EFFECTS OF GILL AND RED CELL CARBONIC ANHYDRASE INHIBITION ON CO_2 EXCHANGE IN THE RESTING AND SWIMMING SHARK, SQUALUS ACANTHIAS.

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Introduction: We have shown that gill carbonic anhydrase plays a major role in acid base homeostasis in the shark. The rates of HCO_3 uptake in respiratory acidosis (Swenson and Claiborne, Bull MDIBL: this volume) and HCO_3 excretion in metabolic alkalosis (Swenson et al Bull MDIBL 24:72, 1984) are greatly reduced when gill enzyme is inhibited. In contrast the role of gill carbonic anhydrase in gas exchange is uncertain. We previously examined the effects of gill CA inhibition by benzolamide, a selective inhibitor of gill enzyme in these fish (Swenson et al Bull MDIBL 22:72-75; 1982) and found a clearly different pattern of HCO_3 and total CO_2 elevation from that occuring in red cell CA inhibited fish. We now present further experiments including quantitative measurements of metabolic activity in resting and swimming fish, that shed further insight into the differences between red cell and gill CA inhibition on CO_2 exchange in sharks.

Material and Methods: Unanesthetized sharks (2 kg) were studied in the resting and swimming states. On the day prior to experimentation, the dorsal artery was cannulated for drug administration and blood sampling. Free swimming fish were transferred to a large circular holding tank (3000 liters). A 36 inch extended catheter made it possible to sample blood and deliver drugs in actively swimming fish without restraining or disturbing the animal. Resting non-swimming fish were transferred to a small darkened box (10 liters). All fish were allowed 24 hours recovery from the catheterization. The effects of 1mg/kg benzolamide were studied in resting and swimming fish and 2mg/kg in swimming fish. Methazolamide (30 mg/kg) was given to swimming fish. Arterial and seawater pH and PO_2 were measured on a blood gas analyzer (IL-213, Instrumentation Laboratories) calibrated with standards at 14°C. Total CO_2 of plasma was measured with a Kopp-Natelson Microgasometer. PCO_2 and HCO_3 were calculated from the Henderson-Hasselbalch equation using a pKa of 6.07 and an α factor of 0.052 for the CO_2/HCO_3 system at 14°C and ionic strength of seawater and dogfish plasma (Pleschka and Wittenbrock, Pflugers Arch 329:186; 1971). Oxygen consumption (VO_2) was measured in resting and swimming fish by following the rate of decline in seawater PO_2 from 130 to 100 mmHg after the box or holding tank was sealed off from atmospheric air. The O_2 α factor of seawater at 14° C is 1.70 μmol/L · mmHg. (Boutilier et al, Fish Physiology, Vol XA 1984; p.420).

Results: The table presents the blood gas values, acid base status and oxygen consumption data in resting fish treated with benzolamide, and swimming fish treated with benzolamide or methazolamide. Benzolamide caused no change in arterial pH but a statistically significant increase in HCO_3 in four hours in both resting and swimming fish. The magnitude of the HCO_3 elevation was greater in swimming fish (3.8 mM vs 0.7 mM). The PCO_2 also rose to maintain an unchanged pH. There was no difference between the two doses of benzolamide in swimming fish. Methazolamide in contrast caused a marked respiratory acidosis with an increase in HCO_3 that approximated the degree of HCO_3 elevation seen in the benzolamide treated fish. Oxygen consumption (VO_2) rose from 1.56 mmol/h · kg at rest to 6.6 mmol/h·kg with swimming. In neither resting nor swimming fish was VO_2 altered by benzolamide.

Discussion: The results with methazolamide (combined red cell and gill CA inhibition) differ strikingly from those with benzolamide (gill CA inhibition). The respiratory acidosis of methazolamide is a consequence of red cell CA inhibition, which causes an elevation of PCO_2 sufficient to maintain steady-state CO_2 output. The severity of the respiratory acidosis is underestimated by measured equilibrium arterial blood gas values. The elevation of HCO_3 is a compensatory response to the acidosis and represents active uptake of HCO_3 by the gills from seawater, albeit at a reduced rate since methazolamide also inhibits gill CA. (Swenson and Claiborne, Bull MDIBL: this volume).

In contrast, benzolamide causes no acidosis even in the swimming fish. In fact while not statistically significant, the data show a small rise in pH. Yet there is an elevation in HCO_3 equivalent to that seen with methazolamide. Since there is no respiratory acidosis which would stimulate gill HCO_3 uptake, we suggest then that gill CA inhibition blocks excretion of endogenously generated HCO_3. Heisler et al (Bull Europ Respir Physiol Pathol 12: 77, 1976) have measured in a related shark (S. stellaris) a gill HCO_3 excretion rate of 0.072 m mol/h · kg, which represents 5% of the measured O_2 consumption in these fish. If we assume a bicarbonate space of ~ 40% in our 2 kg sharks, then a rise in plasma HCO_3 of 0.70 mM at rest and 3.8 mM with swimming over 4 hours equals a HCO_3 retention of 0.07 mmol/h · kg and 0.38 mmol/h · kg respectively. Both figures are 5% of oxygen consumption and match the cited data of Heisler et al. Therefore, inhibition of this small amount of HCO_3 excretion (accounting for only 4% of metabolic CO_2 output) was just barely detectable at rest in four hours, but easily measured in swimming fish whose metabolic rate was increased more than four fold. The minimal elevations in pCO_2 can be explained as secondary to nonbicarbonate buffering of the retained HCO_3. Since fish do not alter their ventilation in response to acid base perturbations (Randall Fish Physiology Vol XA, 1985) and we did not observe any drop in arterial PO_2, it is unlikely hypoventilation explains the slight hypercapnia.

A less satisfactory alternative explanation for the benzolamide results is possible and cannot be entirely ruled out on the basis of our data alone. This is that the drug blocks the excretion of a small fraction (~4%) of metabolic CO_2 elimination that occurs because some plasma HCO_3 moves into gill cells to be dehydrated to CO_2 rather than into the red cell. This would appear to be unnecessary since red cells have five times more CA than do gill cells and are capable of rapid Cl/HCO_3 exchange and have an oxylabile buffer capacity. Additionally if this is a block to metabolic CO_2 elimination (rather than the excretion of base as HCO_3) then one should see some degree of acidosis. Resolution of this issue will require further measurements of $HCO_3/CO_2/H+$ fluxes across the gill and ideally experiments in which there is only red cell CA inhibition.

In summary, we have defined two patterns of CAI in the dogfish. In the first, methazolamide inhibits enzyme in red cells and gill leading to a respiratory acidosis and a secondary uptake of HCO_3 from seawater. In the second, benzolamide acts only on gill enzyme, leading to an elevation of HCO_3 that is either the result of reduced excretion of endogenously produced HCO_3 or inhibition of a small fraction of CO_2 elimination which does not follow the classic pattern of plasma-red cell exchanges in the gill.

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Acid-base and oxygenation status in resting and swimming fish:
Effects of benzolamide and methazolamide

	Hours	pH	pCO_2 mmHg	HCO_3 mM	pO_2 mmHg	$\dot{V}O_2$ mmol/h · kg
Resting fish Benzolamide 1 mg/kg N = 8	0	7.82 ± 0.02	1.6	4.5 ± 0.2	96 ± 10	1.56 ± 0.18
	2	7.82 ± 0.02	1.7*	4.9 ± 0.2*	100 ± 13	—
	4	7.83 ± 0.02	1.8*	5.2 ± 0.3*	102 ± 11	1.50 ± 0.18
Swimming fish Benzolamide 1 mg/kg N = 5	0	7.87 ± 0.02	1.5	4.6 ± 0.1	111 ± 20	6.6 ± 0.6
	2	7.88 ± 0.02	2.0*	6.5 ± 0.3**	109 ± 18	—
	4	7.89 ± 0.02	2.5**	8.4 ± 0.4**	121 ± 15	6.7 ± 0.7
Swimming fish Benzolamide 2 mg/kg N = 4	0	7.85 ± 0.01	1.5	4.6 ± 0.2	117 ± 20	—
	2	7.82 ± 0.02	2.2*	6.2 ± 0.4*	125 ± 15	—
	4	7.88 ± 0.02	2.6**	8.4 ± 0.5**	120 ± 18	—
Swimming fish Methazolamide 30 mg/kg N = 4	0	7.87 ± 0.02	1.5	4.8 ± 0.1	123 ± 10	—
	2	7.53 ± 0.07**	4.6**	6.7 ± 0.3**	129 ± 12	—
	4	7.62 ± 0.06**	4.5**	7.9 ± 0.5**	120 ± 18	—

Values are Means ± SEM
* $p < 0.05$; ** $p < 0.01$; paired t-test versus baseline (hour 0)