Functional Characterization of Rat Brain-specific Organic Anion Transporter (Oatp14) at the Blood-Brain Barrier

HIGH AFFINITY TRANSPORTER FOR THYROXINE

Oatp14/blood-brain barrier-specific anion transporter 1 (Slc21a14) is a novel member of the organic anion transporting polypeptide (Oatp/OATP) family. Northern blot analysis revealed predominant expression of Oatp14 in the brain, and Western blot analysis revealed its expression in the brain capillary and choroid plexus. Immunohistochemical staining indicated that Oatp14 is expressed in the border of the brain capillary endothelial cells. When expressed in human embryonic kidney 293 cells, Oatp14 transports thyroxine (T4; prothyroid hormone) (Km = 0.18 μM), as well as amphipathic organic anions such as 17β estradiol-d-17β-glucuronide (Km = 0.1 μM), cefazolin, and troglitazone sulfate (Km = 0.76 μM). The uptake of triiodothyronine (T3), an active form produced from T4, was significantly greater in Oatp14-expressed cells than in vector-transfected cells, but the transport activity for T4 was 6-fold lower than that for T3. The efflux from Oatp14-expressed cells was more rapid than that from vector-transfected cells (0.032 versus 0.006 min−1). Therefore, Oatp14 can mediate a bidirectional transport of T₄. Sulfobromophthalein, taurocholate, and estrone sulfate were potent inhibitors for Oatp14, whereas digoxin, p-aminophthalein, or leukotriene C₄, or organic cations such as tetraethylammonium or cimetidine had no effect. The expression levels of Oatp14 mRNA and protein were up- and down-regulated under hypo- and hyperthyroid conditions, respectively. Therefore, it may be speculated that Oatp14 plays a role in maintaining the concentration of T₄ and, ultimately, T₃ in the brain by transporting T₄ from the circulating blood to the brain.

Brain capillary endothelial cells are characterized by tightly sealed cellular junctions (tight junctions) and the paucity of fenestra and pinocytotic vesicles, which prevent free exchange between brain and blood (1, 2). Therefore, the uptake of nutrients by the brain occurs through the brain capillary endothelial cells via specific transport systems (3–7). Metabolic enzymes and efflux transporters expressed in the brain capillaries facilitate the elimination of endogenous wastes and xenobiotics from the brain, and restrict their brain accumulation (3–7). Because of these characteristics, the brain capillaries are referred to as the blood-brain barrier (BBB).

The organic anion transporting polypeptides (Oatps in rodents and OATPs in human) belong to the growing gene family of organic anion/prostaglandin transporters that can mediate sodium-independent membrane transport of numerous endogenous and xenobiotic amphipathic compounds (8, 9). Fourteen members of the Oatp/OATP gene family have been identified in rodents and humans, and they are classified within the gene superfamily of solute carriers as the Slc21a gene family (Human Gene Nomenclature Committee DataBase) (8, 9). Several members of the Oatp/OATP family have been identified in the brain (Oatp1–3 and oatp1 in rodents and OATP-A in human) (10–14). Especially, in the BBB, rat Oatp2 and human OATP-A have been shown to be expressed in the plasma membrane of the brain capillary endothelial cells (15, 16). Involvement of rat Oatp2 in the uptake and efflux transport of its substrates was investigated in vivo (17, 18). The uptake of [α-penicillamine²⁻²¹⁴C]-enkephalin (DPDPE) from the blood to the brain was determined by the brain perfusion technique in the presence and absence of Oatp2 inhibitors (17). The brain uptake of DPDPE was increased in Mdr1a (P-glycoprotein) gene knockout mice, and the uptake in Mdr1a knockout mice was inhibited by the substrates and inhibitors of rat Oatp2 such as digoxin and 17β estradiol-d-17β-glucuronide (E₂,17βG). Vice versa, when E₂,17βG was microinjected into the cerebral cortex, the subsequent elimination of E₂,17βG from the brain was carrier-mediated (18), and the elimination of E₂,17βG was completely inhibited by co-administration of taurocholate and probenecid, whereas digoxin had only a partial effect (18). Partial inhibition by digoxin suggested that additional efflux transport system(s) for E₂,17βG, which is taurocholate- and probenecid-sensitive, is involved in the brain capillary.

1 The abbreviations used are: BBB, blood-brain barrier; Oatp, organic anion transporting polypeptide; BSAT, BBB-specific anion transporter; HER293, human embryonic kidney 293; CA, cholate; GCA, glycocholate; LCA, lithocholate; CDCA, chenodeoxycholate; UDCA, ursodeoxycholate; PG₃₂, prostaglandin D₃; PGE₂, prostaglandin E₂; E₃₀₄₀, 6-hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridylmethyl) benzothiazole; PBS, phosphate-buffered saline; DPDPE, [α-penicillamine²⁻²¹⁴C]-enkephalin; E₁₇ΒG, 17β estradiol-d-17β-glucuronide; T₄, thyroxine; TCLS, taurolithocholate sulfate; TRO-S, troglitazone sulfate; RT, reverse transcriptase; MMI, methimazole; T₂, triiodothyronine; ES, estrone sulfate; ESP, sulfobromophthalein; LT, leukotriene; D₂, type 2 iodothyronine deiodinase.

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EXPERIMENTAL PROCEDURES

Chemicals—[3H]Leu-enkephalin was purchased from American Radiolabeled Chemicals (St. Louis, MO). [3H]Pravastatin was kindly donated from Sankyo (Tokyo, Japan). [3H]cerivastatin was from Bayer AG (Wuppertal, Germany), and [14C]E3040 glucuronide and [14C]E3040 glucuronide were from Eisai (Tokyo, Japan). [3H]taurolithocholate sulfate (TRO-S) were synthesized according to a method described previously (22). All steps in the isolation procedure were carried out at 4°C in pregassed (95% O2–5% CO2) solutions. Briefly, pieces of gray matter were gently homogenized in three volumes (v/w) of an artificial extracellular fluid buffer and, after addition of dextran (final concentration 15%), the homogenate was centrifuged at low speed. The resulting pellet was resuspended in saline (2.5 mM CaCL2, 1.2 mM MgSO4, 1.2 mM K2HPO4, and 15 mM HEPES, pH 7.4) and then filtered through a 200-μm nylon mesh. The filtrate was passed over a column of glass beads, and after washing with Buffer B, the capillaries adhering to the beads were collected by gentle agitation.

Northern Blot Analysis—A commercially available hybridization blot containing poly(A+) RNA from various rat tissues (rat multi-tissue Northern blot; Clontech) was used for the Northern blot analysis. A fragment (position numbers 1–807) from Oatp/OATP family (Oatp14). Although the localization at the BBB and the substrates of this isoform remain unknown, BBB-specific expression prompted us to hypothesize that Oatp14 accounts for the efflux of organic anions including E217G via the BBB, together with Oatp2. The purpose of the present study is to characterize the substrate specificity and spectrum of inhibitors of Oatp14, as well as its tissue distribution and localization. Through this study, we found out that thyroxine (T4) is a good substrate of Oatp14, and the expression level of Oatp14 in the brain capillary is affected by plasma thyroid conditions. The results of the present study suggest that Oatp14 plays an important role in regulating the concentration of T4 in the central nervous system and in brain development.

Western Blot Analysis—Antiserum against Oatp14 was raised in rabbits against a synthetic peptide consisting of the 17 carboxyl-terminal amino acids of Oatp14. Antiserum was purified by affinity column chromatography using the antigen and used for subsequent analyses. Choroid plexus, brain homogenate, and isolated brain capillary samples were diluted with Loading Buffer (BioLabs, Hertfordshire, United Kingdom). They were then boiled for 3 min and loaded onto an 8.5% SDS-polyacrylamide electrophoresis gel with a 3.75% stacking gel. Proteins were electroblotted onto a polyvinylidene difluoride membrane (Pall Filtran, Karlstein, Germany) using a blottter (Trans-blot; Bio-Rad) at 15 V for 1 h. The membrane was blocked with TBS-T (Tris-buffered saline, 0.15 M NaCl and 0.015 M sodium citrate) and 0.1% SDS at 65°C and then exposed to Fuji imaging plates (Fuji Photo Film, Kanagawa, Japan) for 3 h at room temperature and examined using an imaging analyzer (BAS 2000; Fuji Photo Film).

Immunohistochemical Staining of Oatp14 for Brain Slices—Frozen sections of rat brain were used for immunohistochemical detection with peroxidase to probe for Oatp14 with a polyclonal antibody. The lined and dotted arrows represent luminal and abluminal sides of brain capillary endothelial cells, respectively. Positive labeling was only found in the border of brain capillary endothelial cells.
Oatp14-transfected HEK293 cells. The uptake of [3H]E217 as template according to the following protocol: 96°C introduced into HEK293 cells by lipofection with FuGENE 6 (Roche) and reverse primer, 5'-ggattccttaaagtcg-3' and reverse primer, 5'-ggaattccgccac-3'. PCR was performed using cDNA prepared from rat brain (19) (GenBankTM accession number NM 053441), and reverse primer, 5'-ggaattccttaaagtcg-3'. PCR was performed using cDNA prepared from rat brain (19) (GenBankTM accession number NM 053441), and reverse primer, 5'-ggaattccttaaagtcg-3'. PCR was performed using cDNA prepared from rat brain (19) (GenBankTM accession number NM 053441), and reverse primer, 5'-ggaattccttaaagtcg-3'. PCR was performed using cDNA prepared from rat brain (19) (GenBankTM accession number NM 053441), and reverse primer, 5'-ggaattccttaaagtcg-3'. PCR was performed using cDNA prepared from rat brain (19) (GenBankTM accession number NM 053441), and reverse primer, 5'-ggaattccttaaagtcg-3'. 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Table I

| Substrates            | Oatp14     | peDNA     | Ratio, Oatp14peDNA |
|-----------------------|------------|-----------|--------------------|
|                       | μg/mg protein | 15 min   |
| CA                    | 5.66 ± 0.30 | 5.24 ± 0.43 | 1.1 ± 0.1          |
| GCA                   | 18.7 ± 2.3  | 15.7 ± 1.6 | 1.2 ± 0.2          |
| TCA                   | 8.09 ± 0.52 | 6.06 ± 0.10 | 1.3 ± 0.1a         |
| LCA                   | 576 ± 6     | 535 ± 18   | 1.1 ± 0.0          |
| CDCA                  | 65.7 ± 6.7  | 84.1 ± 11.5 | 0.8 ± 0.1          |
| UDCA                  | 10.5 ± 0.1  | 9.71 ± 0.35 | 1.1 ± 0.0          |
| TLCS                  | 61.5 ± 1.9  | 43.7 ± 1.7 | 1.4 ± 0.1          |
| Estradiol             | 294 ± 4     | 186 ± 13   | 1.6 ± 0.1          |
| Testosterone          | 52.3 ± 1.5  | 39.2 ± 1.4 | 1.3 ± 0.1b         |
| Dihydropyrostestosterone | 147 ± 15    | 106 ± 5    | 1.4 ± 0.2b         |
| Corticosterone        | 24.7 ± 1.5  | 20.9 ± 0.5 | 1.2 ± 0.1          |
| Estrone               | 303 ± 15    | 248 ± 6    | 1.2 ± 0.1a         |
| DHEAS                 | 9.68 ± 0.24 | 7.72 ± 0.48 | 1.3 ± 0.1          |
| Estrone-sulfate       | 11.1 ± 0.8  | 6.3 ± 0.1  | 1.7 ± 0.1a         |
| E₂17βG                | 50.1 ± 4.7  | 2.4 ± 0.2  | 21.2 ± 2.9b        |
| LTC4                  | 14.5 ± 0.6  | 13.4 ± 0.1 | 1.1 ± 0.0b         |
| LTD4                  | 19.6 ± 0.8  | 18.0 ± 2.0 | 1.1 ± 0.1          |
| LTE4                  | 30.5 ± 1.3  | 26.6 ± 0.9 | 1.2 ± 0.1a         |
| PGD2                  | 3.50 ± 0.1  | 3.61 ± 0.12 | 1.0 ± 0.1          |
| PGE2                  | 6.99 ± 0.46 | 5.80 ± 0.24 | 1.2 ± 0.1          |
| Leu-Enkephalin        | 54.2 ± 2.9  | 43.3 ± 2.5 | 1.3 ± 0.1b         |
| CCK-8                 | 2.58 ± 0.21 | 1.81 ± 0.13 | 1.4 ± 0.2          |
| T3                    | 951 ± 16    | 783 ± 4    | 1.2 ± 0.3b         |
| Reverse T3            | 1397 ± 79   | 71 ± 5     | 19.7 ± 1.7b        |
| T4                    | 1456 ± 10   | 124 ± 3    | 11.8 ± 0.3c        |
| Ketoprofen            | 9.53 ± 0.42 | 6.91 ± 0.26 | 1.4 ± 0.1b         |
| Ibuprofen             | 3.18 ± 0.19 | 3.29 ± 0.56 | 0.8 ± 0.2          |
| Indomethacin          | 31.3 ± 0.9  | 34.3 ± 2.0 | 0.9 ± 0.1          |
| Benzylpenicillin      | 5.76 ± 0.47 | 5.45 ± 0.12 | 1.1 ± 0.1          |
| OchratoxinA           | 8.58 ± 1.1  | 1274 ± 150 | 11 ± 0.1           |
| Quinidine             | 105 ± 3     | 33 ± 1     | 1.3 ± 0.1b         |
| Cerivastatin          | 5.62 ± 0.38 | 3.50 ± 0.75 | 1.6 ± 0.4b         |
| Pravastatin           | 8.70 ± 0.13 | 9.96 ± 0.22 | 0.9 ± 0.0          |
| E3040                 | 106 ± 3     | 93 ± 5     | 1.1 ± 0.1          |
| E3040S                | 10.7 ± 1.0  | 2.12 ± 0.22 | 5.1 ± 0.7b         |
| 4MUS                  | 4.78 ± 0.35 | 1.69 ± 0.26 | 2.8 ± 0.5b         |
| Troglitazone-sulfate  | 64.1 ± 14.3 | 8.4 ± 0.4  | 7.6 ± 1.7b         |

* Statistically significant uptake is indicated. p < 0.05.

Table II

$K_m$, $V_{max}$, and $V_{max}/K_m$ values for Oatp14

The $K_m$ and $V_{max}$ values were determined by nonlinear regression analysis using data shown in Fig. 3.

| Substrate | $K_m$ (μM) | $V_{max}$ (pmol/min/mg protein) | $V_{max}/K_m$ (pmol/min/mg protein) |
|-----------|------------|---------------------------------|-------------------------------------|
| E₂17βG    | 10.7 ± 1.6 | 93.4 ± 10.4                     | 8.73 ± 1.63                         |
| Cerivastatin | 1.34 ± 0.25 | 14.5 ± 2.2                     | 10.8 ± 2.6                         |
| TRO-S     | 0.76 ± 0.09 | 69.0 ± 6.7                     | 91.3 ± 14.2                         |
| T4        | 0.18 ± 0.03 | 32.1 ± 2.5                     | 174 ± 14                           |

could be observed within 2 weeks of the beginning MMI treatment and within 1 week after thyroideectomy. Hyperthyroidism was produced by giving L-T3 (50 μg/100 g body weight, subcutaneously, daily) 4 days before capillary isolation.

RESULTS

Tissue Distribution of Oatp14—The expression of Oatp14 mRNA in rat tissues was investigated by Northern blot analysis (Fig. 1A). A band was detected at 2.6 kbp, predominantly in the brain. No hybridization signals were detected in mRNA isolated from other tissues, including the heart, spleen, lung, liver, skeletal muscle, kidney, and testis.

Immunoblot and Immunohistochemical Staining of Oatp14—The expression of Oatp14 in the choroid plexus, brain homogenate, and brain capillary was examined by Western blot analysis (Fig. 1B). Immunoreactive protein was detected at ~90 kDa in the choroid plexus, brain homogenate, and brain capillary. These bands were abolished when preabsorbed polyclonal antibody for Oatp14 was used, suggesting that the positive bands were specific for the antigen peptide.

To investigate the localization of Oatp14 in brain capillary endothelial cells, immunohistochemical staining was carried out using anti-Oatp14 polyclonal antibody (Fig. 2). Positive signals for anti-Oatp14 polyclonal antibody were detected in brain capillary endothelial cells. The signals were detected along the plasma membrane of brain capillary endothelial cells. The signal was abolished by preincubating the polyclonal antibody of Oatp14 with antigen (data not shown).

Transport Properties of Oatp14—Fig. 3 shows the time profiles of the uptake of [3H]E₂17βG (A), [14C]Cerivastatin (B), [35S]TRO-S (C), and [125I]T₄ (D) by Oatp14-expressed HEK293 cells and vector-transfected HEK293 cells. Their uptake by Oatp14-expressed cells is markedly greater than that by vector-transfected cells. This Oatp14-mediated uptake showed saturation kinetics and followed the Michaelis-Menten equation (Fig. 3, E-H). The kinetic parameters for the uptake by Oatp14 were determined by nonlinear regression analysis and summarized in Table I. The uptake of various organic anions by Oatp14 was investigated, and the results are summarized in Table I. The uptake of [14C]E3040 glucuronide, [14C]E3040 sulfate, [14C]4MUS, and [125I]reverse T₄ by Oatp14-expressed cells was significantly greater compared with that by vector-transfected (Table II). Although the triiodothyronine (T₃) uptake by Oatp14-expressed cells was significantly greater than that by vector-transfected cells, the Oatp14-mediated uptake for T₃ was ~6-fold smaller than that of T₄ and reverse T₄ by Oatp14 (Table II). The difference in the uptake of [3H]taurocholate, [3H]TLCs, [3H]testosterone, [3H]dihydropyrostestosterone, [3H]estrone, [3H]estrone sulfate (ES), [3H]hydroxypropionosterone sulfate, [3H]leukotriene E₂ (LTE₂), [3H]Leu-enkephalin, [3H]cholecytokinin-octapeptide (CCK-8), [125I]T₃, [3H]pravastatin, [3H]ketoprofen, and [3H]ochratoxin A was statistically significant between Oatp14-expressed and vector-transfected cells, although the rates of uptake were very low (Table II).

To investigate whether Oatp14 can mediate bidirectional transport, cells were preloaded with [125I]T₄ for 15 min followed by incubation in the absence of [125I]T₄. The radioactivity associated with cell specimens was rapidly reduced in Oatp14-expressed HEK293 cells compared with that in vector-trans-
Effects of unlabeled probenecid, BSP, trichloroacetic acid, ES, DNP-SG, DPDPE, T3, and T4 on the uptake of \[^{3}H\]E217βG by Oatp14-transfected HEK293 cells. The effects of unlabeled probenecid (A), BSP (B), taurocholate (C), ES (D), DNP-SG (E), DPDPE (F), T3 (G), and T4 (H) on the uptake of \[^{3}H\]E217βG by Oatp14-transfected HEK293 cells were examined at 37 °C. The specific uptake was obtained by subtracting the uptake by vector-transfected cells from that by gene-transfected cells. Open and closed circles represent the uptake by Oatp14- and vector-transfected cells, respectively. Each point represents the mean ± S.E. (n = 3).

**DISCUSSION**

In the present study, we reported the substrate specificity of Oatp14, as well as its tissue distribution and localization in the brain. Oatp14 is expressed in the brain capillary and choroid plexus. It mediated the uptake of T3, T4, and reverse T3, as well as organic anions such as E217βG, cerivastatin, and TRO-S, suggesting its involvement in the membrane transport of these ligands in the brain capillary.

T3 and its prohormone, T4, are produced in the thyroid gland and released into the blood. T3 plays an essential role in brain development via binding to specific nuclear receptors (thyroid hormone receptor) (25). Deficiency of thyroid hormones particularly during fetal and neonatal period in the brain causes mental retardation and cretinism (26, 27). T3 is supplied to the brain and peripheral tissues as T4 from which T3 is enzymatically produced by type 2 iodothyronine deiodinase (D2) (25). Therefore, the brain uptake process of T4 from the circulating blood is the first step in all subsequent reactions of thyroid hormone in the brain. Whether there is a specific transport mechanism(s) for T4 in brain capillary endothelial cells remains controversial. The brain uptake of T4 was saturable in dogs (28) but not in mice (29). Analysis of the transport and molecular properties of Oatp14 should help us resolve this.

Transfection of Oatp14 cDNA into HEK293 cells resulted in a marked increase in the uptake of T4, as well as reverse T3, an inactive metabolite of T4 produced by type 3 iodothyronine deiodinase. Although the uptake of T3 by Oatp14-expressed cells was significantly greater than that by vector-transfected cells (Table II), T3 was extensively taken up by vector-transfected cells (Table II). Whether the uptake in vector-transfected cells is ascribed to specific transport system(s) for T4 in brain capillary endothelial cells remains controversial. The transport activity for T4 from the circulating blood is the first step in all subsequent reactions of thyroid hormone in the brain. Whether there is a specific transport mechanism(s) for T4 in brain capillary endothelial cells remains controversial. The brain uptake of T4 was saturable in dogs (28) but not in mice (29). Analysis of the transport and molecular properties of Oatp14 should help us resolve this.

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**Fig. 5.** Effects of unlabeled probenecid, BSP, trichloroacetic acid, ES, DNP-SG, DPDPE, T3, and T4 on the uptake of \[^{3}H\]E217βG by Oatp14-transfected HEK293 cells. The effects of unlabeled probenecid (A), BSP (B), taurocholate (C), ES (D), DNP-SG (E), DPDPE (F), T3 (G), and T4 (H) on the uptake of \[^{3}H\]E217βG by Oatp14-transfected HEK293 cells were examined at 37 °C. The specific uptake was obtained by subtracting the uptake by vector-transfected cells from that by gene-transfected cells. Open and closed circles represent the uptake by Oatp14- and vector-transfected cells, respectively. Each point represents the mean ± S.E. (n = 3).

Effects of hyperthyroid and hypothyroid conditions on the expression of Oatp14 mRNA in the brain capillary—The effects of hyper- and hypothyroid conditions on the expression of Oatp14 in the brain capillary were investigated by RT-PCR and Western blotting (Fig. 7, A and B). RT-PCR and Western blotting analyses revealed that the expression levels of Oatp14 mRNA and protein were up- and down-regulated under hypothyroid and hyperthyroid conditions, respectively.

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**Involvement of Oatp14 in maintaining homeostasis of T4 in the brain**

In the present study, we reported the substrate specificity of Oatp14, as well as its tissue distribution and localization in the brain. Oatp14 is expressed in the brain capillary and choroid plexus. It mediated the uptake of T3, T4, and reverse T3, as well as organic anions such as E217βG, cerivastatin, and TRO-S, suggesting its involvement in the membrane transport of these ligands in the brain capillary.

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of Oatp14 in the brain capillary under hypo- and hyperthyroid conditions (Fig. 7). The expression of Oatp14 in the brain capillary changed as if Oatp14 was responsible for maintaining the concentration of T$_4$ in the central nervous system: up- and down-regulated under hypothyroid and hyperthyroid conditions, respectively (Fig. 7). This pattern is similar to that observed in D2 expression (25). Increased D2 expression increases the conversion of T$_4$ to T$_3$ to compensate for the decrease in the local brain concentration of T$_4$ and vice versa. Therefore, we hypothesize that Oatp14 is involved in the uptake of T$_4$ through the brain capillaries.

In addition to Oatp14, Oatp2, the other isoform of rat Oatp family, is also the candidate transporter for T$_3$ and T$_4$ uptake by the brain from the circulating blood in rodents. The uptake of both T$_3$ and T$_4$ was significantly increased in Oatp2-cRNA injected oocytes with similar $K_m$ values ($\sim 5-7 \mu M$) (12). Oatp2 has been identified both in the luminal and abluminal membrane of brain capillary endothelial cells (15). It is possible that Oatp14 and Oatp2 serve high and low affinity sites for T$_4$ in the brain capillary, because the $K_m$ values of Oatp2 were 30-fold greater than that of Oatp14 (12). Following uptake from the circulating blood into endothelial cells, T$_4$ has to cross the abluminal membrane to reach the brain interstitial space and brain parenchymal cells. Whether this process is carrier-mediated or not remains unknown. Bidirectional nature of Oatp2-mediated transport has been reported in Oatp2-cRNA-injected oocytes (30). Oatp14 and Oatp2 are candidate transporters involved in the abluminal secretion of thyroid hormones. Further studies are necessary to identify the exact localization of Oatp14 in the brain capillary and to evaluate its contribution to the total brain uptake of T$_4$ into the brain. Partridge et al. (31) demonstrated that the brain uptake of T$_3$ was saturable and inhibited by T$_4$ using carotid arterial bolus injection technique of Oldendorf, and Oatp2 may account for the brain uptake of T$_3$ in the brain capillary.

Oatp14 was detected in the choroid plexus by Western blot analysis (Fig. 1). The choroid plexus is located in the lateral, third and fourth ventricles, and the interface between the cerebrospinal fluid and the circulating blood acting as a barrier to protect the central nervous system, in conjunction with the BBB (32, 33). The brain distribution of T$_3$ and T$_4$ after intracerebroventricular administration is limited to ependymal cells and circumventricular organs and, thus, transport via the choroid plexus could account for the brain distribution near the ventricles (34). As speculated in the case of brain capillary endothelial cells, it is possible that Oatp14 acts as an uptake system to supply T$_4$ to ependymal cells and circumventricular organs in the choroid plexus.

In addition to thyroids, Oatp14 accepts certain types of amphipathic organic anions, such as E$_2$17βG, cerivastatin, and TRO-S, as substrates although their transport activity was markedly lower than that of T$_4$, except TRO-S (see Fig. 3 and Table II). Because Oatp14 can mediate the bidirectional trans-
port, it is possible that Oatp14 is involved in the efflux of organic anions such as $E_2\beta$G from the brain when it is microinjected into the cerebral cortex and possibly in the efflux of excess T4 and reverse T3 from the brain. The spectrum of inhibitors of Oatp14 was consistent with the transporter hypothesized based on in vivo studies (18), but further investigations will be required to confirm this speculation.

Whether the results obtained using cDNA from rodents can be applied to the human situation is an important issue. Human OATP-F, an isoform in which Oatp14 exhibits high homology (84% in amino acid level), has a similar substrate specificity to Oatp14 (35). Northern blot analysis demonstrated abundant expression of OATP-F in the brain and testis and, to a lesser extent, heart, but the localization in the brain remains unidentified. In terms of substrate specificity and homology, OATP-F is supposed to be the human ortholog of Oatp14, and it may be suggested that OATP-F is also involved in the uptake of T4 from the circulating blood into the central nervous system though the brain capillary and choroid plexus. In view of the importance of supplying T4 to the brain during development, it is possible that functional loss of the OATP-F gene may be associated with a thyroid hormone-related neuronal disorder characterized by resistance to thyroid hormone treatment.

In conclusion, we have characterized Oatp14 in terms of its substrate specificity and localization in the brain and demonstrated that Oatp14 accepts T4, as well as organic anions, including certain glucuronide and sulfate conjugates. Oatp14 is localized on the plasma membrane of brain capillary endothelial cells and involved in the uptake of T4 from the blood to the central nervous system. Oatp14 is one of the mechanisms for maintaining homeostasis of T4 and, ultimately, T3 in the brain.

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