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Comparative Biochemistry and Physiology, Part B 140 (2005) 211–218

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Osmoregulatory effects of hypophysectomy and homologous prolactin replacement in hybrid striped bass

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Received 18 June 2004; received in revised form 4 October 2004; accepted 5 October 2004

Abstract

The effects of ovine prolactin (oPRL) and striped bass prolactin (sbPRL; *Morone saxatilis*) on plasma osmolality, electrolyte balance, and gill Na^+,K^+-ATPase activity were investigated in hypophysectomized (Hx), freshwater (FW)-acclimated, hybrid striped bass (*M. saxatilis*×*Morone chrysops*). They were kept in dilute (isoosmotic) seawater for about 10 days after surgery. Seven days after transfer to FW, Hx fish had lower plasma osmolality and lower levels of Na^+, Cl^-, and Ca^{2+} than sham-operated and intact fish. Fish were injected four times with oPRL (1, 5, or 20 μg/g body mass), sbPRL (10 or 100 ng/g), or hormone vehicle (0.9% NaCl) at 48-h intervals (days 0, 2, 4, and 6) in FW and then sampled for blood plasma 24 h after the fourth injection (day 7). In Hx fish, oPRL (5 and 20 μg/g) and sbPRL (10 and 100 ng/g) were effective in maintaining plasma osmolality and levels of Na^+, Cl^-, and Ca^{2+} above values seen in saline-injected controls. Hypophysectomy did not affect branchial Na^+,K^+-ATPase activity, but enzyme activity was significantly reduced in Hx fish receiving oPRL (20 μg/g) or sbPRL (10 or 100 ng/g). These results indicate that PRL acts to maintain plasma osmotic and ionic balance in FW-adapted hybrid striped bass, and that this may involve downregulation of branchial Na^+,K^+-ATPase activity.

Keywords: Teleost; Osmoregulation; Prolactin; ATPase; Hypophysectomy; Osmolality; Na^+,K^+-ATPase activity; Electrolytes; *Morone*; Striped bass

1. Introduction

While prolactin (PRL) has been shown to have diverse actions in teleostean fish, control of osmoregulation is believed to be its primary role (reviewed by McCormick, 2001; Manzon, 2002). As first demonstrated in hypophysectomized (Hx) killifish (*Fundulus heteroclitus*) by Pickford and Phillips (1959), PRL is essential for freshwater (FW) survival and conservation of extracellular ions in some teleosts. Other teleosts (such as eels, goldfish, and rainbow trout) can survive in FW without their pituitary gland with little or no overall osmotic perturbation. It is thus important to determine the impact of hypophysectomy and PRL replacement in a variety of teleosts from diverse taxa in order to determine whether a pattern of the relative importance of PRL in osmoregulation in FW can be established. There are more than 20,000 teleost species with greater diversity than all other vertebrate classes combined, and less than 10 species have been examined to date with regard to the actions of PRL.

PRL receptors are present in all osmoregulatory organs (gill, kidney, intestine, urinary bladder, and skin; Manzon, 2002) and PRL administration reduces fluxes of monovalent and divalent ions and water in these organs

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doi:10.1016/j.cbpc.2004.10.004

(Manzon, 2002). In most studies, heterologous PRL, mainly of mammalian origin, has been applied, although sodium retention by Hx fish has also been used to confirm the bioactivity of purified teleost PRL. The bioassay is rapid, sensitive, and specific for the biological activity of the hormone (Grau et al., 1984). Euryhaline killifish (genus *Fundulus*) have been the test species of choice for most investigators because they are easily Hx through their mouth (Grau and Stetson, 1977). We recently purified PRL from the striped bass (*Morone saxatilis*), an anadromous, perciform teleost native to the east coast of North America and an important fisheries and aquaculture species (Jackson et al., 2000; Fruchtman et al., 2000). Huang and Specker (1994) used immunocytochemical techniques to definitively identify PRL cells in striped bass. The PRL cells appeared most active in FW-acclimated juveniles, showed signs of variable activity in mature adults from FW, and appeared inactive in maturing adults from seawater (SW). These observations suggest a role for PRL in hyperosmoregulation of this species.

The aim of the present study was to examine the biological actions of homologous PRL on the osmoregulatory physiology of hybrid striped bass. We injected striped bass prolactin (sbPRL) into Hx hybrid striped bass (*M. saxatilis*×*Morone chrysops*), thereby performing one of the few hormone replacement studies using fish PRL homologous at least to the level of genus. Specifically, we report here on the effects of injected sbPRL on plasma osmolality and electrolyte levels and branchial Na^+,K^+-ATPase activity in Hx fish. The results of parallel experiments done using ovine prolactin (oPRL) are provided for comparison to prior studies, in which this hormone preparation was employed. Euryhaline hybrid striped bass were chosen instead of striped bass for surgical Hx and hormone injection on the basis of their superior survival, reduced stress response, and resistance to infection after handling (Noga et al., 1994).

2. Materials and methods

2.1. Experimental animals

One-year-old juvenile hybrid striped bass (range 90–230 g body mass) were obtained from FW ponds at the Pamlico Aquaculture Field Laboratory of North Carolina State University (NCSU) and held in a recirculating water system (King et al., 1995) on the NCSU campus. Water conditions acceptable for rearing temperate basses (Nicholson et al., 1990) were maintained as follows: temperature =15 °C, salinity <2 ppt, and hardness=200 ppm. Three treatment groups were established for each experiment; Hx, sham-operated, and intact. Hypophysectomy was performed through the orbit under anesthesia (50 mg/l quinaldine sulfate and 50 mg/l tricaine methanesulfonate [MS-222]) according to the methods of Nishioka (1994). Briefly, the right eye was removed, a hole was drilled through the neurocranium, and the pituitary was removed by aspiration using a modified Pasteur pipette. The empty orbit was filled with a ball of sterile cotton impregnated with triple antibiotic (bacitracin–polymyxin–neomycin) ointment (Eckerd Drug, Clearwater, FL, USA) and the fish were marked with a numbered streamer tag (Floy Manufacturing, Seattle, WA, USA) for identification. Sham-operated fish were treated in a similar manner, except that their pituitary gland was not removed. Intact fish were only anesthesized and tagged. After surgery, the fish were held at near-isoosmotic conditions in artificial seawater (SW) (Instant Ocean; Aquarium Systems, Mentor, OH, USA; salinity=13 ppt, 15 °C) for several days before FW challenge. At the time of transfer to FW (salinity=0 ppt, hardness=50 ppt, 15 °C), hormones were delivered in 0.9% saline vehicle (1.0 μl/g body mass) by intraperitoneal injection. Fish were not fed for 24 h before surgery, during recovery after surgery, or at any other time during the experiments. Completeness of hypophysectomy was confirmed by microscopic examination of the aspirated pituitary gland and reconfirmed at the termination of experiments by postmortem dissection of the fish and examination of their brain and sella turcica under a stereomicroscope.

2.2. Experiment 1

Following a recovery time of 9–11 days after surgery, oPRL (20–50 IU/mg; Sigma, St. Louis, MO, USA) was administered to Hx fish at a concentration of 0, 1, 5, or 20 μg/g body mass and the fish were transferred to FW. Sham and intact control fish were injected with saline vehicle only. The fish were given a total of four injections (days 0, 2, 4, and 6). The experiment was terminated on day 7, when the fish were anesthesized, bled from the caudal vessels, and dissected to retrieve gill tissue and verify Hx. Preliminary experiments with Hx hybrid striped bass indicated that large decreases in plasma osmolality occurred only after 4–8 days in FW (data not shown).

2.3. Experiment 2

This experiment was conducted in the same manner as Experiment 1 with the exception that postsurgical recovery time was 7–10 days. Purified sbPRL (Jackson et al., 2000) was administered four times at 0, 10, or 100 ng/g body mass (days 0, 2, 4, and 6).

2.4. Plasma osmolality and electrolyte levels

At the end of each experiment, fish were anesthesized (100 mg/l quinaldine sulfate) and bled by caudal puncture with 3-ml syringes fitted with 22-gauge hypodermic needles prerinsed with anticoagulant (ammonium heparin;

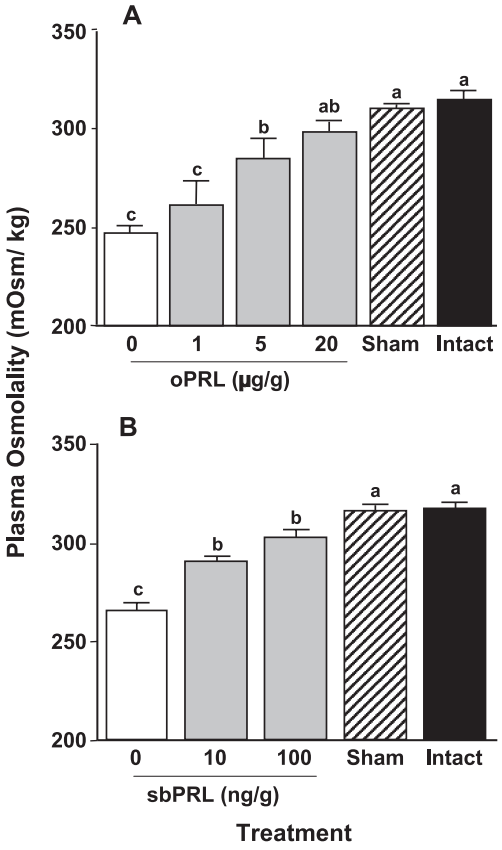

Fig. 1. Plasma osmolality (mOsm/kg) of hypophysectomized hybrid striped bass held in fresh water, injected four times with oPRL (A) or sbPRL (B) at 48-h intervals (days 0, 2, 4, and 6), and bled 24 h after the last hormone injection (day 7). oPRL was administered at doses of 0 (hormone vehicle only), 1, 5, or 20 µg/g body mass. sbPRL was given at doses of 0, 10, or 100 ng/g body mass. Data are expressed as mean±S.E.M. The exact numbers and mean masses of fish in each group are given in Tables 1 and 2. Bars marked with different letter superscripts indicate mean values that are significantly different (ANOVA; $P<0.05$).

10 mg/ml in 0.9% NaCl). The blood was promptly centrifuged at 1000×*g* for 5 min to separate the plasma, which was immediately measured for osmolality. The osmolality of quadruplicate aliquots of each plasma sample was measured on a Model 5100-B vapor pressure osmometer (Wescor, Logan, UT, USA). An aliquot of the remaining plasma sample was frozen in a filled and capped polypropylene microcentrifuge tube at −80 °C for up to 3 months before duplicate measurement of plasma electrolyte (Na^+, Cl^-, Ca^{2+}, and Mg^{2+}) levels on an automated clinical chemistry analyzer (Monarch Plus; Instrumentation Laboratory, Lexington, MA, USA) according to the manufacturer's instructions.

2.5. Branchial ATPase activity

Primary gill filaments were trimmed from ceratobranchials and frozen on dry ice in sucrose–EDTA–imidazole buffer (SEI: 300 mM sucrose, 20 mM Na_2EDTA, 50 mM imidazole, pH 7.3) and stored at −80 °C. Na^+,K^+-ATPase activity (ouabain-sensitive) in homogenates of the gill membranes was measured in duplicate in a NADH-coupled assay, as described previously (McCormick and Bern, 1989; Madsen et al., 1994).

2.6. Statistics

Values for osmolality, plasma electrolyte levels, and branchial Na^+,K^+-ATPase activity are expressed as mean±standard error of the mean (S.E.M.). Differences between means were tested by one-way analysis of variance (ANOVA) followed by Duncan's new multiple range test. Statistical significance was accepted at $P\leq0.05$. When variances were not homogeneous, data were log-transformed to normalize distributions before analysis. All analyses were performed using SuperANOVA (Abacus Concepts, Berkeley, CA, USA).

3. Results

3.1. Plasma osmolality

In both experiments, Hx fish that were not given hormone injections had significantly lower plasma osmolality than intact or sham-operated fish, which did not differ in plasma osmolality (Fig. 1A and B). Hx fish receiving injections of oPRL at 5 or 20 µg/g body mass had significantly higher plasma osmolality than those injected with hormone vehicle, and the higher dose of oPRL restored plasma osmolality to levels seen in intact fish (Fig. 1A). The lowest dose of oPRL (1 µg/g body

Table 1
Plasma electrolyte levels (mmol/l) in hypophysectomized hybrid striped bass held in fresh water, injected four times with ovine prolactin (0, 1, 5, and 20 µg/g) at 48-h intervals, and bled 24 h after the last hormone injection

Treatment	*n*	Mass	Na^+	Cl^-	Ca^{2+}	Mg^{2+}
Intact	6	137.0±5.7^{a}	158±2^{a}	134±3^{a}	2.11±0.04^{a}	0.81±0.01^{a}
Sham	7	123.1±9.6^{ab}	156±1^{a}	134±3^{a}	1.95±0.02^{b}	0.79±0.02^{ab}
20 µg/g	4	149.0±5.3^{c}	148±3^{ab}	108±5^{b}	1.91±0.09^{b}	0.60±0.19^{abc}
5 µg/g	5	151.6±17.4^{abc}	139±8^{b}	107±8^{b}	1.86±0.03^{bc}	0.72±0.07^{ab}
1 µg/g	4	146.6±7.2^{ac}	124±7^{c}	85±6^{c}	1.73±0.09^{c}	0.58±0.06^{bc}
0 µg/g	6	147.5±9.6^{ac}	116±2^{c}	9 0±1^{c}	1.53±0.06^{d}	0.49±0.02^{c}

The number (*n*) and mean mass of fish (in grams) in each group also are shown. Mean values (±S.E.M.) that are not followed by a common letter are significantly different (ANOVA; $P\leq0.05$).

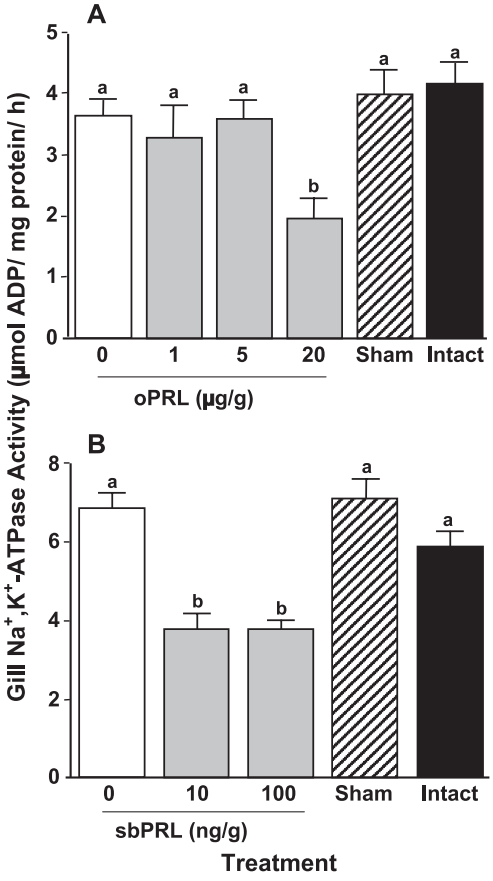

Fig. 2. Gill Na^+,K^+-ATPase activity (µmol ADP/mg protein/h) of hypophysectomized hybrid striped bass held in fresh water and injected four times with oPRL (A) or sbPRL (B) at 48-h intervals (days 0, 2, 4, and 6). Gill tissue was collected for analyses 24 h after the last hormone injection (day 7). oPRL was administered at doses of 0 (hormone vehicle only), 1, 5, or 20 µg/g body mass. sbPRL was given at doses of 0, 10, or 100 ng/g body mass. Data are expressed as mean±S.E.M. The exact numbers and mean masses of fish in each group are given in Tables 1 and 2. Bars marked with different letter superscripts indicate mean values that are significantly different (ANOVA; $P \leq 0.05$).

mass) did not effectively inhibit the drop in osmolality seen in Hx fish. Hx fish receiving sbPRL (10 or 100 ng/g body mass) had significantly higher plasma osmolality than those injected only with hormone vehicle, but the higher dose of sbPRL did not fully restore plasma osmolality to levels seen in intact fish (Fig. 1B). The relative potencies of oPRL and sbPRL were not compared directly in the same experiment. However, sbPRL at a dose of 100 ng/g appeared to be at least as effective in offsetting the drop in plasma osmolality in Hx fish as oPRL at a dose of 5 µg/g, an apparent 50-fold difference in hormone potency (Fig. 1A and B).

3.2. Plasma electrolytes

In Experiment 1, Hx bass that were not injected with oPRL had significantly and substantially lower plasma levels of Na^+, Cl^-, Ca^{2+}, and Mg^{2+} than sham-operated or control fish, and plasma Ca^{2+} levels also were slightly lower in sham-operated fish than in intact control animals (Table 1). Hx fish injected with oPRL at a dose of 5 or 20 µg/g body mass had significantly higher levels of Na^+, Cl^-, and Ca^{2+} than those injected with hormone vehicle. In general, the effects of hypophysectomy and oPRL treatment on plasma levels of Na^+ and Cl^- were mirrored by the response of plasma osmolality to these same treatments. Plasma osmolality was highly correlated with plasma Na^+ (r=0.98, $P \leq 0.001$) and Cl^- (r=0.94, $P \leq 0.001$) levels.

In Experiment 2, Hx fish injected only with hormone vehicle had significantly lower plasma levels of Na^+, Cl^-, and Ca^{2+} than intact or sham-operated bass (Table 2). Injection of Hx fish with sbPRL (10 or 100 ng/g body mass) significantly increased plasma levels of these ions above those seen in bass Hx and injected with hormone vehicle. However, neither dose of sbPRL raised electrolyte levels to those measured in sham-operated or intact control fish. Plasma levels of Mg^{2+} were not significantly altered by hypophysectomy or sbPRL treatment. As in Experiment 1, plasma osmolality was highly correlated with levels of Na^+ (r=0.96, $P \leq 000.1$) and Cl^- (r=0.94, $P \leq 000.1$).

3.3. Branchial ATPase activity

Hypophysectomy did not alter branchial Na^+,K^+-ATPase activity in either experiment (Fig. 2A and B). Na^+,K^+-ATPase activity was significantly and substantially lower in fish receiving the highest dose of oPRL (20 µg/g body mass) or either dose of sbPRL (10 or 100 ng/g body mass) than in all other groups of fish.

Table 2
Plasma electrolyte levels (mmol/l) in hypophysectomized hybrid striped bass held in fresh water, injected four times with striped bass prolactin (0, 10, and 100 ng/g) at 48-h intervals, and bled 24 h after the last hormone injection

Treatment	n	Mass	Na^+	Cl^-	Ca^{2+}	Mg^{2+}
Intact	6	136.3±12.4^a	164±3^a	138±4^a	2.09±0.04^a	0.97±0.11^a
Sham	6	141.2±18.7^a	163±2^a	136±5^a	2.10±0.04^{ab}	0.78±0.12^{ab}
100 ng/g	7	141.0±10.6^a	151±3^{ab}	122±6^b	1.93±0.07^b	0.75±0.05^{ab}
10 ng/g	7	145.1±12.9^a	144±2^b	117±44^b	1.84±0.04^{bc}	0.67±0.03^b
0 ng/g	7	147.3±13.1^a	127±2^c	98±2^c	1.68±0.04^c	0.74±0.07^{ab}

The number (n) and mean mass of fish (in grams) in each group also are shown. Mean values (±S.E.M.) that are not followed by a common letter are significantly different (ANOVA; $P \leq 0.05$).

4. Discussion

The purpose of this study was to evaluate the potential role of PRL in the control of hyperosmoregulation by temperate basses (genus *Morone*). In addition to using commercially available oPRL for hormone replacement in Hx fish, we used a highly purified preparation of homologous *Morone* PRL (Jackson et al., 2000). Our results suggest that the pituitary gland is clearly involved in controlling osmoregulation in hybrid striped bass. Following hypophysectomy and transfer to FW, significantly lower plasma osmolality and levels of Na^+, Cl^-, and Ca^{2+} developed in Hx fish as compared to intact or sham-operated fish. Hx euryhaline *Fundulus* held in FW show a rapid (within 24 h) fall in plasma osmolality and Na^+ levels, which has been exploited to develop a PRL bioassay for verifying the biological activity of newly purified hormone (Grau et al., 1984; Hasegawa et al., 1986; Suzuki et al., 1991). Through preliminary experiments, we determined that significant hydromineral imbalance took much longer to occur (~1 week) in Hx hybrid striped bass. Long-term FW challenge experiments on other Hx teleosts have shown similar results to those reported here (Dharmamba, 1970; MacFarlane, 1974; Bjornsson and Hansson, 1983; Madsen et al., 1996). The difference in timing and severity of effect following hypophysectomy suggests significant variation among teleosts in the importance and time course of PRL action.

Although reductions in plasma Na^+, Cl^-, and Ca^{2+} are consistently measured in hypophysectomy studies, the response of other plasma ions is quite variable in teleosts. In the present study, hypophysectomy affected plasma Mg^{2+} levels inconsistently between experiments. In Experiment 1, but not Experiment 2, Hx bass injected only with hormone vehicle had significantly lower plasma Mg^{2+} levels than controls. Since fish in the first experiment were held after surgery in the recovery tank 2 days longer than fish in the second experiment, the extra time spent in recovery may simply have allowed Mg^{2+} levels to decrease in response to Hx. In neither of our experiments did plasma Mg^{2+} levels in Hx bass respond consistently to PRL injections. Perhaps, there was insufficient scope for action of PRL in this regard on account of the limited recovery time and insignificant drop in plasma Mg^{2+} seen in Hx fish prior to hormone injections. Alternatively, other pituitary-associated factors like cortisol (via ACTH) may be important in regulating plasma Mg^{2+}, as has been shown for plasma Ca^{2+} in trout (Flik and Perry, 1989).

In some teleosts, PRL is known to conserve ions and reduce water loss by affecting the gills, gut, kidney, and urinary bladder, where specific PRL receptors are expressed (reviewed by Manzon, 2002). Hx hybrid striped bass receiving PRL replacement therapy (5 or 20 μg/g oPRL; 10 or 100 ng/g sbPRL) during FW challenge had significantly elevated plasma osmolality and significantly higher plasma levels of Na^+, Cl^-, and Ca^{2+} relative to fish injected with hormone vehicle. In our experiments, both oPRL and sbPRL stabilized ion balance (Na^+, Cl^-, and Ca^{2+}) in Hx bass over the 7-day treatment period. Ovine PRL at the highest dose (20 μg/g) completely restored plasma osmolality and Na^+ and Cl^- levels to control values. But at the doses used, sbPRL did not completely restore plasma osmolality or electrolyte levels to those measured in control or sham-operated fish. We suspect that this is due to our inability to completely optimize our experimental protocol, including dose of sbPRL, length of time between hormone injections (48 h), or the interval between the last injection and the time when the fish were bled (~24 h). In another homologous PRL replacement bioassay, tilapia (genus *Oreochromis*) PRLs ($tPRL_{177}$ and $tPRL_{188}$; alone or in combination) restored plasma osmolality and Na^+ and Cl^- levels in Hx tilapia (*Oreochromis mossambicus*) to values seen in sham-operated fish (Young et al., 1988). The apparent greater potency that we noted for fish PRL versus mammalian PRL in hybrid bass has been reported in several prior studies on *Fundulus*. PRL purified from chinook salmon (*Oncorhynchus tschawytscha*), chum salmon (*Oncorhynchus keta*), and Atlantic croaker (*Micropogonias undulatus*) was more effective than oPRL in sustaining plasma Na^+ levels in Hx *Fundulus* (Grau et al., 1984; Hasegawa et al., 1986; Safford, 1992). The greater potency is likely due to a higher binding affinity of the native hormone to its receptor (Auperin et al., 1994).

PRL's hyperosmoregulatory effect has previously been demonstrated in juvenile striped bass. Injections of oPRL (2 μg/g) significantly elevated mean plasma osmolality of FW-challenged, Hx striped bass over values for saline-injected controls (Madsen et al., 1996). SW-acclimated, intact striped bass receiving injections of oPRL (2 μg/g) or $tPRL_{188}$ (200 ng/g) also had significantly elevated mean plasma osmolality as compared to saline-injected control fish (Madsen et al., 1997).

Excessive Ca^{2+} losses from Hx fish may have been prevented by PRL's hypercalcemic effects. PRL has been shown to regulate extracellular Ca^{2+} homeostasis in a variety of teleosts, including *Fundulus* (Pang et al., 1978), coho salmon (*Oncorhychus kisutch*; Fargher and McKeown, 1989), North American eel (*Anguilla rostrata*; Flik et al., 1989), and common carp (*Cyprinus carpio*; Chakraborti and Mukherjee, 1995). Evidence suggests that PRL promotes Ca^{2+} uptake from water by stimulating branchial Ca^{2+} influx and reducing gill permeability (reviewed by Flik et al., 1996).

The effect of hypophysectomy and PRL replacement on branchial Na^+,K^+-ATPase activity also was explored in this study. Na^+,K^+-ATPase, localized primarily in various chloride cell subtypes (α- and β-) of fish gills, is considered to be the major active mechanism for generating ionic and electrical gradients in transport epithelia relevant to osmoregulation (Hirose et al.,

2003). Alpha chloride cells (secretory type; Perry, 1997), usually associated with SW acclimation, have a high abundance of Na^+,K^+-ATPase, which acts with other transport proteins to secrete excess Cl^-. Previous research has shown that exogenous PRL may reduce the size and density of α-chloride cells (Pisam et al., 1993) and reduce Cl^- extrusion in SW-acclimated *Fundulus* and tilapia, which would be adaptive for FW survival (reviewed by Loretz and Bern, 1982). Foskett et al. (1982) observed a dose-dependent decrease in Cl^- secretion across isolated opercular membranes from SW-acclimated, oPRL-treated tilapia, and proposed that PRL inhibits the active transport pathway in part by promoting dedifferentiation of (α-)chloride cells. This proposal is supported by the results of a study by Herndon et al. (1991), who demonstrated a decrease in chloride cell size and height, but not cell density, following injection of oPRL into SW-acclimated tilapia. The other chloride cell subtype, the β-cell, has less Na^+,K^+-ATPase abundance and is associated with FW acclimation. Accordingly, in several euryhaline teleosts, FW acclimation is associated with a shift in the chloride cell population from α-cells to β-cells and a parallel decrease in specific gill Na^+,K^+-ATPase activity (Shikano and Fujio, 1998).

In the present study, Hx hybrid striped bass receiving PRL injections (20 μg/g oPRL; 10 or 100 ng/g sbPRL) had significantly decreased branchial Na^+,K^+-ATPase activity after 7 days in FW as compared to control animals. The decrease in activity coincided with increased plasma osmolality and electrolyte levels in these same fish. Thus, according to the above model, the reduction by PRL of specific gill Na^+,K^+-ATPase activity may reflect a reduction in chloride cell abundance and/or a shift from α-cell to β-cell subtypes. Injected oPRL decreased gill Na^+,K^+-ATPase activity in Hx Japanese eels and *Fundulus* held in FW, and it was suggested that this action of the hormone assists in retention of monovalent ions during FW acclimation (Pickford et al., 1970; Kamiya, 1972), perhaps by decreasing the number of chloride-secretory cells as described above. Injections of oPRL were effective in reducing opercular chloride cell size and density and gill Na^+,K^+-ATPase activity in SW-acclimated, intact striped bass (Madsen et al., 1997).

As demonstrated previously in the Nile tilapia (Auperin et al., 1995), hypophysectomy alone did not alter branchial Na^+,K^+-ATPase activity in hybrid striped bass. This result is at variance with those reported by Madsen et al. (1996), who found that gill Na^+,K^+-ATPase activity increased in Hx striped bass held at hypoisosmotic conditions. Furthermore, although oPRL injections at a dose of 5 μg/g partly restored plasma osmolality and monovalent ion levels to control values, this dose of hormone did not significantly decrease Na^+,K^+-ATPase activity. Thus, the ability of PRL to offset decreases of plasma osmolality, Na^+, and Cl^- in Hx bass is not obligatorily linked to reduction of branchial Na^+,K^+-ATPase. This dissociation of control is supported by the results of Herndon et al. (1991), who found that PRL injections increased plasma ion levels without changing gill Na^+,K^+-ATPase of SW-acclimated tilapia.

Our results raise the question of whether branchial Na^+,K^+-ATPase activity is normally regulated by the pituitary gland in hybrid striped bass. The lack of a demonstrable effect of hypophysectomy suggests either that pituitary hormones are not important in this regard, or that there is a high constituent level of activity independent of pituitary factors. Madsen et al. (1994) reported that gill Na^+,K^+-ATPase activity and chloride cell density are normally quite high in intact juvenile striped bass and not affected by transfer from FW to SW. These observations do not exclude the possibility that a redistribution of chloride cells and certain associated transport proteins takes place during acclimation to SW. One possibility is that branchial Na^+,K^+-ATPase activity is normally regulated by two or more pituitary or pituitary-dependent factors with antagonistic actions. Thus, a balance between the two or more factors may determine the state of activity of the enzyme, and removal of the opposing influences of these hormones by hypophysectomy may not alter activity from its baseline level. On the basis of its clear ability to downregulate Na^+,K^+-ATPase activity in Hx hybrid striped bass, PRL is a putative inhibitory hormone of this enzyme. Several stimulatory hormones have been demonstrated in teleosts, including growth hormone (GH), cortisol, and insulin-like growth factor-I (reviewed by McCormick, 1995). Recently, it was demonstrated that recombinant striped bass GH stimulated Na^+,K^+-ATPase activity in Hx striped bass in FW and SW (Madsen et al., 1996). The recent availability of purified *Morone* GH and PRL (Jackson et al., 2000), coupled with the methods and information established in this study, sets the stage for elucidation of the role of the pituitary gland in the regulation of branchial Na^+,K^+-ATPase activity during acclimation of temperate basses to changing salinity.

Acknowledgments

We thank Mr. L. Brothers of Carolina Fisheries for providing the hybrid striped bass; Dr. R.G. Hodson for help in producing and procuring the fish; Dr. W. King V and Mr. R.W. Clark for assistance with fish sampling; Ms. D. Covington for help in adapting the Hx protocol to hybrid striped bass; and Dr. G.A. Lewbart for assisting us with analysis of plasma electrolytes. This work was partially sponsored by grant NA46R6007 from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, to the North Carolina Sea Grant College Program. Parts of this work are included in the PhD dissertation research of L.F. Jackson (Department of Zoology, North Carolina State University).

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