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Effects of Salinity, Hypophysectomy, and Prolactin on Whole-Animal Transepithelial Potential in the Tilapia *Oreochromis mossambicus*

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We have examined whether two recently isolated forms of tilapia (*Oreochromis mossambicus*) prolactin exert similar effects on osmoregulatory physiology. The effects of salinity, hypophysectomy, and replacement therapy with tilapia prolactins on whole-animal transepithelial potential (TEP), gill Na^+, K^+-ATPase activity, and plasma ions were determined. When intact fish adapted to 25% seawater (SW) were transferred to different salinities, TEP reached a steady state after 10 hr; TEP increased with increasing salinity from fresh water (FW) to 75% SW but was stable from 75 to 125% SW. Plasma osmolality, $[Na^+]$, and $[Cl^-]$ of these fish 24 hr after salinity change showed that fish in 100 and 125% SW had greater osmotic perturbation than those transferred to lower salinities. Following a 5-day recovery period in 25% SW, hypophysectomized fish transferred to FW for 10 hr had significantly lower TEP and plasma ion levels than either sham-operated fish or intact fish under the same conditions. Injection of hypophysectomized fish with "small" prolactin ($tPRL_{177}$), "large" prolactin ($tPRL_{188}$), or a combination of both (0.5 μg/g body weight) 22 hr and again 20 min prior to transfer from 25% SW to FW, restored TEP and plasma ion levels to those of sham-operated fish. Neither prolactin affected the TEP or plasma ions of sham-operated (intact) fish. Hypophysectomized fish had lower gill Na^+,K^+-ATPase activity than sham-operated fish in FW, but prolactin injections as described above did not affect gill Na^+,K^+-ATPase activity in either hypophysectomized or sham-operated fish. Our results indicate that the two forms of prolactin are indistinguishable with regard to several aspects of tilapia osmoregulation.

Following the experiments of Pickford and Phillips (1959) in which prolactin was found to permit survival of hypophysectomized *Fundulus heteroclitus* in fresh water (FW), the role of prolactin in teleost osmoregulation has been firmly established for several euryhaline species, including the tilapia *Oreochromis mossambicus* (Dharmamba, 1970; Dharmamba and Maetz, 1972, 1976; Dharmamba *et al.*, 1973, 1975; Clarke, 1973; Nagahama *et al.*, 1975; Loretz, 1979; Foskett *et al.*, 1982; Assem and Hanke, 1984). Specker *et al.* (1985) recently isolated two tilapia prolactins, small (20 kDa—177 amino acids) and large (24 kDa—188 amino acids). Although both prolactins were able to prevent the loss of Na^+ from the plasma of hypophysectomized tilapia in FW (Specker *et al.*, 1985), their ability to affect other aspects of tilapia osmoregulation has not been investigated.

Whole-animal transepithelial potential (TEP) is affected by hormone treatment in other teleost species (Iwata and Bern, 1985; Iwata *et al.*, 1987). TEP is generally believed to be the result of active ion transport and passive ion permeability across the chloride cell-containing epithelia of the gills, skin, and opercular membranes (see Potts, 1980, for review; Marshall, 1977; Karnaky *et al.*, 1977; Williams *et al.*, 1988).

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The specific mechanisms responsible have been identified in studies using the isolated opercular membrane. Foskett *et al.* (1981) demonstrated that active Cl^- secretion alone was responsible for the potential difference across the isolated seawater (SW) tilapia opercular membrane bathed with identical Ringer solutions on both sides. Studies on the opercular membranes of other species have shown that passive Na^+ permeability is confined to and dominates the paracellular pathway(s) (Degnan and Zadunaisky, 1980). Further, in tilapia opercular membranes both active Cl secretion and paracellular conductance are decreased in parallel by prior treatment of the fish with prolactin (Foskett *et al.*, 1982) and Dharmamba and Maetz (1976) have shown that prolactin treatment of SW-adapted tilapia decreased both the influx and efflux of Na^+. Thus, the TEP of tilapia in SW is dominated by a combination of an active transport potential due to Cl^- secretion and a Na^+ diffusion potential through the paracellular pathway, both of which tend to make the body fluids of the fish electrically positive with respect to the SW medium.

Although the influence of prolactin on plasma ions and ion fluxes in tilapia has been examined, there is no information on the effect of hypophysectomy and prolactin replacement on whole-animal TEP or on gill Na^+,K^+-ATPase, the major active ion transport enzyme of gill tissue. The intent of the present study is to determine (1) the response of whole-animal TEP of tilapia to rapid changes in environmental salinity and (2) the effects of hypophysectomy and subsequent replacement therapy with two prolactins on TEP, plasma osmolality, [Na^+], [Cl^-], and gill Na^+,K^+-ATPase activity of tilapia adapted to 25% SW and transferred to FW.

MATERIALS AND METHODS

Fish and Maintenance

Adult tilapia *O. mossambicus* of both sexes (20–40 g), which had been reared in fresh water, were maintained in 20-liter tanks with recirculating water at a temperature of 27° and a photoperiod of 12L:12D. Aeration was provided, and water was continuously recirculated through a charcoal and bioactive-uv filter system.

Hypophysectomy

Hypophysectomy was performed on freshwater-adapted tilapia by the orbital method described by Nishioka (1980). Fish were allowed to recover in 25% SW for at least 5 days following surgery and prior to transfer to FW.

TEP Measurement

Electrical measurement of TEP was conducted by the method of Iwata and Bern (1985) with some modifications. Each fish was anesthetized in a shallow pan half-filled with 25% SW containing 0.02% 2-phenoxyethanol, and a small cut was made in the skin between the dorsal and pectoral fins. An internal bridge of 4% agar (in tilapia Ringer solution) in 0.86-mm (i.d.) polyethylene tubing was inserted about 20 mm deep along the dorsal muscle. A similar external bridge was placed in the test water containing 0.015% 2-phenoxyethanol. After 20 min, the fish was transferred into a 350-ml plexiglass chamber with 10 liters of continuously recirculated test water. Both bridges were connected to calomel electrodes attached to a voltmeter/amplifier connected to a chart recorder for continuous recording of TEP. The electrode asymmetry between each pair of electrodes was checked before and after each recording and appropriate compensation was made. TEP values are reported as the potential of the body fluids with respect to the external medium.

All TEP readings were corrected for liquid junction potential at the external Ringer–agar bridge using the method of Barry and Diamond (1970). The ends of agar–Ringer bridges identical to those used to measure the TEP were immersed in separate beakers, one containing tilapia Ringer solution and the other containing a sample of one of the external media used in the experiments. With the potential measuring apparatus previously balanced to eliminate electrode asymmetry, the junction potential between the agar–Ringer bridge and each external medium was measured by connecting the two beakers electrically for brief intervals with a fresh agar–3 *M* KCl bridge. These measurements were performed periodically for 26 hr and the junction potentials are shown in Fig. 1 as the potential of the beaker of Ringer solution (equivalent to body fluids) with respect to the beaker of external medium, i.e., the polarity convention used for the TEP. All TEPs were corrected for the junction potential

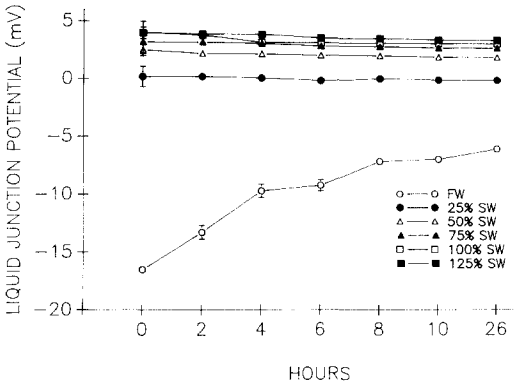

FIG. 1. Junction potential of Ringer–agar bridge with different seawater concentrations from 0 to 26 hr. Vertical bars = means ± SEM (n = 3).

measured at the corresponding time by subtracting the appropriate potential shown in Fig. 1 from the measured TEP. The junction potential between the agar–Ringer bridge and FW was time dependent, taking about 8 hr to stabilize; the junction potentials for all other external media were time independent.

In previous studies on *Gillichthys* and coho salmon (Iwata and Bern, 1985; Iwata *et al.*, 1987), an attempt to control for junction potentials was made by immersion of the agar bridges in appropriate solutions for at least 1 week prior to use. The readings obtained from tilapia by this method are the same as the values corrected by the Barry and Diamond (1970) method at 25 and 50% salinities, but were slightly lower in FW and slightly higher at higher salinities.

For the purpose of determining whether TEP readings taken from the dorsal musculature would differ from those taken from the peritoneal cavity, measurements from both sites were taken from the same fish in 100% SW. An agar bridge was inserted in the dorsal musculature (with a corresponding electrode in the SW medium) and another electrode was inserted in the peritoneal cavity (also with corresponding electrodes in the medium). TEP readings were continuously recorded for 25 hr.

Response of TEP to Different Salinities

Individual tilapia adapted to 25% SW were transferred into SW concentrations from 0 (FW), 25, 50, 75, 100, to 125%, and the TEP reading was taken 10 hr after transfer when the change in TEP had leveled off (n = 4 at each salinity). The various test salinities were prepared by dilution of 100 and 200% artificial SW (Marine Environment, San Francisco, CA) with aerated tap water (100% SW = 34 ppt). After 18 hr of TEP measurement, blood was collected from the caudal vessels into heparinized (ammonium) hematocrit tubes and centrifuged; plasma was stored at −20°.

Effect of Prolactin on TEP

Six hypophysectomized and six sham-operated fish were used for each of the following injections: saline solution, small prolactin, large prolactin, and a combination of both prolactins (1:1). The tilapia prolactins (177-amino acid residues = small; 188 amino acid residues = large) were isolated by HPLC (see Specker *et al.*, 1985; Yamaguchi *et al.*, 1988). For the saline solution controls, 0.7% NaCl was injected in a volume of 0.5 μl/g body weight. For fish receiving small or large prolactin, the hormone was dissolved in 0.7% NaCl at a concentration of 1.0 mg/ml and injected in volumes of 0.5 μl/g body weight. For fish receiving the combination of prolactins, half of the dosage of each hormone was combined and injected. Injections were given intraperitoneally while fish were lightly anesthetized with 0.02% 2-phenoxyethanol at 22 hr and again at 20 min prior to insertion of agar bridges and transfer to FW. TEP was measured for 18 hr in FW, after which blood samples were collected for plasma analyses (osmolality and Na^+ and Cl^- concentrations) and gills were collected for measurement of Na^+, K^+-ATPase activity.

Gill Na^+,K^+-ATPase Activity

In order to determine the effect of salinity on gill Na^+,K^+-ATPase activity, unoperated tilapia were adapted to 0, 25, and 100% SW for a minimum of 3 weeks. The effect of hypophysectomy and prolactin replacement on gill Na^+,K^+-ATPase activity was determined in gill tissue from the same animals upon which TEP measurements were made.

Gill Na^+,K^+-ATPase activity was measured by a modification of the method of McCormick *et al.* (1987). Gill tissue (0.05–0.10 g wet weight) was cut away from the gill arch and placed in 1 ml ice-cold buffer containing 0.3 *M* sucrose, 0.02 *M* ethylenediaminetetraacetic acid, and 0.05 *M* imidazole (SEI buffer, pH 7.3) and then immediately frozen and stored at −80° for 2 to 4 weeks. Gill tissue was homogenized in 0.8 ml SEI buffer with 0.1% sodium deoxycholate in a conical glass homogenizer (0.13 mm clearance) and centrifuged for 5 min at 3000× gravity. Na^+,K^+-ATPase activity of the supernatant was determined by measuring the production of inorganic phosphate following incubation for 10 min at 28° in a solution of 100 m*M* NaCl, 20 m*M* KCl, 5 m*M* $MgCl_2$, and 4 m*M* Na_2ATP at pH 7.6, in the presence or absence of 0.5 m*M* ouabain (cf. Dange, 1985). This concentration of ouabain was found to be a saturating concentration for inhibition of Na^+,K^+-ATPase activity and was equivalent to exclusion of KCl from the incu-

bation media. Inorganic phosphate was measured by the method of Heinonen and Lahti (1981) and protein by the method of Miller (1959). Activity is expressed as micromoles inorganic phosphate per milligrams protein per hour.

Plasma osmolalities were determined using a vapor pressure osmometer. Plasma sodium concentrations were measured with an atomic absorption spectrophotometer. Plasma chloride concentrations were measured using a silver chloride titration chloridometer.

RESULTS

There was no difference in the TEP readings taken from the dorsal musculature and from the peritoneal cavity of fish adapted to 25% SW and placed in 100% SW (Fig. 2). The TEP at the start was −3 mV, gradually increased to 18 mV after 8 hr and remained relatively stable (change of <0.14 mV/hr from 8 to 25 hr). Since it was easier to keep the electrode implanted in the dorsal musculature, TEP readings for all experiments were taken at this site after 10 hr.

Responses of Intact Fish to Salinity

Mean TEP of fish transferred from 25% SW increased with increasing salinity of the medium, from 3 mV in FW to 18 mV in 75% SW (Fig. 3). In 75 to 125% SW the TEP leveled off at 17–18 mV. Although the TEP at 125% SW was slightly lower than the TEP at 100% SW, the difference was not statistically significant ($P > 0.05$; ANOVA, Tukey's test).

Plasma osmolality, $[Na^+]$, and $[Cl^-]$ 18 hr after transfer (Fig. 4) also increased with increasing salinity. Plasma osmolality, $[Na^+]$, and $[Cl^-]$ of fish in 25 to 75% SW were similar and were 5–10% greater than those of fish transferred to FW. Tilapia transferred to 100 and 125% SW had plasma osmolarity and ion levels 25–35% higher than those of fish transferred to FW.

Gill Na^+,K^+-ATPase activity of unoperated tilapia adapted for at least 3 weeks to differing salinities was significantly greater in 100% SW (7.9 ± 0.8 μmol P_i · mg protein^{-1} · hr^{-1}, $n = 6$) than in FW (2.7 ± 0.3) or 25% SW (3.7 ± 0.3; $P < 0.05$; one-way ANOVA, Tukey's test).

Effect of Hypophysectomy and Prolactin Treatment

Saline-injected hypophysectomized fish had a TEP (−6.0 ± 1.1 mV) that was opposite in polarity and significantly lower than those of both sham-operated fish (6.0 ± 1.3 mV) and intact fish (3.1 ± 3.3 mV) transferred from 25% SW to FW ($P < 0.01$; one-way ANOVA, Tukey's test). The TEPs of the sham-operated and intact fish were not significantly different. Treatment of hypophysectomized fish with small or large prolactin, or with a combination of both prolactins, restored TEP to levels seen in sham-operated fish under the same conditions (5–11 mV; Fig. 5). Prolactins had no effect on TEP of sham-operated fish.

Plasma osmolality, $[Na^+]$, and $[Cl^-]$ of hypophysectomized fish injected with saline solution were significantly lower than

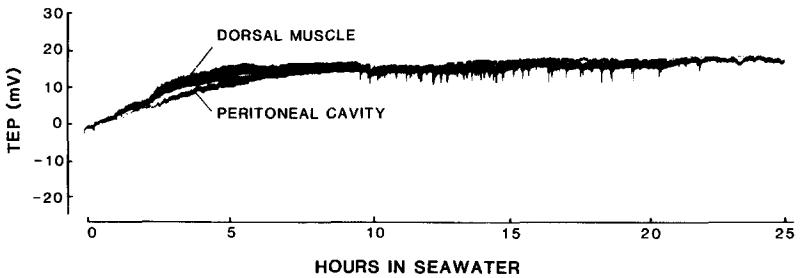

FIG. 2. TEP of tilapia transferred directly from 25 to 100% seawater taken from the dorsal musculature and from the peritoneal cavity for 24 hr ($n = 1$).

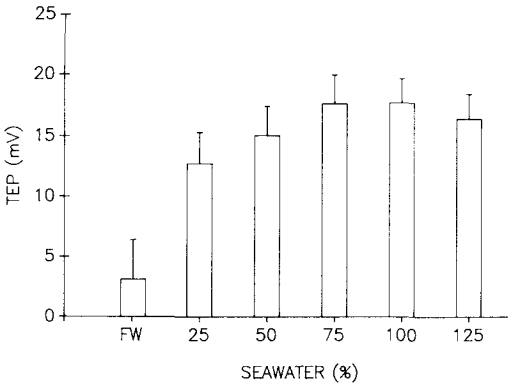

FIG. 3. TEP of tilapia 10–18 hr after being transferred directly from 25% seawater to different seawater concentrations (n = 4).

those of sham-operated fish ($P < 0.05$; ANOVA; Tukey's test; Fig. 6). Treatment of hypophysectomized tilapia with small prolactin, large prolactin, or a combination of both restored plasma osmolality, $[Na^+]$, and $[Cl^-]$ to levels seen in sham-operated fish. Prolactin had no effect on plasma osmolality, $[Na^+]$, or $[Cl^-]$ of sham-operated fish.

Gill Na^+,K^+-ATPase activity of hypophysectomized tilapia (the same fish upon which TEP and plasma ion measurements were made) was significantly lower than that of sham-operated fish ($P < 0.05$; two-way ANOVA; Fig. 7). Prolactin injections (40 and 18 hr prior to sampling of gill tissue) had no significant effect on gill Na^+,K^+-ATPase activity either in hypophysectomized or in sham-operated tilapia ($P > 0.05$; one- and two-way ANOVA).

DISCUSSION

About 8 hr was needed for whole-animal TEP of tilapia transferred from 25 to 100% SW to reach a steady state. In the goby *G. mirabilis* and the coho salmon *O. kisutch*, steady-state TEP is reached in about 10 hr following an abrupt change in salinity (Iwata and Bern, 1985; Iwata *et al.*, 1987). Steady-state readings from the dorsal musculature and from the peritoneal cavity of

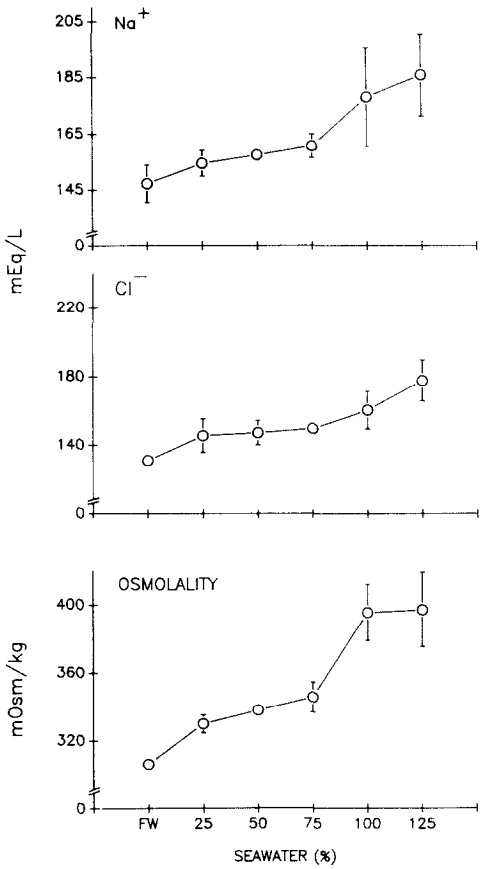

FIG. 4. Plasma Na^+, Cl^-, and osmolality of tilapia 18 hr after being transferred directly from 25% seawater to different seawater concentrations (n = 4).

tilapia were the same (Fig. 2), consistent with the results of Iwata and Bern (1985) in the goby.

The absolute levels of the steady-state TEP and their response to environmental salinity are similar in magnitude to those reported for a variety of other teleosts (Evans, 1980; Iwata and Bern 1985; Iwata *et al.*, 1987). Dharmamba *et al.* (1975) found that TEP of tilapia adapted to 33 and 100% SW were 14.7 and 35.2 mV, respectively. Comparing these values to the present study, abrupt transfer to increasing salinity resulted in similar TEP values at 33% SW (13.2 mV in the present study, by interpolation), but lower values at 100% SW (18.0 mV). It seems likely that the lower TEP in

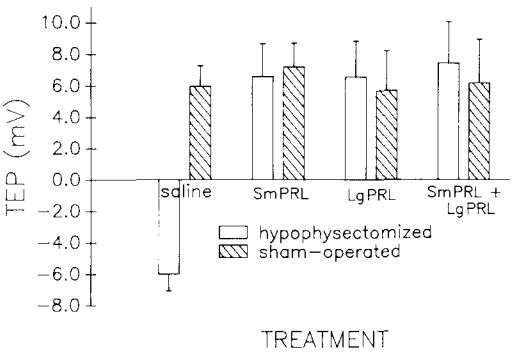

FIG. 5. TEP of hypophysectomized and sham-operated tilapia injected with small prolactin (Sm PRL), large prolactin (Lg PRL), combination of both prolactins, and saline solution (n = 6). Fish were adapted to 25% SW after surgery for at least 5 days, followed by two prolactin injections and transfer to FW.

100% SW is due, at least in part, to the difference between short- and long-term acclimation to SW. Foskett *et al.* (1981) found that tilapia transferred from 33 to 100% SW required several days to reach maximum levels of TEP (of the isolated opercular membrane) and chloride cell size.

Following transfer from 25% SW, TEP increased with increased salinity of the medium from FW to 75% SW (Fig. 3). In salinities from 75 to 125% SW, however, the TEP readings were the same (17–18 mV). In 100 and 125% SW, tilapia had higher plasma osmolality, $[Na^+]$, and $[Cl^-]$ than fish transferred to lower salinities. These values are also higher than those considered normal for SW-adapted tilapia (Dharmamba *et al.*, 1975). The increased plasma ions and osmolality, and the leveling off of TEP at 100 and 125% SW, suggest that at this time point in the acclimation of tilapia to SW, net ion efflux is insufficient to counteract passive influx of ions across the body surface.

The TEP of hypophysectomized fish adapted to 25% SW and transferred to FW was opposite in polarity and significantly lower than that in sham-operated fish (Fig. 5), indicating that without the hypophysis tilapia cannot maintain normal TEP in FW

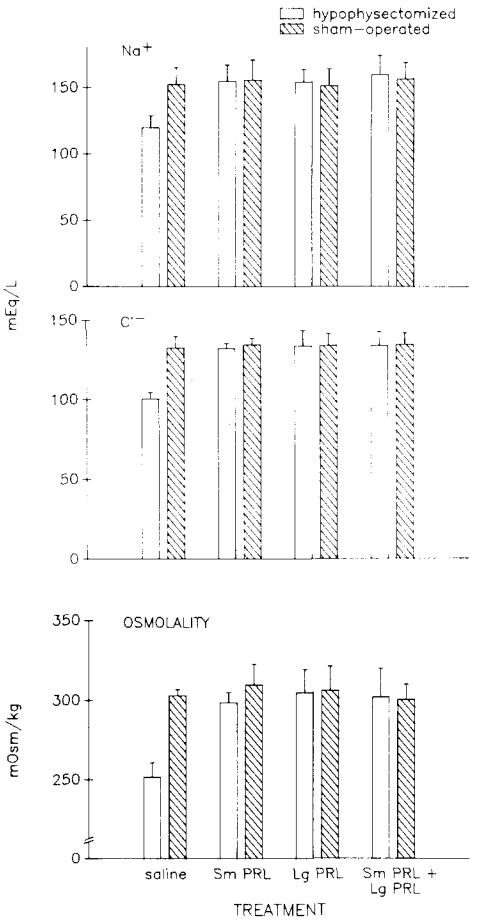

FIG. 6. Plasma $[Na^+]$, $[Cl^-]$, and osmolality of hypophysectomized and sham-operated tilapia after injection with small prolactin (Sm PRL), large prolactin (Lg PRL), combination of both prolactins, and saline solution (n = 6). Fish were adapted to 25% SW after surgery for at least 6 days, followed by two prolactin injections and transfer to FW.

and consistent with the finding that these fish were in negative Na^+ balance (Fig. 6). Iwata and Bern (1985) found that hypophysectomy in *Gillichthys* induced a lower TEP than that in intact fish and RPD-autotransplanted fish in FW. In the present study, treatment of tilapia with either or both prolactin(s) enabled hypophysectomized fish to maintain normal TEP in FW and to maintain plasma osmolality, $[Na^+]$, and $[Cl^-]$. One of the known functions of prolactin (see Foskett *et al.*, 1983; Hirano,

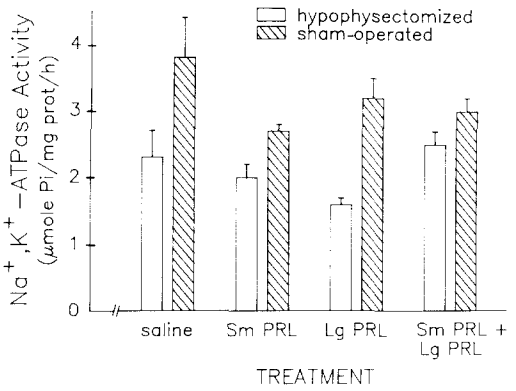

FIG. 7. Gill Na^+,K^+-ATPase activity of hypophysectomized and sham-operated tilapia after injection with small prolactin (Sm PRL), large prolactin (Lg PRL), combination of both prolactins, and saline solution (n = 6). Fish were adapted to 25% SW after surgery for at least 5 days, followed by two prolactin injections and transfer to FW.

1986) is to reduce the permeability of the epithelium to Na^+, thus preventing the loss of Na^+ from the body. Specker *et al.* (1985) found that both tilapia prolactins prevented the loss of Na^+ from the plasma of hypophysectomized tilapia in FW. Our results indicate that both prolactins can also restore plasma osmolality and $[Cl^-]$ to normal levels, as well as return TEP of hypophysectomized tilapia to that seen in sham-operated fish.

Changes in gill Na^+,K^+-ATPase in response to salinity found in the present study are similar to those previously reported for this species (Dange, 1985). Hypophysectomy of a variety of teleosts results in decreased gill Na^+,K^+-ATPase activity which in most instances can be restored by treatment with cortisol (Pickford *et al.*, 1970b; Butler and Carmichael, 1972; Richman *et al.*, 1987). Prolactin, however, may also have a role in regulating gill Na^+,K^+-ATPase; prolactin treatment inhibits gill Na^+,K^+-ATPase activity in SW-adapted *Chelon labrosus* (Gallis *et al.*, 1979) and in hypophysectomized FW killifish *Fundulus heteroclitus* (Pickford *et al.*, 1970a). In the present study, the removal of the tilapia hypophysis reduced gill Na^+,K^+-ATPase (Fig. 7). Treatment with prolactin did not affect gill Na^+,K^+-ATPase activity in either hypophysectomized or sham-operated fish. This indicates that over the time course used in the present study (40 hr from the first injection to the end of TEP recording), prolactin has no effect on gill Na^+,K^+-ATPase activity of tilapia.

In conclusion, we have found that hypophysectomy reduces the ability of tilapia to regulate plasma osmolality, $[Na^+]$, and $[Cl^-]$ in FW and results in reversal of the polarity of whole-animal TEP and decreased gill Na^+,K^+-ATPase activity. Extending the previous results of Specker *et al.* (1985), we have found that both $tPRL_{177}$ and $tPRL_{188}$ have the capacity to restore plasma osmolality, $[Na^+]$, and $[Cl^-]$, as well as whole-animal TEP. We conclude that the two tilapia prolactins have equivalent effects on these aspects of the osmoregulatory physiology of tilapia.

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Note added in proof. Specker *et al.* (*1st Intl. Symp. Fish Endocrinol.*, in press) found a dose-dependent difference between large and small prolactins on salamandrid integumental TEP, using the whole-animal method developed by Brown *et al.* (1985), which is similar to the method used herein on tilabia.

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