Interaction of cations, anions, and weak base quinine with rat renal cation transporter rOCT2 compared with rOCT1

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Arndt, Petra, Christopher Volk, Valentin Gorboulev, Thomas Budiman, Christian Popp, Isabel Ulzheimer-Teuber, Aida Akhoundova, Ernst Bamberg, Georg Nagel, and Hermann Koepsell. Interaction of cations, anions, and weak base quinine with rat renal cation transporter rOCT2 compared with rOCT1. Am J Physiol Renal Physiol 281: F454–F468, 2001.—The rat organic cation transporter (rOCT)-2 was characterized by electrical and tracer flux measurements compared with rOCT1. By applying choline gradients to voltage-clamped Xenopus oocytes expressing rOCT2, potential-dependent currents could be induced in both directions. Tracer flux measurements with seven organic cations revealed similar Michaelis-Menten constant values for both transporters, with the exception of guanidine. In parallel experiments with rOCT2 and rOCT1, inhibition of tetraethylammonium transport by 12 cations, 2 weak bases, corticosterone, and the anions para-amminohippurate, α-ketoglutarate, and probenecid was characterized. The IC₅₀ values of many inhibitors were similar for both transporters, whereas others were significantly different. Mepiperphenidol and O-methylisoprenaline showed an ~70-fold lower and corticosterone a 38-fold higher affinity for rOCT2. With the use of these inhibitors together with previous information on cation transporters, experimental protocols are proposed to dissect out the individual contributions of rOCT2 and rOCT1 in intact proximal tubule preparations. Inhibition experiments at different pH levels strongly suggest that the weak base quinine passively permeates the plasma membrane at physiological pH and inhibits rOCT2 from the intracellular side.

The tissue concentration of endogenous cations, cationic drugs, or xenobiotics is influenced by polyspecific organic cation transporters (OCTs) in kidney, liver, small intestine, and brain (5, 17, 18, 31). In 1994, we cloned the rat OCT1 (rOCT1), which proved to be the first member of a rapidly growing family of polyspecific transporters (9, 18). This family contains a subfamily of electroneutral cation transporters, a subfamily containing OCTs and the sodium-dependent carnitine transporter, and a subfamily of organic anion transporters (OATs) (2, 18). The subfamily of electroneutral cation transporters is composed of the transporter subtypes OCT1, OCT2, and OCT3. OCT1 was first cloned from rat (9) and subsequently isolated from mouse (27), human (6, 41), and rabbit (30). By employing immunohistochemistry, rOCT1 was localized to the sinusoidal membrane of hepatocytes (20) and to basolateral membranes of renal proximal tubules (15, 28). Tracer flux and electrical measurements in Xenopus oocytes showed that OCT1 from rat and human is electronegenic, translocates a variety of small cations, and is inhibited by some large cations that are not transported (3, 4, 9, 22).

OCT2 is a closely related subtype that shares ~70% amino acid identity with OCT1. OCT2 has been cloned from rat (24), human (6), pig (8), and mouse (21). It is mainly expressed in the kidney but has also been detected in neurons (3). After OCT2 from pig, human, and rat was characterized in several papers (6, 10, 11, 15, 24–26, 29, 34), controversial issues concerning localization and function have been solved. Preliminary immunohistological experiments suggested that human OCT2 in the kidney is localized at the luminal membrane of distal tubules (6) and supported the speculation that OCT2 in kidney might be an apical electroneutral proton/cation antiporter that mediates cation efflux into the tubular lumen (8, 10, 11). However, electroneutral proton/cation antiport function was ruled out by functional measurements that showed that OCT2 from human and rat can be driven by the membrane potential (3, 6, 18, 29) and that substrate uptake and efflux via OCT2 from rat (rOCT2) is not influenced by pH gradients (29). Because OCT2 in rat kidney has been localized unequivocally to the basolateral membrane of proximal tubule cells (15, 28), it is reasonable to assume that OCT2 is also localized at the basolateral membrane in humans (for further discussion, see Ref. 15). The overlapping localization of rOCT1 and rOCT2 in the proximal tubule demanded their detailed functional comparison to determine their
METHODS

Cloning of rOCT2. For cloning of rOCT2, the cDNA of rOCT1 (9) was used as a probe to screen a rat kidney cDNA library under high-stringency conditions (6). A positive cDNA with a restriction pattern different from rOCT1 was cloned into pBluescript II SK(−) and sequenced on both strands using the dideoxynucleotide chain-termination method. The sequence was submitted to GenBank/EBI Data Bank with the accession number X98334 (4). The derived amino acid sequence is identical to the sequence reported by Gründemann et al. (8) but differs in two amino acids from the original sequence reported by Okuda et al. (24). In the latter sequence, Asn332 is replaced by Lys, and Phe335 by Ile. For expression in X. laevis oocytes, rOCT2 was subcloned into the pOG2 vector containing untranslated regions of the Xenopus ß-globin gene (4). For the expression in human embryonic kidney (HEK)-293 cells, rOCT2 was subcloned into the pRc-CMV vector (Invitrogen, Groningen, The Netherlands).

Expression of rOCT2 and rOCT1 in Xenopus oocytes and HEK-293 cells. For expression in Xenopus oocytes, pOG2 vector containing rOCT2 and pRSSP vector containing rOCT1 (4) were linearized with NotI and MluI, respectively, and sense cRNAs were transcribed with the use of T7 or SP6 RNA polymerase as described earlier (36). Xenopus oocytes were defolliculated with collagenase A and stored for several hours in Ori buffer [5 mM 3-N-morpholino)propanesulfonic acid-NaOH, pH 7.4, 100 mM NaCl, 3 mM KCl, 2 mM CaCl2, and 1 mM MgCl2] containing 50 mg/l gentamycin. Before mRNA injection, the oocytes were incubated for 5–15 min in hyperosmolar Ori buffer (130 mM NaCl). The oocytes were then injected with 50 nl H2O/oocyte with or without 10 ng of rOCT2 or rOCT1 cRNA. For a comparison between rOCT2 and rOCT1 in side-by-side experiments, cRNAs of rOCT2 or rOCT1 were injected within 3 h into oocytes from the same batch. For transporter expression, the oocytes were incubated at 16°C in Ori buffer containing 50 mg/l gentamycin (2–3 days for tracer flux measurements, or 3–5 days for electrical measurements). For transient expression of rOCT2 in mammalian cells, HEK-293 cells grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum were transfected with the empty vector pRcCMV or with pRcCMV containing rOCT2, using the FuGENE 6 reagent from Roche Molecular Biochemicals (Mannheim, Germany).

Tracer uptake measurements. Uptake measurements of radioactive cations into oocytes of X. laevis and into HEK-293 cells were performed as described (3). To compare transport by rOCT2 and rOCT1 expressed in the same batch of oocytes in side-by-side experiments, uptake by both transporters was measured within 4 h. For uptake measurements, the oocytes were incubated at 19°C for 10 min or 1 h with the radioactive substrates in the absence or presence of inhibitors, and the uptake was stopped by addition of ice-cold Ori buffer. It was verified that the uptake of 400 μM tetraethylammonium (TEA) into oocytes expressing rOCT2 or rOCT1 was linear for 90 min (data not shown). Measurements of choline uptake in transiently transfected HEK-293 cells were performed 2 days after transfection when the cells had reached confluence. The cells were washed with phosphate-buffered saline (PBS), suspended by shaking, collected by 10-min centrifugation at 1,000 g, and suspended at 37°C in PBS. For uptake, the cells were incubated between 1 and 60 s in PBS (37°C) with or without 100 μM tetrapentylammonium (TPEa). Uptake was stopped by addition of ice-cold PBS containing 100 μM quinine (stop solution), and the cells were washed three times with ice-cold stop solution.

Electrophysiology. Electrical measurements were performed on Xenopus oocytes that had been kept at 16°C in Ori buffer, or on oocytes that had been incubated for 12 h in Ori buffer containing 10 mM choline. The two-electrode voltage-clamp measurements were performed as described earlier (3). The oocytes were continuously superfused at room temperature (~3 ml/min). For the determination of current-voltage relationships, steady-state currents were averaged during the last 100 ms of 500 ms of voltage pulses that were applied at a frequency of 0.4 Hz from a holding potential of −50 mV.
Calculation and statistics. In Xenopus oocytes, substrate dependence of uptake and maximal velocity (maximal transport rate; \( V_{\text{max}} \)) values were calculated from 8–10 oocytes incubated without inhibitor minus 8–10 oocytes incubated with inhibitor per data point. The uptake in the presence of different inhibitor concentrations was measured from 8–10 oocytes incubated with a specific inhibitor concentration per data point. In HEK-293 cells, uptake was calculated from four determinations in the absence and four determinations in the presence of inhibitor. For each data point, the mean ± SE was calculated. From individual uptake experiments with different substrate concentrations, apparent Michaelis-Menten constant (\( K_m \)) ± SD values were calculated by fitting the Michaelis-Menten equation to the data. For some substrates, the Michaelis-Menten equation was fit to each of three or four individual experiments, and means ± SD of the \( K_m \) values were calculated. IC\(_{50}\) values were calculated from individual dose-response curves by fitting the Hill equation for multisite inhibition to the data. For inhibitors with largely different affinities, three to five individual inhibition experiments were performed side by side, and means ± SD of the IC\(_{50}\) values were calculated. The unpaired Student’s \( t \)-test was employed to evaluate the significance of differences between means of \( K_m \), IC\(_{50}\), or \( V_{\text{max}} \) values. The straight lines shown (see Figs. 4, 6, 7, and 9) were obtained by linear regression analysis.

Materials. [\(^{3}H\)]choline (2.6 TBq/mmol) and [\(^{3}H\)]histamine (1.9 TBq/mmol) were obtained from Amersham Buchler (Braunschweig, Germany). [\(^{14}C\)]TEA (1.9 TBq/mmol), [\(^{14}C\)]guanidine (2.0 GBq/mmol), [\(^{3}H\)]1-methyl-4-phenylpyridinium (3.1 TBq/mmol), [\(^{3}H\)]quinine (0.56 TBq/mmol), and [\(^{3}H\)]quinidine (0.54 TBq/mmol) were purchased from Biotrend (KölN, Germany). [\(^{3}H\)]N\(^{-}\)-methylisococaine (0.11 TBq/mmol) was purchased from ICN Biochemicals (Meckenheimer, Germany). Unlabeled cyanine-863, decynium-22, quinine, quinidine, desipramine, procainamide, N\(^{-}\)-methylisococaine (NMM), PAH, probenecid, and AKG were obtained from Sigma (Deisenhofen, Germany); tetramethylammonium, tetrathymethylammonium, tetrabutylammonium (TBuA), TPeA, and corticosterone from Fluka (Neu-Ulm, Germany); 3-O-methylnorepinephrine from Boehringer (Ingelheim, Germany); and meperphenidol from Merck Sharp and Dohme (Rahway, NJ). The other chemicals were obtained as described earlier (4).

RESULTS

Electrical properties of choline uptake by rOCT2. Superfusion of \( X. laevis \) oocytes expressing rOCT2 that were clamped to \(-50\) mV with choline, TEA, NMM, and procainamide induced inward currents as above has been described for rOCT1 (4). These currents were not observed in water-injected control oocytes (e.g., Fig. 1A). The inward currents induced by 10 mM choline or other transported cations could be completely inhibited by 200 \( \mu \)M quinine or 100 \( \mu \)M cyanine863 (data not shown). In oocytes with high transport activity that were superfused for 60 s with 10 mM choline, a transient outward current was observed after the removal of choline from the bath solution (Fig. 1C). This outward current was inhibited by 200 \( \mu \)M quinine (see right trace in Fig. 1C). It may represent electrogenic efflux of choline that had been taken up by the oocyte (see Fig. 2). Current-voltage (I-V) curves from the water-injected oocyte in Fig. 1A or from the rOCT2-in-

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Fig. 1. Choline-induced inward currents in Xenopus oocytes expressing rat organic cation transporter (rOCT)-2. Xenopus oocytes were injected with water (control) or 10 ng of rOCT2 cRNA and incubated for 4 days. The oocytes were voltage-clamped and superfused with Ori buffer (○) or with Ori buffer containing 10 mM choline (●). A: steady-state currents in water-injected oocyte clamped at \(-50\) mV. B: current-voltage (I-V) curves from the control oocyte in A. C: steady-state currents in 2 rOCT2-expressing oocytes clamped at \(-50\) mV. The trace on the left is from an oocyte that was superfused with Ori buffer without and with 10 mM choline. The superimposed traces on the right are from another oocyte. In a first superfusion period (solid line), this oocyte was superfused without or with Ori buffer containing 10 mM choline. After 15 min of superfusion with Ori buffer (not shown), the same oocyte was superfused first with Ori buffer containing 10 mM choline and then with Ori buffer containing 200 \( \mu \)M quinine (dotted line). D: I-V curves that were obtained during superfusion of the left oocyte in C.
To circumvent these problems, we tried to demonstrate cation gradient-induced outward currents by rOCT2 in intact oocytes. We preloaded water-injected control oocytes and rOCT2-expressing oocytes with choline by incubating them for 12 h in 10 mM choline. When control oocytes and rOCT2-expressing oocytes were clamped to −50 mV and superfused with 10 mM choline, no significant quinine-inhibitable currents were detected (data not shown). When choline-preloaded oocytes were superfused with choline-free buffer, however, a large outward current was observed in rOCT2-expressing oocytes but not in water-injected control oocytes (Fig. 2, A and C). This rOCT2-mediated outward current was abolished when 100 μM cyanine863 or 200 μM quinine were added to the bath (data not shown). I-V curves from water-injected control oocyte and rOCT2-expressing oocyte of Fig. 2, A and C, are shown in Fig. 2, B and D. The rOCT2-mediated outward current that was induced by the outwardly directed choline gradient and could be inhibited by cyanine863 (see difference curve in Fig. 2D) was decreased at more negative membrane potentials. Taken together, these findings show that rOCT2 is able to mediate electrogenic choline transport in both directions, as has been shown for rOCT1 (22). In rOCT2-expressing oocytes clamped to −50 mV, the outward currents obtained by preloading the oocytes in 10 mM choline and superfusing with choline-free buffer were higher than the inward currents that were obtained by superfusion of nonpreloaded oocytes with 10 mM choline (compare, for example, Figs. 1 and 2).

In two batches of Xenopus oocytes clamped to −50 mV, we compared the induced inward and outward currents by rOCT2. In these oocytes, the choline-induced inward current, measured as in Fig. 1, was 29 ± 3 nA (means ± SD, n = 10), whereas the current induced by removal of choline from the bath solution, measured as in Fig. 2, was 127 ± 22 nA (means ± SD, n = 10). By performing an analogous experiment with two batches of oocytes expressing rOCT1, we obtained choline-induced inward currents of 9 ± 2 nA (means ± SD, n = 4) and currents induced by choline removal of 26 ± 1 nA (means ± SD, n = 5). The data show that rOCT1 and rOCT2 may mediate significant choline efflux at normal membrane potential if the transmembrane concentration gradient is high enough.

Next, we measured the concentration dependence of choline-induced currents at three different membrane potentials. Figure 3 shows an experiment where rOCT2-expressing oocytes that were not preloaded were clamped to 0, −50, or −90 mV and superfused with various concentrations of choline. By fitting the Michaelis-Menten equation to the data, apparent K_m values of 0.70 ± 0.09 mM (0 mV), 0.38 ± 0.05 mM (−50 mV), and 0.27 ± 0.04 mM (−90 mV) and apparent maximal current (I_max) values of 136 ± 15 nA (0 mV), 232 ± 8 nA (−50 mV), and 282 ± 13 nA (−90 mV) were determined. The data indicate that the K_m and I_max for electrogenic choline uptake are potential dependent, as has been described for cation transport by rOCT1 and rOCT3 (4, 16). Inward currents could also be induced.
when rOCT2-expressing oocytes were superfused with other substrates. When rOCT2-expressing oocytes were clamped to −50 mV and superfused with saturating concentrations of TEA (1 mM), guanidine (10 mM), choline (10 mM), procainamide (10 mM), NMN (10 mM), histamine (10 mM), and 1-methyl-4-phenylpyridinium (MPP) (100 μM), the following relative inward currents were obtained (mean ± SD from 3–8 oocytes from 2 oocyte batches, relative to the TEA-induced current): TEA 1.0 ± 0.10, guanidine 2.14 ± 0.24, choline 1.96 ± 0.45, NMN 1.16 ± 0.24, histamine 1.12 ± 0.18, MPP 0.71 ± 0.14, and procainamide 0.12 ± 0.01.

Concentration dependence and Vmax of cation uptake by rOCT2 compared with rOCT1. In Xenopus oocytes injected with rOCT2 mRNA, the uptake of [3H]NMN, [14C]TEA, [3H]MPP, [14C]histamine, and [3H]guanidine was at least 10 times higher than in water-injected control oocytes, showed substrate saturation, and was inhibitable by >95% with specific inhibitors (100 μM quinine) or 100 μM decynium-22. In water-injected control oocytes, the uptake of these cations showed no saturation and was insensitive to cyanine-863 and decynium-22 (data not shown). In control oocytes, a higher uptake was observed for choline compared with the other cations (Fig. 4A). However, this uptake increased linearly with the substrate concentration and was insensitive to cyanine-863. By fitting the Michaelis-Menten equation to cyanine-863-inhibitable uptake of TEA, choline, NMN, and MPP in single experiments performed on different oocyte batches of rOCT2-expressing oocytes, apparent Km values of 91 μM (TEA), 0.6 mM (choline), 0.25 mM (NMN), and 17 μM (MPP) were obtained. These values are similar to those that have been determined for rOCT1 by tracer flux in Xenopus oocytes (7, 9, 17) and are summarized in Table 1. For the uptake of histamine and guanidine by rOCT2 and rOCT1, the substrate dependency was measured in four side-by-side experiments that were performed as described in METHODS (Table 1, values with index “c”). For rOCT2 and rOCT1, apparent Km values for histamine uptake were similar: 0.28 ± 0.05 mM (rOCT2) vs. 0.30 ± 0.12 mM (rOCT1). The Km values for guanidine uptake, however, differed by a factor of 10: 0.17 ± 0.06 mM (rOCT2) vs. 1.7 ± 0.7 mM (rOCT1) (P < 0.05 for difference).

Next, we compared the maximum transport rates of different cations mediated by rOCT2 compared with rOCT1. In each of five side-by-side experiments (see METHODS), oocytes from a single batch were injected within 3 h with 10 ng/oocyte of either rOCT1 or rOCT2 cRNA, and after 3 days, the Vmax values for cyanine-863-inhibitable uptake of TEA, choline, NMN, histamine, guanidine, and MPP were measured within 4 h. During this time period, electrical measurements were performed on five oocytes from each individual experiment. rOCT2-expressing oocytes clamped at −50 mV were superfused with 0.65 mM TEA, and the TEA-induced currents were determined in the absence and presence of 200 μM quinine. In the tracer flux experiments, the uptake rate of 0.65 mM TEA expressed by rOCT2 was three times higher than the uptake of 0.65 mM TEA expressed by rOCT1 [0.47 ± 0.06 (rOCT2) vs. 0.15 ± 0.06 (rOCT1), in nmol-oocyte⁻¹-h⁻¹, P < 0.01.
The ratios of the respective [14C]TEA uptake rates may be rents expressing rOCT2 and rOCT1 compared with the (rOCT1). The greater ratio of the TEA-induced cur-

The Michaelis-Menten equation. Means (TEA), and 0.15 mM 1-methyl-4-phenylpyridinium (MPP) using the near saturation [6 mM guanidine, 2 mM choline and calculated from uptake measurements with substrate concentrations 

The maximal transport rate (Vmax) values were calculated from the respective values of rOCT1 are indicated (d, e, f, g). By superfusion of oocytes expressing rOCT2 or rOCT1, with 0.65 mM TEA clamped at −50 mV, sevenfold higher currents were obtained with rOCT2 [28.6 ± 5.2 nA (rOCT2) vs. 4.0 ± 1.2 nA (rOCT1)]. The greater ratio of the TEA-induced currents expressing rOCT2 and rOCT1 compared with the ratios of the respective [14C]TEA uptake rates may be explained by a more pronounced membrane depolarization in the course of TEA uptake in rOCT2-expressing oocytes. Because the tracer flux measurements were performed with nonclamped oocytes, the degree of oocyte depolarization depends on the magnitude of the expressed electrogenic cation uptake. Membrane depolarization would lead to a reduction of the apparent Vmax.

To compare the uptake rates of different transported cations in a given isoform, we normalized the maximal uptake rates for various individual cations to the maximum TEA uptake rate measured in the respective experiment, which was set to 1.0 (Table 1). For rOCT2, the Vmax values of guanidine, choline, and histamine were significantly higher than the Vmax of TEA, whereas the Vmax of MPP was significantly lower. In contrast, for rOCT1, only the Vmax of guanidine was significantly higher than that of TEA. Making the assumption that substrate binding was not rate limiting in our Vmax measurements, the comparison between the normalized Vmax values by rOCT2 and rOCT1 shows that rOCT2 has a higher turnover num-

Table 1. Apparent kinetic constants for cations transported by rOCT2 and rOCT1

<table>
<thead>
<tr>
<th>Transported Cations</th>
<th>rOCT2</th>
<th>rOCT1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Km, μM</td>
<td>Vmax normalized</td>
</tr>
<tr>
<td>Guanidine</td>
<td>172 ± 57</td>
<td>2.25 ± 0.31</td>
</tr>
<tr>
<td>Choline</td>
<td>604 ± 102</td>
<td>2.16 ± 0.39</td>
</tr>
<tr>
<td>Histamine</td>
<td>275 ± 54</td>
<td>1.73 ± 0.20</td>
</tr>
<tr>
<td>NMN</td>
<td>245 ± 30</td>
<td>1.03 ± 0.22</td>
</tr>
<tr>
<td>TEA</td>
<td>91 ± 10</td>
<td>0.31</td>
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<tr>
<td>MPP</td>
<td>17 ± 2</td>
<td>0.21 ± 0.12</td>
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Apparent kinetic constants for cations that are transported by rat organic cation transporter (rOCT)-2 and rOCT1. X. oocytes were injected with 10 ng of rOCT2 cRNA or rOCT1 cRNA, and the cyanine863-inhibitable uptake was measured after 1 h of incubation with different concentrations of radioactively labeled compounds. a Michaelis-Menten constant (Km) ± SD derived by fitting the Michaelis-Menten equation to individual experiments. b Ranges of Kmax values from new and earlier experiments (7, 17). c Means ± SD of Kmax values that were calculated from 4 separate side-by-side experiments (see METHODS). The maximal transport rate (Vmax) values were calculated from uptake measurements with substrate concentrations near saturation [6 mM guanidine, 2 mM choline and N’-methylnicotinamide (NMN), 1 mM histamine, 0.65 mM tetraethylammonium (TEA), and 0.15 mM 1-methyl-4-phenylpyridinium (MPP)] using the Michaelis-Menten equation. Means ± SD of 5 independent side-by-side experiments are presented. The values were normalized to the Vmax value of the respective transporter obtained with TEA. Vmax and Km values of rOCT2 that are significantly different from the respective values of rOCT1 are indicated (e, f, g). Significant differences between the Vmax values for TEA and the other substrates for a given isoform are indicated (i, j, k). By superfusion of oocytes expressing rOCT2 or rOCT1, with 0.65 mM TEA clamped at −50 mV, sevenfold higher currents were obtained with rOCT2 [28.6 ± 5.2 nA (rOCT2) vs. 4.0 ± 1.2 nA (rOCT1)]. The greater ratio of the TEA-induced currents expressing rOCT2 and rOCT1 compared with the ratios of the respective [14C]TEA uptake rates may be explained by a more pronounced membrane depolarization in the course of TEA uptake in rOCT2-expressing oocytes. Because the tracer flux measurements were performed with nonclamped oocytes, the degree of oocyte depolarization depends on the magnitude of the expressed electrogenic cation uptake. Membrane depolarization would lead to a reduction of the apparent Vmax.

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Fig. 5. Expression of rOCT2-mediated choline uptake in human embryonic kidney (HEK)-293 cells. HEK-293 cells were transiently transfected with the pRcCMV vector containing rOCT2 or with the empty vector. At 2 days after transfection, the time course of [3H]choline uptake was dependent on the expression system, we transiently transfected HEK-293 cells with rOCT2 and measured the time course of [3H]choline uptake in the absence and presence of 100 μM TPeA. Figure 5 shows that the rOCT2-transfected HEK-293 cells exhibited a significantly higher TPeA-inhibitable uptake of 50 μM [3H]choline than HEK-293 control cells that were transfected with the empty vector. The observation that a fraction of the choline uptake into control cells could also be inhibited by TPeA may be explained by the fact that HEK-293 cells express small amounts of endogenous human OCT1 and show low activity of organic cation transport (Ref. 19 and unpublished data).
transport was measured within 4 h. These precautions are important because the affinity of rOCT2 and rOCT1 for cations is influenced by the membrane potential (Fig. 3 and Ref. 4), and the membrane potential may differ between oocyte batches. Inhibition experiments with choline and NMN revealed IC\textsubscript{50} values for rOCT2 and rOCT1 that were in the same range as the apparent K\textsubscript{m} values shown in Table 1 (choline: rOCT2 0.34 ± 0.04 mM, rOCT1 0.35 ± 0.5 mM; NMN: rOCT2 0.27 ± 0.01 mM, rOCT1 0.27 ± 0.02 mM). Comparable IC\textsubscript{50} values have been reported by Okuda et al. (25) for choline, but these authors obtained IC\textsubscript{50} values for NMN that were more than five times higher. For the inhibition of TEA uptake by MPP, we estimated IC\textsubscript{50} values of 46 ± 5 \mu M (rOCT2) and 24 ± 10 \mu M (rOCT1). Similar values have been reported earlier (25). In Table 2, IC\textsubscript{50} values for the inhibition of TEA uptake by nine additional cations and by the weak bases quinine and quinidine are summarized. The mean IC\textsubscript{50} values ± SD presented for TPeA, tetrapropylammonium, quinine, and tetramethylammonium were calculated from individual side-by-side experiments with rOCT2 and rOCT1. Because no large difference between rOCT2 and rOCT1 became apparent, these experiments were not repeated. For cyanine-863, quinine, procainamide, meiperphenidol, and O-methylisoprenaline, we performed three to five side-by-side experiments and calculated the mean IC\textsubscript{50} values. For cyanine-863, quinine, procainamide, meiperphenidol, and O-methylisoprenaline, 5 to 70 times higher IC\textsubscript{50} values were obtained for rOCT2 compared with rOCT1. In contrast, the IC\textsubscript{50} value for guanidine was more than 20 times smaller for rOCT2 than for rOCT1. It is noteworthy that quinine uptake, the uptake of \textsuperscript{[3H]}quinine was measured side by side in water-injected control oocytes, rOCT2-expressing oocytes, and rOCT1-expressing oocytes. In addition to pH 7.4, these measurements were also performed at pH 6 to reduce the nonspecific uptake of quinine that correlates with the fraction of uncharged quinine. Quinine has an acid dissociation constant (pK\textsubscript{a}) value of 8.4 (23), and the fraction of uncharged quinine is 10% at pH 7.4 and <1% at pH 6.0. For the uptake of 0.1 \mu M \textsuperscript{[3H]}quinine in the absence or presence of 100 \mu M cyanine863, the following values (mean values ± SE, in pmol oocyte\textsuperscript{-1}.h\textsuperscript{-1}) were obtained: control pH 7.4, 0.34 ± 0.01 vs. 0.33 ± 0.01; control pH 6.0, 0.06 ± 0.01 vs. 0.02 ± 0.01; rOCT2 pH 7.4, 0.32 ± 0.03 vs. 0.32 ± 0.01; rOCT2 pH 6.0, 0.05 ± 0.01 vs. 0.01 ± 0.01; rOCT1 pH 7.4, 0.83 ± 0.05 vs. 0.32 ± 0.02; and rOCT1 pH 6.0, 0.16 ± 0.02 vs. 0.02 ± 0.01. In water-injected control oocytes, a very small but significant (P < 0.01) cyanine-863-inhibitable uptake of quinine became visible at pH 6.0 that was not detected at pH 7.4. After expression of rOCT2, no additional cyanine-863-inhibitable uptake could be observed. This result was confirmed by electrical measurements. By superfusion with 100 \mu M quinine at pH 7.4 or pH 6.0, no inward currents could be detected in rOCT2-expressing oocytes that were clamped at -40 mV. In the same oocytes, regular-sized inward currents >80 nA were observed on superfusion with 10 mM choline. In rOCT1-expressing oocytes at pH 7.4, significant (P < 0.001) cyanine-863-inhibitable quinine uptake was detected as reported earlier (22). In rOCT1-expressing oocytes at pH 6.0, the cyanine-863-inhibitable quinine uptake was smaller compared with that at pH 7.4, but it was still significantly larger (P < 0.001) than in control oocytes. The data show that rOCT2 does not mediate detectable transport of quinine. In contrast, a small amount of quinine uptake was induced on expression of rOCT1 (see DISCUSSION).

**Investigation of quinine for transport by rOCT2 and rOCT1.** To compare rOCT2 and rOCT1 with respect to quinine uptake, the uptake of \textsuperscript{[3H]}quinine was measured side by side in water-injected control oocytes, rOCT2-expressing oocytes, and rOCT1-expressing oocytes. For the uptake of 0.1 \mu M \textsuperscript{[3H]}quinine, the following results were obtained: control pH 7.4, 0.34 ± 0.01 vs. 0.33 ± 0.01; rOCT2 pH 7.4, 0.32 ± 0.03 vs. 0.32 ± 0.01; rOCT2 pH 6.0, 0.05 ± 0.01 vs. 0.01 ± 0.01; rOCT1 pH 7.4, 0.83 ± 0.05 vs. 0.32 ± 0.02; and rOCT1 pH 6.0, 0.16 ± 0.02 vs. 0.02 ± 0.01. In water-injected control oocytes, a very small but significant (P < 0.01) cyanine-863-inhibitable uptake of quinine became visible at pH 6.0 that was not detected at pH 7.4. After expression of rOCT2, no additional cyanine-863-inhibitable uptake could be observed. This result was confirmed by electrical measurements. By superfusion with 100 \mu M quinine at pH 7.4 or pH 6.0, no inward currents could be detected in rOCT2-expressing oocytes that were clamped at -40 mV. In the same oocytes, regular-sized inward currents >80 nA were observed on superfusion with 10 mM choline. In rOCT1-expressing oocytes at pH 7.4, significant (P < 0.001) cyanine-863-inhibitable quinine uptake was detected as reported earlier (22). In rOCT1-expressing oocytes at pH 6.0, the cyanine-863-inhibitable quinine uptake was smaller compared with that at pH 7.4, but it was still significantly larger (P < 0.001) than in control oocytes. The data show that rOCT2 does not mediate detectable transport of quinine. In contrast, a small amount of quinine uptake was induced on expression of rOCT1 (see DISCUSSION).

**Inhibition of rOCT2 and rOCT1 by quinine, decynium22, and TPeA in the presence of different substrate concentrations.** The concentration dependence of \textsuperscript{[14C]-TEA uptake by rOCT2 and rOCT1 was measured in the presence of various concentrations of NMN, quinine, decynium22, and TPeA (Figs. 6 and 7). Similar results were obtained for rOCT2 and rOCT1. For the inhibition of TEA uptake by the transported cation NMN, a competitive type of inhibition was observed (see Figs. 6A and 7A). In contrast, a noncompetitive type of inhibition was observed for the weak base quinine (see Figs. 6B and 7B). Noncompetitive inhibition of TEA uptake by rOCT2 and rOCT1 was also shown for the permanently charged cations decynium22 (shown for rOCT2 in Fig. 6C) and for cyanine-863 (data not shown). For rOCT1, we demonstrated noncompetitive inhibition of TEA uptake also for the permanently charged cation TPeA (see Fig. 7C).
kinetics for TPeA inhibition of TEA uptake by rOCT2 were not determined. Electrical measurements suggested that neither cyanine-863 nor decynium-22 nor TPeA is transported to a significant extent by rOCT2 or rOCT1, since no inward current could be induced when nonpreloaded oocytes expressing rOCT2 or rOCT1 were clamped at \(-50\) mV and superfused with 100 \(\mu\)M of cyanine-863, decynium-22, or TPeA. In contrast, currents \(>80\) or 7 nA were induced when the oocytes expressing rOCT2 or rOCT1 were superfused with 10 mM choline, respectively.

**Interaction of the weak base quinine with rOCT2.** To elucidate whether quinine may interact with an allosteric binding site, we tried to exclude other reasons for the observed noncompetitive inhibition of rOCT2. We investigated whether the noncompetitive type of inhibition with quinine may result from an irreversible inactivation of rOCT2 by quinine or whether it may result from an intracellular interaction of quinine with rOCT2. This latter possibility was considered, since quinine has a \(pK_a\) value of 8.4 (23), so that \(~10\%\) of quinine is uncharged at \(pH\) 7.4 and may readily cross the lipid bilayer via nonionic diffusion. A quinine-induced inactivation of rOCT2 during the transport measurements in Fig. 6B could be excluded as a reason for the observed noncompetitive type of inhibition. First, electrical measurements in rOCT2-expressing oocytes revealed that the inhibition of TEA-induced inward currents was reversible when the oocytes had been incubated for 1 h with 100 \(\mu\)M quinine (data not shown). Second, when electrical measurements were performed within several minutes, a noncompetitive inhibition of TEA uptake by quinine was also observed. In Fig. 8, rOCT2-expressing oocytes clamped at \(-40\) mV were superfused with various concentrations of

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**Fig. 6.** Kinetics for the inhibition of rOCT2-expressed tetraethylammonium (TEA) uptake by \(N\)-methylnicotinamide (NMN), quinine, and decynium22. *Xenopus* oocytes were injected with rOCT2 cRNA and incubated for 2 days in Ori buffer. The uptake of \([^{14}\text{C}]\)TEA at various bath TEA concentrations was measured after 1 h of incubation in the absence of additional organic cations or in the presence of various concentrations of NMN (A), quinine (B), and decynium-22 (C). The data in A–C were obtained in 3 different oocyte batches. They are plotted according to Hofstee (12). Means \(\pm\) SE from 8–10 oocytes are presented. The lines were calculated by linear regression analysis \((r^2 > 0.94)\). The data show competitive inhibition of rOCT2-expressed TEA uptake by NMN and noncompetitive inhibition of TEA uptake by quinine and decynium22.

**Fig. 7.** Kinetics for the inhibition of rOCT1-mediated TEA uptake by NMN, quinine, and TPeA. *Xenopus* oocytes were injected with rOCT1 cRNA and incubated for 2 days in Ori buffer. \([^{14}\text{C}]\)TEA uptake was determined after 1 h of incubation in the presence of various TEA concentrations plus the indicated concentrations of NMN (A), quinine (B), and TPeA (C). The data are presented as in Fig. 6. They show competitive inhibition of rOCT1-expressed TEA uptake by NMN and noncompetitive inhibition of TEA uptake by quinine and TPeA.
TEA in the absence and presence of 30 μM quinine. The apparent $K_m$ value for TEA was not changed by quinine, and quinine inhibited the inward currents induced by 50, 200, or 2,000 μM TEA by 65, 65, and 67%, respectively. When this experiment was repeated with four other oocytes, similar results were obtained. During superfusion with 50, 200, or 2,000 μM TEA, the degree of inhibition by 30 μM quinine was nearly identical at the three TEA concentrations, showing a maximal difference of 2%.

To evaluate whether quinine may inhibit rOCT2 from the intracellular side, we determined whether the TEA uptake by rOCT2 was correlated to nonionic diffusion of quinine across the lipid bilayer. We measured the passive uptake of quinine at different pH values and tried to correlate passive quinine uptake with the IC$_{50}$ values for the inhibition of expressed TEA uptake by quinine in the bath. At pH 8.4, 7.4, and 6.0, ~50, 10, and <1%, respectively, of quinine is uncharged and may permeate the lipid bilayer by nonionic diffusion. Figure 9A shows the uptake of 0.1 μM [3H]quinine into Xenopus oocytes at different pH values. The uptake rates at different pH values observed in water-injected oocytes and in rOCT2-cRNA injected oocytes were not significantly different. Because the uptake increased strongly with increasing pH in the plasma membrane, it probably includes diffusion of uncharged [3H]quinine through the plasma membrane. The prolonged increase of pH-dependent [3H]quinine uptake is not understood. It may be explained by a slow equilibration of cytosolic quinine with yolk lipids that represent a large lipophilic compartment (see DISCUSSION). In Fig. 9B, we determined the apparent IC$_{50}$ values for quinine inhibition of TEA uptake at bath pH 6.0, 7.4, and 8.4. The IC$_{50}$ values increased with decreasing pH from 1.5 ± 0.3 μM at pH 8.4, over 8 ± 2 μM at pH 7.4, to 38 ± 8 μM at pH 6.0. Because the membrane potential was only slightly affected by these pH changes (60.3 ± 4.6 mV at pH 8.4, 61.3 ± 4.8 mV at pH 7.4, 54.1 ± 2.0 mV at pH 6.0; means ± SD, n = 6), the affinity changes may not be explained by a potential sensitivity of quinine binding to rOCT2. Electrical measurements under voltage-clamped conditions excluded this possibility more unequivocally. Figure 10 shows experiments in which oocytes expressing rOCT2 were clamped at ~40 mV and superfused with Ori buffer adjusted to pH 6.4 or 8.4 that contained 1 mM TEA or 1 mM TEA plus quinine at various concentrations. For the inhibition by quinine, apparent IC$_{50}$ values of 3.9 ± 0.3 and 74 ± 20 μM were estimated at pH 8.4 and 6.4, respectively. This indicates that the pH effects on the quinine inhibition are already observed within 1 min and that they are not induced by changes of the membrane potential. The observation that the IC$_{50}$ values were obtained in the electrical than in the tracer flux measurements may reflect less complete intracellular equilibration of quinine in the electrical measurements. Interestingly, the time for reactivation of transport from quinine inhibition was significantly longer at pH 8.4 than at pH 6.4 (see Fig. 10). The data are
consistent with the interpretation that quinine diffuses in its uncharged form through the lipid bilayer and inhibits rOCT2 from the intracellular side. We wondered whether the high IC_{50} value at pH 6.0 reflects the low intracellular concentration of quinine at this pH or represents low-affinity binding of quinine to rOCT2 from the extracellular side. To approach this question, we measured the concentration dependence of TEA uptake at pH 6.0 without an inhibitor and in the presence of 30 or 100 μM quinine (Fig. 11). When the Michaelis-Menten equation was fitted to the data, a competitive type of inhibition was observed. The apparent V_{max} values were not significantly changed by quinine; the apparent K_{m} values, however, increased with increasing quinine concentration. The following V_{max} values (pmol·oocyte^{-1}·h^{-1}) and K_{m} values (mM) were estimated: absence of quinine, V_{max} = 725 ± 72, K_{m} = 0.21 ± 0.04; 30 μM quinine, V_{max} = 827 ± 64, K_{m} = 0.31 ± 0.05; and 100 μM quinine, V_{max} = 844 ± 80, K_{m} = 0.66 ± 0.09. With the use of the K_{m} of TEA in the absence of quinine, and assuming a competitive type of inhibition, inhibitor constant (K_i) values for quinine of 55 and 45 μM were calculated from the K_{m} values determined in the presence of 30 and 100 μM quinine, respectively. The data suggest that quinine interacts at pH 6.0 with the outward-facing cation binding site of rOCT2.

Interaction of the permanently charged, nontransported cation TBuA with rOCT2. In an attempt to compare the above-described effects of quinine with effects of a permanently charged inhibitor with a comparable affinity that may not permeate the lipid phase of the plasma membrane, we investigated the inhibition of rOCT2-mediated cation uptake by TBuA. To test whether TBuA is transported by rOCT2, we compared the inward currents in rOCT2-expressing oocytes clamped to −50 mV that were superfused with saturating concentrations of choline (10 mM) or TBuA (0.5 mM). Although the choline-induced currents in these experiments were very high (295 ± 5 nA, mean ± SD, n = 3), no currents could be induced by 0.5 mM TBuA (<2 nA, n = 3). The data indicate that TBuA is not translocated by rOCT2 or that it is translocated with a V_{max} that is <1/100 of the V_{max} for choline. The inhibition of TEA-induced inward currents by TBuA in the bath is shown in Fig. 12. Figure 12A shows that 1 mM TBuA partially inhibited inward currents that were induced by 5 or 20 mM TEA, and that the inhibition by TBuA was decreased when the TEA concentration was increased. After removal of TBuA from the bath, the inhibition disappeared within the latency of the buffer exchange (see arrow in Fig. 12A). Figure 12B shows current measurements in Xenopus oocytes clamped to −40 mV that were superfused with different concentrations of TEA either without TBuA or in the presence of 100 or 350 μM TBuA. The curves suggest competitive inhibition of rOCT2-mediated TEA uptake by TBuA. Assuming a competitive type of inhibition, and using the K_{m} value for TEA obtained in the absence of TBuA (150 μM), K_{i} values of 12 and 18 μM were calculated from the measurements performed in the presence of 100 and 350 μM TBuA, respectively. Finally, we determined whether the IC_{50} values for TBuA inhibition of rOCT2-mediated uptake of 10 μM [^{14}C]TEA were dependent on the pH in the bath, as had
Fig. 12. Inhibition of rOCT2-mediated inward currents by tetrabutylammonium (TBUA) induced by various concentrations of TEA. *Xenopus* oocytes were injected with rOCT2 cRNA and incubated as in Fig. 11. A: steady-state currents in an rOCT2-expressing oocyte clamped at −40 mV. The oocyte was superfused with Ori buffer, with Ori buffer containing TEA, or with Ori buffer containing TEA plus TBUA. B: TEA-induced inward currents from oocytes expressing rOCT2 that were clamped at −40 mV. Each oocyte was first superfused with various TEA concentrations in the absence of TBUA. Then the oocytes were superfused with different TEA concentrations in the presence of 100 μM TBUA (n = 3, mean values ± SE) or 350 μM TBUA (1 oocyte). The data were normalized to the currents induced by 20 mM TEA, and the Michaelis-Menten equation was fitted to the data. They show rapid inhibition of TEA-induced currents by TBUA (A) and suggest that the inhibition of TEA-induced current is competitive (B).

been observed for the inhibition of TEA uptake by quinine. With the use of the same experimental conditions and fitting procedure as for the experiments in Table 2, IC₅₀ values of 17.2 ± 3.8 μM at pH 6.0, 24.9 ± 5.7 μM at pH 7.4, and 18.2 ± 6.8 μM at pH 8.4 were estimated. This indicates that the inhibition of rOCT2-mediated TEA uptake by TBUA is not dependent on the pH in the bath. The data suggest that TBUA is a nontransported inhibitor of rOCT2 that interacts with the outward-facing substrate binding site.

Interaction of rOCT2 and rOCT1 with corticosterone and anions. Next, we compared the concentration dependence of inhibition of TEA (10 μM) uptake by the uncharged compound corticosterone. In each of three independent side-by-side experiments, rOCT2 and rOCT1 were expressed, and the inhibition of expressed TEA uptake was measured within 4 h. The IC₅₀ values were determined for each inhibition curve. Significantly different mean values of 4.0 μM (rOCT2) and 151 μM (rOCT1) were calculated (Table 3). The data indicate that corticosterone interacts with both rOCT2 and rOCT1. They confirm an earlier reported IC₅₀ value for rOCT2 (40) and show that corticosterone has a 38-fold higher affinity to rOCT2 compared with rOCT1. Previous measurements in intact renal proximal tubules and isolated plasma membranes showed that organic cation transport may be inhibited by organic anions (14, 32, 33). Because indirect effects on the transport activity could not be excluded in these experiments, we now investigated whether anions may inhibit organic cation transport expressed by rOCT2 or rOCT1 that is structurally similar to the OATs (2, 18). After preliminary experiments had shown that rOCT2- and rOCT1-mediated uptake of [¹⁴C]TEA was partially inhibited by 1 mM probenecid, 1 mM α-ketoglutarate (AKG) or 1 mM para-aminohippurate (PAH), we measured the concentration-dependent inhibition of 10 μM [¹⁴C]TEA uptake by these anions. Similar data were obtained with rOCT2 and rOCT1. When rOCT2 and rOCT1 were expressed in identical oocyte batches (Table 3), the IC₅₀ values calculated for rOCT2 vs. rOCT1 were 0.7 vs. 0.7 mM (AKG), 4.5 vs. 1.3 mM (PAH), and 1.7 vs. 1.6 mM (probenecid). To determine whether the inhibition of cation uptake by anions is due to a competitive type of interaction rather than to an allosteric or indirect effect of the anions, we compared the concentration dependence of TEA-induced inward currents in the absence and presence of 2 mM PAH. This comparison was performed with 16 oocytes from 5 different batches expressing rOCT2 that were clamped at −50 mV. Although the TEA concentration required to induce half-maximal currents in the absence of PAH showed a large variation between different oocyte batches, in all experiments a mixed, mainly competitive type of inhibition by PAH was obtained. At 2 mM PAH, the apparent Kₘ values increased more than twofold (231 ± 34%, P < 0.001), whereas the Vₘₐₓ values decreased slightly (21 ± 3%, P < 0.01).

DISCUSSION

**Functional comparison between rOCT2 and rOCT1.** In this study, two OCTs are compared that exhibit similar sites of expression. In rat kidney, rOCT2 and rOCT1.

### Table 3. Inhibition of TEA uptake by corticosterone and anions

<table>
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<tr>
<th>Inhibitor</th>
<th>IC₅₀, mM</th>
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<tr>
<td></td>
<td>rOCT2</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>0.004 ± 0.0007</td>
</tr>
<tr>
<td>Para-aminohippurate</td>
<td>4.5 ± 1.9</td>
</tr>
<tr>
<td>α-Ketoglutarate</td>
<td>0.70 ± 0.17</td>
</tr>
<tr>
<td>Probenecid</td>
<td>1.70 ± 0.09</td>
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Inhibition of TEA uptake by corticosterone and anions. These experiments were performed and are presented as in Table 2. The IC₅₀ ± SD values of para-aminohippurate, α-ketoglutarate, and probenecid were calculated by fitting the Hill equation to individual side-by-side experiments. The IC₅₀ values of corticosterone for rOCT2 and rOCT1 are mean ± SD values that have been calculated from the IC₅₀ values of 3 side-by-side experiments. They are significantly different for both transporters (P < 0.001).
rOCT1 proteins have been localized to the basolateral membranes of renal proximal tubules (15, 28). rOCT1 protein is mainly expressed in S1 and S2 segments with a low expression in the S3 segment, whereas rOCT2 is expressed in S2 and S3 segments. Because we show in the present study that rOCT2 mediates electrogenic uptake of several structurally different cations, as has been demonstrated for rOCT1, both transporters may participate in basolateral uptake of cations in the proximal tubule. This represents the first step in cation excretion. The efflux measurements from the present study show, however, that rOCT2 can also mediate significant electrogenic efflux of choline at physiological membrane potentials if the outwardly directed choline gradient is high enough. Because electrogenic efflux has been also described for rOCT1 (22), both transporters may also participate in basolateral cation export during cation reabsorption. Our observation that the amount of choline that was taken up during 1-min superfusion of oocytes expressing rOCT2 with 10 mM choline was sufficient to generate a transient outward current on switching to choline-free superfuse at −50 mV (Fig. 1C) suggests a physiological significance of this transport mode.

Our study shows that rOCT2 and rOCT1 have similar basic functional properties but exhibit distinct differences that may entail specific physiological functions. rOCT2 and rOCT1 exhibit similar $K_m$ values for many transported cations, including choline, which has been claimed not to be transported by rOCT2 (11). At variance for guanidine, rOCT2 has a 10-fold lower $K_m$ value compared with rOCT1. Whereas for several organic cations, transport by both rOCT2 and rOCT1 was demonstrated, we observed that some uptake of quinine was expressed by rOCT1 but not by rOCT2. Earlier we explained the small amount of quinine uptake by rOCT1 as binding to the transporter that may be followed by endocytosis (22). Because we observed virtually no uptake of quinine with rOCT2, and recent experiments showed that the permanently charged quinine derivative $[^{3}H]$N-methylquinine is transported by rOCT1 with a $V_{\text{max}}$ of nearly 20 pmol/oocyte$^{-1} \cdot \text{h}^{-1}$ but not by rOCT2 (Ref. 35 and unpublished data), we no longer exclude the possibility that quinine belongs to the transported substrates of rOCT1. Taking together the uptake and electrical measurements with quinine performed so far, we would like to suggest that rOCT1 mediates inwardly directed transport of quinine under trans-zero conditions, but is trans-inhibited by intracellular quinine. This would explain why quinine is a potent inhibitor of the rOCT1-expressed transport of other cations, why no rOCT1-expressed uptake of $[^{3}H]$quinine could be detected with quinine concentrations >3 μM (22), and why we were not able to detect inward currents when we superfused voltage-clamped oocytes expressing rOCT1 with quinine at pH 7.4 (22).

Without knowing the number of functionally expressed transporter proteins, the turnover numbers of rOCT2 and rOCT1 cannot be compared directly. For this reason, we compared the relative $V_{\text{max}}$ values of different cations in side-by-side experiments, injecting the cRNAs of rOCT2 and rOCT1 into the same batch of oocytes and performing the transport measurements within 4 h. Thereby, we tried to ascertain comparable assay conditions, e.g., with respect to membrane potential of the oocytes and concentrations of endogenous compounds that may interact with transport. In normalizing the $V_{\text{max}}$ values to the $V_{\text{max}}$ of the prototypic cation TEA, relative turnover numbers of the two transporters were compared. Our data show that the relative turnover numbers of rOCT2 and rOCT1 depend on the structure of transported cations and that both transporters have different relative turnover numbers for structurally different substrates. For example, the turnover number of rOCT2 for choline is two times higher than for TEA, whereas the turnover number of rOCT1 for TEA and choline is about the same.

Inhibitors and transported cations with 20- to 70-fold different affinities for rOCT2 and rOCT1 have been identified that help to determine the individual contribution of rOCT2 and rOCT1 to cation secretion or reabsorption in vivo. For cation transport measurements in rat kidney, the contribution of three additional cation transporters that are also transcribed in the proximal tubule must be considered. These transporters are the electrogenic cation transporter rOCT3 (16, 38, 40), the cation transporter rOCTN1 (37), and the carnitine/cation transporter rOCTN2 (39). The transcription of these transporters in proximal tubule has been shown by in situ hybridizations, but the subcellular localization of the respective proteins has not been determined. The inhibitor profiles of rOCT3, rOCTN1, and rOCTN2 have been characterized in less detail. Interestingly, our identification of inhibitors with largely different affinities for rOCT2 and rOCT1 and the previously reported functional data on rOCT3, rOCTN1, and rOCTN2 suggest a rationale for experimentally dissecting out the individual contributions of rOCT2 or rOCT1 to cation transport in the intact proximal tubule. It has been reported (1) that rOCTN2 has a high affinity for carnitine (IC$\text{}_{50}$ value 15.5 μM; Ref. 39), 2) that rOCTN1 and rOCTN2 do not interact with 5 and 2.5 mM guanidine, respectively (37, 39), 3) that rOCTN1 has a very low affinity for MPP (IC$\text{}_{50}$ > 5 mM; Ref. 37), 4) that corticosterone inhibits rOCT3 with about the same affinity (IC$\text{}_{50}$ = 4.9 μM) as rOCT2 (40), and 5) that β-estradiol inhibits rOCT3 with a IC$\text{}_{50}$ of 1.8 μM, whereas it inhibits rOCT2 with a IC$\text{}_{50}$ value of 85 μM and does not inhibit rOCT1 at all (40). Thus transport by rOCT2 in proximal tubule may be analyzed by measuring uptake of guanidine at a concentration <0.5 mM (excluding transport by rOCTN1 and rOCTN2) in the presence of 10 μM β-estradiol (inhibition of rOCT3) plus 200 μM O-methylisoprenaline (inhibition of rOCT1). Transport by rOCT1 may be analyzed by measuring uptake of MPP at a concentration <50 μM (excluding uptake by rOCT1) in the presence of 0.5 mM carnitine (inhibition of rOCTN2) plus 15 μM corticosterone (inhibition of rOCT2 and rOCT3). These experimental conditions may be used to
determine initial uptake rates of cation transport by rOCT1 or rOCT2 into proximal tubular cells. To determine the role of the individual cation transporters in transcellular cation movement under steady-state conditions, nonmetabolized substrates should be employed that are common for all transporters that may be involved, and only compounds that are not transported themselves should be used for the inhibition of individual transporters.

Interaction of rOCT2 and rOCT1 with anions and hydrophobic cations. In the present paper, we show that the organic anions PAH, AKG, and probenecid are low-affinity inhibitors of rOCT2 and rOCT1. It has been shown previously through measurements on intact proximal tubules and membrane vesicles that certain anions interact with organic cation transport (14, 32, 33). A direct interaction of anions with cation transporters has not been demonstrated, however. Because the OATs and OCTs belong to the same protein family, share ~30% identical amino acids, and have the same predicted membrane topology (2, 18), the interaction of organic anions with the OCTs is not surprising. The observation that the inhibition by PAH of rOCT2-mediated cation transport was partially competitive with TEA uptake suggests that the substrate binding sites of the OCTs and OATs share structural features. Thus, for future in vivo measurements and for drug therapy, the possibility of cross inhibition between substrates or inhibitors of the organic anion and cation transporters must be considered.

To investigate the possibility that the OCTs contain allosteric cation binding sites that may add to the diversity of their interactions with cations, we performed kinetic studies with hydrophobic cationic inhibitors on rOCT2 and rOCT1. The investigated inhibitors were either permanently charged cations (TPeA, decynium22, or cyanine-863) or the weak base quinine. With both transporters, similar results were obtained. Competitive inhibition of TEA uptake was observed with the transported low-affinity cation NMN, whereas a noncompetitive type of inhibition was obtained with the high-affinity inhibitors quinine, cyanine863, decynium22, and TPeA.

Interaction of rOCT2 and rOCT1 with quinine. Mutagenesis studies with rOCT1 suggested that the non-competitive inhibitor TPeA binds close to the substrate binding site of rOCT1 (discussed in Ref. 7), and recent data with inside-out macropatches from Xenopus oocytes showed to our surprise that quinine inhibits rOCT2 from the intracellular side in a competitive manner (1). Thus we performed a more detailed investigation on the interaction of quinine with rOCT2 to determine the reason for the noncompetitive inhibition by quinine observed with intact oocytes. Quinine represents a weak base that may permeate the lipid bilayer in its uncharged form. This allowed us to change the passive membrane permeation experimentally by varying bath pH. Our findings strongly suggest that quinine permeates the plasma membrane via nonionic diffusion at pH 7.4 or more alkaline pH and inhibits rOCT2 from the intracellular side. We showed that passive quinine uptake into oocytes increased with increasing pH, and that the IC50 value for quinine inhibition of rOCT2-mediated cation transport was decreased from 8 to 1.5 μM when the bath pH was increased from 7.4 to 8.4. At pH 6, where >99% of quinine is positively charged, quinine probably inhibits rOCT2 by interacting with the outwardly facing substrate binding site, which has a relatively low affinity for quinine. At this pH, a competitive inhibition of quinine was observed that could be washed out more quickly than the inhibition at pH 8.4. The intracellular interaction of quinine with rOCT2 probably occurs at an inwardly facing substrate binding site, which may have a higher affinity for quinine than the outward conformation. This interpretation is strongly supported by electrical measurements on inside-out macropatches showing that quinine inhibited electrogenic cation efflux through rOCT2 from the intracellular side with an IC50 value of ~1 μM (1). Because this inhibition was competitive with TEA and choline at pH 7.4, quinine may interact from the aqueous phase with the inward-facing substrate site of rOCT2, rather than uncharged quinine from the lipid phase of the plasma membrane with a hydrophobic domain of rOCT2. Further experiments are necessary to determine whether the inhibition of rOCT2 reflects an interaction of the uncharged or the positively charged form of quinine.

Another unsolved question is why the pH-dependent quinine uptake increases linearly for 1 h, whereas the pH-dependent change of the IC50 values for quinine inhibition of TEA uptake was almost complete after 1 min of incubation (see Fig. 10). Also, the IC50 value for quinine inhibition of TEA uptake at pH 7.4 was similar when the incubation with quinine was performed for 10 min or 1 h (compare Fig. 9B with Table 2). A possible explanation for this apparent discrepancy is that uncharged quinine in the bath equilibrates rapidly with the plasma membrane and the intracellular aqueous phase where quinine may reach a quasi-steady-state concentration within minutes. However, the total amount of quinine in the oocyte increases over a much longer time period, possibly because the uncharged form of quinine from the intracellular aqueous phase, representing ~5% of total quinine at an estimated intracellular pH of 7.1, is slowly trapped by diffusion into the large pool of yolk lipids. Quinine could be also trapped in acidic vesicular compartments. Trapping of quinine in an intracellular compartment may also explain why the concentration of quinine in the oocyte after 1 h of incubation at pH 7.4, exceeds the estimated equilibrium concentration, assuming a homogeneous aqueous compartment within the oocytes, an oocyte volume of 0.5–1 μl, and an intracellular pH of 7.1. It would be helpful to investigate the intracellular distribution of quinine and to determine whether the permanently charged nontransported inhibitors decynium22, TPeA, and cyanine863 may also diffuse through the plasma membrane and interact from the intracellular side of rOCT2, or whether the noncompetitive type of inhibition observed with these compounds has a different reason.
Although several questions have remained open, our data with quinine show that nonionic diffusion of hydrophobic compounds must be taken into account in drug therapy, since intracellular drugs may exhibit high-affinity interactions with inward-facing substrate sites of plasma membrane transporters and may lead to long-lasting inhibition that may alter transporter stability.

In conclusion, the excretion and reabsorption of cations in the kidney are mediated by the successive and parallel action of a set of polyspecific cation transporters with overlapping substrate specificities and membrane distributions. The functional role of the individual transporters for the secretion and reabsorption of specific endogenous and exogenous cations is not understood because the membrane localization of most transporters is not known, their substrate specificities have not been clarified, and the employed transport mechanisms have not been elucidated in sufficient detail. The situation is even more complex, since the transporters may be regulated differentially, nontransported inhibitors may interact from both sides of the plasma membrane, and the transporters may exhibit significant interspecies differences in substrate specificity and localization. In the present study, we showed that the two electrogenic cation transporters rOCT2 and rOCT1 that exhibit an overlapping substrate specificity and membrane localization can be functionally distinguished with the help of inhibitors. In addition, data are presented that suggest that rOCT2 may operate in both directions at physiological membrane potentials and that the weak base quinine inhibits rOCT2 from the intracellular side.

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