SULFATE SECRETION BY FLOUNDER (*PLEURONECTES AMERICANUS*) RENAL EPITHELIUM IS CARBONIC ANHYDRASE DEPENDENT

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The role of carbonic anhydrase (CA) in the renal function of marine fishes has remained a vexing problem since the early work of Hodler et al. (Am. J. Physiol. 183:155-162, 1955) who showed that the typical effects of renal CA inhibition (bicarbonaturia and rise in urine pH) as observed in all vertebrates (except Crocodilia) was not observed in either marine elasmobranch or teleost fishes. It appeared that the enzyme was lacking, or that it had a renal role unlike that in the other vertebrates. Rigorous attempts to assay the enzyme in marine fish were confounded by the presence of hematopoietic tissue in kidneys, but the net result of studies over the years (Gehnrich et al., Bull. MDIBL 33: 61-63, 1994; Gehnrich et al., Bull. MDIBL 34: 96, 1995; Swenson and Maren, Am. J. Physiol. 250: F288-F293, 1986; Swenson et al., Bull. MDIBL 35: 45-46, 1996) suggests that there is CA in renal cells of marine fish despite failure to demonstrate the typical effects of inhibition with large doses of specific sulfonamide inhibitors.

Our preliminary work suggests a hitherto unsuspected role for CA in marine fish, that of subserving sulfate secretion by bicarbonate exchange, the latter formed from enzymatic hydration (or hydroxylation) of carbon dioxide. To compensate for osmotic water loss marine teleosts must ingest seawater. The $SO_4^=$ concentration of normal seawater is 25 mM. Part of this is absorbed by the gut along with the water and is excreted primarily by the kidneys. Flounder plasma inorganic $SO_4^=$ concentration averages 0.6 mM (Renfro and Dickman, Am. J. Physiol. 239: F143-F148, 1980), and renal $SO_4^=$ clearance ratios (clearance of $SO_4^=$/clearance of inulin) exceed 12 (Hickman and Trump, *Fish Physiology*, New York: Academic, Vol. 1, 91-239, 1969). It is thus apparent that a well-developed $SO_4^=$ secretory system is present in the marine teleost renal tubule.

To examine sulfate transport, 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. In the presence of flounder saline (1 mM SO_4) the flux ratio was approximately 20 to 1 in the secretory direction. Net fluxes calculated from the unidirectional fluxes in paired tissues are shown in Table 1. These preliminary data show that at 10 µM methazolamide, a concentration specific for carbonic anhydrase inhibition (i.e., does not significantly inhibit other sulfhydryl containing enzymes), secretory sulfate transport was inhibited by approximately one-half. At 1 mM methazolamide, inhibition increased only slightly, i.e., a 100-fold increase in inhibitor concentration produced a further inhibition of only 0.3-fold.

Table 1. Methazolamide inhibition of Sulfate Transport by Flounder Proximal Tubule Cell Cultures

Treatment	*1 mM*	*100 µM*	*10 µM*
Control	149.8 ± 16.61	151.1 ± 36.64	136.0 ± 22.32
Methazolamide	57.5 ± 4.07 *	77.3 ± 9.07 *	86.8 ± 26.72 *
% Control	40.8 ± 5.96	54.6 ± 7.21	59.6 ± 12.27

Values (nmoles x cm^{-2} x h^{-1}) are mean ± standard error of n = 3 preparations. *Signifcantly different from paired control at $P < .05$.

The action of methazolamide is apparently intracellular since a polymer-linked, high molecular weight (3700) CA inhibitor, polyoxyethylene-aminobenzolamide (10 μM), which is restricted to the extracellular space, had no effect on sulfate transport (% Control = 99.6 ± 18.21).

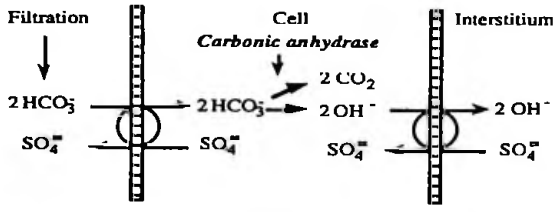

Figure 1

The cellular mechanisms of renal sulfate secretion have been examined in both basolateral and brush border membrane vesicles isolated from two flounder species (*see* Renfro, *Fish Physiology*, New York:Academic, Vol. 14:147-172, 1996). Sulfate entry, interstitium-to-cell, can be driven by the basolateral membrane pH gradient as either proton symport or hydroxyl exchange. In the intact tubule, this process is Na gradient dependent, perhaps secondary to apical membrane Na^+:H^+ exchange. Sulfate exit, cell-to-lumen, is stimulated by counter anion gradients. HCO_3^- SCN^-, Cl^- and $S_2O_3^-$ counter gradients can drive sulfate transport in flounder brush border membrane vesicles. HCO_3^- was the most effective counter anion. The exchanger is inhibited by disulfonic acid stilbenes but is not sensitive to H^+, Na^+ or K^+ gradients. These basolateral and brush border anion exchangers are depicted in Figure 1. In this model the Na^+ electrochemical gradient established by Na^+, K^+-ATPase drives an assumed Na^+:H^+ exchanger which establishes a pH gradient across the basolateral membrane favoring OH^-:$SO_4^=$ exchange. $SO_4^=$ exits apically in an electroneutral exchange for luminal anions, mainly Cl^- and HCO_3^-. The figure also shows the proposed role of carbonic anhydrase in flounder renal tubule sulfate secretion. Filtration provides HCO_3^- in the tubule lumen which is exchanged for cellular sulfate. Inside the cell, the conversion of bicarbonate to CO_2 and OH^- is accelerated by carbonic anhydrase, supplying hydroxyl ions that exchange for extracellular sulfate at the basolateral membranes. This process may account for most of the reabsorption of bicarbonate, as such, at the brush border membranes. For example, a typical value for net sulfate secretion by marine teleosts is about 20 μmoles x kg^{-1} x h^{-1} (Renfro and Pritchard, Am. J. Physiol. 243:F150-F159, 1982) with a typical glomerular filtration rate of 2 ml x kg^{-1} x h^{-1} and urine flow of 0.5 ml x kg^{-1} x h^{-1}. In winter flounder plasma and urine [HCO_3^-] are about 5 and 0.7 mM, respectively, and the amount of HCO_3^- reabsorbed would be about 9 μmoles x kg^{-1} x h^{-1}, considerably less than the amount of $SO_4^=$ secreted. Thus, $SO_4^=$ secretion can account for the near total removal of HCO_3^- from the urine. Catalyzed conversion of reabsorbed HCO_3^- would help maintain a chemical driving force for $SO_4^=$ translocation at the apical membrane as well as contribute to the pH gradient necessary to drive $SO_4^=$ entry at the basolateral membrane. It is likely, however, that $SO_4^=$ secretion is sufficient even in the absence of carbonic anhydrase to reabsorb most of the filtered bicarbonate. The effect of a drug such as methazolamide may thus be reflected more in the amount of sulfate appearing in the urine rather than the urinary [HCO_3^-] or pH.

One consequence of this transport system is the exchange of a weak acid (carbonic acid) for a strong acid (sulfuric acid) anion. Because drinking rate and thus sulfate entry into the animal are likely to be relatively constant, the amount of sulfate secretion is continuous and relatively stable. The consequence of such secretion may account for the relatively stable, acid urine pH of marine teleosts. If, as Marshall and Smith (Marshall and Smith, Biol. Bull. 59: 135-153, 1930) contended, bony fishes re-invaded seawater following extensive evolutionary development of the kidney in freshwater, the role of carbonic anhydrase in renal acid-base control may have been well-established when re-invasion occurred. Thus, renal carbonic anhydrase may have been a "pre-adaptation" useful in the development of the sulfate secretory mechanism which is vital for hypo-osmoregulation in seawater.

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