EXPRESSION OF NHE-LIKE PROTEINS IN THE GILLS OF THE LITTLE SKATE (*RAJA ERINACEA*); EFFECT OF HYPERCAPNIA

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In fishes, compensation to acidosis requires a combination of buffering, metabolism, and the exchange of acid relevant ions with sodium in the external environment (McDonald et al., Can. J. Zool. 67: 3046-3054, 1989). The predominate site of Na^+/proton exchanges occurs across the gills. We have postulated that this transfer is accomplished via Na^+/H^+ exchangers (NHE). Recently, we have demonstrated an increase in NHE-like protein expression in mummichogs exposed to environmental hypercapnia (Wall et al., Bull. MDIBL 38: 50-51, 1999; Wall et al., Bull. MDIBL, this issue). However, there is no data on how NHE-like protein expression is altered in elasmobranchs exposed to environmental hypercapnia. Thus, the goal of this study was to determine what effect hypercapnia has on the expression of two NHE-like proteins (NHE1 and NHE3) in the gills of the little skate (*Raja erinacea*).

Prior to the start of the experiment, skates were placed in an opaque plastic trough containing approximately 3 l of running sea water from Frenchman Bay, ME that was bubbled with normal air for 2 h. At the end of the 2 h period the continuos flow of sea water was stopped and control skates were continually bubbled with normal air while the gas mix to experimental skates was changed to a 1% CO_2 in air gas mix for a period of 6 h. Membrane proteins were prepared as described by Wall et al. (Bull. MDIBL, this issue). 75 µg samples of total protein were loaded on a 7% SDS-PAGE and run for 1.5 h at 130 V. Proteins were then transferred to a PVDF membrane and probed with antibodies made against NHE1 (4E9) and NHE3 (666). Immunoblotting procedures for antibody 4E9 and 666 were as previously described by Claiborne et al. (J. Exp. Biol. 202:315-324, 1999) and Wall et al. (*Op. Cit.*), respectively. Proteins antigenically similar to mammalian NHE1 and 3 were detected by exposing Kodak X-OMAT-AR scientific imaging film to a chemiluminescent signal (SuperSignal System; Pierce, Rockford, IL) according to manufacturer's protocol. Immunoreactive bands were quantified using NIH Image version 1.61 (National Institutes of Health, USA) and differences among groups were detected using unpaired t-test. Differences were considered significant at $p<0.05$.

Exposure to environmental hypercapnia for a period of 6 h, a duration corresponding to elevated H^+ efflux in skates during hypercapnia (Claiborne and Choe, unpublished data) did not cause a significant change in NHE1-like protein expression in the gills of the skate. Figures 1 is a representative Western blot of membrane proteins from skate gills probed with antibodies made against mammalian NHE1 and 3, respectively. Overall, there was a 10% (n=3 pairs) decrease in signal of NHE1-like proteins in hypercapnic skates when compared to control individuals. In contrast, NHE3 immunoreactivity increased in two of the three pairs following hypercapnia while one pair did not change. Thus, the mean 40% increase in expression was not statistically significant ($p > .05$, n=3 pairs).

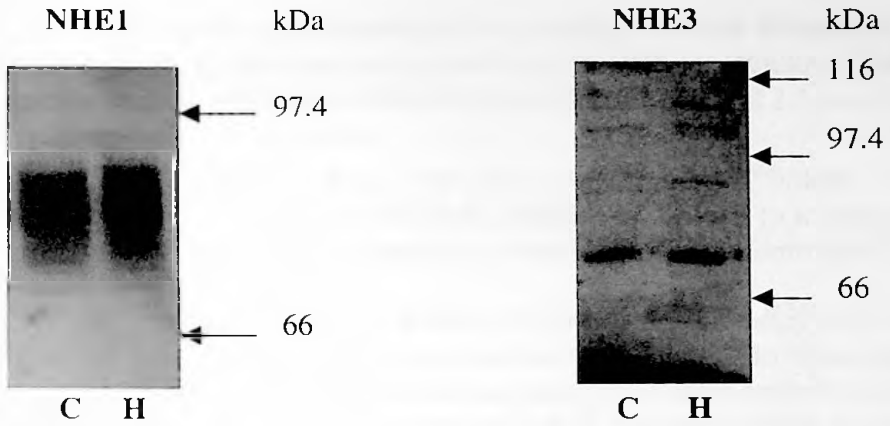

Figure 1. Representative Western blots of gill tissue from control (C) and hypercapnic (H) skates probed with antibodies made against mammalian NHE1 and 3. 75 µg of total protein was separated using SDS-PAGE and transferred to a PVDF membrane at 12 V for 65 min. Blots were digitized and immunoreactive bands were quantified. Molecular weight markers are shown to the right with closed arrows.

Preliminary results from this study show that NHE1-like protein expression does not appear to change following hypercapnia in the skate, but that NHE3 may be modified in some animals. This is in contrast to our previous work on other saltwater adapted fishes (Claiborne et al., *Op. Cit.*; Wall et al., *Op. Cit.*; Wall et al., this issue) which demonstrated adjustments in NHE-like proteins following acidosis. Discrepancies between studies could stem from the different osmoregulatory strategies utilized between species, the experimental timeframe, or the type of acidosis induced. It is also possible that baseline expression of these proteins in the little skate is sufficient to allow compensation for the hypercapnia. Future studies will address variations in isoform expression due to the time course and degree of acidosis. Funding for this work was provided by N.S.F. IBN-9808141 to J.B.C. and A.I.M.S.