Expression cloning in *Xenopus laevis* oocytes was used to isolate an organic anion transport protein from rat kidney. A cDNA library was constructed from size-fractionated poly(A)⁺ RNA and screened for probenecid-sensitive transport of *p*-aminobiphenyl (PAH). A 2,227-base pair cDNA clone containing a 1,656-base pair open reading frame coding for a peptide 551 amino acids long was isolated and named ROAT1. ROAT1-mediated transport of 50 μM [³H]PAH was independent of imposed changes in membrane potential. Transport was significantly inhibited at 4 °C, or upon incubation with other organic anions, but not by the organic cation tetraethylammonium, by the multidrug resistance tetraethylammonium, by the multidrug resistance inhibitor cyclosporin A, or by urate. External glutarate and α-ketoglutarate (1 mM), both counterions for basolateral PAH exchange, also inhibited transport, suggesting that ROAT1 is functionally similar to the basolateral PAH carrier. Consistent with this conclusion, PAH uptake was trans-stimulated in oocytes preloaded with glutarate, whereas the dicarboxylate methylsuccinate, which is not accepted by the basolateral exchanger, did not trans-stimulate. Finally, ROAT1-mediated PAH transport was saturable, with an estimated *Kₘ* of 70 μM. Each of these properties is identical to those previously described for the basolateral α-ketoglutarate/PAH exchanger in isolated membrane vesicles or intact renal tubules.

Renal organic anion transport has been widely studied for more than a century, both as a prototypic transport process and as a primary means for removal of xenobiotics from the body. Because many foreign chemicals, including plant and animal toxins, drugs, and pesticides, are organic anions or are metabolized to organic anions, the renal organic anion secretory system plays a critical role in limiting or preventing their toxicity. Over the last decade, a great deal of progress has been made toward understanding the physiology of this system, particularly its coupling to metabolic energy. Thus, it is now well established that organic anion secretion is a complex process involving distinctly different proteins at the apical and basolateral membranes of the proximal tubule (Fig. 1; for review, see Ref. 1). Transport across the basolateral membrane is energetically uphill. It is accomplished by a tertiary active process in which (a) Na⁺/K⁺-ATPase establishes the out > in Na⁺ gradient, (b) Na⁺/α-ketoglutarate cotransport driven by the movement of Na⁺ down its concentration gradient, in concert with intracellular metabolic α-ketoglutarate generation, sustains an outward Na⁺ gradient, allowing entry of negatively charged substrate against both its chemical concentration gradient and the electrical potential of the cell. Once inside the cell, organic anions are subject to intracellular binding and to sequestration within vesicular structures (4, 5). Finally, luminal exit is thought to occur by anion exchange and/or facilitated diffusion (6-8).

In contrast to the physiology of the organic anion transport system, precise information about the structural properties of the transport proteins that make up this system are not yet available. However, considerable progress has recently been made for a variety of other transport proteins through the application of expression cloning techniques, leading to increased understanding of the regulation of their expression, identification of substrate binding sites, and a much more complete appreciation of their mechanisms of action (9-11). We report here the successful isolation and characterization of a cDNA encoding the organic anion transporter from rat kidney, ROAT1.

**EXPERIMENTAL PROCEDURES**

Preparation of Fractionated Rat Kidney Poly(A)⁺ RNA—Total RNA was isolated from rat kidney using guanidinium thiocyanate extraction followed by cesium chloride gradient centrifugation according to the protocol of Gasser et al. (12). Poly(A)⁺ RNA was separated by running the total RNA fraction over an oligo(dT)-cellulose column twice and eluting with water. The poly(A)⁺ RNA was size-fractionated by centrifugation through a linear sucrose gradient (5-25% w/v) using a modification of the method of Hagenbuch et al. (13). The isolated polyadenylated RNA fractions were checked for organic anion transport activity by expression assay in *Xenopus* oocytes (14). The fraction with the greatest activity (corresponding to 11.1% sucrose) was used to make a cDNA library.

cDNA Library Construction—The cDNA library was constructed using the SuperScript Plasmid System kit (Life Technologies, Inc.). The synthesized cDNA was ligated into pSPORT1 vector with the start site of RNA transcription positioned downstream from a T7 RNA polymerase promoter present in the vector, so that the cDNA insert could be transcribed into cRNA in an *in vitro* synthesis reaction. The cDNA library plasmids were transformed into MAX Efficiency DH5α-competent cells (Life Technologies, Inc.). Transformed bacteria were plated onto Hybond N nylon filters (Amersham), which were overlaid on Luria-Bertani (LB) plates containing 100 μg/ml ampicillin and incubated overnight at 37 °C. To obtain purified plasmid DNA, whole filters (or filter subsections) were placed in 200 ml of LB broth with 100 μg/ml ampicillin and shaken at 225 rpm overnight at 37 °C. The bacteria were pelleted by centrifugation and plasmid DNA was isolated using a Qiagen plasmid kit (Qiagen, Inc., Chatsworth, CA).
FIG. 1. Model for organic anion transport in renal proximal tubule. Organic anion entry across the basolateral membrane is driven by indirect coupling to the sodium gradient through sodium/p-ketoglutarate cotransport followed by organic anion exchange (1). Organic anions are sometimes sequestered in vesicles within the cytoplasm (2). Luminal exit is presumed to occur via exchange for hydroxyl ions (3) or through a facilitated diffusion process (4) down the electrochemical gradient across the brush border membrane.

cRNA Synthesis—Ambion's T7 mMessage mMachine in vitro transcription kit (Ambion, Inc., Austin, TX) was used to synthesize capped cRNA from library plasmid DNA linearized with BamHI. The cRNA products were quantitated in a spectrophotometer and diluted before injection to allow delivery of 20 ng of cRNA/oocyte in 15 nl with a 10-s injection.

**Xenopus Oocyte Isolation**—Adult female *Xenopus laevis* (Xenopus One, Ann Arbor, MI) were anesthetized by hypothermia and decapitated. Ovaries were then removed and stored in Barth's buffer (88 mM NaCl, 1 mM KCl, 330 μM Ca(NO₃)₂, 410 μM CaCl₂, 520 μM MgSO₄, 24 mM NaHCO₃, 10 mM HEPES, pH 7.4). Stage V and stage VI oocytes were manually dissected free of the ovary and the follicles removed by treatment with collagenase A (Boehringer Mannheim) modified from the protocol of Pajor et al. (15). Briefly, oocytes were placed in collagenase solution (5 mg/ml collagenase A, 1 mg/ml trypsin inhibitor type III-O (Sigma) in Barth's) and gently rocked for 1 h at room temperature. After collagenase treatment, the oocytes were rinsed five times with Barth's containing 1 mg/ml BSA¹ and placed in a phosphate/BSA solution for 1 h (100 mM K₂HPO₄, 3H₂O with 1 mg/ml BSA, pH 6.5). Oocytes were agitated every 15 min to remove the follicle and then rinsed five times with Barth's with BSA. The oocytes were maintained at 18 °C in Barth's containing 0.05 mg/ml gentamycin sulfate, 2.5 mM sodium pyruvate, and 5% heat inactivated horse serum. The oocytes were allowed to recover overnight before injection.

**Xenopus Oocyte Uptake Assay**—Two or three days after injection with 20 ng of cRNA, the oocytes were divided into experimental groups (containing 10 oocytes each) and incubated at 18–22 °C for 10 or 60 min in oocyte Ringer's 2 (OR-2; in mM: 82.5 NaCl, 2.5 KCl, 1 Na₂HPO₄, 3 NaOH, 1 CaCl₂, 1 MgCl₂, 1 pyruvic acid, 5 HEPES, pH 7.6) containing 50 μM [³²P]-aminohippurate (4 μCi/ml) in the absence or presence of 1 μM unlabeled aminohippurate, a known inhibitor of organic anion transport in the kidney. After uptake, oocytes were rapidly rinsed three times with ice-cold OR-2 and placed into individual scintillation vials containing 0.5 ml of 1 M NaOH, incubated at 65 °C for 20 min, and neutralized with 0.5 ml 1 N HCl. Finally, 4.7 ml of Econul (ICN Biomedical, Cleveland, OH) was added and oocyte radioactivity measured in disintegrations per minute in a Packard 1600TR liquid scintillation counter with external quench correction. PAH uptake was calculated in pmol/oocyte, i.e., from dpm/oocyte and medium specific activity. Water-injected or uninjected oocytes were included as negative controls in every experiment.

**DNA Sequencing**—The cDNA clone was sequenced at the University of North Carolina Automated DNA Sequencing Facility on a model 373A DNA sequencer (Applied Biosystems, Foster City, CA) using the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems). The full-length sequence of both strands was obtained using M13/pUC forward and reverse primers, as well as synthetic oligonucleotide primers based on the previously determined sequence (Life Technologies, Inc.). All DNA sequence comparisons and data base searches were done with the Wisconsin Package software with default settings (16).

**Topology Analysis**—The amino acid sequence was analyzed using five different modeling programs for predicting potential transmembrane domains: 1) Kyte-Doolittle (17), 2) TMPred (18), 3) DAS (19), 4) PHDhtmTop (20), and 5) TopPred 2 (21), all with default parameters.

**Southern Blotting**—Approximately 1 μg of plasmid DNA from several library fractions was linearized with the restriction enzyme BamHI and separated by electrophoresis on a 1% agarose gel in TBE buffer at 150 V for 2 h. The gel was transferred to a Hybond N nylon membrane under alkaline conditions (0.4 M NaOH). The blot was probed with a 643-bp fragment (positions 782–1425) from the liver organic anion transporter, oatp (22), and with a 1,368-bp fragment (positions 186–1554) from ROAT1, both amplified by PCR from cDNA clones. Primers used for PCR were: r-liv OATcw (5'-CCGTCGACCGACCATAAACCCAGTG-3') and r-liv OATccw (5'-CCGTTACCCTGAGCAGCTACCTT-3') to generate the oatp probe; Anion3cw (5'-GTCTTCGACTGTATCTGAGC-3') and Anion3ccw (5'-TCCTGGAAGCCCTGCTGAC-3') to generate the ROAT1 probe.

The PCR products were gel-isolated and recovered using a Qiagene gel extraction kit (Qiagen, Inc.). The probes were labeled using the ECL kit (Amersham) and hybridized at 42 °C. The blot was washed under conditions of high stringency (0.1 × SSC) and the probes detected with the ECL kit reagents.

**Northern Blotting**—The rat multiple tissue Northern blot was purchased from CLONTECH Laboratories, Inc. (Palo Alto, CA) and probed with the 1,368-bp ROAT1 probe. The probe was labeled using the Gene Images random prime labeling kit (Amersham) and hybridized at 65 °C. The probe was detected with the Gene Images CDP-Star detection kit (Amersham). The blot was stripped in boiling 0.1% SDS and reprobed with the human β-actin control probe included with the blot.

**Statistics**—Data are presented as mean ± S.E. Differences in mean values were considered to be significant when p ≤ 0.05 as determined by one sample or paired Student's t test, as indicated in figure legends. The degree of significance was indicated as follows: * denotes p ≤ 0.05; ** denotes p ≤ 0.01; and *** denotes p ≤ 0.001.

**Chemicals**—[³²P]-hippurate (3.7 Ci/mmol) was obtained from NEN Life Science Products. [¹⁴C]glutaramate (55 mCi/mmol) and [¹⁴C]dextrothymuron (TEA; 55 mCi/mmol) were obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Unlabeled PAH, probenecid, glutaconate, methylsuccinate, and TEA were obtained from Sigma. All other chemicals were obtained from commercial sources and were of the highest grade available.

**RESULTS**

**Screening of cDNA Library**

Total poly(A)⁺ RNA was isolated from rat kidney and size-fractionated on a linear sucrose gradient (14). Fractions corresponding to 12.3% and 11.1% sucrose were shown to support probenecid-sensitive uptake of 50 μM [³²P]-hippurate that was 2–3-fold higher than that observed for the original unfractionated starting material when injected into *X. laevis* oocytes (Fig. 2A). The 11.1% sucrose fraction mRNA was used as template for the construction of a cDNA library, which was screened by expression cloning in *Xenopus* oocytes, yielding a single purified clone that supported PAH uptake (Fig. 2B). In the presence of 1 mM probenecid, PAH uptake by oocytes expressing the cloned transporter was always reduced to the level observed for water-injected oocytes. Electrophoresis of the *in vitro* cRNA synthesis product showed a band ~2.3 kb in size, which corresponds with the previously reported size range for kidney mRNA that supported PAH uptake (14). We refer to this cDNA clone as renal organic anion transporter 1 or ROAT1.
A rat kidney cDNA library was screened for the ability to support PAH uptake. cRNA transcribed from library plasmid DNA was injected into Xenopus oocytes (20 ng/oocyte) and allowed to express. Two days after injection, the oocytes were incubated for 1 h in 50 μM $[^{3}H]$PAH in the absence (No Inhibitor) or presence (Probeneicid) of 1 mM probenecid. Each column shows the mean value ± S.E. for 2 animals (10 oocytes/treatment/animal). A, evaluation of rat kidney poly(A)$^+$ RNA sucrose gradient fractions; B, response from a single positive clone (ROAT1).

**Southern and Northern Analysis**

A Southern blot of cDNA library fractions performed during the course of the screen demonstrated that the positive library fractions containing ROAT1 did not contain any sequence homologous to a 643-bp probe from oatp (22), the only other known organic anion transporter (Fig. 5, left panel). Subsequent reprobing of the same blot with a 1,368-bp ROAT1 probe confirmed that the ROAT1 probe bound only to the library fractions known to support PAH uptake, and did not bind to oatp (Fig. 5, right panel). Therefore, ROAT1 was determined to be unique from oatp. This was confirmed by DNA sequence analysis (see above).

The same ROAT1 probe was used for a Northern blot and detected a strong signal in rat kidney (Fig. 6). ROAT1 transcript was not observed in rat heart, brain, spleen, lung, liver, skeletal muscle, or testis. The major transcript detected in kidney was ~2.4 kb in size; however, a second, far less abundant transcript ~4.2 kb in size was also seen in longer exposures. The blot was stripped and reprobed with a human β-actin probe, confirming that there was viable mRNA present in each lane of the blot (data not shown).

**Functional Characterization of ROAT1 Transport**

**Potential Dependence**—As a first step in determining the identity of ROAT1 (see Fig. 1), the effect of altered membrane potential on PAH transport in ROAT1-injected oocytes was assessed. The luminal facilitated diffusion system is markedly dependent upon membrane potential (7, 8), whereas both the basolateral and luminal exchangers are not (5, 31). Potential was altered by raising external K$^+$, a condition previously shown to depolarize the plasma membrane of the oocyte (11). When ROAT1-expressing oocytes were incubated in OR-2 with a high potassium ion concentration (102.5 mM), there was no reduction in PAH transport (Fig. 7). As a positive control, oocytes injected with OCT2 cRNA, an organic cation transporter known to have a large potential-sensitive transport component (32), showed a 67% drop in transport of $[^{14}C]$TEA when exposed under the same conditions (Fig. 7). Water-injected

Related to the organic cation transporter family (26). A Smith and Waterman (27) alignment of the two DNA sequences showed a 95% identity, whether the 5′- and 3′-untranslated regions were included or not. Needleman and Wunsch (28) analysis of the predicted amino acid sequences for the two proteins yielded a 96% similarity and 95% identity. The two peptides differ at 27 positions, and there is a six-amino acid gap in NKT corresponding to residues 85–90 in ROAT1 (Fig. 4). The only other known cloned organic anion transporter is oatp from rat liver (22). Comparison of ROAT1 and oatp revealed no homology between the two at the DNA or peptide level (27, 28). Comparison between the peptide sequence of ROAT1 and those of the organic cation transporters OCT1 (11) and OCT2 (29), and another liver transporter of unknown substrate specificity, NLT (30), showed some homology: ROAT1/OCT1, 40% similarity and 33% identity; ROAT1/OCT2, 41% similarity and 31% identity; ROAT1/NLT, 48% similarity and 38% identity. No obvious regions of highly conserved sequence were identified, even in the putative membrane-spanning domains. Rather, the peptides seem to have a low level of identity throughout their sequence. There are, however, three motifs conserved among ROAT1, NKT, OCT1, OCT2, and NLT: the positioning of four cysteine residues and a protein kinase C consensus site within the extracellular loop between predicted membrane-spanning domains 1 and 2, and two casein kinase II consensus sites (Table I). Whether any of these features is involved in transporter function is unknown at this time.

### Molecular Characterization of ROAT1

The complete DNA sequence of both strands of ROAT1 was determined, and the sense strand sequence is presented in Fig. 3A. The ROAT1 cDNA is 2,227 bp long, including 253 bp of 5′-untranslated region, a single, large open reading frame 1,656 bp long, and 318 bp of 3′-untranslated sequence. The ATG at position 254 has the strongest correlation with Kozak’s consensus sequence for translation initiation (23), giving a positioning of four cysteine residues and a protein kinase C consensus site within the extracellular loop between predicted membrane-spanning domains 1 and 2, and two casein kinase II consensus sites (Table I). Whether any of these features is involved in transporter function is unknown at this time.

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A BLAST search (24) of the GenBank data base (25) identified a single DNA sequence, NKT (accession number U52842; Ref. 26), with significant homology to ROAT1. NKT was isolated from mouse kidney and described as a gene product.
oocytes showed no uptake under either condition. Therefore, ROAT1-mediated PAH transport is independent of membrane potential.

**Inhibition Profile**—The effects of various compounds and reduced temperature on PAH uptake were also examined (Fig. 8). Incubation at 4 °C reduced uptake to just 3% of that seen at room temperature. The organic anions probenecid (1 mM), α-ketoglutarate (α-KG; 1 mM), bromoresol green (1 mM), and excess unlabeled PAH (1 mM) each reduced [3H]PAH uptake by 80–90%. In addition, like basolateral PAH/α-KG exchange (3, 33), but in contrast with luminal exchange (31), ROAT1-mediated PAH uptake was not inhibited by urate (1 mM).
Transport was also unaffected by the P-glycoprotein inhibitor cyclosporin A (CSA; 10 μM). Unlike urate and CSA, which gave values essentially identical to control, the cation TEA appeared to inhibit slightly, albeit at a high concentration (5 mM). Lower concentrations of TEA were not inhibitory and, at concentrations from 0.05 to 1 mM, actually stimulated uptake 20–40% (data not shown). To establish whether these modest effects might indicate that TEA was a substrate for ROAT1, the uptake of 200 μM [14C]TEA by ROAT1 cRNA and water-injected oocytes was measured. No difference in uptake between the two groups was observed (data not shown).

The ability of α-KG to cis-inhibit PAH uptake (Fig. 8) is potentially diagnostic in that the basolateral dicarboxylate/organic anion exchanger should be inhibited by external α-KG, whereas luminal PAH carriers should not be inhibited by α-KG. Glutarate is also an effective counterion for this exchanger (3). When ROAT1 cRNA-injected oocytes were incubated in OR-2 containing 0–1 mM glutarate, a clear, dose-dependent inhibition of PAH uptake was observed, with significant inhibition at 200 μM glutarate and above (Fig. 9).

**trans-Stimulation**

If ROAT1 is the basolateral dicarboxylate/organic anion exchanger, increasing the intracellular concentration of α-KG (or glutarate) should induce trans-stimulation of PAH uptake (see Fig. 1). For this determination, glutarate is the preferred counterion since, in contrast to α-KG, it is not extensively metabolized (34). Preliminary experiments showed substantial accumulation of 1 mM [14C]glutarate within uninjected Xenopus oocytes over time (170 pmol/oocyte after 90 min; data not shown).
Incubating the oocytes in 1 mM glutarate for 90 min before exposure to PAH (i.e. preloading) significantly stimulated PAH uptake in ROAT1-expressing oocytes, as compared with non-preloaded oocytes (Fig. 10). Moreover, glutarate pre-loading had no effect on water-injected oocytes. Thus, glutarate induced trans-stimulation of ROAT1-mediated PAH uptake. The specificity of trans-stimulation was assessed by comparing the effects of glutarate with the poorly metabolized dicarboxylate methylsuccinate, which cannot substitute for α-KG or glutarate on the α-KG/PAH exchanger (35, 36). As shown in Fig. 11, increasing the preloading concentration of glutarate from 0 to 5 mM increased glutarate’s stimulatory effect on PAH uptake in a dose-dependent fashion, reaching an impressive 250% increase over non-preloaded oocytes at 5 mM. In contrast, methylsuccinate failed to stimulate PAH uptake over the same concentration range. Indeed, at 5 mM, methylsuccinate inhibited PAH uptake by 70% (p ≤ 0.01).

**Kinetics**

PAH uptake by ROAT1-injected oocytes increased steadily with time and was linear for about 1 h (data not shown). Using 10 min to approximate initial rate, the kinetics of ROAT1-mediated PAH transport were assessed by incubating oocytes expressing ROAT1 in medium containing 0.05–1 mM PAH (Fig. 12A). A double-reciprocal plot of the saturation data was constructed and linear regression analysis was performed to obtain estimates for $K_m$ of 70 μM and for $V_{max}$ of 6 pmol/oocyte/10 min (Fig. 12B). It should be noted that, in the oocyte expression assay system, $V_{max}$ reflects the degree of cRNA expression rather than a true measure of maximal uptake rate for a transporter in its native tissue.

**DISCUSSION**

Many toxic anions, whether of endogenous or environmental origin, are eliminated from the body by the organic anion secretory system of the renal proximal tubule. This system has been actively investigated for more than 100 years, in part because of its effectiveness, which can clear the renal plasma of a good substrate like PAH in a single pass, and in part because of its critical role in protecting against the toxic effects of anionic xenobiotics through their rapid excretion via the urine (1). Recent emphasis has been on the specificity of the basolateral carrier, which accepts a remarkably broad spectrum of agents, requiring only a hydrophobic backbone and negative or partial negative charges optimally separated by 6–7 Å (37), and on the energetics of transport, which is driven by a complex tertiary coupling to metabolic energy (1). As a first step toward understanding the molecular basis for these features, we have used expression cloning in X. laevis oocytes to isolate a cDNA that encodes a novel renal organic anion transporter, ROAT1, which mediates transport of the model organic anion, PAH (Fig. 2).

Sequence analysis revealed that the recently described mouse kidney gene NKT (26) is 96% similar and 95% identical to ROAT1 at the amino acid level (Fig. 4). However, Lopez-Nieto et al. (26) were unable to show any transport activity for NKT and, therefore, concluded NKT was related to the organic

![Fig. 7. Effect of membrane potential on substrate uptake.](image)

The 60-min uptake of 50 μM [3H]PAH by water- or ROAT1 cRNA-injected oocytes was compared under normal (2.5 mM K⁺) and membrane depolarizing conditions (102.5 mM K⁺; (11)), in the absence (No Inhibitor) or presence (+ Probenecid) of 1 mM probenecid. We have documented the influence of potential on both ROAT1 and OCT2 in 6 animals; however, the data presented here are from a representative animal because the experiment has only been done with both transporters in the same animal on two occasions. Columns represent mean values ± S.E. from 10 oocytes.

![Fig. 8. ROAT1 substrate specificity.](image)
cRNA from ROAT1 was injected into oocytes and [3H]PAH transport was measured after 60 min in the presence of several organic anions and cations, and at reduced temperature. Data are presented as percent of control uptake. Values are mean ± S.E. for 4–6 animals (10 oocytes/treatment/animal). ** denotes p ≤ 0.01, and *** denotes p ≤ 0.001. BCG, bromocresol green.
cation transporter family based on sequence homology. We believe that NKT is the mouse counterpart of ROAT1. Our Northern tissue distribution results indicated significant quantities of ROAT1 message are expressed only in the kidney (Fig. 6). In contrast to NKT (26), no transcript was detected in the lane containing rat brain mRNA. Additionally, a second band (~4.2 kb) was visible in the kidney mRNA lane, albeit much fainter than the 2.4-kb band. Whether this band represents an isoform of ROAT1, incompletely processed ROAT1 message, or a completely different transporter with significant homology to ROAT1 is unknown. Southern blot analysis indicated that the liver organic anion transporter oatp (22) and ROAT1 are not the same protein and do not represent tissue-specific isoforms of the same transporter (Fig. 5).

The moderate level of homology shared by all of the kidney organic ion transporters identified so far, whether components of the organic anion or organic cation transport systems, may be indicative of some basic properties common to these renal transporters. However, no regions of high homology (e.g., nearly identical sequences in the putative transmembrane domains) were observed. Instead, there was a low level of homology throughout the entire length of the peptides. Interestingly, oatp (22) did not share any homology with ROAT1, despite their similar function (38). This lack of homology may be related to the differing substrate specificities of these two anion transporters (this work and Ref. 22); however, if so, this makes the identity shared between ROAT1 and the cation transporters OCT1 and OCT2 all the more perplexing.

Clearly, ROAT1 is an organic anion transport protein. It mediates probenecid-sensitive PAH transport, which is strongly inhibited by other organic anions, but not by the

**Fig. 9. Effect of external glutarate on PAH uptake.** Two days after injection, oocytes were incubated for 60 min in OR-2 with 50 μM [3H]PAH and 0–1 mM glutarate. Uptake is expressed as percent of uptake when no compound was present, i.e. 0 mM glutarate. Data are mean ± S.E. values for 4 animals (10 oocytes/treatment/animal). * denotes p < 0.05, and *** denotes p < 0.001.

**Fig. 11. Dose response of glutarate trans-stimulation.** Oocytes injected with ROAT1 cRNA were preloaded for 90 min in 0–5 mM glutarate or methylsuccinate, washed briefly in dicarboxylate-free medium, and incubated with 50 μM [3H]PAH for 60 min. Uptake is expressed as percent of uptake with no preloading, i.e. 0 mM glutarate or methylsuccinate. Data are mean ± S.E. values for 4–6 animals (10 oocytes/treatment/animal). * denotes p < 0.05, and ** denotes p < 0.01.

**Fig. 10. trans-Stimulation of PAH uptake.** Water-injected or ROAT1 cRNA-injected oocytes, either non-preloaded (control) or preloaded by a 90-min incubation in 1 mM glutarate, were washed with glutarate-free medium and exposed to 50 μM [3H]PAH for 60 min in the absence (No Inhibitor) or presence (+ Probenecid) of 1 mM probenecid. Data are mean ± S.E. values from a representative animal (10 oocytes/treatment). The experiment was repeated four times and data calculated as percent of control uptake. Uptake by preloaded, cRNA-injected oocytes was 204 ± 19% (p < 0.01). * denotes p < 0.05, using paired Student's t test.
organic cation TEA, or by CSA, which inhibits the multidrug resistance transporter (Fig. 8). However, as summarized in Fig. 1, several renal organic anion transport proteins have been described. Thus, ROAT1 could code for the basolateral α-KG/PAH exchanger (2, 3), either of the luminal carriers (the potential driven carrier (8) and the anion exchanger (31)), or the as yet uncharacterized system responsible for accumulation of organic anions within intracellular organelles (5). To differentiate between these possibilities, the functional properties of ROAT1 were investigated in cRNA-injected Xenopus oocytes. As shown in Fig. 7, ROAT1-mediated PAH transport was independent of changes in membrane potential. This observation demonstrated that the cloned transporter is not the luminal facilitated diffusion carrier, since that carrier shows marked potential dependence (8). Likewise, ROAT1’s lack of inhibition by urate (Fig. 8) would appear to distinguish it from the luminal anion exchanger characterized by Blomstedt and Aronson (31). Thus, the most likely candidate for ROAT1 is the basolateral dicarboxylate/organic anion exchanger. Indeed, all properties of ROAT1 that were examined using the oocyte expression system, including its insensitivity to membrane potential and its lack of inhibition by urate, were entirely consistent with previously documented characteristics of the basolateral exchanger. Its interactions with the dicarboxylates α-KG, glutarate, and methylsuccinate were particularly diagnostic (Figs. 8–11). A variety of evidence from membrane vesicles and intact renal tissue documents that both glutarate and α-KG may act as counterions for the basolateral anion exchanger (2, 3, 35, 39). Thus, the presence of either dicarboxylate on the same side of the membrane as PAH will cis-inhibit PAH transport, and an outwardly directed gradient of either will accelerate (trans-stimulate) PAH accumulation. As shown in Figs. 8–11, ROAT1-mediated uptake behaves exactly like the basolateral system, being both cis-inhibited and trans-stimulated by these compounds. Furthermore, as shown in Fig. 11, methylsuccinate, which is not accepted by the basolateral exchanger (35), failed to trans-stimulate ROAT1-mediated PAH uptake as well. Finally, the $K_m$ for ROAT1-mediated PAH transport was shown to be 70 μM (Fig. 12), which is in good agreement with the value of 80 μM obtained by Ulrich et al. (40) in the intact renal tubules of the rat.

In summary, functional data indicate that ROAT1 is the basolateral dicarboxylate/organic anion exchanger of the renal proximal tubule. Furthermore, both DNA and protein sequence comparisons suggest that ROAT1 and the recently identified mouse homologue, NKT, are species counterparts of the same transporter, although in the absence of functional data on NKT this remains to be conclusively shown. Identification of ROAT1 and its possible mouse homologue provide an important first step in the detailed characterization of the biochemical properties and control mechanisms which determine the functional state of this important excretory protein in the intact tissue.

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