NOCODAZOLE INHIBITION OF ORGANIC ANION SECRETION IN TELEOST (WINTER FLOUNDER, PSEUDOPLEURONECTES AMERICANUS AND KILLIFISH, FUNDULUS HETEROCLITUS) RENAL PROXIMAL TUBULES

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During secretion in the proximal tubule, organic anions must cross the basolateral membranes, the interiors and the luminal membranes of the cells that constitute the tubular epithelium. Studies with intact renal tissue and isolated membrane vesicles have defined the mechanisms responsible for transport across the basolateral and luminal membranes (Pritchard and Miller, Physiol. Rev. 73:765-796, 1993). In contrast, little is known about how organic anions transit the cell interior. In the simplest case, transport could be driven by diffusion through the cytoplasm. However, recent studies from this laboratory suggest other mechanisms. We found that the model organic anion, fluorescein (FL), is compartmentalized within the cells of a variety of organic anion-secreting renal epithelia, including, crab urinary bladder, opossum kidney cell monolayers and teleost and mammalian proximal tubules (Miller et al., Am. J. Physiol. 264:R882-890, 1993; Am. J. Physiol., in press). Intracellularly, FL is distributed over two compartments: one diffuse and cytoplasmic, and the other vesicular. Confocal microscopic analysis of crab urinary bladder, showed that FL-loaded vesicles transit the cytoplasm in the secretory direction and that nocodazole, a drug that specifically disrupts microtubules, both slows the movement of FL-loaded vesicles and inhibits organic anion secretion across the epithelium (Miller et al., Am. J. Physiol., in press). Together, these data suggest that both solute sequestration in vesicles and microtubule-dependent vesicle transport contribute to the flux of organic anions across the cell.

Here we used tissue from 2 teleost fish to examine in isolated proximal tubules the effects of nocodazole on organic anion secretion. Tubular masses from winter flounder and killifish were isolated in a marine teleost saline (containing, in mM:140 NaCl, 2.5 KCl, 1.5 $CaCl_2$, 1.0 $MgCl_2$ and 20 TRIS, at pH 8.25). Uptake of 10 μM ^{3}H-p-aminohippurate (PAH) by flounder masses was measured as described previously (Miller, J. Phamacol. Exp. Therap. 219:428-434, 1981), uptake of 10 μM FL by fluorimetry of tissue extracts. FL uptake and distribution in single killifish proximal tubules were determined using an inverted epi-fluorescence microscope equipped with a video camera and a Macintosh computer (Miller et al., Am. J. Physiol. 264:R882-890, 1993).

Initial experiments indicated that exposing flounder tubular masses to 20 μM nocodazole for 30-60 min reduced steady state accumulations of PAH and FL by 25-30% (not shown). The effects of this drug were abolished when treated tubules were incubated in nocodazole-free medium for an additional 30 min. To determine how nocodazole affected organic anion levels in the lumenal and cellular compartments of the tubules, we followed FL accumulation in single isolated killifish proximal tubules using epi-fluorescence video microscopy and digital image analysis. Control tubules exhibited rapid uptake of dye in both compartments. Indeed, lumen-to-cell fluorescence ratios (L/C) >1 were seen after only a few min, indicating rapid dye secretion into the tubular lumen. As shown in Fig. 1 nocodazole had a profound effect on lumenal accumulation of FL and a smaller but significant effect on cellular accumulation. For example, at the highest concentration of drug used, no uphill secretion of FL into the tubular lumen was observed (L/C, had fallen to unity), but cellular fluorescence was only reduced about 23% (Fig. 1). Similar results were also obtained with colchicine, another drug that disrupts microtubules (not shown).

The effects of nocodazole on killifish proximal tubules were reversible. Thirty min exposure of tubules to 11 μM nocodazole reduced the steady state L/C for FL from 2.8 ± 0.2 (n=8

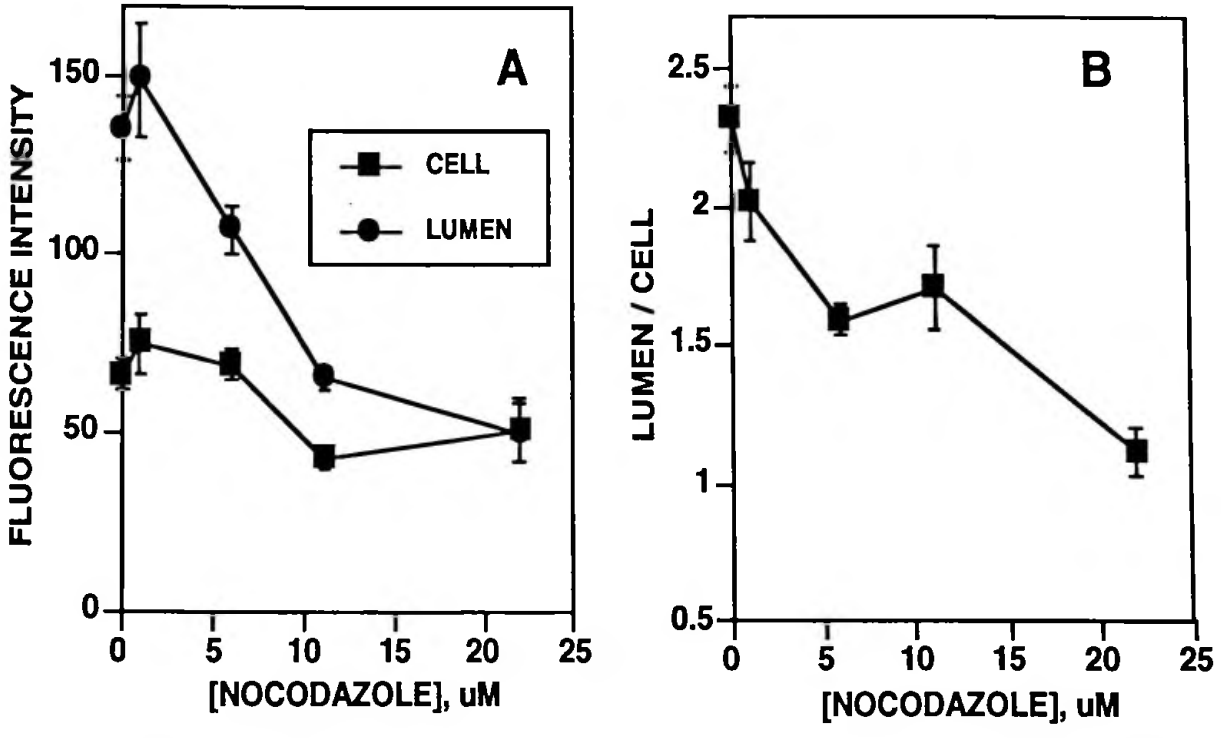

Figure 1. Effects of nocodazole exposure on the uptake and distribution of FL in killifish proximal tubules. Tubules were incubated in medium with 1 μM FL and the indicated concentration of drug for 30-60 min and the regional fluorescence measured (arbitrary fluorescence units; A). As a measure of secretory capacity, paired lumen/cell fluorescence ratios were also calculated (B). Each point represents the mean value for 15-55 tubules from 6 fish; variability is given by SEM bars.

tubules) in controls to 1.7 ± 0.2 in treated tubules (n=9; P<0.01 vs controls). When treated tubules were washed for an additional 30 min in drug-free medium, L/C rose to 3.1 ± 0.4 (n=5; not significantly different from controls). In contrast, tubules incubated 60 min with drug had an L/C of 1.6 ± 0.1 (n=7). A similar reversal was seen when microtubular integrity was monitored using a monoclonal anti-α-tubulin antibody and a fluorescein labeled anti-IgG (both from Amersham). Confocal micrographs of control killifish tubules showed that epithelial cells contained fine, fluorescent filaments, indicating well developed microtubular networks. Exposing the tubules to 11 μM nocodazole completely destroyed these stained filaments and incubating nocodazole-treated tubules in drug-free medium restored them (micrographs not shown).

We show here that disruption of the renal proximal tubule cell cytoskeleton is accompanied by a substantial reduction in organic anion secretion into the lumen. Unpublished observations from this laboratory indicate that the decrease in secretion is not due to nocodazole inhibition of transport at the basolateral or luminal membranes: 1) confocal micrographs of crab bladder and killifish tubule show that the nocodazole decreases cellular FL by disrupting compartmentation rather than reducing cytoplasmic dye levels, 2) nocodazole has no effects on initial rates of FL uptake by flounder tubules, and 3) nocodazole does not inhibit PAH transport in basolateral or lumenal membrane vesicles from rat renal cortex. Together, these findings provide additional support for a role of microtubules in organic anion secretion. Supported in part by the Maine Science and Technology EPSCoR program (J.C.) and the Hearst Foundation (N.B.).