REGULATION OF Na-INDEPENDENT ORGANIC ANION TRANSPORT IN RENAL PROXIMAL TUBULES OF KILIFISH (*Fundulus heteroclitus*)

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The renal proximal tubule is responsible for the excretion of a wide variety of potentially toxic drugs, xenobiotics, and metabolites. Two transport systems which deal specifically with organic anions have been identified. The classical, sodium-dependent and ouabain-sensitive system handles smaller organic anions, such as the model substrates p-aminohippurate (PAH) and fluorescein (FL; Pritchard and Miller, *Kidney Int.* 49:1649-1654, 1996). A recently discovered sodium-independent and ouabain-insensitive system handles larger organic anions (Mw. 600-1000; Masereeuw et al., *Am. J. Physiol.* 271: F1173-F1182, 1996). The basolateral uptake step in this system, is not completely understood. However, excretion into the lumen is probably via an organic anion transporting ATPase, an analog of the canalicular multispecific anion transporter (cMoat or Mrp-2) found in liver. Fluorescein-methotrexate (FL-MTX), a fluorescent probe with a molecular weight of 923, is a model substrate for this system.

Regulation of the Na-dependent organic anion system by protein kinase C (PKC) has recently been demonstrated (Halpin, P. A. and J. L. Renfro, *Am. J. Physiol.* 271:R1372- R1379, 1996.; Takano, M., et al., *Am J Physiol.* 271:F469-F475, 1996; Miller, D.S., *Am. J. Physiol.*, in press). Diacylglycerol and the phorbol ester, PMA, both of which activate PKC, reduce organic anion transport. Staurosporine, a protein kinase inhibitor, blocks these effects. In the present study, we examined effects of PKC modifiers on the Na-independent organic anion transport system. Proximal tubules were isolated from killifish renal masses and 1 μM FL-MTX 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).

Although the amount of FL-MTX transport varied from fish to fish, control tubules always displayed a characteristic distribution of fluorescence intensities. That is, lumens were much brighter than cells, which in turn were brighter than the medium. FL-MTX uptake and secretion were not affected by ouabain, but both were reduced by methotrexate; leukotriene C_4 blocked FL-MTX transport into the lumen. These results are consistent with previous studies in which the Na-independent system for organic anions was first characterized (Masereeuw et al., *Am. J. Physiol.* 271:F1173-F1182, 1996).

The same distribution of fluorescence intensities was found in tubules incubated with the phorbol ester PMA (1-100 nM); however, both cellular and lumenal fluorescence were markedly reduced relative to controls. At 100 nM PMA, lumenal and cellular fluorescence intensities were reduced by 40% ($P<0.01$). A similar effect was found with 100 nM dioctanoyl-*sn*-glycerol (DOG), a physiological activator of PKC. An inactive phorbol ester, 4α-phorbol

12,13-didecanoate, had no effect at 100 nM. If PMA and DOG inhibited FL-MTX transport by activating PKC, then a protein kinase inhibitor should block the PMA and DOG effect. Staurosporine, an inhibitor of protein kinases at nanomolar concentrations, had little effect, with 50 nM staurosporine only increasing lumenal fluorescence by 10% ($P<0.05$), but not affecting cellular fluorescence. Nevertheless, 50 nM staurosporine reversed the inhibitory effects of DOG and PMA.

Which hormones control Na-independent organic anion secretion through PKC? Halpin and Renfro (op. cit.) found that dopamine is a potent inhibitor of Na-dependent organic anion (PAH) transport. Dopamine at 1 μM reduced cellular and luminal accumulation of FL-MTX by 50% inhibition and staurosporine reversed this inhibition. Endothelin-1 (0.5-10 nM), which activates PKC in other renal systems (Nord, E.P., *Clin. Exp. Pharmacol. Physiol.* 23: 331-336, 1996), significantly reduced cellular and lumenal fluorescence. This suggests that endothelin-1 affects renal transport at physiological concentrations, in contrast to dopamine, which appears to act at supraphysiological (micromolar) concentrations. The endothelin-1 receptor antagonist ($ET_{A/B}$-nonselective), PD145065, prevented the inhibitory effect of the agonist ($P<0.01$). Furthermore, endothelin-1 and PMA were not additive in their effects on FL-MTX transport, suggesting that both act via PKC. In contrast, parathyroid hormone, insulin, and glucagon had no effects on FL-MTX transport.

Thus, both proximal tubule organic anion transport systems appear to be controlled by PKC. Miller (*Am. J. Physiol.*, in press) has shown that PKC regulation of transport on the Na-dependent system occurs at the basolateral membrane; transport from cell to lumen was unaffected by PMA or staurosporine. All the data obtained with FL-MTX (present study) suggest that PKC also regulates the basolateral uptake step of the Na-independent system; it is not yet clear whether the luminal step is also affected. The present data also show that the hormones, endothelin-1 and dopamine, acting through PKC, reduced transport on the Na- independent system. Our preliminary data indicate that both hormones reduce transport on the Na-dependent system as well. Additional studies are needed to further characterize the signal transduction chains which lead from hormone receptors to affected transporters, e.g. PKC-isoforms, and to understand the physiological and pathological consequences of the regulation of renal xenobiotic transport. Supported by a travel grant of the Netherlands Organization for Scientific Research (NWO, RM), and by NSF DBI-9531348 (SGL).