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Mechanisms of calcium absorption by anterior and posterior segments of the intestinal tract of juvenile lake sturgeon

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ABSTRACT

Rapid growth in juvenile fish increases calcium demand, and the intestine may play a role in calcium homeostasis at this life stage, in addition to branchial and renal transport. This study examined calcium flux in the gastrointestinal tract (GIT) of freshwater juvenile lake sturgeon acclimated to 0.14, 0.34, and 2.26 mmol L^{-1} environmental calcium. Net Ca^{2+} flux did not differ due to environmental [Ca^{2+}] in either the anterior or posterior intestine. Blocking the apical epithelial calcium channel (ECaC) with ruthenium red (RR, 8.5 μmol L^{-1}) significantly decreased Ca^{2+} influx in the anterior intestine, but exposure to the plasma membrane Ca^{2+}-ATP-ase (PMCA) inhibitor trifluoperazine (TFP, 10 mmol L^{-1}) had no effect at any environmental [Ca^{2+}], nor did inhibition of the Na^+–Ca^{2+} exchanger (NCX) with KB-R7943 (10 μmol L^{-1}). Neither RR nor TFP affected Ca^{2+} uptake by the posterior intestine in any of the treatment groups, but KB-R7943 reduced net calcium flux in the posterior intestine at all environmental [Ca^{2+}]. Thus, basolateral Ca^{2+} influx in the posterior GIT of lake sturgeon relies more heavily on NCX than PMCA. Furthermore, the differing pharmacological effects in the anterior and posterior intestine suggest that the dominant transporters responsible for calcium uptake vary over the length of the GIT in lake sturgeon.

1. Introduction

Calcium is essential in all vertebrates for fundamental physiological processes, such as bone formation and cellular signaling. While the mechanisms for mammalian calcium regulation are fairly well understood, particularly with regard to renal function, these processes inevitably differ between mammals and fish, due to the greater availability of calcium in aquatic compared to terrestrial environments. In terrestrial organisms, exogenous calcium is taken up only via dietary ingestion, whereas in aquatic systems fish obtain calcium from several sources: directly from the environment across the gill, via dietary uptake and accompanying inadvertent ingestion of ambient water (Kristiansen and Rankin, 2001), and in small amounts through deliberate ingestion of ambient water (although drinking by freshwater fish occurs at very low levels). In freshwater teleost fish, dietary uptake is the primary source of calcium entering the intestinal tract, although this calcium source is still secondary to branchial uptake (Bucking and Wood, 2007; Klinck et al., 2012). Certainly, nutritional absorption of calcium in freshwater fish allows for both uptake of this essential metal while also providing a way, in addition to branchial and renal transport, of maintaining ion and water balance (Bucking and Wood, 2006, 2007).

The current model for branchial calcium transport in freshwater fish has been developed primarily using teleost species (Flik and Verbost, 1993). In brief, apical uptake occurs via an epithelial calcium channel (ECaC). Following apical absorption, energetic transport of calcium against the electrochemical gradient across the basolateral membrane into the extracellular fluids can occur via transport by the plasma membrane Ca^{2+}-ATP-ase (PMCA), or by Na^+–Ca^{2+} exchange (NCX), with the latter being driven by the activity of Na^+–K^+-ATP-ase (Hwang and Lee, 2007; Liao et al., 2007). The current model for calcium transport in the gastrointestinal tract in teleost fish, originally developed in tilapia (Flik et al., 1993), relies on similar transport proteins as those functioning in the gill, yet does exhibit distinct differences in transport mechanisms. In contrast to the gill, the epithelial calcium channels in the intestine have been demonstrated to be voltage-gated (Larsson et al., 1998). Furthermore, in branchial transport both Ca^{2+}-ATP-ase and the Na^+–Ca^{2+} exchanger play a role in basolateral transport, but uptake across intestinal enterocytes has been determined to be highly dependent on sodium in teleost species (Flik and Verbost, 1993; Schoenmakers et al., 1993), although PMCA still contributes (Flik et al., 1990) albeit to a lesser extent than in branchial transport. Furthermore, it has been suggested that mechanisms for passive and facilitated calcium transport may vary between the segments of the GIT in rainbow trout (Klinck et al, 2012).

Abbreviations: ECaC, epithelial calcium channel; GIT, gastrointestinal tract; I_{SC}, short-circuit current; NCX, Na^+–Ca^{2+} exchanger; PMCA, plasma membrane Ca^{2+}-ATP-ase; RR, ruthenium red; TFP, trifluoperazine.

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The lake sturgeon (*Acipenser fulvescens*, Rafinesque, 1817) is an exclusively freshwater species, and as such is dependent upon uptake from the surrounding water column and ingestion from diet for its calcium supply. Low environmental calcium levels may limit uptake, although calcium demand is presumably limited in this species by the primarily cartilaginous skeleton, reducing a principal requirement for calcium transport in most fish (and other vertebrates), namely bone formation. However, rapid growth in juvenile sturgeon is accompanied by an increased calcium demand, particularly for formation and maintenance of fin rays, rostrum, and protective dorsal scutes. In juveniles, therefore, the cartilaginous skeleton may serve as a hindrance, as increased calcium demand can only be partly satisfied through uptake from the freshwater environment, and mobilization of the sparse internal calcium stores would be counterproductive, since growth of these structures is one of the primary drivers of the demand.

This study examined the calcium regulatory properties of the gut of a freshwater, cartilaginous species. Juvenile lake sturgeon were acclimated to three environmental calcium concentrations (0.14, 0.34, and 2.26 mmol L^{-1}), and intestinal calcium flux was examined. We hypothesized that given the relatively high calcium demand in the species, but limited available sources, juvenile lake sturgeon would utilize all available uptake pathways, including absorption via the intestinal epithelium in addition to branchial uptake and renal reabsorption, and that this response would be greater in low calcium environments. Furthermore, application of calcium transport protein inhibitors to the apical or basolateral side of isolated intestinal preparations was employed to develop a potential model for calcium transport across the intestine of this ancient freshwater fish. Finally, calcium flux and transport mechanisms were studied in both the anterior and posterior segments of the intestinal tract, in order to determine whether the mode or rate of calcium transport varied over the length of the intestinal tract. As a representative of the phylogenetically ancient chondrosteans, *A. fulvescens* may have unique mechanisms for calcium regulation; alternatively, calcium transport may be similar to that described for the teleost gill and gut. The aim of this study was to provide an evolutionary framework to the present understanding of calcium regulation in a more specialized group of fishes.

2. Materials and methods

2.1. Experimental animals

Sturgeon were hatched and reared at either the Canadian Rivers Institute Field Station hatchery facility (Pinawa, MB, Canada) or the Grand Rapids Fish Hatchery (Grand Rapids, MB, Canada), and fingerling, young-of-the-year lake sturgeon were transported in oxygenated river water to the University of Manitoba (Winnipeg, MB, Canada). Fingerlings were maintained for several months on a 12L:12D light cycle at 16 °C in 170 L aerated holding tanks continually supplied with filtered, dechlorinated City of Winnipeg tap water. All fish were fed daily a mixture of bloodworm (San Francisco Bay Brand Inc., Newark, CA, USA) and 2.5 pt. sinking trout pellets (Martin Mills Ltd., Elmira, ON, Canada) to satiation, until they reached an appropriate size (86.76 ± 3.66 g) for the experiments outlined below. Holding and experimentation were carried out in accordance with an approved University of Manitoba Animal Care Utilization Protocol.

Juvenile lake sturgeon were held in 170 L aerated holding tanks at 14–16 °C and acclimated for two weeks to one of three different environmental calcium concentrations (nominally 0.14, 0.34 and 2.26 mm L^{-1} [Ca^{2+}], see Table 1). The 0.14 and 2.26 mmol L^{-1} [Ca^{2+}] environments were based on a previously published study (Allen et al., 2011) and were made through addition of salts (Fluka; Sigma) to deionized water, using the following recipe (in mmol L^{-1}): 0.11 NaCl, 0.022 KCl, 0.16 $MgSO_4$, 170 nmol L^{-1} Na_2HPO_4, and 0.1, 0.4, or 3.3 mmol L^{-1} $CaCl_2$. Manufactured aquarium water was adjusted to pH 7.60 ± 0.02 with $NaHCO_3$ and cooled to approximately 15 °C prior to use. Water for these treatments was constantly recirculated through a biofilter pump, and approximately 60% of the water was replaced every other day throughout the acclimation period. Juvenile lake sturgeon in the 0.34 mmol L^{-1} [Ca^{2+}] treatment were maintained in flow-through aquaria fed by de-chlorinated City of Winnipeg tap water. See Table 1 for the concentrations of the different ions in the environmental treatments. During the two week acclimation period, fish were fed daily to satiation and were fasted between 48 and 96 h prior to the beginning of experimentation to ensure that calcium absorption occurring in the intestinal tract was related to baseline calcium transport mechanisms as opposed to dietary assimilation.

Table 1
Mean ionic composition (mmol L^{-1}) of acclimation water (n = 4–6). Low and high [Ca^{2+}] treatments (0.14 and 2.26 mmol L^{-1} [Ca^{2+}]) were made from addition of salts to water deionized by reverse osmosis, and were recirculated through a biofilter, with water changed every other day. The intermediate (0.34 mmol L^{-1} [Ca^{2+}]) treatment water was provided by flow-through of dechlorinated City of Winnipeg tap water. NH_4^+ was not detectable (nd) in any treatment. Different letters indicate significant differences ($p < 0.05$) between treatments as assessed by one-way ANOVA.

Treatment	Low	Intermediate	High
Ca^{2+}	0.141 ± 0.050^A	0.342 ± 0.019^A	2.256 ± 0.071^B
Na^+	1.017 ± 0.065^A	2.187 ± 0.060^B	1.591 ± 0.409^{AB}
K^+	0.038 ± 0.004^A	0.268 ± 0.034^B	0.039 ± 0.002^A
Mg^{2+}	0.035 ± 0.017^A	0.131 ± 0.016^B	0.278 ± 0.019^C
Cl^-	0.306 ± 0.007^A	0.671 ± 0.019^A	4.953 ± 0.294^B
SO_4^{2-}	0.130 ± 0.026^A	0.619 ± 0.021^B	0.149 ± 0.009^A
NO_3^{2-}	0.157 ± 0.076	nd	0.127 ± 0.109

2.2. Üssing chamber calcium flux and electrophysiology measurements

Calcium flux across isolated anterior intestinal tissue was examined using Üssing chambers (Physiologic Instruments Inc., San Diego, CA, USA). Lake sturgeon were sacrificed by immersion in water containing an overdose of tricaine methanosulfonate (MS-222, 250 ppt) buffered with equal parts $NaHCO_3$, and the anterior intestine, defined as the segment beginning immediately distal to the pyloric sphincter and ending immediately proximal to the spiral valve, was removed. The tissue segment was opened longitudinally and cut in half. Each flattened half of the segment was then mounted on a tissue holder with 0.25 cm^2 exposed surface area (P2404, Physiologic Instruments, Inc.) between two half chambers. Each half chamber was filled with 3.5 mL of modified sturgeon Ringer's (composition in mmol L^{-1}: 108.0 NaCl, 1.8 KCl, 2.1 Na_2HPO_4, 0.2 KH_2PO_4, 0.8 $MgSO_4$, 1.6 $CaSO_4$ and 8.0 $NaHCO_3$), with the addition of 5 mmol L^{-1} glucose to the luminal saline, and mannitol added as needed to both luminal and serosal salines to increase the osmolality of each solution to the desired level (277 ± 0.7 mOsm) and equalize the osmolality of the luminal and serosal Ringer's. Salines were brought to 15.5 °C in a water bath and gassed at least 30 min with 0.3% CO_2 in O_2 custom gas mix (Praxair, Winnipeg, MB, Canada) prior to use in any experiment, and each half-chamber was continuously mixed throughout the experiment by gas-lifts, using the same 0.3% CO_2 in O_2 gas mix. Üssing chambers were kept at a constant temperature of 15.5 °C by circulation of chilled water through the jacketed chamber block.

Following insertion of the mounted tissue and an initial stabilization period of 15–30 min, each Üssing chamber set up was spiked in one of the half-chambers with 18.5 kBq mL^{-1} [^{45}Ca]$Cl_2 \cdot 2H_2O$ (PerkinElmer). An equivalent concentration of non-radiolabeled $CaCl_2 \cdot 2H_2O$ was added to the opposing half-chamber to prevent formation of a diffusive calcium gradient between the half-chambers. The addition of [^{45}Ca]$Cl_2 \cdot 2H_2O$ to the luminal or serosal half-chamber was alternated between paired anterior intestinal segments, with one segment from a single fish spiked luminally and the other basolaterally. In this way both luminal to serosal transport (influx) as well as serosal to luminal transport (efflux) in the paired segments were determined. The distal and proximal sections of the anterior intestinal segment were alternated