EFFECT OF ENVIRONMENTAL SALINITY AND HYPERCAPNIA ON NHE2-LIKE AND NHE3-LIKE PROTEIN EXPRESSION IN THE GILL OF THE MUMMICHOG (*FUNDULUS HETEROCLITUS*)

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During acidosis in fishes, there is an increase in net acid excretion across the branchial epithelium, which contributes to the compensation of the acidosis. The exact cellular mechanisms responsible for this task have been a topic of debate. We have recently provided evidence that proteins antigenically similar to one or more members of the Na^+/H^+ antiporter (NHE) gene family, may be utilized to accomplish this task (Claiborne, et al., J. Exp. Biol. 202:315-324, 1999). The goal of this study was to determine what effect hypercapnia has on the expression of the epithelia specific isoforms of this protein (NHE2 and NHE3; reviewed by Yun et al., Am. J. Physiol. 269:G1-11, 1995) in the gills of the euryhaline mummichog (*Fundulus heteroclitus*) adapted to fresh and salt water.

Mummichogs were captured by local fishermen in estuaries around Mount Desert Island and adapted to either fresh water (dechlorinated Salisbury Cove, ME tap water) or salt water (33-35 ppt) for 14 days. Experimental protocol was as follows: approximately 2 L of water from tanks were bubbled in a graduated cylinder for a period of 1 h prior to the start of the experiments with either a 1% CO_2 in air gas mix or with air only. This level of hypercapnia is known to elicit an extracellular acidosis in a variety of fishes (Claiborne, The Physiology of Fishes, 2nd Edition, ed. D. Evans, Boca Raton, CRC Press 177-198, 1998). Control and experimental pairs of mummichogs adapted to the same salinity as the water used in the experiment were placed in the graduated cylinders and the water was continuously aerated for 1 h. After the allotted experimental time, mummichogs were pithed and gill tissue was excised for membrane enrichments that were done as previously described by Wall et al. (Bull. MDIBL 39:50-51, 2000). A 75 μg aliquots of total protein were resolved using a 7% SDS-PAGE (1.5 h at 130 V) and then transferred to a PVDF membrane (Immobilon; Millipore) according to manufacturer's protocol. Immunoblotting protocol for antibody 666 and 2M5 was similar to that described by Choe et al. (Choe et al., Bull. MDIBL 39:80-81, 2000). Immunoreactive bands were detected by exposing scientific imaging film (Kodak X-OMAT-AR) to a chemiluminescent signal (SuperSignal System; Pierce, Rockford, IL).

As shown in Table 1, there was an increase in NHE2-like protein expression of 151% in the gills of mummichogs (n=2 pairs) adapted to fresh water that were exposed to environmental hypercapnia. In contrast, no change was observed in the expression of NHE3-like proteins in one matched freshwater pair of fish but detectable levels of these proteins were noted in a total of four fish. NHE3-like protein expression in saltwater

adapted mummichogs increased by 113% (n=4 pairs). Interestingly, no NHE2-like proteins could be detected in any saltwater adapted mummichogs (n=6).

	Control	Hypercapnia	% Change
NHE2 FW	288 (2)	723 (2)	151%
NHE3 FW	181 (1)	182 (1)	0%
NHE2 SW	none (3)	none (3)	-
NHE3 SW	192 (4)	409 (4)	113%

Table 1. Relative band intensities of NHE2-like and NHE3-like proteins in the gills of freshwater (FW) and seawater (SW) adapted mummichogs exposed to control and hypercapnic environments. The number of fish sampled each in condition is shown in parentheses.

To our knowledge, this is the first report of altered expression of NHE-like proteins in the gills of a euryhaline fish adapted to fresh and saltwater during hypercapnia. This suggests that the mummichog may utilize these proteins to compensate for an acidosis. Edwards and coworkers have recently cloned a partial NHE transcript in the mummichog gill that is most homologous to mammalian NHE2 (Susan Edwards, personal communication). mRNA for this isoform is transcribed in both freshwater and saltwater adapted fish. It is of interest to note that the expression of the two isoforms following acidosis in the present study were dependent on ambient salinity. We speculate that the freshwater adapted mummichog utilizes NHE2-like proteins to compensate for an acidosis, while NHE3-like proteins may accomplish this function in seawater animals. Kinetic differences between the two transporters may explain why different isoforms are utilized in the different salinities. This work was funded by N.S.F. IBN-9808141 to J.B.C. and A.I.M.S.