FLOUNDER (*PLEURONECTES AMERICANUS*) RENAL SULFATE CLEARANCE IS CARBONIC ANHYDRASE DEPENDENT

J. Larry Renfro[1], Thomas H. Maren[2], Cristina Zeien[2], and Erik R. Swenson[3]
[1]Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT, 06269,
[2]Department of Pharmacology and Therapeutics, University of Florida, Gainseville, FL 32610,
[3]Department of Medicine, University of Washington,
Seattle, WA 98108

The usual decrease in renal bicarbonate reabsorption and rise in urine pH and titratable acidity caused by carbonic anhydrase (CA) inhibitors in vertebrates are not observed in marine fishes although chemical assays for CA have usually showed that the enzyme is present in the renal tissue (Maren et al., *Am. J. Physiol.* 263:F49-F55, 1992). Evidence from several prior studies has shown that a well-developed sulfate secretory system is present in marine fish kidneys with clearance ratios for sulfate approaching 12 (Hickman and Trump, *Fish Physiology,* New York:Academic, vol. 1:91-239, 1969). Isolated brush border and basolateral membrane vesicles showed that transport of sulfate was linked to anion exchangers in both membranes - bicarbonate in the luminal and hydroxyl ion in the antiluminal (*see* Renfro, *Fish Physiology*, New York:Academic, Vol. 14:147-172, 1996). These observations prompted us to examine the effects of CA inhibitors on sulfate transport by flounder proximal tubule primary cultures (PTCs). Preliminary findings showed that methazolamide, at concentrations specific for CA inhibition, blocked approximately 50% of net sulfate secretion, an action thought to be intracellular since the polymer-linked high molecular weight CA inhibitor, polyoxyethylene-aminobenzolamide (10 μM), which is restricted to the extracellular space, had no effect on sulfate secretion (Renfro et al., *Bull. MDIBL*, 36:48-49,1997). Sulfonamides are in general powerful inhibitors of CA; however, they vary greatly in physicochemical characteristics. To assure that sulfonamides other than methazolamide also inhibited sulfate secretion *in vitro*, we determined the effect of ethoxzolamide on this process in flounder PTCs. Furthermore, we predicted, based on the above noted recent findings, that CA inhibition might alter renal sulfate clearance and, thus, help to explain the lack of effects of CA inhibitors on urinary bicarbonate and pH.

To prepare PTCs, flounder renal epithelial cells were isolated as previously described (Dickman and Renfro, *Am. J. Physiol.* 251:F424-F432, 1986), suspended in modified M-199 and plated to confluence on native rat tail collagen (Dickman and Renfro, *Soc. Exp. Biol. Sem. Series* 52:65-86, 1993). After 12 days the floating collagen rafts were contracted from 35 mm to 17 mm, and the cells forming the epithelial sheet had assumed their normal structure and function. Unidirectional $^{35}SO_4^=$ fluxes across these monolayers were determined in Ussing chambers under short-circuited conditions.

Table 1. Effect of 100 μM ethoxzolamide on secretion of sulfate by flounder proximal tubule primary cultures.

	Sulfate flux (nmoles x cm^{-2} x h^{-1})		Glucose
	Secretory	Reabsorptive	(μA x cm^{-2} x h^{-1})
Control	60.6 ± 1.07	2.7 ± 0.64	3.0 ± 0.56
Ethoxolamide	26.7 ± 1.85*	5.6 ± 1.81	3.1 ± 0.54

Values are mean ± SEM (n = 4 preparations). Fluxes shown were determined at 90 min of incubation. *Significantly different from paired control at $P < 0.05$.

Table 2 . Effect of methazolamide on renal function in winter flounder.

	ml x kg^{-1} x h^{-1}				µmoles x kg^{-1} x h^{-1}	
	GFR	Urine Flow	Serum pH	Urine pH	Phosphate secretion	Sulfate secretion
Control	2.1 ± 0.61	0.7 ± 0.19	7.8 ± 0.05	6.7 ± 0.09	-1.05 ± 2.17	24.7 ± 7.13
Methazolamide	1.7 ± 0.55	0.5 ± 0.15	7.6 ± 0.04**	6.7 ± 0.13	-1.40 ± 0.79	15.3 ± 6.54*

Values are mean ± SEM (n = 8 animals). Values shown are paired and were taken approximately 1 h before (control) and 1 h after drug administration. *,**Significantly different from control at $P < 0.05$ or $P < 0.001$, respectively.

To determine renal clearances, flounder (250-350 g) were anesthetized in MS-222 (1:2000, w/v) while urinary bladder and hemal vein were intubated. Sample collection began 24 hours later. Glomerular filtration rate was determined from the clearance of intramuscularly administered inulin. The latter was assayed with the indole acetic acid method. Inorganic phosphate and sulfate were measured by anion chromatography (Dionex Corp.)

Table 1 shows that 100 µM ethoxzolamide reduced secretory flux to less than one-half that of controls (44% of control). It had no significant effect on reabsorptive flux or on glucose reabsorption as determined from phloridzin-sensitive current. Thus, ethoxzolamide caused no loss of metabolic or structural integrity. This was almost identical to the inhibitory effect of 100 µM methazolamide (55% of control) (Renfro et al., *Bull. MDIBL*, 36:48-49,1997).

Methazolamide was administered intravenously at a dose of 25 mg/kg, about 200 µM in the extracellular fluid, several thousand-fold higher than its K_i. Values shown in Table 2 were obtained in the 1 h just prior to drug infusion and in the 1st hour post-infusion. Glomerular filtration rate and urine flow were not significantly changed by the drug. Serum pH fell 0.2 units, a very highly significant change probably due to inhibition of the branchial CA and slowing of the acid-base transport systems. The rate of urinary phosphate or sulfate secretion was calculated as the difference in the quantity excreted and quantity filtered. There was no effect of CA inhibition on phosphate (minus sign indicates net reabsorption); however, sulfate secretion was inhibited approximately 40%. This is consistent with the effect predicted by the *in vitro* experiments.

These data suggest that a role for renal CA in marine fish is for sulfate excretion. This explains why all previous experiments measuring urinary pH and bicarbonate gave negative results following inhibition of the enzyme.

Supported by NSF IBN 9604070 (JLR), Univ. Florida sponsored research grant (THM) and NIH HL-45571 (ERS). J.L. Renfro was supported by a Salsbury Cove Research Fund Senior Fellowship. Ms. Zeien was supported by a University of Florida Summer Fellowship.