VANADATE INHIBITION OF ORGANIC ANION SECRETION IN KILLIFISH, FUNDULUS HETEROCLITUS, RENAL PROXIMAL TUBULES

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The organic anion transport system in vertebrate renal proximal tubule mediates the transport from blood to urine of a large number of potentially toxic metabolic wastes, xenobiotics and xenobiotic metabolites. Membrane vesicle studies have suggested that secretion of organic anions is driven by indirect coupling to the Na-gradient at the basolateral membrane and PD-dependent facilitated diffusion at the luminal membrane (Pritchard and Miller, Physiol. Rev. 73:765, 1993). Indirect coupling of organic anion influx to Na has been demonstrated in many intact tissue preparations, but little is known about how organic anions move from cell to lumen in intact renal tubules. Last summer, work from this laboratory demonstrated that the transport of several fluorescent organic anions across the lumenal membrane of killifish proximal tubule was not reduced when cells were depolarized in high potassium medium (Miller et al., Am. J. Physiol., submitted). We concluded that a mechanism other than PD-driven facilitated diffusion was responsible for transport across the lumenal membrane and suggested that, in analogy to liver, an organic anion transporting ATPase might be involved. Here we report on initial experiments with vanadate, a potent inhibitor of p-type ATPases.

Renal tubular masses were isolated in a marine teleost saline (MTS; containing, in mM:140 NaCl, 2.5 KCl, 1.5 $CaCl_2$, 1.0 $MgCl_2$ and 20 tris(hydroxymethyl)-amino methane, at pH 8.25). Under a dissecting microscope masses were teased with forceps to remove adherent hematopoietic tissue. Individual killifish proximal tubules were dissected free of the masses and transferred to a foil-covered Teflon chamber (Bionique) containing 1 ml of MTS with 1 μM fluorescein (FL), a fluorescent substrate for the organic anion transport system. The chamber floor was a 4x4 cm glass cover slip to which the tubules adhered lightly and through which the tissue could be viewed by means of an inverted microscope equipped with epi-fluorescence optics and a video camera connected to a Macintosh computer (Miller et al., Am. J. Physiol. 264:R882, 1994).

Previous imaging studies with killifish tubules have shown rapid uptake and secretion of FL into the tubular lumen; steady state is attained within 15 min (Miller et al., Am. J. Physiol. 264:R882, 1994). At steady state, lumenal fluorescence averages 2-3 times cellular fluorescence. Figure 1 shows that addition of vanadate to the incubation medium caused a concentration dependent increase in both cellular and lumenal fluorescence. However, with increasing vanadate concentration, cellular fluorescence increased more rapidly than lumenal fluorescence, so the lumen to cell fluorescence ratio for FL fell from a control value of 3.0 ± 0.4 to 1.1 ± 0.1 with 10 μM vanadate. Additional experiments revealed significant decreases in this ratio with vanadate concentrations as low as 0.5 μM, but with longer exposures (not shown). Time course studies showed that 5 μM vanadate increased cellular fluorescence within 10 min, but that the increase in lumenal fluorescence required 20-30 min of exposure.

Vanadate has been shown to inhibit renal Na,K-ATPase (Nechay, Ann. Rev. Pharmacol. Toxicol. 24:501, 1984). To determine whether the observed pattern of vanadate effects on FL transport could be explained by Na,K-ATPase inhibition, we examined the effects of ouabain, a specific inhibitor of Na,K-ATPase, on FL transport by killifish tubules. In contrast to vanadate, 1-100 μM ouabain caused a concentration dependent decrease in lumenal and cellular fluorescence (not shown). With 100 μM ouabain, a concentration that completely inhibits teleost renal Na,K-ATPase (Miller, J. Pharmacol. Exp. Therap. 219:428, 1981), FL uptake into the cells and secretion into the lumen were abolished. This is the expected result, since Na,K-ATPase inhibition

should collapse the Na gradient that drives organic anion uptake by the cells, which should in turn inhibit transport into the lumen.

The results of the present study show that low concentrations of vanadate have multiple effects on organic anion transport by killifish renal proximal tubules as measured by the distribution of a fluorescent substrate, FL; these effects are not related to Na,K-ATPase inhibition. Vanadate significantly increased both cellular and lumenal fluorescence. It is important to note that with the present nonconfocal optics lumenal fluorescence is expected to increase when cellular fluorescence increases, since at least a portion of the fluorescence measured over the lumen is contributed by the cells above and below. The lumen to cell fluorescence ratio allows us to semiquantitatively correct lumenal values for changes in cellular fluorescence. It is significant that this ratio decreased even though fluorescence in both compartments increased. The fluorescence ratio data suggest that vanadate inhibited transport of FL from cell to lumen. The observed increase in cellular FL could be secondary to inhibition of lumenal exit or it might indicate a separate effect on the basolateral transport mechanism, e.g., inhibited efflux. Supported in part by a fellowship from Burroughs-Welcome and NSF REU 9322221.

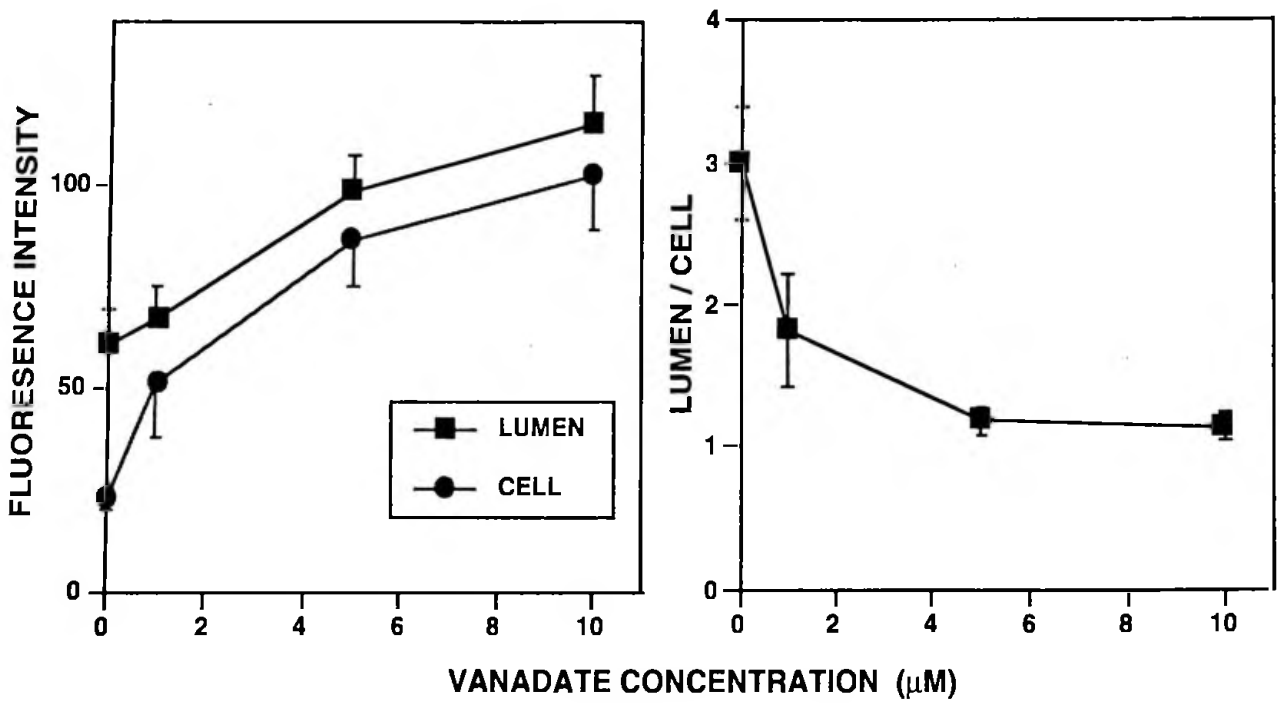

Figure 1. Effects of vanadate on fluorescein (FL) transport by killifish renal proximal tubules. Tissue was incubated in medium with 1 μM FL and the indicated concentration of vanadate for 30 min. Data given as mean ± SE for 24-32 tubules. All concentrations of vanadate significantly increased cellular fluorescence and significantly decreased the lumen to cell fluorescence ratio ($P<0.05$ for 1 μM vanadate and $P<0.01$ for 5-10 μM); 5 and 10 μM vanadate significantly increased lumenal fluorescence, $P<0.01$.