Feeding and PKC activation inhibit sulfate secretion by the intestine of *Pleuronectes americanus*

Ryan M. Pelis and J. Larry Renfro

Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269

Sulfate transport by the marine teleost intestine consists of secretory and absorptive fluxes, and feeding inhibits net active secretion by stimulating the absorptive flux [2]. The purpose of the current study was to determine whether the stimulated absorptive flux is mediated and the second messenger pathway involved in the feeding effect.

Paired Ussing chambers were used to measure unidirectional fluxes of radioactive sulfate across the winter flounder intestine under short-circuited conditions. Sulfate was actively secreted by the intestine of unfed animals at a net rate of 33 ± 4.7 nmoles x cm^{-2} x hr^{-1} (Table 1). Food intake prior to flux measurements reduced net secretion to 4 ± 6.8 nmoles x cm^{-2} x hr^{-1} by increasing the absorptive flux 440%. Feeding did not alter the secretory flux. Feeding lowered the transepithelial potential difference (TPD) 76%, without effecting transepithelial resistance (TER). Addition of 5 mM thiosulfate, a competitive inhibitor of sulfate transporters, to the interstitial and luminal bath fluids of intestines from fed and unfed animals reduced the secretory flux (40-60%) but had no effect on the absorptive flux (Table1). Furthermore, net active secretion was not different from zero when thiosulfate was present. These data suggest that the absorptive flux is non-mediated and may occur through the paracellular pathway.

Table 1. Effects of feeding and thiosulfate on the secretory, absorptive, and net sulfate fluxes by winter flounder intestine.

		Secretory	Absorptive	Net
Treatment	n	nmoles x cm^{-2} x hr^{-1}		
Unfed	9	39 ± 4.8	5 ± 0.4	33 ± 4.7
Fed	4	32 ± 5.4	27 ± 6.5*	4 ± 6.8*
Unfed	3	39 ± 6.5	4 ± 1.1	34 ± 6.6
Unfed + thiosulfate	3	13 ± 5.1*	6 ± 2.1	6 ± 7.1*
Fed	3	46 ± 5.4	25 ± 6.5	20 ± 8.4
Fed + thiosulfate	3	28 ± 2.7*	22 ± 5.5	6 ± 6.3

* Significantly different from control, P< 0.05 (paired or unpaired t-test).

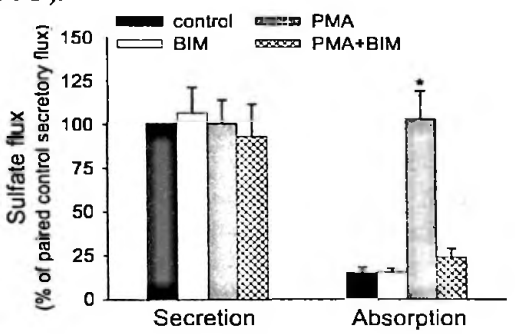

Figure 1. Effects of PMA (1 μM), BIM (1 μM), or both in combination (PMA+BIM) on the unidirectional secretory and absorptive fluxes of sulfate by the intestine of unfed winter flounder (n=5). * Significantly different from control, P< 0.05 (paired t-test).

PKC increases the paracellular permeability of numerous epithelial tissues by modifying tight junctions [1]. Therefore, we tested the effects of phorbol 12-myristate 13-acetate (PMA, 1 μM), a PKC activator, and bisindoylamaleimide (BIM, 1 μM), a PKC inhibitor, on sulfate transport by the intestine of unfed animals. PMA treatment completely abolished net secretion by stimulating the absorptive flux 430%, and pre-treating the tissues with BIM (PMA+BIM) blocked this effect (Figure 1). PMA had no effect on the secretory flux or TER but reduced TPD 63%. The effect of PMA on the secretory flux, absorptive flux, TER, and TPD was similar to feeding suggesting that PKC may be responsible for the reduction in net active sulfate secretion following feeding. Supported by NSF-IBN0078093.

1. Clarke H, Marano CW, Soler AP, and Mullin JM. Modification of tight junction function by protein kinase C isoforms. *Adv Drug Del Rev* 41: 283-301, 2000.
2. Pelis RM and Renfro JL. Active sulfate secretion by the intestine of winter flounder is through exchange for luminal chloride. *Am J Physiol Regul Integr Comp Physiol* 284: R380-R388, 2003.