NEPHROTOXINS RELEASE ENDOTHELIN FROM KILLIFISH (*FUNDULUS HETEROCLITUS*) RENAL PROXIMAL TUBULES BY A CA-DEPENDENT MECHANISM

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Previous studies revealed a new role for endothelin (ET) in the kidney: regulation of ATP-driven drug export pumps in proximal tubule (Masereeuw et al., Mol. Pharmacol., *in press*). The data indicated that ET, acting through a basolateral B-type receptor and through protein kinase C (PKC), negatively regulated two luminal xenobiotic transporters, P- glycoprotein and the multidrug resistance-associated protein2 (Mrp2). These experiments further provided a pathophysiological context in which to view regulation of the drug transporting ATPases by ET. When proximal tubules were exposed to the nephrotoxic radiocontrast agent, iohexol, transport mediated by Mrp2 and P-glycoprotein was reduced. For both transporters, the iohexol-induced reduction was abolished when the tubules were pretreated with a selective ET B-type receptor antagonist (RES-701-1). These results indicate that activation of the ET-B receptor was an intermediate step in the sequence of events by which iohexol reduced transport through Mrp2 and P-glycoprotein. They suggest a way to monitor ET signaling in intact tubules: measuring reductions in Mrp2-mediated transport reversed by RES-701-1. Here we use this transport-based assay system to extend these findings to other nephrotoxic chemicals and to consider the mechanism that signals ET release.

Proximal tubules were isolated from killifish renal masses and 1 μM fluorescein-methotrexate (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). Previous results showed that FL-MTX, a fluorescent organic anion, is a model substrate for teleost Mrp2 (Masereeuw et al., Mol. Pharm. In press). Representatives of three classes of nephrotoxins, *viz.* the radiocontrast agents, diatrizoate and iohexol, the aminoglycoside antibiotics, amikacin and gentamicin, and the heavy metals, cadmium and mercury salts, reduced the transport of FL-MTX from cell to tubular lumen in a concentration-dependent manner with inhibitory constants ranging from 0.1 to 63 μM. For all six nephrotoxins, reductions in luminal fluorescence was abolished by exposing tubules to a PKC-selective inhibitor (bis-indol maleimide, BIM) or to the ET-B receptor anatagonist, RES-701-1; an ET-A receptor antagonist (JKC-301) was without effect. Neither BIM nor the ET receptor antagonists by themselves affected transport of FL-MTX. Finally, the nephrotoxins appeared to release ET, since pretreating tubules with an antibody to ET abolished their effects.

Since increased intracellular Ca causes ET release in capillaries (Rubanyi and Polokof, Pharmacol. Rev. 46:325-414, 1994), we determined whether Ca-based mechanisms could release ET in proximal tubules. A 2-fold elevation of medium calcium concentration reduced Fl-MTX secretion significantly (61%). This reduction was reversed completely by BIM, RES-701-1 and by a specific L-type Ca channel blocker, nifedipine. None of these drugs by themselves affected FL-MTX transport. Importantly, the inhibition of FL-MTX transport caused by each of the 6

nephrotoxins was also abolished when tubules were pretreated with nifedipine, suggesting that all chemicals tested released ET through a common, Ca-dependent mechanism.

We have demonstrated here for the first time a common mechanism in the early response of proximal tubule to three structurally unrelated groups of nephrotoxins. Exposure of teleost proximal tubules to these nephrotoxins induced an influx of calcium through L-type channels, which resulted in a release of ET; ET acting through an ET-B receptor and PKC, subsequently reduced Mrp2-mediated transport. Further research is necessary to determine whether the observed effects on signaling and transport are part of an integrated series of defense mechanisms or of the normal progression of cellular events that occur during renal injury.
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