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Molecular Cloning and Characterization of a New Multispecific Organic Anion Transporter from Rat Brain*

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A cDNA encoding the new member of the multispecific organic anion transporter family, OAT3, was isolated by the reverse transcription-polymerase chain reaction cloning method. Degenerate primers were designed based on the sequences conserved among OAT1, OAT2, and organic cation transporter 1 (OCT1), and reverse transcription-polymerase chain reaction was performed using rat brain poly(A)$^+$ RNA. The 536-amino acid protein sequence encoded by OAT3 showed 49, 39, and 36% identity to those of OAT1, OAT2, and OCT1, respectively. Northern blot analysis revealed that rat OAT3 mRNA is expressed in the liver, brain, kidney, and eye. When expressed in *Xenopus laevis* oocytes, OAT3 mediated the uptake of organic anions, such as *p*-aminohippurate (K_m = 65 μM), ochratoxin A (K_m = 0.74 μM), and estrone sulfate (K_m = 2.3 μM) and a cationic compound, cimetidine. OAT3-mediated uptake of [^{3}H]estrone sulfate was sodium-independent. *para*-Aminohippuric acid, estrone sulfate or ochratoxin A did not show any *trans*-stimulatory effect on either influx or efflux of [^{3}H]estrone sulfate via OAT3. Organic anions such as sulfobromophthalein, probenecid, indocyanine green, bumetanide, piroxicam, furosemide, azidodeoxythymidine, 4,4′-diisothiocyanostilbene-3,3′-disulfonic acid, and benzylpenicillin inhibited OAT3-mediated estrone sulfate uptake, while ouabain and digoxin did not. Organic cations such as tetraethylammonium, guanidine, verapamil, and quinidine did not interact with OAT3. Acidic metabolites of neurotransmitters derived from dopamine, epinephrine, norepinephrine, and serotonin inhibited the uptake of estrone sulfate via OAT3. These results suggest an important role of OAT3 in the excretion/detoxification of endogenous and exogenous organic anions, especially from the brain.

The liver and kidney are organs central to the elimination of endogenous and exogenous organic anions (1–5). Previous physiological and pharmacological experiments have revealed that the uptake of organic anions in the liver and kidney across the basolateral membrane occur via transporters possessing a wide substrate selectivity (1–5). In the liver, the organic anion transporting polypeptide 1 (oatp1)[1] and oatp2 are multispecific organic anion transporters that transport structurally unrelated organic anions in a sodium-independent manner (1, 2, 6, 7).

In regard to the kidney, organic anion transporter 1 (OAT1/ROAT1) has recently been isolated from the rat kidney (8, 9). OAT1 is a multispecific organic anion transporter that interacts with a variety of organic anions such as *p*-aminohippurate (PAH), dicarboxylates, cyclic nucleotides, prostaglandin E_2, urate, β-lactam antibiotics, nonsteroidal anti-inflammatory drugs, and diuretics (8). The transport characteristics of OAT1 are identical to those of the classical organic anion transporter on the basolateral membrane of the proximal tubule (3–5, 8, 9). A search of a DNA data base revealed that the amino acid sequence of novel liver-specific transport protein (NLT) (10) shows 42% identity to that of OAT1 (11). We demonstrated that NLT also mediated the transport of organic anions such as PAH, salicylate and acetylsalicylate, prostaglandin E_2, and dicarboxylates and concluded that NLT is a liver-specific organic anion transporter (OAT2) (11).

In contrast to the liver and kidney, little is known concerning the organic anion transport in the brain. The brain possesses two physiological barriers, namely, blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB), which prevent the entry of xenobiotics into the brain (12–14). The tight junction, an anatomical feature of BBB and BCSFB, connects brain capillary endothelial cells or choroid epithelial cells to each other and minimizes the influx of xenobiotics via paracellular routes (12–14). In addition, *in vivo* and *in vitro* kinetic studies have suggested the presence of efflux transport pathways for organic anions in the BBB and BCSFB. PAH and benzylpenicillin, typical substrates of the renal organic anion transporter, have been demonstrated to be effluxed from the brain (15–17). When injected into the cerebral cortex, the rapid and saturable elimination of PAH from the brain was observed (15). The efflux of benzylpenicillin and cimetidine via BCSFB was also reported to be saturable process (16–18). Based on the results of these studies, the presence of a kidney-like organic anion efflux transporter(s) has been predicted in the brain. OAT1 and

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AB017446.

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[1] The abbreviations used are: oatp, organic anion transporting polypeptide; PAH, *para*-aminohippuric acid; TEA, tetraethylammonium; OAT, organic anion transporter; MES, 4-morpholineethanesulfonic acid; PCR, polymerase chain reaction; OCT, organic cation transporter; NLT, novel liver-specific transport; BBB, blood-brain barrier; BCSFB, blood-cerebrospinal fluid barrier.

OAT2 are expressed specifically in the kidney and liver, respectively (8, 9, 11). Although OAT1 was detected in the brain, its expression level was very small (8). From these observations, we hypothesized the existence of a new member of the OAT family in the brain. In the present study, we report the isolation of the new member of OAT family, OAT3, which is much more strongly expressed in the brain than OAT1.

EXPERIMENTAL PROCEDURES

Materials—[^{3}H]PAH (2.45 Ci/mmol) and [^{3}H]estrone sulfate (53 Ci/mmol) were purchased from NEN Life Science Products. [^{3}H]Ochratoxin A (14.8 Ci/mmol) was purchased from Moravek (Brea, CA). [^{32}P]dCTP and [^{3}H]cimetidine (18.2 Ci/mmol) were obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). All other chemicals and reagents were of analytical grade.

Reverse Transcription-PCR—Degenerate PCR primers were designed based on the amino acid sequences that are conserved among OAT1, OAT2 (NLT) and OCT1 (19): forward primer, 5′-TYMYWGARTCHSCMCGSTGG-3′ (corresponding to the nucleotide 803–822 of OAT1) and reverse primer, 5′-AKSAMWGTRGGGTACARCTC-3′ (corresponding to the nucleotide 1339–1358 of OAT1). One microgram of poly(A)$^+$ RNA prepared from rat brain was reverse-transcribed and used as a template for subsequent PCR with the set of degenerate primers. PCR was performed according to the following protocol: 94 °C for 10 s, 49 °C for 30 s, and 72 °C for 30 s; 40 cycles. PCR products were isolated using TA cloning kit (Invitrogen) and sequenced. A PCR product, whose partial amino acid sequence showed 55% identity to that of OAT1, was labeled with [^{32}P]dCTP by random priming method (T7 Quick Prime Kit, Amersham Pharmacia Biotech) and used for the screening of OAT3 as a probe. Since the preliminary Northern blot analysis revealed that the mRNA detected by this probe was abundantly expressed in the kidney, we used the rat kidney cDNA library for the screening.

Construction of cDNA Library and Isolation of OAT3—A nondirectional cDNA library was prepared from rat kidney poly(A)$^+$ RNA using the Superscript Choice system (Life Techonologies, Inc.), and the cDNAs were ligated into λZipLox *Eco*RI arms (Life Techonologies, Inc.). Replicated filters of a phage library were hybridized overnight at 37 °C in a hybridization solution (50% formamide, 5 × standard saline citrate (SSC), 3 × Denhardt's solution, 0.2% SDS, 10% dextran sulfate, 0.2 mg/ml denatured salmon sperm DNA, 2.5 mM sodium pyrophosphate, 25 mM MES, and 0.01% Antifoam B, pH 6.5), and washed at 37 °C in 0.1 × SSC and 0.1% SDS. The cDNA inserts in positive λZipLox phages were recovered in plasmid pZL1 by *in vivo* excision and further subcloned into pBluscript II SK− (Stratagene) for sequencing and *in vitro* transcription.

Sequence Determination—Deleted clones obtained by KiloSequence deletion kit (Takara, Japan), and specially synthesized oligonucleotide primers were used for sequencing of OAT3 cDNA.

cRNA Synthesis and Uptake Experiments Using X. laevis Oocytes—cRNA synthesis and uptake measurements were performed as described previously (8). The capped cRNAs were synthesized *in vitro* using T7 RNA polymerase from the plasmid DNAs linearized with *Xba*I. Defolliculated oocytes were injected with 10 ng of the capped cRNA and incubated in Barth's solution (88 mM NaCl, 1 mM KCl, 0.33 mM $Ca(NO_3)_2$, 0.4 mM $CaCl_2$, 0.8 mM $MgSO_4$, 2.4 mM $NaHCO_3$, and 10 mM HEPES) containing 50 μg/ml gentamicin and 2.5 mM pyruvate, pH 7.4, at 18 °C. After incubation for 2–3 days, uptake experiments were performed at room temperature in ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.4). The uptake experiment was initiated by replacing ND96 solution with that containing a radiolabeled ligand and terminated by the addition of ice-cold ND96 buffer after 1 h of incubation. Oocytes were washed five times with ice-cold ND96 and solubilized with 10% SDS, and the accumulated radioactivity was determined.

The kinetic parameters for the uptake of PAH, estrone sulfate, and ochratoxin A via OAT3 were estimated from the following equation: $v = V_{max} \times S/(K_m + S)$, where v is the uptake rate of the substrate (picomoles/hour/oocyte), S is the substrate concentration in the medium (micromolar), K_m is the Michaelis-Menten constant (micromolar), and V_{max} is the maximum uptake rate (picomoles/hour/oocyte). To obtain the kinetic parameters, the equation was fitted to the OAT3-specific transport velocity, which was obtained by subtracting the transport velocity in noninjected oocytes from that in OAT3-expressing oocytes, by an iterative nonlinear least squares method using a MULTI program. The input data were weighted as the reciprocal of the observed values, and the Damping Gauss Newton Method algorithm was used for fitting. The fitted line was converted to the v/S *versus* v form (Eadie-Hofstee plot).

Examination of trans-Stimulatory Effect on the Transport via OAT3—In order to examine the *trans*-stimulatory effet on the efflux of radiolabeled substrate, oocytes expressing OAT3 were preloaded with [^{3}H]estrone sulfate (80 nM in the medium) for 90 min and then transferred into the medium with or without unlabeled substrates (PAH, ochratoxin A, and estrone sulfate). Both the radioactivity in the medium and that remaining in oocytes were determined after 90-min incubation. In the experiment examining *trans*-stimulatory effect on the influx of radiolabeled substrate, oocytes expressing OAT3 were preloaded with unlabeled substrates for 5 h and then transferred into the medium containing [^{3}H]estrone sulfate (40 nM). The uptake rate of [^{3}H]estrone sulfate for 1 h was determined.

Northern Blot Analysis—Three μg of poly(A)$^+$ RNA prepared from various rat tissues were electrophoresed on a 1% agarose/formaldehyde gel and transferred onto a nitrocellulose filter. The filter was hybridized in a hybridization solution overnight at 42 °C with a full-length cDNA of OAT3, which was randomly labeled with [^{32}P]dCTP. The filter was washed finally in 0.1 × SSC and 0.1% SDS at 60 °C.

RESULTS

A PCR product was isolated from rat brain mRNA using reverse transcription-PCR method. And its partial amino acid sequence exhibited some identity to OAT1. Using this as a probe, we isolated a novel cDNA (rkl411) from the rat kidney cDNA library. When expressed in *X. laevis* oocytes, rkl411 mediated the uptake of several organic anions; we therefore designated rkl411 as OAT3 (organic anion transporter 3). OAT3 cDNA consists of 2191 base pairs encoding a membrane protein of 536 amino acids. Fig. 1 shows the deduced amino acid sequence of rat OAT3 and the alignment with those of rat OAT1, OAT2, OCT1, and OCT2 (20). The amino acid sequence of OAT3 shows 49, 39, 36, and 35% identity to those of rat OAT1, OAT2, OCT1,and OCT2, respectively (Table I). Recently, a third member of organic cation transporter, rat OCT3, was isolated (21). Its amino acid sequence showed 35% identity to that of OAT3 (Table I). Four putative *N*-glycosylation sites and eight protein kinase C-dependent phosphorylation sites are predicted; two *N*-glycosylation sites in the first loop between transmembrane domains 1 and 2 (54 and 102 residues), and two protein kinase C-dependent phosphorylation sites between transmembrane domains 6 and 7 (259 and 266 residues) are identical with those of OAT1 (Fig. 1). Kyte-Doolittle (31) hydropathy analysis suggests that OAT3 possess 12 putative membrane-spanning domains.

The tissue distribution of OAT3 was investigated by Northern blot analysis using poly(A)$^+$ RNA from various rat tissues (Fig. 2). A strong 2.7-kilobase mRNA band was detected in the brain, liver, kidney, and weakly in the eye. No hybridization signals were detected with mRNA isolated from other tissues. A slightly short transcript (2.2 kilobases) was detected only in the liver (Fig. 2).

Using *Xenopus* oocytes injected with OAT3 cRNA, we investigated the transport characteristics via OAT3. OAT3 mediated the transport of [^{3}H]PAH, [^{3}H]estrone sulfate, [^{3}H]ochratoxin A, and [^{3}H]cimetidine (Fig. 3), but not salicylate, taurocholate, digoxin, tetraethylammonium (TEA), guanidine, and daunomycin (data not shown). The uptake of estrone sulfate via OAT3 was not affected by the replacement of extracellular sodium with lithium, choline, or *N*-methyl-D-glucamine (Fig. 4). We examined whether *trans*-stimulatory effect was observed in the transport via OAT3. Estrone sulfate, ochratoxin A, and PAH, when added to the medium, did not stimulate efflux of the preloaded estrone sulfate via OAT3 (Fig. 5*A*). Vice versa, intracellulary accumulated unlabeled PAH, ochratoxin A, or estrone sulfate did not stimulate the uptake of [^{3}H]estrone sulfate (Fig. 5*B*). Concentration dependence of the uptake of [^{3}H]PAH, [^{3}H]estrone sulfate, and [^{3}H]ochratoxin A via OAT3 was examined (Fig. 6). OAT3-mediated uptake of these com-

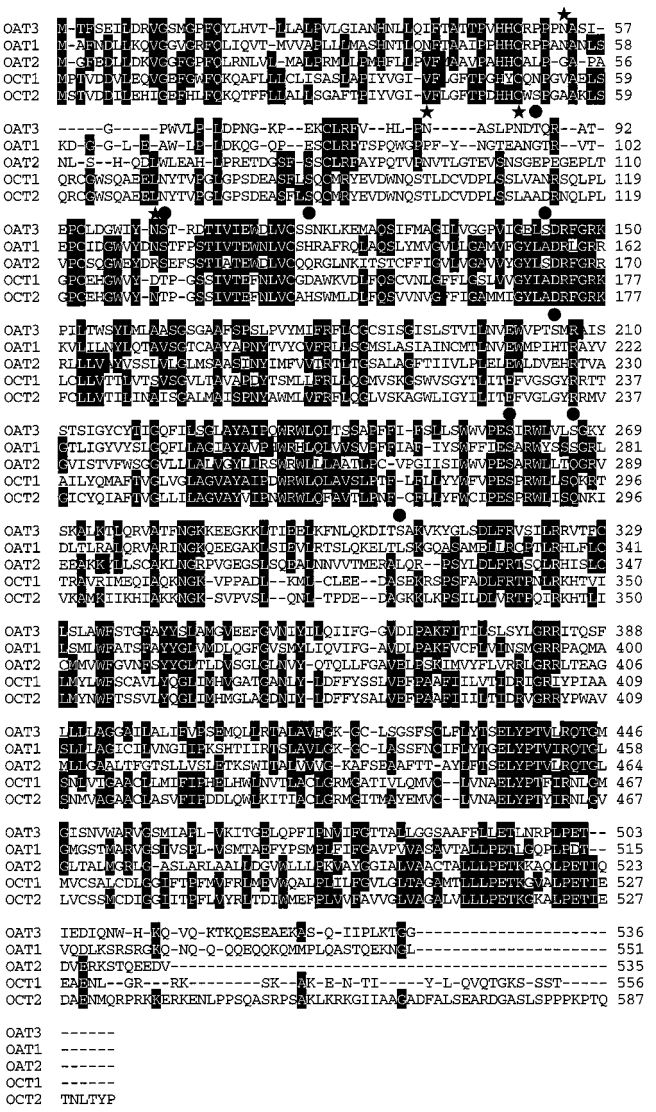

FIG. 1. **The amino acid sequence of OAT3 aligned with those of homologous transporters from rat.** *Boxed* residues denote residues conserved in at least three transporters. Putative *N*-linked glycosylation sites of OAT3 are indicated by *stars;* putative protein kinase C phosphorylation sites (●) are also indicated.

TABLE I
Comparison of amino aid sequence identity between rat OATs and OCTs

	OAT2	OAT3	OCT1	OCT2	OCT3
OAT1	42%	49%	38%	36%	36%
OAT2		39%	36%	36%	37%
OAT3			36%	35%	35%
OCT1				64%	48%
OCT2					48%

pounds showed saturable kinetics and followed the Michaelis-Menten equation. Nonlinear regression analyses yielded K_m values of 64.7 ± 10.0 μM, 2.34 ± 0.20 μM, and 0.739 ± 0.178 μM and V_{max} values of 23.3 ± 2.8 pmol/h/oocyte, 7.60 ± 0.44 pmol/h/oocyte, and 3.08 ± 0.33 pmol/h/oocyte for PAH, estrone sulfate, and ochratoxin A, respectively.

To investigate the substrate selectivity, the inhibitory effect of various compounds on OAT3-mediated [^{3}H]estrone sulfate uptake was determined (Fig. 7). *cis*-Inhibitory effects were observed for structurally unrelated organic anions; sulfobromophthalein, probenecid, indocyanine green, bumetanide, piroxicam, furosemide, azidodeoxythymidine, benzylpenicillin,

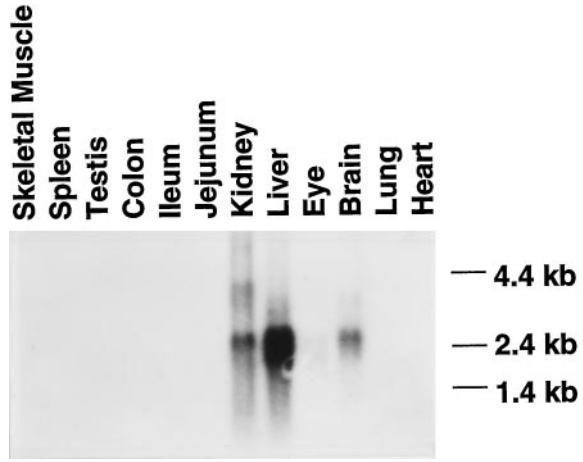

FIG. 2. **Localization of OAT3 mRNA in rat tissues by Northern blot analysis.** Three micrograms of poly(A)$^+$ RNA from various rat tissues (except eye, 1.5 μg) was probed with ^{32}P-labeled cDNA of OAT3. *kb*, kilobases.

and 4, 4′-diisothiocyanatostilbene-2,2′-disulfonic acid potently inhibited OAT3-mediated [^{3}H]estrone sulfate uptake (Fig. 7). Inhibition by taurocholate, cholate, cefoperazone, and methotrexate was moderate, and that by indomethacin, ouabain, digoxin, and cationic compounds, such as TEA, guanidine, quinidine, and verapamil was minimal or not observed at all (Fig. 7). Acidic metabolites of neurotransmitters derived from dopamine, epinephrine, and norepinephrine, such as 4-hydroxy-3-methoxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid, 4-hydroxy-3-methoxymandelic acid, and 3,4-dihydroxymandelic acid, and those from serotonin, such as 5-methoxyindol-3-acetic acid, 5-hydroxyindol-3-acetic acid and 5-methoxytryptophol potently inhibited the uptake of [^{3}H]estrone sulfate by OAT3-expressing oocytes, while 3,4-dihydroxymandelic acid did not (Fig. 8).

DISCUSSION

In the present study, we report the isolation of multispecific organic anion transporter 3 (OAT3). OAT3 encodes a 536-amino acid protein, which shows 49, 39, 36, and 35% identity to rat OAT1, OAT2, OCT1, and OCT2, respectively (Table I). A search of a DNA data base during the revision of this manuscript revealed that OAT3 shows 92% identity to a newly published sequence of Roct (reduced in osteosclerosis transporter) (22) in amino acid level. Although its function has not been elucidated, the reduced expression level of Roct was observed in the oc mouse (a model animal for osteopetrosis) (22). Accordingly, we speculate that Roct is a murine homologue of OAT3.

When expressed in *X. laevis* oocytes, OAT3 mediated the uptake of PAH, estrone sulfate, ochratoxin A, and cimetidine in a sodium-independent manner. In contrast to the fact that OAT1 is apparently an exchanger, we did not observe any *trans*-stimulatory effect in OAT3-mediated transport of estrone sulfate. *trans*-Stimulatory effect was not also observed in the experiments using OAT2 (11); intracellulary accumulated unlabeled dicarboxylate, a substrate of OAT2, did not stimulate the uptake of salicylate via OAT2 (11). These results suggest that the transport characteristic(s) of OAT2 and OAT3 may be different from those of OAT1. There seems to be the other possibility that unidentified endogenous substrate(s), which is abundantly existing in the *X. laevis* oocytes, may mask the *trans*-stimulatory effect. Whether OAT2 and OAT3 surely catalyze one way influx remains to be clarified in further studies.

Since the uptake of estrone sulfate via OAT3 was not *cis*-inhibited by organic cations (TEA, guanidine, verapamil, and quinidine), OAT3 is considered to specifically recognize organic

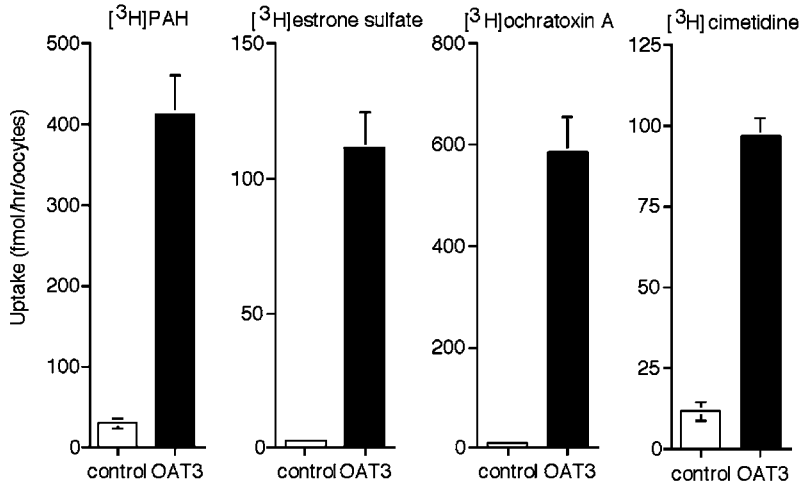

FIG. 3. **OAT3-mediated uptake of radiolabeled compounds.** The uptake rates of radiolabeled compounds ([^{3}H]PAH, 3 μM; [^{3}H]estrone sulfate, 40 nM; [^{3}H]ochratoxin A, 150 nM; and [^{3}H]cimetidine, 150 nM) by control (*open columns*) or OAT3-expressing (*closed columns*) oocytes were measured after incubation for 1 h (mean ± S.E.; n = 10).

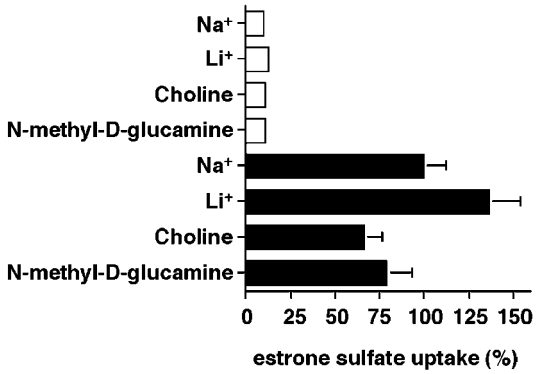

FIG. 4. **Effect of extracellular cation on [^{3}H]estrone sulfate uptake in *X. laevis* oocytes expressing OAT3.** The uptake rate of [^{3}H]estrone sulfate (40 nM) by noninjected oocytes (*open columns*) or OAT3-expressing oocytes (*closed columns*) for 1 h was measured (mean ± S.E.; n = 10) in the presence or absence of extracellular Na^+. Extracellular Na^+ was replaced with equimolar lithium, choline, or *N*-methyl-D-glucamine.

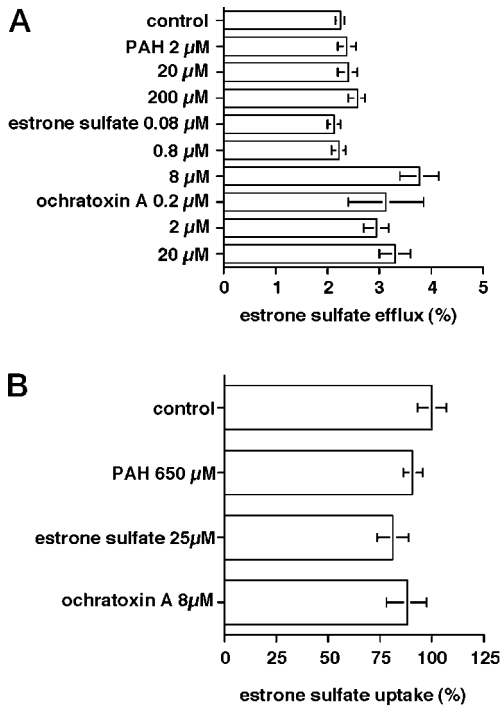

FIG. 5. **Effect of unlabeled substrates on the influx or efflux of [^{3}H]estrone sulfate via OAT3.** *A*, the lack of *trans*-stimulated efflux of [^{3}H]estrone sulfate via OAT3. Oocytes expressing OAT3 were preloaded with [^{3}H]estrone sulfate (80 nM) for 90 min and transferred to the medium with or without unlabeled substrate as indicated. The effluxed amount of estrone sulfate during 90 min was shown as percentage of the preloaded amount. (mean ± S.E.; n = 10) *B*, the lack of *trans*-stimulated influx of [^{3}H]estrone sulfate via OAT3. Oocytes expressing OAT3 was preloaded unlabeled substrates for 5 h at the indicated concentration before starting the uptake experiment. The uptake rate of [^{3}H]estrone sulfate (40 nM) for 1 h by OAT3-expressing oocytes was measured (mean ± S.E.; n = 10).

compounds with anionic moieties. Nonetheless, OAT3 transports cimetidine, which is a weak base (Fig. 3). Cimetidine is known to be a bisubstrate, because the uptake of cimetidine into isolated proximal tubular cells of the rat was inhibited by both probenecid and TEA (23). Ullrich *et al.* (24) investigated the recognition of bisubstrates by the renal organic anion and cation transporters using the stop-flow peritubular capillary microperfusion method. They demonstrated that hydrophobic imidazole analogues with electronegative side group, such as cimetidine, can interact with both transporters, although imidazole by itself did not interact with the renal organic anion transporter. From these results, they emphasized on the importance of hydrophobicity and partial charge, but not net charges of the substrates. Interaction of bisubstrates with both organic anion and cation transporters may be explained by the structural similarity of the OAT family and OCT family (Table I). Based on these hypothesis, a family including both the OAT and OCT family was proposed (8, 25). Now the molecular structures of three organic anion transporters and three organic cation transporters have been revealed. The analyses of the sequence of these transporters may provide clues as to the substrate binding sites of the transporters, especially the sites for charge(s) recognition.

There exist both similarities and differences in substrate selectivity and transport properties among members of the OAT family. OAT1, OAT2, and OAT3 are all multispecific transporters, and PAH is a common substrate; however, their affinity for, and rates of transport of PAH, are different among the OAT family. Kinetic analysis revealed that the K_m value of OAT3 for PAH was 65 μM, which is 4-fold larger than the previously reported value determined using OAT1-expressing oocytes (8). Although the expression level of OAT1 and OAT3 in oocytes is not normalized, V_{max} values for PAH were a 10-fold difference between OAT1 and OAT3 (~240 pmol/h/oocyte for OAT1 and 24 pmol/h/oocyte for OAT3). Thus, the clearance for uptake at tracer concentrations (CL_{uptake}) defined as the V_{max}/K_m of PAH, is 40-fold different between OAT1 and OAT3. Another member of the OAT family, OAT2, also showed relatively small transport activity for PAH compared with that of

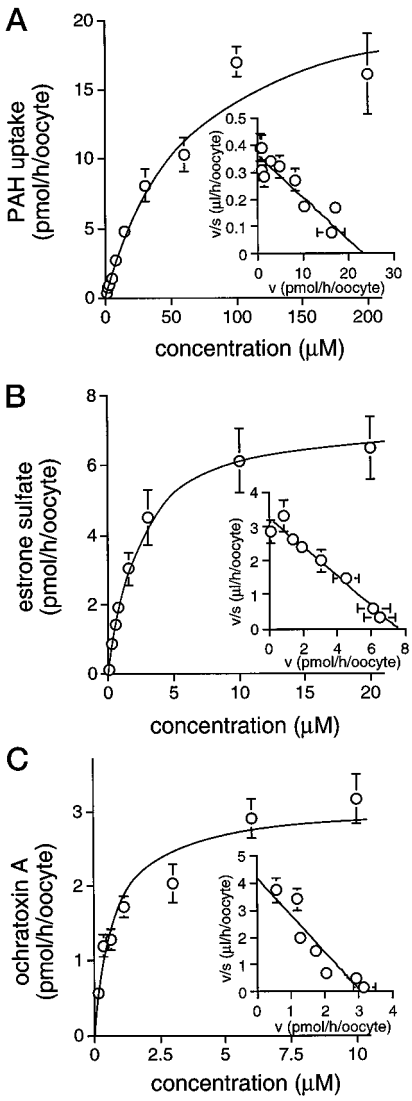

FIG. 6. **Concentration dependence of OAT3-mediated uptake of [^{3}H]*p*-aminohippurate (*A*), [^{3}H]estrone sulfate (*B*), and [^{3}H]ochratoxin A (*C*).** The uptake of radiolabeled compounds ([^{3}H]*p*-aminohippurate, [^{3}H]estrone sulfate, and [^{3}H]ochratoxin A) by control or OAT3-expressing oocytes were measured at the concentration indicated after incubation for 1 h (mean ± S.E.; n = 10). OAT3-mediated transport was obtained by subtracting the transport velocity in noninjected oocytes from that in OAT3-expressing oocytes.

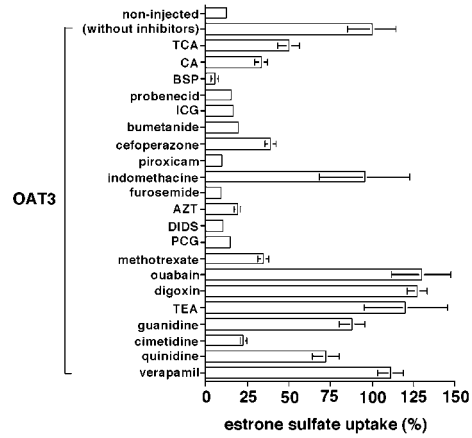

FIG. 7. **Inhibition of OAT3-mediated [^{3}H]estrone sulfate uptake by various compounds.** The concentration used of [^{3}H]estrone sulfate was 40 nM and those of the inhibitors were 1 mM (except for sulfobromophthalein and digoxin, 250 μM). The values were expressed as a percentage of OAT3-mediated [^{3}H]estrone sulfate uptake in the absence of the inhibitor (mean ± S.E.; n = 10).

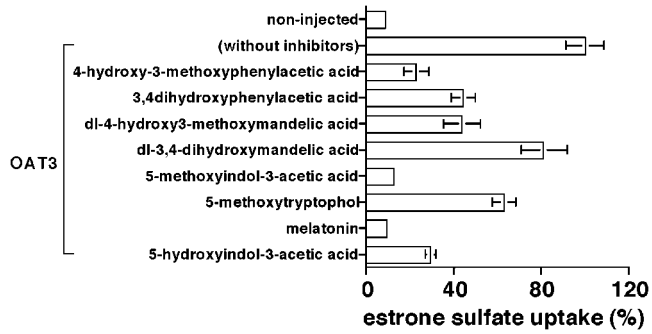

FIG. 8. **Inhibition of OAT3-mediated [^{3}H]estrone sulfate uptake by acidic metabolites of neurotransmitters.** The concentration used of [^{3}H]estrone sulfate was 40 nM and those of the inhibitors were 1 mM. The values were expressed as a percentage of OAT3-mediated [^{3}H]estrone sulfate uptake in the absence of the inhibitor (mean ± S.E.; n = 10).

OAT1 (11). This difference in the transport activity for PAH among the members of the OAT family may be related to the fact that PAH is excreted mainly in the urine. Other than PAH, there are distinct differences in substrate selectivity between OAT1, OAT2, and OAT3. Indomethacin is a potent inhibitor of renal organic anion transporter (OAT1); however, their inhibitory activity on the transport by OAT3 is weak. On the contrary, benzylpenicillin strongly inhibited OAT3-mediated PAH uptake, but only slightly inhibited OAT1-mediated transport, suggesting that the affinity of benzylpenicillin for OAT1 is weaker than that for OAT3. Actually, benzylpenicillin is a good substrate for OAT3 rather than for OAT1 on the basis of K_m value and transport activity of benzylpenicillin (data not shown). OAT2 transports salicylate, but not estrone sulfate, and vice versa, OAT3 transports estrone sulfate but not salicylate. Thus, OAT1, OAT2, and OAT3 have distinct transport properties, despite the fact that they belong to the same transporter family. The relatively low structural similarities among OAT1, OAT2, and OAT3 (less than 50% identity each other) may be relevant to these functional differences.

A Northern blot analysis revealed the expression of OAT3 in the brain. OAT1 and OAT2 are expressed predominantly in the kidney and liver, respectively. The strong expression of OAT3 in the brain suggests its physiological role in the brain such as in the efflux pathway via the BBB and/or the BCSFB. It has been demonstrated that PAH, when microinjected into the cerebral cortex, is eliminated from the brain in a concentration-dependent manner via the BBB (15). Carrier-mediated efflux transport of benzylpenicillin and cimetidine from the cerebrospinal fluid have been also demonstrated (16, 18). Suzuki *et al.* (16, 18) reported that the transport of benzylpenicillin was sodium-independent and inhibited by PAH and that the elimination of cimetidine from the cerebrospinal fluid was inhibited by benzylpenicillin and PAH, but not by N^1-methylnicotinamide, a typical substrate for organic cation transporters. These results suggest that the efflux transport properties of organic anions via the BBB and/or BCSFB are consistent with transport by the OAT family, especially OAT3. Since PAH and cimetidine are transported by OAT3, and benzylpenicillin inhibits the uptake of estrone sulfate by OAT3, OAT3 may be responsible for the efflux transport of these organic anions via the BBB and/or BCSFB. In fact, a preliminary immunohistochemical analysis revealed the positive staining of OAT3 in the choroid plexus (data not shown). In addition to these exogenous compounds, metabolites of neurotransmitters are also candidate substrates for OAT3. After being released from presynaptic neurons, certain amounts of neurotransmitters are metab-

olized by monoamine oxidase and catechol-*O*-methyltransferase. Finally, anionic compounds such as 3,4-dihydroxyphenylacetic acid, 4-hydroxy-3-methoxyphenylacetic acid, 3,4-dihydroxymandelic acid, 4-hydroxy-3-methoxymandelic acid, 5-methoxyindol-3-acetic acid, 5-methoxytryptophol, and 5-hydroxyindol-3-acetic acid, are produced. Since all the anionic metabolites, except 3,4-dihydroxymandelic acid, inhibited organic anion transport via OAT3, they may be endogenous substrates of OAT3. Future immunohistochemical analysis will reveal the distribution/localization of OAT3 in the brain and provide clues to understanding the more precisely physiological role of OAT3.

Oatp1 and oatp2 are also multispecific organic anion transporters expressed in the brain. Their substrate specificities are quite similar except digoxin; oatp2 transports digoxin, but oatp1 does not (7). Oatp1 has been demonstrated to be localized specifically on the brush border membrane of choroid epithelial cells (26), while the localization of oatp2 in the brain has not been determined. There are common substrates for OAT3 and the members of oatp family (*e.g.* estrone sulfate and ochratoxin A); however, the substrate selectivity of these two families overlaps little. The transporter(s) responsible for the efflux transport, via BBB and/or BCSFB, of naphthol glucuronide, dideoxyinosine, azidodeoxythymidine, and valproic acid has not been identified (27–30). The functional analysis and localization of oatp1, oatp2, and OAT3 will clarify the molecular mechanisms of the organic anion transport in the brain.

In conclusion, the third member of the multispecific organic anion transporter family was isolated from the rat brain. Among the members of the OAT family, OAT3 is the most abundantly expressed in the brain. The substrate selectivity of OAT3 is different from the substrate selectivities of OAT1 and OAT2, and the characteristics of transport via this transporter are similar to those of the previously reported efflux transport system in the brain. Localization and further functional analysis of OAT3 will provide important information on the distribution and transport of organic anions, especially in the brain.

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Molecular Cloning and Characterization of a New Multispecific Organic Anion Transporter from Rat Brain

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