RELATIVE EXPRESSION OF mRNA FOR NHE-2 IN THE GILLS OF THE LONG-HORNED SCULPIN, *MYOXOCEPHALUS OCTODECIMSPINOSUS*

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Acid-base homeostasis in fish is accomplished by the transfer of acid-base relevant ions between the aquatic organism and the water (Claiborne, J.B., In: *The Physiology of Fishes*. 2nd Edition, ed.: D.H. Evans, CRC Press, 179-200, 1998). In marine fish, one of the gill transport proteins thought to drive the excretion of H^+ to the water is the Na^+/H^+ exchanger (NHE) (Yun, C., et al., Am. J. Physiol. 269: G1-11, 1995). We recently described the first full-length NHE-2 like sequence in fish (the long-horned sculpin, *Myoxocephalus octodecimspinosus*; Gunning, D., et al., Bull. Mt. Desert Is. Biol. Lab. 40: 71-72, 2001). In order to investigate the functional role of NHE-2 in acid-base balance in the sculpin, we have used relative semi-quantitative RT-PCR to measure changes in the expression of mRNA in the gill tissue following an internal acidosis. Concurrent *in vivo* measurements of net H^+ transfers were also made.

Long-horned sculpin (100-300 g) were cannulated and placed in darkened acrylic boxes (1.8-2.1 liter) with running seawater and allowed to recover for 8-12 hours. Following this recovery period, the chamber was closed to running seawater, aerated, and a control H^+ flux of 8-12 hours was measured. Subsequent to the pre-infusion period, fish were infused with either 0.1N HCl (2 mmol kg^{-1}) or a comparable volume of distilled water. Fish were sacrificed at h 1, 2, 5, or 8 and a 20 ml sample was collected from the seawater at each of these intervals so that cumulative transfers of H^+ between the fish and the water could be calculated as described by Claiborne et al. (J. Exp. Biol. 193: 79-95, 1994). Ambion's QuantumRNA™ Classic 18S Internal Standard kit was used in relative semi-quantitative RT-PCR to determine expression levels of NHE-2 gill mRNA. Specific primers for NHE-2 gill mRNA were developed in the 3' region of the open reading frame (Gunning, D., et al., Bull. Mt. Desert Is. Biol. Lab. 40: 71-72, 2001) to yield an 858 bp product. The ratio of this product to a 488 bp 18S internal standard was then determined. PCR reactions for each animal were run in triplicate and products visualized on ethidium agarose gels. The apparent density of the PCR product bands were digitized for quantitative comparison. The densitometric measurements were used to calculate the mean ratio for all lanes with detectable internal standard expression (NIH Image software).

As we have shown previously (Claiborne, J.B., et al., J. Exp. Biol. 193: 79-95, 1994), sculpin infused with 0.1 N HCl showed a higher net H^+ excretion rate (difference calculated between post-infusion and pre-infusion rates) than those fish infused with distilled water (Table 1). Post-infusion excretion rates were significantly higher than their pre-infusion controls in hour 1, 2, and 8 for acid-infused fish, while control fish were only higher than pre-infusion values at hour 1 (data not shown). Gill mRNA expression of the acid-loaded fish was found to be significantly higher in the first two hours following acid infusion when compared to control fish (Table 2). The mean pixel ratio at hour two was some 2.5x higher in the acidotic animals. At hour 5, mRNA expression in these animals remained high and only decreased significantly ($p<0.01$) in the fish sampled at hour 8. Band densities in the control animals were variable but did not change significantly from each other throughout the experiment.

Table 1: Net excretion of H^+ for water- and acid-infused sculpin. Each time period represents a separate group of experimental animals. Means ± S.E (N). * denotes significance between the respective pre-infusion and post-infusion period for each group ($p<0.05$, one-tailed Student's paired t-test).

Hour	Control (mmole kg^{-1} hr^{-1})	Acid-loaded (mmole kg^{-1} hr^{-1})
1	1.07 ± 0.15 (4)*	1.66 ± 0.70 (5)*
2	0.21 ± 0.61 (4)	1.23 ± 0.45 (5)*
5	0.37 ± 0.14 (3)	0.77 ± 0.37 (4)
8	0.18 ± 0.16 (3)	0.21 ± 0.17 (4)*

Table 2: Mean band density ratio of NHE-2 PCR product and 18S control for control and acid-infused sculpin. A higher ratio indicates increased expression of NHE-2 product. Means ± S.E (N). * denotes significance increase in experimental group over that of comparable control group ($p<0.05$, one-tailed unpaired test). † indicates mean ratio significantly different from animals sampled at other time periods within the same treatment.

Hour	Control (band density ratio)	Acid-loaded (band density ratio)
1	0.39 ± 0.11 (4)	0.63 ± 0.07 (5)*
2	0.22 ± 0.03 (4)	0.55 ± 0.10 (5)*
5	0.40 ± 0.12 (3)	0.56 ± 0.11 (4)
8	0.18 ± 0.04 (3)	0.13 ± 0.01 (4)†

Post-infusion net H^+ excretion increased rapidly in sculpin and 83% of the acid load had been lost to the water in the first hour. As observed previously by Claiborne et al (J. Exp. Biol. 193: 79-95, 1994), sculpin "overexcreted" a total of 3.87 mmol kg^{-1} by hour 8, nearly twice the infused load. Interestingly, water infusion also induced a significant proton loss (perhaps a volume loading or stress response) which returned to pre-infusion levels by hour two. The pattern of excretion following acidosis was mirrored by the measured expression of gill NHE-2 transcripts, as expression in acid loaded animals were higher than controls in the first two hours and decreased only after 8 hours. These data imply that increased mRNA transcription (and presumably the resulting protein translation) plays a role in the observed net H^+ excretion across the gills. The time course was similar to (though perhaps longer lived than) mRNA expression of NHE measured in the gills of the hagfish following an infused acid load (Edwards, S.L., et al., Comp. Biochem & Physiol. 130: 81-91, 2001). Western analysis using heterologous NHE antibodies have demonstrated that the expression of NHE isoforms can increase after one hour of hypercapnic acidosis in *Fundulus heteroclitus* (Edwards, S.L., et al., FASEB Journal (Abstracts) 15: supplement, pg. 11, LB43, 2001), but it remains to be seen if NHE-2 protein expression in sculpin will follow the time course observed for mRNA transcription. Sculpin specific antibodies are currently being developed to test this. This research was supported by NSF IBN-9808141 and IBN-0111073 to J.B.C. and A.I.M.S.