

P&T #21533

**Pathophysiological regulation of renal SLC22A organic ion transporters in acute
kidney injury: Pharmacological and toxicological implications**

Hideyuki Saito *

*Department of Pharmacy, Kumamoto University Hospital; Department of Clinical
Pharmaceutical Sciences, Faculty of Medical and Pharmaceutical Sciences,
Kumamoto University, 1-1-1 Honjo, Kumamoto, Kumamoto 860-8556, Japan*

*Corresponding author. Tel. & fax: +81 96 373 5820
E-mail address: saitohide@fc.kuh.kumamoto-u.ac.jp (H. Saito)

Abstract

The kidneys play a primary role in maintaining homeostasis and detoxification of diverse hydrophilic xenobiotics as well as endogenous by-products. Solute carrier (SLC)22A organic ion transporter family members mediate renal excretion of both endogenous and exogenous substances. Thus, the functional and molecular variations of renal SLC22A transporters under acute kidney injury (AKI) have an impact on systemic clearance of their substrate drugs, resulting in altered pharmacokinetics or unexpected adverse events caused by the accumulation of drugs. Recently, there have been significant advances in our understanding of the regulatory mechanisms for transcription, membrane trafficking and/or kidney-specific expression of SLC22A6/OAT1, SLC22A8/OAT3 and SLC22A2/OCT2. Hepatocyte nuclear factor (HNF)-1 α / β and HNF-4 appear to play key roles in the transcriptional regulation of OAT1 and OAT3. Furthermore, OAT1 activity/function is modulated *via* phosphorylation mediated by protein kinase C (PKC) and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathways. AKI affects renal disposition of organic ions in association with the deteriorated glomerular filtration and tubular transport functions. Thus, dysfunctional regulation of SLC22A transporters during AKI induced by ischemia or toxicants, such as cisplatin, inorganic mercury or uranyl nitrate, cause uremic syndromes or adverse drug reactions. Indoxyl sulfate, a uremic toxin and substrate of OAT1 and OAT3, appears to mediate the progression of AKI evoked by renal ischemia and cisplatin treatment. Precise mechanisms for regulation of the SLC22A transporters in AKI require studies based

on the transcription, trafficking, phosphorylation and endogenous factor-dependent modulation. Such analysis will provide a better understanding of the pathophysiological implications of SLC22A transporters.

Keywords: SLC22A transporter family; Acute kidney injury; Ischemia/reperfusion; Nephrotoxicity; Indoxyl sulfate; Renal tubular secretion.

Abbreviations: AKI, acute kidney injury; ARE, androgen response elements; BUN, blood urea nitrogen; CRE, cyclic AMP-response element; CRF, chronic renal failure; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; ES, estrone sulfate; HNF, hepatocyte nuclear factor; IS, indoxyl sulfate; I/R, ischemia/reperfusion; MAPK, mitogen-activated protein kinase; NSAID, non-steroidal anti-inflammatory drug; MATE, multidrug and toxin extrusion; MEK, mitogen-activated/extracellular-signal regulated kinase kinase; OAT, organic anion transporter; OCT, organic cation transporter; PAH, p-aminohippuric acid; PG, prostaglandin; PKA, protein kinase A; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; SCr, serum creatinine; SLC, solute carrier; TEA, tetraethylammonium; UN, uranyl nitrate.

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1. Introduction

The kidneys play a primary role in homeostasis and detoxification. One key function of the kidneys is the disposition of diverse hydrophilic xenobiotics, including drugs and toxins, as well as endogenous by-products. Epithelial cells in the renal proximal tubules mediate directional solute transport, facilitating urinary secretion of a wide range of organic ions (Ullrich, 1994; Pritchard & Miller, 1996; Inui et al., 2000). Solute carrier (SLC)22A family members mediating transport of organic anions and cations in renal tubular cells appear to play a pivotal role in the efficient transepithelial secretion of substrates (Fig. 1) (Inui et al., 2000; Koepsell & Endou, 2004; Robertson & Rankin, 2006). Therefore, the functional and molecular variations of renal SLC22A transporters in association with kidney diseases and/or injuries have an impact on systemic clearance of their substrate drugs, resulting in altered pharmacokinetics or unexpected adverse events by accumulated drugs. Investigations on regulatory mechanisms involved in functional activities, membrane trafficking, tissue-specific expression, transcription of SLC22A transporters, especially of SLC22A6/OAT1, SLC22A8/OAT3 and SLC22A2/OCT2, have greatly advanced our understanding of their physiological and pharmacological roles.

Acute kidney injury (AKI) is recognized as functional or structural disturbances of the kidney including abnormalities in blood, urine or tissues present for less than three months (Star, 1998). AKI can be brought about by decreased renal blood flow, a toxic or obstructive insult to the renal tubule, tubular interstitial inflammation and edema, or primary decrease in the filtering capacity of the

glomerulus (Shanley et al., 1986). In general, ischemia and several toxicants, including therapeutic drugs, account for the largest number of AKI. Ischemia and toxicants often combine to induce AKI in severely ill patients with conditions such as sepsis, hematologic cancers or acquired immunodeficiency syndrome (Thadhani et al., 1996). Renal ischemia-induced AKI, which is caused by a complicated interaction between renal hemodynamics, inflammatory cytokines, and endothelial and tubular cell injuries, is a major clinical issue (Schrier et al., 2004). The kidney receives about 25% of the cardiac output, and the majority flows into the cortex. Therefore, even a slight decline in renal blood flow results in hypoxic injury of the medullary region.

Ischemia-induced renal medulla injury is of pathophysiological significance in AKI.

Among potential nephrotoxic drugs, including aminoglycoside and glycopeptide antibiotics, contrast mediums, non-steroidal anti-inflammatory drugs and immunosuppressive drugs, cisplatin is known to cause severe AKI during treatment of cancer. Cisplatin is one of the most commonly used antitumor drugs for the treatment of several solid tumors. The nephrotoxic effect of cisplatin is cumulative and dose-dependent, and often requires its dose reduction or cessation of treatment. Therefore, an understanding of the pathological events of cisplatin-induced AKI is a clinical subject for developing adjunctive therapies to prevent AKI. However, there are few reports concerning alteration of renal solute transporters and disposition of xenobiotics or endogenous metabolites, including uremic toxins such as indoxyl sulfate (IS), as a result of cisplatin-induced AKI.

This review article summarizes recent findings concerning the functional and transcriptional regulation of renal SLC22A transporters, and addresses the pharmacological/toxicological implications of the altered regulation of these transporters brought about by AKI.

2. Pharmacology and regulation of renal SLC22A transporters

Rat organic cation transporter OCT1, the first member of the SLC22 family of transporters, was originally identified by expression cloning (Gründemann et al., 1994). A second member of this family of transporters, rat OCT2, was described two years later (Okuda et al., 1996). In 1997, the first organic anion transporter OAT1 was identified from rat and flounder (Sekine et al., 1997; Sweet et al., 1997; Wolff et al., 1997), followed by OAT3 from rat (Kusuhara et al., 1999). Numerous studies on pharmacological and toxicological aspects of these SLC22A transporters have been conducted, thereby providing much information on their functional roles in renal handling and/or disposition of drugs and toxicants. In parallel, the mechanisms of regulation of these renal transporters have advanced from the viewpoint of tissue-specific expression, transcription, membrane trafficking and modulation of functions.

2.1. *SLC22A6/OAT1*

Renal secretion of anionic xenobiotics is performed sequentially by the concerted function of two distinct transport steps in the basolateral membranes and

luminal brush-border membranes of the tubular cells (Inui & Okuda, 1998; Inui et al., 2000). The basolateral transport step of the negatively charged anions requires the energy-dependent uphill uptake, as they must be transported against an electrical potential barrier across the membrane. As the first step towards the molecular characterization of organic anion transporters, Sekine et al. (1997) and Sweet et al. (1997) used an expression cloning technique to isolate a cDNA encoding p-aminohippurate (PAH, a typical substrate for the renal organic anion transport system)/dicarboxylate exchanging protein, which was designated as OAT1. An immunohistochemical study revealed that human (h)OAT1 is exclusively expressed in the renal proximal tubules, being localized to the basolateral membranes (Hosoyamada et al., 1999; Motohashi et al., 2002). The uptake rate of PAH was markedly enhanced in the presence of an outward gradient of dicarboxylate, such as α -ketoglutarate. rOAT1 exhibited a multispecificity for endogenous anions, such as cyclic nucleotides, prostaglandins (PG) and uric acid, as well as toxins and structurally diverse drugs, such as β -lactam antibiotics, methotrexate and nonsteroidal anti-inflammatory drugs (NSAIDs) (Uwai et al., 1998; Tsuda et al., 1999; Sekine et al., 2000; Burckhardt & Burckhardt, 2003; Sweet, 2005; Rizwan & Burckhardt, 2007).

The SLC22A OAT family isoforms possess common structural features, including 12 transmembrane domains flanked by intracellular N- and C-termini; multiple glycosylation sites located in the first extracellular loop between transmembrane domains 1 and 2; and potential phosphorylation sites localized in the intracellular loop between transmembrane domains 6 and 7 and in the C-terminus

(Burckhardt & Wolff, 2000; Dantzler & Wright, 2003). Recent studies concerning structure-function relationship of OAT isoforms suggested that glycosylation is essential for the adequate trafficking of these transporters to the plasma membrane (Tanaka et al., 2004). In addition, protein kinase C (PKC) activation was shown to result in a downregulation of organic anion transport activity in the isolated renal tubules, cultured renal cells (OK) (Nagai et al., 1997) and/or the cells transfected with OAT1 (Lu et al., 1999). Several mechanisms by which PKC could modulate OAT1 activity have been proposed.

Previous reports suggested that PMA and other PKC modulators failed to activate the phosphorylation of OAT1 under various experimental situations, demonstrating that direct phosphorylation may not evoke PKC-induced downregulation of OAT1 activity. Wolff et al. (2003) suggested that PKC-induced hOAT1 downregulation is achieved through carrier retrieval from the cell membrane and does not involve phosphorylation of the predicted classic hOAT1 PKC consensus sites. Kinetic analysis revealed that the decreased transport activity of OAT1 induced by PKC activation was associated with a reduced maximal transport velocity V_{max} , without significant change in the substrate affinity K_m . Direct phosphorylation of OAT1 protein by PKC could cause changes in the turnover of the transporter, i.e., downregulation, without the substrate affinity.

Thus, PKC-mediated downregulation of OAT1 activity could result from internalization of the transporter and/or suppression of the recruitment of the matured transporter into the target membrane. PKC could phosphorylate protein(s) modulating OAT1 in the trafficking pathway. Recently, several hOAT1-interacting proteins have

been isolated (Zhang et al., 2008). They showed that transfection of dominant negative mutant of dynamin-2 into the COS-7 cells reduced the amount of surface-labeled OAT1 internalized both in the presence and in the absence of PMA as compared with that of the control (Fig. 2). The study assumed constitutive OAT1 trafficking between cell membrane and recycling endosomes, PKC activated-downregulation of OAT1 activity, and OAT1 internalization partly through a dynamin and clathrin-dependent pathway (Zhang et al., 2008).

By using cultured renal epithelial OK cells, Sauvant et al. (2003) reported that epidermal growth factor (EGF) stimulates basolateral PAH uptake through the mitogen-activated protein kinase (MAPK) pathway *via* sequential activation of mitogen-activated/extracellular-signal regulated kinase kinase (MEK) and extracellular signal-regulated kinase isoforms 1 and 2 (ERK1/2). The stimulation of PAH uptake induced by EGF was completely inhibited by MEK inhibitors, such as PD98059 or U0126. Phosphorylation of ERK1/2 in OK cells by treatment with EGF was abolished by inhibiting MEK. Indeed, inhibition of MEK results in a similar effect on PAH transport. Using a series of inhibitors and an assay for arachidonic acid release, Sauvant et al. (2003) concluded that EGF activated MEK, ERK1/2 and phospholipase A₂ (PLA₂), resulting in an increased release of arachidonic acid. Subsequently, arachidonic acid is metabolized to prostaglandins by the action of cyclooxygenase 1, which then causes EGF-induced stimulation of basolateral PAH transport. In isolated rabbit S2 proximal tubule segments, prostaglandin E₂ (PGE₂) stimulated basolateral PAH uptake and this stimulation is suppressed by the PKA inhibitor H89. Thus, it was

suggested that basolateral PAH transport, presumably OAT1 activity, is downregulated by activation of PKC and upregulated *via* MAPK through PLA₂, PGE₂, cyclic AMP and PKA activation. EP4 receptor was suggested to be a candidate of PGE₂ action for the regulation of organic anion transporter(s) in renal tubular cells.

It has been proposed that hOAT1 undergoes transcriptional regulation.

Ogasawara et al. (2007) reported that hepatocyte nuclear factor (HNF)-4 α markedly transactivated the OAT1 promoter (Fig. 3). A deletion analysis of the OAT1 promoter revealed that the regions spanning -1191 to -700 base pairs (bp) and -140 to -79 bp appeared to be essential for the transactivation by HNF-4 α . These regions contained a direct repeat separated by two nucleotides (DR-2), which is one of the consensus sequences binding to HNF-4 α , and an inverted repeat separated by eight nucleotides (IR-8), which was recently identified as a novel element for HNF-4 α , respectively. By using an electrophoretic mobility shift assay, HNF-4 α was shown to bind to DR-2 and IR-8 in association with HNF-4 α overexpression. Under normal conditions HNF-4 α binds to IR-8. A mutation in IR-8 markedly reduces the promoter activity, suggesting that HNF-4 α regulates the basal transcription of OAT1 through IR-8. In contrast, Saji et al. (2008) suggested that HNF-1 α plays an essential role in the transcriptional regulation of human and mouse OAT1 expression. They suggested that HNF-1 α /HNF-1 β heterodimer plays an important role in the constitutive expression of human and mouse OAT1 genes. Because the transcriptional activation by HNF-1 α appears to be a common feature of OAT1 and OAT3, this transcription factor might be

involved in the physiological regulation of endobiotics and/or xenobiotics transport in the kidney.

2.2. *SLC22A8/OAT3*

rOAT3 was originally identified from rat brain by using a RT-PCR cloning strategy based on the sequence conserved between rOAT1, rOAT2 and the rat organic cation transporter (Kusuhara et al., 1999). Indeed, rOAT3 exhibits 49, 39 and 36% identity with rOAT1, rOAT2 and rOCT1, respectively. rOAT3 mRNA appeared to be expressed in the liver, brain, kidney and eye. It has been proposed that rOAT3 mediates the uptake of anionic substrates from the blood across the basolateral membrane into the proximal tubule cells in exchange for cytoplasmic dicarboxylates (Sweet et al., 2003).

The physiological function of OAT3 was assumed to be the secretion of both steroid hormones, and their corresponding conjugates, as well as prostaglandins. OAT3 expressed in brain and kidneys could cooperate in the removal of anionic neurotransmitter metabolites. hOAT3 was reported to be localized at the basolateral membranes of human kidney (Motohashi et al., 2002) where it is responsible for the transport of several substrates including the second messenger cAMP; the hormones cortisol, PGE₂ and F_{2 α} ; the conjugated hormones dehydroepiandrosterone sulfate, estrone sulfate (ES) and estradiol-17 β -glucuronide; the bile salt taurocholate; and the purine metabolite urate. The transport ability for corticosterone, ES,

estradiol-17 β -glucuronide and taurocholate distinguishes OAT3 from OAT1 (Burckhardt & Burckhardt, 2003; Rizwan & Burckhardt, 2007).

OAT3-knockout mice are fertile and have a normal appearance, with no detectable tissue abnormalities in the kidneys, liver and brain. The uptake of taurocholate, ES and bromosulfophthalein into kidney slices from the OAT3-knockout mice was reduced, as was the uptake of fluorescein into cells of the choroid plexus (Sweet et al., 2002). rOAT3 mediated uptake of organic anions such as PAH (K_m value of 65 $\mu\text{mol/L}$), ochratoxin A (K_m value of 0.74 $\mu\text{mol/L}$) and ES (K_m value of 2.3 $\mu\text{mol/L}$). Moreover, rOAT3-mediated uptake of ES was inhibited by other anions such as sulfobromophthalein, probenecid, indocyanine green, bumetanide, piroxicam, furosemide, azidodeoxythymidine and benzylpenicillin, but not by cationic compounds such as tetraethylammonium (TEA), guanidine or quinidine (Kusuhara et al., 1999).

OAT3 isoforms in different species consist of 536-542 amino acids, exhibiting 12 trans-membrane domains with a large extracellular loop between transmembrane domain 1 and 2, and a large intracellular loop between transmembrane domain 6 and 7 possessing potential phosphorylation sites to be regulated by protein kinases (Robertson & Rankin, 2006; Rizwan & Burckhardt, 2007). PKC activation resulted in an inhibition of rOAT3 activity probably by induction of internalization. Experiments conducted using intact renal proximal tubules indicated that OAT3-mediated organic anion transport was downregulated by both direct and indirect (physiological) activation of PKC (Soodvilai et al., 2004). In contrast, OAT3 activity was upregulated

by EGF through activation of the MAPK pathway and subsequently through PKA activation (Fig. 4). Using a functional promoter assay of human OAT3, Ogasawara et al. (2006) demonstrated that cAMP-response element (CRE) could be essential for the basal and inducible promoter activity of OAT3. Furthermore, CREB-1 and ATF-1 were found to bind to CRE, thereby activating the transcription of the OAT3 gene for constitutive expression. PKA further activates the transcription of the OAT3 gene through phosphorylation of both CREB-1 and ATF-1 for inducible expression (Fig. 5).

The mechanism underlying the proximal tubule-restricted expression of hOAT3 in the kidney is still not fully understood. Kikuchi et al. (2006) reported that the expression of hOAT3 is positively regulated by HNF-1 α and HNF-1 β and negatively regulated by DNA methylation, suggesting that the coordinated action of genetic and epigenetic factors are involved in the tissue-specific expression of hOAT3.

2.3. *SLC22A2/OCT2*

In the renal tubules, organic cation transporters play physiological and pharmacological roles in the reabsorption and/or excretion of endogenous organic cations such as guanidine, choline, N¹-methylnicotinamide, neurotransmitter monoamines (dopamine, epinephrine, and histamine) (Busch et al., 1998), cationic drugs (TEA, cimetidine, procainamide, and quinidine) and toxins (Inui & Okuda, 1998; Inui et al., 2000). Organic cation transport activity is localized predominantly in renal proximal tubules, but has also been detected in distal tubules and collecting ducts. Functional studies using isolated renal tubules, stop-flow microperfusion of proximal

tubules, and isolated membrane vesicles, suggested that renal secretion of cationic substances is performed by the concerted function of two distinct organic cation transporters: one facilitated by the electrical potential difference at the basolateral membranes and the other driven by the H^+ gradient at the brush border membranes (Holohan & Ross, 1981; Hsyu & Giacomini, 1987; Wright & Wunz, 1987; Miyamoto et al., 1989; Montrose-Rafizadeh et al., 1989; Sokol & McKinney, 1990; Groves et al., 1994). Using isolated brush-border membrane vesicles and cultured renal epithelial cell lines, such as LLC-PK₁ cells, the transport system in these membranes appeared to be mediated by an electroneutral H^+ /organic cation antiporter energized by transmembrane H^+ gradient, which can be sustained by the Na^+/H^+ exchanger and/or H^+ -ATPase (Takano et al., 1984; Inui et al., 1985; Saito et al., 1992; David et al., 1995). The H^+ /organic cation antiporter in renal brush-border membranes has been proposed to mediate directional excretion of cationic drugs or toxins out of renal tubular cells. Recently, the apical type of H^+ /organic cation antiporter, rat multidrug and toxin extrusion (MATE1/SLC47A1) have been identified and functionally characterized (Otsuka et al., 2005; Terada et al., 2005). rMATE1 appeared to be expressed mainly in the kidney, where it mediates the final step of urinary excretion of cationic drugs. In addition, Masuda et al. (2006) identified multidrug and toxin extrusion 2 (hMATE2-K), a novel human kidney-specific H^+ /organic cation antiporter that is responsible for the tubular secretion of cationic drugs across the brush border membranes.

Gründemann et al. (1994) identified the first member of the organic cation transporter family, designated as OCT1, from the rat kidney by expression cloning. rOCT1 is comprised of 556 amino acids with 12 putative transmembrane domains. Immunohistochemical analysis revealed that rOCT1 was localized to the basolateral membranes of S1 and S2 segments of proximal renal tubules and the small intestine and liver. Electrophysiological studies using rOCT1-expressing oocytes under voltage-clamped conditions demonstrated that positive inward currents were induced when TEA, NMN, choline or dopamine was added to the bath medium (Busch et al., 1996). Moreover, electrogenic transport was found for TEA uptake in the renal basolateral membranes (Busch et al., 1996). The presence of a kidney-specific organic cation transporter was deduced, based on pharmacokinetic behavior of various cationic drugs. Hybridization techniques using rOCT2 as a probe identified a cDNA encoding OCT2 from rat kidney (Okuda et al., 1996). rOCT2 is comprised of 593 amino acids with 12 proposed putative transmembrane domains showing a 67% identity to rOCT1. The rOCT2 mRNA transcript was detected predominantly in the kidney, at higher levels in the medulla than the cortex, but not in the liver, lung or intestine (Okuda et al., 1996). TEA uptake by rOCT2-transfected MDCK cells grown on microporous membrane filters was markedly enhanced when TEA was added to the basolateral bath medium, but not to the apical medium (Urakami et al., 1998). Structurally diverse organic cations, including the type 1 cations such as cimetidine, NMN, nicotine and procainamide, and type 2 cations, such as quinine and quinidine, inhibited TEA uptake in the transfectants. Inhibition studies suggested that rOCT1 and

rOCT2 had similar inhibitor binding affinities for many compounds, but showed moderate differences in inhibitor sensitivity for several compounds such as MPP, procainamide, dopamine, and testosterone i.e., by a factor of 2 to 3. rOCT2 and hOCT2, which share 80% amino acid identity, appeared to recognize monoamine neurotransmitters such as dopamine, norepinephrine, epinephrine, 5-hydroxytryptamine and amantadine (Gründemann et al., 1998; Urakami et al., 1998; Koepsell et al., 1999; Okuda et al., 1999). OCT2 has been proposed to play a physiological role in renal disposition of some bioactive monoamines, implying that OCT2 might function as a clearance transporter for these monoamines.

It was reported that the uptake of tetraethylammonium was greater in renal cortical slices of male rats than female rats, suggesting gender differences in the basolateral membrane transport activity for organic cations (Urakami et al., 1999). A previous study reported that the expression level of rOCT2, but not rOCT1 and rOCT3, in the kidney was much higher in males than females (Urakami et al., 1999). This finding suggests that rOCT2 is responsible for the gender differences in renal basolateral organic cation transport activity. Furthermore, treatment of male and female rats with testosterone significantly increased rOCT2 expression in the kidney (Urakami et al., 2000). hOCT2 mRNA levels were increased significantly following exposure of MDCK to the steroid hormones, dexamethasone (2.0-fold), hydrocortisone (2.4-fold) or testosterone (1.8-fold). It was proposed that steroid hormones induce the transcription of OCT2 in the kidney, which plays a pivotal role in the transcriptional regulation of the rOCT2 gene (Shu et al., 2001). Asaka et al. (2007)

reported that rOCT2 promoter activity was stimulated by testosterone. This stimulation was blocked by nilutamide, an antiandrogen agent. Reporter assays using deletion constructs and mutational constructs of putative androgen response elements (ARE) in the rOCT2 promoter region revealed that two AREs, located at approximately 3000 and 1300, respectively, play an essential role in the induction by testosterone (Fig. 6) (Asaka et al., 2006). Testosterone induces the expression of rOCT2, but not of rOCT1 and rOCT3, *via* the AR mediated transcriptional pathway.

Rabbit OCT2-mediated organic cation transport in stable transfectants and intact renal tubules was influenced by MAPK and PKA activity (Soodvilai et al., 2007). Activation of these protein kinases resulted in stimulation of OCT2 activity whereas inhibition led to depression of OCT2 activity. Regulation of OCT2 was similar to that observed for OAT1 and OAT3. These findings suggest that regulation of renal tubular transport of organic anions and cations involves a common MAPK and PKA pathway.

OCT2 appears to be expressed almost exclusively in the kidney, but not in the liver. The regulatory mechanisms involved in this kidney-specific expression of OCT2 has been explored. Aoki et al. (2008) reported the *in vivo* methylation status of the proximal promoter region of OCT2 by using bisulfite sequencing of human genomic DNA extracted from the kidney and liver. All CpG sites in the OCT2 proximal promoter were hypermethylated in the liver, but hypomethylated in the kidney. In contrast, the promoter region of OCT1 was hypermethylated in both the kidney and liver. The level of methylation of the OCT2 promoter was especially low at the CpG site in the E-box, which is the binding site of the basal transcription factor upstream

stimulating factor (USF) 1. *In vitro* methylation of the OCT2 proximal promoter dramatically reduced the transcriptional activity, and an electrophoretic mobility shift assay showed that methylation at the E-box inhibited the binding of USF1 (Aoki et al., 2008). These results suggested that kidney-specific expression of human OCT2 is regulated by the methylation status of the proximal promoter region, which may interfere with transactivation by USF1. In addition, USF-1 appeared to function as a basal transcriptional regulator of the hOCT2 gene *via* the E-box, suggesting the *cis*-elements and *trans*-factors are necessary for regulation.

3. Altered regulation of SLC22A transporters in kidney diseases

Functional disturbances in renal excretion of organic ions may be of clinical importance, especially in the use of drugs with high toxicity or narrow therapeutic range. Serious kidney diseases, such as AKI, have an impact on renal disposition of diverse organic ions in association with decreased glomerular filtration and tubular transport functions. Altered regulation of renal SLC22A transporters and its impact on pharmacokinetics of their substrate drugs have progressed, in conjunction with physiological and pathological roles of uremic toxins in kidney injuries.

3.1. Ischemia/reperfusion-induced AKI

Ischemia/reperfusion (I/R)-induced AKI is a crucial clinical issue (Star, 1998; Schrier et al., 2004). I/R-induced AKI is evoked by a complicated interaction between renal hemodynamics, inflammatory cytokines, and endothelial and tubular cell injury

(Thadhani et al., 1996; Kribben et al., 1999). Although the kidney receives about 25% of the cardiac output, the majority goes to the cortex. Therefore even a slight decline in renal blood flow results in hypoxic injury of the medullary region. I/R-induced injury to the renal medulla plays a significant role in AKI. In fact, the S3 segment of the proximal tubule in the outer medulla has been shown to be the most susceptible portion of the kidney to I/R-induced AKI. The serum level of the uremic toxin indoxyl sulfate (IS) is markedly elevated in uremic patients and in 5/6 nephrectomized rats, a well established animal model for chronic renal failure (CRF) (Enomoto & Niwa, 2007). IS appears to be a substrate for OAT1 and OAT3 (Deguchi et al., 2002, 2004, 2005). In the 5/6 nephrectomized rats, the expression of rOAT1 and rOAT3 was markedly downregulated in the kidney of CRF (Ji et al., 2002). In the clinical situation, patients with renal disease showed a downregulation of hOAT3, which is thought to be responsible for a decreased urinary excretion of cefazolin, an anionic cephalosporin antibiotic (Sakurai et al., 2004, 2005).

Histological examination of kidneys from rats with AKI showed that tubular necrosis occurred in sporadic renal epithelial cells. AKI is characterized by tubular dysfunction with impaired sodium and water reabsorption, which is associated with the shedding and excretion of brush-border membranes and tubular cells into the urine. Following I/R, morphological changes occur in the proximal tubules, including loss of cell polarity and brush border structure, and redistribution of integrins and Na^+/K^+ -ATPase along the apical membrane (Molitoris et al., 1992). Because renal tubular secretion of xenobiotics and endogenous uremic toxins is performed by

organic ion transporters normally localized at the basolateral and brush border membranes, renal tubular dysfunction will adversely affect excretion of these compounds.

Recently, Matsuzaki et al. (2007) suggested that the serum IS level is markedly elevated in ischemic rats with I/R-induced AKI. IS has been shown to induce several genes, including transforming growth factor (TGF)- β 1 and tissue inhibitor of metalloproteinase (TIMP)-1, thereby accelerating the progression of renal sclerosis in subtotal nephrectomized rat kidney (Miyazaki et al., 1997, 2000; Aoyama et al., 2003). The increased serum IS in ischemic AKI rats appeared to enhance acute renal tubular dysfunction. Because >95% of serum IS is bound to albumin, IS is excreted mostly into urine *via* tubular secretion, rather than glomerular filtration. Renal OAT1 and OAT3 have been reported to mediate IS uptake into renal tubular cells (Enomoto et al., 2002).

Inulin and PAH clearance, along with PAH net secretion, were initially diminished after I/R injury but gradually recovered during follow-up. This initial impairment after AKI was accompanied by decreased mRNA and protein levels of OAT1 and OAT3 in clamped rats compared with control rats. The subsequent improvement of renal function was mirrored by an upregulation in the expression of both OAT1 and OAT3 during follow-up. Thus decreased expression of OAT1 and OAT3 could account for the reduced level of PAH secretion after ischemic AKI. As a consequence, the biological effects of endogenous anions may be affected in ischemic AKI. The renal tubular elimination of prostaglandin E₂ appeared to be inhibited after

AKI, presumably preventing a washout of prostaglandin E₂ and thereby suggesting a rescue mechanism for maintenance of renal circulation after ischemic injury.

The expression of rOAT1 and rOAT3 at both the mRNA and protein level were downregulated in ischemic rat kidney (Fig. 7) (Schneider et al., 2007; Matsuzaki et al., 2008). In parallel, organic anion transport activity at the basolateral membrane was significantly reduced in the ischemic rat kidney, which was evaluated by the reduced accumulation of PAH and ES into renal slices. Kwon et al. (2008) reported the altered distribution of OAT1 after ischemia/reperfusion of rat kidney using confocal microscopy with a three-dimensional reconstruction of serial optical images. OAT1 was distributed to basolateral membranes of proximal tubule cells in controls, whereas OAT1 decreased in basolateral membranes, especially in the lateral membrane domain, and appeared diffusely in cytoplasm of ischemia/reperfusion kidney. OAT1 mediates renal tubular uptake of several pharmacological agents, such as methotrexate, β -lactam antibiotics and NSAIDs, whereas OAT3 appeared to efficiently mediate ES transport with relatively high affinity in addition to several anionic compounds such as PAH, methotrexate, PGE₂ and cAMP (Burckhardt & Burckhardt, 2003). Therefore, downregulation of both rOAT1 and rOAT3 should account for the decrease in PAH and ES uptake at the basolateral membrane of tubular cells in ischemic kidney. Alternatively, the driving force of rOAT1 and rOAT3 activity may be unbalanced in renal tubular cells of I/R kidney. rOAT1 and rOAT3 appeared to mediate exchange of anionic substrates with dicarboxylic acids, including α -ketoglutarate, where its cytoplasmic concentration is much higher than in the serum, thereby producing an

outward-directed driving force for influx of anionic substrates. The Na^+/K^+ -ATPase actively pumps Na^+ out of the renal proximal tubule cells thereby sustaining an inwardly directed Na^+ gradient. The inward movement of Na^+ drives the uptake of α -ketoglutarate by a Na^+ /dicarboxylate cotransporter. Decreased expression of Na^+/K^+ -ATPase in the ischemic kidney (Van Why et al., 1994) could collapse the inward Na^+ gradient, thereby reducing uptake of α -ketoglutarate *via* the Na^+ /dicarboxylate cotransporter. In ischemic renal tubules the driving force may be disturbed, thereby resulting in reduced uptake of IS *via* rOAT3. The precise mechanism involved in the relationship between the levels of serum IS and the expression levels of OATs and the I/R-induced downregulation of these transporters remains to be clarified.

A previous report suggested that the clinical pharmacokinetics of famotidine, a histamine H_2 -receptor blocker, is related to renal function (Inotsume et al., 1989). In ischemic rats, the renal excretion of famotidine was significantly reduced (Matsuzaki et al., 2008). A transport study demonstrated that famotidine was a substrate for rOCT1, rOCT2 and rOAT3 (Tahara et al., 2005). Michaelis-Menten constant (K_m) values of famotidine for rOCT1, rOCT2 and rOAT3 were 87, 61 and 345 μM , respectively (Tahara et al., 2005). The maximum plasma concentrations of famotidine in sham-operated and I/R rats were estimated to be 154 and 148 μM , respectively (Matsuzaki et al., 2008). Taking these observations into consideration, the reduced renal excretion of famotidine in I/R may be caused by the diminished organic cation transport activity of the renal basolateral membrane. Using Western blot and Northern

blot analyses, the expression of rOCT2, but not rOCT1, appeared to significantly decrease in I/R rat kidneys, suggesting that rOCT2 was more sensitive to AKI (Fig. 7) (Matsuzaki et al., 2008). It was reported that the expression of rOCT2 was downregulated in rats with chronic renal failure (Ji et al., 2002). Urakami et al. (2000) reported that the expression of rOCT2 was upregulated by testosterone and downregulated by estradiol in rats. The lower plasma concentration of testosterone was responsible for the decreased rOCT2 expression (Ji et al., 2002). Furthermore, testosterone induced the expression of rOCT2, but not rOCT1 and rOCT3, *via* the androgen receptor-mediated transcriptional pathway (Asaka et al., 2006). However, there were no significant changes in plasma testosterone and estradiol in I/R-induced AKI (Park et al., 2004; Shim et al., 2009), although the serum testosterone level was decreased in bilateral ureteral ligation and uranyl nitrate or cisplatin-induced AKI (Masubuchi et al., 2006). Therefore, further studies are required to explore the factor(s) involved in the reduced expression of OCT2 in order to understand its regulation in AKI.

In rats with ischemic AKI, renal clearance of both famotidine and TEA was significantly reduced (Matsuzaki et al., 2008). Renal clearance of famotidine and TEA may be affected by organic cation transport activity not only at the basolateral membranes but also at the brush-border membranes. In the 5/6 nephrectomized rats exhibiting chronic kidney disease, the downregulated expression of apical rMATE1 was correlated with the tubular secretion of cimetidine, in association with the depressed expression of NHE3 (Nishihara et al., 2007). TEA and cimetidine are

substrates for rMATE1 (Terada et al., 2005), although the ability of rMATE1 to recognize famotidine as a substrate has not yet been confirmed. In ischemic AKI rats, expression of rMATE1 at both the protein and mRNA level was markedly reduced. Previously, it was reported that NHE3 expression was markedly depressed in ischemic AKI rats, and the transport activity of organic cations in renal brush-border membranes was decreased in I/R rats (Maeda et al., 1993; Kwon et al., 2000). Therefore, the downregulation of rMATE1 could be involved in the reduced renal clearance of organic cations in I/R rats at the luminal membranes, as well as rOCT2.

3.2. *Drugs and Toxicants-induced AKI*

Renal proximal tubules are primary sites for the active secretion of drugs, toxicants and endogenous metabolites into the urine. During the development of AKI brought about by exposure to nephrotoxic drugs or toxicants, glomerular filtration and/or renal secretion become unbalanced. This often requires reducing the dose and/or lengthening dosing intervals for therapeutics. Among such nephrotoxic xenobiotics, studies into the effects of cisplatin, uranyl nitrate and inorganic mercury on the regulation of renal SLC22A transporters have been performed.

3.2.1. *Cisplatin*

Although cisplatin is an effective antineoplastic drug for the treatment of solid tumors, its clinical use is often limited because of adverse effects on renal function. Nephrotoxicity can be observed in as many as 38% of patients after a single dose of

cisplatin (100 mg/m^2 body surface area) (Shord et al., 2006). This nephrotoxic action often delays subsequent chemotherapy protocols, thereby reducing the overall therapeutic efficacy of cisplatin. It has been demonstrated that exposure to cisplatin leads to necrosis as well as apoptosis of renal proximal tubule cells, with occasional damage to the distal tubules. Previous reports demonstrated the involvement of rat and human OCT2 in the cellular accumulation of cisplatin (Ciarimboli et al., 2005; Yonezawa et al., 2005). Interestingly, administration of a single toxic dose of cisplatin to rats reduces OCT2 mRNA levels after seven days, suggesting a defense response against subsequent exposure and renal accumulation of cisplatin. Aleksunes et al. (2008) reported that cisplatin-induced renal injury increased mRNA and protein levels of the efflux transporters Mrp2, Mrp4, Mrp5, Mdr1a and Mdr1b in mice. By contrast, the expression of Oat1, Oat2, Oct2 and Oatp1a1 mRNA was reduced in cisplatin-treated mice.

Morisaki et al. (2008) suggested the pathophysiological role of IS in the development and progression of cisplatin-induced AKI. Although anionic uremic toxins, such as IS, hippurate, indoleacetate and 3-carboxy-4-methyl-5-propyl-2-furanpropionate, which are derived from dietary proteins, are mainly excreted into the urine, they accumulate to a high degree in the plasma of patients with kidney diseases (Boumendil-Podevin et al., 1975). A recent report suggests that uremic toxins accelerate the progression of renal injury by damaging tubular cells (Niwa et al., 1997). IS is produced enzymatically in the liver from indole, which is generated from tryptophan by the intestinal flora. In normal

kidney function, IS is efficiently excreted into the urine primarily by renal tubular secretion *via* organic anion transporters. This excretion pathway maintains the serum level of IS at approximately 4 μM . Oral administration of IS in uremic rats stimulates glomerular sclerosis, which is accompanied by a reduction in renal function (Niwa & Ise, 1994). Niwa et al. (1997) suggested that IS is involved in the development of CRF, and that AST-120, an oral charcoal adsorbent that reduces the levels of circulating uremic toxins such as IS and indole acetic acid, can delay progression of the condition by reducing the serum and urine levels of IS in undialyzed uremic patients.

Morisaki et al. (2008) found that cisplatin-treated rats exhibited a marked decrease in body weight compared with the untreated control group. Furthermore, the levels of both BUN and SCr values were significantly elevated in cisplatin-treated rats compared with control rats, indicating AKI was induced by cisplatin administration. However, the changes in body weight, BUN and SCr levels in cisplatin-treated rats were significantly reduced by oral administration of AST-120. These results suggested that cisplatin-induced AKI is partially prevented by AST-120 administration, and that IS was involved in the AKI induced by cisplatin (Morisaki et al., 2008). As anticipated during cisplatin-induced AKI, endogenous IS levels in serum and kidney were markedly elevated. By contrast, the cisplatin-associated increase in the IS level in both serum and kidney was significantly suppressed upon administration of AST-120. Cisplatin treatment markedly suppressed mRNA levels of rOAT1 and rOAT3 in the kidney (Fig. 8). In contrast, cisplatin-treated rats administered with AST-120 exhibited significantly higher levels of rOAT1 and rOAT3 mRNA compared with

cisplatin-treated rats. The protein expression levels of rOAT1 and rOAT3 were greatly suppressed in cisplatin-treated rat kidney, whereas administration of AST-120 partially restored the downregulation of rOAT1 and rOAT3 (Fig. 8). In cisplatin-treated rats, rMATE1 mRNA expression was significantly lower than that of control rats. However, administration of AST-120 to cisplatin-treated rats resulted in a slight recovery in the level of rMATE1 mRNA and protein (Morisaki et al., 2008). This suppression in the expression level of rMATE1 could be largely restored by administration of AST-120. Therefore, the expression of rMATE1 is likely to be regulated under cisplatin-induced AKI. Hence, IS appears to be involved in the regulation of rMATE1 expression.

IS appears to be a high-affinity substrate for rOAT1 and rOAT3 (Deguchi et al., 2004, 2005). Therefore, an elevation of renal IS concentration in cisplatin-treated rats might be related to the downregulation of these transporters. IS may play a pathological role, at least in part, in the altered function and expression of organic ion transporters as a mediator. This proposal is supported by the finding that AST-120 can partially restore the decrease in function and expression of organic ion transporters during cisplatin-induced AKI (Morisaki et al., 2008). The fact that AST-120 prevented serum and renal accumulation of IS, in association with significant amelioration of renal function, suggest that IS should be involved, at least in part, in cisplatin-induced AKI. Thus, the downregulation of rOAT1 and rOAT3 caused by cisplatin could accelerate IS accumulation in both serum and kidney, thereby augmenting renal tubular injury and leading to a further reduction in the level of these transporters.

Prevention of cisplatin-induced AKI by concomitant administration of a molecular targeted drug has been reported. Imatinib (STI-571), a potent inhibitor of BCR-ABL tyrosine kinase, is clinically used for the treatment of Philadelphia chromosome-positive (Ph⁺) chronic myeloid leukemia (CML) and gastrointestinal stromal tumor. It has been suggested that leukocytic hOCT1 mediates the uptake of imatinib and the expression status of hOCT1 is an important determinant of the pharmacological response of imatinib in CML patients (Thomas et al., 2004; Wang et al., 2008). Tanihara et al. (in press) reported that imatinib markedly reduced cisplatin-induced cytotoxicity and platinum accumulation in OCT2-expressing HEK293 cells, but almost no change was detected in the cells expressing human MATE1, MATE2-K and rat MATE1. In rats, the renal accumulation of cisplatin together with the subsequent nephrotoxicity were significantly reduced after oral administration of imatinib. The oral imatinib significantly elevated the serum concentration of intravenously administered cisplatin. Histological examinations revealed that concomitant administration of imatinib clearly prevented severe renal injury, suggesting that imatinib ameliorates cisplatin-induced AKI by inhibiting the renal accumulation of cisplatin *via* OCT2.

CDDP-induced AKI is accompanied by disturbances in renal handling of electrolytes. In particular, hypomagnesemia has emerged as a common event associated with CDDP-induced AKI; 76% of patients became hypomagnesemic during CDDP treatment (Schilsky et al., 1982; Lajer & Daugaard, 1999). Mavichak et al. (1985) reported that abnormalities of magnesium (Mg) metabolism were induced

by CDDP administration in rats. In addition, abnormal renal handling of Mg persisted along with residual lesions in the S3 segment of the outer medullary nephrons, indicating that renal tubular reabsorption of filtered Mg is suppressed under CDDP-induced AKI (Yao et al., 2007). Alternatively, hypomagnesemia appeared to be involved in further deterioration of renal tubular dysfunction following treatment with CDDP. Yokoo et al. (in press) observed the up-regulation of the organic cation transporter rOCT2 in hypomagnesemic rats before CDDP administration, but not of rOCT1 or rMATE1 (Fig. 9). TEA uptake by renal slices from hypomagnesemic rats was significantly higher compared with that of control rats. Renal accumulation of CDDP was markedly increased in hypomagnesemic rats. It was proposed that hypomagnesemia could cause dehydration and up-regulation of rOCT2, enhancing renal accumulation of CDDP and the deterioration of AKI (Yokoo et al., in press).

3.2.2. *Uranyl nitrate*

Uranyl nitrate (UN)-induced AKI is a well established experimental model for studying kidney function. Indeed, this method of inducing AKI is particularly attractive due the reproducibly, high survival rate, and relatively consistent interference of renal function. One of the proposed mechanisms in the pathogenesis of UN-induced AKI is its direct effect on hemodynamics with a subsequent decline in glomerular filtration rate. By using a single-pass flow of rat perfused kidney treated with UN, Tanigawara et al. (1990) reported that the uptake of PAH across the basolateral membranes decreased gradually, and that transport across the brush border

membranes remained unchanged after uranyl nitrate treatment. Secretion of TEA from cells to the lumen was initially impaired, followed by interference in the uptake of TEA from blood to the cells. Using rats with UN-induced AKI, Shim et al. (2009) demonstrated that the expression level of OCT2 mRNA and protein in the kidney medulla appeared to decrease, although mRNA expression of the other OCTs was not appreciably affected. The plasma level of testosterone, a suggested modulator of OCT2 expression at the transcription level, was unchanged, suggesting that the downregulation of OCT2 is testosterone-independent. During AKI the steady state tissue-to-plasma ratio of TEA decreased in the kidney medulla by approximately 15-fold. The reduced OCT2 expression in the kidney medulla resulted in a lower distribution of TEA within the kidney medulla. Thus, the renal clearance of TEA in UN-induced AKI rats was less than that in normal rats.

3.2.3. *Inorganic mercury*

Inorganic mercury accumulates preferentially in the kidney, inducing AKI, which is characterized by profound renal vasoconstriction, reduction in glomerular filtration rate and histological injury. Renal tubular cells represent the primary target where highly reactive inorganic mercuric ions are rapidly accumulated, inducing cell injury (Zalups 2000). Several factors may account for this susceptibility, including the specific transport systems mediating the uptake of mercuric ions by the renal tubular cells (Bridges and Zalups. 2004). In rats with HgCl₂-induced AKI, OAT1 protein expression significantly increased in the homogenates and decreased in the basolateral

membranes from kidneys. The exposure to HgCl_2 also decreased the expression of OAT1 mRNA (Di Giusto et al., in press). The increased OAT1 expression in homogenates suggested impairment of its degradation, which could be mediated by the mercury toxicity-dependent inhibition of some specific proteins/enzymes underlying the mechanisms of OAT1 degradation and/or turnover (Zalups 2000). The expression of OAT3 at both the protein and mRNA level was reduced in the kidneys of HgCl_2 -treated rats, indicating downregulation at the transcriptional level. A much greater reduction in the level of OAT3 mRNA (60%) was observed by comparison with the reduction in the level of OAT3 protein (30%) in homogenates, suggesting an alteration in the degradation pathway. Because OAT1 and OAT3 are thought to mediate the accumulation of mercuric compounds in the proximal tubular cells, their downregulation might be one defensive mechanism to protect the kidney against mercury-induced injury. Thus, the pharmacological modulation of the expression and/or the function of OAT1 and OAT3 might be an effective therapeutic strategy for reducing the nephrotoxicity of mercury.

4. Perspective

Functional and transcriptional regulation of renal SLC22A transporters implies that these proteins play an essential physiological role in homeostasis and/or detoxification. Recently, Ahn & Nigam (2009) have proposed a “remote sensing and signaling hypothesis” for communication between different organs expressing organic anion transporters. Namely, factors that impair the clearance of substrates by the OAT

transporters, such as toxins, ischemia or competitive inhibition by other substrates, could disrupt OAT function, thereby leading to perturbed homeostasis. To compensate for the loss of function of OATs and to restore homeostasis, upregulation and/or activated function of other transporter isoforms may occur at the transcriptional, translational or post-transchemical level, either in the injured kidney or other tissues involved in a remote sensing network. Although a great deal of information concerning the regulation of renal SLC22A transporters has been accumulated, the mechanisms based on the altered molecular and functional regulation of renal SLC22A transporters accompanied by ischemia or toxicant-induced AKI still remains to be elucidated. Understanding of such regulatory mechanisms and inter-organ communication network of SLC22A transporters would provide information for their physiological and pharmacological functions in maintaining systemic homeostasis in AKI.

Acknowledgment

This work was supported in part by a Grant-in-Aid for Scientific Research (B) from the Japan Society for the Promotion of Science (JSPS) for Hideyuki Saito (KAKENHI 17390158 & 21390048).

References

- Ahn, S.Y., & Nigam, S.K. (2009). Toward a Systems Level Understanding of Organic Anion and Other Multispecific Drug Transporters: A Remote Sensing and Signaling Hypothesis. *Mol Pharmacol* 76, 481-490.
- Aleksunes, L.M., Augustine, L.M., Scheffer, G.L., Cherrington, N.J., & Manautou, J.E. (2008). Renal xenobiotic transporters are differentially expressed in mice following cisplatin treatment. *Toxicology* 250, 82-88.
- Aoki, M., Terada, T., Kajiwara, M., Ogasawara, K., Ikai, I., Ogawa, O., et al. (2008). Kidney-specific expression of human organic cation transporter 2 (OCT2/SLC22A2) is regulated by DNA methylation. *Am J Physiol Renal Physiol* 295, F165-F170.
- Aoyama, I., Enomoto, A., & Niwa, T. (2003). Effects of oral adsorbent on gene expression profile in uremic rat kidney: cDNA array analysis. *Am J Kidney Dis* 41, S8-14.
- Asaka, J., Terada, T., Ogasawara, K., Katsura, T., & Inui, K. (2007). Characterization of the basal promoter element of human organic cation transporter 2 gene. *J Pharmacol Exp Ther* 321, 684-689.
- Asaka, J., Terada, T., Okuda, M., Katsura, T., & Inui, K. (2006). Androgen receptor is responsible for rat organic cation transporter 2 gene regulation but not for rOCT1 and rOCT3. *Pharm Res* 23, 7697-704.
- Boumendil-Podevin, E.F., Podevin, R.A., & Richet, G. (1975). Uricosuric agents in uremic sera. Identification of indoxyl sulfate and hippuric acid. *J Clin Invest* 55, 1142-1152.
- Bridges, C.C., & Zalups, R.K. (2004). Homocysteine, system b₀,+ and the renal epithelial transport and toxicity of inorganic mercury. *Am J Pathol* 165, 1385-1394.
- Burckhardt, B.C., & Burckhardt, G. (2003). Transport of organic anions across the basolateral membrane of proximal tubule cells. *Rev Physiol Biochem Pharmacol* 146, 95-158.
- Burckhardt, G., & Wolff, N.A. (2000). Structure of renal organic anion and cation transporters. *Am J Physiol Renal Physiol* 278, F853-F866.
- Busch, A.E., Karbach, U., Miska, D., Gorboulev, V., Akhoundova, A., Volk, C., et al. (1998). Human neurons express the polyspecific cation transporter hOCT2, which translocates monoamine neurotransmitters, amantadine, and memantine. *Mol Pharmacol* 54, 342-352.
- Busch, A.E., Quester, S., Ulzheimer, J.C., Waldegger, S., Gorboulev, V., Arndt, P., et al. (1996). Electrogenic properties and substrate specificity of the polyspecific rat

cation transporter rOCT1. *J Biol Chem* 271, 32559–32604.

Ciarimboli, G., Ludwig, T., Lang, D., Pavenstädt, H., Koepsell, H., Piechota, H.J., et al. (2005). Cisplatin nephrotoxicity is critically mediated via the human organic cation transporter 2. *Am J Pathol* 167, 1477-1484.

Dantzler, W.H., & Wright, S.H. (2003). The molecular and cellular physiology of basolateral organic anion transport in mammalian renal tubules. *Biochim Biophys Acta* 1618, 185-193.

David, C., Rumrich, G., & Ullrich, K.J. (1995). Luminal transport system for H⁺/organic cations in the rat proximal tubule. Kinetics, dependence on pH; specificity as compared with the contraluminal organic cation-transport system. *Pflugers Arch* 430, 477–492.

Deguchi, T., Kouno, Y., Terasaki, T., Takadate, A., & Otagiri M. (2005). Differential contributions of rOat1 (Slc22a6) and rOat3 (Slc22a8) to the in vivo renal uptake of uremic toxins in rats. *Pharm Res* 22, 619-627.

Deguchi, T., Kusuhara, H., Takadate, A., Endou, H., Otagiri, M., & Sugiyama, Y. (2004). Characterization of uremic toxin transport by organic anion transporters in the kidney. *Kidney Int* 65, 162-174.

Deguchi, T., Ohtsuki, S., Otagiri, M., Takanaga, H., Asaba, H., Mori, S., et al. (2002). Major role of organic anion transporter 3 in the transport of indoxyl sulfate in the kidney. *Kidney Int* 61, 1760-1768.

Di Giusto, G., Anzai, N., Ruiz, M.L., Endou, H., & Torres, A.M. (2009). Expression and function of Oat1 and Oat3 in rat kidney exposed to mercuric chloride. *Arch Toxicol* 83, 87-97.

Enomoto, A., & Niwa, T. (2007). Roles of organic anion transporters in the progression of chronic renal failure. *Ther Apher Dial Suppl* 1, S27-31.

Enomoto, A., Takeda, M., Tojo, A., Sekine, T., Cha, S.H., Khamdang, S., et al. (2002). Role of organic anion transporters in the tubular transport of indoxyl sulfate and the induction of its nephrotoxicity. *J Am Soc Nephrol* 13, 1711-1720.

Groves, C.E., Evans, K.K., Dantzler, W.H., & Wright, S.H. (1994). Peritubular organic cation transport in isolated rabbit proximal tubules. *Am J Physiol* 266, F450–F458.

Gründemann, D., Gorboulev, V., Gambaryan, S., Veyhl, M., & Koepsell, H. (1994). Drug excretion mediated by a new prototype of polyspecific transporter. *Nature* 372, 549–552.

Gründemann, D., Koster, S., Kiefer, N., Breidert, T., Engelhardt, M., Spitzenberger, F., et al. (1998). Transport of monoamine transmitters by the organic cation transporter type 2, OCT2. *J Biol Chem* 273, 30915–30920.

Holohan, P.D., & Ross, C.R. (1981). Mechanisms of organic cation transport in kidney plasma membrane vesicles: 2. Δ pH studies. *J Pharmacol Exp Ther* 216, 294–298.

- Hosoyamada, M., Sekine, T., Kanai, Y., & Endou, H. (1999). Molecular cloning and functional expression of a multispecific organic anion transporter from human kidney. *Am J Physiol* 276, F122–F128.
- Hsyu, P.-H., & Giacomini, K.M. (1987). The pH gradient-dependent transport of organic cations in the renal brush border membrane. *J Biol Chem* 262:3964–3968.
- Inotsume, N., Nishimura, M., Fujiyama, S., Sagara, K., Sato, T., Imai, Y., et al., (1989). Pharmacokinetics of famotidine in elderly patients with and without renal insufficiency and in healthy young volunteers. *Eur J Clin Pharmacol*, 36, 517-520.
- Inui, K., Masuda, S., & Saito, H. (2000). Cellular and molecular aspects of drug transport in the kidney. *Kidney Int* 58, 944-958.
- Inui, K., & Okuda, M. (1998). Cellular and molecular mechanisms of renal tubular secretion of organic anions and cations. *Clin Exp Nephrol* 2, 100–108.
- Inui K., Saito, H., & Hori, R. (1985). H⁺-gradient-dependent active transport of tetraethylammonium cation in apical-membrane vesicles isolated from kidney epithelial cell line LLC-PK₁. *Biochem J* 227, 199–203.
- Ji, L., Masuda, S., Saito, H., & Inui, K. (2002). Down-regulation of rat organic cation transporter rOCT2 by 5/6 nephrectomy. *Kidney Int* 62, 514–524.
- Kikuchi, R., Kusuhara, H., Hattori, N., Kim, I., Shiota, K., Gonzalez, F.J., et al. (2006). Regulation of the expression of human organic anion transporter 3 by hepatocyte nuclear factor 1 α / β and DNA methylation. *Mol Pharmacol* 70, 887-896.
- Koepsell, H., & Endou, H. (2004). The SLC22 drug transporter family. *Pflugers Arch* 447, 666-676.
- Koepsell, H., Gorboulev, V., & Arndt, P. (1999). Molecular pharmacology of organic cation transporters in kidney. *J Membr Biol* 167, 103–117.
- Kribben, A., Edelstein, C.L., & Schrier, R.W. (1999). Pathophysiology of acute renal failure. *J Nephrol* 12 (Suppl 2), S142-151.
- Kusuhara, H., Sekine, T., Utsunomiya-Tate, N., Tsuda, M., Kojima, R., & Cha, S.H. (1999). Molecular cloning and characterization of a new multispecific organic anion transporter from rat brain. *J Biol Chem* 274, 13675-13680.
- Kwon, T.H., Frokiaer, J., Han, J.S., Knepper, M.A., & Nielsen, S. (2000). Decreased abundance of major Na⁺ transporters in kidneys of rats with ischemia-induced acute renal failure. *Am J Physiol Renal Physiol* 278, F925-F939.
- Kwon, O., Wang, W.W., & Miller, S. (2008). Renal organic anion transporter 1 is maldistributed and diminishes in proximal tubule cells but increases in vasculature after ischemia and reperfusion. *Am J Physiol Renal Physiol* 295, F1807-F1816.
- Lajer, H., & Daugaard, G. (1999). Cisplatin and hypomagnesemia. *Cancer Treat Rev*

25, 47-58.

- Lu, R., Chan, B.S., & Schuster, V.L. (1999). Cloning of the human kidney PAH transporter: Narrow specificity and regulation by protein kinase C. *Am J Physiol* 276, F295–F303.
- Maeda, S., Takano, M., Okano, T., Ohoka, K., Inui, K., & Hori, R. (1993). Transport of organic cation in renal brush-border membrane from rats with renal ischemic injury. *Biochim Biophys Acta* 1150, 103–110.
- Masubuchi, Y., Kawasaki, M., & Horie, T. (2006). Down-regulation of hepatic cytochrome P450 enzymes associated with cisplatin-induced acute renal failure in male rats. *Arch Toxicol* 80, 347-353.
- Masuda, S., Terada, T., Yonezawa, A., Tanihara, Y., Kishimoto, K., Katsura, T., et al. (2006). Identification and functional characterization of a new human kidney-specific H⁺/organic cation antiporter, kidney-specific multidrug and toxin extrusion 2. *J Am Soc Nephrol* 17, 2127-2135.
- Matsuzaki, T., Watanabe, H., Yoshitome, K., Morisaki, T., Hamada, A., Nonoguchi, H., et al. (2007). Downregulation of organic anion transporters in rat kidney under ischemia/reperfusion-induced acute renal failure. *Kidney Int* 71, 539-547.
- Matsuzaki, T., Morisaki, T., Sugimoto, W., Yokoo, K., Sato, D., Nonoguchi, H., et al. (2008). Altered pharmacokinetics of cationic drugs caused by down-regulation of renal rat organic cation transporter 2 (Slc22a2) and rat multidrug and toxin extrusion 1 (Slc47a1) in ischemia/reperfusion-induced acute kidney injury. *Drug Metab Dispos* 36, 649-654.
- Mavichak, V., Wong, N.L., Quamme, G.A., Magil, A.B., Sutton, R.A., & Dirks, J.H. (1985). Studies on the pathogenesis of cisplatin-induced hypomagnesemia in rats. *Kidney Int* 28, 914-921.
- Miyamoto, Y., Tiruppathi, C., Ganapathy, V., & Leibach, F.H. (1989). Multiple transport systems for organic cations in renal brush-border membrane vesicles. *Am J Physiol* 256, F540–F548.
- Miyazaki, T., Aoyama, I., Ise, M., Seo, H., & Niwa, T. (2000). An oral sorbent reduces overload of indoxyl sulphate and gene expression of TGF-β1 in uraemic rat kidneys. *Nephrol Dial Transplant* 15, 1773-1781.
- Miyazaki, T., Ise, M., Seo, H., & Niwa, T. (1997). Indoxyl sulfate increases the gene expressions of TGF-beta 1, TIMP-1 and pro-alpha 1(I) collagen in uremic rat kidneys. *Kidney Int* 52(Suppl 62), S15-22.
- Molitoris, B.A., Dahl, R., & Geerdes, A. (1992). Cytoskeleton disruption and apical redistribution of proximal tubule Na⁺-K⁺-ATPase during ischemia. *Am J Physiol* 263, F488-F495.
- Montrose-Rafizadeh, C., Mingard, F., Murer, H., & Roch-Ramel, F. (1989). Carrier-mediated transport of tetraethylammonium across rabbit renal basolateral

membrane. *Am J Physiol* 257, F243–F251.

- Morisaki, T., Matsuzaki, T., Yokoo, K., Kusumoto, M., Iwata, K., Hamada, A., et al. (2008). Regulation of renal organic ion transporters in cisplatin-induced acute kidney injury and uremia in rats. *Pharm Res* 25, 2526-2533.
- Motohashi, H., Sakurai, Y., Saito, H., Masuda, S., Urakami, Y., Goto, M., et al. (2002). Gene expression levels and immunolocalization of organic ion transporters in the human kidney. *J Am Soc Nephrol* 13, 866-874.
- Nagai, J., Yano, I., Hashimoto, Y., Takano, M., & Inui, K. (1997). Inhibition of PAH transport by parathyroid hormone in OK cells: Involvement of protein kinase C pathway. *Am J Physiol* 273, F674–F679.
- Nishihara, K., Masuda, S., Ji, L., Katsura, T., & Inui, K. (2007). Pharmacokinetic significance of luminal multidrug and toxin extrusion 1 in chronic renal failure rats. *Biochem Pharmacol* 73, 1482-1490.
- Niwa, T., & Ise, M. (1994). Indoxyl sulfate, a circulating uremic toxin, stimulates the progression of glomerular sclerosis. *J Lab Clin Med* 124, 96-104.
- Niwa, T., Nomura, T., Sugiyama, S., Miyazaki, T., Tsukushi, S., & Tsutsui, S. (1997). The protein metabolite hypothesis, a model for the progression of renal failure: an oral adsorbent lowers indoxyl sulfate levels in undialyzed uremic patients. *Kidney Int* 52, S23-28.
- Ogasawara, K., Terada, T., Asaka, J., Katsura, T., & Inui, K. (2006). Human organic anion transporter 3 gene is regulated constitutively and inducibly via a cAMP-response element. *J Pharmacol Exp Ther* 319, 317-322.
- Ogasawara, K., Terada, T., Asaka, J., Katsura, T., & Inui, K. (2007). Hepatocyte nuclear factor-4 α regulates the human organic anion transporter 1 gene in the kidney. *Am J Physiol Renal Physiol* 292, F1819-F1826.
- Okuda, M., Saito, H., Urakami, Y., Takano, M., & Inui, K. (1996). cDNA cloning and functional expression of a novel rat kidney organic cation transporter, OCT2. *Biochem Biophys Res Commun* 224, 500–507.
- Okuda, M., Urakami, Y., Saito, H., & Inui, K. (1999). Molecular mechanisms of organic cation transport in OCT2-expressing *Xenopus* oocytes. *Biochim Biophys Acta* 1417, 224–231.
- Otsuka, M., Matsumoto, T., Morimoto, R., Arioka, S., Omote, H., & Moriyama, Y. (2005). A human transporter protein that mediates the final excretion step for toxic organic cations. *Proc Natl Acad Sci USA* 102, 17923-17928.
- Park, K.M., Kim, J.I., Ahn, Y., Bonventre, A.J., & Bonventre, J.V. (2004). Testosterone is responsible for enhanced susceptibility of males to ischemic renal injury. *J Biol Chem* 279, 52282–52292.
- Pritchard, J.B., & Miller, D.S. (1996). Renal secretion of organic anions and cations.

Kidney Int 49, 1649-1654.

- Rizwan, A.N., & Burckhardt, G. (2007). Organic anion transporters of the SLC22 family: biopharmaceutical, physiological, and pathological roles. *Pharm Res* 24, 450-470.
- Robertson, E.E., & Rankin, G.O. (2006). Human renal organic anion transporters: characteristics and contributions to drug and drug metabolite excretion. *Pharmacol Ther* 109, 399-412.
- Saito, H., Yamamoto, M., Inui, K., & Hori, R. (1992). Transcellular transport of organic cation across monolayers of kidney epithelial cell line LLC-PK₁. *Am J Physiol* 262, C59–C66.
- Saji, T., Kikuchi, R., Kusuhashi, H., Kim, I., Gonzalez, F.J., & Sugiyama, Y. (2008). Transcriptional regulation of human and mouse organic anion transporter 1 by hepatocyte nuclear factor 1 α/β . *J Pharmacol Exp Ther* 324, 784-790.
- Sakurai, Y., Motohashi, H., Ueo, H., Masuda, S., Saito, H., Okuda, M., et al. (2004). Expression levels of renal organic anion transporters (OATs) and their correlation with anionic drug excretion in patients with renal diseases. *Pharm Res* 21, 61-67.
- Sakurai, Y., Motohashi, H., Ogasawara, K., Terada, T., Masuda, S., Katsura, T., et al. (2005). Pharmacokinetic significance of renal OAT3 (SLC22A8) for anionic drug elimination in patients with mesangial proliferative glomerulonephritis. *Pharm Res* 22, 2016-2022.
- Sauvant, C., Holzinger, H., Gekle, M. (2003). Short-term regulation of basolateral organic anion uptake in proximal tubular opossum kidney cells: prostaglandin E₂ acts via receptor-mediated activation of protein kinase A. *J Am Soc Nephrol* 14, 3017-3026.
- Schilsky, R.L., Barlock, A., & Ozols, R.F. (1982). Persistent hypomagnesemia following cisplatin chemotherapy for testicular cancer. *Cancer Treat Rep* 66, 1767–1769.
- Schneider, R., Sauvant, C., Betz, B., Otremba, M., Fischer, D., Holzinger, H., et al. (2007). Downregulation of organic anion transporters OAT1 and OAT3 correlates with impaired secretion of para-aminohippurate after ischemic acute renal failure in rats. *Am J Physiol Renal Physiol* 292, F1599-F1605.
- Schrier, R.W., Wang, W., Poole, B., & Mitra, A. (2004). Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. *J Clin Invest* 114, 5-14.
- Sekine, T., Cha, S.H., & Endou, H. (2000). The multispecific organic anion transporter (OAT) family. *Pflugers Arch* 440, 337-350.
- Sekine, T., Watanabe, N., Hosoyamada, M., Kanai, Y., & Endou, H. (1997). Expression cloning and characterization of a novel multispecific organic anion transporter. *J Biol Chem* 272, 18526-18529.
- Shanley, P.F., Rosen, M.D., Brezis, M., Silva, P., Epstein, F.H., & Rosen S. (1986).

Topography of focal proximal tubular necrosis after ischemia with reflow in the rat kidney. *Am J Pathol* 122, 462-468.

- Shim, W.S., Park, J.H., Ahn, S.J., Han, L., Jin, Q.R., Li, H., et al. (2009). Testosterone-independent down-regulation of Oct2 in the kidney medulla from a uranyl nitrate-induced rat model of acute renal failure: effects on distribution of a model organic cation, tetraethylammonium. *J Pharm Sci* 98, 739-747.
- Shord, S.S., Thompson, D.M., Krempl, G.A., & Hanigan, M.H. (2006). Effect of concurrent medications on cisplatin-induced nephrotoxicity in patients with head and neck cancer. *Anticancer Drugs* 17, 207-215.
- Shu, Y., Bello, C.L., Mangravite, L.M., Feng, B., & Giacomini, K.M. (2001). Functional characteristics and steroid hormone-mediated regulation of an organic cation transporter in Madin-Darby canine kidney cells. *J Pharmacol Exp Ther* 299, 392-398.
- Sokol, P.P., & McKinney, T.D. (1990). Mechanism of organic cation transport in rabbit renal basolateral membrane vesicles. *Am J Physiol* 258, F1599-F1607.
- Soodvilai, S., Chatsudthipong, A., & Chatsudthipong, V. (2007). Role of MAPK and PKA in regulation of rbOCT2-mediated renal organic cation transport. *Am J Physiol Renal Physiol* 293, F21-F27.
- Soodvilai, S., Chatsudthipong, V., Evans, K.K., Wright, S.H., & Dantzler, W.H. (2004). Acute regulation of OAT3-mediated estrone sulfate transport in isolated rabbit renal proximal tubules. *Am J Physiol Renal Physiol* 287, F1021-F1029.
- Star, R.A. (1998). Treatment of acute renal failure. *Kidney Int* 54, 1817-1831.
- Sweet, D.H. (2005). Organic anion transporter (Slc22a) family members as mediators of toxicity. *Toxicol Appl Pharmacol* 204, 198-215.
- Sweet, D.H., Chan, L.M., Walden, R., Yang, X.P., Miller, D.S., & Pritchard, J.B. (2003). Organic anion transporter 3 (Slc22a8) is a dicarboxylate exchanger indirectly coupled to the Na⁺ gradient. *Am J Physiol Renal Physiol* 284, F763-F769.
- Sweet, D.H., Miller, D.S., Pritchard, J.B., Fujiwara, Y., Beier, D.R., & Nigam, S.K. (2002). Impaired organic anion transport in kidney and choroid plexus of organic anion transporter 3 (Oat3 (Slc22a8)) knockout mice. *J Biol Chem* 277, 26934-26943.
- Sweet, D.H., Wolff, N.A., & Pritchard, J.B. (1997). Expression cloning and characterization of ROAT1. The basolateral organic anion transporter in rat kidney. *J Biol Chem* 272, 30088-30095.
- Tahara, H., Kusuhara, H., Endou, H., Koepsell, H., Imaoka, T., Fuse, E., et al. (2005). A species difference in the transport activities of H₂ receptor antagonists by rat and human renal organic anion and cation transporters. *J Pharmacol Exp Ther* 315, 337-345.

- Takano, M., Inui, K., Okano, T., Saito, H., & Hori, R. (1984). Carrier-mediated transport systems of tetraethylammonium in rat renal brush-border and basolateral membrane vesicles. *Biochim Biophys Acta* 773, 113–124.
- Tanaka, K., Xu, W., Zhou, F., & You, G. Role of glycosylation in the organic anion transporter OAT1. (2004). *J Biol Chem* 279, 14961-14966.
- Tanigawara, Y., Saito, Y., Aiba, T., Ohoka, K., Kamiya, A., & Hori, R. (1990). Moment analysis of drug disposition in kidney. III: Transport of p-aminohippurate and tetraethylammonium in the perfused kidney isolated from uranyl nitrate-induced acute renal failure rats. *J Pharm Sci* 79, 249-256.
- Tanihara, Y., Masuda, S., Katsura, T., & Inui, K. (2009). Protective effect of concomitant administration of imatinib on cisplatin-induced nephrotoxicity focusing on renal organic cation transporter OCT2. *Biochem Pharmacol* 78, 1263-1271.
- Terada, T., Masuda, S., Asaka, J., Tsuda, M., Katsura, T., & Inui, K. (2005). Molecular cloning, functional characterization and tissue distribution of rat H⁺/organic cation antiporter MATE1. *Pharm Res* 23, 1696-1701.
- Thadhani, R., Pascual, M., & Bonventre, J.V. (1996). Acute renal failure. *N Engl J Med* 334, 1448-1460.
- Thomas, J., Wang, L., Clark, R.E., & Pirmohamed, M. (2004). Active transport of imatinib into and out of cells: implications for drug resistance. *Blood* 104, 3739–3745.
- Tsuda, M., Sekine, T., Takeda, M., Cha, S.H., Kanai, Y., Kimura, M., et al. (1999). Transport of ochratoxin A by renal multispecific organic anion transporter 1. *J Pharmacol Exp Ther* 289, 1301–1305.
- Ullrich, K.J. (1994). Specificity of transporters for 'organic anions' and 'organic cations' in the kidney. *Biochim Biophys Acta* 1197, 45-62.
- Urakami, Y., Nakamura, N., Takahashi, K., Okuda, M., Saito, H., Hashimoto, Y., et al. (1999). Gender differences in expression of organic cation transporter OCT2 in rat kidney. *FEBS Lett* 461, 339–342.
- Urakami, Y., Okuda, M., Masuda, S., Saito, H., & Inui, K. (1998). Functional characteristics and membrane localization of rat multispecific organic cation transporters, OCT1 and OCT2, mediating tubular secretion of cationic drugs. *J Pharmacol Exp Ther* 287, 800–805.
- Urakami, Y., Okuda, M., Saito, H., & Inui, K. (2000). Hormonal regulation of organic cation transporter OCT2 expression in rat kidney. *FEBS Lett* 473, 173-176.
- Uwai, Y., Okuda, M., Takami, K., Hashimoto, Y., & Inui, K. (1998). Functional characterization of the rat multispecific organic anion transporter OAT1 mediating basolateral uptake of anionic drugs in the kidney. *FEBS Lett* 438, 321–324.

- Van Why, S.K., Mann, A.S., Ardito, T., Siegel, N.J., & Kashgarian, M. (1994). Expression and molecular regulation of Na⁺-K⁺-ATPase after renal ischemia. *Am J Physiol* 267, F75-F85.
- Wang, L., Giannoudis, A., Lane, S., Williamson, P., Pirmohamed, M., & Clark RE. (2008). Expression of the uptake drug transporter hOCT1 is an important clinical determinant of the response to imatinib in chronic myeloid leukemia. *Clin Pharmacol Ther* 83, 258–264.
- Wolff, N.A., Werner, A., Burkhardt, S., & Burckhardt G. (1997). Expression cloning and characterization of a renal organic anion transporter from winter flounder. *FEBS Lett* 417, 287-291.
- Wolff, N.A., Thies, K., Kuhnke, N., Reid, G., Friedrich, B., Lang, F., et al. (2003). Protein kinase C activation downregulates human organic anion transporter 1-mediated transport through carrier internalization. *J Am Soc Nephrol* 14, 1959-1968.
- Wright, S.H., & Wunz, T.M. (1987). Transport of tetraethylammonium by rabbit renal brush-border and basolateral membrane vesicles. *Am J Physiol* 253, F1040–F1050.
- Yao, X., Panichpisal, K., Kurtzman, N., & Nugent, K. (2007). Cisplatin nephrotoxicity: a review. *Am J Med Sci* 334, 115-124.
- Yokoo, K., Murakami, R., Matsuzaki, T., Yoshitome, K., Hamada, A., & Saito, H. (2009). Enhanced renal accumulation of cisplatin via renal organic cation transporter deteriorates acute kidney injury in hypomagnesemic rats. *Clin Exp Nephrol*, DOI: 10.1007/s10157-009-0215-1.
- Yonezawa, A., Masuda, S., Nishihara, K., Yano, I., Katsura, T., & Inui, K. (2005). Association between tubular toxicity of cisplatin and expression of organic cation transporter rOCT2 (Slc22a2) in the rat. *Biochem Pharmacol* 70, 1823-1831.
- Zalups, R.K. (2000). Molecular interactions with mercury in the kidney. *Pharmacol Rev* 52, 113-143.
- Zhang, Q., Hong, M., Duan, P., Pan, Z., Ma, J., & You, G. (2008). Organic anion transporter OAT1 undergoes constitutive and protein kinase C-regulated trafficking through a dynamin- and clathrin-dependent pathway. *J Biol Chem* 283, 32570-32579.

Legends for Figures

Fig. 1. Schematic representation of membrane transporters in renal proximal tubules.

BBM, brush-border membrane; BLM, basolateral membrane; OA^- , organic anion; OC^+ , organic cation; α -KG, α -ketoglutarate; NHE, sodium/proton exchanger. Arrows indicate the direction of movement of substrates and ions *via* each transporter.

Fig. 2. Dominant negative mutant of dynamin 2 blocked constitutive and

PKC-modulated OAT1 internalization. cDNA plasmid encoding human OAT1-tagged with myc at its C terminus was transfected into COS-7 cells. OAT1-expressing cells underwent biotinylation with sulfo-NHS-SS-biotin at 4°C. Following biotinylation, one set of cells was washed with PBS and kept at 4°C to determine the total initial surface OAT1 and stripping efficiencies. **a**, top panel: cells were transfected with cDNA encoding dominant negative mutant of dynamin-2 (Dyn-2 mutant). 48 h later, OAT1 internalization (15 min) was analyzed in the presence and absence of 1 μ M PMA followed by Western blotting using anti-myc antibody (1:100). Bottom panel: as a loading control for the top panel, total expression of OAT1 in cell lysate was measured using anti-myc antibody (1:100). **b**, densitometry plot of results from **a**, top panel as well as from other experiments. Internalized OAT1 was expressed as a percentage of total initial cell surface OAT1 pool. **c**, the effect of Dyn-2 mutant on the expression of OAT1 at the cell surface, intracellular compartments, as well as in total cell lysates.

d, [³H]PAH uptake into cells transfected with Dyn-2 mutant. Uptake activity was expressed as a percentage of the uptake measured in the control cells. (Reprinted from Zhang et al., 2008).

Fig. 3. Schematic model of transcriptional regulation of the human OAT1 gene. In the abundance of HNF-4, HNF-4 transactivates human OAT1 promoter activity *via* DR-2 and IR-8 elements. IR-8 contributes to the activation by HNF-4 more than DR-2 (HNF-4 overexpression). Under normal conditions, IR-8 is responsible for the basal promoter activity of OAT1, and HNF-4 binds to IR-8 (constitutive expression). (Reprinted from Ogasawara et al. (2007) with permission from American Physiological Society).

Fig. 4. Proposed model for the regulatory mechanism of organic anion transport mediated by organic anion transporter 3 (OAT3). Phenylephrine (PE) stimulates PKC leading to downregulation of OAT3 by an unknown mechanism(s). As shown in previous studies and the present study, EGF stimulates OAT3 activity *via* MAPK, MEK and ERK1/2, which activates PLA₂ leading to the release of arachidonic acid (AA). AA is then metabolized to prostaglandins (PGE₂), which activates PKA *via* adenylate cyclase and finally stimulates OAT3 by an unknown mechanism(s). (Reprinted from Soodvilai et al. (2004) with permission from American Physiological Society).

Fig. 5. Schematic model of transcriptional regulation of the human OAT3 gene.

CREB-1 and ATF-1 bind to CRE and activate the transcription of the OAT3 gene (constitutive expression). PKA further activates the transcription of the OAT3 gene through phosphorylation of both CREB-1 and ATF-1 (inducible expression). (Reprinted from Ogasawara et al. (2006) with permission from American Society for Pharmacology and Experimental Therapeutics).

Fig. 6. *Trans*-activation of ARE-mutated rOCT2 promoters by rAR in the presence of testosterone. Constructs were transiently transfected into LLC-PK₁ cells with rAR and pRL-TK. The cells were cultured for 43 h with vehicle or 1 μ M testosterone, and luciferase activity was then measured. Firefly luciferase activity was normalized to Renilla luciferase activity. Black diamonds indicate AREs. *P < 0.05, significantly different from control. (Reprinted from Asaka et al. (2006) with kind permission from Springer Science+Business Media).

Fig. 7. Protein and mRNA expression of basolateral organic ion transporters in the kidney of sham-operated and I/R rats. **A**, antisera specific for rOAT1, rOAT3, rOCT1, rOCT2 or β -actin were used as primary antibodies. **B**, ratio of rOAT1, rOAT3, rOCT1 and rOCT2 density to β -actin density in sham-operated (\square) and I/R (\blacksquare) rats. The values for sham-operated rats were arbitrarily defined as 100%. **C**, mRNA expression of basolateral organic ion transporters in the kidneys of sham-operated and I/R rats. rOAT1, rOAT3, rOCT1 and rOCT2 mRNA

expression levels in sham-operated (\square) and I/R (\blacksquare) rats were determined by real-time PCR analysis. The relative amounts of rOAT1, rOAT3, rOCT1 and rOCT2 mRNA were normalized to that of 18S ribosomal RNA. $***P < 0.001$ versus sham-operated rats. (Reprinted from Matsuzaki et al. (2008) with permission from American Society for Pharmacology and Experimental Therapeutics).

Fig. 8. mRNA and protein expression of rat organic anion transporters in the kidneys of control and cisplatin-treated rats with or without AST-120. mRNA expression levels of (A) rOAT1 and (B) rOAT3 in control and cisplatin-treated rats with or without AST-120 were determined by real-time PCR analysis. (C). $*P < 0.05$, and $**P < 0.01$, significantly different from control rats. $\#P < 0.05$, and $##P < 0.01$, significantly different from cisplatin-treated rats. (Reprinted from Morisaki et al. (2008) with kind permission from Springer Science+Business Media).

Fig. 9. Protein expression levels of transporters in the kidneys of the control group and low Mg group at 0 and 120 h after the administration of cisplatin (3 mg/kg). Immunoblotting was performed by using antiserum specific for rOCT1, rOCT2 and rMATE1 as primary antibodies (a). Relative band intensities were quantified by densitometry for the protein expression levels of rOCT1 (b), rOCT2 (c) and rMATE1 (d). $*P < 0.05$, $**P < 0.01$, significantly different from the control group at the same period. $\dagger P < 0.05$, significantly different from the control group at 0 h.

##P<0.01, significantly different from the control group at 0 h. (Reprinted from Yokoo et al. (2008) with kind permission from Springer Science+Business Media).

Fig. 1

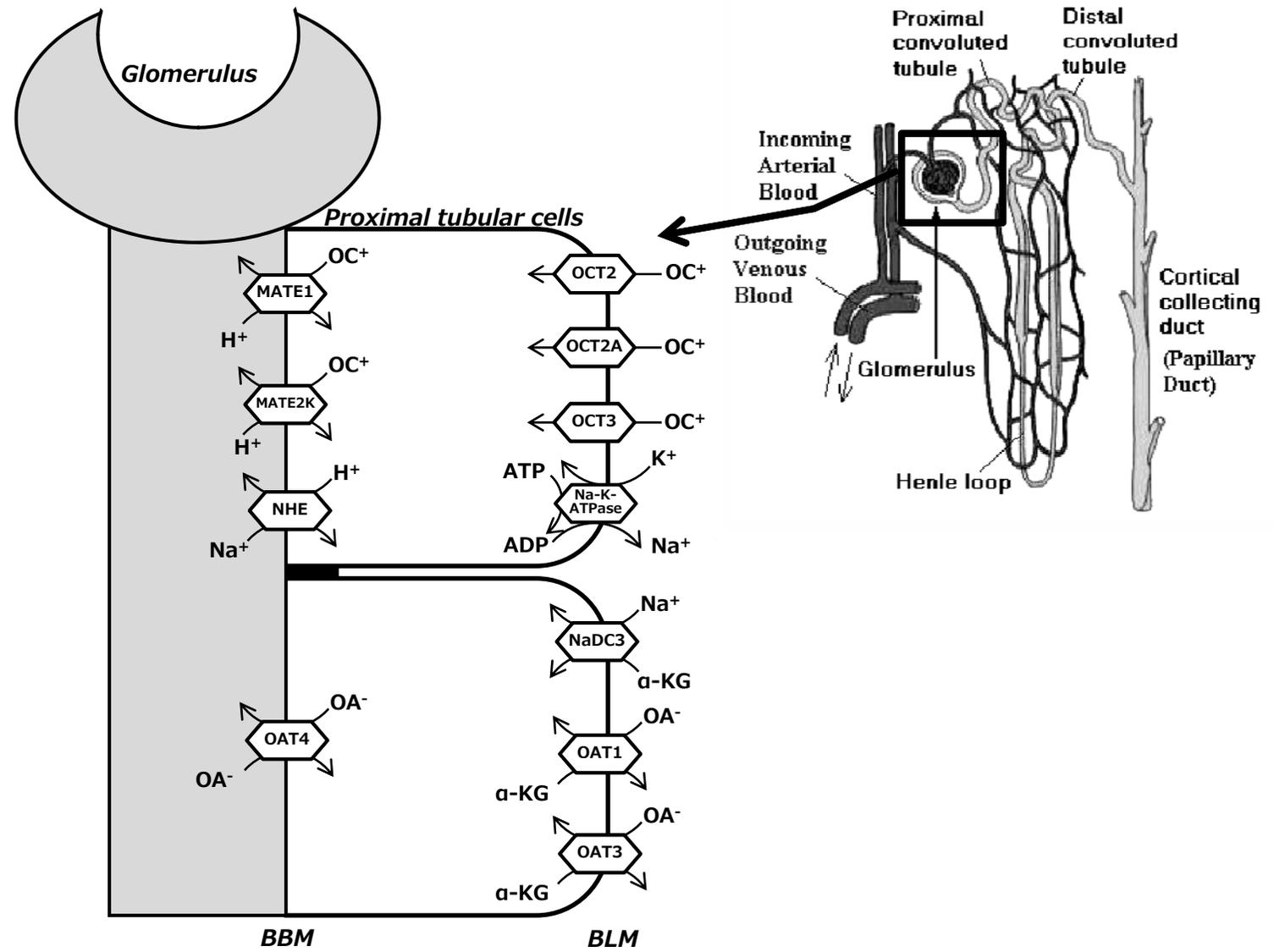
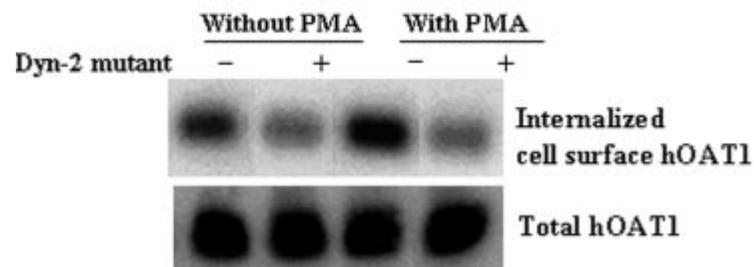
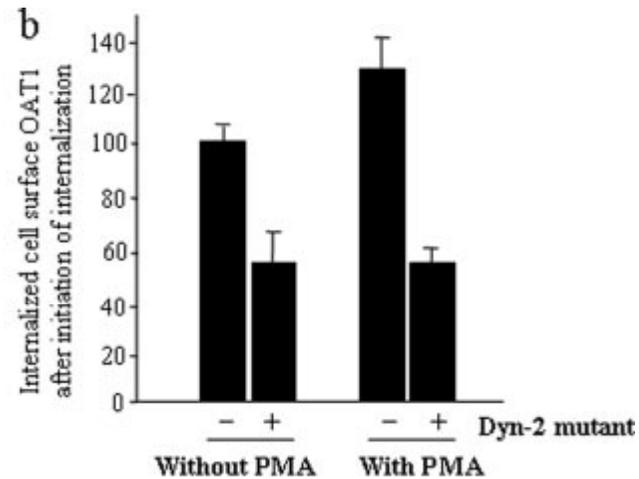


Fig. 2

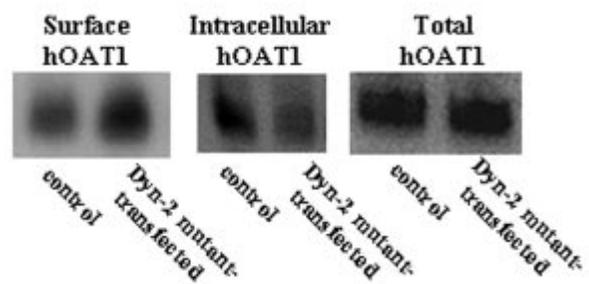
a



b



c



d

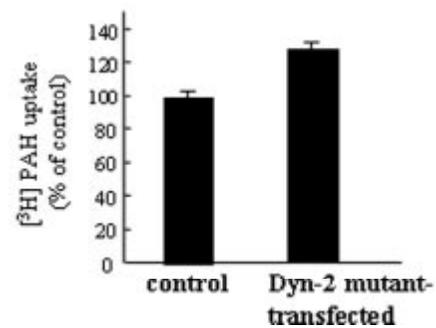


Fig. 3

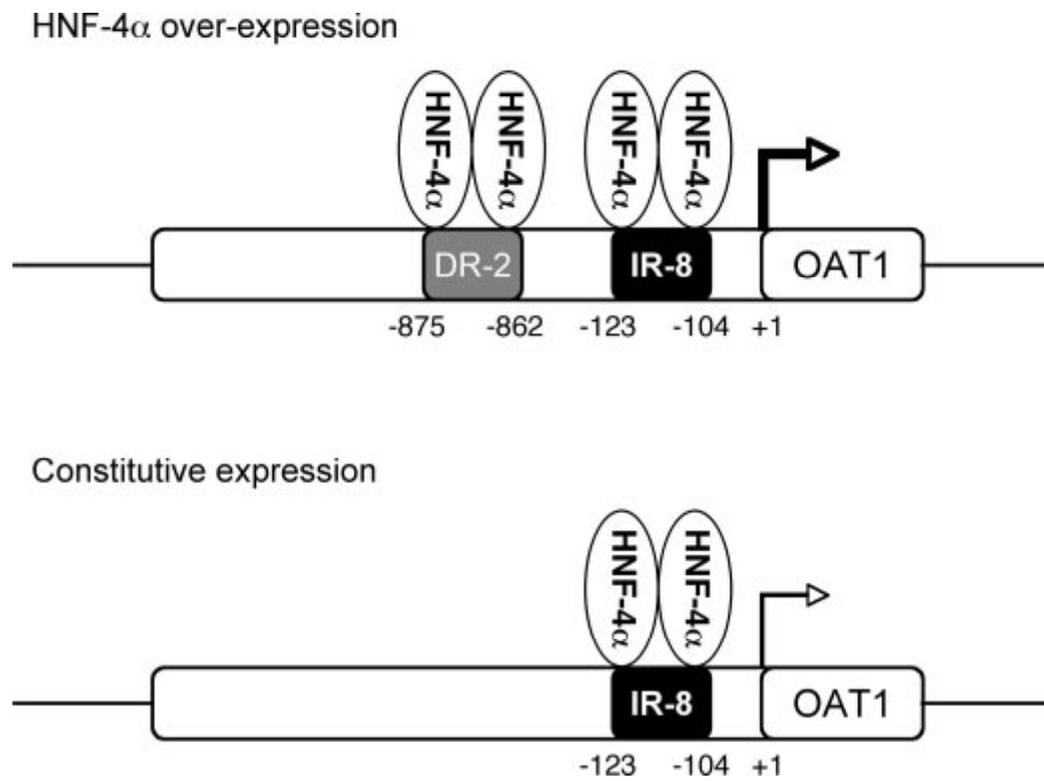


Fig. 4

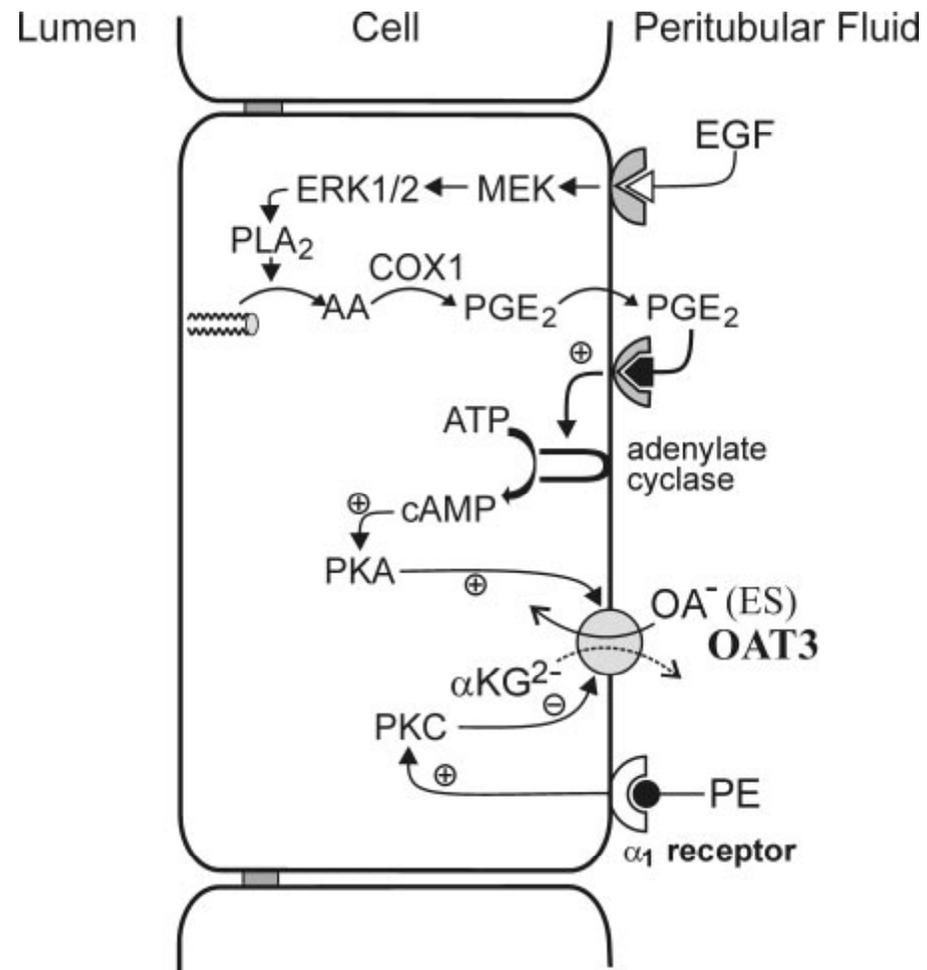


Fig. 5

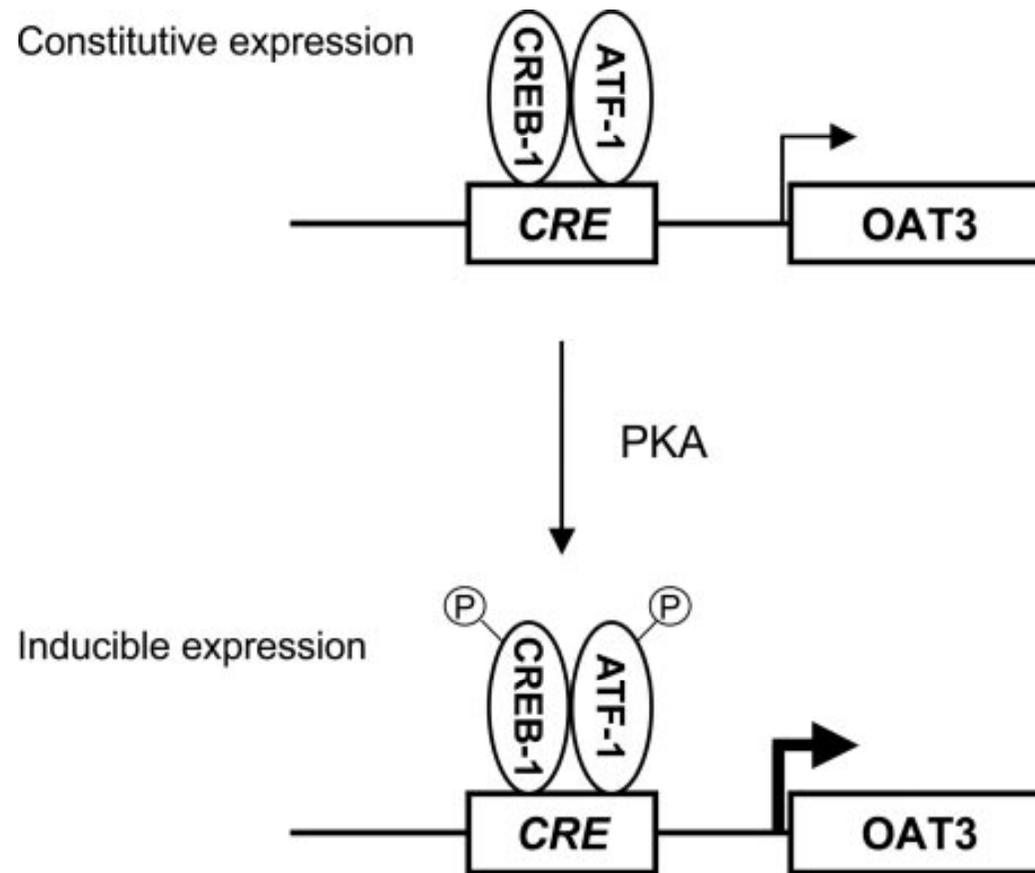


Fig. 6

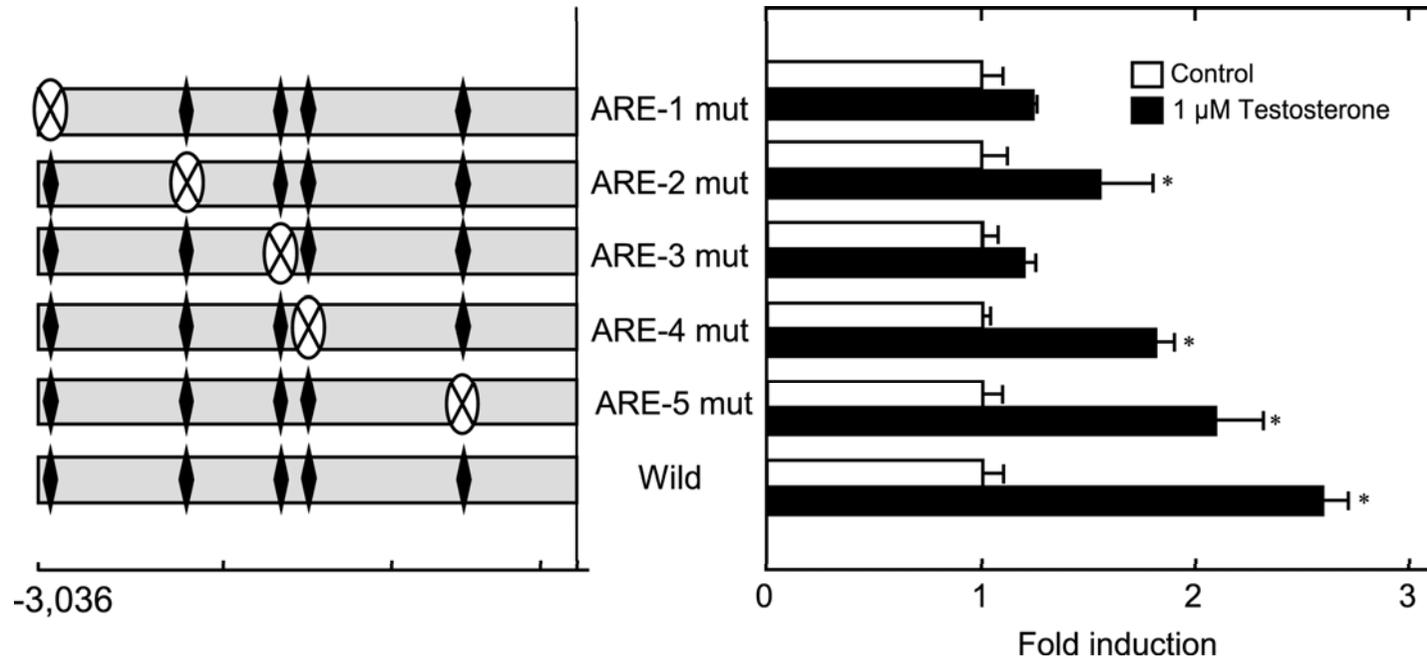


Fig. 7

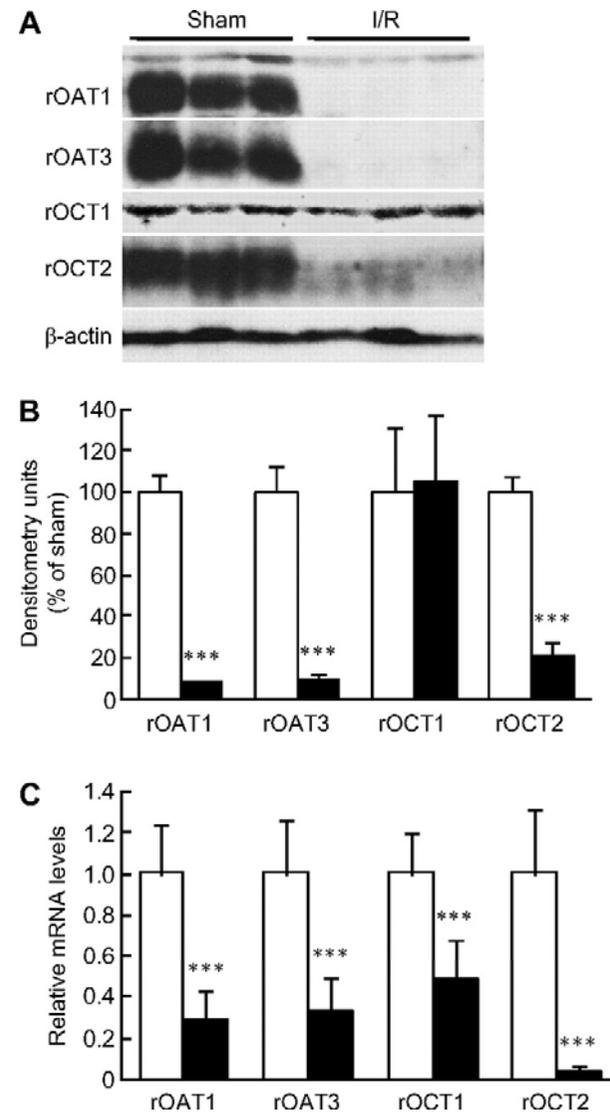


Fig. 8

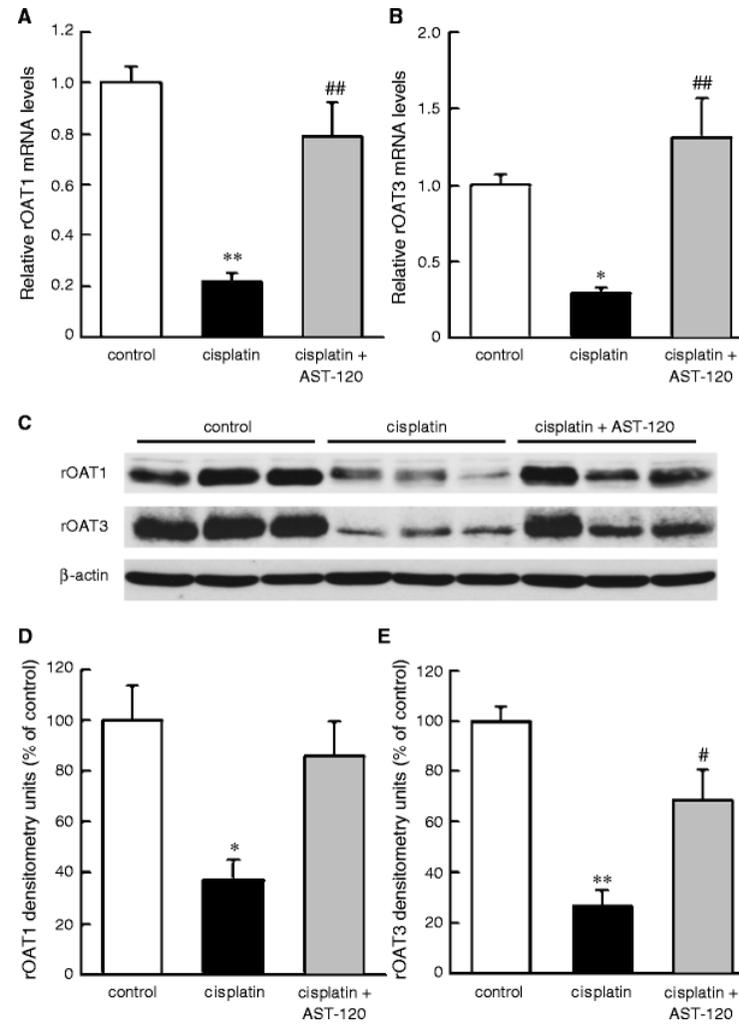


Fig. 9

