Short-Term Exposure of Renal Proximal Tubules to Gentamicin Increases Long-Term Multidrug Resistance Protein 2 (Abcc2) Transport Function and Reduces Nephrotoxicant Sensitivity

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ABSTRACT

We previously showed that the function of renal multidrug resistance protein (Mrp) 2 (Abcc2) is reduced by endothelin (ET)-1 signaling through an ETa receptor, nitric-oxide synthase (NOS), cGMP, and protein kinase C and that this pathway was activated by several nephrotoxicants (Masereeuw et al., 2000; Terlouw et al., 2001; Notenboom et al., 2002, 2004). Here, we determined the long-term effects on Mrp2-mediated transport (luminal fluorescein methotrexate accumulation) of short-term (30 min) exposure to ET-1 and the aminoglycoside antibiotic, gentamicin. Our data show that over the 3 h following exposure, proximal tubules recovered fully from the initial decrease in Mrp2-mediated transport and that transport activity was not changed 9 h later. However, 24 h after exposure, luminal accumulation of an Mrp2 substrate had increased by 50%. Increased transport at 24 h was accompanied by an increased transporter protein content of the luminal plasma membrane as measured by immunostaining. Blocking ET-1 signaling at the ETB receptor or downstream at NOS or guanylyl cyclase abolished both stimulation of transport and increased transporter expression. Thus, regardless of whether signaling was initiated by a short exposure to ET-1 or to a nephrotoxicant, the time course of Mrp2 response to ETB signaling was the same and was multiphasic. Finally, when tubules were exposed to gentamicin for 30 min and removed to gentamicin-free medium for 24 h, they were less sensitive to acute gentamicin toxicity than paired controls not initially exposed to the drug. Thus, short-term exposure to ET-1 or gentamicin resulted in long-term protection against a second insult.

ATP-driven drug efflux pumps, e.g., P-glycoprotein and multidrug resistance protein (Mrp) 2, play an important role in the absorption, distribution, and excretion of endogenous compounds, drugs, and their metabolites (Russel et al., 2002; Schinkel and Jonker, 2003). In the vertebrate renal proximal tubule, Mrp2 has been localized to the luminal pole of the epithelial cells (Schaub et al., 1997), the correct location to provide the final step in transport of many anionic xenobiotics from blood to urine. The efflux pump handles a wide range of substrates, and its activity and expression level in the kidney is influenced by hormones, local mediators, nuclear receptors, protein kinases, and disease conditions (Terlouw et al., 2003). As with drug-metabolizing enzymes, e.g., cytochrome P450s and transferases (Rushmore and Kong, 2002; Korashy et al., 2004), pump activity is regulated in response to exposure to substrates and physiological state.

We previously showed in intact killifish renal proximal tubules that Mrp2 activity, as measured by luminal accumulation of a fluorescent methotrexate (MTX) derivative [fluorescein (FL)-MTX], is rapidly reduced by subnanomolar to nanomolar concentrations of ET-1 acting through an ETa receptor, NOS, cGMP, and PKC. Surprisingly, acute exposure of tubules to several nephrotoxicants, i.e., aminoglycoside antibiotics, radiocontrast agents, and heavy metal salts, also reduces FL-MTX transport, and blocking ET signaling at any point in the chain abolishes both stimulation of transport and increased transporter expression. Thus, regardless of whether signaling was initiated by a short exposure to ET-1 or to a nephrotoxicant, the time course of Mrp2 response to ETa signaling was the same and was multiphasic. Finally, when tubules were exposed to gentamicin for 30 min and removed to gentamicin-free medium for 24 h, they were less sensitive to acute gentamicin toxicity than paired controls not initially exposed to the drug. Thus, short-term exposure to ET-1 or gentamicin resulted in long-term protection against a second insult.

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ABBREVIATIONS: Mrp, multidrug resistance protein; MTX, methotrexate; FL, fluorescein; ET, endothelin; NOS, nitric-oxide synthase; PKC, protein kinase C; L-NMMA, Nω-methyl-L-arginine acetate salt; RES701-1, cyclo(-Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp)-Trp-Phe-Phe-Asn-Tyr-Tyr-Trp-OH; ODQ, oxadiazole quinoxalin; PXR, pregnane X nuclear receptor.
From these experiments, it was also clear that the nephrotoxicants caused Ca\(^{2+}\)-dependent release of ET from the tubules and that released ET activated intracellular signaling by an autocrine mechanism. In contrast, after long-term, continuous exposure (6–24 h) to the nephrotoxic heavy metal salt CdCl\(_2\), transport activity and immunostaining of Mrp2 at the luminal membrane of the proximal tubules had increased (Terlouw et al., 2002). This long-term induction of Mrp2 may function as a compensatory mechanism for the initially reduced efflux of potentially toxic compounds, serving a protective route.

The present study addresses the issue of whether short-term signaling through the ET-activated pathway has long-term consequences to tubular function. Our results show that after 30 min of exposure to ET-1 or the aminoglycoside antibiotic, gentamicin, Mrp2-mediated transport initially declined; this is in agreement with previous studies (Terlouw et al., 2001; Notenboom et al., 2002). When tubules were removed to ET-1- and gentamicin-free medium, transport recovered over the next several hours. Twenty-four hours after exposure, Mrp2-mediated transport and Mrp2 protein expression were significantly higher than controls. These increases in transport and Mrp2 expression were abolished when ET signaling was disrupted. Finally, short-term gentamicin exposure and subsequent recovery for 24 h was protective against acute gentamicin tubular toxicity. Thus, short-term signaling has long-term consequences with regard to transport function and nephrotoxicant resistance.

Materials and Methods

Chemicals. FL-MTX, fluorescein, MitoTracker Red CM-H\(_2\)XRos, and Alexa Fluor 488 goat anti-rabbit IgG (rabbit polyclonal antibodies) were obtained from Invitrogen (Carlsbad, CA). N\(^5\)-Methyl-l-arginine (l-NMMA) was purchased from Alexis Corporation (Lüäufingen, Switzerland). The ET\(_A\) receptor antagonist RES701-1 was purchased from Bachem Biosciences (King of Prussia, PA). Oxadiazole quinoxalin (ODQ; a guanylyl cyclase inhibitor) was obtained from Calbiochem (San Diego, CA). Modified medium 199 with Earle’s salts was purchased from Sigma-Aldrich (St. Louis, MO). Rabbit polyclonal antibodies against Mrp2 (k78 mrp2) and Mrp4 (anti-Mrp4) were obtained as described previously (Van Aubel et al., 1998, 2002). All other chemicals used were obtained at the highest purity available commercially.

Animals and Tissue Preparation. Killifish (Fundulus heteroclitus) were collected by local fishermen in the vicinity of Mount Desert Island, Maine and maintained at the Mount Desert Island clausus was collected by local fishermen in the vicinity of Mount Desert Island, Maine and maintained at the Mount Desert Island Biological Laboratory in tanks with natural flowing seawater. Renal tubular masses were isolated in a marine teleost saline containing 140 mM NaCl, 2.5 mM KCl, tubular masses were isolated in a marine teleost saline based on that Desert Island, Maine and maintained at the Mount Desert Island

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Mrp4, 1:10), and 60-min exposure to secondary antibody (Alexa488labeled goat anti-rabbit IgG, 1:20). Antibody binding was detected with the Zeiss confocal laser scanning microscope using a 20× objective, and staining was quantified using ImageJ version 1.30 (National Institutes of Health, Bethesda, MD). A grid, consisting of 655.02-μm² squares, was placed on top of the confocal images to semiquantify the fluorescent staining. Only there where the grid crossed were luminal staining fluorescence intensities measured. The average of the fluorescence intensities measured for that tubule was used for quantitation.

Data Analysis. Data are given as mean percentage of control fluorescence ± S.E., unless indicated otherwise. Mean values were considered to be significantly different when \( P < 0.05 \) by use of the unpaired Student’s \( t \) test or by a one-way analysis of variance followed by Bonferroni’s multiple comparison test. Software used for statistical analysis was GraphPad Prism (version 3.00 for Windows; GraphPad Software Inc., San Diego, CA).

Results

Isolated renal tubules from killifish have proven to be a powerful model for the study of excretory transport in an intact proximal tubule (Pritchard and Miller, 1991). Unlike mammalian proximal tubules, the broken ends of teleost proximal tubules reseal after isolation, thus, forming a closed fluid filled luminal compartment. This enables study of cellular uptake and luminal accumulation of fluorescent compounds using imaging techniques. As in mammalian proximal tubules, killifish express high levels of Mrp2 in the luminal membrane of renal proximal tubule cells. Moreover, killifish tubules exhibit Mrp2-mediated transport of a number of fluorescent substrates, e.g., FL-MTX, that can be visualized and measured in intact individual tubules using confocal microscopy (Masereeuw et al., 1996, 2000; Miller et al., 1996). Figure 1, A and B, show a typical confocal image of a killifish tubule after 30 min (steady state) incubation in medium with 1 μM FL-MTX following 24 h of recovery. The fluorescence distribution pattern is the same as shown previously after short-term exposure, i.e., fluorescence intensity in lumen > cells > medium (Masereeuw et al., 1996, 2000). We have demonstrated that this pattern is indicative of a two-step process, involving uptake at the basolateral membrane mediated by an as-yet uncharacterized transporter for large organic anions and secretion into the lumen mediated by a teleost form of Mrp2 (for data on substrate and inhibitor specificities as well as immunostaining with Mrp2 antibodies, see Masereeuw et al., 1996, 2000; Terlouw et al., 2001). Using an Sf9 overexpression system, we previously showed that FL-MTX is a substrate for Mrp2 (Terlouw et al., 2002). Although MRPs are known to share many substrates, interference of other members of the MRP family with FL-MTX transport in this model is unlikely. Mrp5 and Mrp6 are located in the basolateral membrane and not in the apical membrane of renal proximal tubules, whereas Mrp1 and Mrp3 are not expressed in renal proximal tubules (Russel et al., 2002). Furthermore, we can exclude the contribution of Mrp4 because results from our group show that FL-MTX is not a substrate for Mrp4 (P. H. E. Smeets and F. G. M. Russel, unpublished data), and MRP4-mediated transport is insensitive to leukotriene C4 (Van Aubel et al., 2002), which is an excellent inhibitor of FL-MTX secretion in killifish proximal tubules (Masereeuw et al., 1996, 2000).

To determine whether short-term ET-1 signaling has long-term effects, we exposed tubules to ET-1 for 30 min, removed them to ET-free medium, and assayed Mrp2 transport function at several times after transfer. Figure 1C shows that 1.5 h after transfer, the tubules exhibit significantly reduced transport. This result is in agreement with previous experi-
ments from this laboratory where tubules were exposed continuously to ET-1 in transport experiments lasting up to 90 min (Masereeuw et al., 2000). However, with time in the ET-free medium, transport increased, and 3- and 12-h values were nearly identical to tubules not exposed to ET-1 (controls). Moreover, 24 h after ET-1 exposure, luminal accumulation of FL-MTX had increased by about 50% (P < 0.001; Fig. 1). Neither short-term exposure to ET-1 nor short-term exposure followed by recovery affected cellular accumulation of FL-MTX. Thus, both the short- and long-term effects of 30-min ET-1 exposure on luminal accumulation were evident with FL-MTX as substrate.

We previously demonstrated that several tubular nephrotoxicants (aminoglycoside antibiotics, radiouroidt agents, and heavy metal salts) were capable of mimicking the effects of ET-1 on FL-MTX transport. Each of these activated the ET signaling pathway by a Ca2+-dependent mechanism causing ET release from the tubules. None of them interacted with Mrp2 directly (Terlouw et al., 2001; Notenboom et al., 2002). When we incubated tubules in medium containing the aminoglycoside antibiotic, gentamicin (10 μM), transferred them to gentamicin-free medium, and monitored FL-MTX transport, we found the same pattern of effects as observed with ET-1 (Fig. 2). Thus, both ET-1 and gentamicin had a triphasic effect on FL-MTX: short-term reduction, followed by recovery, and finally significantly increased transport. Note that when FL was used as a substrate, there was no initial decrease in transport with 10 μM gentamicin (Fig. 3A) and no increase 24 h after gentamicin exposure (Fig. 3B). However, with short-term exposure to higher concentrations of gentamicin (Fig. 3A) and with 24-h continuous exposure to 10 μM gentamicin (preliminary data not shown), a decrease in FL transport was seen, indicating toxicity.

We used a pharmacological approach to further examine the intracellular events signaling the increase in FL-MTX transport 24 h after a short exposure to ET-1 or gentamicin. For this purpose, specific antagonists were used to block steps in the common signaling pathway. Figure 4 shows that blocking the ETB receptor with RES701-1, blocking NOS with l-NMMA, or blocking guanylyl cyclase with ODQ abol-
to gentamicin or ET-1 for 30 min and then assayed 24 h later. In these tubules, Mrp2 immunofluorescence appears to be more intense than in controls (no ET-1 or gentamicin exposure). Quantitation of Mrp2 immunofluorescence bears out this impression. Both ET-1 and gentamicin increased luminal membrane immunofluorescence by about 25% (P < 0.01). In contrast, no such increase was found when tubules were stained for Mrp4, which, as in mammalian renal proximal tubules (Van Aubel et al., 2002), is also localized to the luminal membrane of killifish renal proximal tubule cells (Fig. 5E). Consistent with the transport data presented above, inhibiting NOS with L-NMMA blocked the increase in Mrp2 expression caused by 30-min exposure to ET-1 or gentamicin (Fig. 6).

Thus, one consequence of short-term exposure to ET-1 or gentamicin is an increase in Mrp2 expression and function 24 h later. To determine whether the response to nephrotoxins was also affected, we used MitoTracker Red CM-H2XRos in tubules exposed to 100 μM gentamicin to indirectly assess mitochondrial functional integrity. This concentration of nephrotoxin is an order of magnitude higher than that which alters Mrp2-mediated transport though ET signaling and was found to affect tubular viability (Fig. 3). The advantage of using MitoTracker Red CM-H2XRos is that the probe is highly stable. It is oxidized within cells and then selectively sequestered in mitochondria of actively respiring cells. Probes often used for measuring mitochondrial membrane potential, e.g., JC-1 and Safranine O, cannot be used in our model because they are substrates for efflux carriers present in the apical membrane of the tubular cells. In this experiment, tubules were pre-exposed to 0 (controls) or 10 μM gentamicin for 30 min and then incubated in gentamicin-free medium for 24 h. Subsequently, they were challenged with a 2-h exposure to 100 μM gentamicin, and changes in mitochondrial integrity were measured using 500 nM MitoTracker Red CM-H2XRos and confocal imaging after 30-min incubation with the probe. In tubules not pre-exposed to the low concentration of gentamicin (controls), 100 μM gentamicin significantly decreased mitochondrial integrity (P < 0.05). This effect was roughly half of that seen when control tubules were exposed to 1 mM NaCN for 2 h (Fig. 7). In contrast, no such gentamicin-induced alteration in mitochondrial functional integrity was observed in tubules that had been pre-exposed to 10 μM gentamicin. Thus, at least for one nephrotoxicant and one measure of toxicity, gentamicin pre-exposure and subsequent recovery were protective.

**Discussion**

In killifish renal proximal tubules, ET-1 signals a decrease in Mrp2 transport function by acting through an ET<sub>B</sub> receptor, NOS, cGMP, and PKC. Moreover, exposure to any of several nephrotoxins causes release of ET from the tubules with subsequent activation of signaling and reduced Mrp2 function (Masereeuw et al., 2000; Terlouw et al., 2001, 2002). These inhibitory effects on Mrp2-mediated transport were observed within 15 min of exposure and were maximal within 60 min. After 60 min of exposure, the Mrp2 protein content of the luminal plasma membrane was not changed, suggesting that transporter activity in the luminal plasma membrane was modified, or transporter was removed to a subapical compartment that was not resolved at the light microscope level (Terlouw et al., 2001). In the present study, we investigated the effects of exposing renal proximal tubules to ET-1 or to gentamicin for a short time (30 min) and then returning them to ET-1- and gentamicin-free medium. The data show that over the 3 h following exposure, tubules recovered fully from the initial decrease in Mrp2-mediated transport and that transport activity was not changed 9 h later. However, 24 h after exposure, luminal accumulation of FL-MTX had increased by 50%. Increased transport at 24 h was accompanied by an increased transporter protein content of the luminal plasma membrane measured by immunostaining. This finding could not be confirmed using immunoblots. Immunoblot analysis is not feasible in this situation because of the small amount of membrane protein isolated from killifish tubules. A recent direct comparison of quantitative immunostaining with immunoblot analysis, however, indicates that the results obtained with the two methods are comparable (Bauer et al., 2004). Furthermore, preliminary data in rats treated with gentamicin in vivo showed a clear up-regulation of Mrp2 in the apical membrane of renal prox-
imal tubule confirming the findings of our killifish experiment in a different species (S. Notenboom, F. G. M. Russel, and R. Masereeuw, unpublished data).

Blocking ET-1 signaling at the ET<sub>B</sub> receptor, or downstream at NOS, abolished both stimulation of transport and increased transporter expression. Thus, regardless of whether signaling was initiated by a short exposure to ET-1 or by a nephrotoxicant, the time course of the Mrp2 response to ET<sub>B</sub> signaling was the same. It was multiphasic, involving reduced Mrp2-mediated transport, recovery to control levels, and a delayed increase over control levels 24 h after exposure. Nonspecific leakage of the fluorescent dye can be excluded because luminal FL-MTX accumulation is concentrative with respect to medium, and leakage of the dye at the tight junctions would decrease the luminal concentrating ability of the tubules. Note that FL transport in killifish renal tubules was constant over the entire exposure/recovery time course (Fig. 3) as observed previously (Terlouw et al., 2002). This fluorescent organic anion is avidly transported from bath to tubular lumen by an organic anion transport system that does not include Mrp2 (Masereeuw et al., 1996). Fluorescein may be transported by members of the SLC superfamily of transporters, organic anion transporters and organic anion-transporting polypeptides. However, the finding that fluorescein transport was unchanged argues for the lack of an effect on other carriers. In addition, in agreement with the zebrafish genome, unpublished findings demonstrate that killifish tubules express only one organic anion transporter that is localized to the basolateral membrane (J. B. Pritchard and D. S. Miller, personal communication). Concerning the organic anion-transporting polypeptide carriers, a large species difference exists in this subfamily of transporters, and it is unknown whether an isoform is present in killifish renal proximal tubules. Furthermore, 24 h after

Fig. 5. Increased luminal plasma membrane Mrp2 protein 24 h after 30-min exposure to 10 μM gentamicin or 10 nM ET-1. A, quantitation of luminal plasma membrane immunostaining of Mrp2. B, representative image of control tubule. C, tubule exposed to gentamicin. D, tubule exposed to ET-1. E, quantitation of Mrp4 immunostaining. Functional up-regulation of Mrp2-mediated transport was confirmed by an increased expression of Mrp2 in the luminal membrane of the proximal tubule after 24-h recovery of short-term exposure to 10 μM gentamicin or 10 nM ET-1 (C and D, respectively) compared with control tubules (B). In A, this is semiquantified. Mrp4, however, was not affected by recovery to short-term exposure to 10 μM gentamicin or 10 nM ET-1 (E). Data are given as mean ± S.E. for 31 to 108 tubules from 10 to 31 fish (**, significantly different from the control value; P < 0.01).
exposure, we did not detect any qualitative change in luminal plasma membrane content of Mrp4. This suggests no increase in expression of luminal membrane transporters in general or in the expression of MRP subfamily members in particular.

The increase in Mrp2 transport function (~50%) 24 h after exposure to ET-1 or gentamicin was accompanied by a less than proportional increase in transporter content in the plasma membrane (~25%). Assuming a one-to-one correspondence between transport activity and transporter content, this difference suggests that multiple mechanisms contribute to the increase in transporter function. These might include de novo synthesis of Mrp2, increased insertion of Mrp2 into the apical membrane, reduced Mrp2 retrieval from the apical membrane, and functional activation of membrane-bound transporter. Mrp2 activity is known to be modulated by transcriptional and post-transcriptional mechanisms. For example, several ligand-activated nuclear receptors have been shown to transcriptionally regulate the activity of xenobiotic-metabolizing enzymes and xenobiotic transporters, including Mrp2, in liver, intestine, and blood-brain barrier (Kast et al., 2002; Kullak-Ublick and Becker, 2003; Bauer et al., 2004). These include the pregnane X nuclear receptor (PXR) and the constitutive androstane receptor, both of which are activated by a wide range of xenobiotics. Teleost fish do express a PXR homolog (Moore et al., 2002; Maglich et al., 2003), but it is not known to what extent (if any) gentamicin or ET-1 affect PXR activity in any species. In addition, in mammalian hepatocytes, both PKC and PKA have been implicated in bidirectional, regulated trafficking of Mrp2 between intracellular stores and the canalicular membrane (Roelofsen et al., 1998; Beuers et al., 2001; Kubitz et al., 2001). Furthermore, Hegedus et al. (2003) suggested that PKC is involved in the Mrp2 targeting and recycling through phosphorylation of the PDZ domain, which influences the interaction between Mrp2 and its anchoring PDZ proteins and thereby its transport function. PKA activation does not appear to be involved in short-term exposure, since we previously found no effect of a PKA-selective inhibitor on ET-1 signaling (Masereeuw et al., 2000). Preliminary data using a canine kidney cell line overexpressing MRP2 pointed to an increased insertion of the transport protein in the luminal membrane after 1 h of gentamicin exposure and 24-h recovery or 24-h gentamicin exposure, whereas overall MRP2 expression remained unchanged (S. Notenboom, F. G. M. Russel, and R. Masereeuw, unpublished data). Additional experiments are needed to further characterize in the killifish renal proximal tubule model the mechanisms that link ET-NOS-cGMP-PKC signaling and Mrp2 up- and down-regulation.

Up-regulation of Mrp2 may be interpreted as part of a mechanism called preconditioning. Thus, an up-regulation of Mrp2 and other adaptive mechanisms after recovery protects the tissue against a second exposure to gentamicin. This phenomenon has been observed for ischemia in heart, liver, and kidney (Andreucci et al., 2003; Jaeschke, 2003; Park et al., 2003; Juhaszova et al., 2004). Mrp2 serves a protective function through the elimination of potentially harmful chemicals. Indeed, Mrp2 up-regulation was also observed after long-term exposure to cadmium (Terlouw et al., 2002).
and after ischemia (Laouari et al., 2001). Since Mrp2 handles many potentially toxic compounds, like xenobiotics and their metabolites (Russel et al., 2002), up-regulation of the transport protein may be part of a protective pathway of proximal tubules following harmful events.

Finally, use of gentamicin and other aminoglycoside antibiotics has been associated with severe proximal tubular nephrotoxicity, which limits their clinical use. Although such irreversible toxicity has been historically associated with multiple administration of high doses (Bennett, 1989), gentamicin can alter renal function even at low dose levels (Foster et al., 1992). The present data show for the first time that low-level, short-term, gentamicin exposure could have beneficial side effects, possibly by triggering a survival/protevative pathway. Certainly, ET signaling and gentamicin pre-conditioning affect more than Mrp2 expression and function. It will be important to determine which of these changes in gene expression or enzyme/transporter function confers increased resistance to aminoglycoside antibiotic toxicity. Note that the present experiments, in which Mrp2 transport function was the endpoint, provide an additional example of context-dependent signaling, as demonstrated recently for protein kinases (Bhalla et al., 2002; Ingolia and Murray, 2002). In this regard, we have been able to document three patterns of effects following tubule exposure to gentamicin (and other nephrotoxins): inhibition of transport with short-term exposure (Terlouw et al., 2001, 2002; Notenboom et al., 2002, 2004); toxicity with long-term, continuous exposure (S. Notenboom and D. S. Miller, unpublished data), and increased transport with long-term exposure to low concentrations of nephrotoxants (Terlouw et al., 2002) or with short-term exposure followed by a period of recovery (present study).

In conclusion, renal proximal tubules exposed to gentamicin or ET-1 and allowed to recover for 24 h display an increase in Mrp2-mediated transport and Mrp2 expression in the luminal membrane. These increments are a result of intracellular signaling consequences caused by short-term nephrotoxicant exposure in the renal proximal tubule. Several signaling molecules were identified as participants in this pathway leading to the following sequence of events: Short-term exposure to gentamicin triggers ET-1 release, ET-1 binds to the ET1 receptor, and, subsequently, NOS is stimulated, resulting in the activation of soluble guanylyl cyclase. The produced cGMP might cause de novo synthesis of Mrp2, stimulation of Mrp2 insertion in the luminal membrane, and/or inhibition of Mrp2 retrieval from the luminal membrane, eventually leading to increased Mrp2-mediated transport through increased Mrp2 insertion in the luminal membrane in the renal proximal tubule. Thus, short-term signaling (nephrotoxicant exposure) has long-term consequences for renal proximal tubule functioning.

References


Beuers U, Bilzer M, Chittatutto A, Kullak-Ublick GA, Keppel D, Paumgartner G, and Dombrowski F (2001) Taurochodeoxycholic acid inserts the apical conjugate export pump, Mrp2, into canalicular membranes and stimulates organic anion se-

cretion by protein kinase C-dependent mechanisms in cholestatic rat liver. Hepa-
tology 33:1206–1216.


tes its interaction with PDZ proteins. Biochem Biophys Res Commun 302:454–

461.


tance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor and constitutive androstane receptor. J Biol Chem 277:2908–2915.


thelin B receptor-mediated regulation of ATP-driven drug secretion in renal prox-


F520.


R1470–R1477.


27266.


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