ENDOTHELINS REGULATE MRP2-MEDIATED TRANSPORT IN KILLIFISH (*FUNDULUS HETEROCLITUS*) RENAL PROXIMAL TUBULES

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Renal secretion of anionic xenobiotics is a multistep proces, involving uptake into cells, intracellular distribution and concentrative transport into the urinary space. Two transport systems which deal specifically with the extrusion of anionic drugs have been identified at the molecular level: the organic anion transporter (rOat1) and a multidrug resistance-associated protein (Mrp) analog, probably Mrp2. The first, Na-dependent transport system has been characterized in detail and most of our present knowledge of this system comes from studies with p-aminohippurate and fluorescein as model substrates (Pritchard and Miller, *Kidney Int.* 49:1649-1654, 1996). The Mrp analog handles larger anionic xenobiotics, mainly as their conjugation products with glutathione, glucuronide or sulfate. This transporter belongs to a new branch of the ATP-binding cassette (ABC) superfamily, and it drives primary-active transport, an ATP-dependent process. Fluorescein-methotrexate (FL-MTX), a fluorescent organic anion with a molecular weight of 923, is a model substrate for this system (Masereeuw et al., *Am. J. Physiol.* 271: F1173-F1182, 1996).

Previous studies showed that, as with rOat1-mediated transport (Miller, *Am. J. Physiol.* 274: F156-F164, 1998), the excretion of FL-MTX in killifish renal proximal tubules is regulated by protein kinase C (PKC; Masereeuw et al., *Bull. MDIBL*, 37: 93-94, 1998). In both cases, transport is negatively correlated with PKC activity. Clearly, the hormone(s) initiating this process needed to be defined. Of hormones tested, only dopamine at supraphysiological (micromolar) concentrations, and endothelin-1 (ET-1) at subnanomolar to nanomolar concentrations affected FL-MTX transport. A nonselective endothelin receptor antagonist, PD145065, prevented the ET-1 effect (Masereeuw et al., *Bull. MDIBL*, 37: 93-94, 1998). The present study is concerned with the specificity and selectivity of endothelins in regulating renal FL-MTX transport. Proximal tubules were isolated from killifish renal masses and 1 μM of the fluorescent dye uptake into cells and secretion into the tubular lumen were measured using confocal microscopy and image analysis, as described previously (Masereeuw et al., *Am. J. Physiol.* 271:F1173-F1182, 1996).

To determine whether Mrp2 is a candidate for the Mrp analog in killifish proximal tubules, we visualized the transporter by indirect immunolocalization using rabbit polyclonal antibodies (k78mrp2; Van Aubel et al., *Mol. Pharmacol.* 53: 1062-1067, 1998). A confocal micrograph of immunostained tubules clearly showed specific membrane staining (Fig. 1A). A cross-section generated from a stack of images showed that this staining was predominantly associated with the apical membrane (Fig. 1B), as has been previously shown for mammalian proximal tubules.

To further investigate the role of ET-1 in transport of FL-MTX, tubules were exposed to 10 nM of the hormone and uptake and secretion were analyzed. Cellular fluorescence remained similar to control values, however, luminal fluorescence was 50% lower than controls ($P<0.01$) from 5 min until steady-state (15 min). These results indicate that ET-1 affects FL-MTX secretion potently and rapidly. Other endothelins, ET-2 and ET-3, possessed a similar potency to ET-1, suggesting that the B-receptor subtype is involved.

Consistent with this, suggestion, a selective ET_B receptor antagonist prevented the ET-mediated inhibitory effects on FL-MTX secretion, whereas an ET_A receptor antagonist did not. Immunostaining with an antibody to the mammalian ET_B receptor showed specific localization to the basolateral membrane of the killifish proximal tubules. Finally, the inhibitory effects of the

ETs were reversed by concommittant administration of PKC inhibitors (staurosporine, calphostin C), suggesting that ETs mediate their effect through PKC.

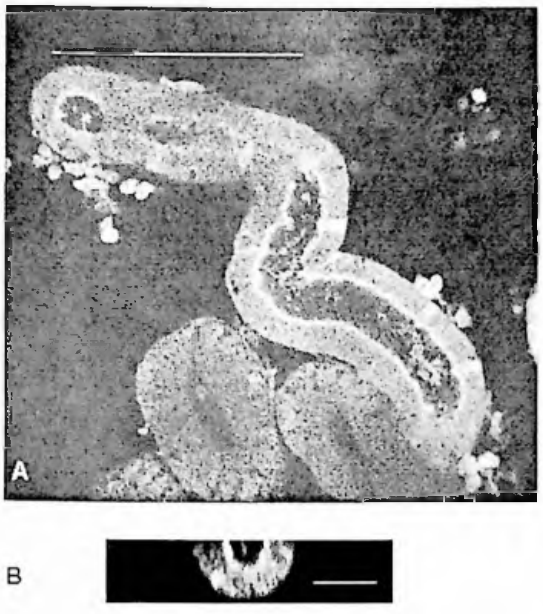

Figure 1. Confocal image of killifish tubules immunostained with rabbit anti-Mrp2 antibody and a fluorescent secondary antibody (A). Optical slice taken in a perpendicular plane showing the apical localization (B). White bar is 100 μm (A) or 25 μm (B).

In summary, we demonstrate here the presence of Mrp2 in the apical membranes of killifish renal proximal tubules. Furthermore, we provide evidence for the regulation of this transporting protein by the vasoactive hormone ET, mediated by the B-receptor subtype through activation of PKC. The basolateral localization of this receptor sub-type in proximal tubule cells is consistent with the rapid action of hormone added to the bath. This research was supported by a grant from the Netherlands Organization for Scientific Research (NWO) to RM.