A Reevaluation of Substrate Specificity of the Rat Cation Transporter rOCT1*

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The substrate specificity of the previously cloned rat cation transporter rOCT1, which is expressed in kidney, liver, and small intestine, was reevaluated. rOCT1 is the first member of a new protein family comprising electrogenic and polyspecific cation transporters that transport hydrophilic cations like tetraethylammonium, choline, and monoamine neurotransmitters. Previous electrical measurements suggested that cations like quinine, quinidine, and cyanine 863, which have been classified as type 2 cations in the liver, are also transported by rOCT1, since they may induce inward currents in rOCT1 expressing Xenopus oocytes. By tracer flux measurements it was shown that rOCT1 mediates transport of structurally different cations, such as tetraethylammonium (TEA), N1-methylquinolinamide, choline, 1-methyl-4-phenylpyridinium (MPP), and dopamine. In voltage-clamped oocytes rOCT1 was also characterized by electrical measurements (2). When TEA, choline, MPP, or dopamine was added to the bath of rOCT1 expressing oocytes, inward currents were induced, and it was concluded that the uptake of these cations is electrogenic. Electrical measurements were also performed with the more hydrophobic cations, quinine, quinidine, d-tubocurarine, and cyanine 863 (2). In transport measurements with isolated rat hepatocytes, these cations had been previously classified as type 2 cations, and it has been postulated that these cations were translocated by a separate transport system to the more hydrophilic type 1 cations like TEA and choline (15, 16). Since, in rOCT1 expressing oocytes, large inward currents were also induced by quinine, quinidine, d-tubocurarine, and cyanine 863, it was proposed that rOCT1 translocates type 1 and type 2 cations (2) to a similar extent. The present paper disproves this hypothesis.

EXPERIMENTAL PROCEDURES

Methods—Oocytes were injected with 10 ng of rOCT1 cRNA per oocyte as described before (1, 2), and were stored 2–4 days in 5 mm Hepes-Tris, pH 7.4, 96 mm NaCl, 2 mm KCl, 1.8 mm CaCl2, 1 mm MgCl2 (ORi) containing 50 mg/l gentamicin. Trace uptake and efflux experiments were performed as described earlier (2). Initial efflux rates were estimated from monoeponential curves that were fitted to the data. For electrical measurements the oocytes were permanently superfused with fresh solution at room temperature (~3 ml/min, 22–24 °C). For the determination of current-voltage relations, steady-state current was measured during the last 100 ms of 500-ms voltage pulses to different potentials in the absence and presence of the agonist. The averages of the currents before and after agonist application were subtracted from the currents during agonist application.

HEK 293 cells were transfected and selected as described (3). A stably transfected single clone was isolated and grown in Dulbecco’s modified Eagle’s medium containing 10% (v/v) fetal calf serum and 0.6 mg/ml Geneticin. Suspended cells were incubated 0, 1, 3, and 5 s at 37 °C in the presence of 137 mm NaCl, 2.7 mm KCl, 8 mm NaHPO4, 1.6 mm KH2PO4, pH 7.4 (PBS) plus the radiactively labeled cations without or with 40 µM cyanine 863 (3). The short term incubations with [3H]MPP showed linearity for only 1 s.

Materials—[3H]Quinine (0.56 TBq/mmol) and [3H]quinidine (0.54 TBq/mmol) were obtained from Biotrend (Köln, Germany) and [3H]methyl-4-phenylpyridinium (3.1 TBq/mmol) from E. I. du Pont de Nemours & Co. (Deireich, Germany). Geneticin G418 was from Life Technologies, Inc. (Eggenstein, Germany). The other chemicals were obtained as described earlier (2, 3).

RESULTS

Transport by rOCT1 Measured with Radioactively Labeled Cations—We determined the uptake of radioactively labeled quinine and quinidine after expression of rOCT1 in Xenopus oocytes and in HEK 293 cells. Fig. 1 shows an experiment in which the time course of the cyanine 863-inhibitable uptake of...
Substrate Specificity of Rat OCT1

FIG. 1. Uptake of MPP and quinine in Xenopus oocytes after expression of rOCT1. After injection and 3 days incubation in OBI, oocytes were incubated with 14 μM [3H]MPP or with 0.3 μM [3H]quinine in the absence and presence of 36 μM cyanine 863. After the indicated incubation times, the radioactivity associated with the oocytes was measured. The cyanine-inhibited fraction of MPP (circles) and quinine uptake (squares) in rOCT1-injected (closed symbols) and water-injected oocytes (open symbols) is indicated. Medians and S.E. from pairs of 8–10 individual oocytes are presented.

[3H]MPP and [3H]quinine was measured in water-injected control oocytes and in rOCT1 cRNA-injected oocytes employing cation concentrations around their apparent Ki cation concentrations around their apparent

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[3H]quinidine no significant cyanine-inhibitable uptake could be observed (data not shown). The data show that rOCT1 does not significantly translocate type 2 cations from the oocytes (data not shown), it probably represents quinine

2.0 0.2

Trans-cyanine

0.0 0.0

MPP without cyanine

0.0 0.0

MPP with cyanine

0.0 0.0

Quinine without cyanine

0.0 0.0

Quinine with cyanine

0.0 0.0

Quinidine with cyanine

0.0 0.0

Quinidine with cyanine

0.0 0.0

Substrate Specificity of Rat OCT1

TABLE I

Attempt to measure transport of quinine and quinidine after expression of rOCT1 in an eukaryotic cell line

HEK 293 cells were stably transfected with rOCT1 and initial uptake rates of 0.3 μM [3H]quinine, 2 μM [3H]quinidine, and 14 μM [3H]MPP were measured in nontransfected HEK 293 cells and in stably transfected HEK 293 cells. The measurements were performed in the absence and presence of 40 μM cyanine 863. Means from three measurements and S.D. values are presented.

| Transport measurement | Uptake rate (pmol × mg protein 1) | Nontransfected |
|-----------------------|----------------------------------|---------------|
|                       |                                   |               |
| MPP without cyanine   | 2.1 ± 0.4                         | 0.01 ± 0.01   |
| MPP with cyanine      | 0.01 ± 0.01                       | 0.01 ± 0.01   |
| Quinine without cyanine| 0.01 ± 0.01                      | 0.03 ± 0.01   |
| Quinine with cyanine  | 0.01 ± 0.01                       | 0.01 ± 0.02   |
| Quinidine with cyanine| 0.3 ± 0.2                         | 0.1 ± 0.1     |
| Quinidine with cyanine| 0.4 ± 0.2                         | 0.2 ± 0.2     |

FIG. 2. Trans-effects of type 1 and type 2 cations on MPP efflux from Xenopus oocytes after expression of rOCT1. Water-injected (control, broken line) and rOCT1 cRNA-injected oocytes (solid lines) were incubated for 3 days in OBI buffer. Thereafter, [3H]MPP was injected, and the oocytes were incubated in OBI alone (trans-zero) or in OBI containing 100 μM MPP (trans-MPP), 40 μM quinine (trans-quinine), 1.4 mM α-tubocurarine (trans-d-tubocurarine), or 40 μM cyanine 863 (trans-cyanine). Under the different experimental conditions similar efflux rates were obtained in water-injected control oocytes (mean value: 0.09 ± 0.01 pmol × oocyte 1 × h 1). After the expression of rOCT1, the initial MPP efflux rate was increased under trans-zero conditions (0.46 ± 0.07 pmol × oocyte 1 × h 1, p < 0.05). Under trans-MPP conditions the initial MPP efflux rate in the rOCT1 injected oocytes was significantly increased over trans-zero conditions (0.74 ± 0.06 pmol × oocyte 1 × h 1, p < 0.05). Compared with the trans-zero condition, the efflux rate of [3H]MPP was decreased (p < 0.05) when the type 2 cations quinine, d-tubocurarine, and cyanine 863 were added to the bath (trans-quinine 0.11 ± 0.01, trans-d-tubocurarine 0.14 ± 0.02, trans-cyanine 0.18 ± 0.03 pmol × oocyte 1 × h 1). Means and S.D. from three cRNA-injected oocytes are presented.

Fig. 2 shows efflux experiments with [3H]MPP, which were performed with rOCT1-injected and water-injected oocytes (control). As reported earlier (2), it was found that rOCT1-induced MPP efflux is observed when no organic cations were present in the bath and is trans-stimulated by extracellular MPP. Fig. 2 also shows that rOCT1-mediated efflux of [3H]MPP is trans-inhibited by quinine, d-tubocurarine, and cyanine 863. Inhibition was also observed for quinidine (data not shown). The stimulatory trans-effect of MPP on the initial efflux rate and the inhibitory trans-effects of the type 2 cations were significant (p < 0.05, see legend of Fig. 2). This suggests that the transporter loaded with type 2 cations exhibits a much slower (if at all) reorientation to its inward orientation.

Electrical Measurements with rOCT1 in Xenopus Oocytes—Fig. 3a shows a typical current recording from an oocyte that had been preincubated for 18 h with 1 mM choline and was clamped at −50 mV. As reported earlier (2) quinine (10 and then 100 μM) induced a large inward current in these oocytes, which might suggest that quinine is transported. Surprisingly, the following application of choline led to a drastically decreased signal which recovered slowly. Fig. 3b shows a current recording from the same oocyte, which was maintained at a holding potential of −100 mV. Whereas the choline-induced
Association of quinine (a, b) days in ORi buffer and for another 18 h in ORi with (five mM choline, inward difference currents were obtained when the rOCT1-expressing oocytes were superfused with 5 mM choline and with saturating concentrations of quinine. When the rOCT1-expressing oocytes were superfused with choline and/or quinine in rOCT1-expressing oocytes that were preincubated without and with choline. Xenopus oocytes were treated as described in the legend to Fig. 3, without (a) or with (b) choline preincubation. The oocytes were continuously superfused, and current was measured before, during, and after application of 5 mM choline or 100 μM quinine at the indicated membrane potentials. Mean difference currents and S.D. from six (a) or three oocytes (b) of one oocyte batch are presented.

**DISCUSSION**

Tracer influx and efflux measurements performed in Xenopus oocytes and stably transfected eukaryotic cells showed that the type 1 cations TEA, choline, N1-methyl nicotinamide, MPP, and dopamine are transported by rOCT1, rOCT2, and hOCT2. Electrical measurements with voltage-clamped oocytes indicated that the cation transport is potential-dependent and electrogenic ([2, 3, 6, 14]. Since rOCT1 is localized in the basolateral membranes of rat hepatocytes, we wondered whether rOCT1 may mediate the two previously distinguished cation transport activities in isolated hepatocytes, i.e. the transport of small relatively hydrophilic type 1 cations like choline, and the transport of larger, more hydrophobic type 2 cations like quinine (17–19). In an attempt to detect rOCT1-mediated trans-
port of nonradioactively labeled type 2 cations, rOCT1 expressing Xenopus oocytes, preincubated with 1 mM choline and clamped at −50 mV, were superfused with type 2 cations and inward currents of similar magnitude to those obtained with TEA or choline were found (2). The present study shows that these results could only be obtained in a limited voltage range (see Fig. 4). We now demonstrate that the type 2 cations quinine, quinidine, cyanine 863, and d-tubocurarine are inhibitors of cation transport by rOCT1 and are not significantly translocated themselves. We suggest that the quinine-induced current is generated by the inhibition of electrogenic choline efflux through rOCT1.

In hepatocytes rOCT1 is responsible for electrogenic uptake of type 1 cations over the basolateral membrane, whereas type 2 cations are taken up by a different nonidentified transport system. In renal proximal tubules type 1 cations may be translocated over the basolateral membrane by OCT2 and in the rat also by rOCT1, whereas no functional evidence for a type 2 cation transporter has been presented (14). Our electrical measurements show that rOCT1 does not only mediate influx of type 1 cations into epithelial cells but may also mediate their electrogenic efflux. Further experiments with excised giant patches (20, 21) from rOCT1-expressing oocytes should help to characterize the different steps in the transport cycle to understand how rOCT1 is operating in vivo.

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