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Modulatory effects of hormones, drugs, and toxic events on renal organic anion transport

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Abstract

The human body is exposed continuously to a wide variety of exogenous compounds, many of which are anionic compounds. In addition, products of phase II biotransformation reactions are negatively charged, viz. glucuronides, sulfate esters, or glutathiones. Renal transport of organic anions is an important defense mechanism of the organism against foreign substances. The combination of the rate of uptake and efflux and the intracellular disposition of organic anions in the proximal tubule determines the intracellular concentration and the nephrotoxic potential of a compound. Modulation of organic anion secretion is observed after exposure of proximal tubules to various hormones, and the subsequent receptor-mediated response is signaled by protein kinases. Transport of anionic compounds across the basolateral as well as the luminal membrane is modified by activation or inhibition of protein kinases. Protein kinase C activation reduces the uptake of organic anions mediated by the organic anion transporter 1 (OAT1/Oat1) and Oat3 and reduces Mrp2-mediated efflux. In addition, activation of protein kinase C has been shown to inhibit transport by the organic anion transporting polypeptide 1 (Oatp1) across the luminal membrane. Additional protein kinases have been implicated in the regulation of organic anion transport, and the role of nuclear factors in xenobiotic excretion is an emerging field. The physiological regulation of organic anion transporters may also be influenced by exogenous factors, such as exposure to xenobiotics and cellular stress. This commentary discusses the current knowledge of endogenous and exogenous influences on renal anionic xenobiotic excretion.

Keywords: Xenobiotic transport; Nephrotoxicity; Protein kinase; Transport regulation

1. Introduction

The kidney plays an important role in the excretion of various endogenous and xenobiotic compounds from the body. Renal disposal includes glomerular filtration, reabsorption, and secretion. The active secretion of waste products to the tubular lumen takes place in the proximal tubule. Three mechanisms are involved in this process: transport of compounds from the blood into the cell across the basolateral membrane, intracellular trafficking, and finally secretion across the apical membrane into the tubular lumen. There are separate transport systems for organic anions and organic cations. Both systems are characterized by a broad substrate specificity. The organic anion system is of particular importance in the process of detoxification because it mediates the final elimination of phase II biotransformation products (e.g. glucuronides, sulfate esters, glutathiones, glycine conjugates) into the urine. Organic anions are taken up into the cell against an electrochemical gradient and leave the cell by an efflux mechanism, which in general is less efficient than basolateral uptake. As a result, some anionic drugs may accumulate in the cell, thereby causing direct toxic effects. The extent of accumulation depends on the relative contribution of tubular uptake, intracellular distribution, and efflux into the tubular lumen. A tight regulation of organic anion transport and the resulting intracellular concentration of potentially harmful compounds are very important. This commentary gives an overview of the regulation of renal organic anion transport across the basolateral and the

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Abbreviations: ABC, ATP-binding cassette; cAMP, cyclic AMP; LLC-PK_1, porcine-derived kidney cell line; MEK, mitogen-activated protein kinase/extracellular regulated kinase; MRP, multidrug resistance protein; NaDC, sodium-dependent dicarboxylate transporter; NPT, sodium/phosphate cotransporter; OAT, organic anion transporter; OAT-K, kidney-specific organic anion transporters; OATP, organic anion transport polypeptide; PAH, *p*-aminohippurate; PEPT, H^+-peptide cotransporter; PKA, protein kinase A; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; and URAT, urate anion exchanger.

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luminal membrane under various conditions. For more detailed information on molecular characteristics of the various renal organic anion transporters the reader is referred to recent comprehensive reviews [1–4].

2. Transport of organic anions

Three separate systems for organic anion uptake have been identified in the basolateral membrane: the PAH/dicarboxylate exchanger (OAT1), the organic anion transporter 3 (OAT3), and the Na^+-dicarboxylate cotransporter (NaDC3; Fig. 1). The organic anion transporter studied most extensively is the PAH/dicarboxylate exchanger, which is cloned from various species and named OAT1 [5–8]. Small organic anions, such as PAH, are taken up at the basolateral membrane via exchange against dicarboxylates (favorably α-ketoglutarate). The outward α-ketoglutarate gradient is maintained by intracellular metabolic generation of α-ketoglutarate, but is also created by Na^+-dicarboxylate cotransport driven by the transmembrane sodium gradient, which is established by Na^+,K^+-ATPase [9,10]. The substrate specificity of OAT1 was demonstrated to be remarkably wide. A variety of endogenous substrates, such as cyclic nucleotides, α-ketoglutarate, and urate [5], products of biotransformation [11], as well as several xenobiotics, such as β-lactam antibiotics [12], nonsteroidal anti-inflammatory drugs [13], nucleoside phosphonates [14], and anti-viral drugs [15,16], were identified. The basolateral Na^+-dicarboxylate cotransporter was cloned recently and named NaDC3 [17]. In contrast to OAT1, NaDC3 has a relatively narrow substrate specificity. Several other OAT isoforms have been detected, including OAT3. This member, which is localized to the basolateral membrane of proximal tubule cells, exhibits a wide substrate specificity and may therefore play, next to OAT1, an important role in the uptake of organic anions [18]. In contrast to OAT1, transport mediated by OAT3 is Na^+-independent, suggesting that organic anion uptake is not driven by exchange against dicarboxylates. Possible other candidates may include glutathione or sulfate. Furthermore, members of the MRP family have been found to be expressed in renal proximal tubules. The MRP family belongs to the ABC superfamily of transporters, and contains at least nine members, MRP1–MRP9. Members thought to be localized to the basolateral membrane of polarized cells are MRP1, MRP3, MRP5, and MRP6, whereas MRP2 and MRP4 have been identified in the apical membrane of renal proximal tubules. MRP1 is expressed in various tissues; however, present data suggest that this drug transporter is not expressed in proximal tubules [19,20]. To date, no information about the three last discovered members, MRP7–MRP9, is available [21]. MRP3 and MRP5 are expressed in the kidney, but their nephron distribution, membrane localization, and role in renal organic anion transport remain to be elucidated. MRP6 has been shown to be localized to the basolateral membrane of the proximal tubule and, recently, characterized as an organic anion efflux carrier [22].

After uptake of organic anions across the basolateral membrane, the compounds are distributed to the apical membrane. Organic anions accumulate in at least two different intracellular compartments. Studies in crab urinary bladder and teleost renal proximal tubules demonstrated that organic anions sequester in a vesicular compartment [23,24]. In addition, anionic drugs appear

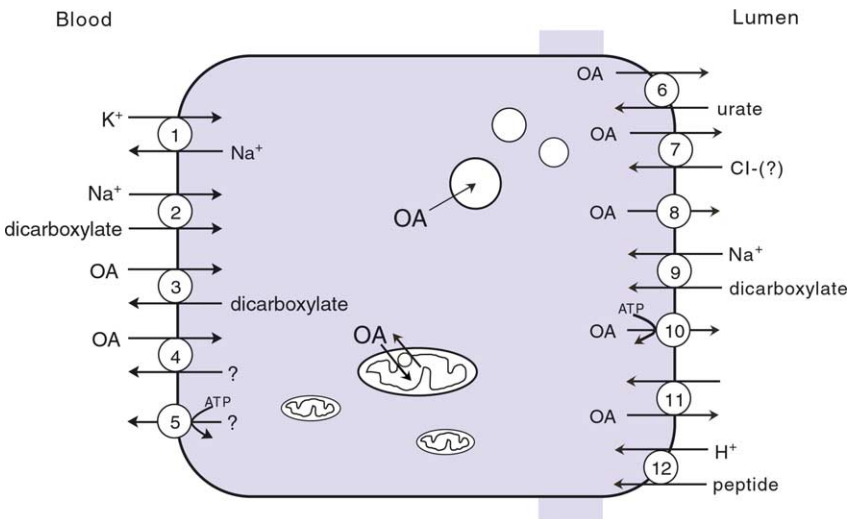

Fig. 1. Schematic model of organic anion transporters in the renal proximal tubule. Uptake of organic anions (OAs), such as PAH, is driven by a Na^+ gradient maintained by Na^+,K^+-ATPase (1) and a Na^+-dicarboxylate cotransport mediated by NaDC3 (2). Eventually, the OA enters the cell through exchange with a dicarboxylate ion via the organic anion transporter, OAT1 (3). OA uptake is also mediated by OAT3 (4). MRP6 (5) may mediate OA efflux. The luminal (apical) membrane contains various transport systems for the efflux of OAs into the lumen or reabsorption from the lumen into the cell. These transporters are: URAT1 (6), the sodium/phosphate cotransporter, NPT1 (7), OAT4 (8), the Na^+-dicarboxylate cotransporter, NaDC1 (9), MRP2 and MRP4 (10), the organic anion transporting polypeptides, OATP1, OAT-K1, and OAT-K2 (11), and the H^+-peptide cotransporters, PEPT1 and PEPT2 (12).

to accumulate in the mitochondria [25]. Direct uptake measurements of fluorescein, using rat renal cortex mitochondria, demonstrated the involvement of at least three anion carrier proteins, viz. the α-ketoglutarate, tricarboxylate, and glutamate–aspartate carriers [26]. In addition, several anionic drugs interact with ligandin or glutathione *S*-transferase B, indicating that this protein may play a role in the reduction of free cytoplasmic drug concentrations [27–29]. However, whether there is a function of binding proteins in transcellular transport is still uncertain. Finally, anionic drugs may be metabolized intracellularly. Although biotransformation mainly takes place in the liver, the kidneys are also able to metabolize drugs during renal secretion. Renal biotransformation reactions involve microsomal oxidation, reduction, or hydrolysis (phase I reactions), and conjugation into glucuronides, sulfates, or glutathiones (phase II reactions) [30–33].

Luminal efflux of organic anions is also carrier-mediated (Fig. 1), although transport is energetically downhill. For small organic anions, like PAH, two transport pathways have been described. First, an anion exchange system is thought to be involved, but this exchanger is only described in urate-reabsorbing species, such as rats, dogs, and humans. The putative transporter for this system, URAT1, was cloned recently and indeed mediates urate uptake in trans-stimulation by various organic anions [34]. The second mechanism is voltage-sensitive facilitated diffusion [35]. A possible candidate might be the sodium/phosphate cotransporter type I (NPT1), which is localized to the brush-border membrane of the renal proximal tubule [36–39]. Additionally, a member of the organic anion transporter family, OAT4, is confined to the apical membrane and mediates organic anion efflux [40]. A sodium-dependent dicarboxylate transporter, called NaDC1, is also expressed in the apical membrane [41]. Furthermore, MRP2 and MRP4 are localized to the brush-border membrane of proximal tubule cells [42,43]. MRP2 transports conjugates of lipophilic compounds and various bulky organic anions [3], whereas MRP4 is most likely an efflux pump for cAMP, cGMP, and nucleoside-based drugs [43]. Several members of the OATP family are also present in the kidney. These include OATP1, OATP3, OATP5, and the kidney-specific OAT-K1 and OAT-K2. In contrast to the liver, OATP1 appears to be located at the luminal membrane of the kidney proximal tubule [44] and mediates sodium- and ATP-independent transport of various organic anions and conjugated compounds. The membrane localization of OATP3 and OATP5 is still unknown [45,46]. OAT-K1 and OAT-K2 are sodium- and ATP-independent transporters that mediate transport of methotrexate and folate [47]. In addition, OAT-K2 also mediates prostaglandin E_2 and taurocholate transport [48,49]. As the OATPs are bidirectional transporters, it is not yet certain whether they are involved in the reabsorption of organic anions from the tubular lumen, rather than in efflux of anionic compounds. Finally, the H^+-peptide cotransporters (PEPT1 and PEPT2) mediate the luminal uptake of small anionic peptides and various peptide-like compounds, such as cephalosporin antibiotics [50,51].

3. Regulation of organic anion transporters

Renal organic anion transporters play an important role in the disposition of potentially harmful compounds in the body. Their activity may influence the degree of accumulation of anionic substrates, indicating that regulation could have toxicological implications. Regulation of transporter activity in response to endogenous and exogenous signals may occur by modifications of gene transcription, mRNA stability, and mRNA translation, and by posttranslational control (Fig. 2). The factors involved in posttranslational control affect either the activity or the amount of transporters in the cell membrane and include insertion into the plasma membrane, turnover by retrieval and proteolysis, modification of transporter activity by phosphorylation, or interaction with regulatory proteins. The modulation of organic anion transporters by protein kinases, hormones, and xenobiotics and cellular stress is summarized in Tables 1–3, respectively, and the species in which the studies were performed are indicated.

3.1. Intracellular signaling by protein kinases

Cellular responses triggered by hormones are often produced through activation of protein kinases, enzymes involved in the phosphorylation of specific proteins. These phosphorylation cascades result in either activation or inhibition of specific enzymes or in activation of the transcription of specific genes. We will first discuss the protein kinases involved in the intracellular signaling leading to regulation of organic anion transport. PKC plays a central role in the signal transduction pathway induced by various hormones. In renal proximal tubules, PKC affects a variety of transport processes at both the basolateral and luminal membranes, such as Na^+,K^+-ATPase (see, for example, Refs. [52,53]). Several recent studies show that PKC is also involved in the regulation of anion transport mediated by OAT1, in which several PKC phosphorylation sites are predicted in the large intracellular loop between transmembrane domains six and seven [54]. Previous studies have suggested that the transport of small organic anions across the basolateral membrane is modulated by PKC. The uptake or transepithelial secretion of small organic anions, such as PAH and fluorescein, in proximal tubule cells was inhibited after exposure to PMA, an activator of PKC. The inhibition was prevented by pretreating the tubular cells with an inhibitor of PKC [55–61]. In addition, uptake of organic anions in proximal tubule cells stably expressing rat Oat3 was inhibited after PMA treatment due to a decrease in V_{max}. Similar to OAT1,

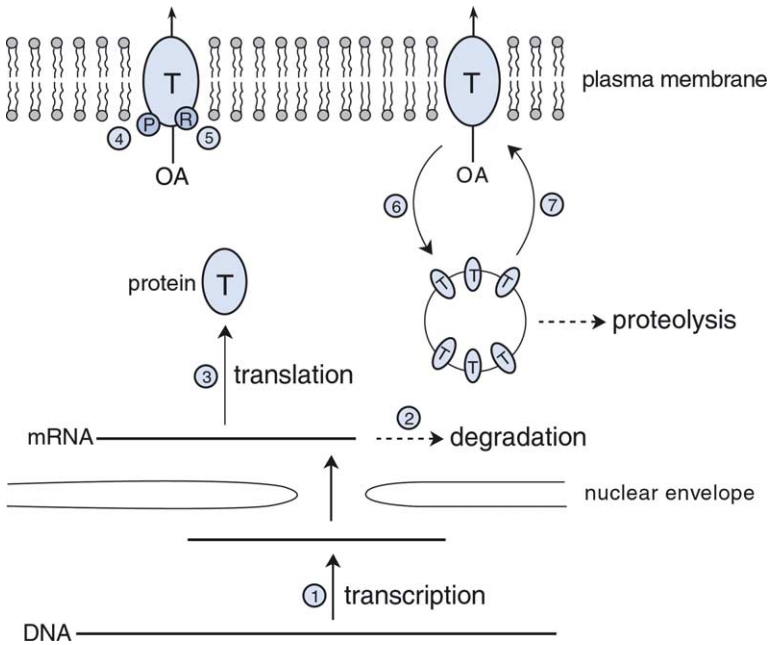

Fig. 2. Schematic model of the different cellular processes possibly modulated in response to endogenous and exogenous signals resulting in the regulation of organic anion (OA) transport. OA transport may be regulated at the transcriptional level, which includes: (1) modulation of gene activity (transcription), (2) stability of mRNA, and (3) translation of mRNA. Furthermore, OA transport may be regulated by changing the activity of the transporter via (4) phosphorylation by protein kinases or (5) allosteric control by regulatory proteins. Finally, regulation of OA transport may occur by modulating the amount of transporters present in the membrane by increasing the retrieval (6) or insertion (7) of the transport proteins.

inhibition in transport activity could be reversed by a selective inhibitor of PKC [62].

The uptake of small organic anions by OAT1 is a tertiary active mechanism (Fig. 1), indicating that inhibition of uptake may not only be the result of a decreased transport by OAT1, but may also be caused by inhibition of Na^+,K^+-ATPase or NaDC3. The reduction of organic anion secretion in primary cultures of winter flounder proximal tubules observed after treatment with PMA is thought to be the result of inhibition of Na^+,K^+-ATPase [63], because PKC activation has been shown to partially deactivate renal Na^+,K^+-ATPase [53]. On the other hand, PMA-induced inhibition of fluorescein uptake in nonperfused rabbit proximal tubules was not due to a decrease in the concentration of counterions, indicating a direct effect of PKC on the PAH/dicarboxylate exchanger [57]. Moreover, Röver *et al*. [64] reported that PKC did not affect transport mediated by the basolateral Na^+-dicarboxylate cotransporter in the rabbit.

PKC can modulate the function of OAT1 by different mechanisms. A recent study in LLC-PK_1 cells transfected with murine *oat1* showed that PKC activation induced a decrease in the maximum PAH transport velocity without direct phosphorylation of the transport protein [65]. Therefore, PKC may regulate OAT1 by a decrease in membrane expression either mediated by internalization of membrane transporters or by inhibition of the recruitment of (preformed) transporters into the membrane.

In killifish renal proximal tubules, PMA also reduced the cellular and luminal accumulation of fluorescein. Again a PKC inhibitor was able to block the inhibition caused by PMA. The inhibition of secretion was caused by changes in the basolateral uptake of fluorescein. Exposure of the proximal tubules to protein kinase inhibitors alone increased the cellular and luminal accumulation of the organic anion [61]. On the other hand, in perfused S2 segments of rabbit proximal tubules, Shuprisha *et al*. [55] did not find an effect of two PKC inhibitors, staurosporine and bisindolylmaleimide, on the secretion of fluorescein.

In contrast to the studies described above, in which a negative coupling was found between PKC activation and basolateral organic anion transport, treatment with PMA of isolated nonperfused S2 segments of proximal tubules, microdissected from rabbit kidneys, resulted in an increased uptake of PAH [66,67]. The effect of PMA could be abolished by pretreatment with staurosporine. PKC inhibition, on the other hand, diminished the uptake of the organic anion across the basolateral membrane [66]. Stärk *et al*. [67] showed that the PMA-induced increase in PAH uptake was due to an increased V_{max} and a decreased K_m for PAH. At present, the reason for the discrepancy between these observations is not clear; however, species differences may be excluded because both a negative and a positive correlation between PKC and basolateral uptake of organic anions were found in rabbits.

The importance of PKC as a signal transducer has also been described for the regulation of Mrp2 in killifish proximal tubules. The cell-to-luminal efflux of fluorescein-methotrexate, a fluorescent substrate for Mrp2, was reduced after treatment of the tubules with PMA. The

Table 1
Protein kinases regulating organic anion transport

	Transporter/mechanism[a]	Effect[b]	Tissue/cell type	Species	Reference(s)
PKC stimulation	FL uptake	Inhibition	Proximal tubule	Killifish	[61]
	PAH uptake + secretion	Inhibition	Kidney	Opossum	[59,60]
	FL uptake	Inhibition	Proximal tubule	Rabbit	[57]
	FL secretion	Inhibition	Proximal tubule	Rabbit	[55]
	2,4-D secretion	Inhibition	Proximal tubule	Winter flounder	[63]
	PAH uptake	Stimulation	Proximal tubule	Rabbit	[66,67]
	Oat1	Inhibition	*Xenopus laevis* oocytes	Rat	[56]
	OAT1	Inhibition	HeLa cells	Human	[58]
	Oat1	Inhibition	Kidney	Mouse	[65]
	Oat3	Inhibition	Proximal tubule	Rat	[62]
	Glutarate uptake	None	Proximal tubule	Rabbit	[64]
	Nadc1	Inhibition	*Xenopus laevis* oocytes	Rabbit	[70]
	Oatp1	Inhibition	*Xenopus laevis* oocytes	Rat	[69]
	FL-MTX efflux	Inhibition	Proximal tubule	Killifish	[68]
PKA stimulation	OAT1	None	HeLa cells	Human	[58]
	PAH uptake + secretion	None	Kidney	Opossum	[59]
	2,4-D secretion	None	Proximal tubule	Winter flounder	[63]
	PAH uptake	Inhibition	Proximal tubule	Rabbit	[67]
	FL-MTX efflux	None	Proximal tubule	Killifish	[68]
	GS-DNP efflux	Stimulation	Hepatocytes	Rat	[71]
	Glutarate uptake	None	Proximal tubule	Rabbit	[64]
	Nadc1	None	*Xenopus laevis* oocytes	Rabbit	[70]
	Oatp1	None	*Xenopus laevis* oocytes	Rat	[69]
PKC inhibition	FL uptake	Stimulation	Proximal tubule	Killifish	[61]
	FL secretion	None	Proximal tubule	Rabbit	[55]
	PAH uptake	Inhibition	Proximal tubule	Rabbit	[66]
	Oatp1	None	*Xenopus laevis* oocytes	Rat	[69]
	FL-MTX efflux	None	Proximal tubule	Killifish	[68]
	Mrp2	Inhibition	Hepatocytes	Rat	[84]
MEK inhibition	PAH uptake	Inhibition	Proximal tubule	Rabbit	[73]
	PAH uptake + secretion	Inhibition	Kidney	Opossum	[60]
TRK inhibition	PAH uptake	Inhibition	Proximal tubule	Rabbit	[73]
	OAT1	None	HeLa cells	Human	[58]
PI3K inhibition	PAH uptake	Inhibition	Proximal tubule	Rabbit	[73]
Cam kinase II inhibition	PAH uptake	Inhibition	Proximal tubule	Rabbit	[123]
	2,4-D secretion	None	Proximal tubule	Winter flounder	[63]

Abbreviations: Cam kinase II, calcium/calmodulin-dependent protein kinase II; 2,4-D, 2,4-dichlorophenoxyacetic acid; FL, fluorescein; FL-MTX, fluorescein-methotrexate; GS-DNP, dinitrophenyl-glutathione; MEK, mitogen-activated protein kinase/extracellular regulated kinase; OAT, organic anion transporter; PAH, *p*-aminohippurate; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKC, protein kinase C; and TRK, tyrosine kinase.

[a] When the specific transporter involved in the organic anion transport is unknown, the studied drug and transport mechanism are indicated. Uptake is transport across the basolateral membrane; secretion is the result of transport across the basolateral membrane in combination with transport across the luminal membrane; efflux is transport across the luminal membrane.

[b] The effect of the indicated protein kinases on organic anion transport was determined through activation or inhibition by specific drugs.

inhibition was prevented by PKC inhibitors [68]. Also, the function of the luminal transporter, Oatp1, was found to be negatively correlated with PKC activity [69].

Contrary to the basolateral Na^+-dicarboxylate cotransporter, the luminal Na^+-dicarboxylate cotransporter, Nadc1, is under the short-term control of PKC. Inhibition of Nadc1 transport by PMA occurs in a concentration-dependent manner, and can be prevented by staurosporine, a PKC inhibitor. Approximately 30% of the inhibition of Nadc1 seen after PKC activation can be accounted for by endocytosis of the transporter from the brush-border membrane. The remaining part should be the result of a direct effect on the activity of Nadc1 [70].

Furthermore, the role of PKA, which is activated by cAMP, in the regulation of renal organic anion transport was studied. Several studies demonstrated that selective activators and/or inhibitors of PKA had no effect on the basolateral uptake of organic anions [58,59,63]. However, treatment of nonperfused rabbit proximal tubules with dibutyryl-cAMP, a cAMP analog, and forskolin, an activator of adenylate cyclase, resulted in an inhibition of PAH uptake due to a decrease in V_{max} [67]. Data on the basolateral Na^+-dicarboxylate cotransporter, the luminal Nadc1, and the luminal drug transporter Mrp2 indicate that these transporters are not regulated by PKA [68,70]. In contrast, results obtained in the rat liver showed that the transport activity of Mrp2 in hepatocyte couplets is regulated by cAMP [71]. In this study, dibutyryl-cAMP stimulated the sorting of transporter-containing vesicles to the apical membrane with a concomitant increase in Mrp2-

Table 2
Receptor-mediated regulation of organic anion transport/transporters by hormones or hormone-like compounds

	Transporter/mechanism[a,b]	Effect	Tissue/cell type	Species	Reference(s)
Catecholamines	PAH uptake	Stimulation	Proximal tubule	Rat	[74]
Phenylephrine	FL uptake[c]	Inhibition	Proximal tubule	Rabbit	[57]
	FL secretion[c]	Inhibition	Proximal tubule	Rabbit	[55]
Oxymetazoline	2,4-D secretion	Stimulation	Proximal tubule	Winter flounder	[63]
Dopamine	2,4-D secretion	Inhibition	Proximal tubule	Winter flounder	[63]
Bradykinin	FL uptake[c]	Inhibition	Proximal tubule	Rabbit	[57]
	FL secretion[c]	Inhibition	Proximal tubule	Rabbit	[55]
Parathyroid hormone	PAH uptake + secretion[c]	Inhibition	Kidney	Opossum	[77]
Endothelin	FL-MTX efflux[c]	Inhibition	Proximal tubule	Killifish	[68]
EGF	PAH uptake + secretion[d]	Stimulation	Kidney	Opossum	[60]
Thyroid hormone	PAH uptake	Stimulation	Kidney	Rat	[79,80]
Testosterone	PAH uptake	Stimulation	Kidney	Rat	[81–84]
	Oatp1 (mRNA)	Stimulation	Kidney	Rat	[85]
	Oatp1 (mRNA)	Stimulation	Kidney	Mouse	[86]
Estradiol	Oatp1 (mRNA)	Inhibition	Kidney	Rat	[85]
	Oatp1 (mRNA)	Inhibition	Liver	Rat	[87]
	Mrp2 (protein)	Inhibition	Liver	Rat	[89]
	Mrp2 (protein)	Inhibition	Hepatocytes	Rat	[88]
Dexamethasone	PAH uptake	Stimulation	Kidney	Rat	[80,91]
	Mrp2 (protein)	Stimulation	Kidney	Rat	[92]
	Mrp2 (protein)	Stimulation	Liver	Rat	[92]
	Mrp2 (mRNA)	Stimulation	Hepatocytes	Rat	[94]
	Mrp2	Stimulation	Hepatocytes	Rat	[93]
	MRP2 (mRNA)	Stimulation	Hepatocytes	Human, rat	[95]

Abbreviations: 2,4-D, dichlorophenoxyacetic acid; EGF; epidermal growth factor; FL, fluorescein; FL-MTX, fluorescein-methotrexate; and PAH, *p*-aminohippurate.

[a] When the specific transporter involved in the organic anion transport is unknown, the studied drug and transport mechanism are indicated. Uptake is transport across the basolateral membrane; secretion is the result of transport across the basolateral membrane in combination with transport across the luminal membrane; efflux is transport across the luminal membrane.

[b] When protein or mRNA is indicated in addition to the transporter, regulation is not measured at the functional level but on the protein or mRNA level, respectively.

[c] Signaling mediated by PKC.

[d] Signaling mediated by MEK.

mediated efflux. Short-term exposure of freshly isolated hepatocytes to stimulators of the cAMP second-messenger system had no influence on Mrp2 transport activity [72], indicating that the increase in Mrp2-mediated secretion was not due to direct modification of Mrp2, but to regulation by insertion into the plasma membrane.

Other signal transducers implicated in the regulation of the basolateral PAH transporter are MEK, tyrosine kinase, phosphatidylinositol 3-kinase, and the calcium/calmodulin-dependent multifunctional protein kinase II. Inhibition of MEK was shown to cause a diminished uptake of PAH in rabbit proximal tubules [73]. Moreover, transepithelial secretion of PAH was reduced after MEK inhibition via reduction of basolateral uptake and apical efflux [60]. Gabriëls *et al*. [73] demonstrated that genistein, a selective inhibitor of tyrosine kinase, reduced basolateral uptake of PAH, whereas the same inhibitor was without effect in HeLa cells transfected with OAT1 [58]. Finally, inhibitors of phosphatidylinositol 3-kinase and the calcium/calmodulin-dependent protein kinase II were shown to induce an inhibition of PAH uptake in rabbit proximal tubules [73], but again the involvement of calcium/calmodulin-regulated enzyme activation in the regulation of small anion transport is contradicted by another study [63].

3.2. Hormonal regulation

Several reports have implicated hormones and agonists of adrenoreceptors in the regulation of proximal tubular organic anion transport. An early study of Jensen and Berndt [74] demonstrated that the catecholamines adrenaline and noradrenaline enhance the uptake of PAH in rat proximal tubular basolateral membrane vesicles. The α-adrenergic agent oxymetazoline was also shown to increase the net secretion of a xenobiotic organic anion in primary cultures of winter flounder proximal tubule cells, whereas administration of dopamine to the basolateral side caused a significant decrease in net secretion [63]. The effects on organic anion transport seen in both studies were suggested to be mediated by an altered activity of basolateral Na^+,K^+-ATPase in response to different agents. In favor of this hypothesis are the results obtained in renal proximal tubules which demonstrate that Na^+,K^+-ATPase activity is increased after oxymetazoline treatment and inhibited after dopamine treatment [75,76]. However, the adrenoreceptors are coupled to the PKC signaling pathway, and as mentioned before, activation of PKC may result in inhibition of small organic anion uptake. Activation of the α-receptor by phenylephrine induced a reversible reduction in organic

Table 3
Exogenous influences on organic anion transport/transporters

	Transporter/mechanism[a,b]	Effect	Tissue/cell type	Species	Reference(s)
Cisplatin	Mrp2 (protein)	Stimulation	Kidney	Rat	[100]
	Mrp2 (protein)	None	Liver	Rat	[100]
	Mrp2 (mRNA + protein)	Stimulation	Hepatocytes	Rat	[104,106]
Arsenite	MRP2 (mRNA + protein)	Stimulation	Hepatocytes	Human	[105]
	Mrp2 (mRNA + protein)	Stimulation	Hepatocytes	Rat	[105]
	Mrp2 (protein)	Stimulation	Hepatocytes	Rat	[111]
2-Acetylaminofluorene	Mrp2 (mRNA + protein)	Stimulation	Hepatocytes	Rat	[104,106]
	MRP2 (mRNA + protein)	Stimulation	HepG2 cells	Human	[109]
Clotrimazol	MRP2 (mRNA + protein)	Stimulation	Hepatocytes	Human	[107]
Hyperforin	MRP2 (mRNA)	Stimulation	Hepatocytes	Human, rat	[95]
Phenobarbital	MRP2 (mRNA + protein)	Stimulation	Hepatocytes	Human, rat	[108]
Prooxidants	MRP2 (mRNA)	Stimulation	HCT116 cells	Human	[110]
Rifampicin	MRP2 (mRNA)	Stimulation	Hepatocytes	Human, rat	[95]
Tamoxifen	Mrp2 (mRNA + protein)	Stimulation	Liver	Monkey	[106]
2,4,5-Trichlorophenoxyacetic acid	Mrp2 (mRNA + protein)	Stimulation	Liver	Mouse	[113]
Cadmium	PAH uptake	Inhibition	Kidney	Rat	[98]
	FL-MTX efflux	Stimulation	Proximal tubule	Killifish	[99]
Cadmium[c]	PAH uptake	Stimulation	Proximal tubule	Rabbit	[97]
	FL-MTX efflux	Inhibition	Proximal tubule	Killifish	[99]
Mercury	FL-MTX efflux	Stimulation	Proximal tubule	Killifish	[99]
Mercury[c]	FL-MTX efflux	Inhibition	Proximal tubule	Killifish	[99]
Hydrogen peroxide[c]	OAT1	Inhibition	Proximal tubule	Human	[96]
Gentamicin[c]	OAT1	Inhibition	Proximal tubule	Human	[96]
Hydrogen peroxide[c]	OAT3	Inhibition	Proximal tubule	Human	[96]
Aminoglycosides[c]	FL-MTX efflux	Inhibition	Proximal tubule	Killifish	[102]
Radiocontrast agents[c]	FL-MTX efflux	Inhibition	Proximal tubule	Killifish	[102]
Chronic renal failure	Mrp2 (mRNA + protein)	Stimulation	Kidney	Rat	[116]
	Mrp2 (mRNA + protein)	Stimulation	Liver	Rat	[116]
Regenerating liver	Mrp2 (mRNA + protein)	Stimulation	Liver	Rat	[117]
	Oatp1 (mRNA + protein)	Inhibition	Liver	Rat	[117]
Cholestasis	Mrp2 (mRNA)	Inhibition	Liver	Rat	[118]
	Mrp2 (mRNA + protein)	Inhibition	Liver	Rat	[89,120]
	Mrp2 (protein)	Inhibition	Hepatocytes	Rat	[119]
	Mrp2 (mRNA + protein)	Inhibition	Hepatocytes	Rat	[120]

Abbreviations: FL-MTX, fluorescein-methotrexate; HepG2, human hepatoblastoma; HCT116, human colorectal carcinoma cell line; MRP, multidrug resistance protein; OAT, organic anion transporter; and PAH, *p*-aminohippurate.

[a] When the specific transporter involved in the organic anion transport is unknown, the studied drug and transport mechanism are indicated. Uptake is transport across the basolateral membrane.

[b] When protein and/or mRNA is indicated in addition to the transporter, regulation in not measured at the functional level but on the protein or mRNA level, respectively.

[c] Short-term exposure.

anion transport activity. Bisindolylmaleimide could prevent the inhibition of fluorescein transport, confirming the role of PKC in this signaling pathway [55,57]. Thus, adrenergic stimulation seems to be one of the physiological stimuli involved in the regulation of organic anion uptake across the basolateral membrane.

There are several hormones known to act via activation of PKC. Bradykinin has been shown to regulate the uptake of fluorescein in rabbit proximal tubules through PKC [55,57]. Parathyroid hormone regulates various cell processes through PKC, but also through PKA. Basolateral uptake of PAH in a monolayer of opossum kidney cells was inhibited in a dose-dependent manner by parathyroid hormone. The inhibition was blocked completely by the PKC inhibitor staurosporine [77]. Furthermore, subnanomolar to nanomolar concentrations of the peptide endothelin reduced the Mrp2-mediated transport of fluorescein-methotrexate from cell to tubular lumen. Pretreatment with a specific B-type receptor antagonist, but not an A-type receptor antagonist, could prevent the reduced efflux. Again, endothelin signaling in proximal tubules occurred via activation of PKC, because the endothelin-induced reduction in fluorescein-methotrexate efflux was blocked by PKC inhibitors [68]. In opossum kidney cells, epidermal growth factor enhanced transepithelial secretion of PAH by stimulation of uptake, whereas the efflux across the luminal membrane was not affected. Epidermal growth factor was hypothesized to act by the successive activation of MEK, extracellular regulated kinase isoforms 1 and 2 (ERK 1/2), and additional downstream signaling steps [60].

The hormones described above do not enter the cell, but rather induce signaling through binding to their plasma membrane receptor followed by activation of a chain of intracellular effector molecules. In contrast, steroid

hormones as well as thyroid hormones exert their effects through binding to intracellular receptors, usually in the nucleus. This activates the receptor to function as a regulating protein of gene transcription. In immature rats, an increase in renal uptake of PAH has been observed following treatment with the thyroid hormones triiodothyronine and tetraiodothyronine [78–80]. Steroid hormones have also been demonstrated to influence organic anion transport. Studies as early as 1955 described sex differences in PAH transport in renal cortical slices. Male rats accumulate PAH to a greater extent than female rats and the accumulation in cortical slices of castrated male rats is reduced, indicating a stimulatory role for testosterone [81–83]. In addition, a more recent study demonstrated that the elimination half-life for PAH is shorter in male than in female rats. Castration of male rats resulted in an increase in the elimination half-life to values comparable to those measured in females. On the other hand, treatment of female rats with testosterone increased the elimination rate to that of males. The stimulatory effect of testosterone on PAH secretion was confirmed *in vitro* using renal cortical slices. The testosterone-induced increase in PAH uptake is thought to be due to an increase in functional transporters at the basolateral membrane [84]. Sex differences are also described for the Oatp1-mediated transport of organic anions across the luminal membrane. The level of renal Oatp1 mRNA expression was lower in female rats than in male rats. Treating female rats with testosterone increased the Oatp1 mRNA expression, whereas estradiol treatment of male rats decreased the total amount of mRNA in the kidney [85]. Furthermore, Oatp1 mRNA was detected only in the kidneys of female mice treated with dihydrotestosterone and not in sham-treated female mice, indicating a strong induction of the *oatp1* gene by androgens [86]. In agreement, estrogens have been shown to reduce the protein content of Oatp1 in the liver [87], and Mrp2 protein decreases after treatment with ethinyl estradiol [88]. However, Mrp2 mRNA levels remained unchanged or were even increased, suggesting posttranscriptional regulation [88,89]. In contrast to the kidney, mRNA and protein levels of Oatp1 were similar in livers of male and female rats [90].

Several studies reported the modulation of renal organic anion transport by the glucocorticoid dexamethasone. The excretion and accumulation of PAH in renal cortical slices increased after exposure to dexamethasone [80,91]. Moreover, the expression of Mrp2 determined by western blotting analysis was increased 2-fold in the rat kidney after long-term *in vivo* administration of dexamethasone [92]. An induction of Mrp2 expression after glucocorticoid treatment was confirmed in the liver. Long-term exposure to dexamethasone caused an elevation at the protein [92,93] and mRNA level [93,94]. In rat hepatocytes cultured in the presence of dexamethasone, a fluorescent substrate for Mrp2 was transported rapidly to the pseudocanaliculi, whereas in untreated hepatocytes no canalicular filling was detectable [93]. Regulation of Mrp2 by dexamethasone is in line with the finding that the 5′-flanking region of the *mrp2* gene contains several glucocorticoid-responsive elements [88], and regulation may involve distinct nuclear receptor signaling pathways [95].

3.3. Exogenous influences

The V_{max} of OAT1-mediated transport was found to be decreased after short-term exposure to hydrogen peroxide and gentamicin [96]. Hydrogen peroxide also induced a reduction in organic anion transport mediated by OAT3, whereas gentamicin had no effect on this transporter [96]. However, short-term exposure of nonperfused rabbit kidney S2 proximal tubule segments to cadmium resulted in an increase in PAH uptake [97]. The cadmium-induced stimulation could not be prevented by staurosporine, suggesting that the effect on PAH uptake was not signaled by PKC. In addition, the effect of long-term *in vivo* exposure of rats to cadmium on PAH transport in renal cortical basolateral membrane vesicles was studied [98]. After 2–3 weeks of cadmium administration, uptake of PAH in basolateral membrane vesicles was reduced markedly due to a decrease in V_{max}. In killifish renal proximal tubules, long-term exposure to cadmium resulted in an increased expression of Mrp2, which was accompanied by an increase in transport function [99]. Furthermore, Demeule *et al*. [100] demonstrated that renal Mrp2 expression was increased by approximately 10-fold after administration of cisplatin to rats, whereas the expression in liver was not affected. Cisplatin reacts with glutathione to form a cisplatin–glutathione conjugate, which is thought to be an Mrp2 substrate [101]. Therefore, the increase in transport protein might protect the cells by decreasing the intracellular concentration of cisplatin or cadmium. In contrast, short-term exposure to aminoglycoside antibiotics, radiocontrast agents, and heavy metals caused a decrease in Mrp2-mediated transport by a calcium-dependent endothelin release and subsequent signaling through PKC [99,102,103].

The regulation of organic anion transporters by xenobiotics in the liver has been studied more frequently. The mechanisms revealed in the liver may give an indication of the responses that can be expected in the kidneys, because several transporters are expressed in both hepatocytes and proximal tubules. Moreover, a number of pathophysiological conditions are comparable in both organs. Enhanced levels of Mrp2 mRNA and protein were detected after treatment with various compounds, such as 2-acetylaminofluorene [88,104], cisplatin [88,104], arsenite [105], tamoxifen [106], the antibiotic rifampicin and the putative anti-depressant hyperforin [95], clotrimazol [107], and phenobarbital [108]. The regulatory mechanism may differ for the various compounds. Induction of *mrp2* gene expression via members of the orphan nuclear receptor superfamily of ligand-activated transcription factors, among

which is the pregnane X receptor (the rodent analog of the human steroid and xenobiotic receptor, SXR), was found following treatment with rifampicin, clotrimazol, hyperforin, and phenobarbital [95,107,108]. Active gene transcription is also required for the induction of Mrp2 by arsenite [105], whereas the up-regulation of MRP2 by 2-acetylaminofluorene is thought to be due to enhanced stability of the mRNA [109]. It could be speculated that, in particular after exposure to a cytotoxic xenobiotic, regulation of MRP2 might be mediated by a mechanism induced by oxidative stress. This hypothesis is in agreement with the up-regulation of MRP2 seen in human colorectal carcinoma cell lines after exposure to prooxidants [110]. In response to oxidative stress, transcription factors, such as nuclear factor κB (NF-κB), or the activator protein 1 (AP-1) family are activated. In support, it was shown that the 5′-flanking region of the *mrp2* gene contains binding sites for AP-1 [88].

A number of compounds need to be conjugated to glutathione to become a substrate for MRP2, suggesting that the enzymes glutathione *S*-transferase and γ-glutamylcysteine synthetase may play a role in the induced MRP2-mediated transport. Glutathione *S*-transferase is a phase II drug-metabolizing enzyme involved in the conjugation of xenobiotics to glutathione, while γ-glutamylcysteine synthetase catalyzes the rate-limiting step in the synthesis of glutathione. Liu *et al*. [111] demonstrated that acquired tolerance to arsenic is obtained by the concerted up-regulation of glutathione *S*-transferase II, Mrp1, Mrp2, and the xenobiotic transporter P-glycoprotein. P-glycoprotein is a member of the ABC superfamily of transporters and handles, in general, uncharged and cationic compounds [112]. The increased rate of gene transcription and the subsequent enhanced level of the protein amount of glutathione *S*-transferase II and the three drug efflux pumps function together to reduce the intracellular concentration of arsenite. Arsenic tolerance was abolished by inhibition of glutathione *S*-transferase II or glutathione synthesis, emphasizing the important role of glutathione *S*-transferase. In addition, a co-induction of the heavy subunit of the γ-glutamylcysteine synthetase and the *mrp2* genes in mouse hepatocytes has been described after xenobiotic treatment [113]. Furthermore, heavy metals co-induce the expression of MRP1 and γ-glutamylcysteine synthetase [114]. A strong correlation between the mRNA levels of MRP1 and γ-glutamylcysteine synthetase was also seen in various drug-resistant cells and untreated tumor cells. However, this study did not find a similar correlation for MRP2 in these cell lines [115]. This may be due to differences between the *in vivo* and *in vitro* situation.

In addition to regulation by xenobiotics, organic anion transport has also been demonstrated to be influenced by pathologic conditions of the kidneys or the liver. During chronic renal failure induced by subtotal nephrectomy, the protein and mRNA expression of Mrp2 are strongly elevated [116]. The induction may be due at least in part to changes in the transcriptional rate and/or in mRNA stability because the elevations in the amount of protein and mRNA were comparable. After a longer period of chronic renal failure, expression of Mrp2 was also induced in the liver [116], indicating a correlation between the function of the kidneys and the liver. In chronic renal failure, the elimination of numerous endogenous and exogenous compounds is reduced, and the accumulation of these compounds in plasma may lead to renal as well as hepatic up-regulation of Mrp2. The expression of the different organic anion transporters was also studied in the regenerating rat liver after partial hepatectomy. The basolaterally localized hepatic Oatp1 was down-regulated at both mRNA and protein levels, while the expression of luminal Mrp2 remained unchanged or was increased slightly [117]. However, Mrp2 is regulated differently during cholestasis. There are several methods used to induce cholestasis, among which are treatment with lipopolysaccharide and bile duct ligation. All models resulted in a decrease in Mrp2 protein and its localization at the canalicular membrane, probably due to enhanced retrieval of the transport protein because Mrp2 was detected in a subapical vesicular compartment [89,118–120]. Beside this early regulatory pathway, the amount of mRNA Mrp2 was also shown to be decreased, suggesting modulation of transport by a transcriptional mechanism [89,118,120]. In contrast, Paulusma *et al*. [119] hypothesized that the down-regulation of Mrp2 is mainly regulated at the posttranscriptional level, because no significant changes in mRNA levels were found. The reason for this discrepancy is unknown.

4. Concluding remarks

Renal transport of organic anions appears to be an important defense mechanism of the organism against foreign substances. This is emphasized by the evolutionary preservation of the system, the wide substrate specificity, and the substrate overlap between the various renal transporters (for details, see Refs. [1,3]). Renal organic anion transport is under the influence of various regulatory mechanisms, and nephrotoxic compounds and acute renal failure affect the regulation. For example, Mrp2-mediated transport is reduced after short-term exposure of killifish proximal tubules to aminoglycoside antibiotics, radiocontrast agents, and heavy metals [99,103]. However, exposing tubules for 6 hr to heavy metals resulted in an increased efflux of the Mrp2 substrate fluorescein-methotrexate [99]. Induced transport was at least partly due to an increase in the amount of transporters in the luminal membrane. Figure 3 summarizes this sequence of events. The underlying mechanisms have yet to be elucidated. In general, an increased amount of transporters in the membrane is obtained by modifications of gene transcription, mRNA stability, mRNA translation, or an increased incorporation by exocytosis. However, an increased insertion

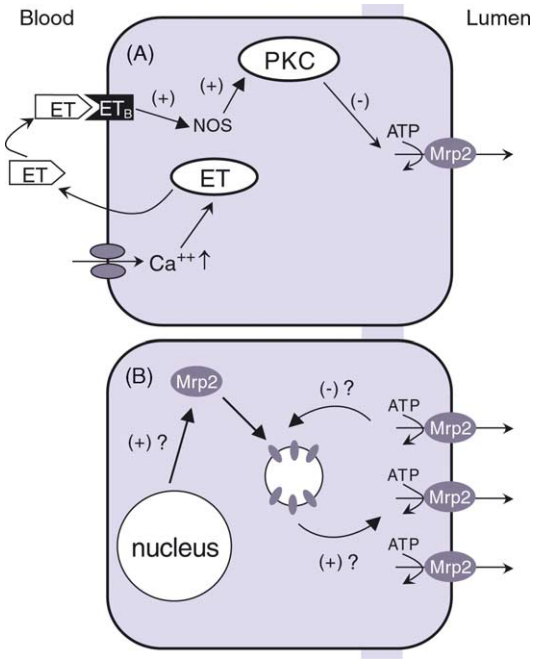

Fig. 3. Scheme illustrating (A) the mechanism of inhibition of Mrp2-mediated transport after short-term exposure to nephrotoxicants, and (B) the induction of Mrp2-mediated transport after long-term exposure to heavy metals. (A) Short-term exposure to various nephrotoxic compounds causes an increase of the intracellular calcium (Ca^{2+}) concentration caused by opening of the Ca^{2+} channels. This stimulates the release of endothelin (ET), which subsequently activates the basolateral endothelin-B receptor (ET_B), NO synthase (NOS), and PKC, finally leading to a reduction in Mrp2-mediated transport. (B) Long-term exposure to heavy metals results in an increased Mrp2-mediated transport due to an enhanced amount of transporters in the luminal membrane. This could be caused by transcriptional or translational regulation or by increased insertion of Mrp2 in the luminal membrane.

of transporters is thought to be a fast response to stimuli, whereas regulation at the level of transcription and translation is considered to mediate long-term effects. An important question that remains unanswered is the reason for the dual regulation. Obviously, Mrp2 is important for the elimination of potentially harmful compounds from the body and for the reduction of the intracellular concentrations of anionic compounds in the proximal tubule. In this way, Mrp2 has a protective function for the body and for the renal proximal tubule in particular. Moreover, additional functions are suggested for P-glycoprotein among which is protection against apoptosis [121]. It could be speculated that Mrp2, as another member of the ABC transporter family, also exhibits such protective functions, supporting the hypothesis that the induction of Mrp2 after long-term exposure to nephrotoxicants is triggered to prevent (further) tubular injury. Then why is the Mrp2-mediated transport initially reduced? The reduction is signaled by endothelin, a hormone responsible for the inhibition of a number of tubular ATP consuming transporters [68]. Therefore, it could be speculated that the function of endothelin release and the subsequent inhibition of the drug efflux pump is to conserve ATP for cellular processes more directly related to cell survival. If the nephrotoxicant were transported by Mrp2, then conditions would be even more deleterious for the cell. Moreover, exposure of cells to toxic compounds generally leads to the production of harmful compounds. Glutathione disulfide is one of the compounds generated during oxidative stress, and efflux of this potentially harmful compound from the cell is mediated by Mrp2 [122]. Therefore, it seems more reasonable to assume that the initial reduction is only part of the pathological events that occur during tubular injury and that the cell needs some time to protect itself by inducing Mrp2-mediated transport. If this is true, the clinical use of endothelin-B receptor antagonists may be a novel approach to prevent some of the early detrimental effects seen after treatment with nephrotoxic drugs.

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