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Characterization of Na^+ transport to gain insight into the mechanism of acid-base and ion regulation in white sturgeon (*Acipenser transmontanus*)

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ABSTRACT

Freshwater fish actively take up ions via specific transporters to counter diffusive losses to their hypotonic environment. While much is known about the specific mechanisms employed by teleosts, almost nothing is known about the basal fishes, such as white sturgeon (*Acipenser transmontanus*) which may offer insight into the evolution of osmo- and ionoregulation in fishes. We investigated Na^+ uptake in juvenile white sturgeon in the presence and absence of transporter inhibitors. We found that sturgeon acclimated to 100 μmol l^{-1} Na^+ have Na^+ uptake kinetics typical of teleosts and that a Na^+/H^+ exchanger (NHE) is the predominant transporter for Na^+ uptake. White sturgeon are tolerant to hypercarbia-induced respiratory acidoses and recover blood pH (pH_e) at 1.5 kPa PCO_2 but not at higher PCO_2 (6 kPa PCO_2) where they preferentially regulate intracellular pH (pH_i). It was hypothesized that during exposure to hypercarbia Na^+ uptake would increase at CO_2 tensions at which fish were capable of pH_e regulation but decrease at higher tensions when they were preferentially regulating pH_i. We found that Na^+ uptake did not increase at 1.5 kPa PCO_2, but at 6 kPa PCO_2 Na^+ uptake was reduced by 95% while low water pH equivalent to 6 kPa PCO_2 reduced Na^+ uptake by 71%. Lastly, we measured net acid flux during hypercarbia, which indicates that net acid flux is not associated with Na^+ uptake. These findings indicate Na^+ uptake in sturgeon is not different from freshwater teleosts but is sensitive to hypercarbia and is not associated with pH_e compensation during hypercarbia.

1. Introduction

Ion homeostasis in freshwater fishes is achieved by using specialized branchial transport mechanisms for active ion uptake (primarily Na^+ and Cl^-) from an environment that is hypotonic to the blood, and a tight gill epithelium to minimize ionic diffusive losses (Evans, 2011; Hwang et al., 2011). At the gills of freshwater fishes, apical Na^+ uptake is coupled to acid excretion and can involve different apical transport proteins, depending on the species and/or the environment they inhabit. The latter may consist of a Na^+ channel or acid-sensing ion channel (ASIC) electrically linked to H^+ extrusion by a V-ATPase (Dymowska et al., 2014), an electro-neutral Na^+/H^+ (or Na^+/NH_4^+ or $H^+ + NH_3$) exchanger (NHE) (Hwang et al., 2011), Cl^--dependent Na^+ uptake via a Na^+-Cl^- co-transporter (NCC) (Hwang et al., 2011) or Na^+-K^+-$2Cl^-$ co-transporter (NKCC) (Brix and Grosell, 2012; Hiroi et al., 2005), or some combination of these mechanisms. Common to these mechanisms for Na^+ uptake is the Na^+/K^+-ATPase (NKA) in the basolateral membrane of gill ionocytes which exports Na^+ into the blood and contributes to the electrochemical gradient that drives Na^+ uptake across the apical membrane (Hwang et al., 2011). In dilute freshwater, a Na^+ channel or ASIC associated with H^+-ATPase is believed to be the most thermodynamically favorable (Parks et al., 2008), however a number of studies have demonstrated that Na^+ uptake occurs in conjunction with an NHE in some freshwater fishes [e.g. zebrafish *Danio rerio* (Kumai and Perry, 2011; Yan et al., 2007); medaka *Oryzias latipes* (Wu et al., 2010); Osorezan dace *Tribolodon hakonensis* (Hirata et al., 2003); and pupfish *Cyprinodon variegatus hubbsi* (Brix et al., 2015; Brix and Grosell, 2012)]. Recently, it has been shown that NHEs may operate in low Na^+ environments (i.e. freshwater) when they are associated with Rhesus (Rh) proteins, transporting ammonia (Kumai and Perry, 2011; Wu et al., 2010; Yan et al., 2007) or with a basolateral membrane sodium bicarbonate co-transporter (NBC). In the latter, carbonic anhydrase (CA) generates H^+ and HCO_3^- from metabolically produced CO_2, and HCO_3^- is exported by the NBC across the basolateral membrane leaving intracellular H^+ to drive the apical NHE (Hirata et al., 2003; Scott et al., 2005).

Abbreviations: ASIC, acid-sensing sodium channel; CA, carbonic anhydrase; DAPI, 4′,6-diamidino-2-phenylindole; DMSO, dimethyl sulfoxide; EIPA, 5-(*N*-ethyl-*N*-isopropyl)-amiloride; kPa, unit of pressure; MRC, mitochondrion-rich cell; NBC, Na^+, HCO_3^- co-transporter; NHE, Na^+/H^+ exchanger; Rh, Rhesus protein; PCO_2, partial pressure of CO_2; pH_e, extracellular pH; pH_i, intracellular tissue pH; PO_2, partial pressure of O_2.

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Most of the studies characterizing the mechanisms of Na^+ uptake in freshwater fishes have been conducted on teleosts, a highly diverse group, comprising the vast majority of extant fish species. Surprisingly, however, nothing is known about the basal fishes, which may offer insight into the evolution of osmo- and ionoregulation in fishes (and vertebrates). We were interested in examining Na^+ uptake in one of these basal fishes, white sturgeon *Acipenser transmontanus* for this reason. Sturgeon belong to the order Acipenseriformes, which are believed to have diverged from the Neopterygii (which includes teleosts) approximately 343–372 million years ago (Betancur-R et al., 2013; Hedges and Kumar, 2009). White sturgeon inhabit river systems along the Pacific coast of North America containing dilute freshwater, where Na^+ concentrations in the Fraser (Swain et al., 1998) and Columbia (City of Trail, 2013; City of Revelstoke, 2010) Rivers, for example, may be <80 μmol l^{-1}. Similar to salmonids, sturgeon spawn in freshwater and juveniles are believed to remain in freshwater for several months or years (McEnroe and Cech, 1985), but depending on the species may spend some of their life in seawater; salinity tolerance appears to be associated with fish size, but there are likely to be considerable differences between populations (Allen et al., 2014; Mojazi Amiri et al., 2009).

Sturgeon are relatively inactive and have low metabolic rates relative to other fishes (Baker and Brauner, 2012; Fitzgibbon et al., 2008). Low metabolic rate is generally associated with low Na^+ loss and therefore, low Na^+ uptake rate in fishes (Gonzalez and McDonald, 1994). Thus, we hypothesized that juvenile sturgeon would have a low Na^+ uptake rate under routine conditions that would be accomplished by a low Na^+ capacity, but high affinity system. We also hypothesized that Na^+ uptake would be driven by a Na^+ channel or ASIC/H^+-ATPase based on observations by Baker et al. (2009) showing weak apical NHE3 staining and the presence of apical V-ATPase at the gills, and because a Na^+ channel or ASIC/H^+-ATPase system would be more thermodynamically favorable (Parks et al., 2008) in the relatively low Na^+ water that sturgeon inhabit. These hypotheses were tested in Series 1 using experiments designed to investigate Na^+ uptake transport mechanisms. Series 1 experiments examined Na^+ uptake kinetics and the effect of specific transporter inhibitors [phenamil (Na^+ channel inhibitor), 5-(N-ethyl-N-isopropyl)-amiloride (EIPA; NHE inhibitor); bumetanide (NKCC inhibitor); ethoxzolamide (CA inhibitor); 4′,6-diamidino-2-phenylindole (DAPI; ASIC inhibitor)] in white sturgeon. Small juvenile fish (~2–5 g) were used, as they are likely to be exclusively freshwater under natural conditions and are a suitable size for radioisotope experiments.

Sturgeon are one of the most CO_2 tolerant fishes known and are able to withstand acute exposure to increased water PCO_2 (hypercarbia) of 12 kPa for 48 h (Baker and Brauner, 2012). Interestingly, at low CO_2 tensions (<1.5 kPa) they are able to compensate for reductions in extracellular pH (pH_e), but not at high CO_2 tensions (>3 kPa) (Baker et al., 2009; Shartau et al., 2016). Respiratory acidoses induced by hypercarbia have been demonstrated to increase Na^+ uptake in some fish, likely in association with increased H^+ efflux and pH_e compensation as observed in in rainbow trout (*Oncorhynchus mykiss*) (Perry et al., 1987), brown bullhead (*Ictalurus nebulosus*) (Goss et al., 1992) and arctic grayling (*Thymallus arcticus*) (Cameron, 1976). Similarly, hypercarbia exposure leads to increased extracellular [Na^+] in blue crab (*Callinectes sapidus*), channel catfish (*Ictalurus punctatus*) (Cameron and Iwama, 1987), Japanese flounder (*Paralichthys olivaceus*), yellowtail (*Seriola quinqueradiata*) and starspotted dogfish (*Mustelus manazo*) (Hayashi et al., 2004) possibly implying an association between Na^+ uptake and H^+ excretion. As there are differences in pH_e regulation during exposure to hypercarbia at low and high CO_2 tensions in sturgeon, we were interested in how Na^+ uptake characteristics would be affected and whether this may contribute to the different strategies of pH_e regulation during different levels of hypercarbia. We hypothesized that Na^+ uptake would increase during hypercarbia exposure at ≤ 1.5 kPa PCO_2 when pH_e compensation occurs, but at higher CO_2 tensions, Na^+ uptake would be reduced due to the effect of low pH hindering H^+ extrusion (Lin and Randall, 1995). These hypotheses were tested in Series 2 using experiments designed to investigate the effect of acid-base disturbances on Na^+ uptake and net acid flux.

Series 2 was organized into three groups of experiments. The first experiment in Series 2 (Series 2.1) was performed to corroborate that there was a difference in pH_e compensation between low (1.5 kPa PCO_2) and high (6 kPa PCO_2) hypercarbia exposure in juvenile white sturgeon. The second set of experiments (Series 2.2) examined Na^+ uptake during various acid-base disturbances. Here, we measured Na^+ uptake during 3 h hypercarbia exposure to 0.75, 1.5, 3 or 6 kPa PCO_2, followed by measurements of Na^+ uptake at 1.5 kPa PCO_2 over 12 h to assess if Na^+ uptake changes during pH_e recovery. Next, since hypercarbia lowers water pH, we wanted to distinguish between the effect of low water pH and hypercarbia on Na^+ uptake; therefore, fish were exposed to the equivalent water pH (via H_2SO_4 addition to the water) as the CO_2 exposures for 3 h and Na^+ uptake was measured. Finally, we wanted to assess if the source of CO_2 [external (hypercarbia) or internal (hypercapnia)] affects Na^+ uptake; to address this, fish were exposed to hyperoxia (high environmental O_2) for 3 h, which induces retention of metabolically produced CO_2 and creates an internally sourced respiratory acidosis (hypercapnia) (Wood and LeMoigne, 1991). The third set of experiments in Series 2 (Series 2.3) measured net acid flux during hypercarbia and low water pH to investigate the relationship between Na^+ uptake and net acid flux during these acidoses.

2. Methods

2.1. Animal acquisition and holding

White sturgeon (*A. transmontanus*) were reared at the International Centre for Sturgeon Studies at Vancouver Island University (Nanaimo, British Columbia, Canada) in dechlorinated water [61 μmol l^{-1} Na^+, 69 μmol l^{-1} Cl^- (City of Nanaimo, 2015), pH ~6.6–6.8 (Mojazi Amiri et al., 2009)] and transported to the Department of Zoology aquatic facilities at the University of British Columbia (Vancouver, British Columbia, Canada) at the juvenile stage (~3 months old, 1–4 g). Fish were held in flow-through dechlorinated City of Vancouver tap water [~55–84 μmol l^{-1} Na^+, 73 μmol l^{-1} Cl^-, 7 μmol l^{-1} Mg^{2+}, 89 μmol l^{-1} Ca^{2+} (Metro Vancouver, 2015), pH 6.3, 3 μmol l^{-1} HCO_3^-] for 5–6 weeks before experiments and fed *ad libitum* every second day and starved for 48 h before experiments unless otherwise indicated. Animal transport was conducted in accordance with federal and provincial regulations (BC ITC Transfer no: 13531). All experiments were approved by the University of British Columbia animal care committee (animal care no: A11-0235).

2.2. Experimental protocols

2.2.1. Series 1: characterization of Na^+ uptake

2.2.1.1. Na^+ uptake kinetics. The Na^+ uptake kinetics of juvenile white sturgeon (1.36–3.52 g) were determined in fish at 15 °C. Uptake rates were measured at eight water Na^+ concentrations ranging from 10 to 890 μmol l^{-1} Na^+. At each Na^+ concentration five fish were placed in 1000 ml of a defined medium (480 μmol l^{-1} $CaSO_4$, 150 μmol l^{-1} $MgSO_4$, 100 μmol l^{-1} $KHCO_3$, pH 7.0) to which the desired concentration of NaCl was added. Flux water was continuously aerated to ensure sufficient oxygenation. Fish were allowed to acclimate for 10 min and then 1–2 μCi of ^{22}Na (depending on the ambient Na^+ concentration) was added to the solution. The flux solution was sampled after 1 min for measurements of [Na^+] (2 ml) and ^{22}Na (1 ml). The total flux exposure time ranged from 1.5 to 2.5 h, depending on the ambient Na^+ concentration used. In all cases, the internal specific activity was $<1\%$ of the external specific activity such that correction for backflux was unnecessary (Maetz, 1956). At the end of the exposure time, water samples for [Na^+] and ^{22}Na activity were collected, fish were removed from the