fish decreased significantly from the control level of 7.97±0.03 to 7.75±0.04 at 5 min, but then recovered quickly. The changes in P_{CO_2} were insignificant. Plasma [HCO_3^-] was significantly lower than the initial level at 5 and 30 min. Plasma somatolactin level increased sharply in response to the acid infusion and became significantly higher

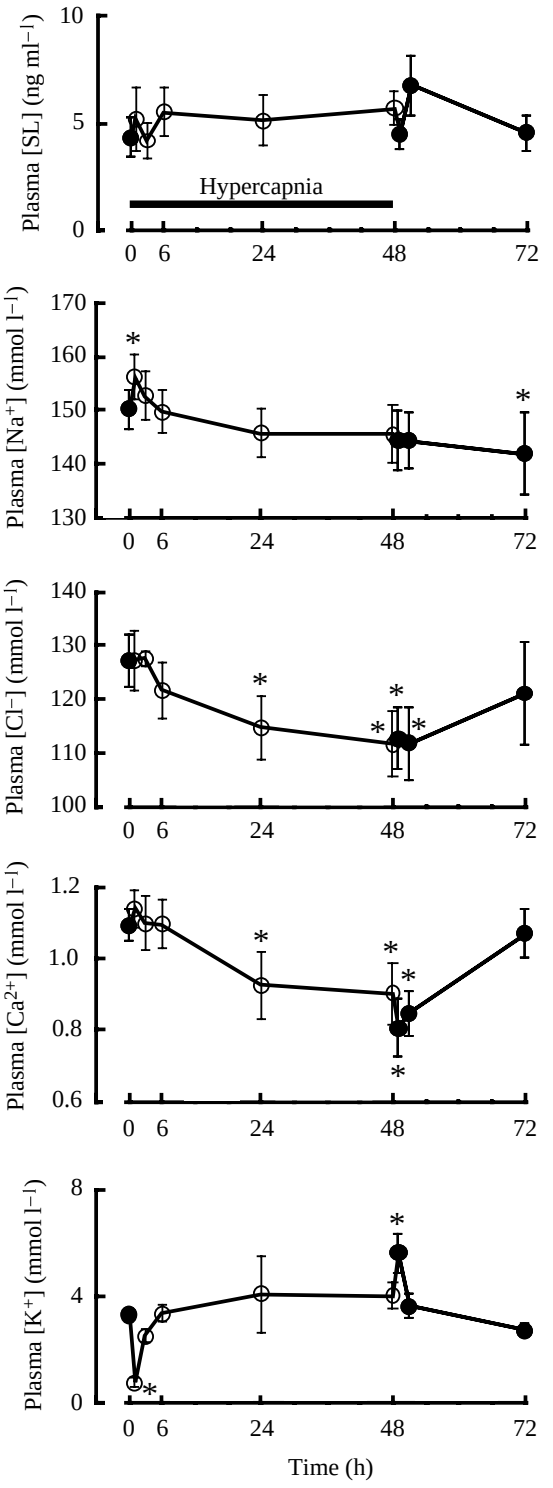

Fig. 2. Effects of environmental hypercapnia on plasma concentrations of somatolactin (SL), Na^+, Cl^-, Ca^{2+} and K^+ in rainbow trout. Each point represents the mean ± S.E.M. (N=6). Error bars are omitted in cases where the values are within the symbols. *Significantly different from control values (time 0), P<0.05.

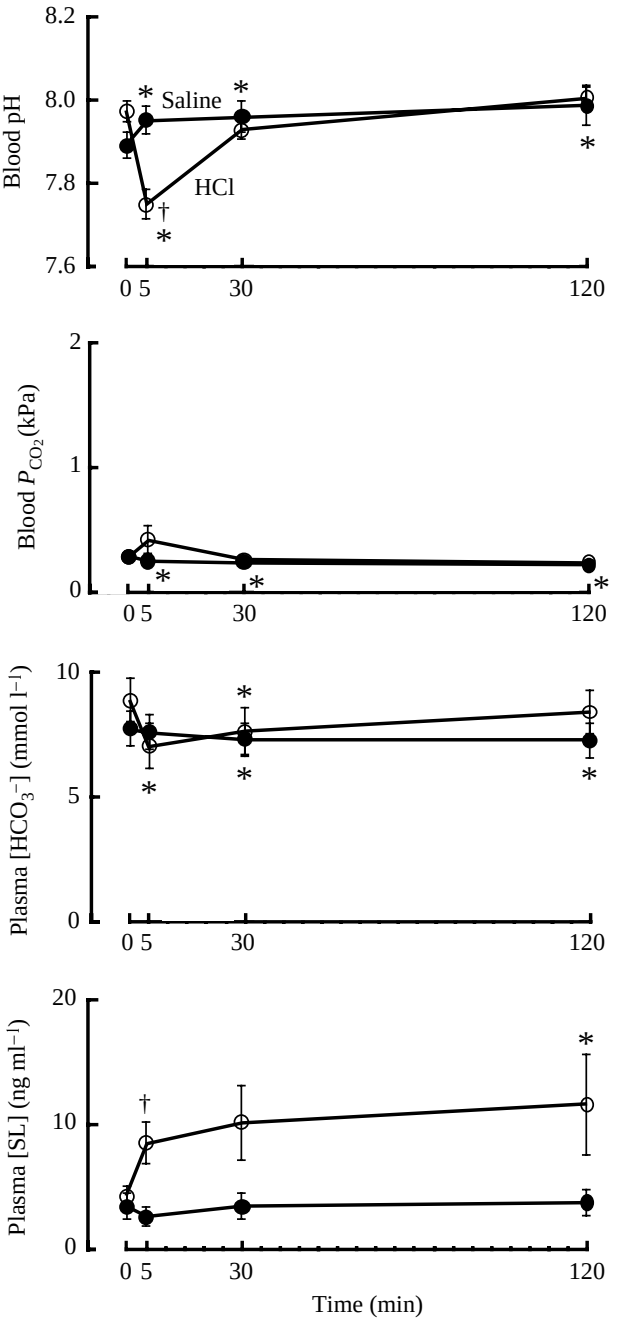

Fig. 3. Effects of saline or acid (HCl) infusion on blood pH and P_{CO_2} and plasma concentrations of HCO_3^- and somatolactin (SL) in rainbow trout. Each point represents the mean ± S.E.M. Filled circles, saline-injected fish (Saline), N=8; open circles, HCl-injected fish (HCl), N=12. Error bars are omitted in cases where the values are within the symbols. *Significantly different from initial values, P<0.05; †Significantly different from corresponding values for the control (saline-infused) group, P<0.05.

than in saline-injected fish at 5 min, but the difference from the initial level was significant only at 120 min. Sham infusion with Ca^{2+}-saline caused small, but significant, changes in plasma pH, [HCO_3^-] and P_{CO_2}, but not in somatolactin level (Fig. 3).

Plasma [K^+] was significantly lower and plasma [Ca^{2+}]

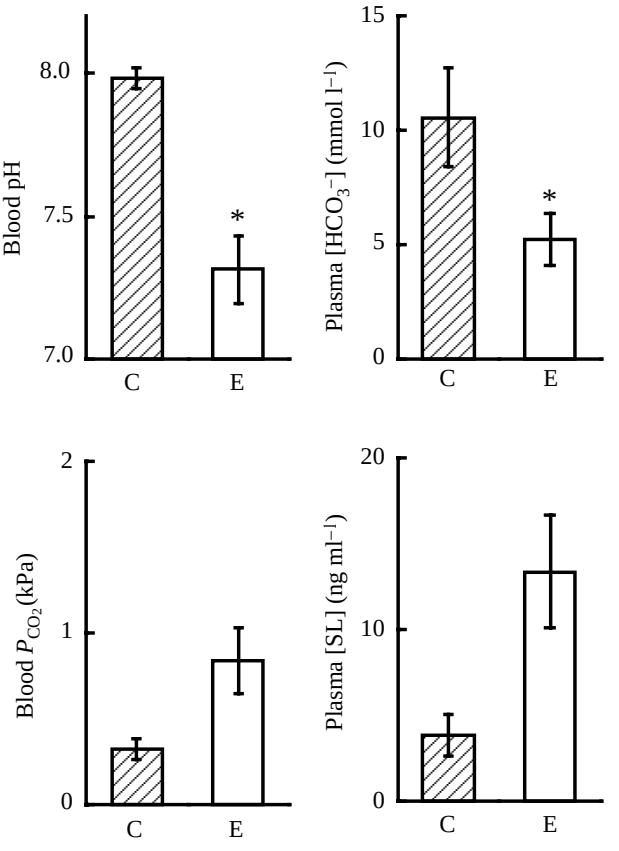

Fig. 4. Effects of exhaustive exercise on blood pH and P_{CO_2} and plasma concentrations of HCO_3^- and somatolactin (SL) in rainbow trout. Each point represents the mean ± S.E.M. (N=4). *Significantly different from the values before exercise (hatched bar), P<0.05. C, control; E, exercised.

significantly higher at 5 min, but neither was significantly different from the control level at 30 min (data not shown).

Exhaustive exercise

Fig. 4 shows effects of exhaustive exercise on blood pH and P_{CO_2} and plasma levels of [HCO_3^-] and somatolactin. The exhaustive exercise resulted in a pronounced and significant decrease in blood pH (from 7.98±0.04 to 7.31±0.12), whereas P_{CO_2} tended to increase (from 0.33±0.06 to 0.85+0.19 kPa), although the increase was not significant (P=0.088). Plasma [HCO_3^-] decreased by more than 50 % (from 10.6±2.2 to 5.2±1.2 mmol l^{-1}). Plasma somatolactin concentration tended to increase from 3.9±1.3 ng ml^{-1} before exercise to 13.4±3.3 ng ml^{-1} immediately after exercise, although the change was not significant (P=0.068). Plasma [Ca^{2+}] also increased from 1.13±0.04 to 1.54±0.16 mmol l^{-1} (P=0.05) (data not shown). In contrast, plasma levels of Na^+, Cl^- and K^+ did not change significantly in response to exhaustive exercise (data not shown).

Environmental hypoxia

The two different levels of environmental hypoxia resulted in different changes in blood gas status and plasma factors.

As is shown in Fig. 5, an environmental hypoxia of 9.3 kPa Pw_{O_2} resulted in significant decreases in blood P_{O_2}, O_2 content and P_{CO_2}. Blood P_{O_2} and P_{CO_2} returned to the initial level by 1 h post-hypoxia, while blood O_2 content was still significantly lower than the control value at this time. Blood pH and plasma [HCO_3^-] and somatolactin levels were not affected by the treatment. Plasma levels of Na^+, K^+, Ca^{2+} and Cl^- were also unaffected (data not shown).

Fig. 6 shows the effects of a more severe environmental hypoxia of 6.1 kPa Pw_{O_2} on blood and plasma factors. Blood P_{O_2} decreased by 90 % and plasma O_2 content by 60–80 % during the hypoxia. Blood pH became significantly lower than the initial level at 1 h after the onset of the hypoxia (a reduction from 7.84±0.03 to 7.56±0.05). Plasma [HCO_3^-] was also lower than the initial level at 2 h (8.7±0.7 mmol l^{-1} initially, 5.1±0.3 mmol l^{-1} at 2 h). There was no change in plasma P_{CO_2}. Plasma somatolactin levels showed a tendency to increase after the onset of the more severe hypoxia and reached a peak at 2 h, although the change was not significant. A significant decrease in plasma [K^+] occurred at 2 h post-hypoxia, but no significant change was seen in plasma Na^+, Cl^- and Ca^{2+} levels throughout the experiment (data not shown).

Acid infusion into immunoneutralized fish

Infusion of anti-somatolactin serum markedly depressed plasma somatolactin level (Fig. 7). The somatolactin level 20 min after the antiserum infusion (time 0, 2.0±0.6 ng ml^{-1}) was significantly lower than the initial (time 0) level in the untreated fish (fish not subjected to antiserum infusion) (4.2±0.8 ng ml^{-1}). Until 30 min after the acid infusion, somatolactin levels in antiserum-infused fish remained significantly lower (1.8±0.9 ng ml^{-1} at 30 min) than in the untreated fish. Somatolactin levels in antiserum-infused fish did not increase significantly from the initial level even 120 min after the acid infusion. Blood pH in both groups was significantly lower than the initial levels at 5 min and then recovered by 30 min. There was no significant difference in either blood pH or P_{CO_2} between the two groups throughout the experiment. Plasma [HCO_3^-] in the immunoneutralized fish was significantly lower than in the untreated fish at 30 and 120 min.

Except for the higher Ca^{2+} level in the immunoneutralized fish at 120 min, there was no difference in plasma levels of Na^+, Cl^-, Ca^{2+} and K^+ between the two groups of fish throughout the experiment. A transient increase in plasma [Cl^-] in immunoneutralized fish and a decrease in plasma [K^+] in both groups occurred at 5 min (data not shown).

In the results described above, plasma somatolactin level was found to be elevated along with the decrease in plasma [HCO_3^-] induced by transient stimulations, namely acid infusion and exhaustive exercise. Thus, the relationship between plasma [HCO_3^-] and somatolactin level at 5 min after saline or acid infusion and just after exhaustive exercise was examined. There was a significant negative correlation (N=24, r=–0.497; P<0.05) between plasma [HCO_3^-] and somatolactin

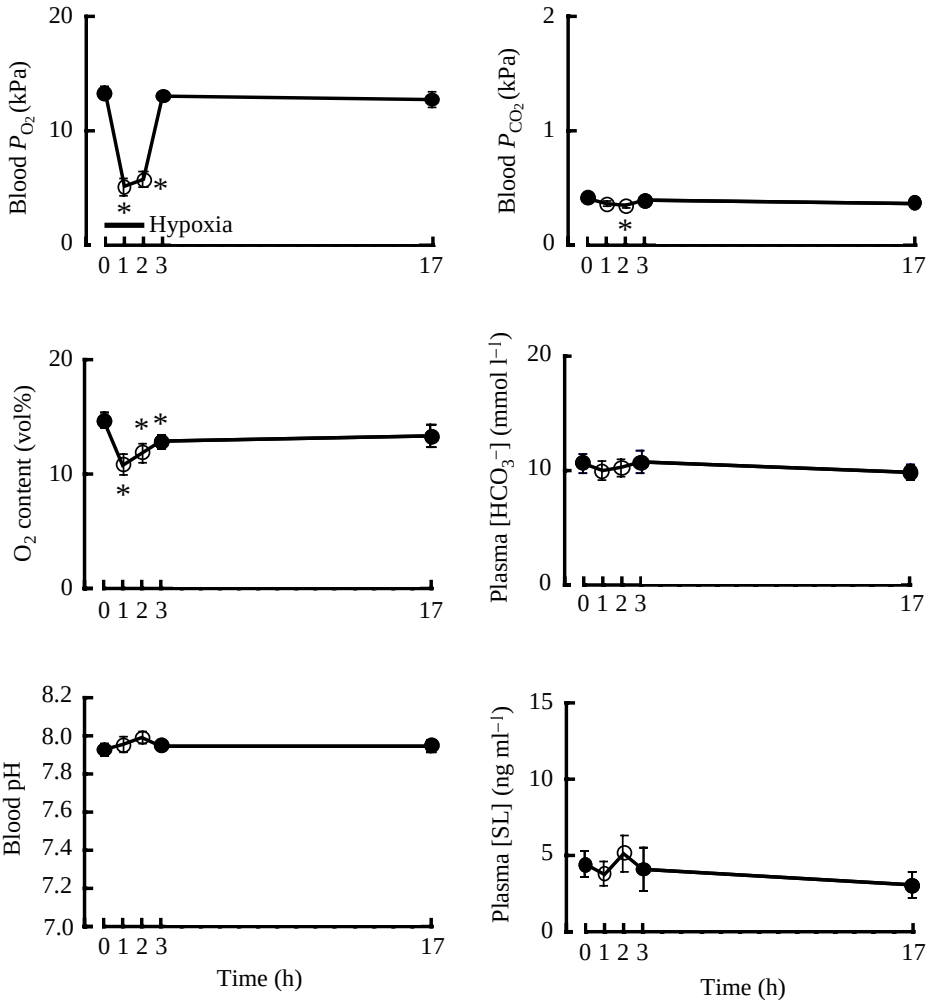

Fig. 5. Effects of an environmental hypoxia of 9.3 kPa P_{WO_2} on blood P_{O_2}, O_2 content, pH and P_{CO_2} and plasma concentrations of HCO_3^- and somatolactin (SL) in rainbow trout. Each point represents the mean ± S.E.M. (N=7). Error bars are omitted in cases where the values are within the symbols. *Significantly different from control values (time 0), P<0.05.

level (each value is given as a percentage of the initial level), as shown in Fig. 8.

Discussion

Elevation of plasma somatolactin level during acidosis in rainbow trout was first observed in our previous study (Kakizawa *et al.* 1996), in which acidosis was induced by water acidification or exhaustive exercise. In the present study, acidosis was also induced by environmental hypercapnia, severe hypoxia, acid infusion or exhaustive exercise. However, plasma somatolactin levels were increased only when metabolic acidosis was induced by acid infusion, exhaustive exercise or severe hypoxia, but not when respiratory acidosis resulted from environmental hypercapnia.

Environmental hypercapnia induced a rise in blood P_{CO_2}. Elevated CO_2 levels resulted in increases in [H^+] and [HCO_3^-], and thereby depressed blood pH with a concomitant rise in [HCO_3^-] (respiratory acidosis). Plasma somatolactin levels were not affected under these conditions. The sustained increase in [HCO_3^-] during the hypercapnic period seems to be due to uptake of HCO_3^- from or proton extrusion into the ambient water, mainly through the branchial epithelium (Heisler, 1993*a*). In contrast, acid infusion, exhaustive exercise or severe hypoxia induced an increase in [H^+] directly or indirectly by dissociation of non-volatile acids. Part of the excess [H^+] is buffered by bicarbonate in the body fluids, thereby depressing [HCO_3^-] (metabolic acidosis). Plasma somatolactin level tended to increase in response to these treatments. The moderate hypoxia did not affect the acid–base status or the plasma somatolactin level. Although the changes in plasma somatolactin levels were not significant after exhaustive exercise or during severe hypoxia, the lack of significance is likely to be due to the small number of fish used. Large individual variations in plasma somatolactin levels resulted in the large standard deviations shown in Figs 4 and 6. However, if the data are examined closely from fish to fish, plasma somatolactin levels increased in all fish during acidosis induced by both exhaustive exercise (150–920 % of the initial levels) and severe hypoxia (140–690 %). Thus, plasma somatolactin levels were found to increase only when a depression of pH was due to metabolic causes where [HCO_3^-] was reduced, but not in response to respiratory causes where [HCO_3^-] was raised. These results suggest that somatolactin release was triggered by the reduction of [HCO_3^-] but not by the fall in pH *per se*.

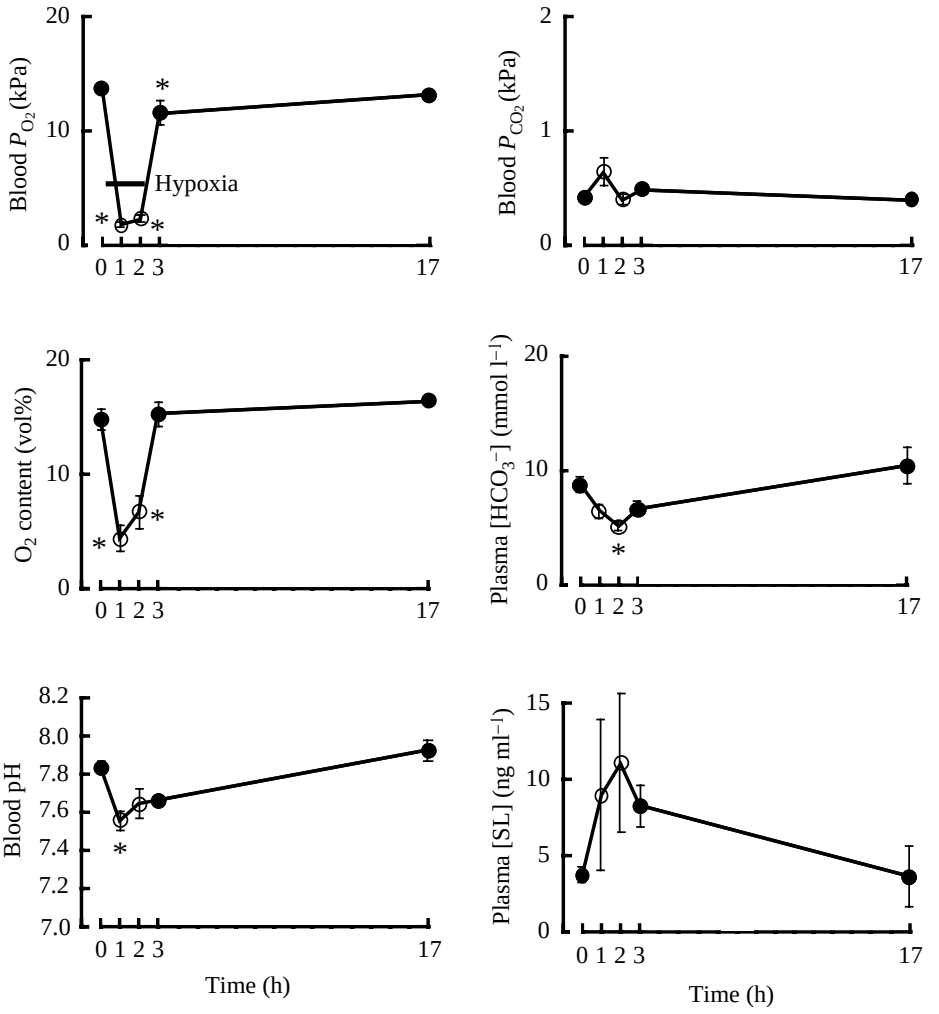

Fig. 6. Effects of an environmental hypoxia of 6.1 kPa Pw_{O_2} on blood P_{O_2}, O_2 content, pH and P_{CO_2} and plasma concentrations of HCO_3^- and somatolactin (SL) in rainbow trout. Each point represents the mean ± S.E.M. (N=3). Error bars are omitted in cases where the values are within the symbols. *Significantly different from control values (time 0), P<0.05.

The hypoxia experiment also allows us to examine whether reductions in blood P_{O_2} and/or O_2 content can influence plasma somatolactin levels. The major differences between mild and severe hypoxia are the extent of the reductions of blood P_{O_2} and O_2 content and the development of metabolic acidosis. Therefore, it is possible that there is a threshold of blood P_{O_2} and/or O_2 content below which a release of somatolactin is enhanced; the drop in blood oxygen level during mild hypoxia may not have been large enough to evoke somatolactin secretion. However, we consider this unlikely for the following reasons. Holeton *et al.* (1983) reported increases in both P_{O_2} and O_2 content in rainbow trout forced to undergo vigorous activity by electrical shock. The fish showed a plasma pH decrease of 0.5 units, with blood lactate levels being considerably elevated until 8 h after exercise. Burst swimming until exhaustion reduced blood P_{O_2} by approximately 4 kPa but maintained O_2 content owing to a 40 % rise in haematocrit. Blood pH dropped by approximately 0.3 units, with a sharp rise in lactate level (Primmett *et al.* 1986). In Atlantic salmon exhausted by manual chasing (the same protocol used in the present study), blood P_{O_2} and O_2 content were decreased transiently by only 1.6 kPa for P_{O_2} and 2.3 vol% (or by 20 % from the control value) for O_2 content. Blood oxygen levels were restored by 0.5 h after exercise, whereas plasma lactate levels remained elevated even at 8 h (Tufts *et al.* 1991). Overall, exhaustive exercise appears to influence blood oxygen levels only slightly. Our finding that plasma somatolactin level was increased by exercise thus appears to contradict the idea that blood oxygen level may be important for the release of somatolactin. The above studies as well as our previous study (Kakizawa *et al.* 1996) have demonstrated consistent increases in blood lactate levels in response to exhaustive exercise. Therefore, we cannot dismiss the possibility that anaerobiosis, not necessarily accompanied by reductions in blood oxygen level, constitutes a stimulus for the release of somatolactin.

The hypothesis that somatolactin release is triggered by the decrease in plasma [HCO_3^-] led us to investigate the effects of somatolactin deficiency on acid–base balance, in particular on plasma [HCO_3^-]. Plasma somatolactin level was decreased 3 min after infusion of anti-somatolactin serum and remained low until 120 min after acid infusion, thus confirming successful immunoneutralization of somatolactin in the antiserum-infused fish. The effect of somatolactin deficiency on acid–base regulation was obvious; plasma [HCO_3^-] in the immunoneutralized fish was significantly lower than in the fish without immunoneutralization 30 and 120 min after acid

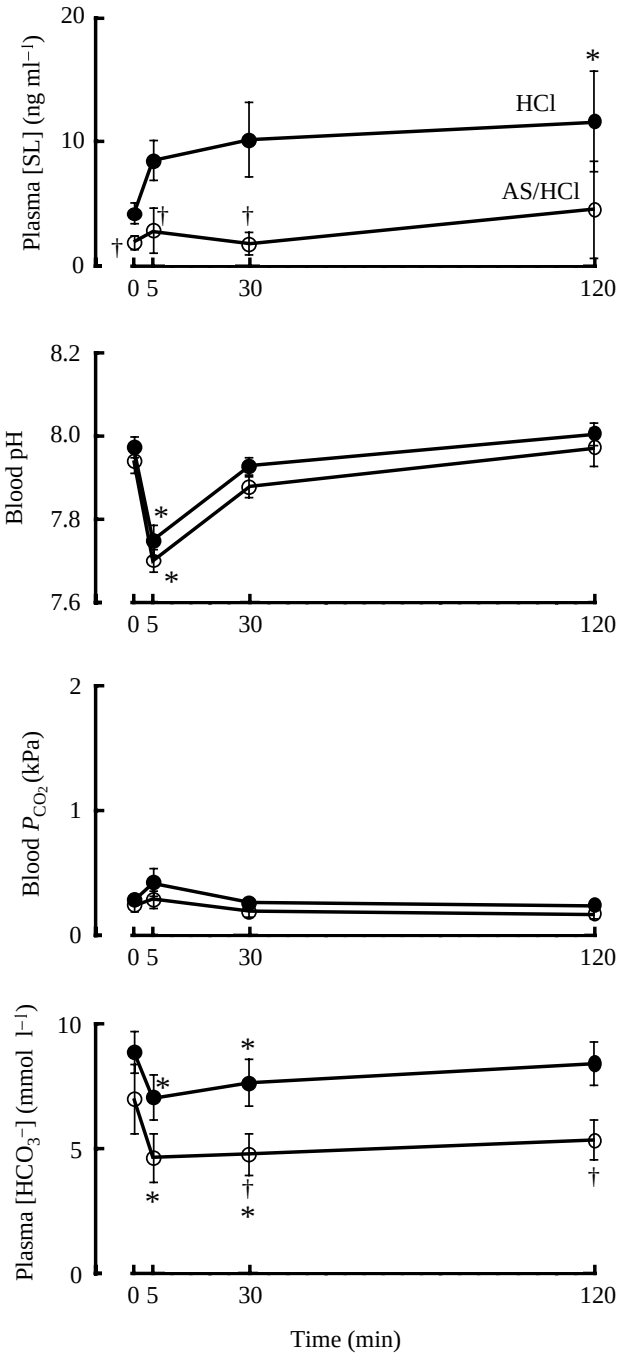

Fig. 7. Changes in plasma somatolactin (SL) concentration, blood pH, blood P_{CO_2} and plasma [HCO_3^-] in rainbow trout before (time 0) and after acid (HCl) infusion into fish infused with anti-somatolactin serum (AS/HCl) or without antiserum infusion (HCl). Data for fish without antiserum injection are the same as for the acid-infused fish in Fig. 4. Each point represents the mean ± S.E.M. (filled circles, HCl, N=12; open circles, AS/HCl, N=4). Error bars are omitted in cases where the values are within the symbols. *Significantly different from initial (time 0) values, †P<0.05; significantly different from the corresponding control values (without antiserum injection), P<0.05.

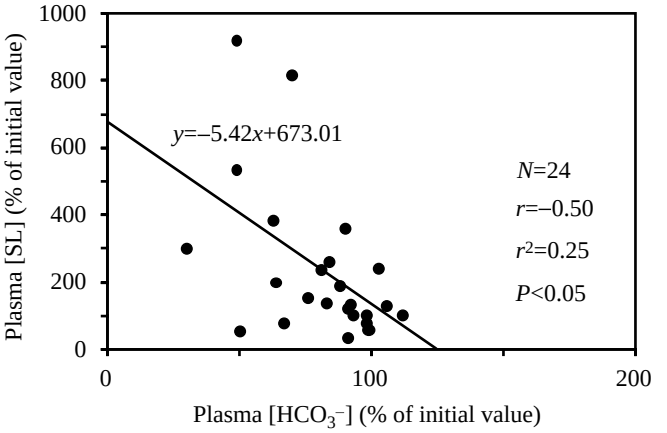

Fig. 8. The relationship between plasma HCO_3^- and somatolactin (SL) concentrations in rainbow trout 5 min after saline or acid infusion or immediately after exhaustive exercise. Each value is expressed as a percentage of the initial value (N=24).

infusion, and no significant recovery in plasma [HCO_3^-] was seen in the immunoneutralized fish. The results also suggest the involvement of somatolactin in the regulation of plasma [HCO_3^-] during acid–base regulation.

The mechanisms of acid–base compensation in fish are still controversial (for recent reviews, see Goss *et al.* 1995; Lin and Randall, 1995). What is evident is that fish manipulate rates of transepithelial ion transfer with the ambient water in order to correct acid–base disturbances (Heisler, 1986, 1993*b*). The gills are the main site responsible for the process, although the types and localization of ion exchangers in the branchial epithelium remain unknown. The determination of the molecular basis for the ionic exchange systems in the gills and the involvement of somatolactin in the regulation of these systems will require further investigation.

A hypercalcaemic action of somatolactin has been indicated in previous studies: somatolactin cells are activated by low environmental Ca^{2+} levels and plasma somatolactin levels are elevated in association with the increase in plasma Ca^{2+} levels in stressed and exercised fish (Kakizawa *et al.* 1993, 1995, 1996). In the present study, however, plasma Ca^{2+} levels in the immunoneutralized fish showed a tendency to increase in parallel with Ca^{2+} levels in the fish without immunoneutralization 5 min after acid infusion, when severe acidosis was observed. The results suggest that the elevation of plasma [Ca^{2+}] during acidosis induced by acid infusion and exhaustive exercise in the present study and in response to stress and exhaustive exercise in previous studies (Kakizawa *et al.* 1995, 1996) may be the result of a dissociation of calcium phosphate in bone and/or scales caused by acidosis (Herrmann-Erlee and Flik, 1989) rather than of the action of somatolactin.

Decreases in plasma [HCO_3^-] resulting from elevated plasma [H^+] induced by acid infusion, exhaustive exercise or severe hypoxia caused an elevation of plasma somatolactin levels. Furthermore, the recovery from the decreased [HCO_3^-] during acidosis was inhibited by somatolactin deficiency induced by immunoneutralization. Thus, somatolactin seems

to be involved in the retentive regulation of plasma [HCO_3^-], especially during acidosis, in rainbow trout.

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