EVIDENCE FOR AN AMILORIDE SENSITIVE GILL Na^+/H^+ EXCHANGE DURING THE RECOVERY FROM ACIDOSIS IN THE LONG-HORNED SCULPIN (MYOXOCEPHALUS OCTODECIMSPINOSUS)

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Acid-base transfers in the long-horned sculpin are altered when ambient $[Na^+]$ is reduced (Claiborne, Walton & Compton-McCullough, J. Exp. Biol. 193:79-95, 1994; Claiborne, Perry & Bellows, Bull. MDIBL 32:95-97, 1993). Transbranchial Na^+/H^+ exchange may assist the animal in compensating for internal acidosis, and an external $[Na^+]$ of 20-30 mmol l^{-1} may be required (Claiborne & Bellows, Bull. MDIBL 34:63, 1995). In contrast, some studies on freshwater trout have indicated that excretion of H^+ is accomplished by an electrogenic H^+ ATPase linked to the 1:1 uptake of Na^+ through apical Na^+ channels (e.g., Lin and Randall, J. Exp. Biol. 161:119-134, 1991). These authors found that H^+ exchange across the gills showed little amiloride sensitivity. Thus, in the present study, we have tested the effect of amiloride and a more specific analog of amiloride (5-N,N-hexamethylene-amiloride; a specific inhibitor of the Na^+/H^+ antiport; Kleyman and Cragoe, J. Mem. Biol. 105:1, 1988) on the net H^+ excretion from the sculpin following acid infusion.

All animals were pre-adapted to dilute seawater (20%; $[Cl^-]$: ~100 mmol l^{-1}) for 9-10 days. Fish were then fitted with an intraperitoneal cannula and following an overnight control period, infused with dilute HCl (2.0 mmol kg^{-1}). External water samples were collected periodically throughout the experiment and analyzed for net transfers of H^+ (ΔH^+) according to the methods of Claiborne et al. (1994). Following the infusion, ΔH^+ was measured during a three hour post-infusion period and then a second three hour period in which amiloride (or hexamethylene-amiloride; HMA) had been added to the water (to a final concentration of 1 x 10^{-4} M). Finally, the ambient water was flushed and ΔH^+ was determined during two recovery periods (1.5 and 5.5 hours in length). Dunnett's tests were used for comparisons of ΔH^+ between control and experimental periods after using the Bonferroni procedure to control for error rate in repeated-measures data (overall protection level, 0.05).

As shown in Figure 1, ΔH^+ was reduced to values not significantly different from control levels following the acid infusion when either form of amiloride was added to the external water. The effect was reversible as ΔH^+ increased once again during the first recovery period when the water was returned to normal. By the end of the second recovery period, approximately 160 and 200% (3.2 and 4.0 mmol kg^{-1}, respectively for the amiloride and HMA series) of the infused load had been excreted by the fish. The "over-excretion" of acid during the recovery periods is not surprising as we have

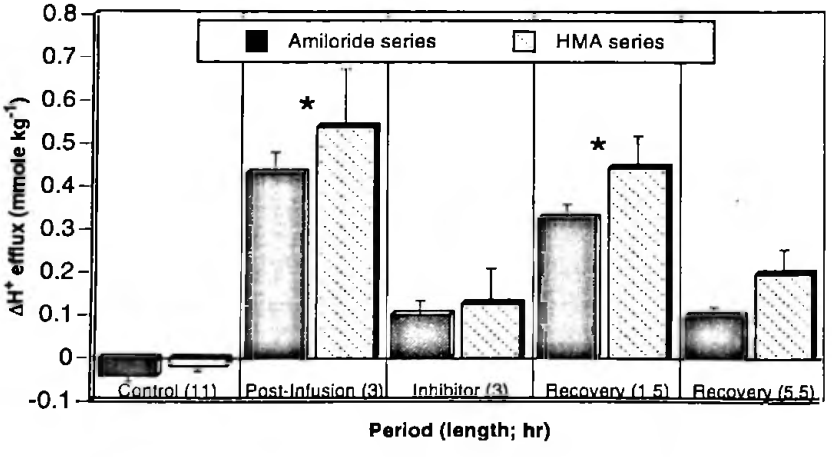

Figure 1. H^+ transfers following acid infusion and the addition of amiloride or hexamethylene-amiloride (HMA) to the external water. A positive transfer rate indicates a net efflux from the animal. An "*" indicates ΔH^+ rates that were significantly different from the control period (mean ± S.E., $p<0.05$, n=6 for amiloride and n=4 for HMA series).

previously hypothesized that once gill H^+ transfer mechanisms are activated, they remain functional after the initial acidosis has been compensated (Claiborne and Perry, Bull. MDIBL 31:54-56, 1992).

Thus, in contrast to some data for freshwater species (Lin and Randall, 1991) amiloride does inhibit H^+ transfers from the sculpin during acidosis. While it is possible that amiloride indirectly reduced electrogenic H^+ transport by blocking Na^+ channels, ΔH^+ inhibition of a similar magnitude was also demonstrated using the HMA analog. As this analog is thought to be a specific inhibitor of the Na^+/H^+ antiporter in mammalian systems, these results point to the presence of a coupled Na^+/H^+ exchange in the sculpin gill. These findings also agree with preliminary Northern blot studies which detected the presence of mRNA transcripts in the sculpin which were homologous to a human cDNA probe for the NHE-1 isoform of the Na^+/H^+ exchanger (Harris, Claiborne, Pouyssegur and Dawson, Bull. MDIBL 32:128-130, 1993). It remains to be seen if regulatory changes of this transporter at the molecular level can be linked to observed physiological adjustments measured in vivo.

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