APICAL GILL MEMBRANE-BOUND CARBONIC ANHYDRASE (CA) INHIBITION AND RESPONSE TO HYPERCAPNIA IN THE SHARK, *SQUALUS ACANTHIAS*

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Marine elasmobranch gills contain cytosolic and membrane-bound CA, both of which contribute to rapid correction of metabolic alkalosis (Swenson and Maren, Am. J. Physiol. 253:R450-R458, 1987 and Swenson et al. Bull. MDIBL 35:47, 1996). We have shown also that compensation to hypercapnia is dependent upon branchial CA (Swenson and Claiborne, Bull. MDIBL. 26:5-8, 1986). In contrast to metabolic alkalosis, we found no effect of selective inhibition of basolateral membrane-bound CA (Patel et al. Bull. MDIBL 36:65-68, 1997) suggesting in hypercapnia either the necessary catalysis of CO_2-HCO_3^- reactions occurs intracellularly or at the apical membrane. To study the latter possibility we used a polymer-linked sulfonamide (F3500), which by virtue of size (3.5 kD) and water solubility remains extracellular (Conroy et al. Bioorganic Chem. 24:262, 1996) and if added to seawater, should inhibit only apical membrane-bound CA.

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 Maren, 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 F3500 (50 mg/l) dissolved into the seawater at the start of CO_2 bubbling. This concentrtation is sufficient to yield maximal effect on gill HCO_3 excretion in metabolic alkalosis (Swenson et al, ibid.). Three fish were given 2 mg/kg benzolamide to inhibit cytosolic CA. Arterial pH, total CO_2 and PO_2 were analyzed at 14° C (Cameron Instruments, Port Aransas,TX).

The table shows the effects of hypercapnia on plasma HCO_3^- (mM, mean ± SD) with F3500 in seawater and i.v. benzolamide in the above described closed (cl) system (cols. 2,4,5). It also shows plasma HCO_3^- increase an open (op) system (cols 1,3) with continuous flowing seawater (Swenson and Claiborne, ibid.). Seawater PO_2, PCO_2 and temperature during hypercapnia remained stable at 150 mm Hg, 7.5 mmHg and 14-15 ° C.

	1	2	3	4	5
Hour	Control (op)	Control (cl)	Benz (op)	Benz (cl)	F3500 (cl)
	(n = 5)	(n = 3)	(n = 5)	(n = 3)	(n = 5)
0	6.1 +/- 0.5	6.7 +/- 0.9	6.4 +/- 0.6	5.9 +/- 0.8	6.5 +/- 0.5
4	20 +/- 1.4	13.5 +/- 1.2 *	12.0 +/- 0.9 *	12.9 +/- 1.9 *	13.8 +/- 1.1 *

$p < 0.05$ vs. Control (op)

The present results show reduced HCO_3^- accumulation in hypercapnia in a closed system (compare cols 1 and 2) which was necessary for the test of F3500 in seawater. In this setting, there is no effect of apical membrane-bound or cytosolic CA inhibition (cols 4 and 5). Inhibition results are similar to those found earlier (col 3, Swenson and Claiborne, ibid.) in an open system. Thus the data do not resolve the role, if any, of apical membrane-bound CA in compensation to respiratory acidosis. Future experiments will be designed to minimize accumulation of other non-volatile metabolites, that may inhibit HCO_3 uptake.

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