Comparative Biochemistry and Physiology, Part C 157 (2013) 236–250

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Gastro-intestinal transport of calcium and cadmium in fresh water and seawater acclimated trout (*Oncorhynchus mykiss*)

Joel S. Klinck *, Chris M. Wood

Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario, Canada L8S 4K1

ARTICLE INFO

Article history:
Received 6 October 2011
Received in revised form 20 November 2012
Accepted 20 November 2012
Available online 29 November 2012

Keywords:
Cadmium
Calcium
Gastro-intestinal tract
Gut sac
Rainbow trout
Steelhead trout

ABSTRACT

Transport of calcium (Ca) and cadmium (Cd) was examined along the gastro-intestinal tract (GIT) of freshwater and seawater *Oncorhynchus mykiss irideus* (FWT and SWTies respectively) using *in vitro* and *in vivo* experiments. Based on known physiological differences between FWT and SWT which aid in regulating ion levels and osmolarity, we hypothesized that SWT would have lower rates of Ca uptake. Also, we predicted that Cd rates would also be lower because Cd is known to share a common transport mechanism with Ca. Kinetics of Ca and Cd transport were determined using mucosal salines of varying concentrations [1, 10, 30, 60, and 100 (mmol L^{-1} for Ca, μmol L^{-1} for Cd)]. Linear and saturating relationships were found for Ca for FWT and SWT, but overall SWT had lower rates. Linear and/or saturating relationships were also found for Cd uptake, but rates varied little between fish types. Elevated Ca had no inhibitory effect on Cd transport, and Ca channel blockers nifedipine and verapamil had little effect on Ca or Cd uptake. However, lanthanum reduced Ca transport into some compartments. A 21 day *in vivo* feeding experiment was also performed where FWT and SWT were exposed to control diets or Cd-spiked diets (552 μg Cd g^{-1} food). Whole body Cd uptake between fish types was similar, but the majority of Cd in SWT remained in the posterior intestine tissue, while FWT transported more Cd through their gut wall. Overall it appears that large differences in Ca and Cd uptake between FWT and SWT exist, with SWT generally having lower rates.

1. Introduction

Oncorhynchus mykiss irideus are euryhaline fish. In fresh water, they are commonly known as rainbow trout (referred to FWT in this study), and when living in seawater they are known as steelhead trout (referred to as SWT in this study). Fish take up metals via two major pathways: their gills and/or their gut, and the contribution of each pathway largely depends on the salinity of its environment. FWT live in a hypo-osmotic environment from which they actively take up ions at their gills and gain water (therefore they do not have the need to drink) (Evans et al., 2005). In contrast, SWT live in environments which are hyper-osmotic, necessitating the constant excretion of ions at their gills, gut, and kidney (Marshall and Grosell, 2006). SWT are continually losing water to their surroundings and compensate for this by drinking copious amounts of seawater. Drinking seawater leads to excessive ion uptake along the gastro-intestinal tract (GIT) which the fish also need to excrete via their gills and kidneys (Folmar and Dickhoff, 1980).

Waterborne Ca concentrations are approximately 10-fold higher in seawater than in fresh water, and GITs of seawater fish are not only exposed to high concentrations of ions in ingested seawater, but also from ingested meals. For example, sardines have an internal Ca concentration 40 times higher than seawater (Taylor and Grosell, 2006). Despite vast differences in environmental conditions in which *O. mykiss* can survive, they maintain a plasma ion content within a narrow range by undergoing major functional changes when they travel between habitats with varying salinities (Folmar and Dickhoff, 1980).

We predict that mechanisms of Ca absorption along the GIT would be generally down-regulated in seawater teleosts relative to freshwater teleosts, so as to protect against excessive, potentially toxic, Ca uptake. There is a small amount of evidence available which supports this idea. In a study by Schoenmakers et al. (1993), net uptake of Ca in the intestine was 71% lower in seawater tilapia compared to freshwater tilapia, probably explained by reduced activity of Ca^{2+}-ATPase (by 28%), and Na^{+}/Ca^{2+} exchanger (by 22%). Furthermore, the intestinal tissues of seawater fish secrete HCO_3^- so as to precipitate Ca as $CaCO_3$, thereby reducing both Ca availability for absorption and luminal osmolality; the latter also aids water absorption (reviewed by Wilson et al., 2002; Ando et al., 2003; Grosell et al., 2009).

Metals (such as Cd) in the environment occur from both natural processes (e.g. erosion, volcanic eruptions, forest fires) as well as from anthropogenic inputs (e.g. mining and manufacturing), and can potentially disrupt ion balance. Compared to freshwater fish, few stud on metal uptake have been conducted on marine fish, and fewer still on dietary uptake (Wood, 2001). It is known that rates of Cd uptake from waterborne exposure decrease with increasing salinity (Zhang and

* Corresponding author. Tel.: +1 905 525 9140x23237; fax: +1 905 522 6066.
E-mail address: klinckjs@mcmaster.ca (J.S. Klinck).

1532-0456/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cbpc.2012.11.006

Wang, 2007b). In freshwater fish it has been found that dietary Cd can be the dominant route of uptake (Dallinger et al., 1987), and the same has been found for seawater fish (> 90% of total metal uptake) (Xu and Wang, 2002; Zhang and Wang, 2005, 2007a).

In the present study, an *in vitro* isolated gut sac technique was used to investigate potential mechanisms of Ca and Cd transport, and their interactions, in FWT and SWT of identical strain and size, originating from the same source. Four distinct gut segments were tested, examining the concentration-dependence of Ca and Cd uptake in SWT and FWT to gain evidence regarding whether saturable transporters were involved, and whether affinity and capacity constants varied among the sites. Three Ca channel blockers were tested for effects on both Cd and Ca uptake, in preparations from both FWT and SWT. An *in vivo* feeding experiment was also carried out to determine tissue burden concentrations and distribution differences of Cd in FWT and SWT. Our overall hypotheses were: (i) that rates of both Ca and Cd uptake would be reduced in SWT relative to FWT, though not necessarily to the same extent; (ii) that tissue Cd burdens resulting from dietary Cd exposure would be lower in SWT; and (iii) that the pharmacological tests and competition experiments would reveal the predominance of different transport mechanisms in SWT versus FWT.

2. Materials and methods

2.1. Experimental animals

Rainbow trout (50–150 g) of the coastal strain, known locally as "steelhead" trout (*Oncorhynchus mykiss irideus*; Robertson Creek Hatchery, Port Alberni, BC, Canada) were held outdoors (in two ~1000 L tanks), with overhead screen netting allowing natural photoperiods (experiments conducted over the months of April – July in 2009 and 2010). Tanks were aerated and individually supplied with either fresh water or seawater (~12 °C). Fish transported to Bamfield Marine Sciences Centre (BMSC) (Bamfield, BC, Canada) were initially kept in fresh water [dechlorinated Bamfield tap water; (in mmol L^{-1}: Na^+ 300, Cl^- 233, K^+ 5, Ca^{2+} 144, Mg^{2+} 48, and titratable alkalinity of 43 μmol L^{-1})] for about 2 weeks, after which, one tank was incrementally increased (~10‰ every 2 weeks) to full strength seawater (~35 ‰, Bamfield Marine Station seawater; (in mmol L^{-1}: Na^+ 452, Cl^- 515, K^+ 9.8, Ca^{2+} 9.5, Mg^{2+} 52, and titratable alkalinity of 2.2 mmol L^{-1}). Fish appeared morphologically to have undergone smoltification (see description in Stefansson et al., 2008), and this was confirmed by conversation with the hatchery manager. Fish were kept on a food ration of ~2% body weight every 2 days of commercial fish food (3 pt floating pellets, EWOS Pacific, Surrey, BC, Canada; with approximate composition of: $Na^+ = 234 \pm 6$; $Cl^- = 197 \pm 4$; $K^+ = 99.2 \pm 3$; $Ca^{2+} = 186 \pm 6$; $Mg^{2+} = 108 \pm 5$ μmol g^{-1} wet weight (from Bucking et al., 2011).

All procedures were approved by McMaster and BMSC Animal Care Committees, and conformed to Canadian Council of Animal Care guidelines.

2.2. In vitro gastro-intestinal 'gut sac' preparation

An *in vitro* 'gut sac' technique was used to determine intestinal Cd and Ca uptake rates and bicarbonate secretion. The method used was identical to that employed by Klinck and Wood (2011) who provide a detailed description of methodology. Very briefly, the entire GIT was removed from anesthetised fish, sectioned into four distinct segments (stomach, anterior-, mid-, and posterior-intestine), then individually tied off using surgical silk forming 'gut sacs' and infused via a catheter with appropriate radiolabeled saline solutions (control and treatment salines are described below). The gut sacs were individually placed in aerated saline filled baths. After ~2 h, the amount of radioisotope appearing in various gut tissues and the bath solution were measured, and flux rates were thereby determined.

2.2.1. Experimental salines

Cortland saline (Wolf, 1963) was used for mucosal and serosal salines, and modified as reported by Ojo and Wood (2008) to prevent Cd and Ca precipitation. The changes were: $Ca(NO_3)_2$ replaced $CaCl_2$ while $NaHCO_3$ and $NaH_2PO_4 \cdot H_2O$ were not added. Therefore the composition of the saline used was, in mmol L^{-1}: NaCl 133, KCl 5, $Ca(NO_3)_2$ 1, $MgSO_4$ 1.9, glucose 5.5; pH = 7.4 (adjusted with NaOH). Additional Cd was added to some treatments (see below) as $Cd(NO_3)_2 \cdot 4H_2O$ with 0.5 μCi mL^{-1} of ^{109}Cd (International Isotopes Clearing House (IICH), Kansas, USA). Additional Ca was added to other treatments (see below) as $Ca(NO_3)_2$ (Fisher Scientific) with 0.5 μCi mL^{-1} of ^{45}Ca (as $CaCl_2$, Perkin-Elmer, Woodbridge, ON, Canada).

Series 1 investigated the concentration-dependence of Ca absorption. Four solutions contained nominal concentrations of 1, 10, 50, and 100 mmol L^{-1} Ca (measured values for FWT exposures: 1.0, 8.0, 49.3, 112.5 mmol L^{-1} Ca; for SWT exposures: 1.0, 8.0, 40.0, 77.5 mmol L^{-1} Ca) in modified Cortland saline (described above) were used for the mucosal saline. Osmolality of all mucosal salines were kept constant (in this series and in the others) by the addition (when needed) of appropriate amounts of mannitol (an inert sugar), and were measured using a Wescor 5520 vapor pressure osmometer (Logan, UT, USA).

Series 2 investigated the concentration-dependence of Cd absorption. Four different solutions containing nominal concentrations of 1, 10, 50, 100 μmol L^{-1} Cd (measured values for FWT exposures: 5.7, 8.0, 59.4, 119.0 μmol L^{-1} Cd; for SWT exposures: 8.0, 15.8, 48.3, 89.4 μmol L^{-1} Cd) were used for the mucosal saline in a concentration kinetics experiment.

Series 3 tested whether there was inhibition of Cd binding and uptake by another divalent metal, Ca. For this experiment, control gut sacs received a luminal saline with the above-mentioned modified saline containing a nominal concentration of 50 μmol L^{-1} Cd (measured concentration: 50.7 μmol L^{-1} Cd). Experimental luminal saline contained the same solution as the controls with the addition of 10 mmol L^{-1} Ca (measured concentration: 8.0 mmol L^{-1} Ca; 51.3 μmol L^{-1} Cd).

Series 4 evaluated effects of three Ca channel blockers: lanthanum, verapamil, and nifedipine, on Ca uptake in preparations from SWT. For each experiment, control gut sacs received a luminal saline with the above-mentioned modified saline containing a nominal concentration of 10 mmol L^{-1} Ca (measured concentration: 10.0 mmol L^{-1}). Experimental luminal salines were made from the control saline with the addition of either 100 μmol L^{-1} lanthanum, 100 μmol L^{-1} verapamil, or 1 mmol L^{-1} nifedipine (Sigma-Aldrich; St. Louis, MO, USA).

Series 5 evaluated possible inhibition of Cd binding and uptake in SWT by the same three Ca channel blockers used in Series 4. Control gut sacs received the above-mentioned luminal saline with the addition of 50 μmol L^{-1} Cd (measured concentration 51.3 μmol L^{-1} Cd). The experimental luminal salines contained the same solution as controls with the addition of either 100 μmol L^{-1} lanthanum, 100 μmol L^{-1} verapamil, or 1 mmol L^{-1} nifedipine.

Series 6 measured HCO_3^- secretion rates in gut sacs of both FWT and SWT when exposed to luminal saline consisting of 10 mmol L^{-1} Ca (measured 8.0 mmol L^{-1} Ca) or to 100 μmol L^{-1} Cd (measured concentration 119.0 μmol L^{-1}Cd). This was to determine whether changes in the rates of uptake of Cd and Ca are related to the secretion rates of HCO_3^-, which may lead to the precipitation of Ca as $CaCO_3$, or Cd as $CdCO_3$.

2.2.2. Gut sac analytical techniques and calculations

Initial samples of the stock mucosal and serosal salines were taken at the beginning of the flux. Ca and Cd concentrations in these