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Studies of Some Molecular Properties of the Vacuolar H^+-ATPase in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: A cloned cDNA fragment of the rainbow trout gill vacuolar H^+-ATPase (H^+ VATPase; proton pump) B subunit was used as a probe to examine: i) its interspecific distribution among marine and freshwater species and ii) its expression during a variety of acid-base and ionic disturbances. Northern blots of gill total RNA, performed under conditions of high stringency, revealed cross hybridisation between the trout probe and 11 of 16 species that were examined. Cross hybridisation was not observed in the pacific hagfish (*E. stoutii*), Lake Magadi tilapia (*Oroechromis alcalicus Grahami*), bigfin eelpout (*Lycodes cortezianus*), blackfin poacher (*Bathyagonus nigripinnus*) or freshwater American eel (*Anguilla rostrata*). Acute (3 h) exposure of trout to external hypercapnia (P_wCO_2 = ~7 mm Hg) was associated with a transient increase after 1 h in gill H^+-ATPase mRNA levels. Thus, the increase in gill H^+-ATPase activity that is known to accompany hypercapnic acidosis in trout may reflect, at least in part, its transcriptional or post- transcriptional regulation. Plasma cortisol levels were elevated in the hypercapnic fish (45±15 to 83±4 ng mL^{-1}) and because 30 cortisol was previously implicated as a regulator of H^+-ATPase activity, mRNA levels were quantified in fish subjected to chronic cortisol elevation. An increase in plasma cortisol concentration from 90±10 (sham implants) to 300±60 ng mL^{-1} (cortisol implants) for 4 days was associated with an approximate doubling of gill H^+-ATPase steady-state mRNA levels. Exposure of trout for 72 h to ion-poor water caused a persistent reduction in the concentration of gill H^+-ATPase steady-state mRNA. The functional significance of this response is unclear but may reflect a reduced rate of Na^+ uptake across the gill. These results are discussed with reference to the physiological role of the branchial H^+-ATPase in both acid-base and ionic regulation.

Key words: Vacuolar H^+-ATPase, proton pump, gill, fish, hypercapnia, cortisol

INTRODUCTION

The vacuolar H^+-ATPase (H^+ V-ATPase) or proton pump is probably expressed in all eukaryote cells where it plays a housekeeping role in the acidification of intracellular organelles (Nelson, 1992). However, in addition to its housekeeping role, the H^+ V-ATPase is thought to be specifically involved in acid-base balance and ionic regulation in a variety of secretory epithelia (Stevens and Forgac, 1997; Forgac, 1998; Nelson and Harvey 1999; Wieczorek *et al*., 1999; AL-Fifi *et al*., 1998; Choe *et al*., 2002, 2004) including the rainbow trout (*Oncorhynchus mykiss*) gill (Lin and Randall, 1995). Avella and Bornancin (1989) first suggested a physiological role for the H^+ V-ATPase in the fish gill. Specifically, it was reasoned using thermodynamic arguments that the traditional model for Na^+ uptake across the freshwater fish gill, involving electroneutral Na^+/H^+ exchange (Krogh, 1938; Choe *et al*., 2004), was not tenable. Instead, it was postulated (Avella and Bornancin, 1989) that Na^+ uptake across the apical membrane of gill epithelial cells was linked energetically to active H^+ extrusion via the H^+ V-ATPase. According to their model, H^+ secretion across the apical membrane establishes a favourable electrochemical gradient that permits the inward entry of Na^+ through epithelial Na^+ channels (AL-Fifi *et al*., 1998, 2002).

Despite scarce empirical evidence for apical membrane Na^+ channels, this newer model for Na^+ uptake is now generally accepted (Marshall, 1995). Less certain, however, is the epithelial location of the fish gill the H^+ V-ATPase. Indeed, arguments have been made for a specific localisation to the chloride cell (Lin and Randall, 1991), the pavement cell (Laurent *et al*., 1994; Sullivan *et al*., 1995; Kultz and Somero, 1995; Sullivan *et al*. 1996) or both cell types (Lin *et al*., 1994; Lin and Randall, 1995; Perry, 1997). Regardless of its location, evidence is accruing that the activity of the H^+ V-ATPase in the fish gill is regulated in accordance with acid-base and ionic uptake requirements (Lin and Randall, 1995; Perry and Fryer, 1997; Choe *et al*., 2002, 2004). For example, H^+ V-ATPase activity is increased in the trout gill during respiratory acidosis