pH-dependent sulfate secretion by the renal proximal tubule of *Pleuronectes americanus*

Ryan M. Pelis[1], Susan Edwards[2], James B. Claiborne[3], and J. Larry Renfro[1]

[1]Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT, 06269
[2]Department of Physiology and Pharmacology, James Cook University, Cairns, Australia
[3]Department of Biology, Georgia Southern University, Statesboro, GA 30460

Ingestion of sulfate-rich seawater (25 mM) by marine teleosts produces a sulfate-burden that is eliminated by renal proximal tubular secretion. Anion exchange mechanisms at both poles of proximal tubule cells facilitate sulfate secretion. Membrane vesicle experiments have demonstrated that sulfate translocation across the basolateral membrane (BLM) is best explained by a SO_4^{2-}/OH^- exchange mechanism[1]. The purpose of the current study was to determine if changes in interstitial pH influence sulfate secretion by confluent monolayers of renal proximal tubule epithelium from winter flounder (fPTCs).

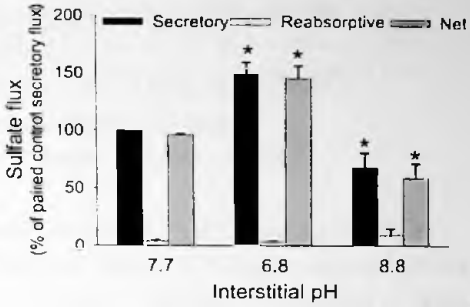

Figure 1. Effect of changing interstitial pH to 6.8 or 8.8 on the unidirectional secretory, reabsorptive, and net sulfate fluxes by fPTCs (n=5). The interstitial bath solution of controls had a pH of 7.7. *Significantly different from paired control, $P < 0.05$ (paired t-test).

Paired Ussing chambers were used to measure unidirectional fluxes of radioactive sulfate across fPTCs under open-circuited conditions. The luminal bath solution always contained flounder saline of pH 7.7 while the interstitial bath solution contained saline of pH 7.7 (control), 6.8, or 8.8. The pH of the flounder saline was adjusted with HCl or NaOH, and NaCl was added to the pH 7.7 saline to balance osmolality. Under control conditions net sulfate secretion was 84 ± 7.4 nmoles x cm^{-2} x hr^{-1}. Acidification of the interstitium (pH 6.8) stimulated net secretion 45% while alkalinization inhibited 42% (Figure 1). Changing interstitial pH only affected the unidirectional secretory flux. These data indicate that tubular sulfate secretion is modulated by interstitial pH.

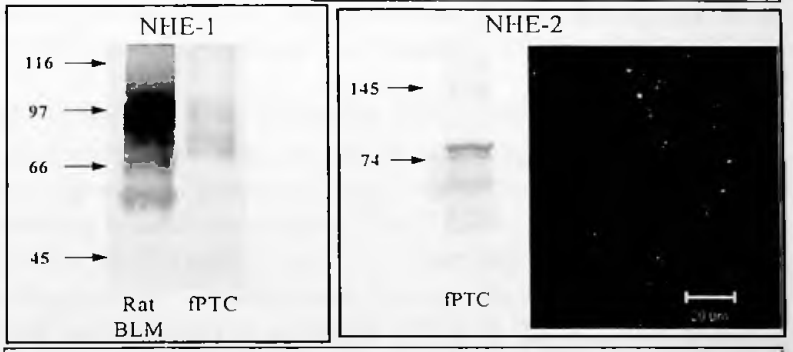

Figure 2. Immunoblot of NHE-1 from rat basolateral membranes (positive control) and fPTCs (left panel), and immunoblot (fPTCs) and immunolocalization (intact tubules) of NHE-2 (right panel). Position of molecular weight markers is shown (arrows). Primary antibodies included a monoclonal against porcine NHE-1 and a polyclonal (rabbit) against shark NHE-2. Approximate molecular weights of NHE-1 and NHE-2 are 90 and 85 kDa, respectively.

The implication of the BLM pH gradient in sulfate secretion prompted an initial characterization of the Na^+/H^+ exchangers in the proximal tubule of winter flounder. Both Na^+/H^+ exchanger isoforms 1 (NHE-1) and 2 (NHE-2) were identified in fPTCs by immunoblotting (Figure 2). NHE-2 was immunolocalized to intact flounder proximal tubules and demonstrated an apical and sub-apical distribution (Figure 2). Future work will be directed at determining if Na^+/H^+ exchange activity is required for maximum tubular sulfate secretion. Supported by NSF IBN-0078093 to JL Renfro, NSF IBN-0111073 to JB Claiborne, and a School of Biomedical Sciences, JCU grant to S Edwards.

1. Renfro JL and Pritchard JB. H^+-dependent sulfate secretion in the marine teleost renal tubule. *Am J Physiol* 243: F150-F159, 1982.