NHE2 expression in the rectal gland of the spiny dogfish *Squalus acanthias*

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Recent physiological studies using isolated dogfish rectal glands have demonstrated the presence of sodium dependant bicarbonate reabsorption and proton excretion mechanisms in rectal gland tubules[1]. Excretion of protons from the rectal gland tubules was strictly sodium-dependant and Na^+/H^+ exchange was inhibited in the presence of HOE694. HOE694 completely inhibits NHE isoforms 1 and 2 and has a minor effect on NHE3. The total absence of exchange in the presence of the inhibitor suggests that the mechanism responsible for the exchange was either NHE1 or NHE2. Electroneutral sodium/hydrogen exchange has been identified in virtually all cell types mediating the exchange of extracellular sodium for intracellular protons. It plays a vital role in the regulation of cell volume, transcellular reabsorption of Na^+ and intracellular pH. Previous investigations have demonstrated the role of carbonic anhydrase on the pH of rectal gland secretions [(3)], however, to date there has been no quantification of net acid excretion in the rectal gland and the role of the rectal gland in systemic pH regulation is unknown. In this study, we have used a homologous shark NHE2 antibody and immunohistochemical techniques to identify an NHE isoform in rectal gland tubular epithelial cells of the spiny dogfish. In addition we have identified a fragment of rectal gland cDNA homologous to dogfish gill NHE2[2].

Shark rectal glands were harvested from pithed dogfish sharks and placed immediately into chilled fixative (3% paraformaldehyde, 0.05% glutaraldehyde, 0.05% picric acid in 10 mmol l^{-1} phosphate-buffered saline, pH 7.3). Glands were rinsed in phosphate buffered saline (PBS) at 4°C, cryoprotected in two changes of 20% sucrose, 5% polyethylene glycol, and frozen in O.C.T. (TissueTek®, Sakura, CA). Cryosections (8-10 mm) were cut with a Reichert-Jung cryostat and dried on positively charged slides (Fisher Scientific). Slides were then thawed at room temperature (RT), and endogenous peroxidase activity was inhibited by incubating with 0.3% H_2O_2 in block (1.5% normal goat serum, 0.09% NaN_3, and 0.1% Tween-20 in PBS at pH 7.3) for 30 min at RT. Non-specific binding sites on the tissues were blocked by incubating with block for 30 min. Sections were then incubated in primary antibody (541-AP),(α5) (T4) (CFTR) diluted in blocking reagent overnight at 4°C, in a humidified chamber and then detected with Vector Laboratories, Vectastain Elite® ABC kit. A rabbit polyclonal antibody was raised against AA 636-654 of the dogfish gill NHE2 sequence (Claiborne et al., in preparation; 541-AP; BioSource International) and affinity purified.

NHE2 immunoreactivity (IR) was abundant and localized to the apical membrane of tubular epithelial cells of the shark rectal gland (Fig 1A). There was a total absence of NHE2-IR in tissue sections incubated in antibody preabsorbed with the shark NHE2 peptide (Fig 1B). Comparison of NHE2 immunoreactive sections were made to sections demonstrating CFTR and Na^+/K^+-ATPase immunoreactivity (Fig 2A & B). The comparison supported the finding the NHE2 immunoreactivity was localized to the apical membrane of tubular epithelial cell similar to the apical staining pattern seen in sections incubated in anti-CFTR (Fig 2A).

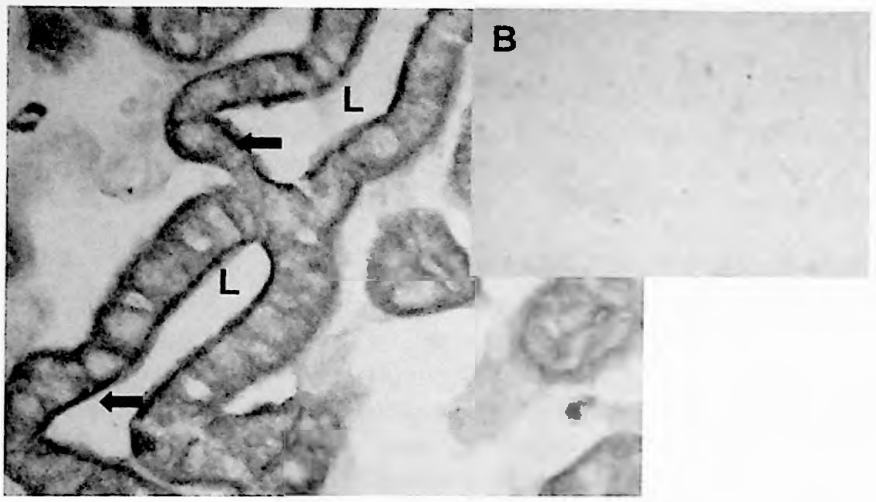

Figure 1. A. Representative high magnification light micrograph of rectal gland in cross section stained against NHE2 (AP541). Arrows indicate apical orientation of NHE2 immunoreactivity. B. Negative AP541-presabsorption control. (L= Lumen)

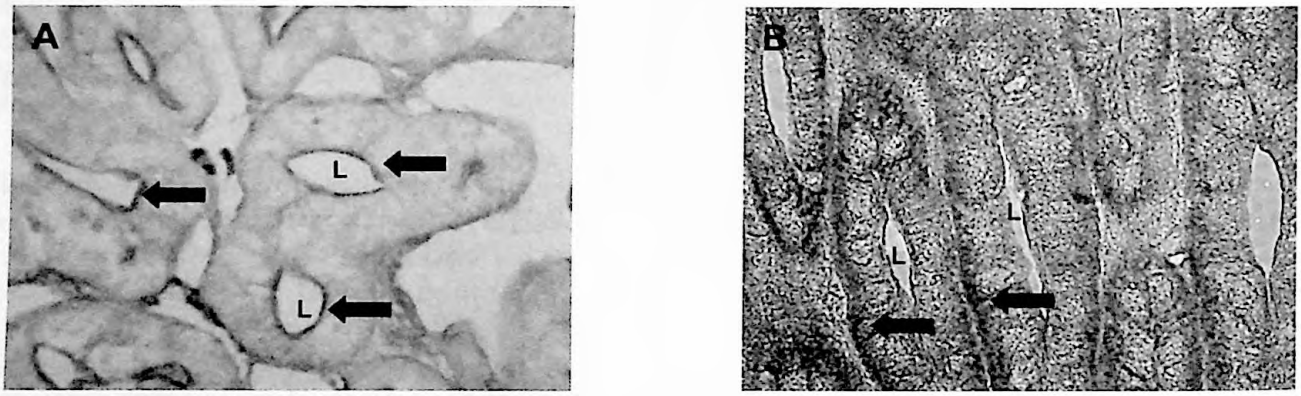

Fig 2 A. Representative high magnification light micrograph of rectal gland in cross section stained for anti-CFTR. Arrows indicate apical orientation of CFTR immunoreactivity. B. Representative high magnification of 5 stained rectal gland arrows highlight the basolateral staining of Na^+/K^+-ATPase immunoreactivity. L= Lumen

Bleich et al (1998) suggested that the presence of a basolaterally positioned NHE (possibly NHE1) could be responsible for intracellular pH regulation. The findings from this study support the presence of an NHE2-like immunoreactive protein located in the apical membrane of the rectal gland epithelial cells. The postulated role of a rectal gland NHE could be to balance intracellular pH during high metabolism periods when rectal gland secretion is stimulated, however further investigations are required into the exact functional significance.

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1. **Bleich, M, Warth, R,Thiele, I, Greger, M** pH regulatory mechanisms in in vitro perfused rectal gland tubules of *Squalus acanthias.* Pfluegers. Arch. Eur. J. Physiol., 1998. **435**(2): p. 248-254.
2. **Freiji A, Claiborne JB. 2004.** mRNA for the NHE2 exchanger is expressed in a variety of epithelial tissues in the spiny dogfish, *Squalus acanthias. Bull Mt Desert Is Biol Lab* 43:106
3. **Swenson E R, Maren, T.H.** Effects on acidosis and carbonic anhydrase inhibition in the elasmobranch rectal gland. *Am. J. Physiol.*, 247: F86 - F92, 1984.