Cellular and Molecular Mechanisms of Renal Tubular Secretion of Organic Anions and Cations

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A wide variety of endogenous organic ions and xenobiotics are secreted into the urine via organic anion and cation transport systems, expressed in brush-border and basolateral membranes of renal tubular cells. Using membrane vesicles isolated from the kidney, cultured renal epithelial cells, isolated renal tubules, and slices of renal cortex, extensive studies have been done regarding the mechanisms of renal tubular secretion of organic ions. Basolateral entry of organic anions is mediated by the organic anion/dicarboxylate exchange system, whereas apical extrusion of organic anions from epithelial cells is mediated by an anion exchanger and/or by membrane potential-sensitive transport systems. Studies using membrane vesicles have made clear the fact that the basolateral transport of organic cations is stimulated by inside-negative membrane potential, whereas the transport of organic cations in brush-border membranes is achieved by a proton gradient. Transport studies using cultured renal epithelial cells have shown other aspects of organic ion transport, such as regulatory mechanisms for transcellular transport of organic anions and cations. The recent development of molecular techniques has greatly advanced our understanding of the molecular aspects of various transport processes. In 1994, a cDNA clone encoding the prototype organic cation transporter was isolated from rat kidney. Within the last 3 years, several organic anion and cation transporters in the kidney have been identified by different cloning techniques. In this review, we describe the mechanisms mediating renal tubular secretion of organic anions and cations, including recent topics in this area.

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The secretion of endogenous metabolites, drugs, and xenobiotics is an important physiologic function of the renal proximal tubules. In general, these compounds are negatively or positively charged organic species. The process of secreting organic anions and cations through the proximal tubular cells is achieved via unidirectional transcellular transport, involving the uptake of organic ions into the cells from blood across the basolateral membranes, followed by extrusion across brush-border membranes into the tubular fluid.[1,2]

A variety of experimental techniques have been used to study organic ion transport in the kidney. The clearance technique is the principal means of studying the renal handling of organic anions and cations in vivo. Slices of renal cortex and tubule suspensions have been studied extensively in vitro. Since the lumina of the tubules are collapsed in slice preparations, the movement of organic molecules into and out of the cells takes place almost exclusively across the peritubular membranes, accumulating levels of organic ions higher than those in the extracellular medium.

It has been difficult, however, to characterize the specific membrane events underlying the transepithelial transport of organic ions because of its complex structure, being composed of 2 distinct membranes, the luminal brush-border and contraluminal basolateral membranes. These 2 membranes differ in their enzyme compositions and in their transport mechanisms for solutes. Isolated brush-border and basolateral membrane vesicles have been used as in vitro model systems for studying renal transport. The advantages of using membrane vesicles are that 1) vesicles are free of metabolic reactions; 2) the composition of the intra and extra vesicular spaces can be manipulated, allowing analysis of the driving forces for transports; and 3) the transport properties

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of the 2 membranes can be studied separately. Furthermore, the development of cell culture techniques has offered advantages for the study of transcellular transport of solutes.

As the renal secretion of organic ions is a transcellular transport event across the proximal tubular epithelial cells, it is essential to study the transcellular transport and its regulation at the cellular level. In 1994, a new era in the study of the tubular transport of organic ions began with the cloning of the rat organic cation transporter, OCT1, by functional expression in *Xenopus laevis* oocytes.[3] Within the last 3 years, several organic anion and cation transporters in the kidney have been identified by different cloning techniques. This review focuses on recent progress in the study of the cellular and molecular mechanisms mediating renal secretion of organic anions and cations.

ORGANIC ANION TRANSPORT SYSTEMS

Organic Anion Transport by Brush-Border and Basolateral Membrane Vesicles

The organic anion transport system has been the subject of extensive investigation, and *p*-aminohippurate is commonly used as the prototype anion. Organic anions are taken up from the blood and secreted into the luminal fluid in the proximal tubules (Fig. 1). Studies of isolated membrane vesicles have helped greatly in understanding the mechanisms of organic anion transport in renal brush-border and basolateral membranes.

The transport of *p*-aminohippurate across basolateral membranes is a carrier-mediated process. It has been reported that *p*-aminohippurate transport in basolateral membranes was indirectly coupled to the Na^+ gradient.[4,5] The transport of dicarboxylates, such as glutarate and α-ketoglutarate, is actively driven by a Na^+ gradient via the Na^+/dicarboxylate cotransporter in renal basolateral membranes, resulting in an outward dicarboxylate gradient. This gradient, in turn, can drive *p*-aminohippurate uptake against its concentration gradient via the *p*-aminohippurate/dicarboxylate exchange system. Conversely, in brush-border membranes, *p*-aminohippurate is transported by an anion exchanger that transports many organic and inorganic anions, such as urate, lactate, OH^-, and Cl^-, and/or by a potential-sensitive transport system.[6–8]

Transcellular Transport of Organic Anions in OK Kidney Epithelial Cells

Cultured epithelial cells derived from the kidney have been useful in studying a variety of renal cellular functions, including transepithelial transport and the regulation of transport by hormones and drugs.[9,10] We recently reported that the transcellular transport of *p*-aminohippurate occurs unidirectionally from the basal to apical side across OK cell (kidney, American opossum) monolayers, and that the *p*-aminohippurate transport system in OK cells showed a substrate specificity similar to that in rat renal proximal tubules.[11,12] In addition, efflux of intracellular α-ketoglutarate from the OK cells to the basolateral side was increased by applying *p*-aminohippurate on the basolateral side of the cell monolayers.[13] Thus, OK cells are a good model system to study the secretory process of anionic drugs across renal epithelial cells.

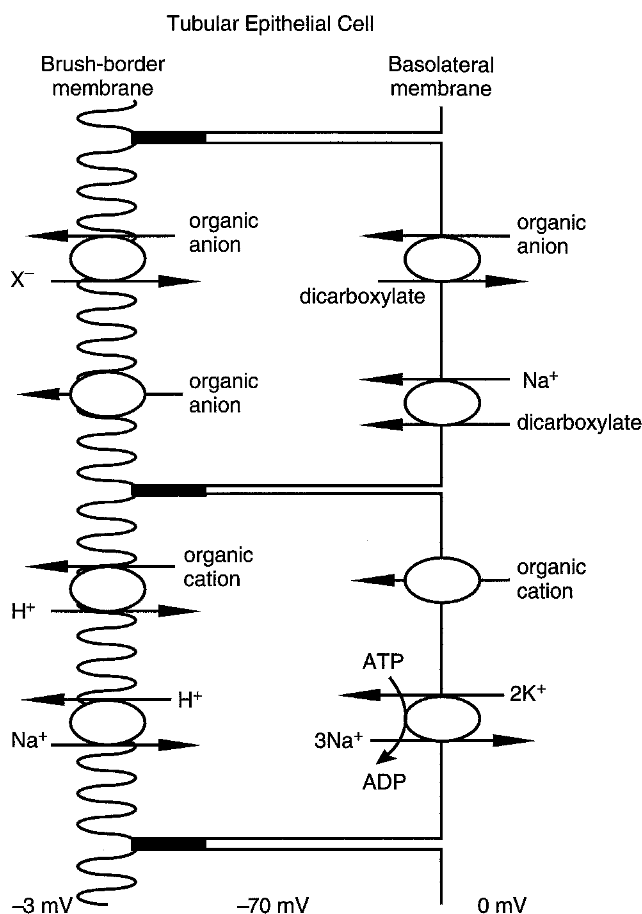

Fig. 1. Transport mechanisms of organic anions and cations in renal tubular cells. ATP, adenosine triphosphate; ADP, adenosine diphosphate; H^+, hydrogen ion; Na^+, sodium ion; K^+, potassium ion; X^-, anion; mV, millivolts.

Numerous studies have demonstrated that protein kinase C plays a central role in signal transduction induced by various hormones, and participates in the modulation of a variety of transport processes.[14,15] There is little information, however, concerning the regulation of organic anion transport by the intracellular signal transduction systems in the kidney. OK cells have specific parathyroid hormone receptors coupled to the protein kinase C and protein kinase A pathways, and several reports have shown that parathyroid hormone regulates the activities of

apically located Na^+/phosphate cotransport,[14] as well as Na^+/H^+ exchange,[15] via the protein kinase A and/or protein kinase C pathways. During the course of studying the regulation of organic anion transport, we demonstrated that active phorbol esters inhibit *p*-aminohippurate transport in OK cells, most likely via the activation of protein kinase C.[16] Parathyroid hormone also inhibited the transcellular transport of *p*-aminohippurate from the basal to the apical side, as well as the accumulation of *p*-aminohippurate in OK cells.[17] Conversely, protein kinase A activators did not affect the transcellular transport or accumulation of *p*-aminohippurate. The parathyroid hormone-induced *p*-aminohippurate transport was blocked by a protein kinase C inhibitor, staurosporine. Thus, protein kinase C may play an important role in the regulation of organic anion transport in the kidney.

Molecular Cloning and Functional Characterization of Organic Anion Transporters

Using the functional expression cloning strategy in *Xenopus laevis* oocytes, a Na^+-independent organic anion transporter of the rat liver was identified (organic anion transporting polypeptide).[18] A human analogue of the organic anion transporter was subsequently isolated by Kullak-Ublick et al.[19] The detection of messenger RNA transcripts, related to the organic anion transporting polypeptide in the rat kidney, suggested the localization of multispecific, organic anion transporters along the nephron.

These findings encouraged us to investigate the expression of transporter proteins, homologous to rat organic anion transporting polypeptide, in the renal tubules. We isolated a cDNA encoding a rat kidney-specific organic anion transporter, designated OAT-K1, which showed a 72% amino acid identity with the rat organic anion transporting polypeptide.[20] In transport studies, methotrexate and folate, but not *p*-aminohippurate or taurocholate, were accumulated by stably transfected renal cells expressing rat OAT-K1. The distribution of OAT-K1 mRNA along microdissected nephron segments was analyzed, and the immunolocalization of this molecule was examined in isolated plasma membranes from rat kidney (Fig. 2).[21] Using reverse transcription polymerase chain reaction (PCR), OAT-K1 mRNA was detected predominantly in the superficial and juxtamedullary proximal straight tubules. Western blotting with antiserum for OAT-K1 showed that the transporter protein, with an apparent molecular mass of 40 kDa, was expressed exclusively in brush-border membranes from rat kidney. Whether or not OAT-K1 transport contributes to secretion and/or reabsorption of methotrexate in renal tubules is presently unknown.

Expression cloning in *Xenopus laevis* oocytes was recently used to isolate an organic anion transport protein from rat kidney and winter flounder kidney by 3 groups, independently. Sekine et al.[22] and Sweet et al.[23] isolated a cDNA from rat kidney that encodes

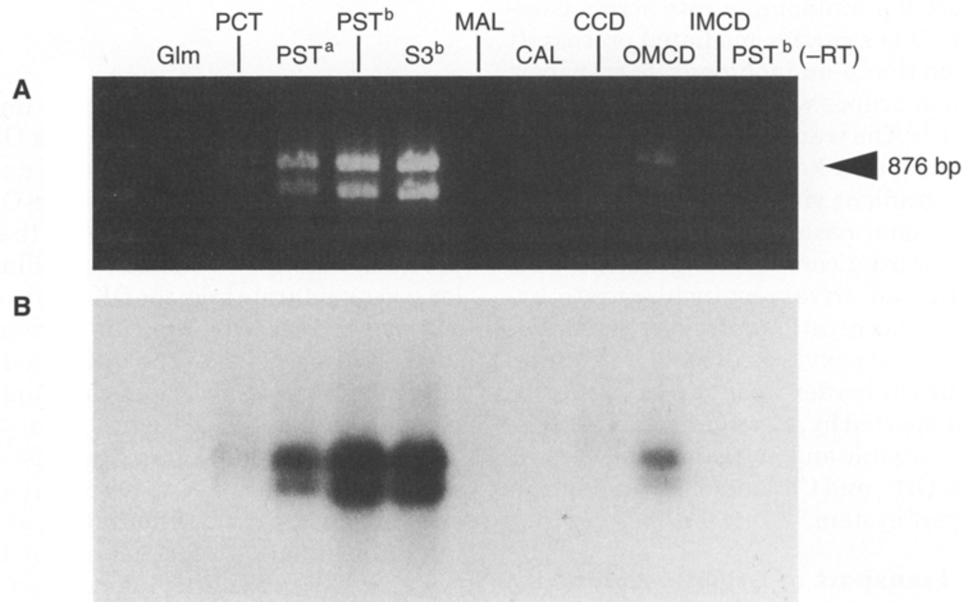

Fig. 2. Distribution of OAT-K1 mRNA in microdissected renal nephron segments. (**A**) Detection by reverse transcription-polymerase chain reaction. (**B**) Autoradiograms of corresponding Southern blots. Glm, glomerulus; PCT, proximal convoluted tubule; PST, proximal straight tubule; S3, late proximal straight tubule; MAL, medullary thick ascending limb; CAL, cortical thick ascending limb; CCD, cortical collecting duct; OMCD, outer medullary collecting duct; IMCD, inner medullary collecting duct; RT, reverse transcription. [a]Superficial; [b]juxtamedullary. Data reproduced, with permission, from Masuda et al.[21]

a 551-amino acid residue protein, with 12 putative membrane-spanning domains, designated OAT1 and ROAT1, respectively. The amino acid sequences of OAT1 and ROAT1 were identical. Both OAT1 and ROAT1 mediated sodium-independent, *p*-aminohippurate uptake, and the uptake rate of *p*-aminohippurate was increased by the outwardly directed dicarboxylate gradient, consistent with the characteristics of a basolateral, organic anion/dicarboxylate exchanger in membrane vesicle studies. Wolff et al.[24] also reported a cDNA encoding a basolateral *p*-aminohippurate transporter from winter flounder kidney, designated fROAT. Characteristics of organic anion transporters identified are listed in Table 1.

ORGANIC CATION TRANSPORT SYSTEMS

Organic Cation Transport by Brush-Border and Basolateral Membrane Vesicles

To characterize organic cation transport systems, the uptake of tetraethylammonium, a prototype cation, was studied, using brush-border and basolateral membrane vesicles.[30,31] The characteristics of carrier-mediated transport for tetraethylammonium were shown in brush-border and basolateral membrane vesicles. The uptake was saturable, was stimulated by the countertransport effect, and showed discontinuity in an Arrhenius plot. In brush-border membrane vesicles, the presence of an outward H^+ gradient ([pH] inside = 6.0, [pH] outside = 7.5) induced the transient uphill transport of tetraethylammonium (overshoot phenomenon), but this was not observed in basolateral membrane vesicles. The presence of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, a protonophore that rapidly dissipates the proton gradient, markedly decreased the H^+ gradient-induced stimulation of tetraethylammonium uptake. The uptake of tetraethylammonium driven by the H^+ gradient was completely inhibited by sulfhydryl reagents, the inhibitory effects of which were also observed in the absence of the H^+ gradient.[32] Tetraethylammonium uptake by basolateral membrane vesicles was stimulated by a valinomycin-induced, inside-negative membrane potential, while no effect on membrane potential was observed in brush-border membrane vesicles.

These results suggest that tetraethylammonium is transported across basolateral membranes via a

Table 1. Characteristics of organic anion and cation transporters.

Transporters	Species	Tissue distribution	Amino acids	Substrates	References
Organic anion transporters					
oatp	rat	Liver, kidney, brain	670	BSP, bile acids, steroid-glucuronides	Jacquemin et al., 1994[18]
	human	Liver, brain, lung, kidney, testes	670	BSP, bile acids	Kullak-Ublick et al., 1995[19]
OAT-K1	rat	Kidney	669	Methotrexate, folate	Saito et al., 1996[20]
OAT1, ROAT1	rat	Kidney	551	PAH, cAMP, cGMP, PGE_2, urate, α-ketoglutarate	Sekine et al., 1997[22] Sweet et al., 1997[23]
fROAT	winter flounder	Kidney	562	PAH	Wolff et al., 1997[24]
Organic cation transporters					
OCT1	rat	Kidney, liver, small intestine	556	TEA, MPP, choline, procainamide, NMN, dopamine	Gründemann et al., 1994[3]
	human	Liver	554	TEA, MPP	Zhang et al., 1997[25]
rOCT1A	rat	Kidney, liver, small intestine	430	TEA	Zhang et al., 1997[26]
OCT2	rat	Kidney	593	TEA, MPP, choline	Okuda et al., 1996[27]
	human	Kidney	555	TEA, MPP, NMN, choline	Gorboulev et al., 1997[28]
OCT2p	pig	$LLC-PK_1$ cells	554	TEA	Gründemann et al., 1997[29]

oatp, organic anion transporting polypeptide; BSP, sulfobromophthalein; PAH, *p*-aminohippurate; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; PGE_2, prostaglandin E_2; TEA, tetraethylammonium; MPP, 1-methyl-4-phenylpyridinium; NMN, N^1-methylnicotinamide.

carrier-mediated system, that this process is stimulated by an inside-negative membrane potential, and that tetraethylammonium transport across brush-border membranes is driven by an H^+ gradient via an electroneutral, H^+/tetraethylammonium antiport system (Fig. 1).[30] Under physiologic conditions, acidic luminal pH can be generated by the Na^+/H^+ antiport system in brush-border membranes. In addition, cimetidine[33] (cation) and amino–β-lactam antibiotics[34] (zwitterions), which are widely used drugs, share a common carrier transport system with tetraethylammonium in brush-border membranes.

Transcellular Transport of Organic Cations in LLC-PK_1 Kidney Epithelial Cells

The pig kidney epithelial cell line,[35] LLC-PK_1, has been used extensively as a model for the analysis of epithelial functions in the proximal tubules.[36,37] These cells form an oriented monolayer with microvilli and tight junctions, and exhibit unidirectional transport of electrolytes and some nutrients. We demonstrated that the apical membranes of LLC-PK_1 cells express the H^+/organic cation antiport system.[38] Fauth and coworkers[39] used LLC-PK_1 cell monolayers grown on permeable supports to examine the transepithelial transport of organic cations. We attempted to clarify the mechanisms of the transcellular transport of tetraethylammonium by LLC-PK_1 cell monolayers, grown on microporous membrane filters.[40] Tetraethylammonium was accumulated progressively in the monolayers from the basolateral side, and was transported unidirectionally to the apical side. The transcellular transport of tetraethylammonium was saturable, temperature-dependent, and sensitive to the pH of the apical side of the monolayers.

As shown in Fig. 3, unlabeled tetraethylammonium, amiloride, procainamide, cimetidine, and choline inhibited the basolateral uptake and transcellular transport of [^{14}C]tetraethylammonium. Interestingly, ofloxacin, a pyridonecarboxylic acid antibacterial drug, had a potent inhibitory effect on the transcellular transport of [^{14}C]tetraethylammonium, but not on its accumulation. A sulfhydryl reagent inhibited the tetraethylammonium transport at both the basolateral and apical membranes of the LLC-PK_1 cells. These findings suggest that LLC-PK_1 monolayers possess unidirectional transport systems for organic cations, and demonstrate the validity of LLC-PK_1 cell monolayers as a model for the renal proximal tubular secretion of organic cations.

Consistent with the inhibitory effect of ofloxacin on tetraethylammonium uptake in brush-border membrane vesicles,[41] levofloxacin, an optical isomer of ofloxacin, interacted with the apical H^+/organic cation antiport system to a greater extent than with the basolateral system.[42] However, transcellular transport of levofloxacin would be mediated by transport systems which are distinct from those for tetraethylammonium in LLC-PK_1 cells. Based on the results of pharmacokinetic analysis of tetraethylammonium transport in the LLC-PK_1 monolayers, we also confirmed that levofloxacin interacts with organic cation transporters, mostly in the apical membranes.[43]

Molecular Cloning and Functional Characterization of Organic Cation Transporters

Although a number of studies regarding the characteristics of organic cation transport in renal tubules have been performed, little information is available about the molecular structures of organic cation transporters. Holohan et al.[44] and Gilsdorf et al.[45] demonstrated that the molecular size of the H^+/organic cation antiporter in brush-border membrane vesicles was 41 kDa, using [^{3}H]azidopine as a photoaffinity-labeling reagent. Using a cimetidine analogue, Kimura et al.[46] identified a 35 kDa protein as a candidate for the organic cation transporter in brush-border membranes.

By functional expression cloning using *Xenopus* oocytes, Gründemann et al.[3] isolated a cDNA encoding an organic cation transporter, OCT1 (556 amino acids), from rat kidney. They deduced that OCT1 is a polyspecific organic cation transporter, mediating the basolateral uptake of organic cations in the liver and kidney. Using cDNA fragments encoding rat OCT1, we isolated a novel organic cation transporter, OCT2, from a rat kidney cDNA library.[27] The full-length, rat OCT2 cDNA clone consisted of 2205 base pairs, including a polyadenylation signal and a poly $(A)^+$ sequence at the 3′ terminus. The open reading frame of rat OCT2 encoded a protein of 593 amino acids, showing a 67% identity with rat OCT1. The hydropathy profile and the secondary structure of rat OCT2 indicated that the transporter has 12 putative, membrane-spanning α-helices. Northern hybridization and reverse transcription PCR analyses showed that the rat OCT2 transcript was expressed predominantly in the kidney, at higher levels in the medulla than in the cortex.

Human analogues of these organic cation transporters, hOCT1[25] and hOCT2,[28] have recently been isolated. Human OCT1 mRNA was expressed primarily in the human liver, whereas hOCT2 mRNA was predominantly expressed in the kidney. Furthermore, Zhang et al.[26] reported the presence of a splicing variant of rat OCT1 (rOCT1A) in the rat kidney, indicating heterogeneity of the organic cation transporter family. It is noteworthy that the amino acid sequences of the recently identified rat renal organic anion transporters, OAT1[22] and ROAT1,[23] showed homology with rat OCT1 and OCT2 (33% and 31% identity, respectively). Protein structure-function analysis of these transporters may

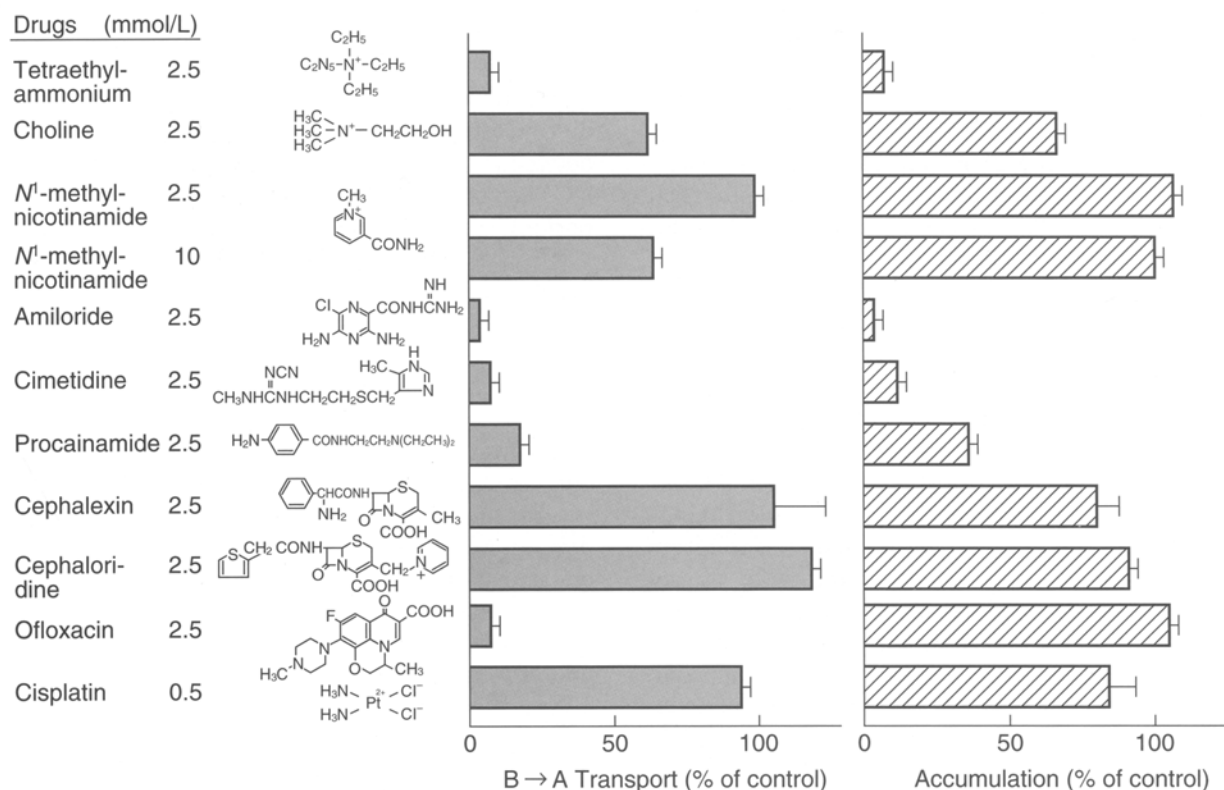

Fig. 3. Effect of various drugs on transcellular transport and accumulation of tetraethylammonium by LLC-PK_1 (pig kidney) monolayers. At 6 days after inoculation, LLC-PK_1 cell monolayers were incubated at 37°C for 60 minutes with 50 μmol/L [^{14}C]tetraethylammonium (pH 7.4) added to basolateral side in the presence and absence of various cationic drugs at same side. Appearance of radioactivity in apical side (pH 7.4) and cellular accumulation were measured. Data were expressed as percentage of control value. Each column represents mean ± SEM of 3 monolayers. Modified data, used with permission, from Saito et al.[40]

provide relevant information regarding the mechanisms of substrate recognition by these transporters.

Transport Characteristics of Organic Cation Transporters OCT1 and OCT2

By comparing the functional characteristics of 1-methyl-4-phenylpyridinium uptake between isolated rat hepatocytes and rat OCT1, Martel et al.[47] demonstrated that rat OCT1 is responsible for the hepatic uptake of small organic cations, which are classified as classical type I substrates. Busch et al.[48] reported tracer uptake studies and electrophysiological measurements, using *Xenopus* oocytes expressing rat OCT1. N^1-methylnicotinamide, 1-methyl-4-phenylpyridinium, tetraethylammonium, and choline were accumulated by OCT1-expressing oocytes. The transport of tetraethylammonium and choline was electrogenic, and independent of extracellular ion composition and pH. They also showed that rat OCT1 is responsible for the tubular secretion of monoamine neurotransmitters, such as dopamine, serotonin, noradrenaline, histamine, and acetylcholine, by using voltage-clamped *Xenopus* oocytes expressing rat OCT1.[49]

When in vitro-synthesized rat OCT2 RNA was injected into *Xenopus* oocytes, tetraethylammonium uptake into the oocytes was markedly elevated, and was saturated at high concentrations.[50] In the uptake buffer at various pHs from 5.4 to 8.0, OCT2-mediated tetraethylammonium uptake did not change significantly, suggesting that the transport of tetraethylammonium by OCT2 is not driven by the H^+ gradient. *Xenopus* oocytes form a transmembrane electrical potential under physiologic conditions, and this potential is decreased by the replacement of extracellular Na^+ with K^+. Uptake of tetraethylammonium by OCT2-expressing oocytes was decreased by high K^+ levels (100 mmol/L), suggesting that OCT2 was dependent on the transmembrane electrical potential. Tetraethylammonium uptake by oocytes expressing OCT2 was also markedly inhibited by cimetidine, procainamide, tetraethylammonium, N^1-methylnicotinamide, guanidine, and choline, although *p*-aminohippurate did not inhibit OCT2-mediated tetraethylammonium uptake (Fig. 4).

These studies suggest that rat OCT2 is a multispecific organic cation transporter, with characteris-

tics of that in the renal basolateral membranes. Gründemann et al.[29] isolated a porcine analogue of the organic cation transporter, OCT2p, from LLC-PK_1 kidney epithelial cells. They reported that tetraethylammonium uptake by OCT2p is pH dependent. In addition, by comparing the order of inhibitory potencies of several organic compounds on cellular accumulation of tetraethylammonium between OCT2p-transfected cells and LLC-PK_1 cells from the apical side, they deduced that OCT2 is an apical organic cation transporter, expressed in LLC-PK_1 cells. These controversial points should be resolved by further studies.

To further clarify the transport characteristics of rat OCT1 and OCT2, we constructed stable transfectants expressing rat OCT1 (MDCK-OCT1) and OCT2 (MDCK-OCT2), respectively.[51] The transfected MDCK cells were cultured on microporous membrane filters, and accumulation of tetraethylammonium by the cell monolayers was determined. When added to the basolateral medium, tetraethylammonium uptake by both MDCK-OCT1 and MDCK-OCT2 cells was markedly enhanced, but the uptake was minimal when tetraethylammonium was added to the apical medium. The efflux of tetraethylammonium from both transfectant lines was not changed by extracellular pH, suggesting that both rat OCT1 and rat OCT2 are basolateral-type organic cation transporters. The Michaelis constant (K_m) values for tetraethylammonium uptake by both MDCK-OCT1 and MDCK-OCT2 cells were similar. The inhibitory potencies of various organic cations, including 1-methyl-4-phenylpyridinium, cimetidine, quinidine, nicotine, N^1-methylnicotinamide, and guanidine on tetraethylammonium uptake were also similar between rat OCT1 and OCT2, suggesting that both rat OCT1 and OCT2 are responsible for the tubular transport of common organic cations. Characteristics of organic cation transporters identified are listed in Table 1.

CONCLUSION

Our understanding of the tubular secretion of organic anions and cations has greatly progressed in recent years, due to the development of new techniques. Molecular studies of organic ion transporters especially have provided new insight into the tubular secretion of drugs and xenobiotics. Transporter protein structure-function relationships are poorly understood at this time, however. The roles of organic ion transporters in physiologic conditions and their relation to the kidney diseases still remain to be clarified. Further studies must be done to achieve sufficient understanding of the renal secretion of organic ions, including the identification of other transporters in brush-border membranes, such as H^+/organic cation antiporter.

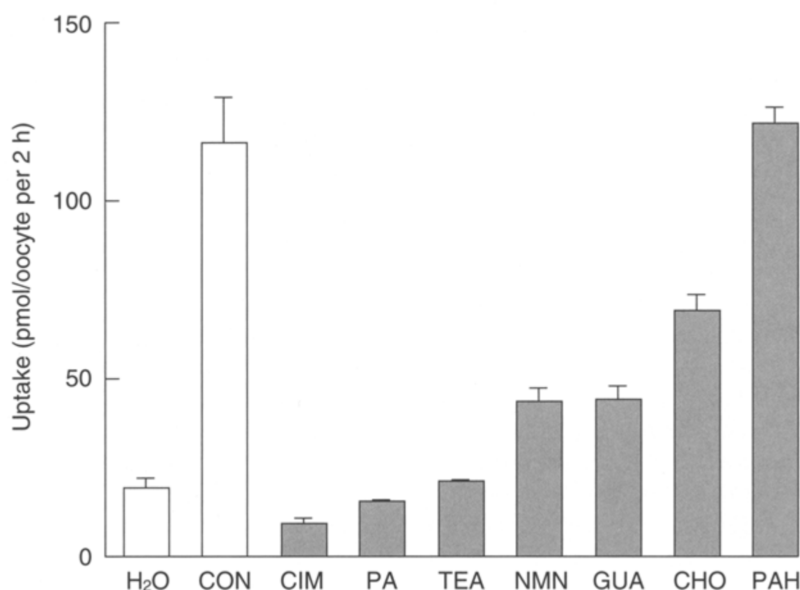

Fig. 4. Effect of various organic cations on tetraethylammonium uptake by OCT2 RNA-injected *Xenopus* oocytes. Uptake by oocytes was assayed at 25°C for 2 hours in incubation buffer containing 250 μmol/L [^{14}C]tetraethylammonium, in the presence and absence of various organic cations (2.5 mmol/L), 1 day after injection of 50 nL of water or OCT2 RNA. Each column represents the mean ± SEM of 4 experiments. Four oocytes were used for each uptake experiment. Con, control; CIM, cimetidine; PA, procainamide; TEA, tetraethylammonium; NMN, N^1-methylnicotinamide; GUA, guanidine; CHO, choline; PAH, *p*-aminohippurate.

ACKNOWLEDGMENTS

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