rOCT2 is a basolateral potential-driven carrier, not an organic cation/proton exchanger

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Sweet, Douglas H., and John B. Pritchard. rOCT2 is a basolateral potential-driven carrier, not an organic cation/proton exchanger. Am. J. Physiol. 277 (Renal Physiol. 46): F890–F898, 1999.—The driving forces mediating tetraethylammonium (TEA) transport were systematically assessed in Xenopus oocytes and MDCK cells expressing organic cation transporter (OCT) 2 cloned from rat kidney (rOCT2). In rOCT2 cRNA-injected oocytes, uptake of [3H]TEA was saturable, with an estimated Michaelis constant (Km) of 393 µM, and was specifically inhibited by organic cations. Furthermore, TEA uptake demonstrated two distinct components, one that was potential sensitive and one that was pH sensitive. When membrane potential was intact, TEA uptake was largely unaffected by changes in medium pH; when the oocyte membrane was depolarized (K+ in = out = 102.5 mM, plus valinomycin), decreasing external medium pH significantly reduced TEA uptake. Consistent with the potential sensitivity of uptake, electrophysiological analysis of rOCT2-injected oocytes demonstrated movement of positive charge into the oocyte upon TEA addition. To further evaluate the nature of the pH effect and assess the properties of rOCT2 in a renal epithelium, rOCT2 was introduced into MDCK cells. A stably transfected single cell line (MDCK-rOCT2) showed mediated, potential-sensitive, pH-sensitive TEA uptake (Km = 48 µM). TEA efflux from preloaded MDCK cells was stimulated by externally applied (trans) tetramethylammonium but was trans-inhibited by H+ (external pH 5.4). The effect of external H+ was to modulate rOCT2-mediated transport. Thus rOCT2 is a potential-driven transporter, not an organic cation/H+ exchanger, consistent with a physiological role in the basolateral entry step in renal organic cation secretion.

rat; kidney; Xenopus laevis; expression cloning; Madin-Darby canine kidney; transfection

ORGANISMS ARE CONTINUALLY exposed to potentially hazardous compounds, including environmental chemicals, drugs, and even endogenous metabolites. Survival in the face of these agents is largely due to efficient metabolic detoxication and excretory transport. The kidney is a major site of these activities and, for certain chemicals, e.g., tetraethylammonium (TEA), is capable of complete clearance from the plasma in a single pass (2, 16–18). For positively charged compounds like TEA, this rapid clearance is mediated by the organic cation transport (OCT) system of the proximal tubule (22). Functionally, this is a multistep process involving potential-driven basolateral entry into the cells (14, 24, 28, 31) and luminal exit via exchange for protons (H+) or by the multidrug resistance transporter (MDR; see Refs. 5, 20, 28, 34).

Because of their very different driving forces, it was assumed that, as the individual transporters were cloned, it should be easy to identify basolateral and luminal carriers. This has not proven completely accurate. For the first of the cloned renal OCTs (rOCT1), it is now accepted that it is a basolateral potential-driven carrier (4, 11, 32). For others, notably OCT2 (8, 10, 19, 21, 26, 32) and OCTN1 (31), considerable uncertainty remains. Both show significant cis inhibition of uptake when the external pH is reduced (H+ increased), raising the possibility that they may function as organic cation (OC)/H+ exchangers (10, 21, 29, 32). Little additional data are currently available on OCTN1 to resolve this issue, but OCT2 has generated substantial attention, unfortunately without resolving the nature of the effect of H+ on OCT2 function. Okuda et al. (19), the first group to characterize the rat homologue, rOCT2, found that, in Xenopus oocytes, rOCT2-mediated TEA uptake was pH independent, indicating that H+ do not provide a driving force, and therefore concluded that rOCT2 is a basolateral transporter (19). However, Grundemann et al. (10) found that uptake mediated by pOCT2 (the porcine OCT2 homologue) expressed in cultured cells was pH dependent, decreasing as the pH of the external buffer was lowered (10). Additionally, they found that the inhibitor specificity of pOCT2 matched the profile they obtained for apical OC transport in LLC-PK1 cells and concluded that pOCT2 is located in the apical membrane and that it mediated OC/H+ exchange. In contrast, electrophysiological measurements made on oocytes expressing the human OCT2 homologue (hOCT2) detected a large inward current induced upon application of TEA (8). TEA uptake by hOCT2 was also found to be pH independent, arguing that hOCT2 does not function as a luminal OC/H+ exchanger. Surprisingly, however, these investigators found that hOCT2 was expressed in the apical membrane of distal tubules in human kidney but not in the basolateral membrane of the proximal tubule, based on immunohistochemistry with an antiserum raised against rOCT1 but which cross-reacts with hOCT2 (8). Recently, another study concluded that rOCT2 was a basolateral transporter that exhibited pH-dependent uptake, but pH-independent efflux, of substrate (32).

Clearly, the issues of the subcellular localization of OCT2 and driving forces have yet to be conclusively resolved. In the studies presented here, we have focused on the driving forces responsible for OC transport mediated by rOCT2, comparing its functional properties when expressed in Xenopus oocytes and in a...
stably transfected renal epithelium (MDCK cells). These studies show conclusively that rOCT2 is a potential-driven carrier. Furthermore, they document that, although rOCT2 transport is modulated by external H+ concentration under both normal and short-circuited conditions, it does not mediate H+/TEA exchange. In fact, it is both cis- and trans-inhibited by external H+. Thus rOCT2 function is entirely consistent with the potential-driven basolateral uptake of OCS in the cells of the renal tubule.

MATERIALS AND METHODS

cDNA library construction and screening. Total RNA was isolated from rat kidney using guanidinium thiocyanate extraction followed by cesium chloride gradient centrifugation according to the protocol of Gasser et al. (6). Poly(A)+ RNA was isolated on an oligo(dT) cellulose column and checked for OC transport activity by expression assay in Xenopus laevis oocytes. A cDNA library was made in the vector pSPORT1 using the SuperScript Plasmid System kit (GIBCO-BRL, Bethesda, MD). Library plasmids were transformed into Max Efficiency DH5α competent cells (GIBCO-BRL), plated onto Hybond-N nylon filters (Amersham, Arlington Heights, IL) that were overlayed on Luria-Bertani (LB) plates containing 100 µg/ml ampicillin, and incubated overnight at 37°C. To obtain purified plasmid DNA, whole filters (or filter subsections) were placed in 200 ml LB with 100 µg/ml ampicillin and were shaken at 225 rpm overnight at 37°C. The bacteria were pelleted by centrifugation, and plasmid DNA was isolated using a QiaGen Plasmid kit (Qiagen, Chatsworth, CA). Plasmid DNA pools were used as template for cRNA synthesis and were checked for OC transport activity by Xenopus oocyte expression assay. After identification of a positive filter, the bacterial colonies were further subdivided until a single positive clone was isolated.

DNA sequencing. The cDNA clone was sequenced using the dideoxy chain-termination method with the Sequenase DNA sequencing kit (United States Biochemical, Cleveland, OH). The full-length sequence of both strands was obtained using M13/prl forward and reverse primers, as well as synthetic sequence comparisons and database searches were done with the Wisconsin Package software with default settings (7).

Xenopus oocyte expression assay. Oocyte isolation procedures and uptake assay were performed as described previously (27). Briefly, adult female Xenopus laevis (Xenopus One, Ann Arbor, MI) were anesthetized by hypothermia and decapitated. Stage V and stage VI oocytes were manually dissected free of the ovary, and the follicles were removed by treatment with collagenase A. Oocytes were maintained at 18°C in Barth’s buffer containing 0.05 mg/ml gentamycin sulfate, 2.5 mM sodium pyruvate, and 5% heat-inactivated horse serum. Oocytes were allowed to recover overnight before injection.

Ambion’s T7 message machine in vitro transcription kit (Ambion, Austin, TX) was used to synthesize capped cRNA from library plasmid DNA linearized with BamHI. The cRNA product was quantitated in a spectrophotometer and diluted before injection to allow delivery of 20 ng of cRNA/oocyte in 15 nl with a 10-s injection.

Three days after injection, oocytes were divided into experimental groups (containing 10 oocytes each) and incubated at 22°C for 30 or 60 min in oocyte Ringer 2 (OR-2) containing 200 µM [14C]TEA (4 µCi/ml) in the absence or presence of inhibitors, and K+ and pH were adjusted as indicated. After uptake, oocytes were rapidly rinsed three times with ice-cold OR-2, placed in individual scintillation vials containing 0.5 ml 1 M NaOH, incubated at 65°C for 20 min, and neutralized with 0.5 ml 1 M HCl. Finally, 4.7 ml of Ecolube (ICN Biomedical, Cleveland, OH) were added, and oocyte radioactivity was measured as dpm in a Packard 1600TR liquid scintillation counter with external quench correction. TEA uptake was calculated as picromoles per oocyte, i.e., from dpm per oocyte and medium specific activity.

Electrical recordings. Three days after cRNA injection, oocyte membrane currents were measured using a conventional two-electrode (3 M KCl; resistance of 1 MΩ) voltage-clamp technique (Genedamp 500; Axon Instruments, Foster City, CA). The oocytes were continuously bathed with OR-2 or OR-2 containing 200 µM quinine (flowing at ~5 ml/min), and oocyte membrane potential was held at ~60 mV. Buffer containing 200 µM TEA was applied to the oocytes in a 10-s pulse via a solenoid-controlled valve. Recordings were sampled at 200 Hz and low-pass filtered at 100 Hz.

Tissue culture. The MDCK (established cell line derived from distal renal tubules of an adult female cocker spaniel) cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA) and was negative for mycoplasma upon receipt from ATCC. All MDCK lines were retested before publication and were found to be negative for mycoplasma. Cells were maintained in MEM supplemented with 10% FBS in a humidified incubator at 37°C with 5% CO2. Cultures were split 1:20 every 3–4 days.

Transfection. For mammalian cell transfection, the fragment containing the full-length rOCT2 cDNA was removed from the isolated library clone, pSPORT1/rOCT2, with the restriction enzymes BamHI and KpnI. The fragment was gel isolated and ligated into pcDNA3.1 (Invitrogen, Carlsbad, CA) cut with BamHI and KpnI, resulting in the plasmid pcDNA3.1/rOCT2. Activity of the new construct was confirmed by Xenopus oocyte expression assay before transfection. One day before transfection, 2 × 105 cells were plated into individual wells of a six-well culture plate (9.1 cm2). Cells were transfected with 10 µg plasmid DNA (pcDNA3.1 or pcDNA3.1/rOCT2) for 3 h at 37°C using SuperFect Reagent (5 µl SuperFect/µg DNA; Qiagen). Transfected cells were washed with PBS, given fresh medium, and maintained at 37°C with 5% CO2. Two days after transfection, cells were lifted, diluted to ~1 cell/ml, and plated in individual wells of a 24-well culture plate containing 1 mg/ml G418 (Invitrogen). Surviving cell clones were maintained with 200 µg/ml G418 and were retested for OC transport activity.

Cell culture transport assay. For filter experiments, 1 × 106 cells were plated onto 30-mm (4.2-cm2) Millicell-PCF 0.4-µm culture plate inserts (Millipore, Bedford, MA). Cell monolayers were cultured for 3 days in MEM supplemented with 10% FBS without G418 (2 ml on each side of the monolayer) in a humidified incubator at 37°C with 5% CO2. The culture medium was changed daily. Before transport experiments, the culture medium was removed from both sides of the monolayers, and the cells were washed two times with 2 ml of Hank’s balanced salt solution (HBSS; Sigma, St. Louis, MO), with a final application of 2 ml HBSS (pH 7.4) to each side. The HBSS was then removed from the basolateral, apical, or both sides and replaced with HBSS containing 100 µM [14C]TEA (2 µCi/ml) in the absence or presence of inhibitors, and K+ and pH were adjusted as indicated. After incubation at 37°C, the medium was removed from both sides of the monolayer, and the cells were rapidly rinsed three times with ice-cold 0.1 M MgCl2. The cells were dissolved in 2 ml 1 M NaOH and neutralized with 2 ml 1 M HCl. Aliquots were removed for protein assay (3) using a Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Hercules, CA) with BSA used as a
standard and liquid scintillation counting using 15 ml of Ecolume (ICN Biomedical). Uptake was calculated as picomoles of substrate per milligram protein.

For solid support experiments, 1 × 10⁶ cells were plated in individual wells (3.5 cm²) of a 12-well tissue culture plate. With the exception that the cells were only cultured for 2 days after plating, they were handled exactly as described above for the experiments done on filter inserts. For the efflux experiments, the culture medium was removed, and the cells were washed twice with 2 ml HBSS and incubated with 100 µM [¹⁴C]TEA in 2 ml HBSS for 60 min at 37°C. The TEA-containing HBSS was then removed, and the cells were rapidly rinsed two times with HBSS and then incubated at 22°C with 2 ml HBSS, HBSS plus 5 mM TMA, or HBSS adjusted to pH 5.4. Duplicate medium samples (25 µl) were removed at the times indicated. At the end of the experiment, the cells were washed, collected, and counted as described above. Total [¹⁴C]TEA cell content was calculated by summing all of the counts removed by medium sampling during the experiment and the counts remaining in the cells.

Statistics. Data are presented as means ± SE, and differences in mean values were considered to be significant at P < 0.05. Data between two experimental groups were compared using unpaired Student's t-test. Trends were evaluated using ANOVA procedures (25) and a nonparametric permutation test (12).

Chemicals. [¹⁴C]TEA (53 mCi/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO). Unlabeled TEA and quinine were obtained from Sigma. All other chemicals were obtained from commercial sources and were of the highest grade available.

RESULTS

Expression cloning. Total poly(A)⁺ RNA was isolated from rat kidney and was shown to mediate quininesensitive TEA uptake when expressed in Xenopus oocytes (33). A cDNA library was constructed and screened by expression cloning, yielding a clone encoding an OC transporter, rOCT2 (19, 33).

Characterization of rOCT2 transport in oocytes. The effect of both OCs and anions, and reduced temperature, on rOCT2-mediated TEA uptake was examined (Fig. 1). TEA uptake was markedly reduced by the presence of excess TEA (5 mM) or N¹-methylnicotinamide (NMN, 5 mM) or by the cation transport inhibitors quinine (200 µM) and quinacrine (200 µM). Incubation at 4°C also abolished transport. Transport was unaffected by the addition of the organic anions p-aminohippurate (1 mM) or probenecid (1 mM) or by the P-glycoprotein inhibitor cyclosporin A (10 µM).

Time course experiments (data not shown) indicated that uptake was linear for ~1 h, and, for kinetic measurements, 30 min was used to approximate the initial rate. Oocytes expressing the transporter were incubated in buffer containing 0.05–2 mM TEA, and uptake was measured (Fig. 2). The diffusion component was determined by measuring uptake at 2 and 3 mM TEA in the presence of 200 µM quinine. Double reciprocal plots were constructed (Fig. 2), and linear regression analysis yielded a mean Michaelis constant (Kₘ) value of 393 µM and a maximal velocity (Vₘₚₓ) of 333 pmol·30 min⁻¹·oocyte⁻¹ (n = 4). However, it must be noted that, due to the nature of the assay (i.e., relying on cRNA expression), Vₘₚₓ largely reflects the degree of cRNA expression (i.e., the number of functional transporters) rather than a true measure of uptake rate for a specific cell or transporter type.

At the normal uptake buffer K⁺ concentration (2.5 mM), oocytes injected with rOCT2 cRNA supported substantial TEA uptake that was largely insensitive to medium pH (Fig. 3A). However, when the external K⁺ concentration was raised to a level previously shown to short circuit the oocyte membrane potential (102.5 mM K⁺, with or without 10 µM valinomycin; see Ref. 11), not only was TEA uptake reduced by at least two-thirds at each pH, but the remaining TEA uptake exhibited significant pH dependence, e.g., P < 0.01 for pH 7.6 vs. pH 6.4 (Fig. 3B). Additionally, the variance among all four pH groups, under short-circuited conditions, was analyzed by a permutation test comparing the ranked order of uptake at each pH for each animal, simultaneously. TEA uptake was found to be significantly inhibited (P < 0.001) as medium pH was reduced, i.e., from ~50 to ~30 pmol/oocyte as pH was reduced from 8.0 to 6.4. Thus membrane potential provides the major...
driving force for TEA uptake mediated by this transporter, but uptake can be modulated by pH even under conditions where the potential can not change.

Further evidence for electrogenic TEA uptake by rOCT2-expressing oocytes was obtained by electrophysiological analysis under voltage-clamp conditions. Water-injected oocytes exposed to 200 µM TEA showed no electrical response to the substrate (Fig. 4A). However, a large inward current was recorded when rOCT2-expressing oocytes were exposed to a pulse of 200 µM TEA, indicating the movement of positive charge across
the oocyte membrane (Fig. 4B). In contrast, when the same oocyte was bathed in OR-2 with 200 µM quinine before reexposure to TEA, no depolarization was detected (Fig. 4C). Membrane potential was held at ~60 mV throughout the experiment. Furthermore, as membrane potential was clamped at progressively more positive potentials, inward current induced by TEA was substantially reduced (data not shown).

Characterization of rOCT2 transport in a stably transfected MDCK cell line. In an effort to establish an improved in vitro model for the study of rOCT2 transport, MDCK cells were transfected with the vector pcDNA3.1/rOCT2, and several stably transfected single cell clones were isolated. When grown on porous membrane filters (Millicell-PCF) and presented with [14C]TEA simultaneously on both basal and apical surfaces, one such clonal cell line (MDCK-rOCT2) showed significantly increased TEA uptake compared with the parental, untransfected MDCK cells or with a control clonal cell line stably transfected with the pcDNA3.1 vector alone (MDCK-pcDNA3.1; Fig. 5A). To determine if the MDCK-rOCT2 cell line was expressing the transporter in a polarized manner, [14C]TEA was applied either basally or apically to cells grown on Millicell-PCF filter inserts. After 60 min, the mediated TEA uptake in the MDCK-rOCT2 cells was significantly greater than in the MDCK-pcDNA3.1 control cells; however, there was no difference between basolateral and apical uptake levels in the rOCT2-expressing cells (Fig. 5B). To confirm this under conditions where “leak” through the cell monolayer could not be a factor, TEA was presented basally, apically, or simultaneously to both sides, and initial uptake rates (5 min uptake) were compared (Fig. 5C). Under these conditions, TEA uptake took place from both the basal and apical sides of the cells, and uptake was additive, i.e., basal only plus apical only equals simultaneous basal and apical. This observation raised the possibility that rOCT2 could be a “bifunctional” transporter, i.e., that it is expressed in both basolateral and apical membranes and is able to sense and respond to its local subcellular environment, functioning as a potential-driven OC transporter in the basolateral membrane and as an OC/H+ exchanger in the apical membrane. This mode of operation would be consistent with the previous observations made in Xenopus oocytes.

To further examine this possibility, the characteristics of apically expressed rOCT2 were determined in MDCK-rOCT2 cells grown on solid support. As shown in Fig. 6, rOCT2-mediated TEA uptake was significantly inhibited by the OCs TEA (5 mM), tetramethylammonium (TMA, 5 mM), and NMN (5 mM) and by the OC transport inhibitors quinine (200 µM) and quinacrine (200 µM). Apical uptake of TEA in MDCK-rOCT2 cells was unaffected by the MDR substrate cyclosporin A (10 µM).

A time course experiment indicated that TEA uptake was linear for at least 20 min; therefore, 3 min was used to approximate initial rate. TEA uptake in MDCK-rOCT2 cells exposed to a range of substrate concentrations from 0.005 to 1 mM was clearly saturable, and double reciprocal analysis yielded an estimated $K_m$ of 48 µM (Fig. 7).

At low $K^+$ concentration (2.5 mM), MDCK-rOCT2 cells showed a significant pH-dependent drop in TEA uptake as the buffer pH was lowered (e.g., $P < 0.001$ for pH 7.4 vs. pH 5.4), whereas TEA uptake in the parental MDCK cells was relatively unaffected by this treatment.
TEA (5 mM), and vector-transfected cells were similarly unresponsive (data not shown). When the buffer K\(^+\) concentration was raised to membrane-depolarizing levels (102.5 mM), TEA uptake in MDCK-rOCT2 cells was reduced significantly at each pH tested, again indicating a large potential-sensitive component to rOCT2 function (Fig. 8A). The parental MDCK cells (Fig. 8) and MDCK-pcDNA3.1 cells (data not shown) were unaffected by this change at pH 7.4. Additionally, as shown for oocytes expressing rOCT2 (Fig. 3), there was a significant decrease in TEA uptake in MDCK-rOCT2 cells as the buffer pH was lowered (e.g., P < 0.01 for pH 7.4 vs. pH 5.4), indicating a large pH-sensitive component. Indeed, ANOVA revealed that TEA uptake by MDCK-rOCT2 cells at low versus high K\(^+\) concentration and TEA uptake at higher versus lower pH (under both buffer conditions) were highly significant (P < 0.001). Permutation analysis also showed a significant correlation between decreased transport by MDCK-rOCT2 cells and a drop in medium pH under both conditions (P < 0.05).

To determine whether this pH-dependent decrease in uptake indicated that rOCT2 could function as an OC/H\(^+\) exchanger, we examined efflux of [\(^{14}\)C]TEA from preloaded cells. As a positive control, we first examined the effect of external TMA on rOCT2-mediated efflux (Fig. 9). As shown above, TMA is an effective inhibitor of TEA uptake by MDCK-rOCT2 cells (Fig. 6), presumably via competition for TEA transport. If so, TMA should trans-stimulate TEA efflux. As shown in Fig. 9, TEA efflux from MDCK-rOCT2 cells was markedly reduced in the presence of TMA (Fig. 9A).

**Fig. 8.** Effect of depolarization and/or medium pH on TEA uptake in MDCK and MDCK-rOCT2 cells. A 60-min uptake assay was performed 2 days after plating 1 × 10\(^6\) cells/well in 12-well culture plates. A: when external K\(^+\) concentration was low (2.5 mM), MDCK-rOCT2 cells supported a high degree of TEA uptake, which, unlike rOCT2 in the oocyte system, was highly pH sensitive. B: when external K\(^+\) concentration was raised to 102.5 mM, TEA uptake was reduced in MDCK-rOCT2 cells. However, depolarization failed to completely abolish TEA uptake, and decreasing buffer pH under this condition caused a corresponding decrease in substrate uptake. Background uptake in the parental MDCK cells was insensitive to depolarization at pH 7.4 but exhibited a similar pH-sensitive drop in TEA uptake as the buffer pH was lowered to 5.4. Data are mean values ± SE from a representative experiment (2 wells/treatment).

**Fig. 7.** Kinetic analysis of rOCT2-mediated TEA uptake in MDCK-rOCT2 cells. A: 2 days after plating, MDCK-rOCT2 cells were exposed to 0.005–1 mM TEA for 3 min, and total TEA uptake was measured. Uptake in the presence of 200 µM quinine was also determined at 0.005, 0.1, and 1 mM TEA concentrations as a measure of diffusion. Mediated uptake curve was generated by subtracting uptake due to diffusion from the total uptake value at each concentration measured. B: double reciprocal plot was constructed using the diffusion-corrected data, and linear regression analysis was performed, yielding an estimated K\(_m\) of 48 µM. Data are mean values ± SE from 4 wells/treatment.
trans-stimulated by the presence of 5 mM TMA in the buffer (Fig. 9A). TEA efflux from MDCK cells was less than that observed in MDCK-rOCT2 cells and was clearly unresponsive to trans-applied TMA (Fig. 9B). We next examined the effect of external H\(^+\) on TEA efflux from MDCK-rOCT2 cells (Fig. 10). In this experiment, TEA efflux from MDCK-rOCT2 cells at pH 7.4 occurred as previously observed, but efflux at pH 5.4 (100-fold increased external H\(^+\)) was markedly inhibited, unequivocally demonstrating that rOCT2 does not function as an OC/H\(^+\) exchanger.

**DISCUSSION**

Initially, the specificity of rOCT2-mediated transport was compared in Xenopus oocytes and stably transfected MDCK cells. TEA transport was saturable and inhibited by a variety of OCs in both systems. A \(K_m\) value for TEA of 393 µM (\(n = 4\)) was obtained in the oocyte system and is similar to the 500 µM value reported elsewhere for rOCT2 (15). This value is quite different from that measured for hOCT2 expressed in oocytes for the same substrate, \(K_m = 76 \pm 13\) µM, indicating that there may be species differences in the affinity of this transporter (8). Additionally, the \(K_m\) value for TEA of 48 µM measured in the MDCK-rOCT2 cells is markedly lower than that obtained in the oocyte system. However, it is in close agreement with that recently reported by Urakami et al. (32) in MDCK cells (45 µM) and with the high-affinity component seen by Grundemann et al. (10) for pOCT2 in HEK-293 cells (20 µM). Studies done on intact tubules have yielded somewhat varied estimates of \(K_m\) for TEA. A \(K_m\) value of 108 µM has been reported in rabbit tubules and 160 µM in tubules from rat (9, 31). These data, like those obtained for the rat and human OCT2 homologues in the oocyte system, may be explained by a species difference in substrate affinity. Alternatively, this apparent discrepancy could be explained by the presence of multiple transporters expressed at different ratios, e.g., OCT1 and OCT2, so that studies on intact renal tissues measure the average \(K_m\) for all functioning transporters, whereas oocyte studies measure the \(K_m\) for whichever individual purified transporter is being expressed.

Previous studies have firmly established that transport of OCs across the basolateral membrane of kidney tubule cells is driven by the potential difference across the membrane (14, 24, 28, 31) and that cation transport across the brush-border membrane involves a H\(^+\)-coupled antiporter (13, 20, 28, 34). To test whether rOCT2 exhibits either of these properties, the effect of membrane potential and/or medium pH on rOCT2-mediated [\(^{14}\)C]TEA uptake was examined (Figs. 3 and 8). Regardless of medium pH, TEA uptake was significantly reduced under depolarizing conditions (102.5 mM K\(^+\)), confirming that the major driving force for rOCT2-mediated uptake is the membrane potential. However, when the large potential-driven component was removed by short circuiting the oocytes, an underlying H\(^+\)- or H\(^-\)-gradient-sensitive component was observed. This result is in contrast to previous reports on rOCT2-, hOCT2-, and rOCT1-mediated TEA uptake in oocytes (8, 11, 19). However, these differences may be largely technical. The rOCT2 studies did not use depolarizing
conditions to isolate potential- and pH-sensitive components (19), whereas the hOCT2 studies used voltage-clamped oocytes but did not examine the effects of decreasing medium pH (8). Although our data showed clear pH dependence, they did not distinguish between modulation of transporter activity by external H+ or OC/H+ exchange. Therefore, we examined the mechanism of rOCT2 transport in a renal cultured cell system in which driving forces could be more easily dissected.

In contrast to the oocyte system, the MDCK-rOCT2 cells showed a marked response to medium pH, even under nondepolarizing conditions (2.5 mM K+). This observation is similar to results for pOCT2 (expressed in HEK-293 cells), rOCT2 (expressed in MDCK and NIH/3T3 cells), rOCT3 (expressed in HeLa cells), and hOCTN1 (expressed in HEK-293 cells) examined in cell culture systems, where TEA uptake was inhibited by H+ (10, 21, 29, 32). Moreover, the inhibition of MDCK-rOCT2 TEA uptake by lowering medium pH was even greater when the cells were short circuited (102.5 mM K+), a condition unexamined by the previous studies. Interestingly, the parental MDCK cells showed little, if any, potential-sensitive uptake and were relatively insensitive to medium pH until they were incubated with high-K+ (102.5 mM) buffer. Therefore, expression of rOCT2 correlates with potential-sensitive, pH-sensitive uptake.

To determine directly whether this cis inhibition involved H+ exchange, the effect of trans-H+ on TEA efflux from MDCK-rOCT2 cells was examined. As shown in Fig. 10, external H+, i.e., trans-H+, produced significant inhibition of TEA efflux, conclusively demonstrating that rOCT2 is not an OC/H+ exchanger, even when expressed in the apical membrane of a renal epithelium. At present, the mechanism mediating inhibition of rOCT2 by external H+ is uncertain but may reflect an allosteric modification of the carrier, similar to control of Na/H+ exchanger activity by internal H+ (1). Nevertheless, it is clear that rOCT2 is not an OC/H+ exchanger. Thus it mediates potential-driven OC transport, precisely the mechanism required for the basolateral entry step in renal OC secretion.

Indeed, examination of the tissue distribution of the OCT family isoforms suggests they may be expressed in a tissue-specific manner. Data on OCT1 are unclear, with Northern blot, RT-PCR, and in situ hybridization yielding conclusions varying from OCT1 being exclusively expressed in the liver to it possibly having a housekeeping function due to its seemingly ubiquitous distribution (8, 10, 11, 23, 30, 32, 35). Thus far, the only consistent finding for OCT1 is its abundant expression in liver. Northern blot and RT-PCR analysis of OCT2, on the other hand, has been very consistent, with strong expression detected in kidney and no detectable expression in the liver (8, 10, 19, 32). Moreover, expression of OCT2 in proximal tubules has been demonstrated both by RT-PCR using mRNA from a cell line derived from proximal tubule (IHKE cells) and by in situ hybridization (8). Currently, only Northern blot data are available for OCT3, but they indicate that it is expressed predominantly in placenta, with weak expression in kidney and none in liver. Thus OCT2 seems poised to fulfill the physiological role of OC uptake at the renal basolateral membrane.

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REFERENCES


