EFFECT OF SALINITY ON THE EXPRESSION OF NHE-LIKE PROTEINS IN THE GILLS OF THE MUMMICHOG (*FUNDULUS HETEROCLITUS*) DURING HYPERCAPNIA

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In order to maintain normal physiological function, fishes must maintain internal pH values between 7.5 and 8.0. Various environmental and intrinsic factors, such as hypercapnia and metabolic by-products, can cause fluctuations in these values. Fishes regulate acid-base disturbances by adjusting the branchial transfer of acid/base equivalents. It is postulated that these transfers are dependent on ambient salinity. In fresh water, fishes are thought to utilize an electrogenic proton pump to excrete H^+ (Lin and Randall, J. Exp. Biol.., 161:119-134, 1991) while marine species may use an electroneutral sodium/hydrogen antiporter (Claiborne et al., J. Exp. Biol., 202:315-324, 1999). Interestingly, some euryhaline fishes like the mummichog (*Fundulus heteroclitus*) move between varying salinities several times a day. It is unknown if these fish employ the same or different mechanisms to compensate for pH changes. The present study examines the effect of salinity on the regulation of NHE-1-like proteins in the gills of the mummichog during acidosis.

Following capture by local fishermen in estuaries around Mount Desert Island, mummichogs were adapted to either seawater (33-35 ppt) or fresh water (dechlorinated Salisbury Cove, ME tap water) for 14 days. Following the adaptation period, individual mummichogs were placed in 2 L graduated cylinders, containing water that was aerated with either a 1% CO_2 in air gas mix or normal air. After a 45 minute exposure, mummichogs were then removed and gill filaments were dissected to make membrane enrichments. Filaments were placed in ice cold buffer (250 mM sucrose, 1 mM EDTA, 2 μg/ml aprotinin, 2 μg/ml leupeptin, 100 μg/ml PMSF, and 30 mM Tris-Cl at pH 7.4) and cells disrupted with a polytron homogenizer (on ice). Whole cells and cellular debris was removed with a low speed spin (3000g for 5 min). Membrane proteins were then pelleted with a high speed spin (50,000g for 30 min). Pellets were air dried and then resuspended in a minimal volume of homogenization buffer. Proteins were solubilized by adding modified Laemmli sample buffer (Laemmli, *Nature* 227:680-685, 1970; no bromophenol blue or β-mercaptoethanol). After total protein concentration was determined (Biorad DC, detergent compatible assay), 2% β-mercaptoethanol and 0.01% bromophenol blue were added to each sample. A 75 μg sample of total protein was then separated in a 7% polyacrylamide gel (2 h at 120 V) and then transferred to a nitrocellulose membrane (GibcoBRL, Life Technologies) according to manufacturer's protocol. Immunoblotting procedures were as follows: Blocking with Blotto (5% nonfat dry milk in TBS, pH 7.4) for one hour at room temperature; primary antibody incubation with monoclonal antibody 4E9 (cultured supernatant diluted 1:1 in Blotto) for 3h at room temperature; primary antibody wash 5 X Blotto and 1 X TBST (0.1% Tween-20 in TBS pH 7.4); secondary antibody incubation, HRP-conjugated goat anti-mouse IgG (diluted 1:2000 in Blotto; Pierce, Rockford, IL) 1 h at room temperature; secondary antibody wash 5 X 1% nonfat dry milk and 1 X TBST. Immunoreactive bands were detected by exposing Kodak X-OMAT-AR scientific imaging film to a chemiluminescent signal (SuperSignal System; Pierce, Rockford IL, USA) according to manufacturer's protocol.

As seen in Figure 1, a band approximately 80 kDa in size was detected in gill tissue of both freshwater and seawater adapted mummichogs using a monoclonal antibody produced against a fusion protein representing the cytoplasmic region of porcine NHE-1 (amino acids 514-818; Biemesderfer et al. *Am. J. Physiol.*, 263:F833-F840, 1992). Acidosis induced by hypercapnia upregulated the expression of NHE-1-like proteins in the gills of the seawater adapted mummichog but not in fresh water adapted mummichogs. This may illustrate that in low salinities, mummichogs may utilize other NHE isoforms or mechanisms other than sodium/hydrogen exchangers to regulate pH.

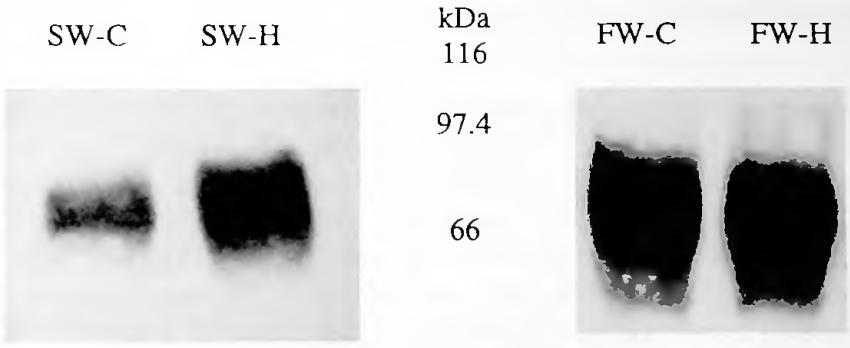

Figure 1: Immunoblot of gill tissue from mummichog adapted to fresh (FW) and seawater (SW) during control (C) and hypercapnia (H) conditions. Seventy-five μg of total protein was loaded in each lane. Equal protein loading was determined by membrane staining (not shown). Blots were probed with an antibody specific for mammalian NHE-1 (4E9). A single immunoreactive band was observed at approximately 80 kDa.

Although preliminary, the present results agree with a previous study from our laboratory (Choe et al., *Bull. MDIBL* 37:38-39, 1998) which demonstrated expression of NHE-1-like proteins in the gills of the long-horned sculpin (*Myoxocephalus octodecimspinosus*) following acidosis. In contrast to the sculpin, the mummichog exhibited an increase in NHE-1-like proteins following hypercapnia. It is possible that differences among protocols employed such as types of acidosis induced, differences in salinities, or membrane localization accounted for the difference in results. NHE-1 has been localized on the basolateral membrane of polarized epithelial tissues. If this is a conserved feature of this protein in fish, an upregulation of NHE-1-like proteins would inhibit the efficiency of net acid excretion. In contrast, mRNA and basolateral activity of NHE-1 in a rabbit kidney cell line was shown to increase during acidosis, perhaps for intracellular pH regulation (Paillard, *Exp. Nephrol.* 5:277-284, 1997). This study was funded by N.S.F. Grant IBM 9808141 to J.B.C. and A.I.M.S.