Effects of phenolic acids on organic anion transport in killifish, *Fundulus heteroclitus*, renal proximal tubules

Natascha A. Wolff[1] and David S. Miller[2]
[1]Institute of Systems Physiology, University of Goettingen, 37073 Goettingen, Germany
[2]Laboratory of Pharmacology and Chemistry, NIH/NIEHS, Research Triangle Park, NC 27709

One function of vertebrate renal proximal tubules is the elimination of potentially toxic small organic anions (OA), including drugs, dietary components and waste products of metabolism. This is accomplished through potent, active secretory transport that involves two concentrative steps: tertiary active uptake at the basolateral membrane of the tubular epithelial cells and efflux into the lumen by an as yet undefined process. The first, rate-determining step in transport (uptake) is mediated by at least one member of the organic anion transporter (OAT) subfamily. In teleosts, basolateral OA uptake is likely to be mediated by only one OAT, possibly representing a common ancestral protein of mammalian OAT1 and OAT3 (Aslamkhan, A. G. et al. The flounder organic anion transporter (fOat): an ancestral ortholog of mammalian OAT1 and OAT3? Manuscript in preparation).

Animals are continuously exposed to OAT substrates, but it is not clear to what extent such exposure can alter renal tubular OA transport. Candidate substrates of plant origin ingested by prey species are various phenolic acids, either found as such in marine algae (caffeic acid, cinnamic acid)[2,3], or derived by degradation from other phenolic compounds, such as gallic acid from the brown algal phlorotannins[1]. Initial experiments had shown that cinnamic acids as well as gallate potently inhibited *p*-aminohippurate transport by the cloned OAT from flounder (fOAT)[4] when expressed in *Xenopus laevis* oocytes. Moreover, 4-hydroxycinnamic acid was found to be an fOAT substrate. Here we report results of preliminary experiments designed to determine the effects of gallate and cinnamate exposure on the transport of fluorescein (FL), a fluorescent OA, by isolated killifish renal tubules.

Renal proximal tubules were isolated from control killifish or from killifish exposed to phenolic acids in seawater. Isolated tubules were transferred to teflon chambers containing teleost Ringer solution (in mM: 140 NaCl, 2.5 KCl, 1.5 $CaCl_2$, 1.0 $MgCl_2$, 20 Tris, pH 8.0) with 1 μM FL. In some experiments with tubules from control fish the medium also contained phenolic acids. After reaching steady-state (1 h), cellular and luminal fluorescence levels were measured using confocal microscopy (Zeiss) and quantitative image analysis (ImageJ 1.32, NIH).

Steady state FL accumulation in tubules from untreated fish was significantly altered by addition of 10 μM gallate or cinnamate to the medium. With 10 μM gallate, cellular accumulation decreased by 17 ± 7% and luminal accumulation decreased by 33 ± 10% relative to gallate-free controls (mean ± SE, n=3 animals). With 10 μM cinnamate, only luminal accumulation decreased significantly (35 ± 13%, n=3 fish). Thus, when applied directly to isolated tubules, 10 μM gallate affected at least the basolateral step in FL transport, while cinnamate appeared to affect predominantly the luminal step.

When killifish were exposed to 10 μM gallate for 30 h and FL transport measured in isolated tubules, cellular accumulation was at control levels, but luminal accumulation was increased (32 ± 7%, n=3 dosed and control fish each). Exposure of fish to 10 or 100 μM cinnamate for 24 h had variable effects; these also suggested increased transepithelial transport. Thus, for gallate, we saw a consistent picture. In vitro exposure inhibited FL transport, suggesting interaction with at least the basolateral OAT. In vivo exposure appeared to increase transepithelial transport, but only at the luminal step. Further studies are clearly needed to evaluate the effects on the individual carriers involved. Supported in part by an MDIBL New Investigator Award to Natascha A. Wolff.

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