MEASUREMENT OF XENOBIOTIC INDUCED NITRIC OXIDE PRODUCTION IN KILLIFISH (*FUNDULUS HETEROCLITUS*) RENAL PROXIMAL TUBULES

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In killifish renal proximal tubules, two ATP-driven xenobiotic export pumps (Mrp2 and p-glycoprotein) are rapidly regulated by endothelin (ET) acting through an ET_B receptor, NO synthase (NOS) and protein kinase C (PKC; Masereeuw et al, Mol. Pharm. 57:59-67, 2000; Notenboom et al, Am. J. Physiol, 282:F458-464, 2002). Several nephrotoxicants, radiocontrast agents, aminoglycoside antibiotics and heavy metal salts, induce ET release from the tubules and "hijack" this signaling pathway (Terlouw et al, Mol. Pharm. 59:1433-1440, 2001; Notenboom et al, Am. J. Physiol, op. cit.; Terlouw et al, submitted). The participation of NOS and NO in this signaling pathway is of particular interest, because of their role in hypoxia and chemical nephrotoxicity (Kone, Am. J. Kidney Dis. 30:311-333, 1997). Here we demonstrate directly generation of NO by killifish tubules after exposure to ET-1 or nephrotoxicants.

NO production was measured using 4,5-diaminofluorescein (DAF), which increases fluorescence upon exposure to NO. For experiments, tubules were loaded for 1 h in medium containing 10 μM DAF-2 diacetate (Molecular Probes). This non-fluorescent derivative is membrane permeant and upon entering cells is hydrolyzed to DAF. After loading, tubules were transferred to confocal chambers containing medium without (control) and with effectors. After 5 min, confocal images of tubules were acquired and saved. In controls, average tubule fluorescence was low and constant, but it increased about 5-fold when tubules were exposed to the NO generator, sodium nitroprusside. Roughly the same magnitude of increase in fluorescence (5-8-fold) was also found for tubules exposed to the nephrotoxicants, diatrizoate, $CdCl_2$, amikacin and gentamicin, at concentrations that reduce transport mediated by Mrp2. Exposure to ET-1 also generated NO and its production was blocked by the NOS inhibitor, L-NMMA. Finally, NO production induced by gentamicin was blocked by the ET_B receptor antagonist, RES-701-1. Together, these results demonstrate that ET-1 and the nephrotoxicants rapidly stimulated NO production, most likely through activation of the ET_B receptor and NOS.

Additional experiments have shed light on signaling by NO. In many systems, NO signals through cyclicGMP and PKG. In killifish tubules, transport on Mrp2 was particularly sensitive to 8-bromo-cyclicGMP (50% reduction at 500 nM), whereas transport on the classical organic anion system was insensitive (no reduction at 10 μM). In initial experiments, KT-5823, a PKG-selective inhibitor, blocked in part the effects of 8-bromo-cyclicGMP on Mrp2-mediated transport, indicating NO signaling through cyclicGMP and PKG. However, we recently found that cyclic nucleotides compete for transport on Mrp2 in shark rectal gland tubules (Miller et al, Am. J. Physiol. 282:R774-781, 2002). Thus, it is also possible that in killifish renal tubules cyclicGMP could reduce Mrp2-mediated transport through competition; this possibility will be investigated in the near future. Supported by the Dutch Kidney Foundation and the MDIBL CMTS (ES 03828).