SHORT-TERM REGULATION OF RENAL SULFATE SECRETION IN WINTER FLOUNDER (*PSEUDOPLEURONECTES AMERICANUS*)

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Winter flounder balance osmotic water loss by ingestion of seawater. The consequent sulfate load absorbed by the gut is actively secreted by the kidneys. Both *in vivo* and *in vitro* studies indicate that long-term (days) regulation of renal sulfate secretion may be controlled by glucocorticoids (Renfro, J.L., *Am. J. Physiol.* 257:R511-R516, 1989). The latter may account in part for changes in sulfate clearance upon acclimation to varying salinities (and resultant sulfate loads). Teleosts clearly have the capacity to alter renal sulfate secretion (Hickman, C.P and B.F. Trump, *Fish Physiology,* New York:Academic, vol. 1:91-239, 1969); however, there is no information on possible *short-term* homeostatic control. We have thus begun to examine the effect of pharmacological manipulation of intracellular signaling systems on rapid adjustments of renal sulfate transport by flounder renal proximal tubule epithelial cell monolayers in primary culture (PTCs).

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

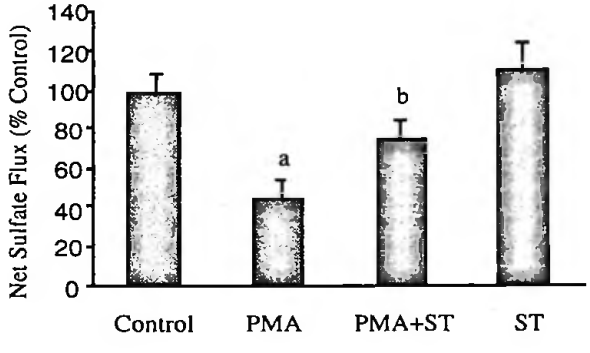

Figure 1. Effect of protein kinase c activation and inhibition on net sulfate secretion by flounder proximal tubule primary cultures. PMA: 1 µM phorbol 12-myristate 13-acetate was added at *time zero*; flux is shown as percentage of paired control unidirectional secretory flux at 90 min. PMA+ST: 1 µM staurosporine was added 0.5 h before initiation of flux measurement (t = -0.5 h), and 1 µM PMA was added at *time zero*. ST: 1 µM staurosporine was added at t = -0.5 h. Values are mean ± SEM, n = 4 preparations. [a]Significantly different from control net flux; [b]significantly different from PMA alone ($P < 0.05$).

The protein kinase C (PKC) activator, phorbol 12-myristate 13-acetate (PMA) at 1 µM inhibited net sulfate secretion 65% (Figure 1). Staurosporine (ST), a PKC inhibitor, partially prevented the PMA effect and had no effect on sulfate secretion itself. Neither transepithelial electrical resistance nor sodium-dependent glucose transport were significantly altered by the treatments. The data indicate that the primary route of sulfate elimination in teleosts may be subject to both short-term as well as long-term regulatory control. Previously we found that sulfate secretion in this tissue is carbonic anhydrase (CA) dependent (Renfro, J.L. et al. *Bull MDIBL* 37:65-66, 1998). The present data raise the question of whether CA activity is part of the regulatory control of transepithelial sulfate transport.

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