INHIBITION OF BRANCHIAL APICAL MEMBRANE-BOUND CARBONIC ANHYDRASE (CA) DOES NOT ALTER THE COMPENSATION TO RESPIRATORY ACIDOSIS IN THE SHARK, *SQUALUS ACANTHIAS*

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Carbonic anhydrase inhibition in elasmobranch gills slows the rate of compensation to respiratory acidosis (Swenson and Claiborne, *Bull. MDIBL.* 25:77-79, 1985). The role of branchial cytosolic vs. membrane-bound CA remains uncertain, although we found no effect of selective inhibition of basolateral membrane-bound CA (Patel et al. *Bull. MDIBL* 36:65-68, 1997) suggesting that in hypercapnia the necessary catalysis of CO_2-HCO_3 reactions occurs either intracellularly or at the apical membrane. Whether the apical membrane contains CA is unknown since definitive histochemical or membrane isolation studies have not been performed. To study the question, we used a polymer-linked sulfonamide (F3500), whose large size (3.5 kD) and water solubility excludes intracellular uptake (Swenson et al., *Bull MDIBL* 37:76, 1998). By adding F3500 in the seawater, we could achieve selective apical membrane-bound CA inhibition. The results, however, were disappointing since we had to use a closed seawater system for administration of the drug. Despite good oxygenation and temperature control, the control rate of serum bicarbonate rise was only half that we found in an open system with constant replenishment of seawater (Swenson and Claiborne, *ibid.*), and F3500 had no effect on this far lower rate.

In order to minimize accumulation of potentially toxic non-volatile metabolites, which may have limited the capacity for bicarbonate retention, we altered the study design in the present experiments in two ways. First, we changed the seawater every hour during the 4 hr experimental period. Second, we used a new, hydrophilic sulfonamide, p-fluoro-benzlamino-benzolamide, pFBB (MW = 454) whose synthesis is sufficiently inexpensive to permit the large amount of drug use required to make hourly seawater changes.

Preliminary studies were done to demonstrate that pFBB would serve as a selective apical membrane-bound CA inhibitor in vivo. The K_I at 0 ° C of pFBB against mammalian CA II is 25 nM and against dogfish red cell CA is 130 nM. The i.v. administration of 50 mg/kg into three fish caused no respiratory acidosis (pH 7.81 $\pm$ 0.05 (SD) vs. 7.79 $\pm$ 0.04 after 4 hr) indicating no red cell CA inhibition. Blood samples taken at 4 hr showed a mean plasma concentration of 27 $\pm$ 4.2 uM (SD) and no detectable red cell pFBB concentration (< 0.05 uM, the detection limit of the assay).

Spiny dogfish, *Squalus acanthias* (wt 1.8 - 2.2 kg) were studied 12-16 hr after caudal artery catheter placement and transfer into small (10 liter) Plexiglas tanks (Swenson and Caliborne, *ibid.*). Hypercapnia was induced by bubbling 1% CO_2 in air (3 l/min) into seawater. At this point, running seawater was stopped for 4 hr to test the effect of pFBB (400 mg/l) dissolved into the seawater. At each hour, 8.5 l of seawater was rapidly drained and recharged with 8.5 l of fresh seawater (the remaining 1.5 l was sufficient for uninterrupted water ventilation by the fish) and this process was repeated. For the treated fish, 400 mg/l of pFBB was again added after the seawater change. The whole procedure took less than 5 minutes. Arterial blood was analyzed hourly for pH, total CO_2 and PO_2 with analyzers calibrated at 14° C (Cameron Instruments, Port Aransas, TX), and for pFBB concentrations in plasma by the micromethod of Maren (*J. Pharmacol. Exp.Therap.* 130:26-29, 1960).

The table shows the effects of hypercapnia on plasma total CO_2 (mM, mean ± SD) in control and pFBB-treated fish. Seawater PO_2, PCO_2 and temperature during hypercapnia remained stable at 150 mmHg, 7.5 mmHg and 14-15 ° C. The data show that hourly replacement of seawater over 4 hr permits equivalent bicarbonate retention in response to hypercapnic seawater as we originally found in an open system (vide supra). Moreover, pFBB did not alter the response. Measurements of plasma at 4 hr showed no detectable drug uptake across the gill in the drug-treated fish.

Hour	0	1	2	3	4
			plasma total CO_2 (mM)		
Control	6.1 ± 0.3	10.8 ± 1.2	15.4 ± 1.6	18.2 ± 2.7	20.3 ± 2.5
pFBB	6.5 ± 0.4	11.5 ± 1.6	15.9 ± 0.9	18.9 ± 1.9	19.8 ± 2.1

values are means ± SD, n = 5 for both groups

The results demonstrate that selective inhibition of a possible apical membrane-bound CA activity in the marine elasmobranch does not limit compensation to respiratory acidosis. In a recent study, Gilmour et al (*J. Exp. Biol.*. 1999, in press) present evidence for a lack of apical membrane CA activity by demonstrating the presence of a disequilibrium pH in the respired seawater of dogfish, that is not altered by externally applied CA inhibitors. These data, in combination with our earlier negative study of selective inhibition of basolateral membrane-bound CA, now establish the primary role of branchial cytosolic CA activity in hypercapnic compensation.

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