EVIDENCE FOR A DIDS SENSITIVE GILL Cl^-/HCO_3^- EXCHANGER IN MARINE LONG-HORNED SCULPIN (*MYOXOCEPHALUS OCTODECIMSPINOSUS*) ADAPTED TO DILUTE SEAWATER

Jennifer Campbell[1], Isaac Darko[2], and J.B. Claiborne[1]
[1]Georgia Southern University, Statesboro, GA 30460
[2]Health Professions High School, Bronx, NY

Previous experiments have shown that a Na^+/H^+ exchanger may be present within the gill of long-horned sculpin, *Myoxocephalus octodecimspinosus* (Claiborne, Campbell, & Long, Bull. MDIBL 35:48, 1996). We have shown that H^+ efflux across the gills shows sensitivity to both amiloride and its analog, hexamethyl amiloride, which are inhibitors of the Na^+/H^+ exchanger. We also believe this exchanger may be working in opposition to a Cl^-/HCO_3^- exchanger for acid-base regulation. When sculpin are exposed to Cl^- concentrations lower than that of the ambient water, they readily excrete an acid load 50% higher than control fish (Claiborne & Bellows, Bull. MDIBL 33:99, 1994). These data imply that the excretion of HCO_3^- is inhibited by low Cl^- concentrations; the mechanism, we believe to be a result of a "band-3" Cl^-/HCO_3^- exchanger (Brosius, Alper, Garcia, & Lodish, J. Biol. Chem. 264:7784-7787, 1989). As an additional test for the presence of this exchanger, fish received an acid load to the peritoneal cavity, and H^+ excretion was monitored before and after exposure to DIDS (4,4 diisothiocyano-stilbene-2,2'-disulfonate), an inhibitor of the Cl^-/HCO_3^- exchanger.

Long-horned sculpin were adapted in 20% seawater ($[Cl^-]$: ~100 mmol l^{-1}) for a period of 9-10 days while temperature was held at the ambient seawater temperature as explained by Claiborne, Walton, & Compton-McCullough (J. Exp. Biol. 193:79-95, 1994). Following this adaptation period, each fish was fitted with an intraperitoneal cannula and left undisturbed for an 11-12 hr control period. A control group of fish was then infused with distilled water (a volume equal to 2% of body mass). Experimental groups were subjected to one of two protocols: (1) fish were infused with 2.0 mmole kg^{-1} 0.1 N HCl after which DIDS was added to the external water or (2) fish were exposed first to DIDS, followed by an acid infusion. Water samples were collected at the beginning and end of each period to measure HCO_3^- and NH_4^+, thus calculating H^+ (Claiborne et al., 1994, ibid.).

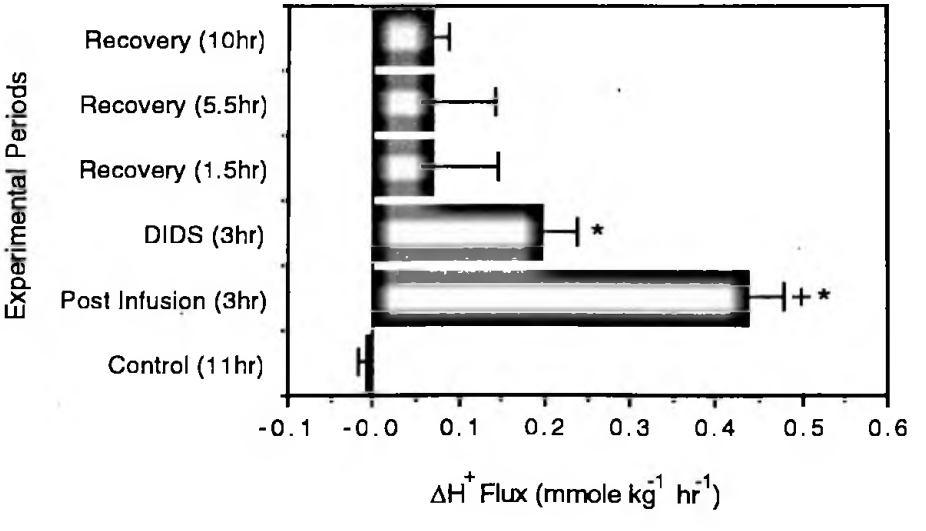

Figure 1: Net H^+ excretion in sculpin which were acid loaded and then exposed to DIDS Length of each period is shown (hr). Bars represent mean ± S.E. (n=5). "*" represents periods significantly different from the previous period. "+" represents periods significantly different from the control period.

Net H^+ transfers in water infused fish did not change significantly from pre-infusion control values. In contrast, when fish were exposed to DIDS, their acid excretion rate decreased significantly from that of the post infusion period (Figure 1; $p < 0.01$). Assuming DIDS blocked Cl^-/HCO_3^- exchangers and that presumptive Na^+/H^+ exchangers remained functional, we expected an increase in H^+ excretion. However we speculate that the decrease in acid excretion may be the result of HCO_3^- accumulation inside cells buffering H^+ and leaving less H^+ to excrete. In contrast, DIDS did increase baseline net H^+ efflux ($p < 0.01$) in control animals that were not acid loaded (Figure 2). Acid infusion after exposure to DIDS did not induce the elevation of H^+ excretion rates observed in fish that had not been exposed to DIDS (Figure 1).

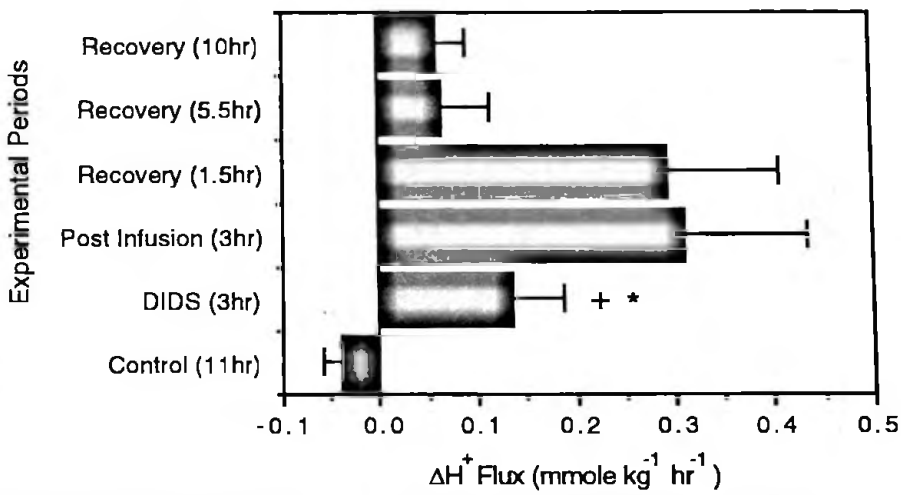

Figure 2: Net H^+ excretion in sculpin which were exposed to DIDS prior to acid infusion. Length of each period is shown (hr). Bars represent mean ± S.E. (n=6). "*" represents periods significantly different from the previous period. "+" represents periods significantly different from the control period.

We conclude that a DIDS sensitive exchange component was indeed inhibited. Further studies may include the measurement of blood acid-base variables in conjunction with molecular studies of this exchanger. Funded by NSF-BIR 95-3134 to J.C. and NSF REU 94-19849 to J.B.C. and NSF 93-22221 (Research Experience for Undergraduates) for I.D.