INVOLVEMENT OF cGMP IN ENDOTHELIN-REGULATED DRUG TRANSPORT IN KILLIFISH (*FUNDULUS HETEROCLITUS*) RENAL PROXIMAL TUBULES

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In intact killifish renal proximal tubules, several nephrotoxicants induce release of endothelin (ET) which acts through an ET_B receptor, to stimulate nitric oxide synthase (NOS), NO release and protein kinase C (PKC). This chain of events signals a decrease in organic anion transport mediated by the multidrug resistance associated protein isoform2 (Mrp2; Masereeuw, M., et al., Mol. Pharmacol. 57:59-67, 2000; Terlouw, S., et al., Mol. Pharmacol. 59: 1433-1440, 2001; Notenboom, S., et al., Am. J. Physiol. 282:F458-64, 2002). Preliminary experiments have shown that cyclic GMP (cGMP) also affects transport on Mrp2, but it was not clear whether cGMP was acting as a messenger of NO signaling or directly interacting with the transporter (Notenboom S., et al, Bull. MDIBL 41: 35, 2002). In the present study, we examined this issue using pharmacological tools to dissect the mechanism of cGMP action. Briefly, isolated tubules were incubated in medium containing 1 μM fluorescein methotrexate (FL-MTX) without (control) or with cGMP analogs or drugs that affect specific steps in signaling. After 30 min, confocal micrographs of the tubules were acquired and analyzed to measure active accumulation of FL-MTX in tubular lumens, a measure of Mrp2 function (Masereeuw M., et al, op. cit.).

Exposing tubules to 0.1-1 μM 8-Br-cGMP, a cell permeable cGMP analog, decreased luminal FL-MTX accumulation by up to 70%. This effect was abolished by bisindolmaleide (BIM), a PKC inhibitor, but not by L-NMMA, an NOS inhibitor. Rp-8Br-cGMP, a cGMP analog that does not activate PKG, also reduced transport, but BIM did not reverse its effects. Oxadiazole quinoxalin (ODQ), an inhibitor of NO sensitive guanylyl cyclase, blocked the effects of ET-1, sodium nitroprusside (NO donor) and nephrotoxicants (gentamicin, amikacin, diatrizoate, $HgCl_2$ and $CdCl_2$) on Mrp2-mediated transport; ODQ did not attenuate the effects of PKC activation by phorbol ester.

These results are consistent with cGMP affecting Mrp2-mediated transport through signaling rather than through direct inhibition of the transporter. Furthermore, guanylyl cyclase appears to be involved in signaling by ET and the nephrotoxins. Apppearently the enzyme is activated by NO and it generates cGMP which in turn activateds PKC. The complete sequence of events induced by exposing tubules to nephrotoxicants includes: 1) transient opening of Ca channels, increasing intracellular Ca concentration and stimulating ET release, 2) binding of ET a basolateral ET_B-receptor, which activates NOS, and generates NO, 3) activation of a soluble guanylyl cyclase, which increases cGMP production and activates PKC, 4) activated PKC then rapidly reduces transport by Mrp2. Supported by the Dutch Kidney Foundation and the Center for Membrane Toxicity Studies (NIEHS P30-ES3828).