Molecular and Cellular Physiology of Renal Organic Cation and Anion Transport

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Wright, Stephen H., and William H. Dantzler. Molecular and Cellular Physiology of Renal Organic Cation and Anion Transport. Physiol Rev 84: 987-1049, 2004; 10.1152/physrev.00040.2003.—Organic cations and anions (OCs and OAs, respectively) constitute an extraordinarily diverse array of compounds of physiological, pharmacological, and toxicological importance. Renal secretion of these compounds, which occurs principally along the proximal portion of the nephron, plays a critical role in regulating their plasma concentrations and in clearing the body of potentially toxic xenobiotics agents. The transepithelial transport involves separate entry and exit steps at the basolateral and luminal aspects of renal tubular cells. It is increasingly apparent that basolateral and luminal OC and OA transport reflects the concerted activity of a suite of separate transport processes arranged in parallel in each pole of proximal tubule cells. The cloning of multiple members of several distinct transport families, the subsequent characterization of their activity, and their subcellular localization within distinct regions of the kidney now allows the development of models describing the molecular basis of the renal secretion of OCs and OAs. This review examines recent work on this issue, with particular emphasis on attempts to integrate information concerning the activity of cloned transporters in heterologous expression systems to that observed in studies of physiologically intact renal systems.

I. INTRODUCTION

Renal secretion of organic electrolytes of broadly diverse chemical structures plays a critical role in limiting the body’s exposure to toxic compounds of exogenous and endogenous origin (including a wide array of compounds of clinical importance). Because of the physiological, pharmacological, and toxicological importance of the renal secretion of “organic cations” (including bases; collectively, OCs) and “organic anions” (including acids; collectively, OAs), the underlying processes have received increasing attention over the last 20 years. The field last received an exhaustive review in 1993 (342), a time at which no transporters associated with the processes of renal OC and OA secretion had yet been cloned. Indeed, schematic models of renal OC and OA secretion, based on the then available functional evidence, typically showed a single peritubular “avenue of entry” into cells for each chemical class of substrate (OC or OA) and one (or two) luminal “avenue(s) of exit.” These functionally characterized pathways were often (and continue to be) referred to as the “classical” pathways for renal secretion of organic cations and anions. As a consequence, however, of the enormous recent growth in information concerning the molecular identity of transporters that accept OCs and OAs as substrates, we now know that the classical processes of renal OC and OA secretion are the products of the concerted activity of an extensive array of discrete molecular entities. These include, but are not restricted to, the entire spectrum of members of what is now referred to as the OCT family of amphiphilic solute transporters, as well as selected members of the multidrug resistance transporters (MDRs and MRPs) and the organic anion transporting polypeptide (OATP) transporters.
In this review we have attempted to compile the novel material on the molecular and cellular physiology of renal OC and OA transport that has accrued since the 1993 review of Pritchard and Miller (342). Readers interested in the material preceding 1993 are directed to that excellent discussion and to several earlier reviews that assessed the historical sweep of studies in this area (292, 355, 514, 515). Here, we limit ourselves to an examination of the several families of transport processes currently suspected of playing significant roles in the renal handling of organic cations and anions. Readers interested in more detail on selected issues should consult excellent recent reviews focused on specific subjects (38, 41, 89, 218–221, 227, 343, 426, 465, 496, 501, 547). We also note that, although there is considerable functional overlap of the OC and OA transport processes expressed in the kidney and the liver (and gastrointestinal tract), we have limited our focus to those processes believed to play major roles in the transport of these compounds in renal cells. Readers are directed to reviews on organic electrolyte transport expressed in the liver (12, 201, 231, 233, 273, 419, 501) and other organs (14, 157, 220, 224, 311, 398, 547).

The review is organized in the following fashion. The molecular and cellular physiology of organic cation secretion is presented first. This reflects the fact that the first of the associated processes to be cloned was a cation transporter (OCT1). The resulting family of transport processes, which includes, as it turns out, processes involved with the classical OA secretory pathway, is referred to as the “organic cation transporter family” (253, 361). For each class of substrate (OC or OA), we initially discuss the general physiology of renal secretion, particularly as it was understood in 1993. This is followed by an extended discussion of the molecular characteristics of individual members of the several families of transporters implicated in renal transport of these substrates. Then, in the light of increasing understanding of the molecular properties of these processes, we consider recent, novel observations on the cellular physiology of renal OC or OA transport, with a particular effort focused on highlighting where “molecular” information has helped clarify “cellular” information, and where physiological observations run counter to expectations arising from the results obtained studying cloned transporters. We close with a discussion of issues that we think require future study. Foremost among these is the need to integrate knowledge obtained on individual transporters into the cellular events that result in renal OC or OA secretion. These secretory processes involve the coordinated function of many separate processes working in the physiological context of intact cells and tissues. Finally, we offer some speculative views on evolutionary aspects of renal OC and OA transport.

II. RENAL ORGANIC CATION TRANSPORT

A. Overview of the Physiological Characteristics of Renal OC Transport

The proximal tubule is the primary site of renal OC secretion, as determined by stop-flow, micropuncture, and microperfusion studies (342, 355). The proximal tubule is also responsible for the reabsorption of a more limited number of cationic substrates (342). In general, substrates for the pathways involved in renal OC transport include a diverse array of primary, secondary, tertiary, or quaternary amines that have a net positive charge on the amine nitrogen at physiological pH. Although a number of endogenous OCs have been shown to be actively secreted by the proximal tubule [e.g., N\textsuperscript{1}-methylnicotinamide (NMN), choline, epinephrine, and dopamine; see Ref. 22], it is generally accepted that the principal function of this process is clearing the body of xenobiotic agents (342), including a wide range of alkaloids and other positively charged, heterocyclic compounds of dietary origin; cationic drugs of therapeutic or recreational use; or other cationic toxins of environmental origin (e.g., nicotine). The secretory process is also a site of clinically significant interactions between OCs in humans. For example, therapeutic doses of cimetidine retard the renal elimination of procainamide (408, 409) and nicotine (17).

Until recently, models of the cellular basis of renal OC secretion typically depicted a single basolateral entry step and a single luminal exit step, a simple view that effectively explained existing physiological data. That view is now known to be an oversimplification of the suite of cellular events that underlies renal OC transport. In developing a model for the functional basis of this complexity, it is useful to consider the “type I” and “type II” classifications for different structural classes of organic cations developed to describe OC secretion in the liver (274). In general terms, type I OCs are comparatively small (generally <400 mol wt) monovalent compounds, such as tetraethylammonium (TEA), tributylmethylammonium (TBuMA), and procainamide ethobromide. Type II OCs, which are usually bulkier (generally >500 mol wt) and frequently polyvalent, include d-tubocurarine, vercuronium, and hexafluorourenium.
Figure 1 shows a model for transcellular secretion of OCs by the proximal tubule that is consistent with observations obtained in studies with isolated renal plasma membranes and intact proximal tubules (342, 343) and that is supported by recent molecular data. OCs enter the cell from the blood across the peritubular membrane. For type I OCs, this entry step involves either an electrogenic uniport (facilitated diffusion), driven by the inside-negative electrical potential difference (PD) (407), or an electroneutral antiport (exchange) of OCs (82, 407) [it is likely that these two mechanisms represent alternative modes of action of the same transporter(s); Ref. 44]. The negative PD is sufficient to account for an accumulation of OCs within proximal cells to levels 10–15 times that in the blood. Studies by Ullrich and colleagues (468, 476) on the structural specificity of inhibition of peritubular OC transport in microperfused rat proximal tubules in vivo indicate a clear correlation between an increase in substrate hydrophobicity and an increase in inhibitory effectiveness, although it is also clear that steric factors influence the interaction of type I OCs with basolateral transporters (e.g., Refs. 15, 470). The marked hydrophobicity of many type II OCs probably results in a substantial diffusive flux across the peritubular membrane, providing an alternative, electrically conductive avenue for entry into proximal cells.

Exit of type I OCs across the luminal membrane involves a carrier-mediated antiport of OC for H\(^+\) (Fig. 1), a process observed in brush-border membrane vesicles (BBMV) isolated from human, rabbit, rat, dog, chicken, and snake kidneys (342). The electroneutral antiport of one OC\(^+\) for one H\(^+\) (533) permits OCs to leave the cells and develop a luminal concentration as large as or larger (depending on the size of the transluminal H\(^+\) gradient) than that in the cytoplasm, resulting in net transepithelial secretion. OC/H\(^+\) antiport is the active step in this process because it depends on the displacement of H\(^+\) away from electrochemical equilibrium, a state maintained through the activity in the luminal membrane of the Na\(^+\)/H\(^+\) exchanger and, to a lesser extent, a V-type H\(^+\)-ATPase (125). Luminal OC/H\(^+\) exchange appears to be the rate-limiting step in transepithelial OC transport (342, 372). Evidence on the structural specificity of luminal OC transport indicates that, as with the peritubular process, binding to the OC/H\(^+\) exchanger is profoundly influenced by substrate hydrophobicity (536). The secretory flux of type II OCs appears to require interaction with the apically located multidrug resistance transporter (MDR1) (102).

We can summarize the current, overall understanding of the cellular processes associated with secretion of organic cations as follows: type I OCs enter proximal cells across the peritubular membrane via electrogenic facilitated diffusion and leave cells across the luminal membrane via electroneutral exchange for H\(^+\). Type II OCs diffuse into proximal cells across the peritubular membrane and are exported into the tubule filtrate via the primary active MDR1 transporter. Importantly, considerable overlap appears to exist in the selectivity of these parallel transport pathways (502; see also Ref. 403). It is also evident, as discussed below, that OC transport across the basolateral and luminal membranes of renal proximal tubules involves the parallel activity of several distinct transport processes.
B. Molecular Characteristics of Renal OC Transporters

1. OCT family of OC transporters

Several alternative nomenclatures are currently in use for describing members of the OCT family of transporters, and we will commonly provide two of these when we introduce each member of the family. The first is the designation within the solute carrier superfamily (SLC), as defined by the Human Genome Nomenclature Committee, in which the OCTs (and the related OATs) are members of group SLC22A. The second reflects the classification system developed by Saier for transport proteins (the Transport Commission; Ref. 361), within which the organic cation transporters are listed as members of the major facilitator superfamily (MFS) and are classified as 2.A.1.19: 2 (electrochemical potential driven porters), A (uniporters, symporters, antiporters), 1 (major facilitator superfamily), 19 (organic cation transporter family). Schömig et al. (382) suggested the alternative name “amphiphilic solute facilitator” (ASF) family for this group of transporters within the MFS, focusing on the characteristics of substrate specificity which link all members of this group. Common structural features of MFS proteins, which are generally shared by the family 19 OCT transporters, include 12 putative transmembrane spanning domains (TMDs) and several highly conserved structural motifs, including a striking degree of conservation for a 13-residue sequence found between TMD2 and TMD3: G-[RKPATY]-L-[GAS]-[DN]-[RK]-[FY]-G-R-[RK]-[RKP]-[LIVGST]-[LIM]. Figure 2 shows the remarkable conservation of this “signature sequence” for 37 of the cation transporting members of the OCT family.

Schömig et al. (382) compared the sequences of nine members of the OCT (ASF) family to determine whether these proteins contained any sequence features that would distinguish them from other members of the MFS.

FIG. 2. Sequence alignment of 37 members of the organic cation transporter family showing the degree of conservation of signature sequence motifs characteristic of the major facilitator superfamily (MFS) and the amphiphilic solute facilitator (ASF) family. The MFS motif (left) is 13 amino acids long, and the ASF motif (right) is 11 amino acids long, as indicated by the shaded regions within the aligned sequences. Open boxes within the shaded regions indicate sequence deviations from the signature motifs.
They suggested the following as possible signature sequences: STIVTEW[D/N]LVC before TMD2; ELYPT after TMD10, and LP[D/E]TI after TMD12. Figure 2 extends this comparison for the first of these motifs to 37 OCT family members. Although a high degree of homology is evident, it is also clear that a degree of conserved degeneracy within the listed motif exists at two residues. Within the 11-residue motif, all the orthologs for OCT1, OCT2, and OCT3 express phenylalanine (instead of tryptophan expressed in OCTNs) at residue seven (as do both of the Caenorhabditis elegans members of the OCT family). Also, whereas orthologs of OCT3, OCTN1, OCTN2, and several of the anion transporters of the OCT family express threonine at residue 2 of this motif, the OCT1 and OCT2 orthologs cloned to date all express serine (instead of threonine). Thus the following "signature motif" for ASP transporters presents itself: S[T/S]IVTE[W/F][D/N]LVC.

Selected amino acids are conserved in all, or virtually all, of the OCT family members cloned to date. These include 4 cysteines and 10 prolines, which suggest an importance for these residues in establishing the secondary structure of this group of proteins. A number of charged amino acids are also conserved, and their potential role in the function of these organic electrolyte transporters is discussed in upcoming sections.

a) OCT1. I) Structure. OCT1 (SLC22A1; 2.A.1.19.1) was cloned in 1994 by Koepsell and colleagues (142) from a cDNA library prepared from rat kidney.1 Orthologs were subsequently cloned from three additional mammalian species (mouse (132), rabbit (451), and human (549)) displaying from 95 to 78% identity with rOCT1. In addition, related sequences have also been cloned from C. elegans (ceOCT1; 28.4% identical; Ref. 537) and Drosophila melanogaster (dOCT; 33.7% identical; Ref. 450). The mammalian isoforms vary in length from 554 to 556 amino acids.

By hydrophy analysis, OCT1 orthologs appear to have 11 or 12 TMDs. It is generally acknowledged that 12 TMDs are likely, in accord with the predicted secondary structure profiles of most MFS members (334), and the recent high-resolution analyses of the structures of the MFS members, LacY (lactose transporter of Escherichia coli; Ref. 1) and GlpT (glycerol-3-phosphate transporter of E. coli; Ref. 174). The secondary structure predicted from a 12 TMD configuration includes intracellular NH2 and COOH termini, a very long extracellular loop between TMD1 and TMD2 (which typically includes three to four N-linked glycosylation sites), and a comparatively long intracellular domain between TMD 6 and TMD 7 (Fig. 3). Conserved motifs within OCT1 across all species include the following (426): 13 cysteine residues, 25 proline residues, 3 N-linked glycosylation sites2 (N 71, 96, 112), 3 protein kinase C (PKC) consensus sites3 (Ser-285, Ser-291, and Ser/Thr-327), 3 protein kinase A (PKA) consensus sites (Thr-235, Thr-296, and Thr-347), 1 protein kinase G (PKG) consensus site (Thr-347), 2 casein kinase II (CKII) consensus sites (Ser-333 and Thr-524), and 1 Ca2+/calmodulin kinase II (CaMII) consensus site (Thr-347). Of these sites, 7 cysteine residues, 17 proline residues, 1 N-linked glycosylation site (N 72), 1 PKA site (Thr-347), 1 PKC site (Ser-285), 1 PKG site (Ser-347), and 2 CKII sites (Ser-333 and Thr-524) are conserved across OCT1, OCT2, and OCT3.

Immunocytochemical evidence supports this postulated secondary structure. Permeabilized and nonpermeabilized HEK 293 cells that stably expressed rOCT1 (276) were exposed to antibodies prepared against the postulated long extracellular loop and the intracellular COOH terminus. Whereas the antibody against the extracellular loop stained both the permeabilized and the nonpermeabilized cells, the antibody against the COOH terminus stained only the permeabilized cells. These observations are consistent with the secondary structure profile presented in Figure 3. The gene for rOCT1 (Roct1) has been mapped to chromosome 1q11–12 (216), and the gene for the human ortholog has been mapped to chromosome 6q26 (217).

The human gene for OCT1 consists of seven exons and six introns (152). Several alternatively spliced variants of OCT1 have been described. The rOCT1A variant (548) arises from a 104-bp deletion between bp 451 and 556 of rOCT1. Although the resulting protein product lacks putative TMDs 1 and 2 and the large extracellular loop that separates those two TMDs, the gene product supports mediated transport of TEA (548). In the human, four alternatively spliced isoforms are present in human glioma cells (152): a long (full-length) form and three shorter forms. Only the long form (hOCT1G/L554) supports transport when expressed in HEK293 cells. The long form and one of the shorter forms (hOCT1G/L506) are present in human liver cDNA (152).

II) Tissue and cellular distribution and localization. OCT1 appears to be expressed in many tissues, although intriguing species differences have been noted. OCT1 is expressed in the liver of all species tested [rat, Northern (142); mouse, cDNA library (132); human,
Northern (129, 549); rabbit, RT-PCR (451); however, expression in the kidney shows more variability among species. In rats, OCT1 is heavily expressed in the kidney (cortex) (402), with substantial expression in the liver (142). It is also well expressed in the colon, small intestine (142), skin, and spleen (402). In rat liver, immunocytochemistry shows that OCT1 expression is restricted to the basolateral (sinusoidal) aspect of hepatocytes (276). This distribution was confirmed in Western blots, which showed a strong 67-kDa band in isolated sinusoidal membranes and a weak reaction (perhaps due to sinusoidal contamination) with isolated biliary membranes (276). Western blots of isolated rat renal membrane vesicles also indicated that OCT1 is restricted to the basolateral domain of cortical cells (489). In a thorough immunocytochemical assessment of OCT distribution in rat kidney

FIG. 3. A: postulated secondary structure of the human ortholog of OCT1. Features common to other members of the OCT transporter family include 12 transmembrane spanning domains (TMDs), a large extracellular loop between TMDs 1 and 2, and a large cytoplasmic loop between TMDs 6 and 7. Indicated in black are the conserved MFS and ASF motifs. (Secondary structure determined using TMpred [http://www.ch.embnet.org/software/TMPRED_form.html]; layout prepared using TOPO2 [http://www.sacs.ucsf.edu/TOPO/].) B: unrooted phylogenetic tree of the OCT family. The scale bar indicates nucleotide substitutions per site (prepared using TreeView 1.6.6).
OCT1 expression was restricted to the basolateral membrane of proximal tubule cells, with the greatest expression apparent in early (S1) segments of superficial, midcortical, and juxtamedullary nephrons. OCT1 was also evident in proximal S2 segments, although not to the same extent as in S1 segments. Expression in late (S3) proximal tubule, although evident, was markedly less than that in the early (S1) proximal tubule. At the transition between outer stripe (S3) and inner stripe of the outer medulla, OCT1 expression was clearly limited to the late S3 segment (and not in the thin descending limb of Henle’s loop). Determination of rOCT1 mRNA in renal tubules by in situ hybridization revealed a distribution for the mRNA qualitatively similar to that for the protein shown by immunocytochemistry. However, the relative abundance of the mRNA in the different tubule segments differed from that of the protein. Whereas the highest protein content apparent was in the S1 segments, the highest concentration of mRNA appeared to be in the S2 and S3 segments (195). No rOCT1 expression (protein or mRNA) was evident in cells of glomeruli or distal tubules (see also Ref. 488). Tissue distribution of rOCT1 during embryonic development appears to differ from that in adults. During early development (up to day E16), expression of rOCT1 mRNA is restricted to liver and brain (335). After E16, rOCT1 mRNA is expressed in the kidney, rising through birth (335) and thereafter (402). The increase in rat OCT1 mRNA from birth through adulthood (which is paralleled by increases in mRNA for OCT2 and OCT3; Ref. 402) coincides with increases in TEA uptake into mouse renal slices through this developmental period (93).

In rabbits, OCT1 is clearly expressed in the kidney (and small intestine), but it appears to be more strongly expressed in the liver (451). In humans, OCT1 expression appears primarily in the liver (129, 549). In one study (300), immunocytochemistry, Western analysis, and quantitative RT-PCR failed to demonstrate the expression of OCT1 in human renal tissue. However, the demonstration in some other studies [by Northern analysis (549) and by RT-PCR (129)] of modest expression of OCT1 in human renal tissue has led to the suggestion that this isoform may play a “housekeeping role” with respect to OC transport in human tissues other than the liver, where it may play a predominant role in secretion of type I OCs (129, 218).

III) Functional characteristics. OCT1-mediated transport activity has been functionally expressed in several heterologous expression systems: *Xenopus* oocytes (43, 44, 61, 88, 129, 130, 132, 142, 304, 307, 451, 502, 548, 549), HEK-293 cells (32, 43, 130, 256, 270, 276), HeLa cells (550–552), Madin-Darby canine kidney (MDCK) cells (488, 489), Chinese hamster ovary (CHO) (191), and COS-7 cells (553). All the orthologs tested supported saturable transport of TEA, with apparent Michaelis constants ranging from 38 to 251 μM. This transport is sensitive to the *trans*-membrane electrical potential. Increasing the external concentration of K⁺ (which depolarizes the oocyte membrane by ~20–50 mV; Refs. 142, 549) reduces the rate of TEA uptake into *Xenopus* oocytes injected with cRNA for rOCT1 (142) and hOCT1 (549). The degree of inhibition can be small, as shown by the observation that in oocytes injected with mOCT1 cRNA, the reduction of TEA uptake associated with elevation of external K⁺ fails to be statistically significant (132).

Direct evidence that OCT1-mediated transport of TEA and other type I cations results in an inward current was obtained in studies using voltage-clamped oocytes injected with rOCT1 (44). The relationship between inward current and the external concentration of substrate shows saturation, with the apparent Michaelis constants varying as a function of the holding potential. In one study, for example, as the holding potential was changed from –90 to –10 mV, the apparent Kᵣ (Michaelis constant; substrate concentration resulting in half-maximal transport) for TEA uptake increased from 14 to 49 μM (44). Interestingly, half-saturation constants derived from saturation of inward currents are typically severalfold lower than those measured using conventional radiotracer techniques. For example, as noted above, the apparent Kᵣ for TEA uptake mediated by rOCT1 in oocytes is on the order of 100–250 μM (61, 142). In addition, the rate of TEA uptake in oocytes inferred from the inward current supported by rOCT1 is about four- to fivefold greater than the rate of radiolabeled TEA uptake measured in parallel experiments (44). This difference may reflect a degree of substrate-induced ion-leak current through rOCT1 (e.g., Ref. 509). It may also reflect the fact that transport of labeled substrates into oocytes (and other cells) that are not voltage clamped can be expected to depolarize the cells, thereby decreasing the driving forces that, in turn, influence (in this case, decrease) the maximal rate of transport (44).

Every OCT1 ortholog tested to date, including mOCT1, supports the saturable electrogenic transport of TEA and other small, type I OCs (e.g., choline, tetramethylammonium, NMN, dopamine) into oocytes (43, 44, 88, 130, 304). Initial studies of electrical currents suggested that rOCT1 also supports electrogenic transport of several large, mono- and divalent type II OCs, including quinidine, quindine, d-tubocurarine, and pancecuronium (44). However, subsequent experiments (304) indicated that the electrogenic currents produced by exposure of rOCT1-expressing oocytes to large type II OCs actually reflect inhibition by these compounds of the rOCT1-mediated electrogenic efflux of endogenous type I OCs (e.g., choline) (304). Nevertheless, additional evidence supports the conclusion that the protonated form of quindine, at least, can serve as a transported substrate of rOCT1 (502).
In addition to operating as an electrogenic unipporter, OCT1 can also mediate OC/OC exchange (44, 88, 304, 550–552). Preloading Xenopus oocytes with unlabeled TEA, for example, stimulates the uptake of [3H]MPP by human, rabbit, mouse, and rat OCT1 (88). The symmetry of this type of trans-effect is apparent in observations of accelerated efflux of preloaded [3H]MPP from rOCT1-expressing oocytes in the presence of inwardly directed gradients of unlabeled TEA (44) or MPP (304). Human OCT1 also supports trans-stimulation of both influx and efflux (of TEA), but quantitative differences in the extent of these stimulated fluxes produced by some substrates (e.g., TBuMA) have led to the suggestion of asymmetrical binding properties on the extracellular versus intracellular face of the transporter (550).

Trans-inhibition of OCT1-mediated transport has also been noted (44, 88, 304, 550–552), usually when cells are exposed to compounds that are also observed to be high-affinity cis-inhibitors of OCT1-mediated activity. For example, although quinine and quinidine are (at best) transported poorly by rOCT1 (304, 502), these substrates cis-inhibit MPP uptake and trans-inhibit MPP efflux in Xenopus oocytes (304). Similar results are seen with the type II OCs d-tubocurarine and cyanine 863 (304), suggesting that when loaded with bulky, hydrophobic substrates, OCT1 undergoes the conformational changes associated with translocation much more slowly (if at all) than the unloaded transporter. At least two alternative explanations can be offered. First, following the conformational change(s) associated with translocation, hydrophobic substrates may dissociate slowly from the binding site, thereby reducing the "physiological turnover" of the substrate-transporter complex (i.e., the sum total of events associated with net substrate transport: binding of substrate to the transporter at the cis-aspect of the membrane, "translocation" of the substrate-transporter complex, dissociation of substrate from the transporter at the trans-face of the membrane, and a second translocation event that returns the transporter to a cis-facing conformation). Second, hydrophobic cations may diffuse across the membrane and exert what amounts to a cis-inhibition of efflux at the cytoplasmic face of the transport protein (also see Ref. 34).

Because of the importance of proton gradients in energizing the active flux of OCs across the luminal membrane of proximal tubules, via OC/H+ exchange, considerable attention has been paid to the effect of pH on the activity of all the cloned OC transporters to determine if one of these could function as such an exchanger. When rOCT1 is expressed in Xenopus oocytes, TEA uptake is unchanged over an external pH range of 6.5–8.5 (142). Moreover, when rOCT1 is stably expressed in MDCK cells, TEA uptake across the basolateral membrane shows a fivefold increase as external pH is increased from 5.4 to 8.4 (489), whereas rOCT1-mediated efflux of TEA from these cells is not influenced at all over this same range of extracellular pH. These data indicate that mediated exchange of TEA for H+ is an unlikely operational mode for rOCT1 (489).

The other cloned orthologs of OCT1 show a varied, and variable, response to changes in extracellular pH. At one extreme, the comparatively distantly related OCT1 ortholog from C. elegans, when expressed in HRPE cells, shows an almost complete elimination of mediated TEA transport as pH is decreased from 8.5 to 5.5 (537). Mouse OCT1 expressed in Xenopus oocytes shows a more modest (~40%) decrease in TEA uptake over the range of 8.5 to 6.5 (132), and human OCT1, also expressed in oocytes, shows little (549) or no (129) response to extracellular pH. The failure of extracellular pH or transmembrane pH gradients to systematically influence OCT1 activity, combined with the previously discussed electrogenecity of OCT1-mediated OC transport, supports the conclusion that OCT1 operates as an electrogenic unipporter that can also support electroneutral OC/OC exchange.

IV) Substrate structural specificity. The issue of substrate selectivity of OCT1 has been examined in three ways: 1) directly, through measurement of transmembrane flux of labeled compounds (or transport-induced current); 2) indirectly, either through determination of the extent of inhibition of transport of a model substrate (e.g., TEA) produced by coexposure to a test agent, or as noted above, by gauging the stimulatory (or inhibitory) effect on transport of the model substrate following imposition of a trans-gradient of the test agent; and 3) by means of introducing mutations into the transporter sequence to gauge the influence of physicochemical alterations in protein structure on interaction with transported substrates. The latter approach, which has provided valuable insights into the mechanisms of transport protein activity (190), has been used sparingly, to date, in studies of OCT activity. Gorboulev et al. (130), reasoning that the binding of cationic substrates likely involves interaction with anionic residues on the surface(s) of OCTs, considered the effect on rOCT1 of mutating each of the six acidic residues found in all orthologs of OCT1, OCT2, and OCT3 (but not in the OCTNs or in the OATs). They observed a 15-fold decrease in the $K_r$ for MPP uptake into oocytes injected with mRNA coding for a mutant rOCT1 in which Asp-475 was converted to Glu-475. In addition, the IC$_{50}$ values for inhibition of TEA uptake into oocytes expressing the mutant transporter, measured for several n-tetraalkylammonium (n-TAA) compounds, all decreased, but the ratio of the IC$_{50}$ measured for the wild-type versus mutant transporter increased with alkyl chain length. They concluded that rOCT1 contains a large cation-binding pocket with several interaction domains that may be responsible for high-affinity binding of structurally different cations and that Asp-475 is located close to one of these domains. A study by Chen et al. (60) employ-
ing chimeras of rOCT1 and rOCT2 implicated TMDs 2–7 as the locus of differences in the interaction of these homologs with selected nucleosides. Future studies assessing the influence of other regions of and selected residues in the sequence of OCT1 (and the other members of the family of OCT transporters) can be expected to add to the understanding of the molecular basis of substrate-transporter interaction.

The results of studies employing the first two approaches outlined above support the general conclusion, as discussed below, that OCT1 is a polyspecific carrier that transports a diverse array of cationic compounds. However, detailed conclusions of the type required to advance our understanding of the role this process plays in, for example, renal excretion of selected cationic drugs, are frequently complicated by confusion concerning the extent to which results have been influenced by choice of “model system,” including both the specific ortholog (e.g., human versus rat) and the heterologous cell system in which the cloned transporter is expressed (e.g., Xenopus oocyte versus cultured mammalian cell).

Measurements of substrate transport, using either radiochemical or electrical methods, show that OCT1 accepts a broad array of type I organic cations, i.e., monovalent OCs with a molecular weight typically less than 400. This includes, in addition to what have become prototypical substrates for renal OC transporters, i.e., TEA, MPP, choline, and NMN, a variety of monoamines (e.g., dopamine, serotonin, and epinephrine) and nucleosides [e.g., 2-deoxytubercidin, cytosine arabinoside, and azidothymidine (AZT)]. Although there are clear similarities in the structural specificity displayed by the several OCT1 orthologs tested to date, there are also significant species differences in substrate selectivity. For example, electrophysiological measurements show that rat, mouse, human, and rabbit OCT1 orthologs all support mediated uptake of the n-TAA compounds, TMA and TEA (88). However, whereas hOCT1 and rbOCT1 also support uptake of tetrapropylammonium (TPrA) and tetrabutylammonium (TBUA), the two rodent orthologs do not (88).

The selectivity of OCT1-mediated transport has also been probed in studies measuring the inhibitory effectiveness of a vast array of cationic compounds (44, 142, 256, 318, 489, 549, 553). It is, however, difficult to discern a pattern in the molecular determinants associated with interaction of (putative) substrates with OCT1. Nevertheless, studies of the effect of increasing alkyl chain length on the inhibitory interaction of n-TAA ions with hOCT1 have revealed a strong correlation between increasing chain length (increasing hydrophobicity) and decreasing IC$_{50}$ values for inhibition of TEA transport into HeLa cells transiently expressing hOCT1 (15, 550). This observation coincides closely with reports of a strong correlation between increasing hydrophobicity and increasing inhibitory interaction of test agents with contraluminal OC transport in intact rat (468) and rabbit (135) proximal tubules and with OC/H$^+$ exchange in rabbit renal BBMV (536), at least for structurally related compounds (e.g., Ref. 534).

Three-dimensional variations in substrate structure produce steric constraints on substrate binding, although for OCT-mediated transport there has been little attention paid to this issue. In a study employing a computational approach to the development of a putative pharmacophore of substrate binding to hOCT1, Bednarczyk et al. (15) observed a systematic effect of planar hydrophobic mass on binding to the receptor: 4-phenylpyridinium compounds were more effective inhibitors of TEA transport than were 3-phenylpyridinium compounds, which were, in turn, more effective than quinolinium compounds. The deduced pharmacophore consisted of a single cationic recognition site and three hydrophobic features. Inhibitors that interacted most effectively with hOCT1 contained all these features, and weaker inhibitors generally displayed fewer of these sterically defined features. It was telling, however, that despite a strong correlation between predicted and measured IC$_{50}$ values ($r = 0.86$), a number of outliers were noted, as in the IC$_{50}$ values for a “test set” of eight compounds. Algorithms used to develop a pharmacophore typically seek a single, best-fit structure for interaction with a receptor that is assumed to possess a marked degree of structural specificity. However, OC transporters do not display the narrow specificity of the typical receptor, which generally accepts a “best” structure to the exclusion of most others. OC transporters, in contrast, apparently because of the protective role they play, must accept a broad array of substrate structures, including compounds to which the host organism may never have been exposed (e.g., dietary toxins or synthetic drugs). Consequently, one might predict a selective advantage arising from a transport process that can interact effectively with a diverse array of environmental chemicals, thus making it desirable for OC transporters to accept chemical structures that fit a generalized format, rather than one represented by a classical pharmacophore. QSAR analysis, which depends less on steric elements and more on physicochemical properties of the members of the training set, resulted in a model of the basis of substrate binding to hOCT1 ($r = 0.95$) that more adequately described the members of the test set (15).

Before proceeding further with our discussion of substrate specificity, however, we need to highlight the following issue. There is sufficient variability in reported values for the kinetic interaction of substrates and inhibitors of OCT1 (and other cloned transporters) to render suspect most conclusions concerning the quantitative basis of substrate-transporter interaction. There are at least three sources of this variability in results that may obscure the true structural selectivity of OCT1-mediated transport. These include differences in 1) species, 2) ex-
experimental technique or methodology, and 3) expression system. The potential influence of each of these sources of variability must be considered.

The possible influence of species differences in both quantitative and qualitative aspects of substrate specificity of OCT1 (and all other transporters, as well) is obvious. There is ample evidence that changes in a single amino acid residue can exert profound differences in transporter selectivity (e.g., Ref. 512). As noted earlier, substitution of a glutamate for an aspartate at amino acid 475 in rOCT1 decreases the $K_t$ for TEA transport by 15-fold (130). The fact that the orthologs for OCT1 cloned to date generally differ by at least 15% from each other with respect to amino acid identity (despite conservation of many specific residues at what are likely to be key sites for transporter function; Refs. 41, 130, 426), makes it unlikely that any one of these proteins will be quantitatively identical to any other with respect to functional characteristics. Dresser et al. (88) examined this directly by comparing the kinetics of n-TAA interaction with the human, rabbit, rat, and mouse orthologs of OCT1 (expressed in *Xenopus* oocytes). Significant differences in the inhibition of OCT1-mediated MPP uptake were noted for all the orthologs, with the greatest differences noted between human OCT1 and the two rodent transporters; rabbit OCT1 showed intermediate properties. In addition, whereas both hOCT1 and rbOCT1 supported mediated transport of TMA, TEA, TPrA and tetrabutylammonium (TBA), the rodent orthologs transported only TMA and TEA (although, interestingly, TPrA and TBA were more potent inhibitors of MPP transport mediated by rOCT1 and mOCT1, than by hOCT1 or rbOCT1). Thus the kinetic and selectivity characteristics noted in one species cannot be assumed to hold for any other species, a fact that can have important implications in efforts to extrapolate from animal transport models to humans.

The degree of functional variation among OCT1 orthologs (i.e., species differences) is difficult to assess given the extent of variation in the literature regarding the kinetic characteristics of individual orthologs. As noted above, this variation may reflect differences in technique between laboratories. Values for the $K_t$ or inhibition constant ($K_i$) for MPP interaction with rOCT1 expressed in oocytes ranges from ~3 μM (130) to ~60 μM (318); for NMN, the values range from ~125 μM (130) to >2 mM (318). The basis of such variation is not clear, but it could reflect differences in the level of expression, methods of analysis, or other aspects related to the technique of measuring transport. Unfortunately, there are no clear “benchmark” values for the kinetic constants of OCT1-mediated transport in oocytes (or any other expression system); the database is not yet large enough.

The other potential source of variability that has not received sufficient attention is the influence of the expression system on the quantitative characteristics of expressed transport. Again, the database is small and examination of the kinetic constants for different OCT1 orthologs expressed in different systems reveals sufficient variability that systematic influences of the expression system used are not clear (Fig. 4). However, a pair of studies by Inui and colleagues (318, 489) suggests that systematic differences between expression systems may exist, at least with respect to the activity of OCT transporters. These studies examined a battery of compounds as inhibitors of the rat orthologs of OCT1 and OCT2 when transiently expressed in *Xenopus* oocytes (318) or stably expressed in MDCK cells (489). For every test compound, the apparent $K_t$ (for inhibition of TEA transport) was lower in the mammalian cell line than in the oocytes. The extent of the difference was not, however, “predictable.” For example, whereas the $K_t$ for TEA self-inhibition decreased about 3-fold when the transporter was expressed in MDCK cells rather than oocytes, the $K_i$ for cimetidine inhibition of TEA uptake decreased more than 50-fold. Indeed, whereas in oocytes the $K_t$ for inhibition of radio-labeled TEA transport by cimetidine was greater than the $K_t$ for inhibition by unlabeled TEA (329 versus 129 μM; Ref. 130), in MDCK cells it was much less (5.7 versus 38 μM). Interestingly, the pattern of these differences was quite similar for rOCT1 and rOCT2. Are such differences noted for all substrates or for all OCT transporters? There are not sufficient data to draw a conclusion. Neither is it known if systematic differences exist between transporters expressed in different mammalian cell lines (e.g., epithelial cells versus nonpolarized cells).

![Graph](image.png)

**FIG. 4.** Graphic demonstration of the range of literature values for kinetic parameters ($K_t$ or $IC_{50}$) for substrate interaction with OCT1. Solid symbols represent measurements made using the *Xenopus* oocyte expression system; open symbols represent measurements made using various cultured mammalian cells as the expression system. Symbol shapes represent different orthologs of OCT1: squares, human; circles, rat; triangles, mouse; diamonds, rabbit. The variation is not clearly correlated with differences in either species or expression system.
Resolution of the issues discussed above is of paramount importance for at least two reasons. First, if the study of expressed OCT transporters is to provide data leading to predictions of, for example, drug interactions, then an understanding of which expression system(s) and experimental methodology(ies) produce observations closest to what actually occurs in humans in vivo is of obvious relevance. Second, if data obtained on the isolated activity of cloned transporters are to be used as the basis of quantitative hypotheses for the behavior of these processes in native renal tubules, knowledge of which expression system(s) provide “reliable” data is clearly important.

b) OCT2. I) Structure. OCT2 (SLC22A2; 2.A.1.19.5) was isolated originally from a rat renal library (317). However, orthologs have now been cloned from human (129), mouse (296), pig (141), and rabbit (553). The initial rOCT2 clone had an open reading frame that coded for a 593-amino acid protein (317). The human, mouse, pig, and rabbit orthologs, in contrast, are 555, 553, 554, and 554 amino acids in length, respectively. Gründemann et al. (144) isolated a clone of rOCT2 with a 555-amino acid open reading frame and suggested that the last 38 amino acids of the original clone were erroneously included due to difficulties associated with sequencing a poly(A) region of the 3’-end of the cDNA. The sequences of the several OCT2 orthologs are 68–70% identical to that of hOCT1 and 82–92% identical to one another. Conserved motifs within OCT2 across all species include 13 cysteine residues, 24 proline residues, 1 N-linked glycosylation site (N72), 2 PKA consensus sites (Thr-348, Thr-489), 1 PKC consensus site (Ser-286), 1 PKG consensus site (Thr-348), and 3 CKII consensus sites (Ser-334, Ser-472, Thr-525).

Human kidney expresses at least one splice variant of OCT2. Designated hOCT2-A, it is characterized by the insertion of a 1,169-bp sequence arising from the intron found between exons 7 and 8 of hOCT2 (486). This insertion adds an in-frame stop codon resulting in a truncated protein of 483 amino acids that is missing the last three putative TMDs (i.e., 10, 11, 12). Despite the absence of the last three TMDs, hOCT2-A retains the capacity to transport TEA and cimetidine, although guanidine transport is lost. The affinity of hOCT2-A for cationic substrates differs markedly from that of OCT2. In side-by-side experiments of expression in HEK 293 cells, the apparent $K_P$ for TEA was about sevenfold lower in hOCT2-A (63 versus 431 μM). The inhibitory profiles of the truncated and parent transporters also suggest that, for most compounds (including cimetidine, procainamide, and nicotine), hOCT2-A has a higher affinity for substrate than does hOCT2. However, for a few inhibitors (e.g., noradrenaline and dopamine), hOCT2 displays a higher apparent affinity than does hOCT2-A. The physiological role of two closely related OCT2s expressed in the kidney is not evident, although possible differences in regional distribution or functional regulation have been suggested (486).

II) Tissue and cellular distribution and localization. Northern blots show expression of rOCT2 in the kidney, and in neuronal tissue, but not in the liver (42, 317); hOCT2 and mOCT2 show a similar tissue distribution (129, 296). This restricted distribution was confirmed by RT-PCR: rat OCT2 is expressed in rat kidney cortex and medulla, but not in rat liver (489). RT-PCR of adult mouse tissue gives a similar distribution (296). Intriguingly, RT-PCR on isolated tubule segments from rat kidney showed expression of OCT2 not only in superficial and juxtamedullary proximal straight and convoluted tubules, but also in medullary thick ascending limbs, distal convoluted tubules, and cortical collecting ducts (488). OCT2 mRNA levels in male rat kidneys are approximately four times greater than those in female rat kidneys when determined using branched DNA signal amplification (402). This correlates with greater TEA transport into renal slices from male than from female rat kidneys (487).

In rats, in situ hybridization revealed that OCT2 mRNA is expressed throughout the proximal tubules within the cortex and outer stripe of the outer medulla, with the heaviest hybridization in cells of the outer stripe (143, 195).

Like OCT1 expression, OCT2 expression in the kidney appears to be restricted to the basolateral membrane of proximal tubule cells. Immunocytochemistry showed that rOCT2 is restricted to the basolateral membrane of mid to late proximal tubules (S2 and S3 segments) (195, 415). Similarly, in the human kidney, OCT2 expression is restricted to the basolateral membrane of proximal tubule cells (300), although it is not clear if the levels of expression differ along the length of the tubule. Functional mapping of OCT1 and OCT2 transport activity in single isolated rabbit renal proximal tubules (RPT) showed that TEA uptake in the S2 segment is dominated by OCT2-mediated transport (553). An early report of OCT2 expression in the apical membrane of human distal tubules (129) may have reflected complications associated with the quality of the tissue. Another line of evidence consistent with the basolateral localization of OCT2 in renal epithelia arises from observations obtained with a fusion protein construct consisting of rOCT2 plus green fluorescent protein (GFP) (423). When rOCT2/GFP was transiently transfected into polarized MDCK cells, fluorescence was localized to the basolateral membrane (423). Moreover, transient transfection of rOCT2/GFP into intact, isolated killifish renal proximal tubules resulted in expression of GFP fluorescence in the basal and lateral aspects of the tubule cells (423). It is noteworthy that in the porcine renal cell line, LLC-PK1, OCT2 appears to be expressed in the apical membrane. This localization is based on the comparison of the selectivity characteristics of pOCT2 expressed in oocytes with those observed for apical
versus basolateral OC transport in the LLC-PK₁ cells (91, 141).

III) Functional characteristics. OCT2 has been functionally expressed in Xenopus oocytes (5, 34, 42, 61, 129, 317, 318, 423, 425), HEK-293 cells (42, 143), NIH3T3 cells (333), MDCK cells (423, 425, 489), COS-7 cells (553), and CHO cells (13). The characteristics of all the OCT2 orthologs studied to date are qualitatively similar to those of OCT1. All OCT2 orthologs support mediated transport of TEA, with Michaelis constants ranging from 20 to 393 μM. Transport is electrogenic, with inwardly directed gradients of substrate resulting in the generation of inward currents (5, 34, 129, 425). These currents are saturable, with apparent Michaelis constants that are similar to those measured using radiolabeled substrates. Isolated giant patches of rOCT2-expressing Xenopus oocytes have been used to study the electrogenic operation of this process in some detail (34). The patch configuration permits measurements of currents that correspond to the efflux mode of transporter operation. Small, type I substrates, including TEA and choline, produce saturable efflux currents with \( K_i \) values for efflux (160 μM and 2 mM for TEA and choline, respectively) that are similar to those measured for the mediated uptake of these compounds. This result is consistent with the assumptions that only membrane potential and concentration gradients, i.e., the electrochemical potential, of an organic cation serve as driving forces for rOCT2-mediated transport and that this transport shows little rectification.

Larger, type II cations, including quinine and TBuA, typically do not produce OCT2-mediated currents (5, 34), but they do frequently inhibit OCT2-mediated transport activity, indicating some type of interaction with OCT2. The nature of this interaction is, however, difficult to predict. For example, the weak base quinine exerts a noncompetitive inhibition of TEA uptake in Xenopus oocytes expressing rOCT2 (5), although the results of inhibition experiments performed at different pH values suggest that this effect reflects nonionic diffusion of the uncharged substrate into the oocyte and a subsequent competitive interaction at the cytoplasmic face of the transporter (5). In contrast, the quaternary ammonium cation TBuA, which is not transported by rOCT2, is a competitive inhibitor of TEA transport by rOCT2. This inhibition presumably reflects TEA’s interaction at the extracellular face of the transporter. Decynium-22, cyanine-863, and tetraptalamin, all of which have fixed cationic charges and are not transported by rOCT2 (5), are presumably restricted to accessing the extracellular face of the transporter. However, each exerts a noncompetitive inhibition of transport activity, suggesting the presence on the transporter of an allosteric site that may interact with hydrophobic cations.

The OCT2 binding site appears to have different affinities for (at least) selected compounds depending on whether it faces the outward (extracellular) or inward (cytoplasmic) face of the membrane. When rOCT2 was expressed in Xenopus oocytes, the nontransported compounds TBuA and corticosterone blocked both inward and outward currents generated by TEA and choline, as measured in whole cells (inward currents) and excised giant patches (outward currents) (507). However, the apparent affinity of the OCT2 binding site for TBuA was four times higher when the transporter was oriented to face the extracellular face of the membrane, whereas the affinity for corticosterone was 20-fold lower. The data suggest that the substrate-binding site of rOCT2 is like a pocket containing overlapping binding domains for ligands, and these binding domains may undergo separate structural changes.

OCT2, like OCT1, can support OC/OC exchange. Inwardly directed gradients of unlabeled MPP, TEA, and amantidine, for example, accelerate efflux of labeled MPP from hOCT2-expressing oocytes (42).

The influence of proton gradients on OCT2-mediated transport has received considerable attention because of early functional evidence that this transporter resides in the apical, rather than basolateral, membrane of selected (cultured) cells and, in addition, may act as an OC/H⁺ exchanger (141, 144). The initial studies indicating that pOCT2 may be located in the apical membrane (mentioned above) used decynium-22 as a means to discriminate between apical and basolateral transport of OCs in LLC-PK₁ cells (141). Decynium-22 inhibits TEA uptake in oocytes mediated by the pOCT2 ortholog cloned from LLC-PK₁ with a \( K_i \) of 5.1 nM. This \( K_i \) corresponds closely to the measured \( K_i \) of 6.7 nM for inhibition of apical TEA transport in confluent monolayers of LLC-PK₁ (381) (basolateral TEA uptake in LLC-PK₁ cells is not blocked by 30 nM decynium-22; Ref. 381), thus supporting the contention that OCT2 is expressed in the apical membrane of these cells (141). This conclusion has been supported by independent observations based on the selectivity profile of OC transport in LLC-PK₁ cells (91).

This functional information on localization has been evaluated in the light of evidence that (1) LLC-PK₁ cells can support net transepithelial transport of TEA (364) and cimetidine (16) and (2) apical membrane vesicles isolated from LLC-PK₁ cells can support mediated OC/H⁺ exchange (176). The apical localization of pOCT2 in LLC-PK₁ cells would be consistent with the hypothesis that OCT2 is an OC/H⁺ exchanger involved in active OC secretion in LLC-PK₁ cells. This hypothesis was extended from porcine to renal kidneys on the basis of the selectivity profiles of the rat orthologs of OCT1, OCT2, and OCT3 (EMT) that were interpreted as supporting an apical, rather than basolateral, location of rOCT2 (144). Weakening this last conclusion and calling into question the general interpretation of studies relying on comparisons of substrate selectivity to determine subcellular location of individual transporters.
processes is the clear demonstration by the use of immunocytochemistry and OCT/GFP constructs that rOCT2 is restricted to the basolateral membrane of native proximal tubule cells (see above) (195, 415).

In addition, OCT2 fails to show convincingly the physiological characteristics of the apical OC/H$^+$ exchanger: 1) changes in extracellular H$^+$ concentration exert a modest effect on OCT2-mediated transport (rat, Ref. 317; pig, Ref. 141); 2) trans-gradients of H$^+$ do not stimulate rOCT2-mediated transport (425); and 3) OCT2-mediated transport (unlike OC/H$^+$ exchange, Ref. 533) is electroneutral, as assessed by the observation of transport-induced currents in rOCT2-expressing oocytes (5, 34). In addition, the K$^+$-induced collapse of the apical membrane potential inhibits apical metoprolol uptake in LLC-PK$_1$ cells (91). It has been suggested that the discrepancy between the behavior of OC/H$^+$ exchange as measured in membrane vesicles and the transport activity of OCT2 in heterologous expression systems reflects differences in the experimental procedures involved in measurement of transport (e.g., different buffer systems; Ref. 144). However, inwardly directed H$^+$ gradients do stimulate TEA efflux and outwardly directed H$^+$ gradients do stimulate TEA uptake across the apical membrane of LLC-PK$_1$ cells (364, 430). Nevertheless, all the data taken together suggest that, although OCT2 may be expressed in the apical membrane of some renal cells (or some cultured cells of renal origin), it is unlikely to work as a secondarily active secretory process and, instead, in those cells may be limited to mediating trans-apical fluxes in response to the prevailing electrochemical gradient of the substrate in question.

III) Substrate structural specificity. OCT2 is a polyspecific carrier with selectivity characteristics that are very similar to those observed for OCT1. All OCT2 orthologs tested to date (rat, human, rabbit, and pig) support the mediated transport of TEA, with Michaelis constants ranging from 20 to 400 μM, with an average value of ~150 μM. The observation that, in addition to expression in the kidney, hOCT2 is expressed in neuronal tissues (42) has led to the study of the interaction of monoamine transmitters with this process. The human ortholog of OCT2 transports (in order of increasing $K_c$) serotonin (80 μM), dopamine (390 μM), histamine (1.3 mM), and norepinephrine (1.9 mM) (42). Since these comparatively low affinity substrates are all relatively small, polar molecules, these findings are consistent with the general view that hydrophobicity is an important criterion in the binding of substrates and inhibitors to OCT transporters (e.g., Ref. 465). However, as in the case of the substrate selectivity of OCT1-mediated transport, the database is too small and the variability in apparent binding and transport constants that exists within and between different orthologs and expression systems is too large to permit any specific conclusions concerning the molecular determinants involved in the transport activity of OCT2.

Of particular importance to future studies of the relative role of the various OCTs in the kidney is finding one or more substrates/inhibitors that can effectively discriminate between the activities of different homologs when expressed in native tissue (where multiple homologs may be coexpressed in the same cell). The parallel selectivity of OCT2 and OCT1 for at least some OCs is evident in the side-by-side comparisons, in two different expression systems, referred to previously (191; see also Ref. 488). It is, however, also evident that some substrates show strikingly different affinities for these two processes. Koepsell and colleagues (5) compared the selectivity of the rat orthologs of OCT1 and OCT2 when expressed in Xenopus oocytes. Whereas several compounds interacted very similarly with both orthologs (e.g., TEA, MPP, NMN), other compounds interacted very differently. Most notably, mepiperphenidol and O-methylisoprorenaline had IC$_{50}$ values for inhibition of transport by rOCT1 that were 60 times lower than the values for inhibition of transport by OCT2. On the other hand, guanidine and corticosterone were 25–35 times more potent in their inhibition of rOCT2 than rOCT1. It is also noteworthy that selected nucleotides, including 2-deoxytubercidine, 2-chlorodeoxyadenosine, and cytosine arabinoside, are transported by rOCT1 but not by rOCT2 (60–62). Similarly, human OCT1 and OCT2 display markedly different relative affinities for n-TAA compounds (90). The rabbit orthologs of OCT1 and OCT2 also display markedly different affinities for selected substrates (553). Whereas the affinities of these transporters for TEA is approximately the same, the apparent affinity of OCT2 for cimetidine and the fluorescent cationic substrate [2-(4-nitro-2,1,3-benzoxadiazol-7-yl)aminoethyl]trimethylammonium (NBD-TMA) is 35–100 times higher than that of OCT1 (when expressed in COS-7 cells).

Exploiting the marked differences in apparent affinity that different OCT homologs have for selected substrates can provide a means to map the functional distribution of OC transport activity in renal cells. The IC$_{50}$ of 13 μM for cimetidine’s inhibition of peritubular TEA transport in isolated single S2 segments of rabbit proximal tubules is close to the IC$_{50}$ of 6 μM for cimetidine inhibition of TEA transport by heterologously expressed rbOCT2, but differs radically from the IC$_{50}$ of >500 μM for such inhibition of transport by rbOCT1 similarly expressed (553). Although such comparisons require extrapolation of kinetic values obtained with heterologous expression systems to the quantitative behavior of transporters expressed in native tubules (with the attendant caveats noted previously), these data argue that TEA transport in the S2 segment of rabbit proximal tubule is dominated by activity of OCT2 (see also Ref. 191). Extending the demonstration of functional distribution of
transporter activity to different regions of the nephron remains a critical aspect of integrating molecular information into the context of cellular and organ-level physiology.

The recent discovery of altered transport function of hOCT1 and hOCT2 containing single nucleotide polymorphisms present in different ethnic populations (237, 238, 393) has underscored the importance of understanding structure-activity relationships for these processes. For example, 28 variable sites in the hOCT2 gene were discovered in a collection of 247 ethnically diverse DNA samples (Caucasian, African-American, Asian-American, Mexican-American, and Pacific Islander). Eight of these polymorphisms caused nonsynonymous amino acid changes, of which four were present in at least 1% of an ethnic population. These four displayed altered transporter function as seen by a threefold change in $K_v$ values for MPP and TBA, changes that could result in differences in the pharmacokinetics of renal drug excretion between individuals expressing different variants of hOCT2. However, population-genetic analysis suggests that selection has acted against amino acid changes to hOCT2, which may reflect a necessary role of OCT2 in the renal elimination of endogenous amines or xenobiotics (238).

c) oct3. I) Structure. OCT3 (SLC22A3; 2.A.1.19.6) was initially cloned from a rat placental cDNA library (199). However, orthologs have now been cloned from mouse (521) and human [originally referred to as the extraneuronal monoamine transporter (EMT) (145)]. OCT3 is a 551-amino acid peptide with 12 putative TMDs. The three orthologs of OCT3 display 47–48% sequence identity with hOCT1 and 48–49% sequence identity with hOCT2. Conserved motifs within OCT3 across all species include the following (426): 15 cysteine residues, 25 proline residues, 3 N-linked glycosylation sites (N 72, 99, 119), 2 PKA consensus sites (Thr-351, Thr-437), 2 PKC consensus sites (Ser-291, Thr-297), and 3 CKII consensus sites (Thr-327, Ser-337, Thr-528).

II) Tissue and cellular distribution and localization. Northern analysis indicates that OCT3 is expressed most heavily in the placenta and intestine, but it is also found in the kidney, heart, brain, and lung (199). Using a branched DNA signal amplification assay, Slitt et al. (402) found a very broad distribution of OCT3 mRNA in rat tissues, with highest expression in blood vessels, skin, and thymus; intermediate expression in kidney, lung, intestine, spleen, and muscle; and low expression in liver, bladder, and prostate. In mice, OCT3 is also expressed in retinal pigment epithelium (349). In humans, Northern analysis shows OCT3 to be expressed strongly in the liver, placenta, kidney, and skeletal muscle (539), although by quantitative RT-PCR, OCT3 expression in human kidney appears to be <10% that of OCT2 (with OCT1 expression virtually undetectable; Ref. 300). Within the mouse kidney, OCT3 expression is confined to proximal and distal tubules in cortex, and little expression is evident in medullary tissues or in glomeruli (539).

III) Functional characteristics. OCT3 has been functionally expressed in Xenopus oocytes (199, 539, 541), HeLa cells (199, 541), HRPE cells (539, 541), and ARPE-19 cells (349). The qualitative characteristics of OCT3-mediated transport are similar to those for its homologs, OCT1 and OCT2: rOCT3 supports saturable transport of TEA and MPP (199) and transport is electrogenic. rOCT3-mediated transport activity shows sensitivity to external pH similar to that seen in selected studies of OCT1 and OCT2 (199). However, in oocytes expressing rOCT3, clamping the membrane potential to prevent it from changing with changes in the $H^+$-gradient completely eliminates the effect of external pH on OCT3-mediated transport (pH 6.5–8.5), thereby supporting the contention that OCT3 is a potential-driven electrogenic uniporter (199).

IV) Substrate structural specificity. In addition to transporting TEA and MPP, rOCT3 also transports guanidine (199) and dopamine (541). Although a polyspecific OC transporter like OCT1 and OCT2, OCT3 displays substantial quantitative differences from them with respect to substrate specificity. OCT3 has a much lower apparent affinity for TEA ($K_v$ values of 1–6 mM, Refs. 199, 539) than either OCT1 or OCT2 ($K_v$ values of 20–400 µM). The profile of substrate specificity matches closely that displayed physiologically by the extraneuronal monoamine transporter, suggesting that OCT3 is the molecular structure involved in this latter process (541). Side-by-side comparisons of rOCT2 and rOCT3 show that the IC$_{50}$ values for inhibition of TEA uptake by dopamine and norepinephrine (in transfected HRPE cells) are 6- and 25-fold higher in OCT2 than in OCT3, respectively (541). Also, β-estradiol blocks rOCT3 activity with an IC$_{50}$ of 1.1 µM, compared with 85 µM for OCT2 (541). These observations, along with the differences between selectivities of OCT1 and OCT2, offer potential strategies for defining the activity and distribution of the several OCTs expressed in native tissues (5).

D) octN1. I) Structure. OCTN1 (SLC22A4; 2.A.1.19.2) was first isolated, using PCR, from a fetal human liver cDNA library (445). Rat (538) and mouse (443) orthologs have also been cloned. OCTN1 is a 551- to 553-amino acid peptide and shares with the other OCT family members a predicted secondary structure that includes 12 putative TMDs. The three orthologs show 30–31% sequence identity to hOCT1. Conserved motifs within OCTN1 across all species include the following (426): 7 cysteine residues, 24 proline residues, 3 N-linked glycosylation sites (N 57, 64, 91), 1 PKA consensus site (Ser-472), 4 PKC consensus sites (Ser-164, Ser-225, Ser-280, and Ser-286), and 1 CKII consensus site (Thr-514).

II) Tissue and cellular distribution and localization. OCTN1 is widely expressed in human tissues. Northern analysis shows the most marked expression in fetal
liver, kidney, and lung and in adult kidney, trachea, and bone marrow, with weaker signals evident in adult skeletal muscle, prostate, lung, pancreas, placenta, heart, uterus, spleen, and spinal cord (445); OCTN1 is not evident in adult human liver by Northern analysis. In one study, quantitative mRNA analysis of expression in human kidney cortex reported barely detectable levels of OCTN1 (a few percent of that measured for OCT2, and much less than that measured for OCTN2) (300). The rat shows a slightly different distribution. Quantitative mRNA analysis indicated that the highest expression of rOCTN1 is in the kidney, with comparatively high levels also in intestine, brain, lung, heart, and skin (402; see also Ref. 538). Western analysis of the distribution of mOCTN1 indicated that the greatest expression is in the kidney, followed by the heart and liver (538). Within the kidney, in situ hybridization revealed rOCTN1 expression in cortical and medullary tissues, with expression most abundant in the outer stripe of the medulla. Expression occurs in glomeruli and in proximal and distal tubules, being most evident in the straight portion of the proximal tubules. There is currently no direct information concerning the subcellular distribution of OCTN1. There is, however, reason to postulate a luminal localization of this process. OCTN1 and OCTN2 have a very high degree of homology (76% identity between hOCTN1 and hOCTN2, for example), and OCTN2 definitely appears to be expressed in the luminal membrane of proximal tubule cells (540). Additionally, OCTN1 and OCTN2 exist within the human genome as a tightly linked gene pair (100). Therefore, it is reasonable to propose that OCTN1 is also expressed at the apical pole of renal cells.

III) Functional characteristics. OCTN1 has been functionally expressed in Xenopus oocytes (543), HEK 293 cells (445, 543), and HRPE cells (538). All orthologs tested support saturable TEA transport with Michaelis constants ranging from 200 to 400 μM for the human and mouse orthologs (445, 543) to ~1 mM for the rat ortholog (538). As with many of the homologous OCTs, increases in the extracellular H+ concentration inhibit OCTN1-mediated transport (445, 538, 543). However, whereas in the other homologs these effects do not appear to reflect a coupled interaction between OC and H+ (i.e., via mediated OC/H+ exchange), the current evidence, albeit limited, supports the contention that OCTN1 can function as an OC/H+ antiporter. First, unlike the other homologs, OCTN1-mediated transport does not appear to be electrogentic; changes in membrane potential brought about by raising extracellular K+ concentrations have no effect on the rate of hOCTN1-mediated TEA uptake (543). Second, efflux of TEA from hOCTN1-expressing HEK 293 cells is stimulated by inwardly directed H+ gradients (543). Both observations suggest that OCTN1 operates as an electroneutral OC/H+ exchanger. However, despite the evidence supporting an OC/H+ exchanger mode of activity for OCTN1, it is not clear that this cloned transporter is the process that dominates transport activity as measured in isolated renal luminal membranes. OC/H+ exchange activity as functionally expressed in renal cells, or more specifically, mediated exchange of TEA for H+, appears to be restricted to the kidney and the liver (299); it is not found in the placenta or intestine, although both of these organs do express a transporter that mediates exchange of guanidine for H+ (121, 289). OCTN1, however, is expressed in many tissues, including the placenta and intestine (538). The comparatively low level of expression of OCTN1 in human kidney (300) also appears to be inconsistent with the observation that OC/H+ exchange is the dominant mechanism for OC (i.e., TEA and NMN) flux across isolated human renal BBMV (322). In addition, the kinetic/selectivity characteristics of OCTN1 (discussed below) also appear to be inconsistent with this process playing a major role in luminal OC transport. There are, however, too few data on the function of OCTN1 to draw a firm conclusion about its role in renal cells.

IV) Substrate structural specificity. OCTN1 is a polyspecific OC transporter, as shown by its inhibition by a diverse set of cationic substrates (538, 543). Comparison of the specificity of hOCTN1 with the selectivity of OC/H+ exchange as expressed in native renal cells should provide insight into the role OCTN1 may play at the luminal membrane. Although there are few data upon which to make such a comparison, they render suspect the conclusion that OCTN1 serves as the principal route for OC/H+ exchange at the luminal membrane. Whereas the transport activity of the human OCTN1 ortholog, for example, shows no inhibition when exposed to 5 mM NMN (543), BBMV isolated from human kidney transport MN with an apparent Ki of 0.44 mM (322). Also, although the rat ortholog of OCTN1 shows a very weak interaction with MPP (<40% inhibition of TEA uptake produced by exposure to 5 mM MPP, Ref. 538), MPP transport across the luminal membrane of microperfused rat proximal tubules in vivo has an apparent Michaelis constant of 120 μM (83).

It should be emphasized that the two methods that were used to examine transport properties of native cells typically reveal properties of outer cortical proximal tubule segments; the transport properties of the pars recta are typically not evident in studies with isolated cortical membrane vesicles (unless material from the outer medullary stripe is specifically included) or with rat tubules microperfused in vivo. Therefore, in view of the evidence for the differential distribution of renal organic cation (295) and organic anion transporters along the renal tubules, OCTN1 could be expressed in the luminal membrane of late proximal tubule segments and have a selectivity that differs from that of an OC/H+ exchanger found in early proximal segments.
e) OCTN2. 1) Structure. OCTN2 (SLC22A5; 2.A.1.19.3) was first cloned from a cDNA library prepared from human choriocarcinoma placental cells (JAR cells) (542). Subsequently, orthologs from rat and mouse (540) have been described. These cDNAs code for a protein of 557 amino acids with 12 putative TMDs. The three orthologs share 31–33% sequence identity with hOCT1 and ~85% sequence identity with each other. Conserved motifs within OCTN1 across all species include the following (426): 7 cysteine residues, 24 proline residues, 3 N-linked glycosylation sites (N 57, 64, 91), 3 PKA consensus sites (Thr-344, Ser-402, Ser-474), 3 PKC consensus sites (Ser-164, Ser-225, Ser-280), and 1 CKII consensus site (Thr-311).

II) Distribution and localization. By Northern blot analysis hOCTN2 appears to be most heavily expressed in kidney, heart, placenta, skeletal muscle, and pancreas; expression is also evident, although much weaker, in brain, lung, and liver (542). hOCTN2 is also expressed in several human cell lines, including HeLa, Caco-2, JAR, BeWo, MCF-7, and HKPT (542). Within the rat kidney, in situ hybridization revealed expression of OCTN2 predominately in the cortex with very little expression in the medulla (540). Within the renal cortex, rOCTN2 appears to be expressed in both proximal and distal tubules and in glomeruli (540). To date, there is no direct evidence (e.g., immunocytochemical data) concerning the subcellular localization of OCTN2. However, as discussed below, OCTN2, in addition to being an organic cation transporter, is a Na\(^+\)-dependent carnitine transporter, and lesions in OCTN2 have been linked to systemic carnitine deficiency (SCD) that is associated with failure of tubular reabsorption of carnitine (447). This observation strongly supports the conclusion that OCTN2 is expressed in the apical membrane of renal tubule cells. In addition, the tissue-to-plasma TEA ratio in kidney tissue from OCTN2-deficient mice (jos mice) is increased more than twofold compared with wild-type, supporting the probable brush-border location of OCTN2 (312).

III) Functional characteristics of OCTN2. OCTN2 has been functionally expressed in HeLa cells (120, 387, 542), HEK 293 cells (313, 441, 443, 444), HRPE cells (540), and CHO cells (513). In addition to transporting TEA (with an apparent Michaelis constant of 60–200 \(\mu\)M; Refs. 443, 540), OCTN2 also supports Na\(^+\)-dependent transport of the zwitterion carnitine (\(K_{\text{Na}} \approx 4–20 \mu\)M; Refs. 120, 313, 443, 513, 540) and various acylcarnitine derivatives (313). Indeed, OCTN2 is unique in that no other transporter has been shown to transport some substrates in a Na\(^+\)-dependent manner and other substrates in a Na\(^+\)-independent manner (387). Whereas OCTN2-mediated uptake of TEA is completely independent of the presence of Na\(^+\) in the extracellular medium (540), uptake of carnitine (and closely related structural analogs) is almost completely eliminated by removal of Na\(^+\) from the external medium (313, 387, 441, 510, 540). Nevertheless, the maximal rate of uptake of each substrate (measured in the presence of Na\(^+\)) is similar. For example, in rOCTN2-expressing HRPE cells, the maximal rate of TEA uptake is 1.5 nmol \cdot 10^6 cells\(^{-1}\) \cdot 30 min\(^{-1}\), whereas the maximal rate of carnitine uptake is 1.1 nmol \cdot 10^6 cells\(^{-1}\) \cdot 30 min\(^{-1}\) (540). Chimeric transporters made from different regions of human and rat OCTN2 cDNAs revealed that the primary sequence sites responsible for TEA and carnitine transport are spatially separated. A chimera that includes the 5’-half of hOCTN2 and the 3’-half of rOCTN2 displays “humanlike” Na\(^+\)-dependent carnitine transport and “ratlike” TEA transport. A reverse chimera showed ratlike carnitine transport and humanlike TEA transport (387). Nevertheless, the binding of TEA to the rat and human orthologs of OCTN2 competitively inhibits Na\(^+\)-dependent carnitine transport (387). Thus, whereas the binding sites may be physically distinct, binding to one site appears to preclude binding to the second site.

Analysis of mutations in human patients reveals that primary carnitine deficiency can arise from several qualitatively different types of OCTN2 failure. Expression in HRPE cells of cDNAs containing the naturally occurring truncation mutation (Trp132Stop) or the missense mutation P475L fails to result in measurable rates of carnitine transport (447). In contrast, the natural mutation E452K increases the concentration of Na\(^+\) required to half-maximally stimulate carnitine transport \((K_{\text{Na}})\) into CHO cells from the physiological value of ~12 to 187 mM (513). Substitution of glutamine, aspartate, or alanine for Ghu\(^{\text{E452}}\) causes intermediate increases in the \(K_{\text{Na}}\) for carnitine transport.

Both the Na\(^+\)-dependent and Na\(^+\)-independent modes of OCTN2 operation result in inwardly directed currents. For the Na\(^+\)-dependent transport of carnitine, which is a zwitterion and carries no net charge at physiological pH, the inward current presumably reflects cotransport of carnitine with cationic Na\(^+\) (510). For the Na\(^+\)-independent transport of TEA, this current presumably reflects the electrogenic uniport of this cationic substrate (510). Both modes of OCTN2 operation also display a modest sensitivity to extracellular pH, with higher rates of transport of TEA (Na\(^+\) independent, Ref. 542) and carnitine (Na\(^+\) dependent, Refs. 313, 510) observed at alkaline pH.

IV) Substrate structural specificity. When operating as a Na\(^+\)-independent OC uniporter, OCTN2 displays a polyspecific selectivity that is the usual hallmark of OCT transporters. For example, rOCTN2-mediated transport of TEA is inhibited by >60% by 5 mM concentrations of unlabeled TEA, cinetidine, procainamide, dimethylamiloride, clonidine, tetrahexylammonium, desipramine, and MPTP (540). Poor inhibitors of rOCTN2-mediated TEA transport (<30% inhibition by 5 mM concentrations) include guanidine, TMA,amphetamine, methamphet-
amine, dopamine, serotonin, norepinephrine, and thiamine (540). A similar pattern of OC interaction is seen for hOCTN2 (542).

The transport of OCs such as TEA is inhibited by carnitine and selected other zwitterions, but such interaction is effectively dependent on the presence of Na⁺ in the medium. For example, whereas the IC₅₀ for unlabeled TEA’s inhibition of [¹⁴C]TEA transport by rOCTN2 is the same in the presence or absence of Na⁺ (106 versus 107 μM; Ref. 540), the IC₅₀ for carnitine’s inhibition of TEA transport is reduced 50-fold by the presence of Na⁺ (15.5 versus 787 μM; Ref. 540).

The interaction of selected β-lactam antibiotics with OCTN2 may provide some insight into the basis for the interaction of zwitterionic substrates with this unique transporter. Cephaloridine, cefoselis, and cefepime all block carnitine transport by the human and rat orthologs of OCTN2 (120). However, the interaction of cephaloridine is Na⁺ dependent, whereas the interaction of cefoselis and cefepime is Na⁺ independent. Similarly, the inhibition of TEA transport by cephaloridine is Na⁺ dependent, whereas inhibition of TEA transport by the cefoselis and cefepime is Na⁺ independent. The structure of cefoselis and cefepime includes an amino group and a modified imino group as substituents in the cepham nucleus, and it has been suggested that these groups may occupy the Na⁺-binding site, occupancy of which is typically required for transport of zwitterions. Consequently, the requirement for Na⁺ may be eliminated for the interaction of these selected zwitterions with OCTN2 (120). In addition, whereas OCTN2 has an affinity for cefepime and cefoselis several orders of magnitude lower than for cephaloridine in the presence of Na⁺, it displays a similar affinity for all three compounds in the absence of Na⁺. This might be explained if the binding of cephaloridine to OCTN2 leaves the Na⁺ binding site accessible to Na⁺, whereas the binding of the other two compounds to OCTN2 makes the Na⁺ binding site inaccessible to Na⁺ (120). The interaction of β-lactam antibiotics with OCTN2 may explain the carnitine deficiency that is associated with therapeutic use of cephaloridine (120).

f) OCTN3. I) Structure. OCTN3 has been cloned from mouse embryo mRNA using RT-PCR (443). The cDNA for mOCTN3 codes for a 564-amino acid peptide that is 34% identical to hOCT1, 66% identical to hOCTN1, and 69% identical to mOCTN1.

II) Tissue and cellular distribution and localization. By both RT-PCR and Western blot analysis, mOCTN3 is strongly expressed in adult testis and weakly expressed in kidney, with virtually no expression in other tissues (443).

III) Functional characteristics. mOCTN3 has been functionally expressed in HEK 293 cells (443), in which it transports both TEA and carnitine. Uptake of TEA is, however, very modest compared with that measured for carnitine, with the relative rate of carnitine-to-TEA transport being on the order of 750:1 (443). Interestingly, mOCTN3-mediated carnitine transport is completely independent of the presence of Na⁺ in the medium and is scarcely affected by changes in the extracellular pH (443).

IV) Substrate structural specificity. mOCTN3 has a comparatively low affinity for TEA (indeed the rate of TEA transport is sufficiently low that accurate measurement of the Kᵢ for TEA has proven to be difficult, but it appears to be on the order of 500 μM; Ref. 443). However, the affinity of mOCTN3 for carnitine and acyl-analogs of carnitine is higher than that displayed by mOCTN2. For example, whereas 5 μM concentrations of l-carnitine, acetyl-l-carnitine, and butyryl-l-carnitine inhibit OCTN3-mediated uptake of l-[¹⁴H]carnitine by >50%, this concentration of these substrates reduces OCTN2-mediated carnitine transport by <50% (measured in the presence of Na⁺) (443).

2. MDR family of OC transporters

MDR1 (also called the P-glycoprotein, or P-gp; ABCB1; 3.A.1.201.1) was first characterized within the context of its role in the development of cross-resistance of cancer cells to a structurally diverse range of chemotherapeutic agents. The normal expression of MDR1 in barrier epithelia, including the intestine, liver, and kidney, supports the conclusion that it plays a role in limiting absorption (in the intestine) and facilitating excretion (by the liver and kidney) of xenobiotic compounds. The following is a brief overview of the molecular, cellular, and physiological characteristics of MDR1 and the current understanding of the role it plays in the renal transport of organic electrolytes. The reader is directed to recent reviews that consider the molecular biology and physiology of MDR1 (P-gp) in more depth (2, 23, 154).

A) Molecular aspects of MDR1. MDR1 is a member of the ATP-binding cassette (ABC) superfamily of transport proteins (3.A.1.201; Ref. 362). The human ortholog of MDR1 is a protein of 1,279 amino acids (141 kDa) and is composed of two homologous halves, each containing six TMIs and an ATP-binding domain, separated by a “linker” polypeptide.

The molecular mechanism of MDR1-mediated transport is not clear. The purified protein displays both basal and drug/substrate-stimulated ATPase activity. In general, most compounds transported by MDR1 stimulate ATPase activity of the purified protein. Although MDR1 has been purified and functionally reconstituted into lipid bilayers (e.g., from human and hamster; see Ref. 388), efforts to develop kinetic models describing the interaction of substrates with MDR1 have often come to contradictory conclusions. Nevertheless (as reviewed in Ref. 388), MDR1-mediated transport 1) clearly appears to be coupled to ATP hydrolysis, 2) involves conformational changes of
MDR1/substrate complexes, and 3) is capable of directly generating substantial substrate concentration gradients arising from the active efflux of substrates from cells (or into inside-out membrane vesicles). Current theories for the mechanism of MDR1 activity include variations of the traditional “pump model” in which the conformation of the transport protein alternates between forms that allow access to one or more binding sites on one side of the membrane or the other. An alternative hypothesis has MDR1 displaying a “flip-flop” mode of action in which substrate accesses the transporter from within the lipid bilayer, with subsequent conformational changes of the protein shifting the substrate to the outer leaflet of the bilayer, thereby creating a steady-state concentration gradient across the thickness of the membrane (155).

We will limit the present discussion of MDR1 to three subjects. The first two of these, i.e., the location within the kidney of MDR1 expression and its substrate specificity, are addressed in this section. The third subject, i.e., the physiological role of MDR1 in mediating OC secretion, is dealt with in section II C.

b) LOCATION OF MDR1 EXPRESSION IN RENAL TISSUES. By immunocytochemistry, MDR1 is expressed in the apical membrane of proximal tubule cells in human (455) and mouse kidneys (101). MDR1 is also expressed, albeit at apparently lower levels, in the mesangium, thick ascending limb of Henle’s loop, and collecting tubule of the normal human kidney (103).

c) SPECIFICITY OF MDR1. MDR1 supports ATP-dependent export of a structurally diverse range of comparatively bulky, hydrophobic cationic substrates that, in general, fall within the type II OC classification. These “traditional” substrates include the vinca alkaloids (e.g., vinblastine, vincristine), cyclosporine, anthracyclines (e.g., daunorubicin, doxorubicin), and verapamil. In addition, MDR1 mediates the transport of a number of relatively hydrophobic compounds that are either uncharged or are neutral at physiological pH, including digoxin, colchicine, propafenone, and selected corticosteroids. Many of these compounds also inhibit the renal secretion of smaller type I OCs (464, 465). Consequently, the extent to which the transporters generally viewed as being specific for type I OCs (e.g., the OCTs previously discussed) accept these bulky type II compounds as substrates is not clear. Indeed, there is evidence in intact tubules that at least some of the traditional MDR1 substrates [e.g., rhodamine-123 in the rat (3, 359); daunomycin in killifish (277)] can also be transported by OCTs.

There is even greater confusion concerning the extent to which MDR1 can accept type I OCs as substrates. The contradictory evidence concerning the interaction of cimetidine with MDR1 will serve as a case in point. Cimetidine (mol wt 252) is actively secreted by renal proximal tubules (267, 269) and is transported by both the basolateral (267, 269) and apical (266) transport processes acknowledged as being largely specific for type I OCs. However, several studies have reported enhanced transepithelial cimetidine secretion across cultured cell monolayers that express MDR1 [e.g., Caco-2 (72), LLC-PK1 (92), MDCK (332)]. Indeed, such observations have been used as the basis for other studies using cimetidine as a tool for probing MDR1 distribution (156). However, other reports seem to contradict this conclusion. For example, in one study, although verapamil, vincristine, and quinidine enhanced cellular accumulation of doxorubicin by LLC-PK1 (presumably by blocking pMDR1-mediated doxorubicin export), cimetidine had no effect (85). In another, cimetidine had no effect on rhodamine-123 (Rho) accumulation in doxorubicin-resistant colon carcinoma cells and failed to reverse resistance of these cells to anticancer hMDR1 substrates (223). In another, Rho clearance by the liver was blocked by the rMDR1 inhibitors cyclosporin A and colchicine, but not by cimetidine (3). Similar contradictory observations have been made concerning the possible interaction of MPP+ with human and mouse MDR1 (e.g., Refs. 24, 411).

Although the basis for these contradictory observations is not clear, it could be influenced by the cultured cell lines used for such studies. Caco-2 cells, which clearly do express hMDR1 in the apical membrane (24), also appear to express a number of other OC-selective processes (24, 47, 291). This expression could complicate interpretation of inhibition studies. LLC-PK1 cells have been used in some studies because they apparently express (pig) MDR1 (e.g., Refs. 85, 242) and in others because they apparently either do not express MDR1 (e.g., Ref. 75) or express it at comparatively low levels (e.g., Refs. 405, 446). Different behaviors have been noted for OC transport in LLC-PK1 cells before, with some studies reporting the presence of OC/H+ exchange in the apical membrane (176), including cimetidine/H+ exchange (16), whereas others found no evidence for such a process (92). Again, the basis for such differences is not clear, but it could reflect differences in culture methods, cell passage number, or other selection criteria. Nevertheless, Smit et al. (405) used LLC-PK1 cells to provide strong evidence that MDR1 can accept at least some type I OCs as substrates. Using several clonal lines of LLC-PK1 cells that stably expressed transfected human or mouse MDR1, they measured the influence of MDR1 expression on the transepithelial flux of selected type II and type I OCs. There was a modest net secretory flux in the wild-type (nontransfected) cells of two type II substrates, vinblastine and vercuronium, and two type I substrates, TBuMA and azidoprocainamide (APM). Expression of MDR1 in the apical membrane of the transfected cells significantly increased transepithelial flux of all the substrates, supporting the conclusion that MDR1 can support transport of at least some type I OCs. A similar study employing MDCK cells showed that cells that stably express human
MDR1 can support enhanced secretion of cimetidine (332), thus providing the least ambiguous evidence to date for the contention that cimetidine, in addition to TBUmA and APM, is an MDR1 substrate.

C. Physiological Integration of Renal OC Transporters

Although great strides have been made in understanding the molecular and cellular physiology of renal OC transport through studies employing cloned transport proteins in heterologous expression systems, the observations so obtained must be interpreted with the caveat that they indicate how a process works outside its normal physiological milieu. In the light of the growing list of processes known to contribute to renal transport of OCs, we believe that determination of the net result of the concerted activity of suites of these processes is essential if we are to understand renal secretion within the context of physiologically intact systems. In this section, we examine the results of studies employing “native” transport systems. These include studies of transport by renal tubules in vivo, isolated tubules and cells, isolated renal membrane preparations, and cultured cell systems.

It is convenient to organize the great diversity of such studies into groups that focus on particular areas of general emphasis. These include 1) the functional organization of renal OC transporters and 2) the structural specificity of renal OC transport. The first category can be subdivided further into studies primarily focused on understanding mechanisms and regulation of renal OC transport in general and studies primarily focused on understanding mechanisms of transport of a particular compound of major physiological, pharmacological, or toxicological interest. The second category includes efforts to establish both the molecular determinants associated with binding to and translocation by renal OC transporters and the presence of multiple processes operating in series in the basolateral or apical poles of renal cells. When possible, we will attempt to correlate observations made with intact or native systems with evidence obtained using cloned transporters in an initial effort to establish the molecular basis of the integrated processes of renal OC transport. As noted earlier, the emphasis here is on studies conducted since 1993; reference to earlier studies is largely restricted to those that form a necessary backdrop to more recent work. The reader is directed to previous reviews that effectively summarize the earlier literature (76, 342, 352, 355).

1. Cellular organization and mechanisms of renal OC transport

Physiological characteristics of OC transport in renal proximal tubules have been studied with intact tubules and isolated membrane vesicles from a wide variety of species, including representative mammalian (rat, rabbit, dog), avian (chicken), reptilian (snake), and teleost (flounder) species. The physiological profile of OC transport observed in these studies suggests that the cellular mechanism of renal OC secretion is phenotypically conserved over many phyla. The secretion of type I OCs involves (indeed, may be dominated by) electrogenic, facilitated diffusion at the basolateral membrane, and electroneutral OC/H+ exchange at the apical (luminal) membrane. Secretion of type II OCs, on the other hand, appears to involve a largely diffusive flux across the basolateral membrane, followed by P-glycoprotein-mediated export at the luminal membrane.

A) BASOLATERAL/CONTRALUMINAL TRANSPORT MECHANISM.

Studies with isolated renal basolateral membranes and intact renal tubules have clearly implicated electrogenic, facilitated diffusion as a principal element in the renal secretion of organic cations. Uptake of NMN, TEA, and other prototypic type I OCs into isolated mammalian renal basolateral membrane vesicles (BLMV) (211, 294, 407, 429, 533) consistently reveals that transport is saturable and stimulated by intravesicular electrical negativity. Importantly, an inside negative membrane potential has been shown to be sufficient to support the transient uphill accumulation of TEA in isolated renal BLMV (294, 407). The characteristics of basolateral OC uptake in intact tubules are also consistent with an electrogenic mode of transport. In vivo micropuncture of rat proximal tubules, depolarization of the basolateral membrane potential significantly inhibits NMN uptake (468). Similarly, depolarization of the basolateral membrane inhibits uptake of TEA across the basolateral membrane of isolated single snake and flounder proximal tubules (205, 207, 406). Furthermore, addition of TEA to the medium bathing isolated snake (205) or flounder (406) renal proximal tubules causes a depolarization of the basolateral membrane, supporting the contention that basolateral OC transport includes electrogenic facilitated diffusion. Interestingly, efflux of TEA from preloaded snake proximal tubules is not sensitive to depolarization of the membrane potential, leading to the suggestion that basolateral OC efflux may involve a process distinct from the pathway(s) that dominates influx (205, 206). It should be noted that the electrogenicity of OCT1-, OCT2-, and OCT3-mediated transport in heterologous expression systems, discussed previously, is typically used as support for the probable basolateral location of these processes.

OC transport across the basolateral membrane can also operate in an OC/OC exchange mode. For example, outwardly directed gradients of the OCs TEA and mepiperphenidol (Darstine) support the transient, uphill accumulation of labeled TEA in voltage-clamped rabbit renal BLMV (294). Also, in isolated single rabbit proximal tubules, an inwardly directed gradient of TMA, TEA, or...
choline (82, 135) significantly stimulates efflux of labeled TEA from preloaded tubules. Operationally, this mode of activity is similar to that observed for the mediated exchange of OCs supported by OCT1 and OCT2 (34, 44).

B) INFLUENCE OF INORGANIC IONS ON BASOLATERAL OC TRANSPORT. Na\(^+\) does not exert a direct effect on basolateral transport of OCs in proximal cells. Uptake of TEA into rat renal BLMV is not influenced by the removal of Na\(^+\) (429), and imposition of either an outwardly or inwardly directed Na\(^+\) gradient has no effect on TEA transport in rabbit renal BLMV (294). In microperfused rat renal proximal tubules in vivo, removal of Na\(^+\) causes a modest inhibition of NMN uptake (468). However, the degree of inhibition observed is similar to that noted when extracellular K\(^+\) is elevated or bicarbonate is removed, so this effect has been ascribed to the associated depolarization of the membrane potential caused by these three manipulations (468).

Although H\(^+\) concentrations and trans-membrane H\(^+\) gradients have been found to influence basolateral OC transport in some experiments, these effects do not appear to indicate a direct coupling of H\(^+\) in the transport process. Hydrogen ion gradients typically have little or no effect on OC transport in isolated renal BLMV (168, 429, 533), and when such effects have been observed, they generally have been found to reflect the indirect effect of H\(^+\) gradients on membrane potential (407). In this regard, the effect of H\(^+\) gradients on OC transport in BLMV appears to parallel the pH sensitivity of TEA transport into oocytes expressing rOCT3 that disappears when the oocyte membrane potential is clamped (199).

In intact tubules, changes in ambient pH do influence basolateral OC transport in some cases (207), although whether this reflects a direct effect on basolateral transporters, or indirect effects arising from changes in trans-membrane potentials or other factors that influence transport, is not clear. In studies on isolated snake renal tubules, alterations in extracellular and intracellular pH provided no evidence for trans-stimulation of either TEA uptake or efflux (207). However, in these tubules there appeared to be an optimal intracellular H\(^+\) concentration for TEA uptake corresponding to that found at the physiological intracellular pH of 7.1 and an optimal extracellular H\(^+\) concentration for TEA efflux corresponding to that found at the physiological extracellular pH of 7.4 (207). Although the mechanism involved in this relationship is unknown, these data support the concept noted above that OC efflux may occur by a pathway distinct from the pathway(s) involved in uptake in these tubules.

Basolateral transport of selected OCs can be markedly influenced by extracellular bicarbonate concentration. In suspensions of isolated rat proximal tubules, replacement of bicarbonate with phosphate, Tris-HEPES, or acetate buffers inhibits basolateral uptake of the organic base amantidine. The inhibition reflects both a two- to threefold increase in the apparent \(K_p\) for amantidine transport and a two- to threefold reduction in maximum flux (\(J_{\text{max}}\)) as well (106). This is a much more pronounced effect than that noted for contraluminal NMN transport in rat proximal tubules in vivo noted previously (468). The effect of bicarbonate on amantidine transport is also present in suspensions of rat distal tubules (106), which is one of the few observations of OC transport in a nephron segment other than the proximal tubule (105, 106).

Significantly, the bicarbonate-sensitive component of amantidine uptake appears to involve a process distinct from the well-characterized transporter for TEA, NMN, and other OCs. rOCT1 and rOCT2 both support amantidine transport that is blockable by TEA (126). However, whereas amantidine is a potent inhibitor of TEA uptake into suspensions of both proximal and distal rat renal tubules, TEA has no effect on amantidine uptake in these preparations (127). Bicarbonate-sensitive amantidine is not influenced by treating tubules with acetazolamide, suggesting that the intracellular bicarbonate pool exerts little or no effect on the rate of basolateral amantidine transport (106). Unlike basolateral transport of TEA, NMN, and other OCs, bicarbonate-sensitive amantidine uptake is not responsive to changes in the extracellular K\(^+\) concentration and, therefore, does not appear to be electrogenic (105). The amantidine transport pathway shows a marked stereoselective interaction with quinine and quinidine (105). The bicarbonate-sensitive component of amantidine transport in proximal tubules is blocked with threefold greater potency by quinine than by its diastereomer, quinidine. In distal tubules, however, amantidine transport is blocked equally well by quinine and quinidine. Lactate inhibits the bicarbonate-sensitive component of amantidine transport in both proximal and distal tubules (104). Acute increases in blood bicarbonate concentration are correlated with a decrease in renal amantidine clearance (128), suggesting that the effect of bicarbonate on renal OC clearance is influenced by the effects of bicarbonate on a battery of bicarbonate-sensitive cellular events. These observations, combined with those discussed in an upcoming section, support the conclusion that there are multiple, mechanistically distinct OC pathways in the basolateral membrane of renal proximal (and distal) tubules.

Basolateral OC transport is also influenced to variable degrees by the extracellular concentrations of other inorganic ions. Reducing the extracellular Ca\(^{2+}\) concentration from 2.5 to 0.25 mM has no effect on the rate of amantidine uptake in rat renal proximal tubules (105). Similarly, removal of extracellular Ca\(^{2+}\) has no effect on the initial rate of TEA uptake by isolated snake renal proximal tubules, but it does reduce the 1-h, steady-state accumulation of TEA and increases TEA efflux (206). Exposure to Ba\(^{2+}\) inhibits TEA uptake into isolated snake proximal tubules, but this appears to be secondary to
depolarization of the membrane potential (205, 206). However, exposure to Ba\(^{2+}\) also inhibits TEA efflux, even in the face of the concomitant depolarization of the basolateral membrane potential (206). These data suggest that TEA flux across the basolateral membrane in snake renal tubules, as noted above, is kinetically and, perhaps, mechanistically asymmetrical (206). Removal of Cl\(^-\) from the medium bathing isolated rabbit proximal tubules (S2 segment) virtually eliminates TEA accumulation (163), with approximately half activity noted at an external concentration of between 30 and 70 mM Cl\(^-\). Bromide can replace chloride, but iodide, sulfate, and nitrate cannot (163). The presence of TEA in the extracellular solution has no effect on the basolateral membrane (163). The mechanistic basis of these observations has received considerable attention. Early studies on substrate selectivity, involving renal OC clearance (131) and transport in renal slices (353), revealed a correlation between inhibition of transport and increasing hydrophobicity of test agents (90, 550; W. Barendt, unpublished observations). It should, however, be emphasized that studies of the interaction of test agents with OC transporters have largely been limited to an assessment of the effectiveness of the test compounds as inhibitors of OC transport. Generally, it is not known if the agents are themselves transported, nor is it known if the inhibition is, in fact, competitive. Ulrich speculated that high-affinity inhibitors (i.e., those with very low \(K_i\) values) may bind to the transporter but are translocated through the membrane slowly, if at all (468). Consistent with this suggestion, Groves et al. (135) found that n-TAA compounds of comparatively low affinity (TMA and TEA, with \(IC_{50}\) values of 1.3 and 0.08 mM, respectively) trans-stimulated efflux of radiolabeled TEA from preloaded rabbit proximal tubules, whereas high affinity n-TAAs (TBA and TPeA, with...
IC50 values of 4 and 0.8 μM trans-inhibited efflux (135). The interaction of TPeA with basolateral OC transport appears to have both high- and low-affinity components, with the high-affinity component (Ki of <1 μM) being competitive (140). Interestingly, basolateral TEA transport in isolated rabbit proximal tubules continues to be reduced for 10 min following only a brief (<10 s) exposure of the basolateral membrane to TPeA (140). These data, which further support Ullrich’s speculations (468), suggest that high-affinity, hydrophobic cations may dissociate slowly from their binding site(s), thereby reducing physiological turnover of OC transporters, a concept discussed previously in the context of trans-inhibition noted in studies with cloned transport proteins.

As for basicity, a strong correlation has been observed between the inhibitory interaction of weak bases with basolateral OC transport and their pKa values, the latter being defined as the strength of the partial ionic charge of the basic nitrogen (e.g., Refs. 269, 474, 475). The interaction of a number of compounds with hOCT2, an increase in external pH markedly diminished the interaction of weak bases (cimetidine, trimethoprim, and 4-phenylpyridine) with this transporter. Increases in external pH also decreased the rate of hOCT2-mediated uptake of labeled cimetidine. In addition, whereas the interaction of these charged compounds with hOCT2 was competitive, inhibition produced by the neutral steroid corticosterone was noncompetitive and, therefore, may not have reflected an interaction at the transport binding site. Consequently, the very effective inhibitory interaction of several nonionizable steroid hormones with basolateral OC transport in rat proximal tubules, including corticosterone and testosterone, should not be used as evidence that noncharged substrates can bind effectively to the transport site of basolateral OCTs (474).

A number of organic electrolytes (anionic, cationic, or zwitterionic) have been found to interact (to some degree) with both OC transporters and transporters that typically interact with organic anions. For example, the prototypic inhibitor of renal OA transport, probenecid, also inhibits, albeit with comparatively low affinity, renal OC transporters (e.g., Ref. 269). Ullrich et al. (474, 475) compared the interaction of a number of compounds with rat renal basolateral OC and OA transport, and substrates that interacted with both processes were referred to as “bisubstrates.” Three groups of bisubstrates were noted: 1) zwitterions (containing both potential anionic and cationic groups), including hydrophobic dipeptides, amino cephalosporins and gyrase inhibitors; 2) substances with a potential cationic N-group and one or more electronegative (electron-accepting) or hydrogen bonding groups (e.g., Cl−, Br−, or NO3− groups); and 3) substances with one or more partially charged or hydrogen-bond forming groups in a suitable spatial arrangement (this latter group includes a number of noncharged steroids). Typically, bisubstrates that have a comparatively high affinity for one pathway have a comparably low affinity for the other. In other words, compounds with a combination of steric and physico/chemical characteristics that render them high-affinity substrates of, for example, basolateral OC transport, generally interact with renal OA transport with low affinity. There are, however, a number of compounds, including the azepines (e.g., chlorimidine, chloropromazine, and flurazepam) and many of the...
quinolines, which have moderately high affinities for both OC and OA transport at the basolateral membrane (475). Finally, a few molecules, including a number of the non-ionic steroid hormones (e.g., corticosterone, aldosterone, and testosterone), display comparatively high affinity for both OC and OA transporters (474). As noted previously, in at least some cases, the inhibitory interactions these compounds exert with OC and/or OA transport are not competitive (13). In particular, extremely hydrophobic compounds might be expected to interact with hydrophobic regions of transport proteins distinct from the transport receptor.

Steric (spatial) parameters appear to play a comparatively minor role in influencing affinity of basolateral OC transport for type I substrates. This has been examined most extensively in studies comparing the relative interaction of a number of chiral cations with OC transport in rat and human renal tissue (173, 410, 527). Comparisons of the relative affinity for basolateral OC transport in rat tubules of 10 pairs of cationic enantiomers/diastereomers, including \((R)-(\pm)-\) and \((S)-(\pm)\)-pindolol, quinine/quinidine, and several analogs of ephedrine, revealed differences no greater than a factor of three (410). This is the same degree of difference reported for quinidine and quinine as inhibitors of the amantidine-selective OC transport pathway in rat proximal (105) and distal (526) tubules. Studies of the renal clearance of stereoisomers by human kidney also show the influence of structure on rates of clearance, but these effects are generally very modest (<2-fold; Refs. 410, 463, 527). In addition, owing to the number of events associated with the process of renal clearance, it is not certain where the influence of substrate structure is occurring, i.e., at the basolateral membrane, the luminal membrane, and/or at one or more intracellular events (e.g., sequestration). Evaluation of three-dimensional models of the interaction of ephedrine enantiomers/diastereomers with basolateral OC transport in the rat suggests that the spatial orientation of the amino group and the OH group is important. The importance of the three-dimensional location of OH groups on the interaction of morphine analogs with the basolateral OC transporter in rat kidney (470) further supports the conclusion that steric interactions can influence (usually modestly) the binding of substrates to basolateral OC transporters. We discussed previously the systematic influence of selected steric parameters on the binding of substrates to hOCT1 (15), results that underscore the fact that three-dimensional structure, and general hydrophobicity, both influence the interaction of substrates with basolateral OC transporters.

b) MULTIPLECTY OF BASOLATERAL OC TRANSPORTERS. Although the observations of Ullrich et al. (468) are consistent with the operation of a single mediated pathway for OCs in the basolateral membrane, other observations are not. For example, the observation mentioned above that amantidine blocks TEA transport into isolated rat proximal tubules but TEA does not block amantidine transport (126, 127) is strong evidence that at least two parallel OC transport processes must be coexpressed in these tubules. In addition, there is strong evidence, both physiological and immunocytochemical, for the potential presence of at least three OCT homologs (OCT1, OCT2, and OCT3) in the basolateral membrane of renal proximal tubule cells (e.g., Refs. 195, 415). Thus it is likely that the basolateral OC uptake measured in native renal tissues (including intact proximal tubules and isolated BLMV) is influenced, if not dominated, by the activity of some combination of these cloned transport proteins. So, to what extent can we compare the quantitative and qualitative characteristics of the cloned transporters (i.e., their kinetic and selectivity characteristics) to those measured in renal tubules? Can conclusions be drawn concerning the functional distribution and integrated activity of the several OC transporters that are expressed in the kidney based on transport behavior observed in intact tubules? We deal first with the issue of evidence that multiple OC transporters must be expressed in the basolateral membrane of native tissues/cells.

c) MULTIPLE TRANSPORTERS IN NATIVE RENAL CELLS. The profile of inhibitor interactions with the transport of prototypic OC substrates (e.g., TEA, NMN, MPP) in intact rat and rabbit proximal tubules is consistent with the presence of a single pathway for small, monovalent (type I) OCs (135, 468), although immunocytochemical data support the expression, at least in the rat, of significant levels of at least two similar transporters, i.e., OCT1 and OCT2 (195, 415). The results of studies with OCT1 and OCT2 knockout mice provide the most direct evidence that both of these processes play a role in OC clearance in rodents. Interestingly, OCT1−/− mice show an increase (rather than a decrease) in renal TEA clearance. But this paradoxical effect presumably reflects the ability of OCT2 in the kidney to deal with the increase in plasma TEA concentration resulting from the marked decrease in hepatic uptake and clearance of TEA in OCT1−/− mice (184). Significantly, deficiency in OCT2 by itself has little effect on renal TEA clearance, indicating the presence of sufficient OCT1 in the kidney to handle the load (185). However, simultaneous elimination of both OCT1 and OCT2 virtually eliminates renal TEA clearance (185), providing the most direct evidence for the parallel importance of both OCT1 and OCT2 in the rodent kidney. This latter observation suggests that OCT3 expression in the kidney is not likely to play a quantitatively significant role in TEA (or other type I OC) secretion. Thus it is not surprising that elimination of OCT3 has no effect on the renal accumulation of MPP+ (554).

Studies employing the cultured cell line LLC-PK1 are generally interpreted as being consistent with the presence in these cells of a single pathway for basolateral
uptake of type I OCs (111, 115, 268, 364, 428, 430, 458). The snake proximal tubule shows a different pattern; whereas TEA transport displays the routine characteristics of OC transport described above, NMN transport appears to be dominated by a distinct set of pathways that are Na⁺ dependent and that result in net reabsorption (rather than secretion). Another example of distinct basolateral pathways for type I OCs is the suite of processes responsible for amantidine versus TEA transport in rat proximal tubules, described previously (127). There is also evidence for a basolateral transport process (in rabbit proximal tubules; Ref. 137) for small, divalent OCs (e.g., paraquat) that is distinct from the process(es) that accept monovalent OCs (54, 55, 250).

**D) COMPARISON OF CLONED TRANSPORTERS TO TRANSPORT ACTIVITY IN NATIVE RENAL CELLS.** Although phenotypic evidence mostly suggests the presence of a single transport process for type I OCs in the basolateral membrane of proximal cells, there is ample evidence to suggest that, in at least some species (e.g., the rat), multiple OCT processes may be expressed. Functional data with intact or native (i.e., vesicular) experimental systems have generally not lent themselves to conclusions concerning the relative contributions to cellular OC transport of the different OCTs expressed in the kidney. Whereas the kinetics of transport of prototypic OC substrates, including TEA, NMN, and MPP, show no evidence of being influenced by interaction with more than one mediated pathway (468, 475), such evidence is only expected if parallel pathways display markedly different affinities for these substrates (306). Indeed, in practice, apparent affinities of parallel pathways for a common substrate must differ by a factor of 5–10 or more if kinetic evidence for the presence of separate process is to be evident. The apparent affinities for TEA, NMN, and MPP, though rather different from one another, are very similar in OCT1 and OCT2. Thus it is unlikely that the parallel expression of these processes in renal tubules would be evident from the kinetic profile of substrate transport.

Inhibitors that selectively block individual OC transporters are useful in assessing the extent of coexpression of multiple OCTs that display similar affinities for prototypic substrates. Several substrates that discriminate between rat OCT1 and OCT2 expressed in oocytes have been identified (5). These include mepiperphenidol and O-methylisoprenaline (O-methylisoproteranol), which selectively interact with rOCT1 over rOCT2, and guanidine and corticosterone, which selectively interact with rOCT2 over rOCT1. These compounds may permit the functional assessment of coexpression of rOCTs in native renal cells.

The largest database currently available for comparing the characteristics of transport in native tubules with those of cloned transporters is that provided by the observations of Ullrich and colleagues (83) using in vivo microperfusion techniques. Their work included measures of the inhibitory interactions of guanidine and corticosterone with basolateral OC transport (474, 475). Corticosterone’s apparent $K_i$ in intact rat tubules is 200 μM (474). When this value is compared with the IC$_{50}$ values of 150 and 4 μM for rOCT1 and rOCT2, respectively, as expressed in oocytes (5), we could conclude that OC transport in the rat tubule is dominated by OCT1. Similarly, the interaction of guanidine with basolateral OC transport (Apparent $K_i$ of 1 mM; Ref. 475), is more consistent with an interaction dominated by OCT1 ($K_i$ for guanidine transport of 1.6 mM when expressed in oocytes; Ref. 5) than with OCT2 ($K_i$ of 170 μM when expressed in oocytes; Ref. 5).

The microperfusion method is limited to tubule segments at the surface of the kidney and, consequently, is effectively restricted to early (S1) and mid (S2) segments of proximal tubule. By immunocytochemistry, these regions of the proximal tubule are expected to contain both OCT1 (prevalent in the S1 segment) and OCT2 (prevalent in the S2 segment). Thus the apparent absence of transport activity dominated by or, at least, influenced by activity of OCT2 in the in vivo studies is, perhaps, surprising. But, how comparable are kinetic values obtained with native cell/tissue preparations to those obtained with cloned transporters in expression systems? We emphasized earlier the caution that should be employed when comparing kinetic parameters for cloned transporters obtained with different expression systems. In this regard, it is relevant to compare results obtained with cloned transporters to those obtained in native tissues in which we can be reasonably confident that transport activity is strongly influenced, if not completely dominated, by activity of that protein. Figure 5 compares apparent $K_i$.
values determined in intact rat proximal tubules with \( K_i \), \( K_p \), or \( IC_{50} \) values determined for the same compounds used as substrates or inhibitors of transport mediated by rOCT1 in heterologous expression systems. Included in the comparison are results obtained using both the oocyte and mammalian cultured cell expression systems. It is evident that there is not a strong correlation between kinetic observations made in intact tubules with those made with cells expressing rOCT1.

Despite the absence of such a correlation, OC transport in the studies with intact rat tubules is probably strongly influenced, if not dominated, by rOCT1 activity. Why, then, are apparent \( K_i \) values measured in intact tubules routinely much higher than those for the cloned transporter measured in various expression systems? At least two possibilities suggest themselves. First, the techniques associated with use of the different experimental systems are very different (e.g., microperfusion versus uptake in single oocytes or cultured cells) and could lend themselves to introduction of analytical artifacts. Second, and almost certainly a contributor to the apparent discrepancies highlighted in Figure 5, the physical, chemical, and regulatory environments of the native tubule, including the complicating influence of extracellular binding of substrates to blood proteins, differ from that of each of the various expression systems used to characterize cloned transporters.

Indeed, the strength of expression systems, i.e., the ability to physiologically isolate a process from the complexity of its native environment, is also the basis of an important “weakness” of such studies. Whereas observations of transport activity in an expression system provide information on how a cloned transporter can act, they do not necessarily provide precise information on how the transporter does act within the physiological context of the native cell or tissue. Nevertheless, as noted previously, kinetic parameters obtained using multiple expression systems have been used as the basis for quantitative tests of the functional distribution of OCT1 and OCT2 in rabbit proximal tubules (553), leading to the conclusion that OCT1 activity predominates in the S1 segment and that OCT2 activity predominates in the S2 and S3 segments (553; K. Evans, unpublished observations).

3. Regulation of basolateral OC transport

Comparatively little attention has been paid to the regulation of peritubular OC transport. OC transport appears to be influenced by acute activation of protein kinases, although the profile of such responses is not yet clear. Activation of PKA or PKC [via treatment with either forskolin or 1,2-dioctanoylglycerol (DOG), respectively] resulted in a marked stimulation in rOCT1-mediated uptake of the cationic dye, 4-(4-(dimethylamino)steryl)-N-methylpyridinium (ASP\(^+\)) into HEK293 cells stably expressing this transporter (270). These effects were blocked by coexposure to inhibitors of PKA (KT5720) or PKC (tamoxifen). The increase in uptake was associated with an increase in the apparent affinity of rOCT1 for TEA, TPA, and quinine, and with an increase in phosphorylation of rOCT1 (270). Interestingly, kinase activation produced different effects in two renal cell lines that phenotypically display OC transport. Whereas activation of PKC also increased ASP\(^+\) uptake into IHKE-1 cells, it decreased uptake into LLC-PK\(_1\) cells (164). Different regulatory profiles in these cells were also noted following activation of PKA and PKG; ASP\(^+\) uptake was stimulated in IHKE-1 cells, but remained unchanged in LLC-PK\(_1\) cells (164). The authors noted that these differences could be caused by differences in protein trafficking or expression of different isoforms in the different cell lines. Changes in the trafficking of organic anion transporters at the level of the plasma membrane in response to activation of PKC has been shown to influence OA uptake (150, 331) and may represent a means of influencing the level of OC transport as well.

In one study with isolated, nonperfused S2 segments of rabbit renal proximal tubule, activation of PKC (via exposure to phorbol ester) produced a marked stimulation of TEA uptake (162). The effect was time and dose dependent and was blocked by coexposure to the nonspecific PKC-inhibitor staurosporine. In contrast, activation of PKC, as well as PKA and PKG, in single, isolated human proximal tubules resulted in an inhibition of ASP\(^+\) uptake (337). The authors noted that the selectivity profile of ASP\(^+\) uptake into isolated human tubules suggested activity of hOCT2, rather than hOCT1, an observation consistent with immunocytochemical and mRNA expression data discussed previously (300). Thus the difference in the response to activation of PKC in human and rabbit tubules could reflect differences in the isoforms expressed in these species. However, OCT2 appears to be the only homolog expressed at appreciable levels in the human kidney (300), and OC transport in the S2 segment of rabbit proximal tubule is dominated by expression of OCT2 as well (553). Both the human and rabbit orthologs share consensus sites for PKC-mediated phosphorylation, including one (Ser-285/286) that is shared by all five of the cloned OCT2 orthologs (426, 553).

Activation of G protein-coupled receptors by carbachol results in a decrease in hOCT2-mediated transport activity when expressed in HEK293 cells (51). This effect does not appear to involve activation of PKC because the permeable diacylglycerol analog DOG has no effect on hOCT2 activity. Interestingly, this contrasts with the observation, noted above, that activation of PKC in human tubules causes an apparent downregulation of OCT2 activity (337), suggesting the influence of different regula-
tor profiles in different tissues or cell types.] Instead, carbachol-induced activation of G protein receptors appears to involve phosphatidylinositol 3-kinase. Additional evidence suggests that OCT1 (rat) and OCT2 (human) are also downregulated by cGMP (378) and by activation of PKA (51). Activation of calmodulin-dependent kinase, instead, stimulates hOCT2-mediated transport (51). Future studies of the acute effect of kinase activation on both OC transport activity mediated by cloned OCTs and OC transport by native renal cell systems will be required to determine the basis, and the physiological role, of acute OCT regulation.

There is strong evidence that expression of OCT activity in the kidney is under the control of steroid hormones. Early studies of OC accumulation in renal slices showed a marked gender difference, with TEA uptake in male rats being greater than that in females (27). This has been confirmed in more recent studies (487, 490). These latter studies compared levels of OCT mRNA in male and female rat kidneys, in an effort to determine the basis for the sex difference in renal OC transport. Whereas no sex-based differences were observed in the renal expression of either OCT1 (402, 487) or OCT3 (402, 487) mRNA, the OCT2 mRNA level was approximately three- to fourfold higher in kidneys from male rats than in kidneys from female rats (402, 487). Treatment of male and female rats with testosterone significantly increased expression of OCT2 mRNA (490). This was correlated with an increase in OCT2 protein expression and increased rate of TEA uptake into renal slices from both sexes (490). Neither male nor female rats showed a significant decrease in OCT2 mRNA expression following treatment with estradiol (490). However, treatment of male rats with estradiol resulted in a decrease in OCT2 protein expression, and this was correlated with a decrease in TEA uptake in renal slices from treated males (but not females) (490). Gonadectomized adult male rats showed a marked decrease in expression of OCT2 mRNA; this treatment had no effect on expression of OCT2 mRNA in females (402). As noted previously, TEA uptake is substantially greater in renal slices from male rat kidney (487), and this is correlated with greater expression of OCT2 mRNA (487, 490), and greater expression of OCT2 protein (487) in male rat kidney. Consistent with this observation is the increase of both TEA transport and OCT2 expression in MDCK cells following treatment with testosterone (392). Treatment of MDCK cells with dexamethasone and corticosterone significantly increases TEA transport and OCT2 expression as well (392). In line with the above observations, the reduction in renal secretion of cimetidine by male rats that is associated with chronic renal failure (following 5/6 nephrectomy) is correlated with a reduction of both plasma testosterone and renal expression of OCT2 (183).

4. Apical/brush border/luminal OC transport: mechanism

The luminal step in renal secretion of type I OCs appears to be dominated by carrier-mediated exchange of extracellular H⁺ for intracellular OC. This scheme was first put forward by Holohan and Ross in 1981 (168) and has not changed appreciably since then (342). The activity of OC/H⁺ exchangers has been identified in isolated luminal membrane vesicles from every mammalian species examined, including dog, rat, rabbit, and human (e.g., Refs. 168, 322, 429, 533), and in all nonmammalian species examined, including birds (504, 505) and snakes (81). The exchange of small, monovalent (type I) OCs for H⁺ is electroneutral (429, 533). Because intracellular H⁺ is kept far from electrochemical equilibrium (through the activity of luminal Na⁺/H⁺ exchange and the V-type H⁺-ATPase), the downhill influx of H⁺ serves as the driving force for active transepithelial OC secretion. At least two separate OC/H⁺ exchangers have been identified in isolated membrane preparations. Of the two, the process that accepts TEA and MPP is generally accepted as being the major route of luminal type I OC efflux (342). The second process displays much narrower substrate specificity, with guanidine being the prototypic substrate (290) (although studies with LLC-PK1 cells have implicated a guanidine-sensitive transporter in the apical flux of quinolone derivatives; Ref. 366). In addition to these two OC/H⁺ exchangers, the presence of at least three other OC transporters is generally accepted. The first is an electrogenic facilitated diffusion process with a comparatively narrow selectivity for choline and structurally related analogs (471, 535). This process has been suggested to play a role in the routine reabsorption of choline from the tubular filtrate (80, 342, 535). The second is the Na⁺-dependent carnitine cotransporter, which has proven to be the cloned transporter OCTN2 (314, 350, 413, 540). Although OCTN2 plays a clear role in the physiological reabsorption of carnitine (441), it can also operate as an OC transporter, suggesting a possible role in the reabsorption of selected OCs (312, 387, 540). OCTN2 also supports mediated carnitine/TEA exchange (312), and mice lacking functional OCTN2 show depressed rates of TEA clearance (312), suggesting a physiological role for OCTN2 in net tubular OC secretion as well. The third luminal transport process involved in renal OC secretion is the multidrug resistance transporter MDR or P-glycoprotein, which is postulated to play a significant role in the renal secretion of a wide array of type II OCs (377).

To date, efforts to clone a transporter with characteristics paralleling those of luminal OC/H⁺ exchange have been unsuccessful. As discussed earlier, OCTN1 does display pH sensitivity consistent with OC/H⁺ exchange activity (543). However, the tissue distribution and kinetic profile of OCTN1 are quite different from the
physiological profile of OC/H⁺ exchange activity observed in most experimental systems. Indeed, mRNA for OCTN1 was effectively absent from samples of human renal mRNA studied using quantitative real-time PCR (300). Thus OCTN1 is an unlikely candidate for the principal OC/H⁺ exchanger implicated in renal OC secretion. Several studies have attempted to characterize the biochemical profile of the luminal OC/H⁺ exchanger. Treatment of isolated membrane vesicles or the apical membrane of cultured OK and LLC-PK₁ cells with various group-selective modifying reagents implicates one or more histidine (197), lysine (319), carboxyl (208), and sulfhydryl (197) groups as being important in the activity of luminal OC/H⁺ exchange. Cationic photoaffinity labels have also been used in efforts to identify protein associated with luminal OC/H⁺ exchange. Treating rat renal BBMV with an azido group-containing analog of cimetidine irreversibly reduces TEA transport, and transport activity is protected by coexposure with TEA (210). This radiolabeled photoaffinity probe labeled a 36-kDa protein, which was decreased by coexposure during labeling with TEA, NMN, and several other OCs, thereby implicating the 36-kDa protein in OC/H⁺ exchange. A similar approach has been used to label a 41-kDa protein in dog renal BBMV with radiolabeled azidopine (124, 169). Both these proteins are much smaller than the 61- to 63-kDa size of all current cation transporters in the OCT family of proteins. These data suggest that a separate family of transport proteins may be responsible for luminal OC transport.

5. Specificity of luminal OC transport

In the absence of cloned candidates for the role of the luminal OC/H⁺ exchanger, the emphasis in recent years has been on the use of native cell and membrane preparations to extend the understanding of the luminal efflux step in renal secretion of type I OCs. These studies, in turn, have tended to be dominated by efforts to understand the molecular determinants associated with substrate binding and transport by the luminal OC/H⁺ exchanger, using isolated BBMV as well as intact renal tubules.

The physicochemical parameters that influence interaction of OCs with the luminal OC/H⁺ exchanger are similar to those discussed previously for basolateral OC transport. Ulrich et al. (83) compared the interactions of a large number of OCs with luminal MPP transport (presumably reflecting interaction with the luminal OC/H⁺ exchanger) with those observed in their previous work on the basolateral NMN transporter (reflecting activity of one or more basolateral OC transporters). Again, the most general observation was the positive correlation between increasing substrate affinity and increasing substrate hydrophobicity, confirming and extending an earlier assessment made for TEA transport in isolated rabbit renal BBMV (536). Ulrich and co-workers (83) also noted three quantitative differences in the interaction of OCs with the two sets of transport processes. First, the luminal transporter interacts more readily with large OCs than does the basolateral OC transporter. Second, there is a more marked relationship between substrate hydrophobicity and apparent affinity for luminal transport than for basolateral transport. Third, there is a more marked relationship between substrate pKₐ and apparent affinity for luminal transport than for basolateral transport. The consequence of these relationships is that, for a common set of substrates, there is a broader range of affinities observed for luminal OC transport than for basolateral OC transport.

A principal characteristic of renal OC transporters, and one shared by the luminal OC/H⁺ exchanger, is their ability to accept an extremely diverse array of chemical structures as substrates. This characteristic implies that steric factors play a relatively modest role in stabilizing the binding of substrate on (within) the receptor region of the OC/H⁺ exchanger. This issue was examined in a study with rabbit renal BBMV that compared the relative interaction of a set of pyridinium/quinolinium analogs with the OC/H⁺ exchanger (534). These compounds all had a cationic nitronium moiety at the 1-position of a six-member aromatic ring and varied sterically in the position of a planar (a flat aromatic ring) hydrophobic mass. Intriguingly, the position of the planar hydrophobic mass had no effect on the IC₅₀ values displayed by these compounds against the transport of TEA. Instead, affinity of the transporter for planar hydrophobic OCs is predicted by their relative hydrophobicity. This information was used to postulate the presence of a 9 Å × 12 Å planar receptor surface with which OCs interact to stabilize binding to the OC/H⁺ exchanger (534).

The influence of steric factors on luminal OC transport has also been assessed by determining the relative inhibitory effect of stereoisomers on activity of the OC/H⁺ exchanger. In vivo micropuncture of rat renal proximal tubules found that a wide array of enantiomers/diastereomers (including several ephedrine derivatives, deprenyl, transcycloporomine, disopyramide, verapamil, pindolol, and quinine/quinidine) have IC₅₀ values for luminal OC transport that differ by less than twofold (410). Consistent with this observation is the finding that in rat renal BBMV the IC₅₀ values for several stereoisomers (verapamil, hydroxychloroquine, and quinine/quinidine) differ by less than twofold (134). Similar results have been reported for rabbit renal BBMV and isomers of ephedrine, nicotine, pindolol, and quinidine/quinidine (321, 534). In contrast, however, are results obtained with dog renal BBMV and cultured OK cells. In the former, IC₅₀ values for inhibition of apical TEA transport differed by 3-fold for quinine and quinidine and by >10-fold for S-(−)- and
$R(\pm)$-verapamil (320). These results, which may reflect species differences in the selectivity of the apical OC/H$^+$ exchanger, suggest that relatively modest differences in the structure of the transport protein may lead to substantial differences in the influence of steric factors on substrate binding.

There is comparatively little information on the relationship between binding of substrate to a luminal OC transporter and subsequent turnover of the substrate-transporter complex. Ullrich et al. (475) observed in their studies using in situ microperfusion methods in the rat kidney that initial rates of OC transport increased with decreasing $K_i$ (or $K_i$), though, importantly, they noted that this general trend reflected transport from “low substrate concentrations.” Potentially pertinent to this observation, there is an inverse relationship between affinity of the OC/H$^+$ exchanger for substrate and actual turnover of the substrate-transporter complex, i.e., substrates for which the OC/H$^+$ exchanger has a high affinity have comparatively low $J_{\text{max}}$ values (maximal rates of transport). It is, however, instructive to consider the ratio of $J_{\text{max}}$ to $K_i$ as a means for comparing the relative rates of carrier-mediated solute flux from the low concentrations (i.e., <<$K_i$) to which transporters are most likely to be exposed. The traditional units of $J_{\text{max}}$ are moles per unit time per unit area, and those for $K_i$ are moles per unit volume. The ratio of $J_{\text{max}}$ to $K_i$, therefore, has units of velocity (e.g., cm/s) and, so, is a measure of the “carrier-mediated permeability” of the membrane when rates of transport are nearly first-order functions of substrate concentrations. Viewed in this context, “high affinity” substrates are predicted to cross the membrane more efficiently (i.e., higher rates at low concentrations) than “low affinity” substrates, consistent with Ullrich’s observation. Importantly, as also suggested by Ullrich (475), compounds for which OC transporters display an extremely high affinity ($K_i$ or $K_i$ <<1 M) may dissociate from the transporter sufficiently slowly to be transported poorly, if at all.

6. Role of MDR1 in renal OC secretion

As discussed earlier, MDR1 supports active export of type II and at least some type I OCs. Expression of MDR1 in the apical membrane of proximal tubule cells suggests that it is likely to play a role in the secretion of at least some OCs. Indeed, there is clear evidence that MDR1 dominates the secretion of daunomycin (277) and fluorescent analogs of cyclosporin (383) and rapamycin (282) by intact, isolated renal proximal tubules. There are, however, contradictory indications of the quantitative role played by MDR1 in renal secretion of some OCs. In one study (259), renal clearance of Rho by the intact isolated rat kidney was blocked by MDR1 inhibitors known to also inhibit type I transporters (quinidine and verapamil), but not by inhibitors that do not influence type I transporters (cyclosporine A and digoxin). Rho clearance was also blocked by cimetidine, a known substrate for type I OC transporters, but one for which interaction with MDR1 is less conclusive. The authors concluded that, in the intact kidney, Rho secretion is dominated by type I transporters, rather than by MDR1. Net secretion of Rho across LLC-PK$_1$ cells also appears to involve type I transporters, rather than MDR1 (493a). The results of these studies are, however, difficult to reconcile with measured rates of Rho efflux in single isolated perfused proximal tubules from wild-type mice and mice failing to express either of the murine MDR1 isoforms (mMDR1a or mMDR1b) (MDR1a/1b$^{−/−}$ mice) (460). The half-time for Rho efflux from tubule cells was increased from the control value of 34 to 407 s in the MDR1a/b$^{−/−}$ tubules. This latter value correlated closely with the 434 s $t_{1/2}$ measured in control tubules when verapamil was added to the perfusate. Importantly, addition of TEA to the perfusate had no effect on efflux of Rho from control tubules, suggesting that, in the native proximal tubule, type I transporters play, at best, a modest role in mediating Rho secretion. However, it is increasingly apparent that Rho can interact with multiple OC transport processes, making it difficult to draw firm conclusions about the quantitative role of distinct, parallel transport processes.

As noted previously, digoxin is a substrate for MDR1 expressed in cultured cells (179, 316, 448) and, importantly, digoxin clearance by rat kidney is blocked by traditional MDR1 inhibitors, including cyclosporin, quinidine, and verapamil (87, 170, 316), but not by the type I OC TEA (170). These data suggest that, in the normal kidney, digoxin clearance is dominated by its interaction with MDR1. Conclusions drawn from studies employing MDR1 knockout mice are, however, more equivocal. Although MDR1a/1b$^{−/−}$ mice show a profound reduction in hepatic and intestinal clearance of digoxin, renal clearance of digoxin is actually enhanced (198, 404) (although these mice also show an increase in renal digoxin content; Ref. 460). Importantly, the MDR1a/1b$^{−/−}$ animals also show an increase in renal clearance of the type I MDR1 substrates, TBuMA and APM (404), which are likely substrates for both MDR1 and type I OC secretory pathways (405). Taken together, the enhanced renal secretion of selected OCs in MDR1a/1b$^{−/−}$ animals may reflect (over)-expression of additional mechanisms for renal OC secretion (198). Interestingly, there appears to be no upregulation of either OCT2 or OCT3 in OCT1$^{−/−}$ mice (184).

MDR1 activity in renal proximal tubules appears to be regulated acutely. Activation of PKC, either through exposure to phorbol esters (286) or via an endothelin receptor-mediated response (261), reduces luminal secretion of fluorescent cyclosporin A. This effect is blocked by inhibition of PKC (286).

In summary, it is likely that MDR1 plays a quantitatively significant role in the secretion of type II OCs and
some hydrophobic neutral substrates. It is not clear, however, if MDR1 plays a significant role in the renal excretion of type I OCs, including the vast array of cationic drugs known to interact with the several OCTs described previously.

7. Intracellular OC sequestration

Trans epithelial OC secretion across renal tubules involves three compartments: peritubular, cellular, and luminal. Substantial evidence suggests that a fourth compartment, i.e., an intracellular “vesicular” compartment, could also influence the flux dynamics of OCs. The potential influence of intracellular vesicular sequestration on reducing the toxic effect of chemotherapeutic agents has received considerable attention (e.g., Refs. 258, 347, 348). Endosomes isolated from rat renal cortex show an ATP-dependent accumulation of TEA that is linked to an outwardly directed H+ gradient generated by the V-type H+-ATPase (344). The carrier-mediated TEA transport was suggested to reflect the presence in the endosomal membrane of the OC/H+ exchanger, possibly arising from cycling of luminal membrane through the early endosomal compartment (344). Accumulation of OCs within an intracellular vesicular compartment could play at least two roles. First, sequestration could spare sensitive cytoplasmic constituents from exposure to potentially toxic OCs. This would only be effective if the intravesicular compartment were “emptied” (to the outside of the cell) rapidly or often enough that steady-state is not achieved. However, the parameters of endosomal turnover in some cells, at least, suggest that this could occur and, theoretically, reduce the maximal intracellular concentration (348). Second, sequestration of OCs could play a role in transcellular OC transport by a mechanism similar to that proposed for the demonstrated transcellular movement of sequestered organic anions in renal epithelium (see below) (284). Indeed, the transcellular flux of the fluorescent OC quinacrine across choroid plexus includes a component that resides within a vesicular compartment that can be visualized as it releases its contents following fusion with the basolateral membrane (287). The two postulated roles for OC sequestration are not mutually exclusive; sequestration of OCs within a population of vesicles that moves across the cells could increase the rate of active secretion (by increasing intracellular OC content) while simultaneously sparing sensitive cytoplasmic constituents from exposure to potentially toxic levels of selected compounds.

It should be noted that ATP-dependent TEA transport has also been observed in preparations of renal BBMV (265). Whereas it is unlikely that the characteristics of transport observed in the cortical endosomal preparation represent contamination with brush-border membranes (344), the reverse is certainly possible.

III. RENAL ORGANIC ANION TRANSPORT

A. Overview of the Physiological Characteristics of Renal OA Transport

As with OCs, the proximal tubule is the primary site of renal OA secretion, as determined by studies employing stop flow, micropuncture, and microperfusion (see Refs. 292, 342). Substrates for the pathways involved in renal OA transport include a diverse array of weak acids that have a net negative charge on carboxylate or sulfonyl residues at physiological pH. Although a number of endogenous OAs have been shown to be actively secreted by the proximal tubule (e.g., 5′-hydroxyindoleacetic acid, riboflavin), it is generally accepted that the principal function of this process is clearing the body of xenobiotic agents (292, 342), including many of the products of phase I and phase II hepatic biotransformation, as well as anionic drugs of therapeutic or recreational use (41, 420).

As discussed in section II, dealing with renal transport of OCs, until recently, models of renal OA secretion typically depicted a single basolateral entry step and a single luminal exit step, a simple view that adequately explained existing physiological data. As in the case of the renal handling of OCs, that view is now known to oversimplify a process that entails a suite of cellular events that underlies renal OA transport. We previously made use of the type I and type II classifications for OCs to assist in the discussion of the multiple transporters with distinct selectivities for different structural classes of substrate. Although a parallel classification of OAs has not been formally recognized, it is useful to acknowledge that OAs can be separated into different structural divisions, based largely on molecular weight, net charge, and hydrophobicity. Therefore, we will define type I OAs as being comparatively small (generally <400 mol wt) and typically monovalent compounds, such as p-aminobenzoic acid (PAH), probenecid, and fluorescein. Type II OAs are bulkier (generally >500 mol wt) and frequently polyvalent and include, for example, calcein and estradiol-17β-D-glucuronide (E17β-D). As we shall see, OA transporters can be distinguished, at least in part, on the basis of the substrates with which they most efficiently interact.

1. Peritubular OA transport

In the 1980s and 1990s, Ullrich and colleagues (465, 477) characterized what has frequently been referred to as the “classical” OA secretory pathway, i.e., the one associated with the active secretion of a wide variety of xenobiotic compounds exemplified by the type I OA PAH. The peritubular entry of a type I OA is generally recognized as the active step in the transepithelial secretion of these compounds. In this regard, steady-state secretion of PAH has been modeled as reflecting the concerted behavior of...
three basolateral transport processes arranged in parallel. Figure 6 shows a model for transepithelial secretion of type I OAs by the proximal tubule that reflects an attempt to integrate observations obtained in studies with isolated renal plasma membranes and intact proximal tubules (342, 343) with those supported by recent molecular data. Type I OAs are taken up into the cell via mediated exchange with intracellular α-ketoglutarate (α-KG) (342). The outwardly directed α-KG gradient is maintained, at least in part, through the activity of a basolateral Na$^+$-dicarboxylate (NaDC) cotransporter. The Na$^+$ gradient that supports activity of the NaDC cotransporter is maintained, in turn, through activity of the Na$^+$-K$^+$-ATPase. Molecular candidates for each of the OA transport processes have been cloned, including three OA-selective members of the “OCT” family of transport proteins (OAT1, OAT2, and OAT3) that are expressed in the basolateral membrane of renal proximal tube cells,[489, 425, 569, 485] of which (OAT1 and OAT3) serve as OA/DC exchangers with functional characteristics that correlate closely with those observed in physiological studies of OA secretion in intact renal systems (386, 421, 427) and an NaDC cotransporter (NaDC-3; Refs. 63, 200, 511) expressed in the basolateral membrane of proximal tubules (153).

2. Luminal OA transport

In contrast to the current consensus on the mechanism of peritubular OA transport, comparatively little is known about luminal OA flux (Fig. 6). Transport into the lumen can be inhibited (59), implicating a mediated mode of efflux. Luminal secretion of the type I OA fluorescein has been shown to be saturable and described by Michaelis-Menten kinetics (397). There is, however, little agreement on the mechanism for efflux of PAH and other “classical” OAs, with evidence in some species (dog and rat) consistent with carrier-mediated anion exchange, and in other species (pig and rabbit), consistent with electrogenic facilitated diffusion (342). In human and bovine luminal membranes, the presence of demonstrable OA/α-KG exchange activity (357, 380) further complicates efforts to develop an integrated view of how peritubular and luminal events work in concert to produce transepithelial OA secretion. Recent evidence has implicated the multidrug resistance-associated transport (MDR2) in the active, ATP-dependent export of some OAs, including PAH (201). Also, the type I Na$^+$-phosphate cotransporter, NaPi-1 or NPT1 (45, 462), has been shown to support the electrogenic transport of selected organic anions, and a homolog of NPT1 (i.e., OATV1; Ref. 188) has been cloned that displays a number of the properties characteristic of a postulated electrogenic OA pathway. Immunocytochemical data have placed OATV1, as well as OAT4 and several members of the distinct “organic anion transporter family” of proteins (rOAT1, OAT-K1 and OAT-K2) in the luminal membrane of proximal cells, where they have been suggested to play roles in mediating OA transport. It is, however, not clear if their mode of activity will support net import or export of anionic substrates.

B. Molecular Characteristics of Renal OA Transporters

Renal OA transport involves the concerted activity of a suite of organic electrolyte transport processes belonging to at least five different families of transport protein. The “active step” in the classical OA secretory pathway,
i.e., peritubular uptake of anionic substrates, appears to involve at least two members of the OCT family of transporters: OAT1 and OAT3. Their activity is functionally linked to that of the several NaDC transporter homologs that are expressed in renal proximal tubules, and the reader is directed to several excellent reviews on the molecular physiology of the NaDCs (326–328). Two additional OCT family members have been identified (OATs 2 and 4), although their roles in renal OA transport remain speculative. The physiological basis of the luminal step in renal OA secretion, as noted above, is still unclear. Nevertheless, there are multiple candidates for transporters that could play a role in exporting selected OAs into the tubular filtrate. These processes include homologs 2 and 4 of the multidrug resistance associated protein, MRP2 and MRP4, as well as members of the OCT, OAT, and NPT families of membrane transporter.

1. OCT family of OA transporters

OAT1 was the first of the transport proteins cloned from the kidney to express the “physiological fingerprint” indicative of a role in the active secretion of OAs by renal proximal tubules, i.e., transport of PAH driven by an oppositely oriented gradient of α-KG. The primary sequence of this protein contains sufficient homology (~30%) to, and similarity of predicted secondary structure with, OCT1 to support its classification within the OCT family of the MFS (2.A.1.19). This is also true of the primary sequence of the several homologs of OAT1 (OAT2–4 and URAT1) subsequently cloned. All four OCT homologs share the MFS and ASF structural motifs described previously (Fig. 2).

a) OAT1. 1) Structure. OAT1 (SLC22A6; 2.A.1.19.4) was cloned from rat kidney and functionally identified as an organic anion transporter in 1997 by two independent groups (OAT1, Ref. 386; ROAT1, Ref. 427). It proved to be the rat ortholog of a protein originally named NKT, for which no function was suspected previously (Fig. 2). The 526-amino acid protein, and OAT1–4, have been cloned in both the human (9, 172) and pig (149). In the human, four splice variants were identified, OAT1–1, OAT1–2, OAT1–3, and OAT1–4 (9, 172). OAT1–1 and OAT1–2 show comparable rates of PAH transport (172). OAT1–1 is a 563-amino acid protein, and OAT1–2 has a 13-amino acid deletion within the putative intracellular COOH terminus following TMD 12 (W523-R535). These two variants arose as a consequence of the use of alternative 5′-splice sites in exon 9 (175). A 132-bp deletion (Q455-S498), which results in loss of TMD 11 and half of TMD 12, produces the splice variants OAT1–3 (which also lacks the 13 amino acids in the COOH terminus) and OAT1–4 (9), neither of which transports PAH (A. Bahn, personal communication). The 550-amino acid peptide hOAT1–2 was independently cloned by four groups and is commonly referred to as the human ortholog of OAT1 (172, 248, 346, 351). The hOAT1 gene is localized to chromosome 11 (11q13.1-g13.2) and spans 8.2 kb with 10 exons divided by 9 introns (9; see also Refs. 175, 346).

Conserved motifs within OAT1 include 12 cysteine residues, 25 proline residues, 4 N-linked glycosylation sites (N39, 56, 92, 97), 3 PKA consensus sites (Ser-195, Thr-456, and Ser-469), and 3 CKII consensus sites (Ser-325, Thr-515, and Ser-543).

II) Tissue and cellular distribution and localization. Northern blots indicated that the mRNA coding for OAT1 in human, rat, and mouse is almost exclusively expressed in the kidney, although long exposures typically reveal weak expression in the brain and skeletal muscle (69, 172, 246, 248, 346, 386). Quantitative measurements of mRNA in rat tissues using branched-DNA signal amplification confirmed the observation that rOAT1 expression is largely limited to the kidney (35). Expression in rat kidney increases shortly after birth, and this is correlated with a threefold increase in PAH accumulation in renal slices from adult rat kidneys, compared with uptake in slices from neonatal rat kidneys (305). Quantitative real-time RT-PCR revealed that OAT1 expression in human renal cortex is greater than five times that of either OAT2 or OAT4; expression of hOAT3 mRNA, however, is about three times that of hOAT1 (300). Immunocytochemical studies indicated that OAT1 expression in the kidney is restricted to the basolateral membrane of proximal tubule cells in the human (172, 300) and rat (457), and GFP-OAT1 fusion proteins are expressed in the basolateral membrane of cultured cells and native proximal tubule cells (422, 545). Initial efforts to resolve the distribution of OAT1 along the length of the rat proximal tubule concluded that expression was restricted to the basolateral membrane of cells in the S2 segment (457). Subsequent studies, however, revealed positive, albeit weaker, signals in the basolateral membrane within the S1 and S3 segments as well (222). This distribution of OAT1 protein is consistent with previous physiological data showing highest PAH secretion in the S2 segment of rabbit renal proximal tubules (390, 391, 528).

III) Functional characteristics. OAT1 has been functionally expressed in Xenopus oocytes (4, 40, 69, 172, 177, 181, 338, 346, 386, 427, 459, 491–493, 508, 523) and in HeLa...
(123, 177, 248), CHO (68, 138, 159), LLC-PK1 (545), Cos-7 (138, 235), mouse S2 (186, 209, 432, 434, 435), and mouse S3 cells (181, 437). OAT1-mediated uptake of PAH is saturable, with apparent Michaelis constants ranging from 5–20 μM for human OAT1, to 15–70 μM for rat OAT1, to 0.43 mM for C. elegans OAT1. The activity of OAT1 is influenced by α-KG, glutarate, and selected other divalent anions. cis-Concentrations of these compounds inhibit uptake of PAH and other anions (68, 69, 172, 235, 346, 386, 427, 491), whereas elevation of the trans-concentration of these compounds (for example, by preloading OAT1-expressing cells with glutarate) stimulates uptake of PAH and other OAs (4, 6, 69, 123, 248, 386, 427, 459, 493, 508). These functional characteristics of OAT1-mediated transport in heterologous expression systems are sufficiently similar to those observed for basolateral uptake of PAH and other OAs in native renal tubules to support the conclusion that OAT1 is a principal contributor to the classical renal OA secretory pathway.

The stoichiometry of PAH/α-KG exchange mediated by hOAT1 is 1:1, as determined by static-head experiments employing membrane vesicles isolated from hOAT1-expressing MDCK cells (6). The exchange of a monovalent substrate (e.g., PAH) for a divalent substrate (e.g., α-KG) suggests that the translocation process may be electrogenic. In fact, evidence on the electrogenicity of OA/DC exchange in renal basolateral membrane vesicles has been equivocal, with some studies exhibiting an effect of an imposed diffusion potential on the rate of exchange (252), whereas others failed to obtain such effects (340). PAH uptake into oocytes that are expressing rOAT1 is not influenced by increases in extracellular K⁺, suggesting that the rate of exchange is not sensitive to membrane potential (427). However, uptake of PAH (and other selected monovalent OAs) into oocytes expressing rOAT1 (6) or flOAT1 (37, 40) results in an inward current. Extracellular concentrations of glutarate inhibit these currents, and the application of glutarate by itself causes no currents, consistent with the electroneutral exchange of divalent anions (40). The PAH-induced current and the current calculated from the flOAT1-mediated uptake of [³H]PAH are of similar magnitude. These observations support the contention that OAT1 typically supports uptake of monovalent OAs through the mediated exchange for intracellular divalent OAs (physiologically, α-KG) and that this process is energetically supported by the inside-negative membrane potential that drives the net efflux of negative charge.

IV) Regulation of OAT1. The regulatory influence of PKC activation has been examined for several OAT1 orthologs. In all cases examined [hOAT1 expressed in HeLa cells (248); rOAT1 expressed in oocytes (491); mOAT1 expressed in LLC-PK₁ cells (545)], activation of PKC through exposure to phorbol ester resulted in a time- and dose-dependent inhibition (~30–70%) of PAH transport. This inhibition was reduced or eliminated by exposure to the PKC inhibitor staurosporine. Exposure of mOAT1-expressing LLC-PK₁ cells to the phosphatase inhibitor okadaic acid also resulted in a downregulation of PAH uptake and was associated with an increase in phosphorylation of OAT1 (545). Interestingly, however, the inhibition of mOAT1 activity resulting from activation of PKC was not correlated with an increase in phosphorylation of OAT1 protein, but from a decrease in the J_{\text{max}} for PAH transport (with no effect on K_{\text{m}}) (545). These observations led to the suggestion that activation of PKC may increase internalization of membrane transporters, or inhibit recruitment of preformed transporters from a cytoplasmic pool (545). In fact, the decrease in hOAT1-mediated PAH transport that follows activation of PKC (in Xenopus oocytes) is correlated with an internalization of the transport protein (524). Interestingly, although consensus motifs for phosphorylation mediated by PKC are conserved in OAT transporters (41, 426), elimination of these sites in hOAT1 had no effect on the decrease in transport activity that followed activation of PKC (524). Consensus motifs for phosphorylation mediated by PKA and CKII are also conserved in OAT transporters (41, 426), but whether any of these sites are involved in the regulation of OAT1 is not known.

Sex steroids also influence the level of expression of OAT1 mRNA. Although Northern blot analysis indicates no apparent difference in the level of OAT1 mRNA in male and female (Wistar) rat kidneys (214, 230), quantitative analysis of OAT1 mRNA levels using branched-DNA signal amplification reveals a trend toward higher OAT1 levels in the kidneys of male than of female (Sprague-Dawley) rats (35). This is consistent with Western blot analyses that found ~50% more OAT1 protein in basolateral membranes from kidneys of males than of females (Sprague-Dawley) rats (48). Treating female rats with testosterone results in a significant increase in the OAT1 mRNA in their kidneys, to a level equal to that in male rat kidneys (230).

Transcriptional regulation of OAT1 (or other OATs) has received little attention to date. However, examination of phylogenetic footprints (evolutionarily conserved regions within noncoding sequences) of related OATs has revealed a number of conserved transcription factor binding sites that are suggested to be potential sites of regulation (100). These include Pax1, Pbx, Wt1, and Hnf1. Additionally, Oat1 and Oat3, and other members of the OCT transporter family, typically occur within the human and mouse genomes as tightly linked pairs of closely related paralogs (e.g., OAT1 and OAT3; OAT4 and URAT1; OCT1 and OCT2; OCTN1 and OCTN2). This pairing correlates well with the observation that pair members generally have similar profiles of cellular and tissue distribution.

V) Substrate structural specificity. OAT1 interacts with a diverse structural array of OAs, supporting the
frequent use of the descriptor “polyspecific” or “multispecific” for this process. For the most part, evidence for “interaction” has been limited to demonstration that a test agent inhibits OAT1-mediated transport. With the use of this criterion, OAT1 has been shown to interact with variety of endogenous compounds, including cGMP (386), prostaglandin E2 (386), urate (172, 175, 386, 427), and α-KG (172, 235, 248, 386, 427, 491). Inhibitory criteria have been used to implicate OAT1 in the transport of an extremely diverse array of drugs, toxins, and other xenobiotic compounds. In many cases, OAT1 has actually been shown to mediate transport of these compounds. These studies include the intriguing observation that OAT1 can support the transport of the nonionized form of the weak protonated substrate L-glutamate (98). Also, furosemide, although a potent inhibitor of PAH transport mediated by the flounder ortholog of OAT1, does not evoke an inward current, suggesting that it is transported slowly, if at all, by flOAT1 (40). Indeed, differences in OAT1 substrate specificity observed between species, combined with the presence of multiple OAT transporter isoforms (as discussed in an upcoming section), have led to suggestions that the renal secretion of a diverse array of anionic compounds may be the result of several different transporters operating in parallel. These processes, including OAT1, may individually have narrower selectivity than implied in studies of inhibition of PAH transport in intact renal preparations (248, 465).

Site-directed mutagenesis of the flounder OAT1 ortholog identified two cationic residues that may play a role in the binding of anionic substrates to OAT1. Wolff et al. (523) reasoned that the homology that exists between members of the OCT transporter family would be reflected in conservation of sites involved in the basic elements of substrate recognition. If the binding of cationic substrates to OCTs involves interaction with one or more anionic residues (as suggested by the study of Gorboulev et al., Ref. 130), then OATs might be expected to display conservation of cationic residues at homologous sites. Three sites fulfilling these criteria were identified: H34, K394, and R478. These were exchanged individually for the corresponding amino acids present in OCTs. Each of these mutations retained PAH transport, albeit at rates reduced compared with the wild type. However, mutations K394A and R478D resulted in complete insensitivity of PAH transport to the presence of glutarate and loss of the PAH/glutarate exchange. Interestingly, R478 in fOAT1 is homologous to the D475 site in OCT1–3 that appears to play a role in the binding of cationic substrates to these transporters (130). It is tempting to speculate that comparatively modest changes in the primary sequence of an archetypal member of the OCT family resulted in the dramatic differences in specificity (e.g., for anions versus cations) and underlying energetic mechanisms found today across this large family of transport proteins.

b) OAT2–4/URAT1. These homologs are 535 to 565 amino acids in length and have 6–12 predicted TMDs (depending on the algorithms upon which the prediction is based). Given the strong homology among the OCT transporters, it is generally assumed that, like OAT1, OATs 2–4 and URAT1 probably share the 12 TMD structure associated with most members of the MFS transporter families. In addition, OATs 2 through 4 and URAT1 share the sequence motifs common to MFS and ASF transporters, as discussed previously.

i) OAT2 (SLC22A7). This was cloned initially from a rat liver library and named NLT (novel liver transporter; Refs. 385, 399). Orthologs from the human (98) and mouse (215) have subsequently been cloned. There is a marked gender-based difference in the level and tissue distribution of OAT2 expression. The mRNA for OAT2 is predominantly expressed in the liver of male rats, with very modest expression in the kidney (35, 214). In contrast, the level of OAT2 mRNA in female rat kidneys is substantially larger than that in female liver (the latter value being comparable to that in male rat liver) (35, 214, 230). Expression of OAT2 mRNA is significantly increased in kidneys from castrated male rats, and treating castrated rats with testosterone reverses this increase (230). The sex/tissue profile is very different in the mouse. OAT2 expression in the male mouse is virtually restricted to the kidney, whereas in the female, there is substantial expression of OAT2 mRNA in both kidney and liver (215).

Just as there are marked species differences in the organ distribution of OAT2, there are also marked species differences in the subcellular localization of OAT2 within the kidney. In the rat kidney, OAT2 appears to be localized to the apical membrane of medullary thick ascending limb and the cortical and medullary collecting ducts (222). However, in the human kidney, OAT2 appears to be restricted to the basolateral membrane of the proximal tubule (98).

OAT2 has been functionally expressed in Xenopus oocytes (215, 385), LLC-PK1 cells (298), and mouse S2 cells (98, 99, 203, 209). OAT2 transports a variety of substrates, including PAH, methotrexate, ochratoxin A, PGE₂, PGF₂α, salicylate, α-KG, and glutarate (98, 215, 298, 385). The affinity for PAH, however, is very low (compared to OAT1 and, as we shall see, OAT3), with an IC₅₀ for inhibition of salicylate transport of >1 mM (298). The affinity of OAT2 for PGE₂, on the other hand, is comparatively high, with Kₑ values for transport being 5 nM, 713 nM, and 39 μM for mOAT2 (215), hOAT2 (209), and rOAT2 (298), respectively. OAT2 shows little or no
interaction with tetraethylammonium, cholate, taurocholate, and estrone sulfate (385). Significantly, efforts to show trans-stimulation of OAT2 activity with gradients of glutarate have failed to show an effect, leading to speculation that, unlike OAT1 (and OAT3), OAT2 does not operate as an OA exchanger (385). Consequently, the energetic basis of OAT2-mediated OA transport is not clear. However, if OAT2 were to operate as an electrogenic uniporter, its expression in the basolateral membrane of renal proximal tubules (in the human, at least; Ref. 98) makes it unlikely that OAT2 plays a major role in the secretory flux of OAs. Expression of OAT2 in the apical membrane of selected regions of the nephron, as appears to be the case in rat kidneys (222), would, in contrast, permit an electrogenic mode of operation to support a role in secretion of anionic substrates.

II) OAT3 (SLC22A8; 2.A.1.19.7). The cDNA for OAT3, cloned from rat kidney, encodes for a protein 536 amino acids long (232). It has subsequently been cloned from mouse (424), rabbit (X. Zhang and A. Bahn, unpublished observations), and human (52) kidney. Two OAT3 orthologs have been cloned from human kidney (52, 346). The second of these, hOAT3, codes for a 542-amino acid protein that supports transport of a wide range of OAs, including PAH, estrone sulfate, and methotrexate (52). In contrast, the first reported clone of human OAT3 (346; referred to here as hOAT3) encodes for a 568-amino acid protein. This longer cDNA, however, does not appear to support transport. The difference between these hOAT3 clones lies in four specific regions of the sequence, including the presence of 25 extra amino acids inserted in the sequence in the near portion of the COOH terminus (52). These sequence differences may alter the conformation of the protein in a manner that precludes transport function. It remains to be determined if hOAT3 and hOAT3\* represent examples of alternative splicing of the same gene or are encoded by different genes.

Northern-blot analysis indicated that rOAT3 is most heavily expressed in kidney, liver, and brain (214, 232). Quantitative analysis of rOAT3 mRNA expression using branched-DNA signal amplification showed the highest levels of rOAT3 expression in rat kidney, with no difference in renal expression between male and female rats (35). The distribution of OAT3 in humans differs from that in rats, with Northern blots showing expression to be virtually restricted to the kidney (52, 346). Interestingly, quantitative analysis of the mRNA levels of OATs 1, 2, 3, and 4 in the human kidney revealed OAT3 expression to be the highest, about 3-fold that of OAT1 and >10-fold higher than either OAT2 or OAT4 (300). Within human and rat kidney, OAT3 is expressed in the basolateral membrane of S1, S2, and S3 segments of the proximal tubule (52, 222, 300). In the human kidney, expression levels in the proximal tubule appear to run S1 > S2 = S3 (52). In the rat kidney, OAT3 is also expressed at the basolateral aspect of the medullary and cortical thick ascending limbs, the connecting tubules, and the cortical and medullary collecting duct (222).

OAT3 has been functionally characterized in Xenopus oocytes (52, 86, 112, 232), mouse S2 cells (187, 209, 436), and LLC-PK1 cells (416). Transported substrates include estrone sulfate, indoxyl sulfate, PAH, ochratoxin A, PGE2, benzylpenicillin, cephaloridine, E17β-D, and glutarate (86, 112, 187, 209, 232, 416). Although it is evident that the selectivity of OAT3 overlaps that of OAT1 (and OAT2), affinities for several substrates appear to permit discrimination between OAT3 and these other transporters. Estrone sulfate (ES) has been frequently used as a test substrate in studies of OAT3 activity. OAT3 displays a moderately high affinity for ES (K_i of 3–35 μM; Refs. 52, 112, 232, 436), whereas OAT1 interacts little with ES (424). Salicylate is transported by mouse and rat OAT2 (215, 298, 385), but not by rat and human OAT3 (52, 232).

Other candidate substrates that have the potential for permitting discrimination between homologous anion transporters include citrinin (186) and taurocholate (424).

Interestingly, OAT3 transports the weak base cimetidine (pK_a 6.9) (52, 112). Indeed, the affinity of human and rat OAT3 for cimetidine (K_i of 40–60 μM; Refs. 52, 112) is comparable to that for PAH (60–90 μM; Refs. 52, 232). Whereas the hydrophilic quaternary ammonium compound TEA has little effect on rOAT3 activity (52, 112), the hydrophobic cation quinidine has generally been found to exert a more marked inhibitory effect on OAT3 activity (52, 112, 232).

Giacomini and colleagues (112) examined the influence of two cationic amino acid residues within the primary structure of rOAT3 on the interaction of substrates with this transporter. Their results supported the following conclusions. First, substrate recognition by the organic anion and organic cation transporters of the OCT family of proteins resides in the COOH-terminal half of the transporters. Second, multiple domains within the COOH-terminal half of the protein differentially contribute to the interaction of rOAT3 with hydrophilic versus hydrophobic organic anions. Third, two conserved amino acids within this region, arginine-454 and lysine-370, play important roles in substrate-transporter interaction. Fourth, changing these amino acids (R454D and K370A) shifts the specificity of the transporter from preferential transport of the organic anion PAH to the organic cation MPP.

In a separate study, Giacomini and colleagues (113) identified several aromatic amino acid residues in TMD 7 of rOAT3 that exerted a specific effect on the affinity of the transporter for the hydrophilic substrates PAH and cimetidine, but had little effect on interaction with the hydrophobic substrate ES. They concluded that rOAT3 does not have a single binding site but, rather, has multiple binding domains that can participate in binding and
translocation of different substrates depending on their structural and physical properties. Interestingly, the several residues that correlated with the ability of rOAT3 to interact with both PAH and cimetidine are all conserved in the rabbit ortholog of OAT3 (which transports cimetidine and ES but does not interact well with PAH; Zhang and Bahn, unpublished observations), indicating that these residues alone are not sufficient to account for selectivity of the OAT3 binding site(s). Nevertheless, the results of these studies suggest how substrate/charge selectivity of the homologous OCT transporter family may have evolved by mutations of a very few amino acids.

The mechanism of OAT3-mediated OA transport has been the source of some confusion. Because inwardly directed gradients of unlabeled PAH and ES did not stimulate efflux of labeled ES from rOAT3-expressing oocytes in initial studies, the investigators concluded that, in contrast to OAT1, OAT3 does not operate as an anion exchanger (52, 232). However, its location in the basolateral membrane of proximal tubule cells and its overlapping specificity with OAT1 substrates (e.g., PAH) that are actively secreted by the proximal tubule make an electrogenic mode of action unlikely. Additionally, renal slices from kidneys of OAT3-knockout mice show a >60% reduction in uptake of PAH, as well as profound decreases in uptake of ES and taurocholate (424). Thus it was not surprising that a more focused examination of the mechanisms of OAT3 activity revealed that it can operate as an OA/dicarboxylate exchanger. When rOAT3-expressing oocytes were preloaded with glutarate, the rate of ES and PAH uptake was markedly stimulated (421). Moreover, oocytes that coexpressed rOAT3 and rNaDC1 showed enhanced ES uptake in the presence of glutarate, and this effect was blocked by the addition of Li+ or methylsuccinate (421), known inhibitors of NaDC-1 activity. The human and rabbit orthologs of OAT3 also support exchange of glutarate for ES (and urate as well; Ref. 10 and Zhang and Bahn, unpublished observations). Collectively, these observations implicate OAT3 as a principal contributor, along with OAT1, in the classical renal OA secretory process.

The parallels between OAT3 and OAT1 include the regulatory response to PKC activation. As seen with OAT1, treatment of OAT3-expressing oocytes with phorbol ester resulted in a time- and dose-dependent downregulation of transport activity (436). rOAT3-mediated ES transport was decreased by ~40% by pretreating oocytes for 10 min with 10−7 M phorbol 12-myristate 13-acetate (PMA), and this effect was blocked by coexpression of oocytes to PMA plus the PKC inhibitor chelerythrine chloride. The kinetic basis of this downregulation of rOAT3 activity was a decrease in \( V_{\text{max}} \) with no change in \( K_v \), just as in the response of mOAT1 to exposure to PMA (436).

III) OAT4 (SLC22A9; 2.A.1.19.10). OAT4 was cloned from a human renal cDNA library (53). Northern-blot analysis indicated that OAT4 is most heavily expressed in the kidney but is also expressed in the placenta (53). Within the human kidney, however, OAT4 mRNA expression is only 5–10% of OAT1 and OAT3 mRNA expression and is comparable to OAT2 expression (300). In contrast to OATs 1, 2 and 3, which are typically restricted to the basolateral aspect of the proximal tubule, OAT4 protein appears to be localized to the luminal membrane of human proximal tubule cells (7). Activity of hOAT4 has been studied in Xenopus oocytes (53) and in mouse S2 cells (7, 98, 209). Human OAT4 displays a wide range of apparent affinities for the substrates transported. For example, the \( K_v \) values for ES, dehydroepiandrosterone, PGE2, and PGF2\(_a\) are all \( \approx 1 \) \( \mu \)M (53, 209); the \( K_v \) for ochratoxin A is ~20 \( \mu \)M (7); and for PAH, although it is transported, the \( K_v \) is >1 mM (53). Potent inhibitors of OAT4-mediated ES transport include sulfobromophthalain and probenecid, whereas taurocholate (a substrate for mOAT3), glutarate (a trans-exchanging substrate for OAT1 and OCT3), and the cationic TEA have little or no interaction with hOAT4 (53).

The energetic basis of OAT4-mediated transport is not clear. Mouse S2 cells that express hOAT4 can accumulate ochratoxin A (OTA) to levels well above that in the surrounding solution, although, in the absence of information on intracellular binding of OTA, it is not clear if this represents net flux against an electrochemical gradient (7). Transport mediated by hOAT4 is Na+ independent, and trans-concentrations of unlabeled ES do not stimulate efflux of radiolabeled ES from hOAT4-expressing oocytes (53). The presence of OAT4 on the luminal membrane lends support to a role for this process in transepithelial secretion of OAs, or luminal reabsorption of selected substrates, or both. If OAT4 is an electrogenic process, as existing evidence implies, then a potential role in supporting luminal efflux of anionic substrates is evident. However, the rather narrow selectivity of OAT4, including its poor interaction with PAH, makes it unlikely that OAT4 is the principal transporter associated with the classical renal secretory process. However, the fact that OAT4 can transport substrates like OTA, suggests that it can serve as an avenue of luminal entry of selected anionic toxins, thus making OAT4 potentially important in influencing the nephrotoxicity of selected compounds (7).

IV) URAT1 (SLC22A12; 2.A.1.19.11). URAT1 was cloned from human kidney and is 52% identical with hOAT4 (97). In the human, URAT1 mRNA appears to be restricted to the kidney, and immunocytochemistry indicates that expression of URAT1 is limited to the apical membrane of proximal tubules cells. When expressed in Xenopus oocytes, hURAT1 supports urate transport, and it has been suggested that URAT1 represents the principal pathway in the human kidney for urate reabsorption (97). URAT1-mediated urate transport is neither electrogenic nor dependent on Na+. Instead, URAT1 supports an elec-
troneutral urate transport that is trans-stimulated by gradients of Cl\(^{-}\) (and other halides, including Br\(^{-}\) and I\(^{-}\)) and by gradients of lactate, nicotinate, and by the uricosuric drug pyrazinocarboxylate. Three different mutations of URAT1 found in patients with idiopathic renal hypouricemia were shown to express proteins that did not support urate transport, consistent with a central role of URAT1 in renal urate reabsorption (97).

RST (renal-specific transporter; Ref. 297) was isolated from mouse kidney. Although no function has been reported for this protein, it was originally suspected to be an organic cation transporter, based on its 30% homology with rOCT1 (297). However, its sequence similarity with hURAT1 (74% identical; see also Fig. 2) suggests it may be the mouse ortholog of URAT1.

2. OATP family of OA transporters

The family of transport proteins usually referred to as organic anion transporting polypeptides (OATPs) is classified as 2.A.60 (the organic anion transporter (OAT) family) within the Transport Commission Protein Database (361), and also as the solute carrier gene family Slc21a/SLC21A. Members of the OATP family generally share a common postulated secondary structure that includes 12 TMDs and several long extracellular and intracellular loops (147), although there is no primary sequence homology with members of the OCT family. The research on this group of transport proteins has focused on their well-documented role in mediating the Na\(^{+}\)-independent transport of bile salts in the liver, and the reader is directed to recent reviews that deal extensively with this subject (147, 148, 272, 456, 501). Several members of the OATP family are, however, expressed in the kidney and have been suggested to play a role in the secretion/reabsorption of selected anionic substrates.

a) OATP1 (Slc21a1; 2.A.60.1.1). Originally cloned from rat liver (180), OATP1 consists of 670 amino acids with a calculated molecular mass of 74 kDa. Expressed primarily in the liver and kidney, it is also expressed in brain, lung, skeletal muscle, and proximal colon (180, 244). In rat liver, expression is restricted to the basolateral (sinusoidal) membrane of hepatocytes (18). However, in rat kidney, OATP1 expression is restricted to the apical membrane of cells in the S3 segment of the proximal tubule (18). The differences in subcellular localization of rOATP1 in liver and renal cells may reflect differences in the biosynthetic processing of this protein in these two tissues (18). When SDS-PAGE gels were run under standard reducing conditions, immunoblots of liver cell membranes using a polyclonal rOATP1 antibody revealed a single 80-kDa band. Membranes from rat kidney run under the same conditions revealed two bands, 33 and 37 kDa. Immunoblots of renal membranes separated under nonreducing conditions (to maintain disulfide bonds) revealed a single 80-kDa rOATP1 band (18).

The regulatory response of rOATP1 to sex steroids also differs markedly in liver and renal tissues. Whereas there is no difference in expression of OATP1 mRNA in male and female rat liver, expression in male kidneys is ~20 times greater than that in female kidneys (244). Administering testosterone to female rats increases, and administering estradiol to male rats decreases, renal OATP1 mRNA expression (247). These treatments have no effect on OATP1 expression in rat liver (247).

The range of rOATP1 substrates is extremely broad and includes anionic, cationic, and unchanged substrates (94, 180, 193, 271, 500). Of relevance to the issues raised here, OATP1 substrates include taurocholate, ES, and OTA (193). Of equal relevance is the observation that PAH, α-KG, and fluorescein have little or no interaction with rOATP1 (296). rOATP1 supports mediated exchange of anionic substrates for HCO\(_3\)\(^{-}\) (367) and glutathione (GSH) (243). Whereas the prevailing electrochemical gradient for HCO\(_3\)\(^{-}\) across hepatic cell membranes would not typically support net uptake of anionic substrates from the blood, the exchange of extracellular substrates for intracellular GSH is consistent with the postulated role of rOATP1 in the liver, i.e., uptake of a broad array of endogenous and exogenous anions that are then secreted (via separate mechanisms) across the canalicular membrane into the bile (243). Proximal tubule cells contain a relatively large (5 mM) concentration of GSH (236) that could serve to support uphill accumulation of anions, consistent with a role for rOATP1 in reabsorption of selected substrates from the tubular filtrate. It has also been suggested that rOATP1 could mediate the electrogenic secretion of E17β-D, a physiological metabolite of estradiol (194), and there is evidence that rOATP1 can support an electrogenic mode of activity (288).

The several homologs of rOATP1 identified in human kidney are not orthologous to those identified in the rat (442). On the basis of criteria drawn from differences in sequence homology and substrate specificity, it has been concluded that the human orthologs for rOATP1 and several other rat OATPs have not been identified (293). The current list of human homologs includes two, OATP-D and OATP-E, which are expressed in the kidney (442). Both proteins support transport of ES and PGE\(_2\) (442). However, their mechanism of transport and subcellular distribution within the kidney are not known.

b) OAT-K1/OAT-K2. In addition to rOATP1, three other members of the organic anion transporting polypeptide family, i.e., Oatp5, OAT-K1, and OAT-K2, are highly expressed in rat kidney. Expression in mouse of OATP5 (Slc21a13) appears to be effectively restricted to the kidney (67), but it has not been functionally characterized. OAT-K1 (Slc21a4; 2.A.60.1.4) was cloned from rat kidney and is 72% identical to rOATP1 (363). OAT-K2
OAT-K1 that eliminates the LLC-PK1 cells indicated that the transporter was restricted to the apical pole of stably transfected MDCK cells (262, 264), the initial functional characterization of rOAT-K1 in the apical pole of stably transfected MDCK cells (262, 264), whereas expression of both transporters is restricted to proximal tubule cells (262, 263). It is of interest that, and OAT-K2 expression is limited to the apical pole of the proximal convoluted tubule (262). Western blots of the straight portion of proximal tubule in rat kidneys (262, 264, 438) showed that the mRNA for both transporters is expressed in TMDs. The mRNA for both transporters is expressed in rat kidney (262). It is noteworthy that in the initial characterization of rOAT-K1 (363), where functional expression of transport activity in stably transfected LLC-PK1 cells was restricted to the basolateral membrane (see above), MTX transport was supported but taurocholate transport was not. Similarly, rOAT-K1 expressed in Xenopus oocytes transports MTX and folate, but not taurocholate (264). However, when expressed in MDCK cells, where biosynthetic processing results in apical localization of the transporter (263) (see above), rOAT-K1 supports the transport of taurocholate and a wide variety of other anionic substrates as well (438). It was suggested that the membrane sorting mechanism and the proteolytic processing and/or excision of rOAT-K1 found in renal tubular cells are retained in MDCK cells, but not in LLC-PK1 cells and Xenopus oocytes (438). These observations underscore the caveats we introduced previously concerning the care that must be exercised when interpreting quantitative or qualitative aspects of cloned transporter function as determined in heterologous expression systems.

3. NPT family of Pi/OA transporters

NPT1 (SLC17A1; 2.A.1.14.6), or NaPi-I, is in one of the three current families of Na+-phosphate cotransporters. It is most heavily expressed in the luminal membrane of renal proximal tubules and is also expressed in liver and brain (301, 302). Originally cloned from rabbit kidney (519), and subsequently cloned from rat, mouse, and human tissues (301), NaPi-I is a 465-amino acid protein with 6–8 TMDs. Although it supports Na+-PO42- cotransport when expressed in Xenopus oocytes (519), it is not believed to play a major role in renal phosphate handling (518). In addition to supporting Na+-PO42- cotransport, however, rbNPT1 also supports an anionic conductance and the mediated uptake of several organic anions, including penicillin G and probenecid when expressed in oocytes (45). Interestingly, the human ortholog of NPT1 (but not rbNPT1) accepts PAH as a transported substrate (45, 462).

OATV1 (voltage-driven organic anion transporter 1; 2.A.1.14.15) is a protein cloned from pig renal cortex that shares 60–65% sequence identity with human, rat, rabbit, and mouse NPT1 (188). OATV1 supports voltage-dependent transport of PAH. Although the Kt for PAH uptake into oocytes that express pOATV1 is comparatively high (4.4 mM), it interacts with a structurally diverse array of anionic compounds (including probenecid, furosemide, ES, and penicillin G). Immunocytochemical localization placed OATV1 in the apical membrane of pig proximal tubules. The physiological role of OATV1 (and NPT1) is not clear, but its characteristics indicate that it could be an element in the conductive transport of organic anions observed in the luminal membrane of some species (301, 462).

4. MRP family of OA transporters

The multidrug resistance-associated proteins, or MRPs, are members of the ABCC subfamily of the ABC transporter superfamily (conjugate transporter-2 or CT2; 3.A.1.208). Nine MRPs have been identified, of which six have been functionally characterized. The MRPs are large proteins (1,300–1,500 amino acids) compared, for exam-
ple, with the OCTs and OATs (~550 amino acids). MRPs 1, 2, 3, 6, and 7 have a predicted secondary structure that contains 17 TMDs. MRPs 4 and 5 are smaller owing to the absence of TMDs 1–5. The MRPs that have been functionally characterized share the ability to support primary active (ATP-dependent) export of a broad array of anionic substrates from cells. The interested reader is directed to recent reviews that discuss in detail the molecular and functional aspects of this group of transporters (25, 26, 166, 224, 241). We will limit our discussion to observations that pertain to the potential role of MRPs in the renal handling of organic anions.

a) MRP1 (ABCC1; 3.A.1.208.1). First cloned from multidrug-resistant human lung cancer cells in 1992 (71), MRP1 (ABCC1) is broadly expressed in many cell types (66, 225), with the highest expression generally found in the basolateral membrane of epithelial cells (241). When expressed in the renal cell line LLC-PK1, hMRP1 is localized to the basolateral membrane (241). When expressed in the renal cell line LLC-PK1, hMRP1 is localized to the basolateral membrane (110). Studies of the distribution of MRP1 in mouse kidney (i.e., mMRP1) (522) showed greatest expression in the loop of Henle and collecting duct. Interestingly, there was no evidence of mMRP1 expression in proximal convoluted tubules.

hMRP1, like MRPs 2, 3, and 4, supports the ATP-dependent transport of a diverse array of amphiphilic type II organic anions. In particular, hMRP1 generally displays a high affinity (K<sub>i</sub> generally < 1 μM) for glutathione, glucuronide, and sulfate conjugates, including those of many toxic xenobiotics (241). Nonconjugated hMRP1 substrates include selected nonconjugated type I OAs, such as fluorescein (418) and PAH (201), glutathione disulfide (GSSG; Ref. 240), and vinca alkaloids (178). Transport of nonconjugated substrates is typically sensitive to the intracellular presence of physiological concentrations of GSH, leading to suggestions of modes of activity that include cotransport of substrates with GSH and/or an allosteric GSH activator site on the transport protein (241). It has been suggested that hMRP1 has a multipartite binding pocket that permits interaction with and transport of conjugated substrates (in particular, GSH conjugates) and interaction with and transport of selected nonconjugated substrates when the appropriate binding pocket is occupied by GSH (241). The issue of whether hMRP1 supports the transport of un conjugated GSH, in the absence of other substrates, is less clear. Evidence supporting this contention includes the observations that hMRP1-expressing cells show enhanced release of GSH to the surrounding medium (25, 241) and that inside-out membrane vesicles isolated from hMRP1 expressing cells accumulate GSH, albeit at low rates and with low affinity, in an ATP-dependent fashion (345). Although the apparent affinity for GSH is quite low, the relatively high concentration of GSH in renal cells suggests that hMRP1 may play a role in maintaining the normal redox status of the renal tubular cells in which it is expressed, in addition to protecting these cells from the toxic effects of endogenous and exogenous compounds (241).

b) MRP2 (ABCC2; 3.A.1.208.2). Long before MRP2 was cloned, there was physiological evidence for the presence of an ATP-dependent transporter in the canilicular (apical) membrane of hepatocytes (323). A protein capable of supporting this process, originally cloned and characterized as the canilicular multispecific organic anion transporter (cMOAT; Ref. 449), is now referred to as MRP2 (ABCC2). Orthologs of MRP2 have been cloned from a number of mammals, including the human (449), rat (33), mouse (116), and rabbit (495, 499). Of particular relevance to the present report, MRP2 has been shown to be expressed in the apical membrane of both human (373) and rat (374) renal proximal tubule cells [in addition to the apical membrane of liver (33) and intestinal (494) cells]. In addition, hMRP2 is localized to the apical pole of MDCK cells where it supports active secretion of a wide array of type II OAs (73, 74, 109, 365).

Like MRP1, MRP2 supports the ATP-dependent export of a structurally diverse array of GSH conjugates (202). There is also strong evidence supporting the contention that MRP2 mediates the ATP-dependent cotransport of GSH with a number of nonconjugated substrates (see Refs. 11, 46, 108, 239, 308, 497). Interestingly, MRP2 has been shown to support ATP-dependent transport of the type I OA PAH (human, Ref. 239; rabbit, Ref. 497). MRP2’s location on the apical membrane and its secretory mode of action raises the question as to what fraction of the luminal efflux of PAH (and other type I OAs) could be supported by MRP2. We think it unlikely, given current evidence, that MRP2 represents a major avenue for type I OA secretion. This issue is discussed in more detail in section III C.

c) MRP3 (ABCC3; 3.A.1.208.9). Although MRP3 is expressed in the kidney, its principal site of expression in the rat (66) and human (225) is the intestine. In the human kidney, MRP3 is expressed in the basolateral membranes of distal convoluted tubule cells, but not in proximal convoluted tubule cells (376). Whereas MRP1 and MRP2 display a broad selectivity for anionic conjugates, MRP3 shows a marked preference for glucuronate conjugates as substrates, including E17β-D (158). The physiological function of MRP3 remains to be established.

d) MRP4 (ABCC4; 3.A.1.208.7). MRP4 and MRP5 are the “short” MRPs, lacking TMDs 1–5. Interestingly, MRP4 (ABCC4) is expressed in the apical membrane of human renal proximal tubule cells (498), which immediately implicates it in the secretion of selected OAs. Although MRP4 transports a broad range of anionic conjugates, including GSH, glucuronate, and phosphate conjugates (64, 498), the quantitative aspects of MRP4 selectivity differ rather markedly from those of MRP1, MRP2, and MRP3 (241). hMRP4 transports methotrexate and has been suggested to play a role in mediating efflux of folate.
derivatives (65). hMRP4 also supports export of a variety of cyclic nucleotides (64, 498), and it has been suggested that it may play a role in the release of cAMP and cGMP into the urine, thereby supporting their role as paracrine modulators of renal fluid homeostasis (498).

e) MRP5 (ABCC5). MRP5 supports the transport of a wide array of anionic conjugates, including cAMP and cGMP (182). The distribution of MRP5 in the kidney is, however, not yet established.

f) MRP6 (ABCC6; 3.A.1.208.10). This member of the family is most heavily expressed in liver and kidney (226). Within human kidney, MRP6 is localized to the basolateral membrane of proximal tubule cells (375). Transport function has been difficult to establish for MRP6, although at least one compound, the endothelin receptor antagonist BQ-123, is clearly transported in an ATP-dependent fashion by MRP6 (and MRP2) (251). Although the physiological function of MRP6 is not clear, it has been suggested that it may play a housekeeping role (251).

C. Physiological Integration of Renal OA Transporters

Here we discuss the results of studies that have examined the renal transport of organic anions by “native” systems, including renal tubules in vivo, isolated tubules and cells, isolated renal membrane preparations, and cultured cell systems. As with the previous discussion of renal OC transport, we have organized this section into categories that focus on particular areas of general emphasis. Areas that received particular attention in recent years include I) function of renal OA transporters and II) regulation of renal OA transport. The first category can be subdivided further into studies primarily focused on issues that pertain to the mechanism(s) of renal OA transport, the specificity of renal OA transporters, the physiological organization of renal OA transporters, or selected aspects of transport of a particular compound of major physiological, pharmacological, or toxicological interest. The second category includes efforts to assess the extent to which activation of signal transduction pathways results in modulation of renal OA transport. We will close with a presentation of the evidence documenting the carrier-mediated sequestration of OAs in renal proximal tubule cells and the possible physiological role(s) intracellular sequestration can play in the renal handling of OAs. When possible, we will attempt to correlate observations made with intact or native systems with evidence obtained using cloned transporters in an initial effort to establish the molecular basis of the integrated processes of renal OA transport. Again, the emphasis here is on studies conducted since 1993; reference to earlier studies is largely restricted to those that form a necessary backdrop to more recent work. The reader is directed to previous reviews that effectively summarize the earlier literature (76, 292, 339, 342, 352, 355).

1. Mechanisms, selectivity, and functional organization of renal OA transport

Physiological characteristics of OA transport in renal proximal tubules have been studied with intact animals, intact tubules, and isolated membrane vesicles from a variety of species, including representative mammalian (e.g., rat, mouse, rabbit), reptilian (snake), and teleost (flounder, killifish) species. The physiological profile of OA transport observed in these studies suggests that the mechanism of peritubular (basolateral) OA secretion is phenotypically conserved over many phyla and involves (indeed, may be dominated by) carrier-mediated exchange of intracellular α-KG for an extracellular substrate anion. The secretory flux of type I OAs across the luminal (apical, brush border) membrane appears to involve a collection of processes that are more mechanistically diverse and that show more species diversity than transport into the cells across the peritubular membrane. The secretion of the bulkier type II OAs presumably involves diffusion across the basolateral membrane, followed by MRP2- or MRP4-mediated export at the luminal membrane.

A) BASOLATERAL/PERITUBULAR TRANSPORT. I) Mechanisms. Following the demonstration in 1987 (339, 389) that PAH transport in isolated renal BLMV can be driven by oppositely oriented gradients of selected dicarboxylates, considerable attention was directed toward defining the physiological characteristics of “OA/DC exchange.” Of particular importance were the initial studies showing that manipulation of intracellular dicarboxylate concentrations resulted in changes in basolateral (peritubular) PAH transport, i.e., demonstration that conditions capable of driving OA transport in isolated membrane vesicles are also operative in intact native renal cells, as well. Pritchard (341) showed that the rate of PAH uptake into rat renal slices is directly correlated with the intracellular concentration of α-KG when the latter is manipulated by preincubating slices in increasing concentrations of α-KG. In a separate study, elevation of intracellular α-KG concentrations using the transaminase inhibitor aminooxycetate increased uptake of fluorescein in rat renal slices (309). In both of these studies, the stimulatory influence of exogenous α-KG was linked to the activity of Na-α-KG cotransport in the peritubular membrane of renal cells through the demonstration that addition of lithium to experimental solutions blunted or eliminated the stimulation of OA uptake caused by preincubating tissue with α-KG, an effect associated with lithium’s inhibition of basolateral Na-α-KG cotransport (95, 234). Similarly, the inhibitory effect of valproic acid on OA uptake into iso-
lated rat renal proximal tubules appears to reflect, in large part, reduction of cellular α-KG (546).

Experiments with isolated single perfused and non-perfused tubules (56, 57) provided further support for the developing contention that α-KG is the primary intracellular counterion used by intact proximal tubules to drive the active accumulation of extracellular OA. Preloading isolated single S2 segments of rabbit proximal tubules with α-KG increased peritubular uptake of PAH (57) by three- to sixfold. Importantly, preloading tubules with α-KG also resulted in a three- to sixfold increase in the rate of transepithelial secretion of PAH (56, 57) by single perfused proximal tubules. This observation is consistent with basolateral entry’s being rate-limiting in active transepithelial secretion of OAs.

Although elevation of intracellular α-KG (as well as glutarate, suberate and adipate; Ref. 417) clearly results in stimulation of basolateral OA/DC exchange activity, the physiological contribution to basolateral OA transport of exogenous α-KG was not evident from the initial studies that typically used large dicarboxylate concentrations, nonphysiological buffers, and extended preincubations to show that such coupling can occur. Mitochondrial metabolism produces endogenous α-KG (400), and the routine observation of active renal accumulation of PAH (and other organic anions) in the absence of exogenous α-KG (e.g., Ref. 212) clearly shows that metabolic sources of α-KG are sufficient to support active OA uptake. Welborn et al. (517) used an optical method to monitor peritubular fluorescein transport in real-time to determine the extent to which uptake of exogenous α-KG results in an increase in basolateral OA transport. Acute exposure of single S2 segments of rabbit renal proximal tubules to 10 μM α-KG in the bathing medium (a representative plasma concentration), in the presence of physiological bicarbonate concentrations, increased peritubular fluorescein transport by ~75% within 7–10 s, an effect that was completely blocked by the presence of lithium. The stimulatory effect of extracellular α-KG is even larger when applied in a HEPES-based, bicarbonate-free bathing medium (57, 517), presumably reflecting the decrease in intracellular metabolic production of α-KG that occurs upon removal of bicarbonate (401). Real-time measurements of fluorescein transport also revealed the extent to which reaccumulation of endogenous α-KG (i.e., that which is lost to the peritubular extracellular space following exchange for a transported OA substrate) supports renal OA transport (517). In the absence of added α-KG in the bathing medium, blocking basolateral NaDC cotransporter resulted in an immediate and reversible 25% reduction in the initial rate of fluorescein uptake. The authors concluded that the combined influence of accumulating exogenous extracellular (i.e., plasma) α-KG and recycling α-KG lost from proximal cells following OA/DC exchange is responsible for supporting about 60% of peritubular OA transport.

The stimulation of OA transport produced by exposure to α-KG in the aforementioned studies involved activity of a basolateral NaDC cotransporter (presumably NaDC-3) (153, 200). However, proximal tubule cells also express a robust NaDC cotransporter in the luminal membrane (NaDC-1) (324) for reabsorption of dicarboxylates, including α-KG, from the tubular filtrate (327). Addition of 100 μM α-KG to the perfusate results in a twofold increase in steady-state secretion of PAH by single perfused S2 segments of rabbit renal proximal tubule (79). This stimulating effect was blocked by the addition of lithium to the perfusate. Whereas this effect initially was only noted in tubules incubated in a bicarbonate-free buffer (i.e., a condition in which intracellular α-KG production is low), a subsequent study of the differential effect of bath versus luminal exposure to α-KG implicated both luminal and peritubular NaDC cotransport in the stimulation of OA secretion under physiological conditions. Steady-state fluorescein secretion by single S2 segments of rabbit renal proximal tubule was increased by ~15% by the presence of either 10 μM α-KG in the bathing medium or 50 μM α-KG in the luminal perfusate, and these effects were additive in the presence of both bath and luminal α-KG (395). Taken together, these observations suggest that the combined influence of luminal and peritubular NaDC co-transport activity (including uptake of exogenous α-KG and the recycling of originally intracellular α-KG) is responsible for supporting between 30 and 60% of the steady-state rate of transepithelial OA secretion.

B) SPECIFICITY AND MULTIPLICITY OF BASOLATERAL OA TRANSPORT. I) SPECIFICITY OF BASOLATERAL OA TRANSPORTERS. Ulrich and colleagues (464, 465) described the presence of three principal pathways for the basolateral entrance of anionic substrates in renal proximal tubule cells: 1) a PAH/dicarboxylate exchange system, 2) a NaDC cotransport system, and 3) a sulfate/anion exchange system (464, 465). The presence of these three processes was inferred based on patterns of inhibition of basolateral uptake of either PAH, succinate, or sulfate, produced by a chemically and structurally diverse array of substrates during in vivo situ microperfusions in the rat kidney (refer to Ref. 465). The last of these three processes mediates the efflux of SO42− from proximal tubule cells in exchange for the entry of bicarbonate, hydroxyl, thiosulfate, and sulfate ions, but not chloride, phosphate, lactate, or PAH. Its narrow selectivity makes it a minor contributor to the renal secretion of OAs, although it may contribute to the secretion of selected sulfamoyl compounds (473, 482). The candidate transport protein for this process in the rat, SAT-1 (sulfate-anion transporter; SLC26A1; 2.A.53), has been cloned and characterized (255), and the interested reader is directed to a recent review of the molecular and cellular characteristics of renal sulfate transport (254).

The second of these processes, i.e., basolateral NaDC cotransport, also probably plays only a minor role in
directly mediating renal OA secretion. However, as indicated previously, basolateral NaDC cotransport plays a quantitatively important role in supporting active OA secretion by maintaining the large, outwardly directed α-KG gradient that, in turn, supports the first of the three processes listed above, PAH/dicarboxylate exchange. Interested readers are directed to several recent reviews on the cellular and molecular physiology of NaDC cotransport (326–328). Here, we provide an overview of those kinetic and selectivity characteristics of the basolateral NaDC cotransport process and its suspected molecular transporter, NaDC-3, that are of particular relevance to renal OA secretion.

II) NaDC cotransport selectivity. It is pertinent to compare the kinetic and selectivity characteristics of the basolateral NaDC cotransporter to the functionally similar process found in the luminal membrane. Kinetically, the two processes are clearly distinguishable: the basolateral transporter has a markedly higher affinity for its prototypical substrates (succinate and α-KG) than does the luminal transporter. In the rabbit, for example, the apparent \( K_a \) for succinate transport in renal BLMV is 10 \( \mu M \), compared with 0.6 mM in luminal BBMV (532); and in the rat, the apparent \( K_a \) for α-KG transport in renal BLMV is 15 \( \mu M \), versus 158 \( \mu M \) in BBMV (95). The physiological significance of high-affinity basolateral NaDC cotransport becomes evident when it is considered that the circulating concentration of α-KG is on the order of 10–20 \( \mu M \) (354), and exposure of intact proximal tubules to α-KG concentration as low as 10 \( \mu M \) is sufficient to increase basolateral OA uptake by >50% (517). The NaDC cotransporter NaDC-1 (SLC13A1; 2.A.47.1.1) has been cloned in human (325), rabbit (324), rat (2937), and mouse (414) and localized to the luminal membrane of proximal tubule cells by immunocytochemistry (330, 384). The similarity of its \( K_a \) for succinate (0.4 mM; Ref. 324) to that observed in luminal BBMV supports the contention that NaDC-1 is the NaDC cotransporter, the function of which has been characterized in isolated BBMV and intact, microperfused tubules (117, 466). NaDC-3 (SLC13A3; 2.A.47.1.4) has been cloned in human (511), rat (63, 200), mouse (329), and flounder (412) and has also been shown by immunocytochemistry to be in the basolateral membrane of proximal tubule cells (153). In addition, the similarity between the affinity of NaDC-3 for succinate (\( K_a \) of 2 to 20 \( \mu M \); Refs. 63, 511) and the affinity of BLMV for contraluminal succinate transport (\( K_a \) of 10–90 \( \mu M \); Refs. 479, 532) supports the contention that NaDC-3 is the molecular candidate most likely to be the basolateral NaDC cotransporter (200).

The selectivities of basolateral and luminal NaDC cotransport show marked similarities and a few significant differences. Indeed, the differences in selectivity of these two processes lend further support to the contention that NaDC-3 plays a dominant role in basolateral dicarboxylate transport. Neither the luminal nor the basolateral NaDC cotransporters interact effectively with monocarboxylates, and the affinity of both is largely influenced by the distance between the two carboxylate residues (465). Both processes display comparatively high affinities for succinate and glutarate (with carboxylate residues separated by 2 or 3 carbons, respectively), but they have virtually no affinity for oxalate and malonate (carboxyl residues separated by 0 or 1 carbon, respectively). Longer chain dicarboxylates (e.g., adipate and pimelate) show a systematic decrease in their inhibitory interactions with both processes (479). However, the basolateral NaDC cotransporter is more tolerant of substitutions (=O, -OH, -SH, or -CH3) on internal carbons than is the luminal transporter (465). Of particular interest is the observation that, whereas 2,3-dimethylsuccinate is a potent inhibitor of basolateral NaDC cotransport, it has comparatively little interaction with the luminal transporter (531). It is significant, therefore, that succinate and dimethylsuccinate appear to be equally effective at interacting with rNaDC-3 (200). The ability of rNaDC-3 to interact with disubstituted dicarboxylates is the probable basis for the recent observation that the heavy metal chelator 2,3-dimercaptosuccinic acid (DMSA) is transported by the flounder ortholog of NaDC-3 (30). This finding suggests that this transporter may play a clinically significant role in clearing renal cells of heavy metals.

III) PAH/dicarboxylate exchange selectivity. Ulrich and co-workers (470, 473–475, 478–485) used the in vivo stopped-flow capillary microperfusion technique in the rat kidney to examine the selectivity of basolateral PAH transport. Factors that influence selectivity of PAH/dicarboxylate exchange were deduced from the patterns of inhibition of basolateral PAH transport produced by a diverse array of chemical structures, including monocarboxylates; aliphatic dicarboxylates; mono- and polysubstituted benzene analogs; phenolphthaleins; sulfophthaleins; fluoresceins; amino acids; di- and oligopeptides; methyl-, acetyl-, and berizoylderivatives; glutathione and cysteine conjugates; cyclic nucleotides; eicosanoids; corticosteroids; β-lactam antibiotics; sulfamoyl compounds and sulfurylurea derivatives; oxazaphosphorines; analgesics (including salicylates); aniline derivatives; weak organic acid derivatives; mercapto compounds and their Hg-complexes; and several other chemicals and drugs which act as bisubstrates [i.e., they interact with both the basolateral PAH- and NMN-transporter(s)]. These studies were extended by observations from other groups that used a variety of intact renal systems to study the interaction of selected groups of compounds with basolateral renal OA transport, including anionic pesticides (84), herbicides (506), and nucleoside derivatives (151, 279). The inhibitory profiles indicated that interaction with the basolateral PAH transporter is primarily influenced by 1) the
number and location of anionic charges or partial charges and 2) the size and placement of hydrophobic regions.

Ullrich (465) developed a model of the chemical and spatial features of a PAH transporter binding site. The general requirements for interaction with the putative binding site are a single (or partial) anionic charge and a hydrophobic domain with a length of at least 4 Å. A second full or partial anionic charge located 6–7 Å from the first is also acceptable, thereby accounting for the interaction of dicarboxylates such as α-KG with the transporter. The affinity increases with increasing hydrophobicity, up to a hydrophobic domain length of 8–10 Å (located slightly off-axis to that of the two anionic charges).

IV) Multiplicity of basolateral OA transporters. Ullrich’s model should be viewed in the context of current knowledge that there are multiple OA transporters coexpressed in the basolateral membrane of the proximal tubule. Furthermore, there appear to be marked species differences in the relative distribution of different OAT proteins within renal tubules, complicating the development of models that attempt to explain or predict the basis of the selectivity of renal OA secretion. For example, the human kidney expresses OAT1, OAT2, and OAT3 in the basolateral membrane of renal proximal tubule (RPT) cells, although OAT2 expression appears to be comparatively low, and OAT1 appears to be more broadly distributed than OAT3 along the length of the RPT (300). In contrast, in rat kidney OAT1 and OAT3 are restricted to the basolateral membrane of the RPT and OAT2 is limited to the apical membrane of the thick ascending limb and collecting duct (222). In addition, within the rat RPT, OAT1 expression is largely restricted to the S2 segment, whereas OAT3 appears to be expressed in the basolateral membrane of S1, S2, and S3 segments (222). Finally, marked differences between kinetic values obtained with intact tubules and those obtained with cloned transporters in heterologous expression systems (Fig. 7) further complicate development of models of the mechanistic basis of tubular OA transport.

The rabbit kidney offers an opportunity to compare functional expression of different OAT transporters in different regions of the proximal tubule. PAH secretion is 4- to 10-fold higher in the S2 segment than in the S1 or S3 segments (390, 391, 528), which is qualitatively consistent with the distribution of OAT1 protein in the rat noted previously (222). Unlike the rat and human, rabbit OAT3 interacts very weakly with PAH, and transport of PAH appears to be effectively restricted to OAT1 in the rabbit kidney (249). Consequently, the profile of PAH secretion along the length of the rabbit proximal tubule probably reflects the distribution of OAT1. The apparent specificity of PAH for OAT1 in rabbit kidney clarifies interpretation of studies that have used PAH as an inhibitor of secretion of other anionic substrates. Whereas inhibition by PAH of OA transport in rat renal tubules could reflect interaction with OAT1 and/or OAT3, inhibition by PAH of transport in rabbit tubules implies an interaction with OAT1. Thus the ability of PAH to effectively block secretion of, for example, riboflavin (544), the angiotensin II receptor antagonist losartan (96), and the artificial sweetening agent stevioside (189) and its metabolite, steviol (58), is strongly suggestive that secretion of these compounds in the rabbit is dominated by an interaction with OAT1 and not OAT3.

OAT1 and OAT3 are coexpressed in rabbit RPT (249). OAT3 is expressed in (at least) the S1 and S2 segments of rabbit RPT. ES is a relatively specific, high-affinity (Kᵢ of 4.5 μM) substrate for OAT3 in the rabbit (Zhang and Bahn, personal communication). Thus the observation that single S2 segments of rabbit RPT display high-affinity ES transport (Kᵢ of 3 μM) that is only weakly inhibited by PAH (249) probably reflects the functional expression of OAT3 in S2 cells. Interestingly, whereas the J_max for ES transport in single S2 segments is only ~10% that for PAH, the J_max values for ES and PAH transport are virtually identical in isolated rabbit cortical RPT, a preparation enriched in both S1 and S2 segments (249). Consequently, whereas OAT1 may be expressed at higher levels in S2 cells, OAT3 expression may be predominant in S1 cells.

The profile of basolateral transport of the mycotoxin OTA into rabbit RPT is consistent with the contention of differential expression of OAT1 and OAT3 in different regions of RPT. OTA is accumulated by isolated S2 segments of rabbit RPT (516), suspensions of cortical RPT (136), and primary cultured cells derived from rabbit cortical RPT (139). In a study with isolated S2 segments of rabbit RPT, 1 mM PAH blocked ~90% of total OTA accu-
mulation, suggesting that, in the S2 segment, OTA transport is dominated by its interaction with OAT1. However, in the studies with cortical tubule suspensions and primary cultured cells, the block of OTA transport produced by 2.5 mM PAH was not complete (~40%), although other anionic compounds, including probenecid and octanoic acid, blocked OTA uptake by 80–90%. These observations suggest that OTA uptake into these preparations involved interaction with OAT1 and (at least) one more process. Thus the differential distribution of multiple OAT transporters with overlapping, but nevertheless distinct specificities, must be considered when modeling the renal secretion of anionic compounds.

OAT1 and OAT3 share the same energetic mechanism of transport (i.e., mediated exchange of OAs for intracellular dicarboxylates; Refs. 386, 421, 427) and, as noted above, are coexpressed in at least some renal proximal cells (249, 300). Moreover, although OAT1 and OAT3 clearly have overlapping substrate specificities (e.g., human, rat and mouse OAT3 all transport PAH; Refs. 52, 232, 424), they can also discriminate between selected substrates (whereas, for example, mOAT3 and rbOAT3 transport ES, mOAT1 and rbOAT1 do not; Ref. 424). Ulrich’s model for the molecular basis of selectivity by the PAH/dicarboxylate exchanger must, therefore, be viewed as representing “average” physical and structural criteria that influence the physiological secretion of organic anions by a suite of processes working in parallel. The ability to predict the extent to which any one of these processes influences total secretion of a given compound is critically important to efforts to predict and thereby prevent unwanted drug interactions at the level of OAT transporters (e.g., Refs. 203, 204, 433). Accurate predictions will, however, require an understanding of both the degree of interaction of specific substrates with each member of the entire suite of transporters expressed in the RPT and the profile of distribution within the tubule of each of the transporters that interact significantly with the selected agents. Therefore, additional studies that focus on the selectivity characteristics of the individual transporters, in conjunction with their functional distribution along the length of nephron, will be required before a physiological model of the structural basis of renal OA secretion can be developed.

V) Apical/Brush border/luminal OA transport. Although mediated entry of type I OAs across the basolateral membrane appears to be largely restricted to two or three processes, each of which (i.e., OAT1, OAT2 and OAT3) has been cloned and characterized, luminal OA efflux, and the processes underlying it, is less well understood. Evidence based on immunocytochemical and Western blot data discussed previously indicates that OAT4, OAT-K1, OAT-K2, OATP1, MRP2, NaPi-1, and OATV1 are expressed in the luminal membrane of renal proximal tubules. Thus each could play a role in mediating the secretory flux of selected organic anions. Following is a discussion of recent studies employing isolated membrane vesicles and intact renal tubules that address issues of mechanism, selectivity, and multiplicity of luminal OA efflux.

B) Mechanism of luminal PAH (i.e., type I) OA transport. In isolated renal tubules, the apparent permeability of the luminal membrane to OAs is several times greater than the apparent passive permeability of the basolateral membrane (76, 461). This favors movement into the tubule lumen of OAs that have entered the tubule cells at the basolateral side. Although this movement from the cells into the lumen could involve simple passive diffusion, the apparent permeability of the luminal membrane is much greater than would be expected for simple passive diffusion of a charged molecule across a biological membrane. Moreover, transport can be inhibited by probenecid and other inhibitors in isolated tubules and BBMV from a number of species (21, 77, 78, 211). Unidirectional luminal efflux of fluorescein has been measured in isolated single perfused rabbit tubules (397) and was found to be saturable with a $K_v$ of ~500 $\mu$M and a $J_{\text{max}}$ of 635 fmol · min$^{-1}$ · mm$^{-1}$. Comparison of these values with those measured for net transepithelial fluorescein secretion in the same system (maximal rate of secretion of ~280 fmol · min$^{-1}$ · mm$^{-1}$, half-saturated at ~4 $\mu$M; Ref. 395) reveals the luminal efflux step to be of both lower affinity and higher capacity than the basolateral influx step, consistent with the view that the active basolateral uptake step is rate limiting during transepithelial secretion. The comparatively low affinity of the luminal efflux process(es) for what is usually considered to be a “high-affinity” substrate (i.e., fluorescein) also lends credibility to the potential role in secretion of several cloned transporters, including MRP2 and OATV1, that have been shown to transport type I OAs but to have a low (millimolar) affinity for these substrates (e.g., Refs. 188, 239).

The energetic basis of the luminal OA efflux step is not clear. Although an OA exchanger that can be shared by PAH, urate, Cl$^-$, and OH$^-$ has been physiologically characterized in the luminal membrane of some mammalian species (e.g., dog, rat; Refs. 146, 192, 211), changes in luminal pH do not appear to influence efflux of PAH from proximal cells in intact rat tubules (472). Also, replacement of Cl$^-$ in the luminal fluid does not eliminate PAH secretion by isolated perfused rabbit proximal tubules (59). Isolated BBMV from human (358) and bovine (379, 380) kidney display mediated PAH/α-KG exchange with characteristics similar to those observed in BLMV. However, studies employing the doubly perfused rat renal proximal tubule method (472) failed to demonstrate a trans-stimulation of luminal PAH efflux in the presence of inwardly directed gradients of either PAH or α-KG. Interestingly, the presence in the luminal fluid of substrates for which the luminal pathway was suspected to have a high
Although luminal OA flux may, in some species, involve anion exchange, it may also involve a potential-driven conductive pathway. Indeed, given the presence of active accumulation of PAH (and other type I OAs) at the basolateral membrane via OA/DC exchange, the simplest luminal transport mechanism consistent with net secretion is an electrogenic PAH transporter or “channel.” Studies with pig (228, 520), rabbit (213, 257), and rat (171, 315) BBMV have described an inhibitable conductive pathway for PAH (and urate). In pig BBMV, for example, mediated PAH and urate efflux clearly involve a saturable, low-affinity (half-saturated at 12 mM) electrogenic process that is distinct from the conductive pathway for Cl\(^-\) in the same membrane (228). The cloning of an electrogenic PAH transporter from pig kidney that is expressed in the apical membrane of proximal tubule cells (OATV1; Ref. 188) offers a novel new molecular candidate for this process. Conductive pathways may exist in parallel with exchange pathways, as suggested by studies with isolated rat renal BBMV (315). However, experiments with intact rat (472) and flounder (283) proximal tubules failed to show any change in luminal OA efflux following depolarization of the luminal membrane (via elevation of extracellular [K\(^+\)]). It has been suggested that species that display net urate secretion (e.g., rabbit, pig) may depend on potential-driven, conductive pathways to support luminal OA efflux, whereas species that exhibit net urate absorption (rat, dog, human) may typically have both voltage-driven and exchanger/mediated modes of luminal PAH flux (356).

C) SPECIFICITY AND MULTICLIVITY OF LUMINAL OA TRANSPORTER PATHWAYS. The molecular identity of the luminal pathway for PAH efflux is not clear. As noted above, there is evidence, in different species, supporting the presence of both conductive processes and those that involve mediated PAH/anion exchange. OAT-K1 (363), OAT-K2 (262), and OATP1 (416) display little or no interaction with PAH and other type I OAs and so are unlikely to be major elements within the classical OA secretory pathway. OAT4 does support PAH transport, albeit with comparatively low affinity (7). Although the human ortholog of NPT1 (NaPi-1) does accept PAH as a substrate (462), rabbit NaPi-1 does not (45), making it unlikely that NaPi-1 is the principal luminal element in the OA secretory pathway. The NPT1 homolog from pig kidney, OATV1 (188), supports electrogenic PAH transport and is localized within proximal cells to play a potential role in transepithelial OA secretion. Human and rabbit MRP2 both support the ATP-dependent transport of PAH, and the potential role of this process in renal OA transport is discussed at greater length below.

There is physiological evidence that luminal efflux of at least some OAs can involve the parallel activity of multiple transport processes. The fluorescent sulfonated anion Lucifer yellow (LY) is actively secreted by flounder proximal tubules (260). Whereas basolateral LY uptake in flounder RPT appears to be limited to an interaction with an OA/DC exchanger (probably including fIOAT1), secretion of LY across the luminal membrane is partially blocked by leukotriene C\(_4\) (LTC\(_4\)), a stereotypic inhibitor of MRP2, suggesting that \(\sim 50\%\) of luminal LY efflux involves MRP2. LY is a divalent anion of molecular weight 443 and, so, is on the cusp of the loose definition of type I versus type II OAs employed here. Nevertheless, luminal secretion of fluorescein was not affected by LTC\(_4\), consistent with the failure of fluorescein to inhibit rbMRP2-mediated transport (495). This further supports the contention that multiple processes play a role in luminal OA efflux.

PAH has also been shown to be a substrate for MRP2 (human, Ref. 239; rabbit, Ref. 497). The apparent affinity of MRP2 for PAH is comparatively low: the \(K_m\) values for ATP-dependent PAH transport are 0.9 and 1.1 mM for the human and rabbit orthologs of MRP2, respectively. These values contrast sharply with the \(K_m\) values of 1.5 \(\mu\)M for vinblastine transport by rbMRP2 (495) and 7.2 \(\mu\)M for E17\(\beta\)-D transport by hMRP2 (73). However, as noted above, given that the apparent \(K_m\) for luminal fluorescein efflux in intact rabbit tubules is \(\sim 560 \mu\)M (397), the comparatively low affinity of MRP2 for PAH should not be interpreted as being indicative of a quantitatively insignificant role for this process in luminal secretion of PAH. Indeed, for hMRP2 there is less than a 10-fold difference in the \(J_{\text{max}}/K_m\) ratio for PAH and the prototypic MRP2 substrate E17\(\beta\)-D (239), suggesting that PAH is at least a modestly effective substrate for MRP2.

The unanswered question is, “What percentage of total luminal efflux of PAH (or any other type I OA) is carried by each of the growing list of candidate transporters?” On the basis of reported interactions with PAH (albeit, in some cases, weak), this list now includes MRP2, OATV1/NaPi-1, OAT4, and OAT-K2. Unfortunately, with the exception of the study on LY secretion in flounder tubules mentioned previously, no studies have determined the effect on total tubular substrate secretion of selectively blocking one or more of these potential pathways. Such studies will be difficult to design and interpret because few compounds are known to selectively interact with a restricted population of suspected luminal transport processes. Nevertheless, the issue under question, i.e., mapping the contribution to luminal OA efflux of different transport proteins, is important enough to the overall understanding of renal OA secretion to warrant the identification (e.g., using heterologous expression sys-
2. Regulation of tubular OA transport

Organic anion transport in the kidney is influenced by a wide array of regulatory processes (19, 453). Regulation of PAH transport has generally been the focus of study, and the potential influence of multiple transporters that accept PAH as a substrate in both basolateral and luminal membranes complicates the interpretation of much of the work to date. Nevertheless, it is evident that renal secretion of organic anions can be dynamically modulated. The following is a discussion of observations that pertain to mechanisms of regulation of renal transport of type I and type II OAs that appear to involve rapid, kinase-mediated events, and long-term, steroid hormone-mediated events.

A) KINASE-MEDIATED MODULATION OF RENAL BASOLATERAL OA TRANSPORT. Activation of PKC influences transport of type I OAs in intact renal tubules (122, 161, 165, 278, 396) and in cultured OK cells (303, 369, 431). Transport has been influenced by both direct activation of PKC using phorbol esters or diacylglycerol analogs (122, 161, 278, 369, 396, 431), and by indirect, receptor-mediated activation (122, 303, 396). In all these cases, the effects of PKC activation were reversed or blunted by preexposure of cells to PKC inhibitors. Although in one study activation of PKC caused a stimulation of PAH uptake into intact rabbit RPT (161), the profile observed in all other studies has involved a dose-dependent decrease in basolateral OA uptake. For example, exposing isolated single perfused S2 segments of rabbit RPT to bradykinin or phenylephrine caused a dose-dependent decrease (25–40%) in basolateral uptake (122) or trans-tubular secretion (396) of fluorescein. This effect occurred within 10 min of exposure, was maximal within 60 min, and was blocked by preexposure to the PKC inhibitor bisindolylmaleimide I (122).

Depending on the substrate involved, the molecular basis of the regulation of basolateral type I OA transport in native tubules or cultured cells could reflect modulation of either OAT1 or OAT3 (or both). Activation of PKC with phorbol esters has been shown to decrease activity of both OAT1 [human, expressed in HeLa cells (248); rat, *Xenopus* oocytes (491); mouse, LLC-PK1 cells (545)] and OAT3 [rat, *Xenopus* oocytes (436)]. The decrease in transport noted in rabbit tubules (122, 396), for example, reflected changes in measured rates of accumulation of fluorescein, which in the rabbit is a substrate for both OAT1 (8) and OAT3 (Zhang and Bahn, personal communication). Consequently, how much these effects reflect modulation of one or both of these processes is not clear.

The extent to which PKC-mediated regulation of type I OA transport involves direct phosphorylation of the transporter(s) is not known. In a study employing LLC-PK1 cells that stably expressed mOAT1 (545), exposure to okadaic acid (to inhibit phosphatase activity) resulted in both a downregulation of PAH uptake and an increase in phosphorylation of mOAT1. However, the quantitatively similar decrease in transport activity that followed exposure to phorbol ester was not associated with a change in mOAT1 phosphorylation. The decrease of mOAT1-mediated PAH reflected a decrease in the *J*max for transport and not a change in *K*1 (545), leading to the suggestion that the regulation of mOAT1 activity induced by activation of PKC may involve changes in the trafficking of transport protein, an observation confirmed in studies of the PKC-mediated downregulation of hOAT1 activity expressed in *Xenopus* oocytes (524).

Other kinases have also been implicated in the regulation of renal transport of type I OAs. Inhibition of CKII activity has been shown to decrease PAH uptake into isolated rabbit RPT (118). Activation of PKA with forskolin has also been shown to inhibit PAH uptake in isolated rabbit RPT (133). This latter observation is, however, at odds with the recent observation that basolateral PAH uptake into OK cells is stimulated by exposure to forskolin (371). The basis for this apparent discrepancy is not clear, but it may reflect the different concentrations of forskolin employed in these two studies (100 versus 5 μM, respectively). Indeed, the stimulatory influence of PKA activation on basolateral OA transport is consistent with the observed stimulation of PAH transport in OK cells (369, 370) and intact rabbit RPT (368) by exposure to epidermal growth factor (EGF). Furthermore, inhibition of the mitogen-activated protein kinase (MAPK) pathway (through exposure to PD98059) inhibits basolateral PAH uptake in intact rabbit RPT (119). These data support a cascade of events that includes EGF receptor activation of the MAPK, MEK (mitogen-activated extracellular signal-regulated kinase kinase) and ERK1/2 (extracellular signal-regulated kinase isoforms 1 and 2), and the subsequent activation of phospholipase A2 and an increase in cytoplasmic arachidonic acid. Cyclooxygenase-1 (COX-1) mediates metabolism of AA to prostaglandin E2 (PGE2) that then activates adenylate cyclase. The resulting rise in cAMP leads to activation of PKA and finally to the previously noted increase in basolateral OA transport.

The activity of basolateral OA/DC exchange is functionally linked to the activity of NaDC cotransport (340, 389, 517). Consequently, regulation of NaDC cotransport offers an alternative means to influence basolateral uptake of OAs. Flounder NaDC-3 activity (expressed in *Xenopus* oocytes) responds to activation of PKC with a marked downregulation of transport that occurs in parallel with the endocytosis of NaDC-3 protein (150). As noted earlier for PKC-induced downregulation of OAT1 activity,
mutation of the five consensus PKC phosphorylation sites of flNaDC3 had no effect on the downregulation of transport activity that followed exposure of oocytes to phorbol ester. The extent to which this regulatory response to PKC activation occurs in intact tubules is not clear in light of the observation that exposure of isolated rabbit S2 segments to phorbol ester appears to have no effect on the rate of Na⁺-dependent glutarate transport (359). In addition, exposure to PD98059 (MAPK inhibitor), genistein (tyrosine kinase inhibitor), wortmannin (phosphatidylinositol 3-kinase inhibitor), or KN93 (CaMII inhibitor) has no effect on basolateral glutarate uptake in intact tubules (118, 119).

B) KINASE-MEDIATED MODULATION OF RENAL LUMINAL OA TRANSPORT. Twenty-five years ago, E. Wright likened the existing knowledge of transport in the basolateral aspect of the intestinal epithelium to the previous decade’s knowledge of “the dark side of the moon” (530). The substantial difference in understanding of transport processes of the luminal versus basolateral side of intestinal cells reflected, in large part, the ease of experimental access to the former, compared with the latter. An inverse relationship exists with respect to the study of renal transporters; whereas the basolateral (peritubular) side of renal tubules is easily accessed, the luminal aspect is the “dark side” of the renal epithelium. Generally speaking, experimental access to the luminal membrane of living renal cells is limited to technically challenging studies that employ microperfusion methods. Even then, assessment of the contribution of luminal processes to transepithelial secretion is typically complicated by the necessity that substrates cross the basolateral membrane before they can interact with luminal membrane transporters. Although such complications have plagued all aspects of the study of secretory processes, they have had a particular impact on the study of the regulation of luminal transport processes simply because of the difficulty of testing the influence of signal cascade activation on the initial rate of transport across the luminal membrane.

Two studies have examined the influence of activation of PKC on the steady-state transepithelial flux of the type I OA fluorescein in intact renal proximal tubules. Treating isolated fluorid proximal tubules with phorbol ester (PMA) reduces intracellular accumulation of fluorescein but has no effect on the lumen-to-cell ratio of fluorescein distribution across the apical membrane (278). Whereas the former effect is consistent with the well-documented downregulation of basolateral OA/dicarboxylate exchange, the latter observation suggests that activation of PKC has no effect on luminal fluorescein efflux. Similarly, the decrease in transepithelial fluorescein secretion measured in isolated perfused rabbit proximal tubules following activation of PKC (396) can be explained by the downregulation of the basolateral entry step (122) without the need to invoke a PKC-mediated change in the luminal exit step.

Transepithelial PAH secretion across confluent monolayers of OK cells shows a different profile in which activation of PKC results in a downregulation of both basolateral and luminal exit (369). Similarly, inhibition of MEK resulted in a downregulation of both the basolateral and luminal transport steps associated with PAH secretion in OK cells (369). It is not clear if the apparent difference in apical response to PKC activation in cultured OK cells versus intact rabbit and flounder tubules reflects species differences in luminal exit mechanism(s), differences in substrate studied [i.e., luminal FL exit may involve pathway(s) different from those for PAH], or simply the technical difficulty of clearly assessing functional activity of luminal efflux processes (particularly in intact tubules).

MRP2, which mediates luminal secretion of type II OAs (260) and may play a role in the luminal efflux of at least some type I OAs (239, 497), is also regulated by PKC. Activation of PKC in human hepatoblastoma (HepG2) cells results in a downregulation of MRP2-mediated efflux of fluorescent type II substrates by a process that involves internalization of transport protein from canalicular membrane (229). Miller and colleagues (261, 280, 310, 452) have also linked activation of PKC in intact teleost renal proximal tubules to the rapid downregulation of MRP2-mediated efflux of the type II OA fluorescein-methotrexate. Their studies documented a cascade of events involving endothelin (ET), nitric oxide (NO), and PKC (see Refs. 280, 310). The scheme involves 1) release of ET from proximal tubule cells, 2) the autocrine/paracrine activation of ETB receptors on proximal tubule cells, 3) activation of NO synthase and production of NO, and 4) NO-dependent activation of PKC. Activation of PKC then downregulates MRP2, although it is not clear if PKC changes MRP2 trafficking in the kidney, as seen in the liver (229). ET is frequently released in response to renal injury, including nephrotoxic insults (70, 160). The short-term response of this regulatory scheme, including as it does the PKC-mediated downregulation of basolateral OA accumulation, may play a protective role for renal cells. However, the parallel decrease in MRP2-mediated luminal efflux may also place renal cells at increased risk of the effects of toxic anions that are no longer exported from the cells across the luminal membrane (280, 454).

NaDC-1 responds to physiological regulation in a manner similar to that noted earlier for NaDC-3. Activation of PKC caused a 95% decrease in Na⁺-dependent succinate uptake into oocytes expressing NaDC-1, and this effect was blocked by preexposure to the PKC inhibitor staurosporine (331). Mutations of two consensus PKC sites within the NaDC-1 sequence did not prevent the PKC-mediated downregulation of succinate transport. Oocytes treated with PMA displayed a 30% decrease in
NaDC-1 protein in the plasma membrane, suggesting that the downregulation of transport activity involves (but may not be restricted to) internalization of transport protein. Downregulation of NaDC-1 by activation of PKC may also play a small role in the downregulation of basolateral OA uptake in perfused tubules observed with PKC activation because α-KG transport by NaDC-1 apparently accounts for a small portion of the outwardly directed α-KG gradient at the basolateral membrane (395, 396). Activation of PKA appears to have no effect on NaDC-1 activity (331).

C) Steroid-mediated modulation of renal OA transport. Tubular transport of PAH is influenced by glucocorticoids (30), 1,25-dihydroxycholecalciferol (31), and sex steroids (29, 48–50). PAH accumulation in human renal slices is increased by exposure to dexamethasone (114). Treatment of rats (particularly 5- to 10-day-old rats with immature kidney function) with the glucocorticoids prednisolone or dexamethasone results in increased renal excretion of PAH and increased PAH accumulation in renal cortical slices (30). In contrast, treatment with mineralocorticoids (desoxycorticosterone, aldosterone) had no effect (30). Synergistic interactions were also observed following treatment with multiple hormones. Calcitriol (1,25-dihydroxycholecalciferol), which by itself reduces PAH accumulation in renal cortical slices in rats of various age groups, can prevent the increase in renal PAH transport that follows treatment with triiodothyronine (31).

Sex steroids have a marked influence on renal PAH transport. Cortical slices from adult male rat kidneys display higher tissue-to-medium ratios of PAH accumulation than do slices from female rat kidney (29), and this difference is correlated with a more twofold higher expression of OAT1 protein (by Western analysis) in BLMV from male than from female rat kidneys (48). Whereas castration of male rats results in a significant decrease in renal accumulation of PAH, ovariectomy has no effect on PAH transport in female rat kidneys (29). This relative refractoriness of sex steroid regulation of PAH transport in female rat kidneys is also observed when treating rats with sex steroids; whereas PAH accumulation in male rat kidneys increases significantly following treatment of the animals with testosterone, it does not change in female rat kidneys with the same treatment.

Generally, the higher rates of PAH transport in kidneys of male rats are correlated with higher levels of expression of mRNA for OAT1 and OAT3 in renal tissue from male rats (compared with female rats) (35, 36, 196, 230). The most extreme sex difference in expression, however, is the difference in mRNA for OAT2, which is four- to eightfold higher in female rat kidney (35, 36, 196, 230). The functional consequences of these differences in OAT expression to actual rates of renal OA uptake or secretion are not clear, but sexual dimorphism of OA transport could result in sex-based differences in rates of drug excretion or exposures of renal tissues to anionic drugs and nephrotoxicants.

OATP1 expression in the kidney, where it appears to be restricted to the luminal membrane of the S3 segment of renal proximal tubules (18), is under the control of sex steroids. In Northern blots of renal total mRNA, male rats display much higher levels of OATP1 mRNA than do female rats (247). A similar profile of OATP1 mRNA expression was observed in a study employing the branched-DNA signal amplification method (244). Administering testosterone to female rats increases, and administering estradiol to male rats decreases, renal expression of OATP1 mRNA (247). In contrast to the marked sex steroid sensitivity of renal OATP1 expression, sex steroids do not influence expression of OATP1 in rat liver (18). It has been suggested that increased expression of OATP1 in the apical membrane of proximal tubule cells in male rats facilitates clearance of glucurononlated steroids, including conjugated estradiol, thereby assisting in the maintenance of “maleness.”

3. Intracellular OA sequestration

In addition to the basolateral entry and luminal exit steps that are requisite elements of net transepithelial OA transport, secreted substrates must traverse the cell cytoplasm. Although this process is frequently tacitly assumed to involve simple, passive diffusion through an aqueous cytoplasm, a number of observations indicate that secreted OAs are not uniformly distributed within the intracellular compartment. Binding to cytoplasmic constituents has been known for sometime to contribute to intracellular accumulation of selected OAs, including phenol red, PAH, and probenecid (e.g., Refs. 20, 107, 167; see also Ref. 342). However, in addition to passive binding of OAs, several observations support the contention that at least some OAs can be actively sequestered within intracellular vesicles. Optical methods were used to demonstrate directly the “punctate” sequestration of FL within intact flounder proximal tubules, cultured OK cells, and crab urinary bladder (281, 285). When FL was directly injected into the cytoplasm of OK cells, its accumulation within these punctate compartments was energy dependent and blocked by coinjection of PAH. Treating tubules with the microtubule inhibitor nocodazole significantly reduced transepithelial secretion of fluorescein into the lumen of isolated flounder proximal tubules (284). Studies of the effect of nocodazole on fluorescein distribution in crab urinary bladder (281) offered some insight into the possible mechanistic basis of the reduction in secretion observed in flounder tubules. Accumulated fluorescein within control bladder cells was found in two discontinuous compartments: one that was diffuse and evenly distributed through the cytoplasm and one that was punct-
tate. Accumulation in both compartments was blocked by addition of PAH or glutarate to the bath, implicating entry into bladder cells via OA/dicarboxylate exchange. Moreover, whereas treating the cells with nocodazole had no effect on the level of accumulation in the diffuse distributed compartment, it significantly reduced accumulation in the punctate compartment. Confocal microscopy revealed that the individual sites of punctate accumulation displayed a net basolateral-to-apical movement of ~0.8 μm/min. Nocodazole nearly abolished this movement and significantly reduced net transepithelial secretion of fluorescent across crab bladder (281). These data suggest that intravesicular sequestration can play a quantitatively significant role in the transepithelial flux of selected OAs in (at least) some epithelial systems. It should be stressed, however, that active sequestration of OAs has not been shown to play a role in the secretion of OAs by physiologically intact mammalian renal tubules. Consequently, the potential role of intracellular compartmentalization to the processes of secretion and detoxification of OAs by the kidney remains to be established.

IV. SPECULATIONS AND CONCLUSIONS

Our understanding of the renal handling of organic cations and anions has been profoundly influenced by the introduction of molecular methods for the study of transport proteins. Since the cloning of OCT1 in 1994, at least 12 distinct members of the OCT family of organic electrlyte transporters (alternatively, amphiphilic solute facilitators) have been identified, and selected orthologs cloned in some nine species, including six mammalian species. In addition, functional evidence has been obtained implicating members of at least five other transport protein families in the secretion of selected OCs and OAs.

Studies employing these cloned transporters and associated molecular tools have established that the “classical” pathways for renal secretion of organic cations and anions consist of multiple processes arranged in parallel and in series, processes that involve different energetic mechanisms with overlapping yet distinct substrate specificities. Indeed, that very complexity of organization with multiple redundancies speaks to the physiological importance of efficient xenobiotic excretion.

It now seems reasonable to speculate on “why” renal OC and OA secretion is organized the way it appears to be. Extant transport processes probably arose, in evolutionary terms, in response to the extraordinarily diverse array of secondary plant products to which heterotrophs are routinely exposed (now including man-made “variants” of these plant products). The chemical/structural diversity of these agents was the probable “selective pressure” that led to the retention within evolving genomes of multiple transport processes that represented an increasing number of “variations” on a general theme. The central element of this theme may have been a binding site that 1) interacted with molecules that shared a limited set of physical determinants, i.e., a degree of hydrophobicity plus a cationic or anionic charge center, and 2) imposed a limited (but finite) set of steric constraints on binding to a structurally diverse population of substrates. Inclusion within genomes of multiple transport homologs with overlapping yet distinct selectivity characteristics would then have increased the structural range of xenobiotic agents that could be effectively handled by animals that were, in turn, exposed to an ever-increasing array of plant-derived products.

The requirement for transport processes that could interact efficiently with a structurally diverse array of substrates may also have been the pressure that led to reliance on exchange processes, rather than cotransport processes, as the principal strategy for moving (type I) organic anions and cations against their electrochemical gradients. The binding of a given substrate to a cotransporter apparently occurs when a conformational change in the cotransporter, consequent to its binding of one or more activator ions (typically Na⁺), increases its affinity for the substrate in question (245, 275, 336, 529). The magnitude of such conformational changes is not clear, but it seems reasonable to infer that a structural change that, for example, increases the affinity of SGLT1 for D-glucose but not for L-glucose is comparatively subtle. It seems likely that the conformational changes required by this cotransporter paradigm are ill suited for transport processes that must be capable of the multiselectivity shown by OCTs and OATs. However, future studies on the molecular nature of substrate binding and translocation will be required before firm conclusions can be drawn about this issue.

The advent of molecular approaches to the study of renal OC and OA transport should result in continuing advances in understanding of both the molecular and systems physiology of these processes. As noted above, the ability to examine the activity of single transport proteins should lead to a clearer understanding of the structural basis of the multiselectivity of renal OC and OA transporters, which has been a hallmark of their secretory activity. Application of molecular tools is also enhancing studies of the mechanistic basis of the physiological and developmental regulation of OA and OC transport. At the cellular and organ level, the challenge is now to integrate the information being obtained from the study of individual molecular processes into the physiological context of intact renal tissues. Particular areas of emphasis will need to involve establishing the functional distribution of these distinct processes and their profiles of physiological and developmental regulation. Given the importance of these processes for human health, we believe that other focal points for future studies should include the influence of
sex differences on expression of renal OC and OA transporters and the consequences of polymorphisms of OC and OA transporters on the pharmacokinetics and toxicokinetics of renal secretion.

NOTE ADDED IN PROOF:

The reader is directed to several recently published studies that expand and/or clarify topics discussed in this review. 1) Human OAT4 was shown to support mediated exchange of selected OAs, including PAH and estrone-3-sulfate for dicarboxylates (i.e., glutarate) (96α). 2) RST was shown to support exchange of urate for Cl− or glutarate, supporting the contention that RST is the mouse ortholog of URAT1 (171α). 3) Phylogenetic analysis of members of the SLC22 family suggests that organic anion and cation transporter-like genes already existed before the divergence of vertebrates and invertebrates. The authors suggest that subsequently, within the vertebrate lineage, this gene family expanded through independent tandem duplications in each of the OAT, OCT, and OCTN subfamilies, producing multiple tandem gene pairs. The argument is developed that this reflects a requirement for redundancy or broader substrate specificity in vertebrates (compared with invertebrates), due to their greater physiological complexity and thus potentially broader exposure to organic ions (100α).

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