Drug excretion mediated by a new prototype of polyspecific transporter

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CATIONIC drugs of different types and structures (antihistaminics, antiarrhythmics, sedatives, opiates, cytostatics and antibiotics, for example) are excreted in mammals by epithelial cells of the renal proximal tubules and by hepatocytes in the liver[1–4]. In the proximal tubules, two functionally disparate transport systems are involved which are localized in the basolateral and luminal plasma membrane and are different from the previously identified neuronal monoamine transporters and ATP-dependent multidrug exporting proteins[1–3,5–12]. Here we report the isolation of a complementary DNA from rat kidney that encodes a 556-amino-acid membrane protein, OCT1, which has the functional characteristics of organic cation uptake over the basolateral membrane of renal proximal tubules and of organic cation uptake into hepatocytes. OCT1 is not homologous to any other known protein and is found in kidney, liver and intestine. As OCT1 translocates hydrophobic and hydrophilic organic cations of different structures, it is considered to be a new prototype of polyspecific transporters that are important for drug elimination.

Using functional expression cloning in *Xenopus* oocytes, we isolated a 1,882-base-pair (bp) cDNA from a rat kidney library, which induces high activity of N^1-methylnicotinamide (NMN)-inhibitable ^{14}C-tetraethylammonium (^{14}C-TEA) uptake (Fig. 1*a*). This clone, OCT1, contains an open reading frame encoding

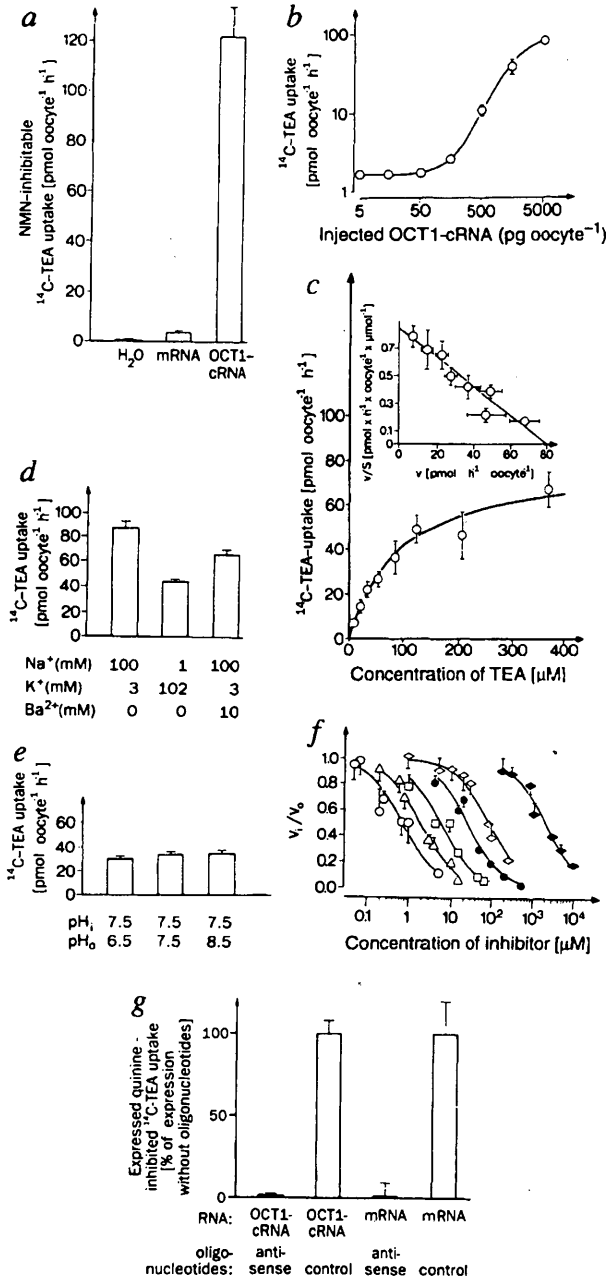

FIG. 1 Expression of OCT1 in *Xenopus* oocytes. Oocytes were injected with 3 ng (*c*, *d*, *e*), 5 ng (*f*, *g*) or 10 ng OCT1-cRNA (*a*). The indicated ^{14}C-TEA-uptake rates represent the medians of 10–20 oocytes ±s.e.m. *a*, Comparison of NMN-inhibited ^{14}C-TEA uptake after injection of water, 20 ng rat kidney mRNA or 10 ng OCT1-cRNA; ^{14}C-TEA and NMN concentrations in incubation medium were 200 µM and 10 mM respectively. Under these conditions 77 ± 4% of the total uptake was inhibited by NMN. *b*, Uptake rates of 200 µM ^{14}C-TEA after injection of different amounts of OCT1 cRNA. The curve was calculated by fitting the Hill equation to the data ($n = 1.9 \pm 0.2$). *c*, Substrate dependence of ^{14}C-TEA uptake expressed by OCT1-cRNA. Uptake measured with cRNA-injected oocytes was corrected for that obtained with water-injected control oocytes, which increased linearly with substrate concentration (30 fmol h^{-1} oocyte^{-1} µM^{-1}). The curve was fitted to the Michaelis–Menten equation ($K_m = 95 \pm 10$ µM; $V_{max} = 81 \pm 5$ pmol h^{-1} oocyte^{-1}). *d*, Dependence of expressed ^{14}C-TEA uptake on membrane potential. In oocytes injected with OCT1 cRNA, the uptake of 95 µM ^{14}C-TEA was measured in the presence of Na^+, K^+ and Ba^{2+} at the indicated concentrations and corrected for uptake in water-injected control oocytes. Membrane potentials measured by conventional two-microelectrode techniques ranged between −40 and −60 mV (100 mM Na^+, 3mM K^+), 0 and −10 mV (1 mM Na^+, 102 mM K^+), and −18 and −22 mV (100 mM Na^+, 3 mM K^+, 10 mM Ba^{2+}). *e*, Uptake of 95 µM ^{14}C-TEA in the absence and presence of proton gradients in OCT1-cRNA-injected oocytes. To prevent proton-gradient-induced changes in membrane potential, measurements were made in the presence of 102 mM K^+ and 1 mM Na^+. Measurements with an ion-sensitive microelectrode showed that the internal pH changed by less than 0.1 units during the 30-min uptake recording. *f*, Inhibition of OCT1-mediated uptake of 95 µM ^{14}C-TEA by decynium-22 (○), quinine (△), desipramine (□), procainamide (●), *O*-methylisoprenaline (◇) and tetramethylammonium (◆). *g*, Inhibition of quinine-inhibitable ^{14}C-TEA uptake, expressed by mRNA from rat kidney, by an antisense oligonucleotide directed against OCT1. Poly(A)$^+$ mRNA and OCT1 cRNA were incubated without and with 40 µM antisense oligonucleotide (5′-AGG ACA TCA TCC ACG GTG GG-3′, nucleotides 60–40 of OCT1) or with a non-interacting control oligonucleotide (control) as described[17]. 50 ng mRNA per oocyte was injected, the uptake of 200 µM ^{14}C-TEA measured in the absence and presence of 25 µM quinine, and the quinine-inhibited uptake calculated.

METHODS. *Xenopus laevis* oocytes were selected and expression measured as described[24]. After RNA injection, oocytes were incubated for 3 days with 5 mM Tris-HCl, pH 7.8, 100 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ (ORi buffer). Uptake was measured for 90 min at 22 °C by incubating the oocytes with ^{14}C-TEA in ORi buffer, ORi with altered Na^+ and K^+, ORi plus Ba^{2+}, ORi with altered pH (HEPES substituted for Tris), or ORi plus inhibitors. OCT1-expressed TEA uptake was linear for more than 90 min. For measurements with altered concentrations of Na^+, K^+, H^+ and with inhibitors, oocytes were pre-incubated for 30 min under the respective buffer conditions and the uptake rate was then determined during a 30-min incubation with ^{14}C-TEA.

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a membrane protein of relative molecular mass 61,528, which shares no similarity with any protein in the data banks (Fig. 2*a*). Expression of ^{14}C-TEA uptake in oocytes was dependent on the amount of injected OCT1 cRNA (Fig. 1*b*), and the substrate dependence of OCT1-mediated ^{14}C-TEA uptake followed Michaelis–Menten kinetics (Fig. 1*c*). The estimated K_m value of 95 ± 10 μM was similar to the K_m (160 μM) for cation transport through the basolateral membrane of rat renal proximal tubules[13] and was more than ten times lower than the apparent K_m of the H^+–cation antiport through the luminal membrane[2,14]. To determine whether OCT1 could be responsible for potential-dependent organic cation transport over the basolateral membrane or the luminal H^+–organic cation antiport[1,3,8,11], we investigated whether OCT1-mediated uptake is potential- or proton-gradient-dependent and studied the inhibition of ^{14}C-TEA uptake using a variety of inhibitors. Figure 1*d* and *e* shows that

a

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1     GCAGGCCTGGCTAAACTGGTGAGGGCCCTACCCAGCCATGCCCACCGTGGATGATGTCCT
                                      MetProThrValAspAspValLeu

61    GGAGCAAGTTGGAGAGTTTGGCTCGTTCCAGAAACAAGCCTTCCTGTTGCTATGCCTGAT
9      GluGlnValGlyGluPheGlyTrpPheGlnLysGlnAlaPheLeuLeuLeuCysLeuIle

121   CTCAGCTTCTTTAGCTCCCATCTATGTGGGCATCGTCTTCCTGGGCTTCACCCCTGGACA
29     SerAlaSerLeuAlaProIleTyrValGlyIleValPheLeuGlyPheThrProGlyHis

181   TTATTGCCAGAATCCTGGGGTGGCTGAGCTGAGCCAGCGGTGTGGCTGGAGCCAGGCAGA
49     TyrCysGlnAsnProGlyValAlaGluLeuSerGlnArgCysGlyTrpSerGlnAlaGlu

241   GGAGCTGAACTACACTGTGCCGGGCCTGGGACCTTCGGACGAGGCCTCCTTCCTCAGCCA
69     GluLeuAsnTyrThrValProGlyLeuGlyProSerAspGluAlaSerPheLeuSerGln
              *

301   GTGCATGAGGTATGAGGTGGACTGGAACCAGAGCACCCTTGACTGTGTGGACCCACTGTC
89     CysMetArgTyrGluValAspTrpAsnGlnSerThrLeuAspCysValAspProLeuSer
                          *

361   CAGCCTGGTTGCCAACAGGAGTCAGTTGCCATTGGGCCCCTGCGAGCATGGCTGGGTATA
109    SerLeuValAlaAsnArgSerGlnLeuProLeuGlyProCysGluHisGlyTrpValTyr
          *

421   CGACACTCCCGGCTCCTCCATCGTCACTGAGTTTAACCTGGTGTGTGGAGACGCCTGGAA
129    AspThrProGlySerSerIleValThrGluPheAsnLeuValCysGlyAspAlaTrpLys

481   AGTGGACCTTTTTCAGTCCTGTGTGAACTTGGGCTTCTTCCTGGGCTCCCTGGTTGTGGG
149    ValAspLeuPheGlnSerCysValAsnLeuGlyPhePheLeuGlySerLeuValValGly

541   TTACATTGCAGACAGGTTTGGCCGTAAGCTCTGTCTCTTGGTGACCACGCTGGTCACATC
169    TyrIleAlaAspArgPheGlyArgLysLeuCysLeuLeuValThrThrLeuValThrSer

601   TGTGTCCGGTGTGCTAACAGCGGTGGCCCCAGACTATACATCCATGTTGCTCTTTCGCCT
189    ValSerGlyValLeuThrAlaValAlaProAspTyrThrSerMetLeuLeuPheArgLeu

661   GCTGCAGGGCATGGTCAGCAAGGGCAGCTGGGTGTCCGGCTATACCTTGATCACAGAGTT
209    LeuGlnGlyMetValSerLysGlySerTrpValSerGlyTyrThrLeuIleThrGluPhe

721   TGTCGGCTCTGGCTACAGGAGAACGACGGCCATTTTGTACCAGATGGCCTTCACAGTGGG
229    ValGlySerGlyTyrArgArgThrThrAlaIleLeuTyrGlnMetAlaPheThrValGly

781   GCTAGTGGGGCTTGCCGGGGTGGCCTATGCCATTCCAGACTGGCGCTGGCTCCAGCTAGC
249    LeuValGlyLeuAlaGlyValAlaTyrAlaIleProAspTrpArgTrpLeuGlnLeuAla

841   TGTGTCCCTGCCTACCTTCCTCTTCCTGCTGTATTACTGGTTTGTCCCAGAATCCCCCCG
269    ValSerLeuProThrPheLeuPheLeuLeuTyrTyrTrpPheValProGluSerProArg
                                                    *

901   GTGGCTGTTGTCCCAGAAGAGAACCACGCGAGCTGTCAGGATAATGGAGCAAATTGCACA
289    TrpLeuLeuSerGlnLysArgThrThrArgAlaValArgIleMetGluGlnIleAlaGln
          *                 *

961   GAAGAACGGGAAGGTGCCTCCTGCTGACCTGAAGATGCTCTGCCTTGAGGAGGATGCCTC
309    LysAsnGlyLysValProProAlaAspLeuLysMetLeuCysLeuGluGluAspAlaSer
                                    *

1021  AGAAAAGCGAAGTCCTTCGTTTGCCGACCTGTTCCGCACTCCCACCCTGAGGAAGCACAC
329    GluLysArgSerProSerPheAlaAspLeuPheArgThrProThrLeuArgLysHisThr
                                    *

1081  CGTCATCCTGATGTATCTATGGTTCTCTTGTGCTGTGCTGTACCAGGGTCTCATCATGCA
349    ValIleLeuMetTyrLeuTrpPheSerCysAlaValLeuTyrGlnGlyLeuIleMetHis

1141  CGTGGGAGCCACAGGGCCAACCTCTACCTGGACTTCTTTTATTCTTCTCTGGTGGAATT
369    ValGlyAlaThrGlyAlaAsnLeuTyrLeuAspPhePheTyrSerSerLeuValGluPhe

1201  CCCCGCGGCCTTCATCATCCTGGTCACCATTGACCGCATTGGCCGCATCTACCCAATAGC
389    ProAlaAlaPheIleIleLeuValThrIleAspArgIleGlyArgIleTyrProIleAla

1261  GGCCTCGAATCTGGTGACGGGGGCAGCCTGCCTCCTCATGATCTTTATCCCGCATGAGCT
409    AlaSerAsnLeuValThrGlyAlaAlaCysLeuLeuMetIlePheIleProHisGluLeu

1321  GCACTGGTTGAACGTTACCCTCGCCTGTCTTGGCCGTATGGGGGCCACCATTGTGCTGCA
429    HisTrpLeuAsnValThrLeuAlaCysLeuGlyArgMetGlyAlaThrIleValLeuGln
          *

1381  GATGGTCTGCCTGGTGAACGCTGAGCTGTACCCTACATTCATCAGGAATCTTGGGATGAT
449    MetValCysLeuValAsnAlaGluLeuTyrProThrPheIleArgAsnLeuGlyMetMet

1441  GGTATGCTCTGCCCTGTGTGACCTGGGTGGGATCTTCACCCCCTTCATGGTGTTCAGGCT
469    ValCysSerAlaLeuCysAspLeuGlyGlyIlePheThrProPheMetValPheArgLeu

1501  GATGGAAGTTTGGCAAGCCCTGCCCCTCATTTTGTTTGGGGTTTTGGGCCTGACTGCTGG
489    MetGluValTrpGlnAlaLeuProLeuIleLeuPheGlyValLeuGlyLeuThrAlaGly

1561  GGCCATGACTCTTCTTCTCCCAGAGACCAAGGGTGTGGCTTTGCCTGAGACTATTGAAGA
509    AlaMetThrLeuLeuLeuProGluThrLysGlyValAlaLeuProGluThrIleGluGlu

1621  AGCAGAGAACCTGGGGAGGAGGAAATCAAAGGCCAAAGAAAACACGATTTACCTTCAGGT
529    AlaGluAsnLeuGlyArgArgLysSerLysAlaLysGluAsnThrIleTyrLeuGlnVal

1681  CCAAACAGGCAAGTCCTCAAGTACCTGACAGGGATGCTGTGCCAGGAGCTGAGTGGCAGA
549    GlnThrGlyLysSerSerSerThr
          *

1741  GAGAAAGGAGGACTTGCCACTTGGAGGATTCCCAGAAGCCTTTGCCTTTCCAGACTCTTG

1801  TATATATGCACCAGGTTCCAAATGAACTACCAACCTTAAAGACTTTTCTGAAAGCCCAAA

1861  AAAAAAAAAAAAAAAAAAAAA
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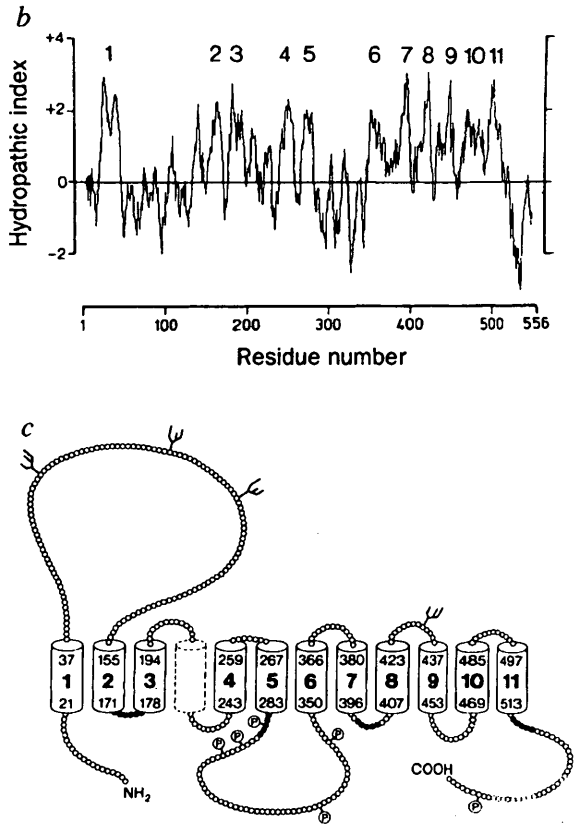

FIG. 2 *a*, Nucleotide and deduced amino-acid sequence of OCT1. Predicted transmembrane domains are underlined and intracellular transporter signatures[20] are boxed. Potential *N*-glycosylation sites of the type NXT/S are indicated by asterisks and potential protein kinase C phosphorylation sites by points. *b*, Kyte–Doolittle hydropathy analysis of OCT1 using a window of 9 (ref. 25); putative transmembrane domains are numbered. *c*, Schematic representation of OCT1 in which an additional presumed transmembrane domain is included. Amino acids of the intracellular transporter signature are shown in black and the potential glycosylation and protein kinase C phosphorylation sites are indicated. METHODS. Using poly(A)$^+$ RNA from rat kidney, double-stranded, blunt-ended cDNA was prepared with the aid of a *Not*I–oligo(dT) primer for first-strand synthesis. After *Eco*RI adaptors containing an SP6 RNA polymerase promoter were added, the cDNA was digested with *Not*I, size-fractionated in agarose (1.5–2.3 kb), ligated into the *Not*I/*Eco*RI restriction sites of pBluescript SK(−) (Stratagene) and electroporated into *E. coli* DH10B. Sublibraries of the transfected bacteria were amplified on agar plates and stored. By testing sublibrary fractions with decreasing numbers of colonies, significant transport was obtained with a mixture of 2,000 colonies. Subpopulations of this mixture were tested over several rounds of screening until OCT1 was isolated. The pools of colonies to be tested were first amplified on agar plates and then in liquid culture. Plasmid DNA was isolated, linearized with *Not*I, and transcribed using SP6 RNA polymerase. cRNA was purified by poly(A)$^+$ selection and injected with 20–40 ng per oocyte. NMN-inhibitable ^{14}C-TEA-uptake was measured as for Fig. 1*a*. For DNA sequencing[26], overlapping restriction fragments of OCT1 were recloned and completely sequenced on both strands using universal and specific primers with Sequenase version 2.0 kit (US Biochem.).

the ^{14}C-TEA uptake expressed by OCT1 is potential-dependent but is not significantly altered by inwardly or outwardly directed proton gradients of 1 pH unit. Thus OCT1 has virtually the same transport characteristics as organic cation transport measured over the basolateral membrane of renal proximal tubules. Figure 1*f* and Table 1 show that OCT1-mediated ^{14}C-TEA uptake is inhibited by cations having different molecular structures, including several common drugs. Unlike the multidrug transporter, which is inhibited exclusively by hydrophobic substances[7], OCT1 is also inhibited by hydrophilic compounds like tetramethylammonium (TMA) and NMN. We found that desipramine inhibited OCT1 700-fold less effectively than the neuronal plasma-membrane noradrenaline transporter[15] and 5 μM of reserpine did not alter OCT1-mediated transport, whereas the vesicular monoamine transporters are inhibited by subnanomolar reserpine concentrations[5]. Comparing the low-affinity inhibitors TMA and NMN, we found that the K_i values of OCT1-expressed transport (~1 mM) were identical to the K_i values (TMA, 1.4 ± 0.4 mM; NMN, 1.1 ± 0.2 mM) estimated for the basolateral TEA uptake in rat renal proximal rubules[13], but differed significantly from the K_i values (TMA, 74 ± 48 mM; NMN, 8.3 ± 2.7 mM) estimated for luminal TEA uptake (ref. 14 and K. J. Ullrich *et al.*, unpublished results). The connection of OCT1 with basolateral transport is also supported by the K_i of 0.4 μM for inhibition of OCT1-mediated uptake by decynium-22: in LLC-PK1 kidney cells, K_i for the inhibition of TEA transport was 6 nM apically and >0.1 μM basolaterally[16]. To verify that structurally different organic cations can be transported by OCT1, uptake of *N*-methyl-4-phenylpyridinium (MPP) was measured. After injection of 8 ng OCT1 cRNA per oocyte in the same batch of oocytes, similar V_{max} values were estimated for the expressed, quinine-inhibitable uptake of ^{14}C-TEA (148 ± 4 pmol oocyte^{-1} h^{-1}) and ^{3}H-MPP (97 ± 5 pmol oocyte^{-1} h^{-1}). We obtained ~100% inhibition by antisense oligonucleotides to OCT1[17] of the quinine-inhibitable uptake of 200 μM ^{14}C-TEA expressed by rat kidney mRNA (Fig. 1*g*). As luminal transport activity is less than 10% in the transport assay, our results suggest that OCT1 mediates organic cation transport across the basolateral membrane.

The nucleotide and derived amino-acid sequence of OCT1 is shown in Fig. 2*a*. The open reading frame is preceded by stop codons and a Kozak-type[18] initiation site of translation (CAGCCATG). Hydropathy analysis of OCT1 (Fig. 2*b*) suggests that there are eleven putative transmembrane protein regions (TM). Because three *N*-linked glycosylation sites are predicted in the first hydrophilic loop, the amino terminus may lie in the cytoplasm. Another putative transmembrane domain

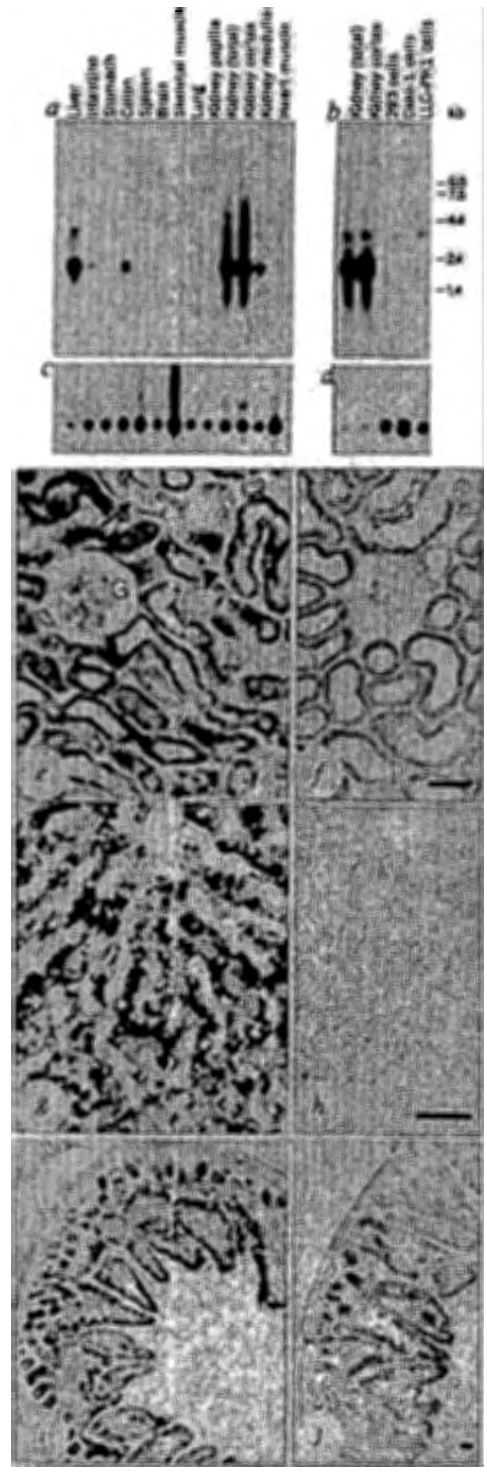

FIG. 3 Localization of OCT1-homologous mRNA analysed by northern blotting (*a–d*) and *in situ* hybridization (*e–j*). Northern blot hybridizations with the cDNA encoding OCT1 are shown in *a* and *b*, and control hybridizations with a cDNA encoding glyceraldehyde-3-phosphate dehydrogenase in *c* and *d*. For *in situ* hybridization, rat kidney cortex (*e*, *f*), liver (*g*, *h*) and small intestine (*i*, *j*) were hybridized with antisense (*e*, *g*, *i*) and sense probes of OCT1 cRNA (*f*, *h*, *j*). Specific signals were detected in renal proximal tubules, hepatocytes and small intestinal enterocytes. In kidney (*e*) no specific signals were detected in glomeruli (G), distal tubules (arrowhead) and collecting ducts (arrow). Scale bars, 100 μm. METHODS. Total RNA was isolated by the acid guanidinium–phenol–chloroform method[27] and mRNA purified using oligo(dT)-cellulose chromatography. For northern blotting, mRNA (tissues and 293 cells (5 μg), Caki-1 cells and LLC-PK1 cells (1.5 μg)) was fractionated on a formaldehyde–agarose gel, transferred to Hybond-N membrane (Amersham) and hybridized to a random-primed ^{32}P-labelled cDNA fragment of pOCT1 (nucleotides 285–1,196) for 18 h at 42 °C in 50% formamide, 5 × SSPE, 5 × Denhardt's solution, 0.5% SDS and 20 μg ml^{-1} salmon sperm DNA. The blot was washed to a final stringency of 0.25 × SSPE, 0.1% SDS at 60 °C. The lanes with mRNA from cells were exposed for 24 h and the other lanes for 6 h. The position of RNA standards (0.14–9.5 kb ladder; GIBCO/BRL) is indicated. To test the loading of mRNA analysed, the ^{32}P-OCT1 probe was removed and the filters were hybridized with ^{32}P-labelled human glyceraldehyde-3-phosphate dehydrogenase cDNA probe (ITC Biotechnology, Heidelberg). *In situ* hybridization was done on 7-μm cryostat sections of tissue fixed with 4% paraformaldehyde using digoxigenin-labelled sense and antisense cRNA probes. The probes were derived from nucleotides 396–835 and 396–1,205 of OCT1, respectively. For hybridization, sections were incubated for 12 h with the labelled cRNA probes (3–5 μg ml^{-1}) dissolved in 1 × Denhardt's solution containing 50 mM Tris-HCl, pH 7.6, 1 mM EDTA, 0.3 M NaCl, 50% deionized formamide, 10% dextran sulphate and 0.5 mg ml^{-1} yeast tRNA. Sections were washed to a final stringency of 0.5 × SSC, 50% formamide (50 °C), incubated (for 4 h at 22 °C) with alkaline-phosphatase-labelled anti-digoxigenin antibodies, and developed with alkaline phosphatase substrate.

TABLE 1 Inhibitor sensitivity of ^{14}C-TEA uptake in *Xenopus* oocytes injected with cRNA of the renal organic cation transporter OCT1

Inhibitor	K_i (μM)
Cyanine-863	0.13 ± 0.02
Decynium-22	0.36 ± 0.08
Tetrapentylammonium	0.43 ± 0.09
Quinine	0.93 ± 0.08
Desipramine	2.8 ± 0.6
Mepiperphenidol	5.2 ± 0.3
Procainamide	13 ± 2
1-Methyl-4-phenylpyridinium	13 ± 2
Corticosteron	> 10
Reserpine	> 20
O-methyl-isoprenaline	43 ± 5
Tetramethylammonium	1,000 ± 100
N^1-methylnicotinamide	1,000 ± 200

Xenopus oocytes were injected with 5 ng OCT1 cRNA and the effect of 5–8 different inhibitor concentrations on the uptake of 95 μM ^{14}C-TEA into the oocytes was measured as for Fig. 1*f*. Inhibition curves were fitted by nonlinear regression analysis and the K_i values (±s.e.m.) calculated.

between TM3 and TM4 has been found in an OCT1-homologous cDNA from human kidney (our unpublished results) which may also exist in OCT1 (Fig. 2*c*). With this transmembrane domain included, there are several possible intracellular protein kinase C phosphorylation sites which may be involved in transport regulation[19]. Also, three short sequence motifs in OCT1 that have been identified in the cytoplasmic domains of transport proteins from different families[20] become localized intracellularly. Northern blot analysis (Fig. 3*a–d*) showed bands of 1.9, 3.4 and 4.8 kilobases (kb) in samples of kidney cortex, kidney medulla, liver, intestine and colon of rat. Porcine LLC-PK1 cells gave a single hybridization band at 3.4 kb. Extra-neuronal noradrenaline transport in heart and Caki-1 cells has been described that has the functional properties of renal luminal H^+-cation antiport[21–23], but as we detected no hybridization in heart and Caki-1 cells, OCT1 probably belongs to a different genetic family. We amplified a cDNA fragment from rat liver by polymerase chain reaction which was identical to nucleotides 400 620 of OCT1, indicating that OCT1 is probably also expressed in liver. The uptake of organic cations in primary cultured rat liver hepatocytes has been found to be almost exclusively performed by a system with K_i values nearly identical to OCT1 for the inhibitors *O*-methyl-isoprenaline, MPP, quinine, decynium-22 and cyanine-863 (E. Martel, H. Russ, I. Azevedo and E. Schömig, manuscript in preparation). This OCT1 fingerprint of inhibitory constants indicates that OCT1 could be the main transporter responsible for organic cation uptake into hepatocytes. *In situ* hybridization in rat kidney showed that OCT1 is expressed in proximal tubules but apparently not in distal tubules, collecting ducts and glomeruli (Fig. 3*e*, *f*). OCT1 is expressed in hepatocytes in liver (Fig. 3*g*, *h*) and in enterocytes of villi and crypts in small intestine (Fig. 3*i*, *j*).

Our results indicate that OCT1 is a new type of polyspecific transporter which is involved in the elimination of cationic drugs. It shares common features with organic cation uptake over the basolateral membrane of renal proximal tubules and appears to be identical to the main organic cation uptake system in hepatocytes. Expression of the human OCT1-homologous gene and of the renal luminal organic cation transporter in epithelial cell lines will provide *in vitro* test systems for the development of drugs with optimized excretion and minimized nephrotoxicity. □

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Systemic and mucosal immunity induced by BCG vector expressing outer-surface protein A of *Borrelia burgdorferi*

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THE bacillus Calmette–Guerin (BCG) is a live attenuated strain of *Mycobacterium bovis* which offers potential advantages as a vector for mucosal delivery of antigens[1–3]. Recombinant BCG elicits protective humoral immune responses to a variety of antigens[4]. Furthermore, BCG binds specifically to microfold cells[5] present in the epithelium overlying lymphoid follicles throughout the mucosal immune system[6–8]. Here we show that a single intranasal vaccination with recombinant BCG expressing the outer-surface protein A antigen from *B. burgdorferi*[9] results in a prolonged (more than one year) protective systemic IgG response and a highly sustained secretory IgA response which is disseminated throughout the mucosal immune system. Furthermore, intranasal immunization induces marked, organized lymphocyte accumulation in the proximal nasopharyngeal lymphoid tissue as well as at distal mucosal sites; the appearance and persistence of lymphoid aggregates correlates with the secretory immune responses. Thus

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