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Effects of salinity and prolactin on gene transcript levels of ion transporters, ion pumps and prolactin receptors in Mozambique tilapia intestine

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ABSTRACT

Euryhaline teleosts are faced with significant challenges during changes in salinity. Osmoregulatory responses to salinity changes are mediated through the neuroendocrine system which directs osmoregulatory tissues to modulate ion transport. Prolactin (PRL) plays a major role in freshwater (FW) osmoregulation by promoting ion uptake in osmoregulatory tissues, including intestine. We measured mRNA expression of ion pumps, Na^+/K^+-ATPase α3-subunit (NKAα3) and vacuolar type H^+-ATPase A-subunit (V-ATPase A-subunit); ion transporters/channels, $Na^+/K^+/2Cl^-$ co-transporter (NKCC2) and cystic fibrosis transmembrane conductance regulator (CFTR); and the two PRL receptors, PRLR1 and PRLR2 in eleven intestinal segments of Mozambique tilapia (*Oreochromis mossambicus*) acclimated to FW or seawater (SW). Gene expression levels of NKAα3, V-ATPase A-subunit, and NKCC2 were generally lower in middle segments of the intestine, whereas CFTR mRNA was most highly expressed in anterior intestine of FW-fish. In both FW- and SW-acclimated fish, PRLR1 was most highly expressed in the terminal segment of the intestine, whereas PRLR2 was generally most highly expressed in anterior intestinal segments. While NKCC2, NKAα3 and PRLR2 mRNA expression was higher in the intestinal segments of SW-acclimated fish, CFTR mRNA expression was higher in FW-fish; PRLR1 and V-ATPase A-subunit mRNA expression was similar between FW- and SW-acclimated fish. Next, we characterized the effects of hypophysectomy (Hx) and PRL replacement on the expression of intestinal transcripts. Hypophysectomy reduced both NKCC2 and CFTR expression in particular intestinal segments; however, only NKCC2 expression was restored by PRL replacement. Together, these findings describe how both acclimation salinity and PRL impact transcript levels of effectors of ion transport in tilapia intestine.

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1. Introduction

Euryhaline fishes have the capacity to maintain a narrow range of internal osmolality in response to a wide range of environmental salinities. While fish acclimated to fresh water (FW) face a hyposmotic environment, in which equilibrium is challenged by osmotic water gain and diffusive ion loss, fish acclimated to seawater (SW) face dehydration. To manage such challenges, teleost fishes, including the euryhaline Mozambique tilapia (*Oreochromis mossambicus*), have evolved complex physiological functions at the level of osmoregulatory organs, such as the gill, kidney and intestine, which are governed in large part by the endocrine system (see Marshall and Grosell, 2006; McCormick, 2011).

In FW, teleosts eliminate excess water by producing a copious amount of dilute urine, while in SW, fish must compensate for water loss by drinking SW, which in turn must be desalinated to establish an osmotic gradient that is favorable to water absorption (Lin et al., 2001; Smith, 1930). In most teleosts, desalination of the ingested SW begins in the esophagus, where ions are removed, both actively and passively, from the luminal fluid through the activities of ion pumps and transporters (Grosell, 2006; Hirano

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and Mayer-Gostan, 1976; Parmelee and Renfro, 1983). The active transport of ions is energized by Na^+/K^+-ATPases (NKA) located in the basolateral membrane of epithelial cells of the gastrointestinal (GI) tract. Ion movements through apical epithelial transporters and channels takes place following the electrochemical gradient established by NKA (Grosell, 2006). The transport of Na^+ across intestinal epithelia has been mainly attributed to the $Na^+/K^+/2Cl^-$ co-transporter (NKCC) and the Na^+/Cl^- co-transporter (NCC), whereas Cl^- has been shown to enter intestinal epithelial cells through an electrogenic HCO_3^-/Cl^- exchanger (SLC26a6), in addition to NCC and NKCC (Bayaa et al., 2009; Gregorio et al., 2013; Grosell et al., 2009b; Marshall and Singer, 2002; Watanabe et al., 2011). The proton pump vacuolar type H^+-ATPase (V-ATPase), extrudes H^+ through the apical membrane, which contributes to the driving force for HCO_3^-/Cl^- exchange, and thereby Cl^- uptake (Grosell et al., 2009a,b; Guffey et al., 2011). Exit of Cl^- down a concentration gradient across the basolateral membrane takes place through Cl^- channels including the cystic fibrosis transmembrane conductance regulator (CFTR) (Loretz and Fourtner, 1988; Marshall and Singer, 2002). Transcription of the catalytic α1 and α3 subunits of NKA and specific isoforms of NKCC and NCC have been described in multiple tissues of Mozambique tilapia (Feng et al., 2002; Hiroi et al., 2008; Lee et al., 1998; Tipsmark et al., 2011). Of the NKCC isoforms, NKCC2 was most highly expressed in the intestine, with low or absent expression in other tissues, whereas mRNA levels of NKCC1a, NKCC1b and NCC were relatively low in the intestine (Hiroi et al., 2008). In addition, robust expression of V-ATPase mRNA was observed in the intestine of FW-acclimated fish (Hiroi et al., 2008). Together, these pumps, transporters and channels participate in maintaining osmotic homeostasis following changes in environmental salinities, and therefore, are potentially under endocrine control.

Osmoregulatory capabilities of the intestine are partly regulated by prolactin (PRL), a hypophyseal hormone that plays a major role in FW acclimation in teleosts (see Hirano et al., 1987; Manzon, 2002; McCormick and Bradshaw, 2006). The hyperosmoregulatory actions of PRL are consistent with experiments where hypophysectomized euryhaline fish were able to survive in FW only after PRL was administered (Breves et al., 2010b; Dharmamba, 1970; Pickford and Phillips, 1959; Young et al., 1988). Also consistent with its essential role in FW osmoregulation, PRL gene expression and release are directly stimulated by a fall in extracellular osmolality, and baseline levels are higher in tilapia acclimated to FW than those acclimated to SW (Grau et al., 1981; Seale et al., 2002, 2012b). While PRL is known to increase Na^+ and water absorption in the anterior intestine of SW-acclimated Mozambique tilapia (Mainoya, 1982), little information is available on how PRL regulates specific ion pumps, transporters and channels along the GI tract. Two PRL receptors, PRLR1 and PRLR2, have been identified in tilapia; both receptors are highly expressed in the various osmoregulatory organs, including intestine (Fiol et al., 2009). While the relationships among environmental osmolality, PRL and its receptors in the intestine are unknown, branchial PRLR1 is regulated by PRL (Breves et al., 2010b), whereas PRLR2 mRNA levels in the gill and pituitary rise with increases in extracellular osmolality (Fiol et al., 2009; Seale et al., 2012a). Therefore, it is plausible that acclimation salinity may exert a strong influence on intestinal ionoregulatory function, via the modulation of PRL receptor expression. We hypothesize that the expression patterns of effectors of ion transport and PRLRs along the tilapia intestine are distinct between FW- and SW-acclimated fish and potentially under pituitary control. Thus, the aims of this study were to (1) compare intestinal gene expression of NKA (NKAα3), V-ATPase (V-ATPase A-subunit), NKCC2, CFTR, PRLR1 and PRLR2 between FW- and SW-acclimated tilapia and, (2) examine the capacity for PRL to regulate expression of these transcripts *in vivo*.

2. Materials and methods

2.1. Fish

Mature Mozambique tilapia (*O. mossambicus*) of both sexes (45–140 g), were selected from a population maintained at the Hawaii Institute of Marine Biology, University of Hawaii. Fish were reared in outdoor tanks (700 L) with a continuous flow of either FW or SW (35‰; Kaneohe Bay, HI) under natural photoperiod. SW-acclimated tilapia employed in this experiment were spawned and reared in SW, having never been previously exposed to FW. FW-acclimated tilapia, on the other hand, were spawned and reared in FW, having never been previously exposed to SW. Water temperature was maintained at 24–26 °C. Animals were fed approximately 5% of their body weight per day with Silver Cup Trout Chow (Nelson and Sons, Murray, UT) until experimentation. All experiments were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee, University of Hawaii.

2.2. Collection of intestinal segments from FW- and SW-acclimated fish

Tilapia of both sexes ranging in size from 45 to 140 g were sampled from FW and SW tanks, and food was withheld for 1 day prior to sampling. At the time of sampling, fish were anesthetized in 2-phenoxyethenol (2-PE; 0.3 ml/L; Sigma, St. Louis, MO). Blood was collected from the caudal vasculature by a needle and syringe coated with ammonium heparin (200 U/mL, Sigma). Plasma was separated by centrifugation and stored at −20 °C for later analyses. Measurements of plasma osmolality were made using a vapor pressure osmometer (Wescor, Logan, UT). Anesthetized fish were euthanized by rapid decapitation and the entire length of the intestine was removed. The intestine was then divided into five sections based on structural morphology (Smith et al., 2000); hepatic loop (HL), proximal major coil (PMC), distal major coil (DMC), gastric loop (GL), and terminal segment (TS) without the rectum. Within these five sections, the intestine was further subdivided and the tissues were collected from a total of eleven segments (Fig. 1). After a wash in saline (0.9% NaCl), each tissue (1 cm) was frozen in liquid nitrogen and stored at −80 °C prior to RNA extraction and analysis of gene expression by qRT-PCR.

2.3. Hypophysectomy and PRL replacement

To determine the effects of PRL on intestinal ion pumps, transporters and PRLRs, hypophysectomy followed by PRL replacement therapy was carried out using FW-acclimated male tilapia weighing 80–100 g. Food was withheld from animals from 1 day prior to surgery through the 3-day recovery period. Hypophysectomy was performed by the transorbital technique described by Nishioka (1994). Sham-operated fish were subjected to the same surgical procedure as hypophysectomized animals except for removal of the pituitary. Following surgery, fish were allowed to recover in isosmotic brackish water (BW; 12‰) treated with kanamycin sulfate (National Fish Pharmaceuticals, Tucson, AZ). Fish (n = 6–7) were kept in recirculating aquaria containing aerated BW for 3 days and then injected intraperitoneally with 5 μg/g body weight ovine PRL (oPRL; Sigma) dissolved in saline (0.9% NaCl; 0.01 M $NaHCO_3$) under anesthesia (2-PE; 0.3 ml/L), and placed back into recirculating aquaria containing BW. Sham-operated fish were injected with saline vehicle and intact fish were only handled at the time of sampling. Fish were sampled 24 h after injection. After fish were anesthetized in 2-PE (0.3 ml/L) blood was collected and plasma osmolality measurements were obtained as described

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