The previously cloned rat cation transporter rOCT1 detected in renal proximal tubules and hepatocytes (Gründemann, D., Gorboulev, V., Gambaryan, S., Veyhl, M., and Koepsell, H. (1994) Nature 372, 549–552) was expressed in Xenopus oocytes, and transport properties were analyzed using tracer uptake studies and electrophysiological measurements. rOCT1 induced highly active transport of a variety of cations, including the classical substrates for cation transport, such as N-1-methylnicotinamide, 1-methyl-4-phenylpyridinium (MPP), and tetraethylammonium (TEA), but also the physiologically important choline. In oocytes rOCT1 also mediated efflux of MPP, which could be trans-stimulated by MPP and TEA. Cation transport via rOCT1 was electrogenic. In voltage-clamped oocytes, transport of TEA and choline via rOCT1 produced inwardly directed currents, which were independent of extracellular ion composition or pH. The choline- and TEA-induced currents were voltage-dependent at nonsaturating concentrations, and the apparent affinity of these cations was decreased at depolarized voltages. Other substrates transported by rOCT1 were the polyamines spermine and spermidine. Interestingly, the previously described potent inhibitors of rOCT1, cyanide 863, quinine, and t-tubocurarine were substrates themselves. The data indicate that rOCT1 is an effective transport system that is responsible for electrogenic uptake of a wide variety of organic cations into epithelial cells of renal proximal tubules and hepatocytes.

Drugs and xenobiotics are excreted into the urine and bile by epithelial cells of proximal renal tubules and hepatocytes (1–4). Translocation steps across two plasma membranes are involved, first, the uptake across the basolateral membrane into the cells and second, the secretion across the luminal membrane into urine or bile. In vivo measurements as well as measurements with plasma membrane vesicles have demonstrated translocation of anions, cations, and uncharged compounds by polyspecific transport systems. In renal proximal tubules three polyspecific cation transport systems with overlapping substrate specificity have been described, distinct potential dependent systems in the basolateral and luminal membrane and an electroneutral H⁺-cation antiporter in the luminal membrane (2, 5–12). In the liver a H⁺-cation antiporter (13), a transport system for small cations (type I) and a transporter for larger cations (type II) have been proposed on the basis of functional studies (1, 4, 14). In these studies, different transport systems with overlapping substrate specificities may have not been distinguished. Thus, these classifications must remain hypothetical until the involved transport systems have been cloned, functionally characterized, and checked for their in vivo contribution.

We identified the primary structure of a polyspecific cation transporter from rat kidney using expression cloning (15). This new membrane protein named rOCT1 is also expressed in liver and contains 12 potential membrane-spanning α-helices (15). Since tetraethylammonium (TEA) uptake expressed by rOCT1 was inhibited by a variety of cations with different molecular structures, and preliminary experiments also suggested the induction of 1-methyl-4-phenylpyridinium (MPP) transport, rOCT1 was defined as a polyspecific cation transporter. Previous observations indicated that rOCT1-induced TEA uptake may be potential-dependent and that the apparent Kₐ was nearly identical to the Kₐ value that had been estimated for potential-dependent TEA uptake over basolateral membranes of rat renal proximal tubules (5, 6, 16). Therefore, the hypothesis was raised that rOCT1 is responsible for basolateral cation uptake in renal proximal tubules. In the present work the functional properties of rOCT1 were further investigated in an attempt to elucidate (i) whether rOCT1 transfers net charge over the membrane, (ii) whether a variety of inhibitors of rOCT1 are also transported, (iii) whether rOCT1 may be identical to either the hypothesized type I or type II liver transporter, (iv) whether rOCT1 can be trans-stimulated by organic cations, and (v) whether the mode of action of OCT1 is consistent with the established unipporter model.

**EXPERIMENTAL PROCEDURES**

Expression of rOCT1 in Oocytes of Xenopus laevis—To yield high expression, rOCT1 was subcloned in a pRSP vector which contained 5′- and 3′-untranslated regions of the Xenopus β-globin gene (pRSSP vector was a kind gift of Dr. R. Schöfer, Heidelberg, Germany). cRNA encoding rOCT1 was synthesized in vitro as described previously (15). Dissection of X. laevis ovaries and collection and handling of the oocytes has been described in detail (17). The oocytes were stored in 100 mM NaCl, 5 mM MOPS-NaOH, 3 mM KCl, 2 mM CaCl₂, pH 7.4 (ORi buffer) containing gentamicin (50 mg/liter), sodium pyruvate (2.5 mM), and choline chloride (1 mM). Unless otherwise indicated, the experiments

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The nomenclature sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) X98334.

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1 OCT1 from rat (15) is designated as rOCT1.

2 The abbreviations used are: TEA, tetraethylammonium; MPP, 1-methyl-4-phenylpyridinium; MOPS, 4-morpholinepropanesulfonic acid; NMN, N-1-methylnicotinamide; MR1, multidrug resistance protein 1.
were performed on oocytes injected with 10 ng of cRNA/oocyte.

Tracer influx Measurements—3 days after cRNA injection, the oocytes were incubated 15 min (19°C) with 200 µl of ORI buffer in the absence or presence of inhibitors. Then radioactively labeled substrates (6–9 kBq/200 µl) were added, and the oocytes were incubated for 60 min at 19°C. During this time period linear uptake rates were observed. Transport was stopped with ice-cold ORI buffer. The oocytes were washed four times with ORI buffer (0°C), solubilized with 100 µl 5% (w/v) SDS, and analyzed for radioactivity. The indicated uptake rates represent medians of 8–10 oocytes ± S.E.

Tracer Efflux Measurements—3 days after cRNA injection the oocytes were injected with 50 nl of [3H]MPP (0.4 kBq, 0.1 pmol) and immediately transferred to a test well containing 100 µl of ORI buffer either without addition of cations or with addition of 164 µM MPP or 1 mM NaCl. Within 1, 2, 3, 4, and 5 min, the respective incubation media were removed for determination of radioactivity and were replaced by 100 µl of the respective incubation media. Finally, the oocytes were solubilized with SDS and analyzed for radioactivity as described above. For each experimental condition three to four oocytes were analyzed independently, and mean values ± S.D. were calculated. The amounts of MPP in the oocytes for time 0 and for the different incubation times are indicated in Fig. 2. The initial efflux rates were estimated from monoeponential curves which were fitted to the data.

Electrophysiological Measurements—Two-electrode current or voltage-clamp recordings were performed at room temperature 3–8 days after cRNA injection. The data were filtered at 10 Hz and recorded with a MacLab D/A converter and software for data acquisition and analysis (AD Instruments, Castle Hill, Australia). The external control solution (superfusate) contained 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, and 5 mM HEPES. It was titrated with NaOH or HCl to the indicated pH values. To study the Na⁺ and Cl⁻ dependence of cation-induced currents, NaCl was replaced by 200 mM D-glucose (0 NaCl). In these experiments LiOH was used for titration. For one set of experiments all extracellular Na⁺ was replaced by an equimolar concentration of K⁺. The flow rate of superfusion was 20 ml/min, and a complete exchange of the bath solution was reached within about 10 s. The reported currents and depolarizations represent maximal values, which were measured during a 30–45-s period (as indicated) of substrate superfusion. The data are given as means ± S.E., n indicating the number of experiments. The size of cation-induced currents varied significantly, depending on the time period after cRNA injection and on the batch of oocytes (from different animals). Data are shown for sets of experiments which were each obtained on the same day. All experiments were repeated with two to three batches of oocytes, and qualitative similar results were obtained. A paired Student’s t test was used to test for statistical significance.

Materials—[3H]Choline chloride (2.6 TBq/mmol) was obtained from Amersham Buchler (Braunschweig, Germany), [3H]-1-methyl-4-pyridinium acetate (3.1 TBq/mmol) from Du Pont de Nemours (Dreieich, Germany), and [3H]-N-1-methylnicotinamide (0.11 TBq/ mmol) from ICN Biochemicals (Meckenheim, Germany). MOPS, TEA, NMN, cyanine 863 chloride, quinine hydrochloride, quinidine hydrochloride, lidocaine hydrochloride, pancuronium bromide, D-tubocurarine chloride, spermine tetrahydrochloride, and spermidine trihydrochloride were supplied by Fluka (Neu-Ulm, Germany) and [3H]choline acetate (3.1 TBq/mmol) from Amersham Buchler (Braunschweig, Germany).

RESULTS

Uptake and Efflux Measurements with Radioactively Labeled Substrates of rOCT1—To characterize the transport kinetics of substrates other than TEA (15), uptake measurements with radioactively labeled MPP, NMN, and choline were performed in X. laevis oocytes which had been injected with cRNA of rOCT1. The expressed total uptake rates were corrected for uptake observed in the presence of 36 µM cyanine 863, a specific inhibitor of organic cation transport with high affinity to rOCT1 (15, 18). Water-injected control oocytes showed some endogeneous uptake of MPP, NMN, and choline which increased linearly with the substrate concentration (r² > 0.98). The endogeneous uptake was not significantly inhibited by 36 µM cyanine 863 (Fig. 1) and was not electrogeneous. The endogeneous uptake rates were 41 ± 2 (MPP), 20 ± 1 (NMN), and 103 ± 3 (choline) pmol × oocyte⁻¹ × h⁻¹ × mm⁻². We observed that rOCT1 is also able to transport MPP, NMN, and choline. Fig. 1 shows the substrate dependence of [3H]MPP (a), [3H]NMN (b), and [3H]choline (c) was measured in the absence and presence of 36 µM cyanine 863. Cyanine-inhibitable uptake rates are presented that were observed after injection of water (open symbols) or rOCT1 cRNA (closed symbols). The medians from 8–10 individual oocytes and S.E. values that exceed the symbol size are presented.

Fig. 1. Substrate dependence of rOCT1-mediated cation uptake measured by tracer influx. After injection of rOCT1 cRNA or water into Xenopus oocytes, the uptake of different concentrations of [3H]MPP (a), [3H]NMN (b), and [3H]choline (c) was measured in the absence and presence of 36 µM cyanine 863. Cyanine-inhibitable uptake rates are presented that were observed after injection of water (open symbols) or rOCT1 cRNA (closed symbols). The medians from 8–10 individual oocytes and S.E. values that exceed the symbol size are presented.
Functional Properties of Rat OCT1

Electrical Properties of Organic Cation Uptake—Previous tracer flux measurements showed that TEA uptake expressed by rOCT1 was decreased when the membrane potential was reduced (15). Employing the two microelectrode voltage-clamp technique in oocytes we investigated whether rOCT1-mediated transport carries a net electrical charge over the plasma membrane. Superfusion of noninjected oocytes with 1 mM TEA at a holding potential of −50 mV did not induce a significant change in the holding current (ΔI was −0.1 ± 0.1 nA; n = 20). In contrast, in oocytes expressing the cation transporter rOCT1, TEA produced a concentration-dependent inward current. Fig. 3 shows that the TEA-induced currents occurred with an apparent $K_v$ value of $35 ± 7 \mu M$ at −50 mV with a Hill coefficient of 0.92 (n = 6). The apparent $K_v$ for TEA was voltage-dependent. At −90 mV and −10 mV, the estimated $K_v$ values were $14 ± 1 \mu M$ and $49 ± 3 \mu M$, respectively (n = 6). The data were best fitted with Hill coefficients of 0.79 ± 0.01 and 1.07 ± 0.02 at −90 mV and −10 mV (difference p < 0.01), respectively. In parallel experiments with identical batches of oocytes, we tested how the current induced by 1 mM TEA compares with tracer uptake of 1 mM TEA. In these experiments the electrical measurements were performed at the mean membrane potential which was measured in the respective oocyte batch. The tracer uptake was approximately 4–5 times smaller ($345 ± 43 pmol \times oocyte^{-1} \times h^{-1}$) than the net positive charge transfer which was calculated from the induced currents ($1584 ± 313 pmol \times oocyte^{-1} \times h^{-1}$). These data could indicate the presence of a cation leak (slippage) in rOCT1-expressing oocytes as has been described for a great variety of mammalian transporters (19). However, simple technical reasons may also contribute to this difference. For example, in voltage-clamped oocytes the membrane potential is stable, while the oocytes in uptake studies are expected to depolarize during cation uptake (see also Fig. 4c) which could in part explain a decreased substrate uptake.

Substrate Specificity of rOCT1—In search for other physiologically relevant cations, we tested the organic cations spermine, spermidine, and the dibasic amino acids L-arginine and L-lysine (all at 1 mM) for electrogenic transport via rOCT1. The polyamines spermidine and spermine induced inward currents of different proportions, while the dibasic amino acids had no significant effects. There was no significant difference between spermine- and spermidine-induced currents (−4.6 ± 0.5 nA versus −4.3 ± 0.4 nA; n = 5 for both compounds). However,
both compounds (1 mM) produced significantly smaller currents than choline (−12.4 ± 2.0 nA; n = 5). Next we investigated whether the high affinity cation transport inhibitors quinine, quinidine, and cyanine 863 are transported by rOCT1. We also analyzed the muscle relaxants D-tubocurarine and pancuronium. Quinine, pancuronium, and D-tubocurarine had previously been defined as type II substrates of cation transport in the liver (1, 4, 14). Quinine and its diastereomer quinidine produced inward currents. At a holding potential of −50 mV, for quinine and quinidine half-maximal currents were observed at 0.27 ± 0.05 μA and 2.2 ± 0.62 μA, respectively (Fig. 5; n = 5). Measuring choline-induced currents in the absence of the high affinity inhibitor cyanine 863 (15) currents induced by 1 mM choline were half-maximal inhibited by cyanine 863 concentrations of 63 ± 5 nM (n = 6). When superfused alone 1 μM cyanine 863 produced an inward current of −20.3 nA (n = 5). This current was not different from the maximal current induced by TEA in the same batch of oocytes (−23.7 ± 1.8 nA; n = 5). With 0.1 mM D-tubocurarine and pancuronium, the current induced by 1 mM choline was reduced to 49.7 ± 5.8% (n = 6) and 33.5 ± 3.6% (n = 5), respectively. Superfusion of 0.1 mM D-tubocurarine and pancuronium alone (−50 mV holding potential) produced inward currents of −17.3 ± 6.2 nA (n = 6) and −8.9 ± 1.0 nA (n = 6). In this batch of oocytes the inward current produced by 1 mM TEA was −27.2 ± 3.7 nA. For the analysis of D-tubocurarine-induced currents at −50 mV an apparent K_0.5 of 2.9 ± 0.6 μM was estimated (Fig. 5).

Following the demonstration that rOCT1 is able to translocate small and large mono-, di-, and polyvalent cations we were interested in determining how rOCT1 can transport the charged and uncharged form of a weak base. For this purpose we analyzed the local anesthetic lidocaine (pK_a of 7.9) at pH 9 (fraction of unprotonated lidocaine, >90%) and 6.5 (fraction of protonated lidocaine, >90%). At pH 6.5, lidocaine produced maximal currents (at 3 mM) that were not significantly different from the maximal currents induced by TEA. However, increasing the pH value to 9.0 shifted the concentration-current relation for lidocaine to higher lidocaine concentrations. For the currents induced at pH 6.5 and 9.0, apparent K_0.5 values of 180 ± 29 μM and >1 mM were estimated. The K_0.5 for lidocaine at pH 9 can be considered only as a rough estimate, because the highest superfused lidocaine concentration (3 mM) is clearly not saturating (see Fig. 6). When these currents were plotted against the calculated concentrations of protonated lidocaine (assuming a pK_a of 7.9) apparent K_0.5 values of 180 ± 28 μM (pH 9.0) and 110 ± 23 μM (pH 6.5) were estimated. The data suggest that current is generated by transport of protonated lidocaine and that noncharged lidocaine may bind to the substrate binding site of rOCT1. This interpretation is supported by the observation that 10, 30, and 100 μM lidocaine did not produce significant inward currents at pH 9.0 but inhibited TEA-induced currents by 11.9 ± 3.1%, 22.9 ± 2.0%, and 26.9 ± 2.1% (n = 5), respectively (see Fig. 6, inset).

**DISCUSSION**

In this study we investigated the transport specificity of the organic cation transporter rOCT1 (15) and further characterized its functional properties. rOCT1 has been cloned from rat kidney and belongs to a new transporter family with 12 putative membrane-spanning α-helices. Previously we have shown that rOCT1 mediates uptake of TEA, which could be inhibited by many structurally different cations. Thus, we raised the hypothesis that rOCT1 is a polyspecific cation transporter. The following results from our laboratory suggest that rOCT1 is an important cation transporter at the sinusoidal membrane of rat hepatocytes and at the basolateral membrane of rat renal proximal tubules. 1) Performing Northern blots with an rOCT1-specific probe, which does not hybridize with a recently isolated rOCT1-homologous cDNA from rat (rOCT2), we demonstrated that rOCT1 is significantly transcribed in kidney, liver, colon, and small intestine. 2) Immunological data indicated that rOCT1 is localized at the sinusoidal membrane of rat hepatocytes. 3) Employing microdissected nephron segments from rat, transcription of rOCT1 could be demonstrated in all three segments of renal proximal tubules. 4) The localization of rOCT1 at the basolateral membrane of rat proximal tubules is

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3 The sequence of rOCT2 has been submitted to the GenBank™ EMBL Data Bank with the accession no. X98334.
4 S. Gambaryan, V. Gorboulev, and H. Koepsell, unpublished data.
suggested from the functional comparison of organic cation transport expressed by rOCT1 with that obtained by microperfusion experiments with rat renal proximal tubules. Thus, rOCT1-mediated uptake is electrogenic and pH-independent, and the apparent $K_m$ values for transport of TEA, NMN, and choline are nearly identical with the apparent $K_m$ and $V$ values obtained for potential dependent uptake across the basolateral membrane of renal proximal tubules (5, 6, 16). However, the $K_m$ values described here differ by one order of magnitude from the respective values which have been determined for potential-dependent uptake over the luminal membrane (16). Altogether, the data indicate that rOCT1 mediates the cellular uptake of endogenous and exogenous cations representing the first step in renal and hepatic excretion of cationic metabolites, xenobiotics and drugs.

This report describes that rOCT1 translocates electrical charge over the plasma membrane and can be functionally characterized by electrical measurements. We show that cation uptake by rOCT1 is sodium-independent and not altered by pH gradients or alterations of other ion concentrations. Our hypothesis that rOCT1 is a polyspecific transporter was verified. Diverse cations that were previously shown to inhibit TEA uptake (15) were also found to be transported by rOCT1. This was even true for the high affinity inhibitor cyanine 863 which has an apparent $K_m$ value of less than 0.2 $\mu$M. We demonstrated that rOCT1 translocates monovalent, divalent (pancuronium), and even polyvalent cations (spermine has at physiological pH four positive charges) of different structures, which may be small and hydrophilic or large and also hydrophobic. We showed that noncharged hydrophobic molecules such as corticosterone (previous study) and lidocaine (present study) inhibit cation transport and are translocated by rOCT1. Whether they interact at the cation binding site and are translocated by rOCT1 must be elucidated in future experiments.

For the following monovalent cations apparent $K_m$ values were determined by tracer flux measurements (underlined) and/or by electrical measurements with holding potentials of $-50$ mV: NMN (0.4 $\mu$M), choline (0.2–1.1 $\mu$M), TEA (0.05–0.1 $\mu$M), lidocaine at pH 6.5 (0.2 $\mu$M), MPP (10 $\mu$M), quinidine (0.3 $\mu$M), quinine (2.2 $\mu$M). The apparent $K_m$ values range over three orders of magnitude and the observed uptake rates are remarkably high and do not decrease in parallel with the $K_m$ values. In this respect polyspecific transport via rOCT1 can be compared with polyspecific transport mediated by the multi-drug resistance protein MDR1 (20). In contrast some classical substrate-specific transporters may allow some low rate, low affinity transport of related compounds (for example the Na$^+$-D-glucose cotransporter SGLT1 (21, 22)). Unlike MDR1, which is an ATP dependent extrusion pump for lipophilic intracellular molecules, rOCT1 is an electrogenic import system for organic cations into cells. Whereas MDR1 probably removes lipophilic compounds which have been solved in the lipid bilayer (23), rOCT1 accepts cations from the aqueous phase of the extracellular fluid.

To understand the functional role of rOCT1 in cation excretion in liver and kidney, it is not sufficient to know the cellular localization and membrane topology of rOCT1 and other cation transporters in the same cells. In addition, the transport mechanism must be further elucidated, including clarification of transport asymmetry, driving forces, and substrate specificity. In this respect our present data allow only a limited understanding. The efflux measurements indicate some symmetry of rOCT1-mediated cation transport and show that, in the efflux mode, rOCT1 may function as a unipporter since the unloaded transporter may switch from an outside to the inside conformation. Further, the observation that MPP efflux was trans-stimulated by MPP or by TEA is consistent with a unipporter model. We have shown that rOCT1 mediates electrogenic transport of cations since electrical currents were induced by the cations TEA, choline, NMN, and MPP which were identified as substrates by tracer uptake experiments and for TEA and choline similar apparent $K_m$ values were estimated by both methods. A comparison of the currents induced by TEA with the tracer uptake of TEA suggests some non-specific charge transfer during the transport cycle. The potential dependence observed at low substrate concentrations suggests that the driving force for cation transport by rOCT1 may be provided by the membrane potential. However, tracer uptake of TEA (15) and cation induced currents (see Fig. 4d) were also observed when the membrane potential was zero. Because the transport was apparently independent of any extracellular ions or pH the driving force may be also provided by the chemical gradients of the transported substrates. Further studies employing more sophisticated methods in which the substrate composition on both membrane sides can be varied independently and individual transport steps can be resolved may help in the understanding of polyspecific transport of organic cations mediated by rOCT1.

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![FIG. 6. Current-concentration relationship for rOCT1-mediated currents induced by lidocaine at distinct pH values. The experiment was performed, and the data were analyzed as in Fig. 3. The data for each oocyte were normalized at the maximal induced currents at the individual pH. At pH 9 the apparent affinity of lidocaine is decreased, and 3 mM is not sufficient to saturate rOCT1. The inset shows the inhibition of TEA (1 mM)-induced current by lidocaine at pH 9.0.](image-url)
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