

**NaCl AND ACID/BASE REGULATORY MECHANISMS IN THE GILLS
OF A EURYHALINE ELASMOBRANCH (*Dasyatis sabina*)**

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Introduction

The cellular mechanisms associated with NaCl and acid/base regulation in gills of elasmobranch fishes have not been well established, compared to those of teleost fishes (reviewed by Evans et al. 1999). Moreover, the influence of environmental salinity on the expression of these mechanisms in elasmobranch gills is unknown. The goals of this study are to 1) determine what mechanisms are present in the gill epithelium of a euryhaline elasmobranch (Atlantic stingray, *Dasyatis sabina*); and 2) determine if environmental salinity influences expression of these mechanisms. The specific ion transporters examined in this study are Na⁺,K⁺-ATPase, vacuolar proton-ATPase (V-H-ATPase), and pendrin.

In teleosts, Na⁺,K⁺-ATPase is localized to the basolateral membrane of mitochondrion-rich chloride cells, where it indirectly energizes NaCl secretion by the seawater teleost gill (reviewed by Evans et al. 1999). This transporter is also present in chloride cells of freshwater teleosts where it is presumably involved with active ion uptake (see Perry 1997). In seawater elasmobranchs, Na⁺,K⁺-ATPase-rich cells have been detected in the gills (Conley and Mallatt 1988), but the influence of salinity on Na⁺,K⁺-ATPase expression and function of the Na⁺,K⁺-ATPase-rich cells are unknown.

In teleosts, V-H-ATPase has been most extensively studied in gills of freshwater teleosts, where it is found on the apical membrane of chloride and pavement cells (Wilson et al. 2000). This transporter is hypothesized to actively excrete H^+ and establish a favorable gradient for Na^+ entry from freshwater (reviewed by Evans et al. 1999). In elasmobranch gills, V-H-ATPase has been detected in mitochondrion-rich cells of a seawater species (Wilson et al. 1997), but the influence of salinity on V-H-ATPase expression and function of the V-H-ATPase-rich cells are not known.

Pendrin is a newly described anion exchanger in mammals and has not been investigated in fishes or any other lower vertebrate. This exchanger functions as an apical Cl^-/HCO_3^- exchanger in V-H-ATPase-rich, bicarbonate-secreting intercalated cells in collecting ducts of the mammalian nephron (Royaux et al. 2001).

Materials and Methods

Ten Atlantic stingrays were captured from freshwater lakes of the St. Johns River, Florida, USA. Five individuals were held captive in fresh water (freshwater stingrays), while the other five were acclimated to 32 parts per thousand (ppt) seawater for 1 week (seawater-acclimated stingrays). In addition, a group of 5 marine Atlantic stingrays was captured from Cedar Key, Florida, USA, and held captive in 32 ppt seawater (seawater stingrays).

From each animal, gill filaments were snap frozen in liquid nitrogen for immunoblot analysis and were fixed in 10 mmol/L phosphate buffered saline (pH 7.3) containing 3% paraformaldehyde, 0.05% glutaraldehyde, and 0.05% picric acid for immunohistochemical analysis.

For immunoblots, proteins from gill homogenates were separated by molecular weight using gel electrophoresis. The proteins were transferred to PVDF membranes that were incubated with the following antibodies: 1) a monoclonal to the α -subunit of avian Na^+,K^+ -ATPase; 2) a polyclonal to the B-subunit of insect V-H-ATPase, and 3) a polyclonal to human pendrin. Binding of antibodies to the PVDF was detected with an ECL detection kit.

For immunohistochemistry, sections of paraffin-embedded gill tissue were incubated singly with the above antibodies. Binding of antibodies was detected with an immunoperoxidase detection kit using DAB (brown stain) as the substrate. A double-labeling immunohistochemical procedure was also used to

detect binding of 2 antibodies on a tissue section, in which case DAB and another substrate (Vector SG; blue stain) were used to detect the first and second applied antibodies, respectively.

Results and Discussion

Immunoblotting detected Na^+, K^+ -ATPase, V-H-ATPase, and pendrin immunoreactivity in gills of freshwater, seawater-acclimated, and seawater stingrays. Densitometry analysis of the immunopositive bands revealed that relative abundance of the transporters was greatest in gills of freshwater stingrays, compared to gills of seawater-acclimated and seawater stingrays.

Immunohistochemistry detected Na^+, K^+ -ATPase-rich, V-H-ATPase-rich, and pendrin positive cells in gills of freshwater, seawater-acclimated, and seawater stingrays. Immunostaining for all three transporters occurred in cells of gill lamellae and interlamellar regions in freshwater stingrays, but was primarily found in cells of interlamellar regions in seawater-acclimated and seawater stingrays. Regardless of salinity, Na^+, K^+ -ATPase and V-H-ATPase immunoreactivity occurred in separate mitochondrion-rich cell types and were localized to the basolateral region of their respective cells. Pendrin immunoreactivity colocalized to the apical region of V-H-ATPase-rich cells.

In conclusion, results from this study provide immunochemical evidence for 1) the presence of Na^+, K^+ -ATPase, V-H-ATPase, and pendrin in gills of the Atlantic stingray; and 2) an influence of salinity on expression of these transporters. The pattern of immunostaining for V-H-ATPase and pendrin is remarkably similar to bicarbonate-secreting cells of the mammalian nephron (Royaux et al. 2001), and we suggest that these cells are sites of apical $\text{Cl}^- / \text{HCO}_3^-$ exchange in the stingray gill. In contrast, we propose that Na^+, K^+ -ATPase-rich cells are sites of apical Na^+ / H^+ exchange through a currently unidentified transporter(s).

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