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Osmoregulatory ability and stress responses during freshwater adaptation of black porgy (*Acanthopagrus schlegeli*) treated with exogenous prolactin

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Abstract

The effects of ovine prolactin (oPRL) on osmoregulatory ability (electrolyte balance, plasma osmolality and activity of gill chloride cells and gill Na^+/K^+-ATPase) and stress responses (plasma cortisol, glucose, aspartate aminotransferase: AST and alanine aminotransferase: ALT) were investigated in black porgy transferred to freshwater (FW). Fish in seawater (SW) were injected twice at a 24 h interval with oPRL (at 1, 3, or 5 μg g^{-1} body weight) or vehicle (0.9% NaCl) and then transferred to FW. They were sampled 3 days after the transfer. With oPRL at 5 μg g^{-1}, levels of plasma Na^+ and Cl^- and osmolality were significantly higher than in saline-treated fish, whereas gill CCs number and Na^+/K^+-ATPase activity were lower. Also, the 5 μg g^{-1}oPRL treatment led to significantly lower plasma cortisol levels than did saline treatment. However, there were no significant differences in plasma AST and ALT between groups. These results support the positive osmoregulatory role of PRL in black porgy during FW adaptation.

Keywords: black porgy, freshwater adaptation, osmoregulation, stress response, exogenous prolactin

Introduction

Prolactin (PRL), growth hormone and cortisol play important osmoregulatory roles in teleosts. PRL, secreted by the pituitary gland, is essential for survival and the retention/uptake of Na^+ of vertebrates in fresh water (FW) (Loretz & Bern 1982; Hirano 1986; McCormick 2001). Hyperosmoregulatory role of PRL has been studied in several teleosts. Plasma PRL level was found to decrease in many salmonids when they were transferred from FW to seawater (SW) (Prunet & Boeuf 1989; Young, Bjornsson, Prunet, Lin & Bern 1989; Yada, Takahashi & Hirano 1991), however, tilapia (*Oreochromis mossambicus*) showed increased levels of plasma PRL when transferred from SW to FW (Yada, Hirano & Grau 1994; Shepherd, Sakamoto, Hyodo, Ball, Nishioka, Bern & Grau 1999). In gilthead seabream (*Sparus aurata*) PRL-secreting cells had significantly increased after transfer from SW to FW (Mancera, Perez-Figares & Fernandez-Llebrez 1993). Likewise, the levels of PRL mRNA expression and plasma PRL were increased in hypoosmotic media (Yamauchi, Nishioka, Young, Ogasawara, Hirano & Bern 1991; Morgan, Sakamoto, Grau & Iwama 1997; Lee, Kaneko & Aida 2006).

Treatment with exogenous PRL diminishes the permeability of the skin in teleosts to ions and water (Hirano 1986). It decreases gill Na^+/K^+-ATPase activity in salmonid and non-salmonid; the activity of gill Na^+/K^+-ATPase decreased when PRL was injected into rainbow trout **(***Oncorhynchus mykiss***)** (Madsen & Bern 1992), and ovine PRL (oPRL) also reduced gill Na^+/K^+-ATPase activity in SW and BW-adapted silver seabream (*Sparus sarba*) (Kelly, Chow & Woo 1999).

Black porgy (*Acanthopagrus schlegeli*) is a marine aquaculture species in Korea, but its low salinity culture is also being investigated in view of the superior osmoregulatory ability of this species. Low salinity culture of black porgy may produce advantages to the conventional SW culture. Firstly, higher growth could be expected in fish adapted to isosmotic media due to amount of energy available for fish growth by altering the energetic cost for osmoregulation (Iwama 1996), Diseases such as parasites and bacteria are also controlled by osmotic shock (Min, Jeong, Noh, Lim, Choi & Chang 2006). In aquaculture, salinity changes cause a variety of physiological stress responses including plasma hormones, energy metabolism and electrolyte equilibrium reactions (Barton & Iwama 1991). Cortisol plays a role of mediating stress related gluconeogenesis (Mommsen, Vijayan & Moon 1999). Hyperglycaemia is known to satisfy the increased energy requirements due to stress (Vijayan, Pereira, Grau & Iwama 1997). Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are amino transfer enzymes and a general index of liver function in vertebrates. High AST and ALT generally, but not definitively, indicate the weakening or damage of normal liver function (Pan, Chien & Hunter 2003). Aminotransferases are also used to evaluate the stress responses and health in several fish (De Smet & Blust 2001; Almeida, Diniz, Marques, Faine, Ribas, Burneiko & Novelli 2002; Choi, Min, Jo & Chang 2007).

Salinity stress can depress the physiological condition and homeostasis of fish and affect growth and survival (Wada, Aritaki & Tanaka 2004). Thus, hormonal treatment such as PRL to reduce osmoregulatory stress is being considered as a means of adaptaion to hypoosmotic media.

The aim of this study was to verify the hyperosmoregulatory action of PRL on plasma ions, osmolarity, gill CCs number and size and gill Na^+/K^+-ATPase activity in black porgy, and to investigate the correlation between PRL and stress on the basis of plasma cortisol, glucose and aminotransferases.

Materials and methods

Experimental fish

The 60 black porgy (average length 18.0 ± 1.1 cm, weight 113.1 ± 26.3 g) were obtained from the culture cages of the Marine Science Technology Center (Pukyong National University, Busan, Korea) and held in a recirculating SW (33 psu) system in the laboratory for 2 weeks. The fish were divided into six 400 L square aquariums containing SW, with 10 fish in each, and were adapted for 24 h. They were fed daily commercial extrude pellets (42% protein, 7% fat, 4% fibre, 17% ash and 2.7% phosphorus, Jeilfeed, Daejeon, Korea) at 1–2% body weight. They were fasted for 24 h before hormone injection and before blood was sampled. FW (or SW) temperature was maintained at 18°C, and the photoperiod was a 12L/12D cycle for the experiment.

Experimental design

Fish were anaesthetized with 200 mg L^{-1} tricaine methanesulfonate (MS-222) (Sigma, St. Louis, MO, USA) and weighed. Ovine prolactin (oPRL) (20–50 IU mg^{-1}, Sigma) dissolved in 0.9% saline was injected intraperitoneally at 1, 3, or 5 μg g^{-1} body weight and the fish were placed back in SW. These doses are same or similar to those of hypeosmoregulatory effects of oPRL in other teleost (Seidelin & Madsen 1999; Mancera, Carrión & Río 2002; Jackson, McCormick, Madsen, Swanson & Sullivan 2005). Sham fish were injected with saline only. Control fish were taken out and handled and anaesthetized in the same way as the sham and experimental fish without hormone or saline. Fish were returned to their SW tanks, with each tank holding 10 fish that had all received the same treatment. A second injection was given 24 h after the first injection, and the fish were then transferred immediately to five 400 L square aquariums containing FW (underground water containing in mg L^{-1}; 58 Na^+, 4 K^+, 21 Ca^{2+}, 28 Mg^{2+}, 44 $C1^-$, 590 HCO_3^-, 29 SO_4^{2-}, 7.6 pH). No mortality was observed during the experiments.

Blood and tissue sampling

Three days after transfer to FW, fish were anaesthetized with 200 mg L^{-1} MS-222 prior to collection of blood and gill tissue. Blood was collected from the caudal vasculature in a 3 mL syringe coated with heparin. Plasma samples were separated by centrifugation for 5 min at 9800 × *g* and 4°C and were stored at −80°C until analysis. Fish were killed by spinal transection for the collection of the gill sample. For measurement of

Na^+/K^+-ATPase activity, left gill filaments were placed in SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) and frozen at −80°C. For measurement of CCs number and size, right gill filaments were fixed in Champy–Maillet solution (0.4% OsO_4 + 2.0% ZnI_2) for 24 h at room temperature.

Analysis of plasma parameters

Plasma cortisol was analysed using a commercially available competitive radioimmunoassay (Coat-a-count, Diagnostics Product, Los Angeles, CA, USA) and an automatic gamma counter (1470 Wizard Automatic Gamma Counter, Perkin-Elmer, Turku, Finland). The lower detection limit of the assay was 0.5 ng mL^{-1}. Intra- and inter-assay coefficients of variation were 8.9% and 7.6% respectively. Plasma glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), Na^+ and Cl^- were analysed with a Biochemistry Auto analyzer (model 7180; Hitachi, Tokyo, Japan). Plasma osmolality was examined with a Vapor Pressure Osmometer (Vapro 5520; Wescor, Logan, UT, USA).

Analysis of gill Na^+/K^+-ATPase, and CCs number and size

Gill Na^+/K^+-ATPase activity was determined using the method of McCormick (1993) and expressed as μmol ADP mg $protein^{-1}$ h^{-1}. The tissue fixed in Champy–Maillet solution was embedded in paraffin, and cut in 7 μm sections serially. The sections were examined using light microscopy. The number and size of CCs were analysed with an image analysis system (Matrox Inspector 2.0 Program, Matrox Electronic System, QC, Canada). About 90 sections were examined from gill tissue. CCs number was measured on two filaments per fish, CCs size was measured for biggest size of each cell.

Statistics

All data were analysed with the SPSS statistical package (version 10.0; SPSS, Chicago, IL, USA). One-way ANOVA followed by a *post hoc* multiple comparison test (Duncan's test) was used to compare differences in the data of FW. Results were considered significantly different at $P < 0.05$. Differences between SW group and each FW group were analysed using Student's *t*-test.

Results

Osmoregulatory ability

Transfer from SW to FW induced significant reduction in plasma Na^+, Cl^- and osmolality. In FW, black porgy injected with oPRL at 5 μg g^{-1} body weight had significantly higher plasma Na^+ and Cl^- than those injected with saline alone. Fish injected with oPRL at 3 μg g^{-1} or 5 μg g^{-1} body weight had significantly higher plasma osmolality than those injected with saline and the control fish. However, oPRL at 1 μg g^{-1} had no effect on plasma osmolality (Table 1).

Gill Na^+/K^+-ATPase activity was lower in FW- than SW- adapted fish. In FW, the enzyme activity of fish injected with oPRL at 5 μg g^{-1} body weight was significantly lower than that in sham-treated or control fish (Fig. 1).

With gill tissue fixed in Champy-Maillet solution, ZnI_2 stains the mitochondrial membrane of CCs, which are generally found around the epithelium in the interlamellar space. Three days after transfer from SW to FW, number of CCs decreased in fish injected with oPRL (> 3 μg g^{-1}), however, no

Table 1 Levels of plasma Na^+, Cl^- and osmolality after transfer from seawater (SW) to freshwater (FW) of black porgy injected twice with ovine prolactin (oPRL)

		FW				
				oPRL (μg g^{-1})		
	SW	Control	Sham	1	3	5
Na^+ (mEq L^{-1})	178.2 ± 1.3	155.5 ± 2.0^{a,***}	159.6 ± 2.8^{ab,***}	162.0 ± 1.1^{abc,***}	164.7 ± 1.4^{bc,***}	169.0 ± 1.6^{c,**}
Cl^- (mEq L^{-1})	155.5 ± 2.0	136.0 ± 2.5^{a,*}	138.5 ± 4.0^{a,*}	137.5 ± 2.5^{a,*}	144.0 ± 1.6^{ab,*}	148.5 ± 1.3^{b,*}
Osmolality (mOsm kg^{-1})	350.0 ± 1.4	324.3 ± 2.3^{a,***}	324.7 ± 3.0^{a,***}	326.6 ± 1.5^{a,***}	336.8 ± 1.3^{b,***}	340.3 ± 0.6^{b,**}

Values are mean ± SEM. (n = 8–10). Same letters indicate no difference among groups after transfer to FW ($P < 0.05$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with SW group.

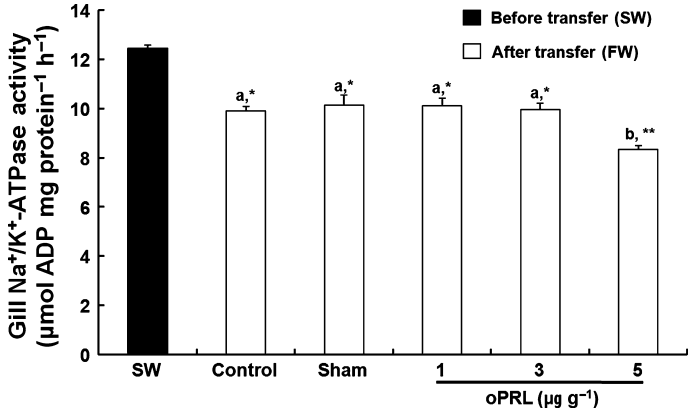

Figure 1 Gill Na^+/K^+-ATPase activity after transfer from SW to FW of black porgy injected twice with oPRL. Values are mean ± SEM. (n = 8–10). Same letters indicate no difference among groups after transfer to FW ($P < 0.05$). *$P <$ 0.05, **$P < 0.01$ compared with SW group.

Table 2 Number and size of chloride cells (CCs) after transfer from seawater (SW) to freshwater (FW) of black porgy injected twice with ovine prolactin (oPRL)

		FW				
				oPRL (μg g^{-1})		
	SW	Control	Sham	1	3	5
Number of CCs/filament	137.0 ± 5.3	130.7 ± 3.5^{a}	128.0 ± 4.2^{a}	126.0 ± 4.3^{a}	111.7 ± 6.4^{a,*}	76.0 ± 7.2^{b,**}
Size of CCs (μm)	11.9 ± 0.4	12.5 ± 0.8^{a}	11.1 ± 0.5^{a}	13.2 ± 0.7^{a}	9.8 ± 0.5^{a}	10.1 ± 0.4^{a}

Values are mean ± SEM. (n = 10). Same letters indicate no difference among groups after transfer to FW ($P < 0.05$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with SW group.

effect of oPRL treatment on size of the CCs was observed (Table 2).

Stress responses

Transfer from SW to FW caused significant increase in plasma cortisol (except fish injected with oPRL at 5 μg g^{-1} body weight) and glucose. In FW, plasma cortisol levels were significantly lower after injection with oPRL or saline than those in the controls, plasma glucose levels followed a similar pattern to that of cortisol, with fish injected with oPRL at 5 μg g^{-1} body weight had significantly lower plasma glucose (Fig. 2).

The transfer to FW did not induce significant changes in AST and ALT. Fish injected with oPRL at 5 μg g^{-1} body weight had lower AST than that in the control fish in FW. However, oPRL injection had no effect on ALT (Fig. 3).

Discussion

In SW, teleost have osmoregulatory mechanisms to replace water lost through osmosis to the ambient water and to discharge salt that is absorbed, whereas in FW, fish need to replace salt lost through diffusion to the surrounding water and to eliminate excess water absorbed; in this way, a stable hydromineral balance is maintained. In marine euryhaline teleost, transfer from SW to hypoosmotic environments induces changes in osmotic plasma parameters and consequent activation of an osmoregulatory system to recover the original values (Mancera *et al.* 2002). However, the salinity stress extends the 'acute phase', which is an unstable phase of physiological response to changes in salinity, during which the physiological activity of the fish is lowered (McCormick & Bradshaw 2006). Thus, reduction in the acute phase is the key for the low salinity culture of marine euryhaline teleosts such as black porgy. In this study black porgy, a SW species, was treated with PRL to strengthen osmoregulatory ability and to shorten the acute phase in FW.

The osmoregulatory role of PRL facilitating adaptation to hypoosmotic environments is well established in teleost (McCormick 1995; Manzon 2002). In this study, injection with oPRL (at 5 μg g^{-1} body weight) before transfer of the fish from SW to FW increased plasma ions and osmo-

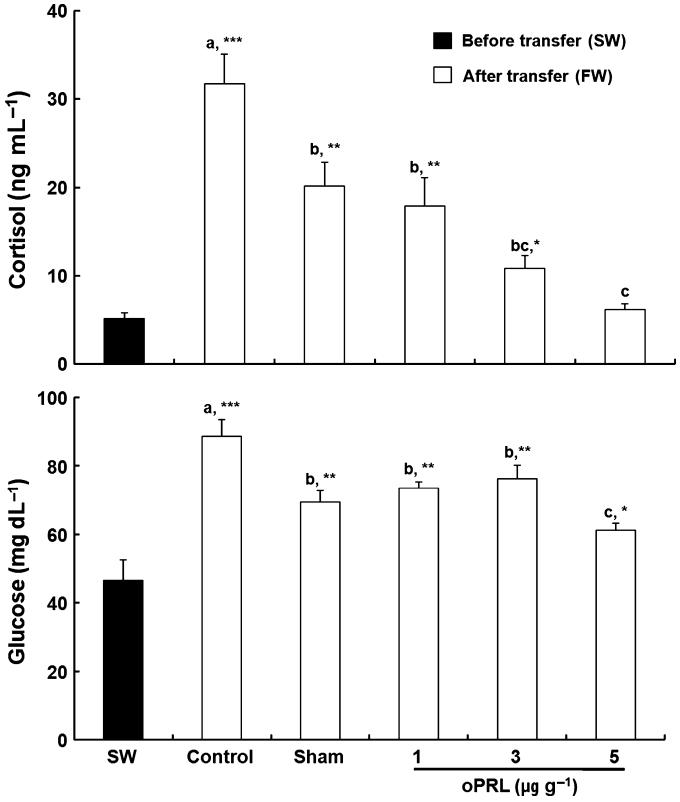

Figure 2 Levels of plasma cortisol and glucose after transfer from SW to FW of black porgy injected twice with oPRL. Values are mean ± SEM. (n = 7–10). Same letters indicate no difference among groups after transfer to FW ($P < 0.05$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with SW group.

lality and decreased Na^+/K^+-ATPase activity in the gills in FW; this indicates that PRL facilitates hyperosmoregulation in FW-adapted black porgy. This agrees with previous reports on euryhaline teleosts. PRL injections inhibited gill Na^+/K^+-ATPase of hypophysectomized killifish (*Fundulus heteroclitus*) in FW (Pickford *et al.* 1970). PRL also decreased gill Na^+/K^+-ATPase of grey mullet (*Chelon labrosus*; Gallis, Lasserre & Belloc 1979), catfish (*Heteropneustes fossilis*; Parwez & Goswami 1985), rainbow trout (Madsen & Bern 1992) and tilapia (*O. mossambicus*; Sakamoto, Shepherd, Madsen, Nishioka, Siharath, Richman, Bern & Grau 1997) in FW, silver seabream (Kelly *et al.* 1999) gilthead sea bream (Mancera *et al.* 2002) and hybrid striped bass (*Morone saxatilis* × *M. chrysops*: Jackson *et al.* 2005) in SW. Actually, this hyperosmoregulatory action of PRL should allow most euryhaline marine fish to live in hypoosmotic media with plasma osmolality similar to that in fish adapted to SW. In this study, oPRL at 3 μg g^{-1} increased plasma osmolality, although this dose did not significantly decrease Na^+/K^+-ATPase activity. This supports previous suggestions that plasma osmolality may not always be regulated by Na^+/K^+-ATPase activity in the gills: Herndon, McCormick and Bern (1991) found that PRL injections increased plasma ion levels without changing gill Na^+/K^+-ATPase activity in tilapia (*O. mossambicus*), and Jackson *et al.* (2005) reported that the ability of PRL to offset decreases in plasma osmolality and ions is not necessarily linked to reduction in gill Na^+/K^+-ATPase activity in hybrid striped bass.

The gill is the primary site of osmoregulation in teleosts. Within the gill epithelium, CCs (or mitochondria-rich cells) are involved in salt secretion in SW and ion uptake in FW (Foskett & Scheffey 1982; Hiroi, Kaneko, Seikai & Tanaka 1998). The number and size of CCs and the expression levels of their protein components such as Na^+/K^+-ATPase, and ion channels and transport, are influenced by the environmental osmotic and hormonal signals (Hirose, Kaneko, Naito & Takei 2003). For instance, cortisol can increase the density and size of branchial CCs in salmonids (Madsen 1990), and increase expression of the Na-K-2Cl co-transporter in the gill of Atlantic salmon (*Salmo salar*) acclimated to FW (Pelis & McCormick 2001). Similarly, growth hormone and

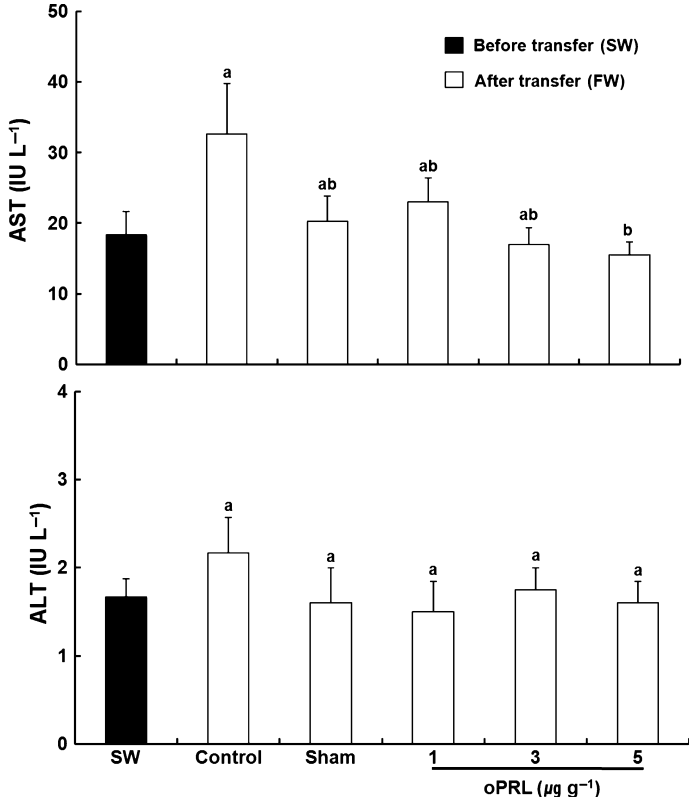

Figure 3 Levels of plasma AST and ALT after transfer from SW to FW of black porgy injected twice with oPRL. Values are mean ± SEM. (n = 8–10). Same letters indicate no difference among groups after transfer to FW ($P < 0.05$).

insulin-like growth factor I can increase the density and size of CCs (McCormick 1995, 2001). On the other hand, PRL can reduce the density (or size) of gill CCs in teleosts acclimated to FW (Gallis *et al.* 1979; Evans 2002), this is similar to our result, in which exogenous PRL (at 5 μg g^{-1} body weight) reduced CCs number in the gills of black porgy, although it had no effect on CCs size. In SW, PRL also reduced CCs number in striped bass (*Morone saxatilis*; Madsen, Nishioka & Bern 1997) or CCs size in tilapia (*O. niloticus*; Pisam, Auperin, Prunet, Rentier-Delrue, Martial & Rambourg 1993).

Mammalian hormones such as oPRL and ovine growth hormone have been frequently used to research the osmoregulation system of teleost (McCormick 1995; Seidelin & Madsen 1999; Manzon 2002). Jackson *et al.* (2005) showed that gill Na^+/K^+-ATPase activity in hypophysectomized hybrid striped bass was significantly lower in fish receiving homologous and heterologous oPRL at a dose 10 ng g^{-1} and 20 μg g^{-1} body weight respectively. This result indicates that heterologous hormones are not well affined with the hormone receptors of teleosts (Mancera *et al.* 2002), thereby the biological activity of these hormones is unpredictable. Use of oPRL decreases gill Na^+/K^+-ATPase activity in killifish and rainbow trout, but increases kidney Na^+/K^+-ATPase activity in killifish (Pickford *et al.* 1970; Madsen & Bern 1992) and increases gill Na^+/K^+-ATPase activity in Atlantic salmon (Boeuf, Marc, Prunet, le Bail & Smal 1994). In tilapia (*O. mossambicus*), oPRL had no effect on gill Na^+/K^+-ATPase activity (Herndon *et al.* 1991; Auperin, Rentier-Delrue, Martial & Prunet 1995). In the present study, the concentration of oPRL required for effective hyperosmoregulation was 5 μg g^{-1} body weight; this was also the dose required to increase plasma osmolality in hypophysectomized hybrid striped bass (Jackson *et al.* 2005), whereas 2 μg g^{-1} was sufficient in striped bass in FW (Madsen, Nishioka & Bern 1996). Thus, the efficacy of oPRL may depend on fish species, size (or age), physiological conditions, target organs and dose.

When fish are exposed to a stressor, the activity of the hypothalamus-pituitary-interrenal axis is increased, resulting in secretion of cortisol in the blood (Perry & Reid 1993; Wendelaar Bonga 1997; Chang & Hur 1999). Secondary responses include

imbalances in the concentrations of ions in the blood, increases in AST and ALT, increased cardiac pulse, increased oxygen consumption and energy stimulus, i.e. increased blood glucose (Eddy 1981; Carmichael, Tomasso, Simco & Davis 1984; McDonald & Milligan 1997). In the present study, fish transferred to FW without hormone treatment or vehicle had higher plasma cortisol (318 ng mL^{-1}) and glucose (89 mg dL^{-1}) levels than those reported for non-stressed black porgy in SW (cortisol, 10–35 ng mL^{-1}; glucose, 52–55 mg dL^{-1}) (Min, Kim, Hur, Bang, Byun, Choi & Chang 2003; Chang, Min & Choi 2007; Choi *et al.* 2007). The fish that received oPRL at 5 μg g^{-1} body weight had significantly lower plasma cortisol (34 ng mL^{-1}) than did control or sham-treated fish. According to a previous study, transfer from SW to FW induced an increase in plasma cortisol that reached its peak within 24 h and then started to decrease, at which time PRL mRNA increased (Chang *et al.* 2007). These results strongly suggest that endogenous or exogenous PRL reduces stress responses during adaptation of marine euryhaline teleosts to a hypoosmotic environment, because this hormone enhances hyperosmoregulation through ions retention by reduced gill Na^+/K^+-ATPase activity, and CCs number during adaptation to FW.

Acknowledgments

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References

Almeida J.A., Diniz Y.S., Marques S.F.G., Faine L.A., Ribas B.O., Burneiko R.C. & Novelli E.L.B. (2002) The use of the oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to *in vivo* cadmium contamination. *Environment International* **27**, 673–679.

Auperin B., Rentier-Delrue F., Martial J.A. & Prunet P. (1995) Regulation of gill prolactin receptors in tilapia (*Oreochromis mossambicus*) after a change in salinity or hypophysectomy. *Journal of Endocrinology* **145**, 213–220.

Barton B.A. & Iwama G.K. (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases* **1**, 3–26.

Boeuf G., Marc A.M., Prunet P., le Bail P.Y. & Smal J. (1994) Stimulation of parr-smolt transformation by hormonal treatment in Atlantic salmon *Salmo salar L. Aquaculture* **121**, 195–208.

Carmichael G.J., Tomasso J.R., Simco B.A. & Davis K.B. (1984) Characterization and alleviation of stress associated with hauling largemouth bass. *Transaction of American Fisheries Society* **113**, 778–785.

Chang Y.J. & Hur J.W. (1999) Physiological responses of grey mullet (*Mugil cephalus*) and Nile tilapia (*Oreochromis niloticus*) rapid changes in salinity of rearing water. *Journal of Korean Fisheries Society* **32**, 310–316.

Chang Y.J., Min B.H. & Choi C.Y. (2007) Black porgy (*Acanthopagrus schlegeli*) prolactin cDNA sequence: mRNA expression and blood physiological responses during freshwater acclimation. *Comparative Biochemistry and Physiology* **147B**, 122–128.

Choi C.Y., Min B.H., Jo P.G. & Chang Y.J. (2007) Molecular cloning of PEPCK and stress response of black porgy (*Acanthopagrus schlegeli*) to increased temperature in freshwater and seawater. *General and Comparative Endocrinology* **152**, 47–53.

De Smet H. & Blust R. (2001) Stress responses and changes in protein metabolism in Carp *Cyprinus carpio* during cadmium exposure. *Ecotoxicology and Environmental Safety* **38**, 255–262.

Eddy F.B. (1981) Effects of stress on osmotic and ionic regulation in fish. In: *Stress and Fish* (ed. by A.D. Pickering), pp. 77–102. Academic Press, London, UK.

Evans D.H. (2002) Cell signaling and ion transport across the fish gill epithelium. *Journal of Experimental Zoology* **293**, 336–347.

Foskett J.K. & Scheffey C. (1982) The chloride cell: definitive identification as the salt-secretory cell in teleosts. *Science* **215**, 164–166.

Gallis J.L., Lasserre P. & Belloc F. (1979) Freshwater adaptation in the euryhaline teleost, *Chelon labrosus.* I. Effects of adaptation, prolactin, cortisol and actinomycin D on plasma osmotic balance and (Na+–K+)ATPase in gill and kidney. *General and Comparative Endocrinology* **38**, 1–10.

Herndon T.M., McCormick S.D. & Bern H.A. (1991) Effects of prolactin on chloride cells in opercular membrane of seawater-adapted tilapia. *General and Comparative Endocrinology* **83**, 283–289.

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

Hiroi J., Kaneko T., Seikai T. & Tanaka M. (1998) Developmental sequence of chloride cells in the body skin and gills of Japanese flounder (*Paralichthys olivaceus*) larvae. *Zoological Science* **15**, 455–460.

Hirose S., Kaneko T., Naito N. & Takei Y. (2003) Molecular biology of major components of chloride cells. *Comparative Biochemistry and Physiology* **136B**, 593–620.

Iwama G.K. (1996) Grwoth of salmonids. In: *Principles of Salmonid Culture* (ed. by W. Pennell & B.A. Barton), pp. 467–516. Elsevier, Amsterdam, the Netherlands.

Jackson L.F., McCormick S.D., Madsen S.S., Swanson P. & Sullivan C.V. (2005) Osmoregulatory effects of hypophysectomy and homologous prolactin replacement in hybrid striped bass. *Comparative Biochemistry and Physiology* **140B**, 211–218.

Kelly S.P., Chow I.N.K. & Woo Y.S. (1999) Effects of prolactin and growth hormone on strategies of hypoosmotic adaptation in a marine teleost, *Sparus sarba*. *General and Comparative Endocrinology* **113**, 9–22.

Lee K.M., Kaneko T. & Aida K. (2006) Prolactin and prolactin receptor expressions in a marine teleost, pufferfish *Takifugu rubripes*. *General and Comparative Endocrinology* **146**, 318–328.

Loretz C.A. & Bern H.A. (1982) Prolactin and osmoregulation in vertebrates. *Neuroendocrinology* **35**, 292–304.

Madsen S.S. (1990) Effect of repetitive cortisol and thyroxine injections on chloride cell number and Na^+, K^+-ATPase activity in gills of freshwater acclimated rainbow trout, *Salmo gairdneri*. *Comparative Biochemistry and Physiology* **95A**, 171–175.

Madsen S.S. & Bern H.A. (1992) Antagonism of prolactin and growth hormone: impact on seawater adaptation in two salmonids, *Salmo trutta* and *Oncorhynchus mykiss*. *Zoological Science* **9**, 775–784.

Madsen S.S., Nishioka R.S. & Bern H.A. (1996) Seawater acclimation in the anadromous striped bass, *Morone saxatilis*: strategy and hormonal regulation. In: *The Physiology of Migratory Fish Symp. Proceedings of the International Congress on the Biology of Fishes* (ed. by S. McCormick, M. Sheridan, R. Patiño & D. MacKinlay), pp. 167–174. American Fisheries Society, San Francisco, CA, USA.

Madsen S.S., Nishioka R.S. & Bern H.A. (1997) Prolactin antagonizes seawater acclimation in the anadromous striped bass, *Morone saxatilis*. In: *Advances in Comparative Endocrinology*, Vol. II (ed. by S. Kawashima & S. Kikuyama), pp. 1011–1015. Monduzzi Editore, Bologna.

Mancera J.M., Perez-Figares J.M. & Fernandez-Llebrez P. (1993) Osmoregulatory responses to abrupt salinity changes in the euryhaline gilthead sea bream (*Sparus aurata* L.). *Comparative Biochemistry and Physiology* **106A**, 245–250.

Mancera J.M., Carrión R.L. & Río M.P.M. (2002) Osmoregulatory action of PRL, GH, and cortisol in the gilthead seabream (*Sparus aurata* L.). *General and Comparative Endocrinology* **129**, 95–103.

Manzon L.A. (2002) The role of prolactin in fish osmoregulation: a review. *General and Comparative Endocrinology* **125**, 291–310.

McCormick S.D. (1993) Methods for nonlethal gill biopsy and measurement of Na^+, K^+-ATPase activity. *Canadian Journal of Fisheries and Aquatic Science* **50**, 656–658.

McCormick S.D. (1995) Hormonal control of gill Na^+,K^+-ATPase and chloride cell function. In: *Fish Physiology*, Vol. XIV (ed. C.M. Wood & T.J. Shuttleworth), pp. 285–315. Academic Press, New York, USA.

McCormick S.D. (2001) Endocrine control of osmoregulation in teleost fish. *American Zoologist* **41**, 781–794.

McCormick S.D. & Bradshaw D. (2006) Hormonal control of salt and water balance in vertebrates: minireview. *General and Comparative Endocrinology* **147**, 3–8.

McDonald D.G. & Milligan C.L. (1997) Ionic, osmotic and acid base regulation in stress. In: *Fish Stress and Health in Aquaculture* (ed. G.W. Iwama, A.D. Pickering, J.P. Sumpter & C.B. Schreck), pp. 119–144. University Press, Cambridge, UK.

Min B.H., Kim B.K., Hur J.W., Bang I.C., Byun S.K., Choi C.Y. & Chang Y.J. (2003) Physiological responses during freshwater acclimation of seawater-cultured black porgy (*Acanthopagrus schlegeli*). *Korean Journal Ichthyology* **15**, 224–231.

Min B.H., Jeong M.H., Noh G.A., Lim H.K., Choi C.Y. & Chang Y.J. (2006) Hyposmotic treatment for control of the parasitic copepod, *Alella macrotrachelus* on the gill of cultured black porgy. *Journal of Aquaculture* **19**, 19–24.

Mommsen T.P., Vijayan M.M. & Moon T.W. (1999) Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Fish Biology and Fisheries* **9**, 211–268.

Morgan J.D., Sakamoto T., Grau E.G. & Iwama G.K. (1997) Physiological and respiratory responses of the Mozambique tilapia *Oreochromis mossambicus* to salinity acclimation. *Comparative Biochemistry and Physiology* **117A**, 391–398.

Pan C.H., Chien Y.H. & Hunter B. (2003) The resistance to ammonia stress *of Penaeus monodon* Fabricius juvenile fed diets supplemented with astaxanthin. *Journal of Experimental Marine Biology and Ecology* **297**, 107–118.

Parwez I. & Goswami S.V. (1985) Effects of prolactin, adrenocorticotrophin, neurohypophysial peptides, cortisol and androgens on some osmoregulatory parameters of the hypophysectomized catfish, *Heteropneustes fossilis* (Block). *General and Comparative Endocrinology* **58**, 51–68.

Pelis R.M. & McCormick S.D. (2001) Effects of growth hormone and cortisol on Na^+–K^+–$2Cl^-$ cotransporter localization and abundance in the gills of Atlantic salmon. *General and Comparative Endocrinology* **124**, 134–143.

Perry S.F. & Reid S.D. (1993) β-Adrenergic signal transduction in fish: interactive effects of catecholamines and cortisol. *Fish Physiology and Biochemistry* **11**, 195–203.

Pickford G.E., Griffith R.W., Torretti J., Henfletzh E. & Epstein F.H. (1970) Branchial reduction and renal stimulation of Na^+, K^+-ATPase by prolactin in hypophysectomized killifish in freshwater. *Nature* **228**, 378–379.

Pisam M., Auperin B., Prunet P., Rentier-Delrue F., Martial J. & Rambourg A. (1993) Effects of prolactin on alpha and beta chloride cells in the gill epithelium of the saltwater adapted tilapia *Oreochromis niloticus*. *The Anatomical Record* **235**, 275–284.

Prunet P. & Boeuf G. (1989) Plasma prolactin levels during smolting in Atlantic salmon, *Salmo salar*. *Aquaculture* **82**, 297–305.

Sakamoto T., Shepherd B.S., Madsen S.S., Nishioka R.S., Siharath K., Richman N.H. III, Bern H.A. & Grau E.G. (1997) Osmoregulatory action of growth hormone and prolactin in an advanced teleost. *General and Comparative Endocrinology* **106**, 95–101.

Seidelin M. & Madsen S.S. (1999) Endocrine control of Na^+,K^+-ATPase and chloride cell development in brown trout (*Salmo trutta*): interaction of insulin-like growth factor-I with prolactin and growth hormone. *Journal of Endocrinology* **162**, 127–135.

Shepherd B.S., Sakamoto T., Hyodo S., Ball C., Nishioka R. S., Bern H.A. & Grau E.G. (1999) Is the primitive regulation of pituitary prolactin (tPRL177 and tPRL188) secretion and gene expression in the euryhaline tilapia (*Oreochromis mossambicus*) hypothalamic or environmental? *Journal of Endocrinology* **161**, 121–129.

Vijayan M.M., Pereira C.E., Grau E.G. & Iwama G.K. (1997) Metabolic responses associated with confinement stress in tilapia: the role of cortisol. *Comparative Biochemistry and Physiology* **116C**, 89–95.

Wada T., Aritaki M. & Tanaka M. (2004) Effect of low-salinity on the growth and development of spotted halibut *Verasper variegatus* in the larva-juvenile transformation period with reference to pituitary prolactin and gill chloride cell responses. *Journal of Experimental Marine Biology and Ecology* **308**, 113–126.

Wendelaar Bonga S.E. (1997) The stress response in fish. *Physiological Reviews* **77**, 591–625.

Yada T., Takahashi K. & Hirano T. (1991) Seasonal changes in seawater adaptability and plasma levels of prolactin and growth hormone in landlocked sockeye salmon (*Oncorhynchus nerka*) and amago salmon (*O. rhodurus*). *General and Comparative Endocrinology* **82**, 33–44.

Yada T., Hirano T. & Grau E.G. (1994) Changes in plasma levels of the two prolactins and growth hormone during adaptation to different salinities in the euryhaline tilapia, *Oreochromis mossambicus*. *General and Comparative Endocrinology* **93**, 214–223.

Yamauchi K., Nishioka R.S., Young G., Ogasawara T., Hirano T. & Bern H.A. (1991) Osmoregulation and circulating growth hormone and prolactin in hypophysectomized coho salmon (*Oncorhynchus kisutch*) after transfer to fresh water and seawater. *Aquaculture* **92**, 33–42.

Young G., Bjornsson B.T., Prunet P., Lin R.J. & Bern H.A. (1989) Smoltification and seawater adaptation in coho salmon (*Oncorhynchus kisutch*): plasma prolactin, growth hormone, thyroid hormones and cortisol. *General and Comparative Endocrinology* **74**, 335–345.