ORGANIC ANION SECRETION IS NOT SENSITIVE TO MEMBRANE POTENTIAL IN ISOLATED KILLIFISH (FUNDULUS HETEROCLITUS) 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 shown 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 several intact tissue

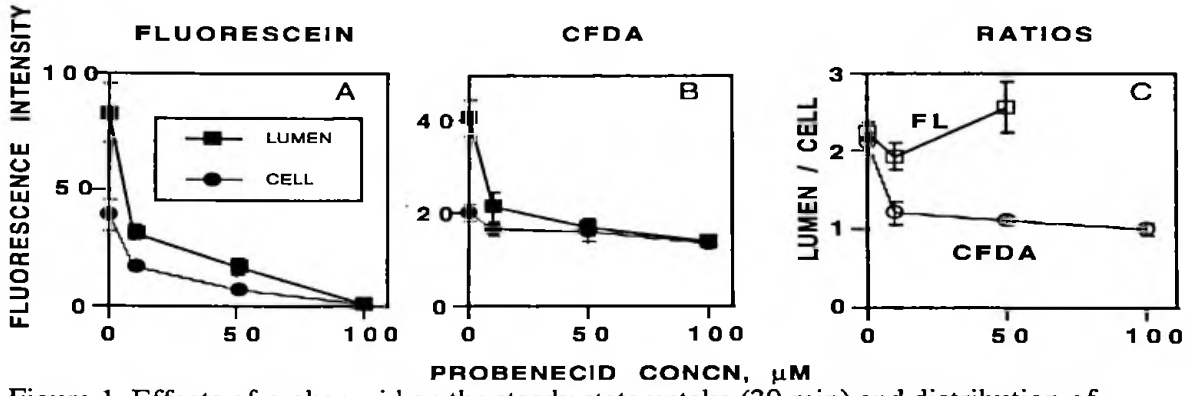

Figure 1. Effects of probenecid on the steady state uptake (30 min) and distribution of fluorescein (FL; A) and carboxyfluorescein diacetate (CFDA; B). Tubules were incubated in medium with 1 μM substrate and the indicated concentration of probenecid and regional fluorescence measured (arbitrary units). Paired cell/lumen fluorescence intensities are given in (C). Each point represents the mean value for 6-10 tubules; variability is given by SEM bars.

preparations, but the role of the proposed lumenal transporter has not been similarly evaluated. We do so here using epifluorescence microscopy and digital image analysis to measure the transport of fluorescent organic anions in individual killifish proximal tubules.

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 fluorescent substrate and added effectors. 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 and Macintosh computer (Miller et al., Am. J. Physiol. 264:R882, 1994). Three substrates were used: 1) fluorescein (FL), a fluorescent substrate for the renal organic anion system (Miller and Pritchard, Am. J. Physiol. 267:R695, 1994), 2) carboxyfluorescein diacetate (CFDA), a non-fluorescent ester that is hydrolyzed intracellularly to a fluorescent organic anion (carboxyfluorescein, CF), and 3) monochlorobimane

(MCB), a nonfluorescent electrophile that forms a fluorescent glutathione conjugate intracellularly.

Figure 1 shows the patterns of transport for two of the substrates. In both cases, control tubules (no probenecid) exhibited lumen-to-cell fluorescence ratios of 2 or greater. With FL as substrate, the organic anion, probenecid, caused a concentration dependent decline in both cellular and lumenal fluorescence. The lumen-to-cell fluorescence ratio for FL was not affected by probenecid. With CFDA as substrate, probenecid caused cellular fluorescence to decrease slightly and lumenal fluorescence to fall dramatically. The lumen-to-cell fluorescence ratio for CF in probenecid-treated tubules fell to unity (Fig. 1). With MCB as substrate, probenecid greatly reduced lumenal fluorescence, but had at most a small effect on cellular fluorescence (data not shown). Finally, with MCB as inhibitor and FL or CFDA as substrate, lumenal fluorescence was greatly reduced, but cellular fluorescence was only slightly affected (not shown). These data indicate two patterns of transport for the substrates studied. For FL, mediated, i.e., inhibitable, transport was found at both the basolateral and lumenal membranes of the tubule cells. For CFDA and MCB, both of which are metabolized to fluorescent organic anions, mediated transport was only found at the lumenal membrane; the small reduction in cellular accumulation appeared to be related to altered compartmentalization rather than inhibited basolateral uptake (not shown).

Smith et al. (Am .J. Physiol. 255:R492, 1988) have shown that raising MTS K from 2.5 mM

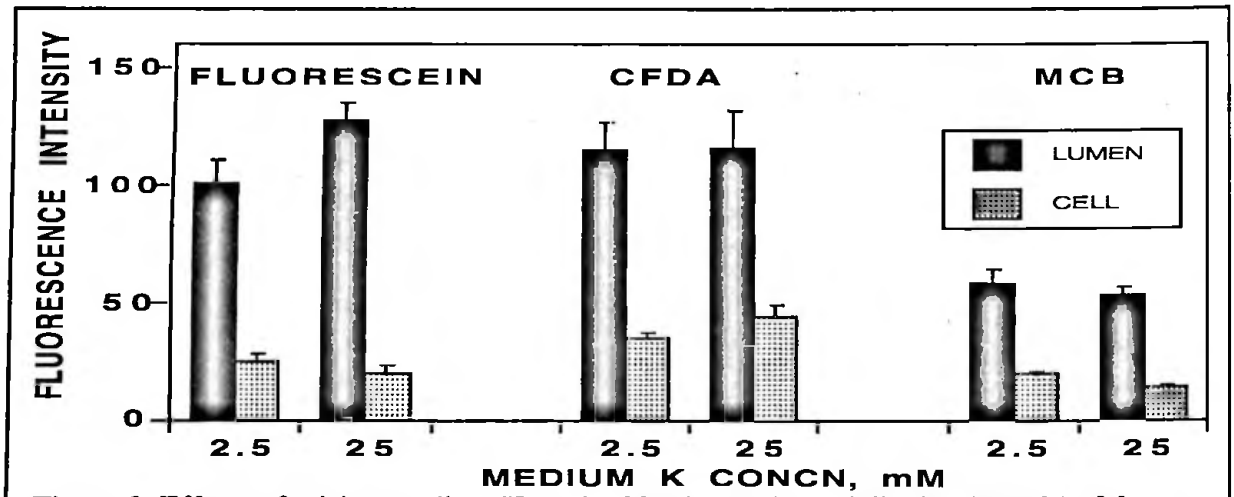

Figure 2. Effects of raising medium K on the 30 min uptake and distribution of 1 μM fluorescein, 1 μM carboxyfluorescein diacetate (CFDA) and 5 μM monochlorobimane (MCB). Tubules were incubated in medium with substrate and the indicated concentration of K and regional fluorescence measured (arbitrary units). Each point represents the mean value for 6-10 tubules; variability is given by SEM bars.

to 25 mM (isoosmotic replacement of NaCl with KCl) depolarizes killifish proximal tubule cell membrane potential (PD) from -66 mV to -34 mV and substantially reduces the influx of tetraethylammonium (TEA) through the membrane organic cation transporter. When we raised MTS K and measured the uptake and distribution of FL, CFDA and MCB, we found no effects of depolarization (Fig. 2). In addition, experiments with the K-channel blocker, Ba, also showed no effects. In contrast, raising medium K reduced the uptake of the organic base, daunomycin (data not shown), in agreement with the TEA data of Smith et al. (op. cit). These findings indicate that the transport of two organic anions from cell to tubular lumen is unaffected by membrane depolarization. This result is not consistent with facilitated diffusion playing a major role in organic anion transport from cell to tubular lumen. It suggests the involvement of other processes, e.g., different carriers or vesicular transport. Supported in part by the Hancock County Scholars Fund (SL) and the Blum/Halsey Scholar Fund (DSM).