EVIDENCE FOR GILL Na^+/H^+ EXCHANGER (NHE3) IN LONG-HORNED SCULPIN (*MYOXOCEPHALUS OCTODECIMSPINOSIS*)

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Gill Na^+/H^+ antiporters are thought to be one of the primary mechanisms driving acid-base transfers in marine fish (Claiborne et al., J. Exp. Zool. 293: 302-319, 2002). In mammalian epithelial tissues, NHE1 is located on the basolateral membrane and functions in intracellular pH and volume regulation. NHE2 and NHE3 are normally apical and drive transepithelial Na^+ reabsorption and acid secretion. NHE2 is primarily expressed in the gastrointestinal tract, kidneys and skeletal muscle of mammals, whereas NHE3 is found in the greatest concentration in the renal proximal tubule, followed by intestines and stomach (Wakabayashi, S. et al., Physiol. Rev. 77: 51-74,1997). We have recently cloned a full-length open reading frame for NHE2 in the long-horned sculpin, *Myoxocephalus octodecimspinosus* (Gunning, D. et al., Bull. MDIBL 40: 71-72, 2001) and mRNA for this isoform is upregulated following acidosis (Hair, N. et al., Bull. MDIBL 41: 21-22, 2002). NHE3 has been detected in gills of the extremely acid tolerant freshwater dace (Hirata, T. et al., In: Control and Diseases of Sodium Dependent Transport Proteins and Ion Channels. Ed.:Y. Suketa et al., Amsterdam, Elsevier Science, pp. 127-128, 2000), but to date, there have been no reports on the molecular identification of NHE3 in the branchial epithelium of marine fish.

Isolation of total RNA (TRI reagent, Invitrogen) from gill homogenates of long-horned sculpin was used in reverse transcription reactions to generate cDNA. Degenerate forward and reverse primers from NHE3 conserved regions of Osorezan dace (*Tribolodon hakonensis*; BAB83083) and pufferfish (*Fugu rubripes*; NC_004299) were used in PCR amplification of sculpin cDNA (Fast taq DNA polymerase system, Roche). Agarose gel electrophoresis revealed an ~1000 bp PCR product. The PCR product was cloned (pGEM-T Easy Vector System, Promega) and sequenced at the MDIBL. Sequence comparison showed a highly significant 49% identity and 67% total homology to the corresponding segment (putative membrane spanning and cytoplasmic 3' end) of rat NHE3 (Fig. 1.).

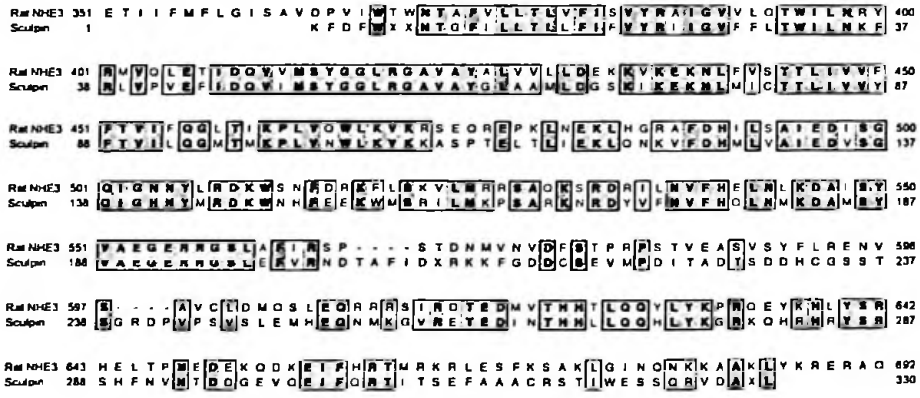

Figure 1. Sculpin NHE3 amino acid sequence alignment with rat NHE3 (P26433). Dark gray areas indicate identity. The total amino acid homology between the two sequences is 67%.

This study represents the first isolation of a partial sequence for Na^+/H^+ exchanger isoform-3 (NHE3) in marine fish gill epithelia. We are currently using 3'-5' RACE in an attempt to resolve the complete sculpin NHE3 open reading frame. Funded by NSF IBN-0111073 to JBC.