MOLECULAR IDENTIFICATION OF Na^+/H^+ EXCHANGER cDNA IN THE GILLS OF THE EURYHALINE MUMMICHOG (*FUNDULUS HETEROCLITUS*).

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The current models for branchial acid excretion in fishes include Na^+/H^+ exchange and the electrogenic excretion of H^+ via an H^+-ATPase. The predominant route of acid excretion in some freshwater fishes is widely thought to be via the H^+-ATPase/Na^+ channel system (Sullivan et al., Can. J. Zool. 74:2095-2103, 1996; Perry et al., J. Exp. Biol. 203:459-470, 2000). In contrast, the hyperosmotic environment of seawater provides a situation in which proton efflux, powered by passive inward Na^+ exchange diffusion, could be more favorable than the proton pump model of freshwater animals that requires direct ATP hydrolysis. The current marine model for H^+ excretion is centered upon Na^+/H^+ exchange across the apical membrane of the branchial epithelium (Claiborne, In: *The Physiology of Fishes*. 2nd Edition, ed.: D.H. Evans, CRC Press, 179-200, 1998). We hypothesize that in marine and some brackish water fishes the predominant mechanism of acid excretion is via a Na^+/H^+ exchanger (NHE). This study utilized RT-PCR techniques to obtain the full-length sequence of NHE cDNA in the gills of the mummichog.

Total RNA from the gills of SW adapted mummichogs (*Fundulus heteroclitus*) was isolated using the Tri-Reagent methodology (Sigma, St Louis). First strand cDNA was synthesized from gill total RNA with either oligo-dT or GeneRacer™ Oligo-dT using Superscript™ II RNase H-reverse transcriptase according to the manufacturer's protocol (Invitrogen). Degenerate oligonucleotide primers based on conserved regions of several vertebrate Na^+/H^+ exchanger sequences were used to amplify a discrete 750 bp product using mummichog gill cDNA as the template. The initial fragment showed a 60% amino acid homology to the rat NHE2. To isolate the entire mummichog gill NHE cDNA, a 5′ and 3′ RACE protocol (Generacer™kit, Invitrogen) was employed. Primers were designed from the initial 750 bp fragment. In the 5′ direction, the cDNA sequence was extended about 850 bp in several independent clones. In the 3′ direction, a 1500 bp cDNA sequence was extended to a poly $(A)^+$ tail also in a number of independent clones. Both the 3′ and 5′ RACE generated fragments overlapped with the original 750 bp fragment. Together, these clones generated a composite cDNA of 2481 nucleotides. The cDNA contained an open reading frame encoding a polypeptide of 717 amino acid residues. In a pairwise comparison the *F. heteroclitus* gill NHE showed a 58-62% amino acid homology to other known mammalian NHE2 amino acid sequences. A relationship analysis based upon multiple alignment showed that the mummichog NHE was more closely related to the rat NHE2 and NHE4 than other epithelial NHE isoforms (Fig. 1). This research was funded by NSF grants IBN-9808141 & IBN-0111073 to JBC & AIMS.

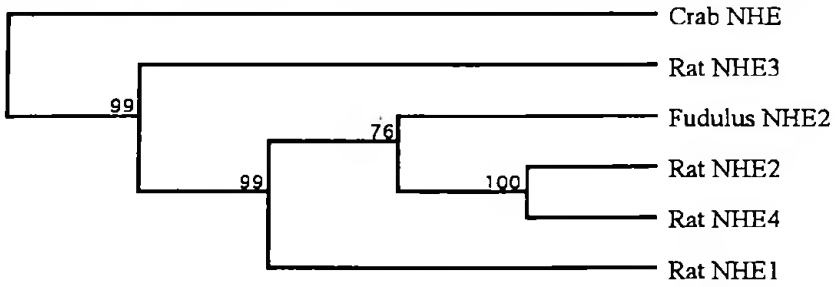

Figure 1. Relationship tree of NHE isoforms using the derived gill NHE amino acid sequence from the mummichog and other known NHE sequences, GenBank accession numbers: M85299 NHE1 (*Rattus norvegicus*), L11004 NHE2 (*R. norvegicus*), M85300 NHE3 (*R. norvegicus*), M85301 NHE4 (*R. norvegicus*) & U09274 crab NHE (*Carcinus maenas*). Bootstrap values are indicated at the nodes.