IMMUNOLOGICAL LOCALIZATION OF NA^+, K^+, ATPASE IN THE KIDNEY TUBULES OF THE KILLIFISH, *FUNDULUS HETEROCLITUS*

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In the kidneys of euryhaline teleosts, Na^+, K^+-ATPase (NKA) activity has been shown to be as much as 110% greater in freshwater-adapted fishes than seawater-adapted ones (Epstein, F.H. et al. Yale J. Biol. Med. 41:388-393, 1969; Jampol L.M. et al. Am. J. Physiol. 218:607-611, 1970; Lasserre, P. Life Sciences 10:113-119, 1971) and freshwater fish species generally have a higher NKA activity than seawater species (Jampol L.M. et al. Am. J. Physiol. 218:607-611, 1970). Kidneys from the little skate (*Raja erinacea*) have been shown to express NKA with Western blots (Piermarini, P. et al. Bull. MDIBL 38:35-36, 1999), yet there are no published studies localizing the enzyme in the fish kidney. In mammalian kidneys, NKA has been localized in the basolateral membrane of the epithelia cells from the proximal and distal tubules, collecting ducts (Ernst , S.A. J. Cell Biol. 66:586-608, 1975; Rostgaard, J. and Moller, O. Cell Tissue Research, 212:12-28, 1980), and ascending thick limb of the loops of Henle (Baskin, D.G. and Stahl, W.L. Histochemistry 73:535-548, 1982), thus we hypothesize that the NKA also will be localized in the basolateral membrane of the fish kidney tubules.

In this study we used killifish adapted to full strength seawater (32‰, 15 °C). To localize the NKA, we fixed freshly dissected killifish kidneys in Bouin's (150ml saturated picric acid, 50 ml 37% formaldehyde and 10ml glacial acetic acid) for 60 minutes at room temperature. The kidneys were washed in 10mM PBS, placed in cryobuffer (20% sucrose) for 30 minutes, and snap frozen in a drop of Tissue Freezing Medium (Triangle Biomedical Sciences). The tissue was then sectioned (seven microns) with a cryostat, positioned on poly-L lysine coated slides, and kept at –70 °C. Sections were incubated with normal goat serum for 20 minutes, washed in PBS, and incubated overnight at 4 °C, with the NKA primary antibody (α5, 1:100, Developmental Studies Hybridoma Bank, see Piermarini, P. and Evans, D.H. J. Exp Biol. 203:2957-66, 2000). The primary was then washed off and the secondary was incubated for 2 hours, at room temperature, in the dark (Alexa Fluor 488, Molecular Probes) and the sections were viewed with an Olympus FluoView inverted confocal laser-scanning microscope. Kidneys were also fixed, sectioned, stained and visualized using the method mentioned in Hyndman and Evans, (Bull. MDIBL, this issue). The α5 was used at a 1:2000 dilution on these sections.

As seen in figures 1 and 2, the NKA is localized in the basolateral membrane of the renal tubules of the killifish, as has been seen in mammalian kidneys. In the killifish, 90% of the kidney tubules are proximal tubules (Edwards, J.G., and Schnitter, C. Am. J. Anat. 53:55-87, 1933), so we assume that the majority of the sections in figures 1 and 2 are proximal tubules. Future work will study how NKA expression changes with varying

environmental salinity and how hormones, such as endothelin, affect NKA expression, in the fish kidney. (Supported by NSF IBN-0089943 to DHE)

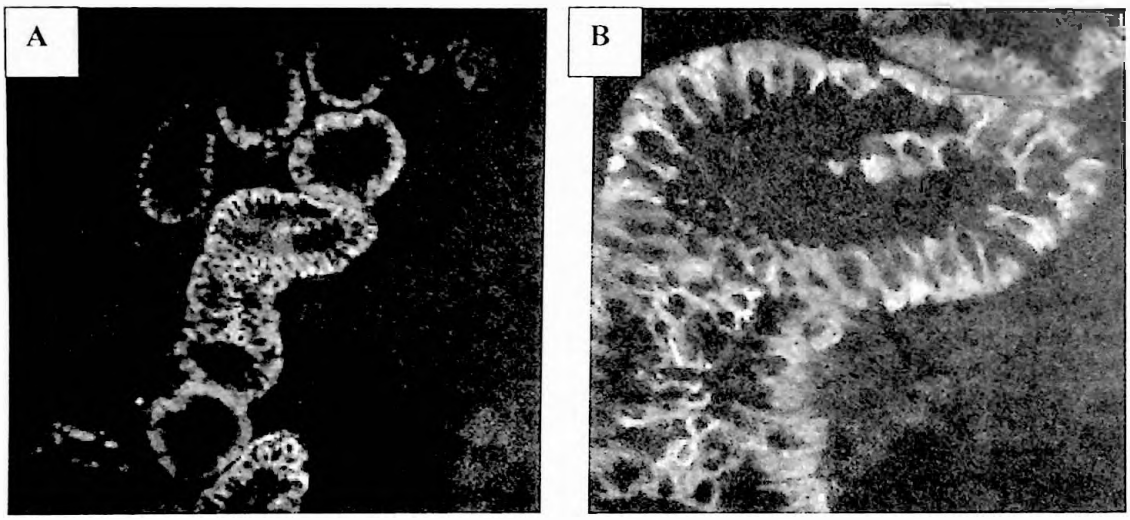

Figure 1: Basolateral localization of the Na^+, K^+, ATPase in the killifish kidney tubules using confocal microscopy. **A.** is at a magnification of 400X and **B.** is the same slide at a 600X magnification.

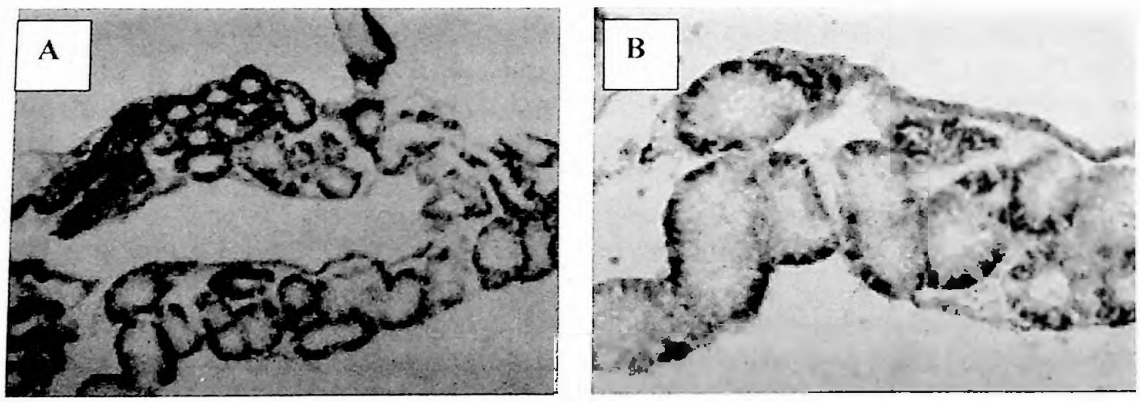

Figure 2: Basolateral localization of the Na^+, K^+, ATPase in the killifish kidney tubules using horseradish-peroxidase-labeled streptavidin and 3,3`-diaminobenzidine tetrahydrochloride to visualize antibody binding. **A.** is at a magnification of 200X and **B.** is the same slide at a 400X magnification.