SODIUM/PROTON EXCHANGER (NHE) PROTEIN EXPRESSION IN THE GILLS AND KIDNEYS OF THE LITTLE SKATE (*RAJA ERINACEA*)

Keith P. Choe[1], Tim Jensen[2], Brian P. Wall[3], Alison I. Morrison-Shetlar[3], and James B. Claiborne[3]
[1]Department of Zoology, University of Florida, Gainesville, FL 32611, USA
[2]Mayo Clinic Scottsdale, Scottsdale, AZ 85259
[3]Georgia Southern University, Statesboro, GA 30460, USA

Fishes elevate bicarbonate in their blood to compensate for extracellular acidosis (Heisler, in *Fish Physiology* ed. W. Hoar and D. Randall, Academic Press, Orlando 10:315-392, 1984). Marine fish accomplish this by elevating net acid excretion from their gills. This transport process is expected to take place via Na^+/H^+ exchangers (NHE). Claiborne et al. and Edwards et al. recently provided direct evidence for expression of multiple NHE isoforms in the gills of teleosts (Claiborne, et al., *J. Exp. Biol.* 202:315-324, 1999, Edwards, et al., *J. Anat.* 195:465-469, 1999) and agnathans (Edwards, et al., *Bull. MDIBL* 38:16-18, 1999). NHE-1, the ubiquitous, housekeeping isoform of mammalian cells, is expressed in the gills of the long-horned sculpin where it appears to play a role in systemic pH regulation (Claiborne, et al., op. cit.) but direct evidence for NHE-1 expression in an elasmobranch has not been reported. Thus, the first goal of this study was to screen for NHE-1 expression in gill and kidney tissue of the little skate. NHE-3 is an apical isoform of mammalian renal proximal tubules that is responsible for bicarbonate reabsorbtion. This isoform is also thought to function in fish gills. Thus, the second goal of this project was to screen for NHE-3 expression in gill tissue.

Total membrane protein enrichments were made from little skate (*Raja erinacea*) gills and kidneys as described by Choe et al. (*Comp. Biochem. Physiol.* 124 A:161-168, 1999). All immunoblotting was done as previously described by Claiborne et al. (op. cit.) with a few modifications. NHE-1 immunoreactivity was detected with monoclonal antibody 4E9 at a dilution of 1:1 (culture supernatant:blotto), and NHE-3 immunoreactivity was detected with polyclonal antibody 666 at a dilution of 1:3000 (serum:blotto). These primary antibodies were detected with horse radish peroxidase conjugated goat antibodies (anti-mouse for 4E9 and anti-rabbit for 666; Pierce, Rockford, IL) diluted 1:3000 for 4E9 and 1:40,000 for 666. Mouse monoclonal antibody, 4E9, was graciously provided by Bliss Forbush and Daniel Biemesderfer at Yale University School of Medicine. Rabbit polyclonal antibody, 666, was graciously provided by Mark Musch at the University of Chicago, Department of Medicine.

Antibody 4E9 (anti-NHE-1) bound to a protein of 70 to 80 kDa from crude membranes of gills and kidneys (Fig. 1A). Antibody 666 (anti-NHE-3) bound to a crude membrane protein that was 80 kDa in gills (Fig. 1B). When pre-immune serum was substituted for antibody 666, bands were absent.

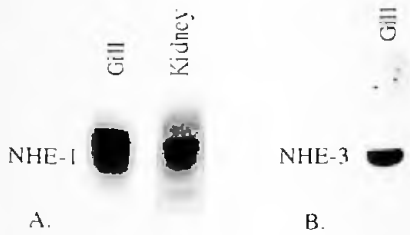

Figure 1. Immunoblots of total membrane proteins isolated from little skates using (A) monoclonal antibody 4E9 (anti-NHE-1) and (B) polyclonal antibody 666 (anti-NHE-3). Fifty µg of crude membrane proteins were separated in each lane of 7% polyacrylamide gels and transferred to supported nitrocellulose membranes. Antibody binding was detected by exposing film to an enhanced chemiluminescent signal.

We have provided the first immunological evidence for expression of Na^+/H^+ exchangers in gills and kidneys of an elasmobranch. Interestingly, preliminary results from a cell fractionation experiment suggest that the majority of gill NHE-1 is expressed basolaterally (Choe et al., unpublished results). This result is consistent with mammalian and reptilian literature, and indicates that basolateral membrane targeting is a conserved characteristic for NHE-1 (Biemesderfer, et al., *Am. J. Physiol.* 263:F833-840, 1992, Bookstein, et al., *J. Clin. Invest.* 93:106-113, 1994, Harris, et al., *Am. J. Physiol.* 272:G1594-G1606, 1997). NHE-3-like protein expression in the gills is consistent with the current model of branchial acid excretion in seawater fishes (Claiborne, in *The Physiology of Fishes* , 2nd Edition, ed. D. Evans, Boca Raton, CRC Press 177-198, 1998). Future work will evaluate membrane targeting and regulation of NHE-1 and 3 expression during systemic acidosis. This research was supported by NSF IBN-9808141 to JBC and AIMS and Sigma Xi Grant-in-Aid to KPC.