ION REGULATION IN ION POOR, ACIDIC WATERS OF THE RIO NEGRO

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Introduction

The waters of the Rio Negro, a major tributary of the Amazon River, drain extremely mineral poor soils and are very dilute. Typical cation concentrations of the river in μmol L^{-1} are: $Na^+ = 16.5 \pm 5.3$, $K^+ = 8.2 \pm 2.7$, $Ca^{2+} = 5.3 \pm 1.6$, $Mg^{2+} = 4.7 \pm 1.4$ (Furch 1984, and small forest streams that feed the Rio Negro can be even more dilute. These waters have a very low buffering capacity and the presence of organic acids from decaying vegetation (giving the water its tea color), make it acidic ($\leq$ pH 4.5). Ion poor water of low pH such as that found in the Rio Negro pose multiple challenges for ion regulation in teleost fish inhabiting these waters.

To better understand the nature of the challenge to ion regulation posed by the waters of the Rio Negro we must first briefly review the basic model of ion regulation (we will focus on Na^+). Teleosts in freshwater are hypertonic to there environment. Consequently they experience a diffusive loss of Na^+ across the gills, largely through paracellular tight junctions (McDonald 1983) which is countered by active uptake across the gill epithelium (Fig. 1). Two mechanisms for Na^+ uptake have been proposed. The older model calls for an electroneutral exchange of Na^+ for H^+ (or NH_4^+) across the apical membrane (Meatz & Garcia-Romeau 1964). Once inside the cell Na^+ is actively transported into the plasma in exchange for K^+. Alternatively, in recent years there has been growing support for an electrogenic extrusion of H^+ across the apical membrane which creates a negative potential that draws in Na^+ through an ion specific channel (Lin & Randall, 1991). Despite the very different methods of uptake, both mechanisms exhibit apparent Michaelis-Menten type saturation kinetics (Potts 1994) in which uptake increases with external concentration, but levels off when some external level that saturates the uptake mechanism is reached.

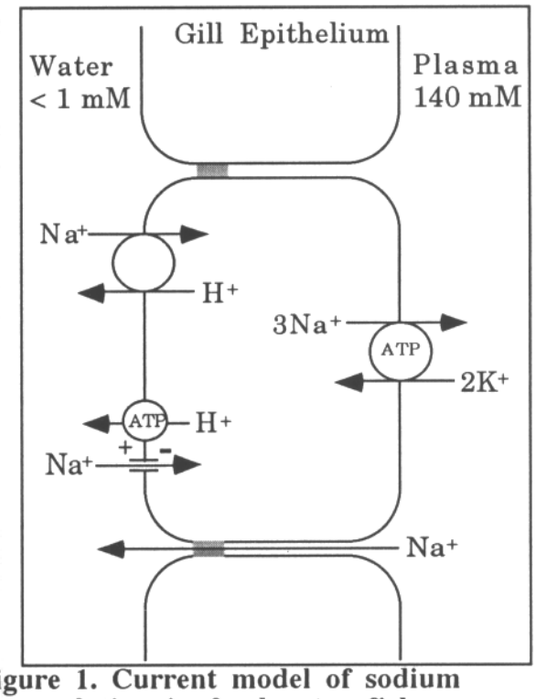

Figure 1. Current model of sodium regulation in freshwater fish.

Dilute, acidic waters act to reduce active Na^+uptake in two ways. At extremely low environmental Na^+ levels, uptake can be reduced simply because of the extreme scarcity of Na^+ in the bulk

medium. At the same time, high concentrations of H^+ can act to inhibit uptake; although the specific details depend upon the mechanism. If a Na^+/H^+ antiport is responsible for uptake, then inhibition is thought to be competitive (Potts 1994). Alternatively, if a proton pump is responsible then high external H^+ concentrations would create a gradient too steep for further extrusion of protons (Lin & Randall 1991). Regardless of the mechanism, the theoretical low pH limit for active uptake is believed to be about pH 4.5 - 5.0.

While inhibiting uptake, ion poor, acidic waters also stimulate diffusive efflux by disrupting paracellular tight junction integrity. The permeability of the tight junctions to ions is correlated with the binding of Ca^{2+} ions to the membrane bound junctional proteins (Madara 1988, Gonzalez & Dunson, 1989). When Ca^{2+} is bound to the tight junctional proteins their permeability to ions is low, and when Ca^{2+} is removed permeability is high. When ambient Ca^{2+} levels are low or H^+ concentrations are high, Ca^{2+} is leached from tight junctions causing an increase in branchial permeability and a rise in efflux of Na^+.The result of the inhibition of uptake and acceleration of efflux is a net loss of sodium If the rate of loss is too high or the total amount lost too great (around 50%) then serious, potentially fatal, internal osmotic disturbances result (Milligan & Wood 1982). The basis of tolerance of low pH and low ion concentrations, then, would appear to be related to the ability to maintain ion balance, by some combination of continued influx and/or control of efflux.

Results and Discussion

In my laboratory I have been addressing this question by studying the neon tetra (*Paracheirodon innesi*), which inhabits the upper reaches of the Rio Negro. Our studies clearly indicate that it is exceptionally tolerant of low pH and possesses a suite of ionoregulatory specializations that allow it to maintain ion balance and thrive in these harsh waters.

Neon tetras proved to be extremely tolerant of low pH. They were able to maintain ion balance at pH 3.5. In fact, fish exposed to pH 4.0 and 3.5 for a 2 week period showed no depression of whole body sodium concentration compared to fish held at pH 6.5. A closer examination of unidirectional Na^+ fluxes while at pH 3.5 revealed that tetras did experience an initial disturbance, but it was small and short-lived. Upon exposure to pH 3.5 net sodium flux becomes significantly negative (Fig 3), but by 24 h ion balance is restored. The cause of the disturbance was a slightly more than doubling of Na^+ efflux that returned to control levels within 24 h. Interestingly sodium influx showed a rapid, strong stimulation in response to the sodium loss. It was not until the pH was lowered to 3.25 that the disturbance was great enough, largely due to a massive stimulation of efflux (active uptake is unaffected), to cause death. From these findings it is clear that these fish are able to avoid the stimulation of efflux until the pH is very low.

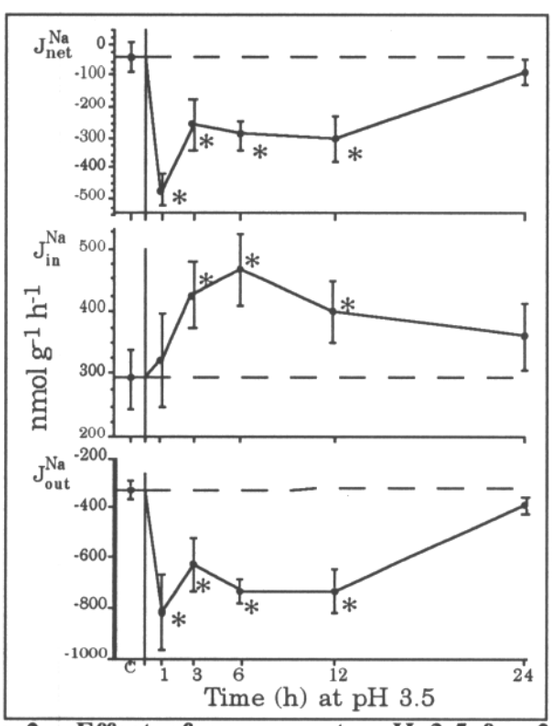

Figure 2. Effect of exposure to pH 3.5 for 24 h on net sodium flux, sodium influx, and sodium efflux of neon tetras

At this stage the mechanism for control of Na^+ efflux is not clear.

One possible mechanism is an exceptionally high branchial affinity for Ca^{2+} that would resist leaching. We have tried a couple of tests to examine this possibility with mixed results. Measurements of Na^+ efflux at pH 3.5 in water with varying Ca^{2+} concentrations did not show any effect. However, Na+ efflux was strongly stimulated by exposure to equimolar concentrations of $LaCl_3$, a strong competitor for Ca^{2+} binding sites on the branchial epithelium.

The mechanism for Na+ uptake also proved to be highly specialized for ion poor, acidic waters. Kinetic analysis revealed an uptake mechanism with an extremely high affinity for Na^+ (K_m was estimated at 10.5 ± 4.0 μmol l^{-1}), and high capacity (V_{max} = 441.1 ± 33.6 nmol g^{-1} h^{-1}) as one might expect from a fish that inhabits ion poor waters (Fig. 3). Further, the uptake parameters were completely insensitive to low pH. At pH 3.5 the uptake kinetics were virtually identical. Even at pH 3.25, the lowest pH tested and where fish were dying from the high rates of Na^+ loss, uptake was unaffected.

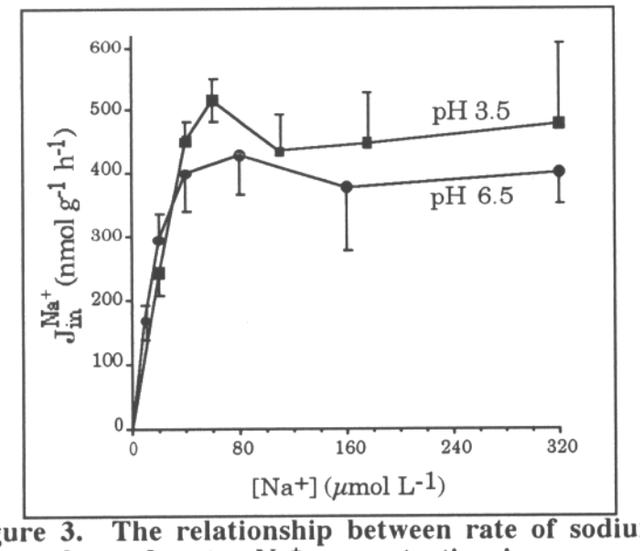

Figure 3. The relationship between rate of sodium uptake and water Na^+ concentration in neon tetras at pH 6.5 and 3.5.

The nature of the uptake mechanism remains to be clarified. It is only very slightly sensitive to amiloride, a general blocker of Na^+ uptake. At a concentration (0.1 mmol l^{-1}) that almost completely blocked uptake in other species of fish, uptake in tetras was reduced only about 10%. This may simply be a consequence of the extremely high affinity of the transporter protein for Na^+. Further work is needed to clarify this point.

These tests clearly demonstrate the exceptional tolerance these fish possess for ion poor, acidic waters and identify the specializations responsible for their great tolerance. However, the specific details of these mechanisms remain to be uncovered.

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