Distribution of Rat Organic Anion Transporting Polypeptide-E (oatp-E) in the Rat Eye

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Purpose. To examine the protein and mRNA expression levels of the recently cloned rat multifunctional Na^+-independent organic anion transporting polypeptide (rat oatp-E), which is involved in the transport of thyroid hormone in the rat, the distribution and function of this transporter were investigated in the retina.

Methods. Real-time quantitative reverse transcription–polymerase chain reaction (RT-PCR) was performed with gene-specific primers for oatp-E in rat ocular tissues. Western blot analysis was performed by raising a specific antibody against oatp-E in rat ocular tissues. Immunohistochemistry was performed with a specific antibody for oatp-E in paraffin sections of rat eyes. The expression of oatp-E in isolated and cultured rat retinal pigment epithelial (RPE) cells was confirmed by RT-PCR, Western blot analysis, and immunohistochemistry. In addition, oatp-E function was analyzed in cultured rat RPE cells by measuring the uptake of triiodothyronine (T_3), which is a known substrate for oatp-E.

Results. Using real-time quantitative RT-PCR, oatp-E mRNA was detected, in order of highest to lowest concentration, in the rat retina, cornea, and ciliary body-iris. A single band for oatp-E was observed by Western blot analysis in the rat brain, retina, cornea, and ciliary body-iris. oatp-E immunostaining was predominantly expressed in the corneal epithelium, in the pigmented and nonpigmented epithelium of the ciliary body, and in the iris of the rat eye. In the rat retina, intense immunostaining was detected in the RPE, inner and outer nuclear layers, ganglion cell layer, and nerve fiber layer. In addition, oatp-E immunoreactivity in cultured rat RPE cells was expressed in the cell membrane and cytoplasm of RPE cells, a finding that was also confirmed by RT-PCR and Western blot analysis. RPE cells, which were shown to express high levels of oatp-E, transported T_3 in a saturable and dose-dependent manner. Moreover, this uptake was significantly inhibited by sulfobromophthalein (BSP), an inhibitor of oatp, suggesting that oatp-E may in part contribute to this uptake.

Conclusions. Results from the present study revealed that rat oatp-E is localized mainly to the corneal epithelium, ciliary body, iris, and retina. Furthermore, the findings appear to suggest that transport of T_3 in the RPE may have a functional role for organic anion (i.e., thyroid hormone) transport in the rat eye. (*Invest Ophthalmol Vis Sci.* 2003;44:4877–4884) DOI: 10.1167/iovs.02-1108

The organic anion transporter polypeptide (oatp) family plays important roles in the elimination of a variety of endogenous and exogenous substances from the body. This family of transporter peptides consists of membrane proteins with 12 putative membrane-spanning domains that function as sodium-independent exchangers or facilitators. Within the past decade, several members of multispecific oatps that mediate the elimination of organic anions in the liver and other organs have been identified.[1–7] Recently, we have isolated two Na^+-independent oatps, termed oatp2[2,3]and oatp3,[2] that transport thyroid hormones from the rat retina. The tissue distribution patterns show that oatp2 and oatp3 are widely expressed.[8] Thyroid hormone plays a key role in the neural function of the mammalian central nervous system, particularly during critical periods of its development.[9,10] In the eye, the absence of thyroid hormone causes serious damage to the structural development and organization of the retina.[11] However, in humans the molecular organization of organic anion transporters has diverged, and thus the responsible molecule for thyroid hormone transport in the human eye has not been clarified.

Recently, we isolated and characterized a novel human organic anion transporter, OATP-E from the human brain.[12] The isolated cDNA encodes a polypeptide of 722 amino acids with 12 transmembrane domains. A rat counterpart, oatp-E, was also isolated from the rat retina. The overall amino acid sequence homology between rat oatp-E and human OATP-E was found to be 72.6%, where the transmembrane domain and the surrounding area are highly conserved. Human OATP-E transports thyroxine (T_4), triiodothyronine (T_3), and reverse T_3 (rT_3) in a Na^+-independent manner.[12] Although OATP-E was isolated from the human brain, OATP-E mRNA has also been shown to be abundantly expressed in various peripheral tissues, such as the heart, placenta, liver, skeletal muscle, kidney, pancreas, brain, spleen, thymus, prostate, testis, and small intestine. The rat counterpart, oatp-E also transports T_3. However, little is known about the existence or cellular localization of oatp-E/OATP-E in the eye. In this study, we examined the

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Supported in part by the Ministry of Education, Science, Sports and Culture, the Novartis Foundation, the Osaka Cancer Research Foundation, the Japan Health Sciences Foundation, and Precursory Research for Embryonic Science and Technology, Japan Science and Technology Corporation.

Submitted for publication October 30, 2002; revised March 25 and April 22, 2003; accepted May 29, 2003.

Disclosure: **A. Ito**, None; **K. Yamaguchi**, None; **H. Tomita**, None; **T. Suzuki**, None; **T. Onogawa**, None; **T. Sato**, None; **H. Mizutamari**, None; **T. Mikkaichi**, None; **T. Nishio**, None; **T. Suzuki**, None; **M. Unno**, None; **H. Sasano**, None; **T. Abe**, Novartis (F); **M. Tamai**, None

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TABLE 1. Primer Sets for Real-Time Quantitative RT-PCR

cDNA		Sequence (5′-3′)	Position	Reference
oatp-E	Sense primer	CTGGTTCTCGTGTTCGTTGTAATT	1874–1898	12
	Antisense primer	CACAGACACATCGTAGAGTAGCAGTTAG	1926–1955	
	*Taq*Man probe	CTTTACATTCCTCAGCAGCATTCCCG	1900–1926	

expression and function of oatp-E in the rat eye and discuss its role in this specialized tissue.

MATERIALS AND METHODS

Animal Procedures

All experiments were performed on 8-week-old male Sprague-Dawley rats (Charles River Japan Inc., Yokohama, Japan), weighing 200 to 250 g. All animals were kept under conditions of constant temperature and humidity in a 12-hour light–dark cycle in the animal house. Rats were killed with a lethal dose of pentobarbital sodium. All animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Cell Culture

RPE cells were isolated, with slight modification, as described by Wang et al.[13] and cultured in 75 cm^2 flasks with Dulbecco's modified Eagle's medium (DMEM)/nutrient mixture F12 (1:1) with HEPES containing 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO), 0.348% sodium bicarbonate, 1% (by volume) 200 nM glutamine, 0.1 mg/mL streptomycin, and 100 U/mL penicillin. The culture medium was replaced with fresh medium every other day, and cells were allowed to grow until they reached approximately 80% confluence. Subconfluent cultures were passed by dissociation in 0.05% trypsin and 0.02% EDTA in calcium-magnesium–free phosphate-buffered saline (PBS).

Isolation of RNA from Rat Ocular Tissues and RPE Cells

Animals ware killed as described, and five eyes from three rats were immediately enucleated and prepared as previously reported.[8] The cornea, ciliary body together with the iris, and the major part of the retina and RPE were homogenized. Total RNA was isolated from homogenized tissues using extraction reagent (TRIzol; Invitrogen-Gibco, Gaithersburg, MD). Total RNA (5 μg) was prepared from the cornea, ciliary body-iris, retina, and RPE cells.

cDNA Synthesis, PCR, and Real-Time Quantitative RT-PCR

Reverse transcription (RT) was performed with a kit (Superscript RT; Amersham-Pharmacia, Piscataway, NJ) used according to the manufacturer's instructions. cDNA was synthesized from 5 μg total RNA extracted from the cornea, ciliary body-iris, retina, and RPE cells. Polymerase chain reaction (PCR) was set up in a reaction volume of 25 μL, in which a 1:40 volume (0.5 μL) of synthesized cDNA was included. Each reaction contained master mix (Universal Master Mix; Perkin Elmer, Boston, MA), 900 nM forward primer, 900 nM reverse primer, and 200 nM *Taq*Man (Applied Biosystems, Foster City, CA) probe. Primers for rat oatp-E were designed according to the sequence in GenBank (accession number AF239262; http://www.ncbi.nlm.nih.gov/Genbank; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD). Specific oatp-E primer sets for RT-PCR are summarized in Table 1. DNA amplifications were performed in a 96-well reaction plate format in an automated sequence-detection system (GeneAmp 5700; Applied Biosystems). Each sample was analyzed in triplicate. Endogenous control rat GAPDH (Applied Biosystems) was used to normalize for mRNA quantity. Thermal cycling was initiated with a 2-minute incubation at 50°C, followed by an initial denaturation step for 10 minutes at 95°C and then 40 cycles at 95°C for 15 seconds and 60°C for 1 minute.

To compare the relative abundance of oatp-E mRNA, standard curves for both oatp-E and GAPDH were generated from cDNAs synthesized from 10-fold dilutions of cRNA (5, 0.5, and 0.05 pg, and 5, 0.5, and 0.05 fg), as described previously.[14,15] For each rat ocular sample, the concentrations of oatp-E and GAPDH were determined from those standard curves. The resultant oatp-E concentration was divided by the concentration for GAPDH to obtain a normalized value. Normalized oatp-E values (oatp-E/GAPDH ratio) were subsequently divided by the normalized retinal oatp-E value to generate the relative expression levels of oatp-E mRNA.

Gene specific primers for oatp-E used in RT-PCR are listed in Table 2. After reverse transcription from the rat retina and RPE cells, PCR amplification was performed according to the following parameters: 94°C for 3 minutes, followed by 94°C for 45 seconds, 57°C for 1 minute, and 72°C for 2 minutes for 35 cycles, with a final cDNA elongation step at 72°C for 10 minutes. PCR products were electrophoresed in a 1% agarose gel, transferred onto a nylon membrane, and subsequently hybridized with a ^{32}P-labeled oatp-E–specific cDNA probe,[12] respectively. The PCR product was subcloned and sequenced, to confirm the identity of the fragment. Samples without reverse transcriptase were used in the RT-PCR procedure as a negative control.

Preparation of Rabbit Antibodies

A 14-amino acid peptide (SDGLEASLPSQSSA) corresponding to position 697-711 of the carboxyl terminus of rat oatp-E was synthesized.[12] The oatp-E peptide was linked to a maleimide-activated key hole limpet hemocyanin (KHL; Pierce, Inc., Rockford, IL). The KHL-linked peptide (1 mg/injection) was emulsified by mixing with an equal volume of Freund's complete adjuvant and injected into female rabbits. Booster injections were performed every 2 weeks, and the animals were killed at 10 weeks. The antibodies were affinity purified using CNBr-activated Sepharose CL-4B (Amersham Pharmacia Biotech, Piscataway, NJ) coupled with synthetic peptides according to standard procedures.[16,17]

Western Blot Analysis

Six rat eyes of three rats were enucleated and prepared as previously described.[8] The cornea, ciliary body together with the iris, and the retina and RPE cells were homogenized in a buffer containing 0.23 M sucrose, 2 mM EDTA, 5 mM Tris-HCl (pH 7.5), 0.1 mM phenylmethylsulfonyl fluoride, 2 μg/mL aprotinin, 2 μg/mL leupeptin, and 2 μg/mL pepstatin A. The homogenate was centrifuged at 1000*g* for 15 minutes and 7500*g* for 15 minutes. The supernatant was further centrifuged at

TABLE 2. Primer Sets for RT-PCR

cDNA		Sequence (5′-3′)	Position	Reference
oatp-E	Sense primer	GATGGCAATATAGATGGGCG	181–200	12
	Antisense primer	CAGGTTGAGAAACGGAGCAG	797–816	12

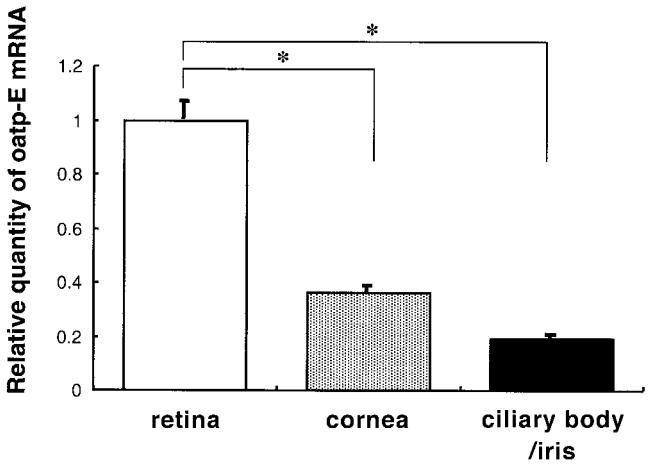

FIGURE 1. Real-time quantitative RT-PCR. cDNA was synthesized from 5 μg of total RNA obtained from retina, cornea, and ciliary body-iris. The oatp-E mRNA level was quantified using real-time quantitative PCR. oatp-E mRNA concentration is expressed relative to that of the retina (n = 3, ±SD).

150,000*g* for 60 minutes at 4°C. The resultant pellet, referred to as the crude membrane fraction, was resuspended in a buffer of 0.23 M sucrose, 2 mM EDTA, 5 mM Tris-HCl (pH 7.5), and 2 mM EDTA.[16] Western blot analysis was performed, with some modification, as previously described.[17,18] Briefly, 30 μg of crude membrane was solubilized in a sample buffer (2% SDS, 125 mM Tris-HCl [pH 7.4], 20% glycerol, and 2% 2-mercaptoethanol) at room temperature for 5 minutes and then applied to a 10% SDS-polyacrylamide gel. After electrophoresis, proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA). The blots were blocked with 5% nonfat dry milk in PBS-T (80 mM Na_2HPO_4, 20 mM NaH_2PO_4, and 100 mM NaCl [pH 7.5], containing 0.1% Tween 20) at 4°C overnight and incubated with oatp-E/OATP-E antibody (2 μg/mL) for 1 hour at room temperature. The blots were then washed and incubated with anti-rabbit IgG conjugated with horseradish peroxidase (1:5000 dilution; Amersham Pharmacia Biotech) at room temperature for 1 hour. An enhanced chemiluminescence kit was used for detection (Amersham Pharmacia Biotech). To confirm antibody specificity, the antibody was incubated with 10 μg of the antigen peptide before use. Rat brain served as a positive control.

Immunohistochemistry

We prepared six eyes from three rats, and performed three replications. The systemic circulation was perfused through an intra-aortic administration of 4% periodate-lysine-4% paraformaldehyde in PBS. The whole eye was removed and immersed in the same fixative overnight at 4°C, followed by dehydration. The eyes were embedded in paraffin wax, and then thin sectioned at 3 μm. After incubation in PBS containing 1% bovine serum albumin and 0.05% Triton X-100, sections were incubated with oatp-E antibody, at a final concentration of 2 μg/mL at 4°C overnight. The sections were then incubated in 0.3% H_2O_2 in methanol for the inhibition of endogenous peroxidase activity. Subsequently, the sections were incubated with anti-rabbit horseradish peroxidase (EnVision+; Vector Laboratories, Burlingame, CA) for 40 minutes. The sections were then washed three times with PBS and treated with DAB solution (0.01% 3′,3-diaminobenzidine tetrahydrochloride, Tris-HCl [pH 7.5], and 0.002% H_2O_2). In control experiments, sections were incubated with primary antibody preabsorbed with 10 μg/mL antigen peptide overnight before use. For immunofluorescence chemistry, after sections were washed, fluorescein isothiocyanate–labeled anti-rabbit IgG was applied, and 4′,6′-diamino-2-phenylindole (DAPI) was counterstained to reveal the nucleus. The cultured RPE cells were also fixed and incubated with primary antibody, as described earlier. Stained RPE cells were analyzed by confocal laser microscopy (MRC 600; Bio-Rad).

Uptake Measurements in Cultured RPE Cells

For uptake experiments, RPE cells were seeded at a density of 0.5×10^6 cells/well in 24-well culture plates and cultured in the presence of 1 mL/well culture medium. The medium was replaced every other day. Cultures were used for uptake measurements 3 days after seeding. Uptake of [^{125}I] T_3 (NEN Life Science Products, Boston, MA) into RPE cells was measured at room temperature for 15 minutes. The uptake buffer was composed of 142 mM NaCl, 4.83 mM KCl, 23.8 mM $NaHCO_3$, 0.96 mM KH_2PO_4, 1.2 mM $MgSO_4 7H_2O$, 1.53 mM $CaCl_2$, 5 mM glucose, and 12.6 mM HEPES (pH 7.3). To assess for experimental inhibition, the uptake buffer contained 0.1 μM T_3 and 100 μM sulfobromophthalein (BSP). After incubation, transport was terminated by aspiration of the uptake buffer followed by three washes with 2 mL ice-cold uptake buffer. The cells were then solubilized with 0.5 N NaOH and transferred to vials for quantification of the radioactivity associated with the cells. All experiments were repeated at least three times, with each experiment performed in triplicate.

RESULTS

Real-Time Quantitative RT-PCR

Quantitative estimates of the relative abundance of oatp-E mRNA were obtained using real-time RT-PCR. mRNA levels for rat oatp-E were evaluated by real-time quantitative RT-PCR. oatp-E was expressed, in order of highest mRNA concentration, in the rat retina, cornea, and ciliary body-iris (Fig. 1). The rat retina expressed significantly higher levels of oatp-E mRNA—more than two times that of the cornea and five times that of the ciliary body-iris. Data are expressed as the mean ± SE (n = 3). Statistical significance was determined by an unpaired t-test ($P < 0.05$).

Western Blot Analysis

To analyze the expression of oatp-E at the protein level, we performed Western blot analysis. As shown in Figure 2, anti-oatp-E antibody recognized an apparent band in the rat retina and brain (68 kDa) and a faint band in the cornea and ciliary body/iris (68 kDa). These bands completely disappeared when the antibody was preabsorbed with the antigen peptide (data not shown), indicating the specificity of the detected band.

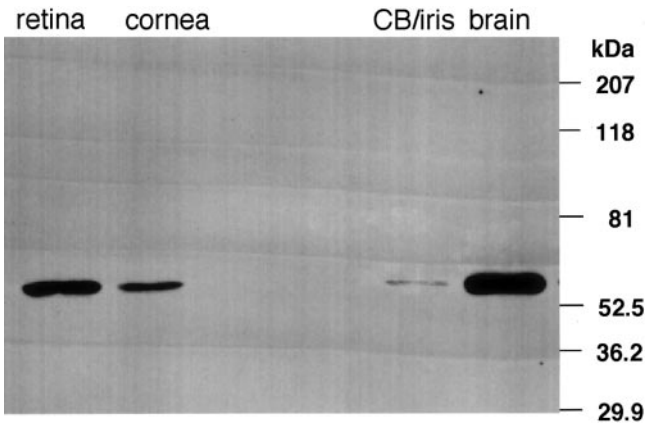

FIGURE 2. Western blot analysis for oatp-E. Western blot analysis of 30 μg crude membrane of the rat retina, cornea, ciliary body-iris, and brain was performed with antibodies against rat oatp-E. CB, ciliary body.

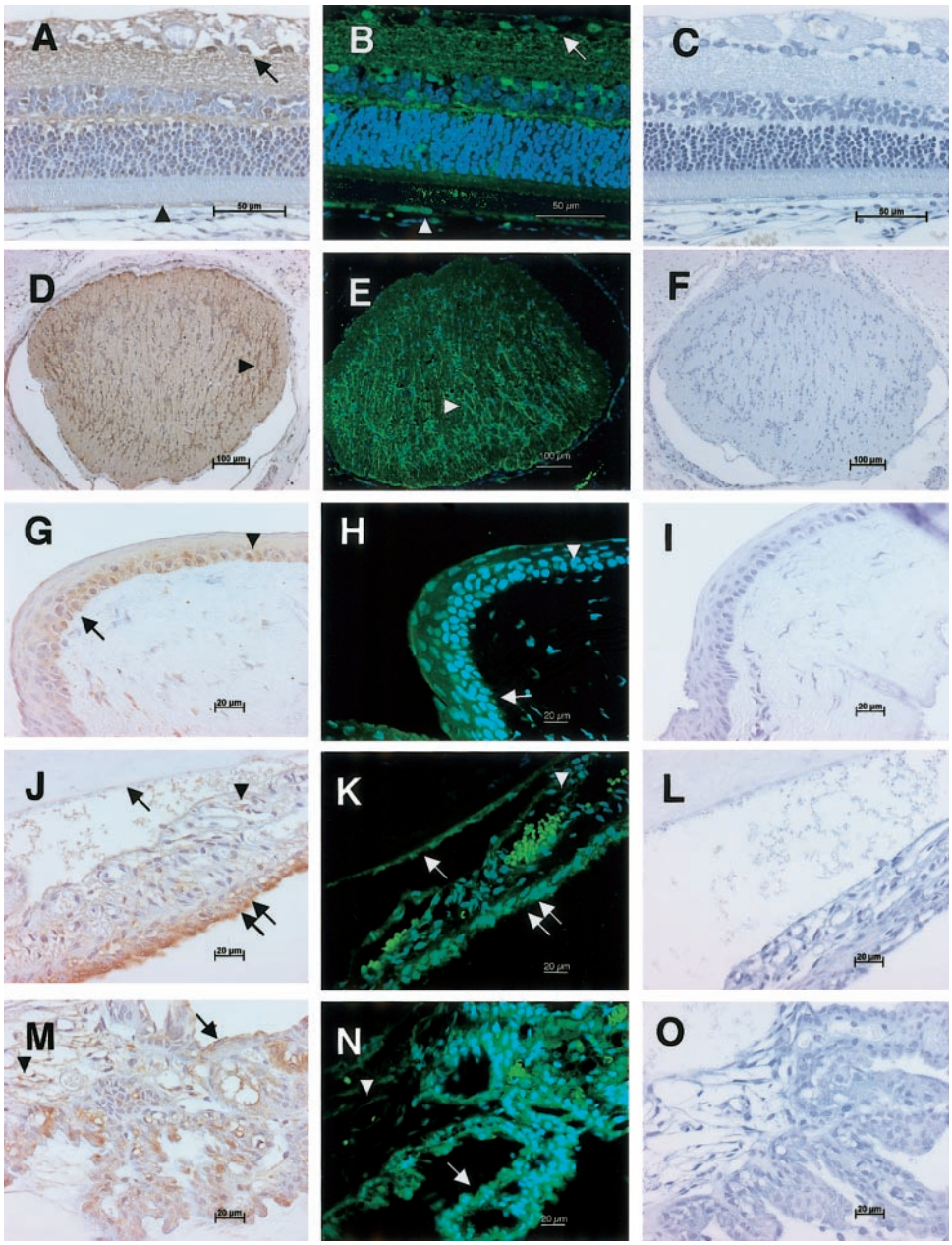

FIGURE 3. Immunohistochemical localization of oatp-E in the rat eye. In the rat retina (**A**, **B**), oatp-E immunoreactivity was observed in the RPE (*arrowhead*), outer and inner plexiform layers, nerve fiber layer, cytoplasm of ganglion cells (*arrow*), and inner nuclear layer. In the optic nerve, which was sectioned longitudinally, staining was seen in the glial cell processes (**D**, **E**, *arrowhead*) and nerve fibers. Apparent immunostaining was observed in the basal cells of the corneal epithelium (**G**, **H**, *arrowhead*) and corneal limbus (*arrow*), and faint staining was seen in the corneal endothelium (**J**, **K**, *arrowhead*). Immunostaining for oatp-E was detected in both the pigmented and nonpigmented epithelium of the iris (**J**, **K**, *double arrows*) and ciliary body (**M**, **N**, *arrow*). The muscular tissues of the ciliary body (**M**, **N**, *arrowhead*) and iris (**J**, **K**, *arrowhead*) were shown to be weakly immunopositive. (**C**, **F**, **I**, **L**, **O**) Negative control sections. For immunofluorescent chemistry, DAPI was counterstained to reveal the nucleus (*blue*).

Immunohistochemistry

In the rat retina, oatp-E immunostaining was found to be widely expressed (Figs. 3A, 3B). In addition to the apparent immunostaining for oatp-E observed in the RPE (arrowhead), intense immunostaining for this peptide was also localized in the outer and inner plexiform layers and nerve fiber layer. In addition, oatp-E immunostaining was observed in the cytoplasm of ganglion cells (arrow) and the inner aspect of the inner nuclear layer. Moreover, oatp-E immunoreactivity was detected in the optic nerve (Figs. 3D, 3E). Apparent immunostaining was present in the glial cell processes (arrowhead), and diffuse immunostaining was seen in the whole nerve fibers.

Immunostaining for oatp-E was seen in all layers of the corneal epithelium (Figs. 3G, 3H). Apparent immunostaining was observed especially in the stroma of basal cells and wing cells of the corneal epithelium (arrowhead). In the limbus, immunostaining was also identified in basal cells (arrow). In contrast, no immunostaining was observed in the stroma of the cornea (Figs. 3J, 3K). However, faint immunostaining was seen in the corneal endothelium (arrow).

In the iris (Figs. 3J, 3K), intense oatp-E immunostaining was broadly detected in both the pigmented and nonpigmented epithelium of the iris (double arrows). In the ciliary body (Figs. 3M, 3N), a similar result was seen in the iris, with prominent oatp-E immunostaining detected in both the pigmented and nonpigmented epithelium (arrow). In addition, muscular tissues of the ciliary body (Figs. 3M, 3N, arrowhead) and iris (Figs. 3J, 3K, arrowhead) were shown to be weakly immunopositive. These patterns of immunostaining were specific for oatp-E, because preabsorption of the antibody with excess antigen peptide resulted in the complete abolition of immunostaining (Figs. 3C, 3F, 3I, 3L, 3O).

oatp-E Expression in Cultured RPE Cells

Before characterizing the function of oatp-E in the eye, we confirmed the expression of oatp-E in rat RPE cells by RT-PCR.

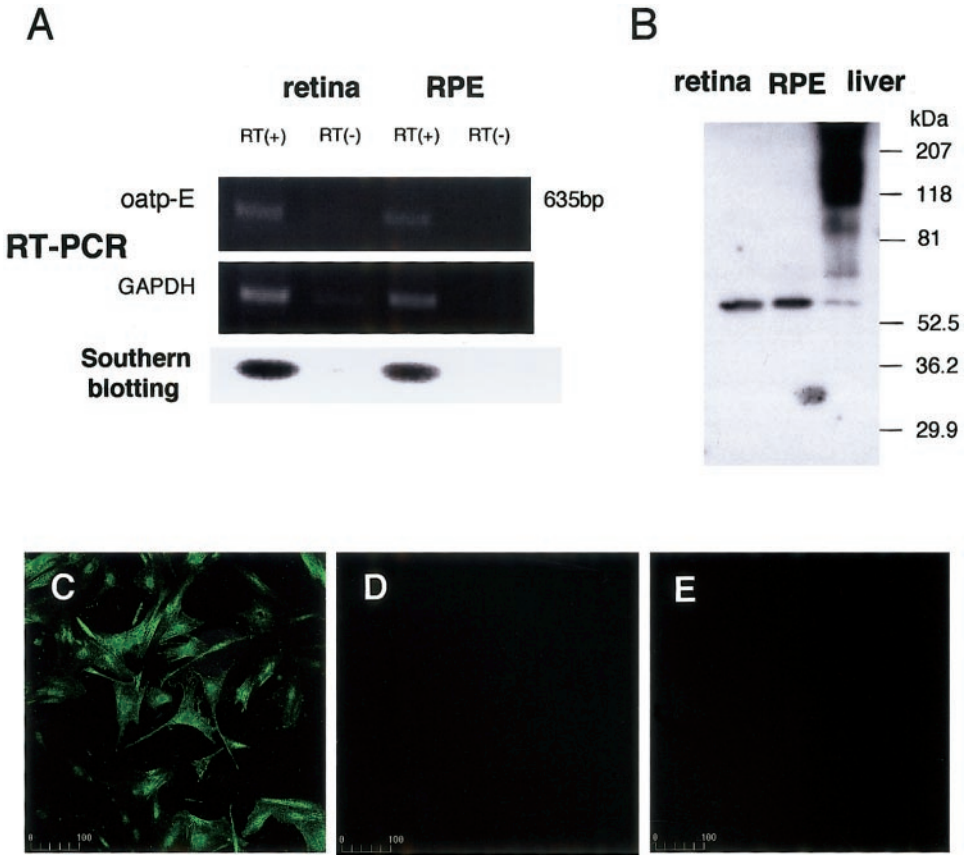

FIGURE 4. oatp-E expression in RPE cells. (**A**) RT-PCR and Southern blot analysis of oatp-E from cultured rat RPE cells and rat retina. cDNA (5 μg) from both RPE cells and rat retina samples were used for RT-PCR. Samples without reverse transcriptase were used as a negative control. PCR products were hybridized with a ^{32}P-labeled fragment of oatp-E–specific cDNA probe.[13] (**B**) Western blot analyses of rat retina, cultured RPE cells, and liver membrane were performed with antibodies against rat oatp-E. Crude membrane (30 μg) was used for each sample. Confocal immunofluorescence study of cultured RPE cells: Permeabilized (**C**) and nonpermeabilized (**D**) cells were treated with oatp-E–specific antibody. (**E**) Specimens were treated with antibody preabsorption with excess immunogen peptide.

In brief, RT-PCR was performed on total RNA isolated from rat RPE cells and rat retina (positive control). A gene-specific band for oatp-E, 635 bp in size, was identified in both of these tissues (Fig. 4A, top panel). When the RT-PCR products were subjected to Southern blot analysis and hybridized with an oatp-E–specific cDNA probe, gene-specific bands for oatp-E were detected in the retina and RPE cells (Fig. 4A, bottom panel). These data confirm that the RT-PCR product from rat RPE is indeed identical with oatp-E.

Western blot analysis revealed oatp-E expression at the protein level. oatp-E–specific bands (68 kDa) were detected in the rat retina and isolated RPE cells (Fig. 4B). However, a faint band was seen in rat liver. These bands completely disappeared when the antibody was preabsorbed with the antigen peptide (data not shown), indicating the specificity of the detected band.

To confirm the expression and membrane topology of oatp-E in RPE cells further, immunohistochemical analysis was performed. oatp-E was localized mainly to the plasma membrane and perinuclear portion of the cells (Fig. 4C). Because oatp-E–specific antibody recognizes the C terminus of oatp-E, only cells permeabilized by detergent were stained. No staining was seen without membrane permeabilization, which may explain the membrane's topology (Fig. 4D). Preabsorption of the antibody with excess immunogen peptide resulted in the complete abolition of immunostaining for oatp-E (Fig. 4E).

Uptake of [^{125}I] T_3 in Cultured RPE Cells

T_3 uptake by OATP-E and oatp-E was determined by using a *Xenopus* oocyte expression system.[12] Because of the consistency of expression of oatp-E, we next examined T_3 uptake by RPE cells. T_3 uptake in RPE cells was dose dependent and saturable, according to saturation kinetics. The apparent K_m was 3.7 $\pm$ 1.1 μM (Fig. 5A). To confirm the contribution of oatp-E T_3 uptake in RPE cells, an inhibition study was performed. In RPE cells, T_3 uptake (0.1 μM) was significantly inhibited by BSP (100 μM), which was evidenced by oatp-E-mediated T_3 uptake ($P < 0.05$; Fig. 5B).

DISCUSSION

In a previous study, we demonstrated the mRNA and protein expression of multifunctional Na^+-independent oatps, termed oatp2 and oatp3, involved in the transport of thyroid hormone in the rat retina.[8] We also reported that oatp2 was localized mainly in the RPE of the rat retina, whereas, oatp3 was localized mainly in optic nerve fibers.[8] In this study, results from quantitative RT-PCR demonstrated the expression of high levels of oatp-E mRNA in the rat retina rather than in the cornea and ciliary body-iris. Because thyroid hormones play an essential role in the neural function of the mammalian central nervous system,[9–11] it is not surprising to find oatp-E mRNA expressed most abundantly in the rat retina.

To date, there have been a few reports regarding thyroid hormone uptake in the central nervous system.[19–23] The reported K_m for thyroid hormone uptake varies from the nanomolar level in mouse neuroblastoma cells[19] to the micromolar level in rat glia cells.[20] The K_m for oatp-E obtained in this study was found to be within the same range of values as for cultured cerebrocortical neurons and rat glial cells.[20] The K_m for thyroid hormone uptake from rat RPE cells in this study was found to be similar to the rate for that of oatp-E–expressing *Xenopus* oocytes[12] and also significantly inhibited by BSP. These findings appear to suggest that thyroid hormone uptake may play a role in the transport of thyroid hormone into the retina.

By immunohistochemistry, we found oatp-E to be expressed at detectable levels throughout almost all retinal layers. Similar to oatp2,[8] oatp-E immunostaining was also localized to

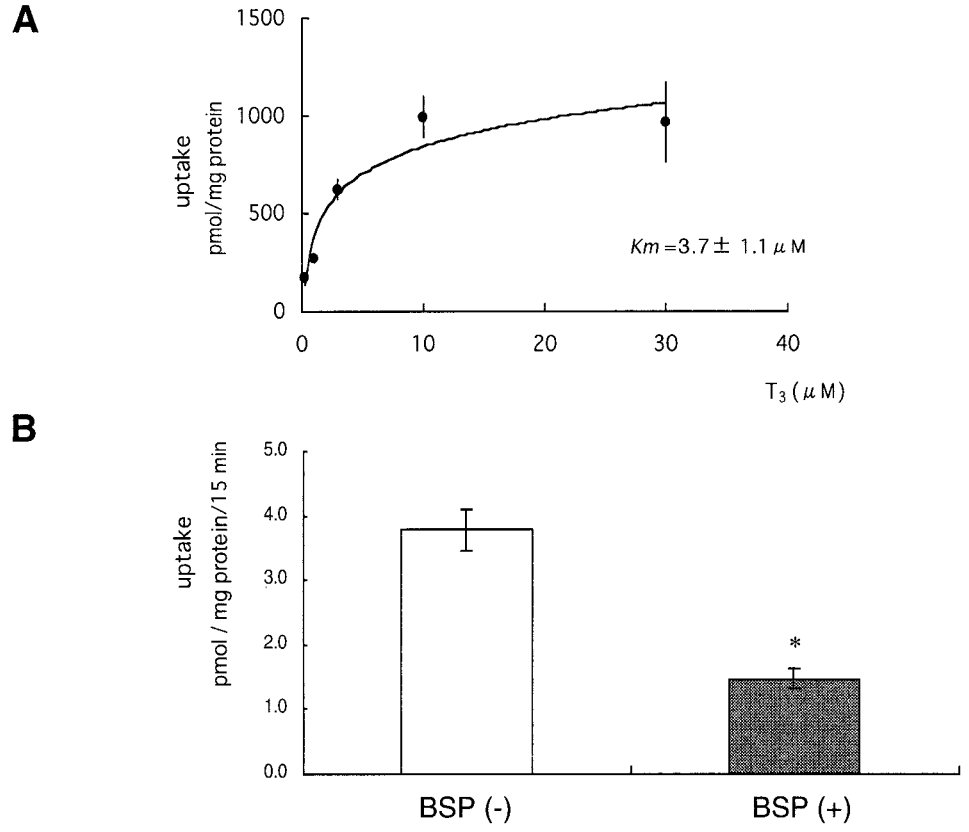

FIGURE 5. Uptake of [^{125}I] T_3 in cultured RPE cells. (**A**) Transport of [^{125}I] T_3 in RPE cells was measured for 15 minutes. RPE cells showed T_3 uptake in a dose-depended and saturable manner. (**B**) Inhibition by BSP of T_3 uptake in RPE cells. T_3 uptake (0.1 μM) was significantly inhibited.

the RPE. The RPE is also a unique source of transthyretin (TTR) synthesis.[24,25] TTR is an important plasma transport protein for thyroid hormone. It is synthesized and secreted in the liver, the choroid plexus, and RPE. Intense immunostaining for TTR has been identified in the RPE, ciliary epithelium, iris epithelium, corneal endothelium, nerve fiber layer of the retina, and lens capsule.[26] TTR protein has been suggested to be responsible for the transport of thyroid hormone in the eye. Based on the findings of TTR subcellular localization in cultured and native human retinal pigment epithelium, TTR labeling was observed in both mitochondrial and nuclear compartments and in close apposition to both apical and basal membranes, suggesting that TTR may function as a cytoplasmic carrier protein for thyroxine in the human RPE.[27] Because the endothelium of the choriocapillaris was found to possess a system for binding and receptor–mediated transport of TTR,[28] thyroid hormone may be transported from the choriocapillaris to the RPE by oatp-E and/or an unknown carrier protein. It is assumed that interphotoreceptor retinoid-binding protein (IRBP) is the major protein present in the interphotoreceptor matrix (IPM) capable of shuttling visual-cycle retinoids between photoreceptors and the RPE.[29] Adler et al.[30] found that serum albumin may participate in visual cycle transport. Considering the conjugation of retinol binding protein to TTR in plasma, such a complex of proteins may be formed within RPE cells, which also incorporate thyroid hormones.[31] It is possible that thyroid hormone may be transported from the RPE to the interphotoreceptor space of photoreceptor cells by oatp 2 and oatp-E cotransport, which may be involved in retinol cycling within the eye.

We also observed intense immunoreactivity in the outer plexiform layer, specifically within the synaptic interactions of photoreceptors, horizontal cells, and bipolar cells, and in the inner plexiform layers, which contain the cell processes and synaptic connections of retinal cells. Based on these observations, oatp-E may play an important role in transporting thyroid hormone in this specialized milieu. Thyroid hormone is essential for normal development of the vertebrate brain, influencing diverse processes such as neuronal migration, myelin formation, axonal maturation, and dendritic outgrowth.[32] Even in the mature rat brain, increased RNA polymerase I activity stimulated by T_3 in the neuronal nuclei has been detected.[33] Thus, oatp-E similar to oatp 3 in the optic nerve may function as a means by which various organic anions are kept from reaching high concentrations in the optic nervous system and thyroid hormone–mediated aspects of ocular growth and development. In hypothyroid mice, the activity of both carbonic anhydrase and Na+,K(+)-ATPase was affected more in the myelin than in other subcellular fractions by deficiency of thyroid hormones.[34] In addition, impairment of myelin compaction was demonstrated by experiments with 2′3′-cyclic nucleotide 3′-phosphodiesterase immunohistochemistry in hypothyroid rats.[35] Therefore, oatp-E may assume responsibility as an ordinary requirement of thyroid hormones to be restored in these ocular tissues. oatp-E immunostaining was also observed in the inner nuclear layer, which contains the nuclei of Müller cells, which are major retinal glia and the cellular component of optic nerves. Thyroid hormone has been reported to be transported into rat glial cells in primary culture through a saturable, stereospecific, and energy-independent carrier system.[20] Our results also suggest that thyroid hormone is transported in the glial cells of the optic pathway by oatp-E.

In the cornea, oatp-E immunostaining was present in the epithelium. Moreover, the immunostaining was most prominent in the basal cells at the limbus. This structure, which is five to seven cells thick in the rat, is composed of three cell types: basal cells (innermost), which undergo mitosis to produce daughter cells that move toward the surface of the cornea; wing cells (middle), which are in an intermediate state of differentiation; and superficial cells (outermost), which are

terminally differentiated and in the process of degenerating. We found that immunoreactivity for oatp-E tended to be greater in the basal cells of the corneal epithelium than in the superficial cells. This pattern may be related to the fact that the basal cells are more metabolically active than superficial ones.[36,37] It is well known that corneal stem cells exist among the basal cells at the limbus, which plays a major role in the regeneration of the corneal epithelium. Coulombre et al.[38] have provided experimental proof of the role of thyroid hormone in corneal dehydration and transparency. Masterson and Edelhauser[39] have reported that the development of both the endothelial and epithelial cell layers of the cornea is thyroxine dependent. The function of oatp-E in the cornea may relate to its ability to transport thyroid hormone into the cornea to maintain optical transparency. The corneal epithelium has been described as a "tight" ion-transporting cell layer that functions both as a protective barrier and as an accessory fluid-secreting layer that augments the endothelial regulation of stromal hydration.[40] The most likely mechanism for the delivery mechanism of retinol to the cornea is by uptake, which has been shown to be time dependent and substrate saturable from the circulation and/or tears.[41] oatp-E may be involved in the transcellular movement of amphipathic compounds, including thyroid hormone and other organic anions.

In the ciliary body and iris, oatp-E was found to be abundant throughout both the pigmented and the nonpigmented epithelium. The oatp-E in these structures may be related to their roles in transporting thyroid hormone and other organic anions into the secretion of the aqueous humor. Moreover, the expression of oatp-E in these epithelia form the barrier between the blood, and the aqueous and vitreous chambers of the eye. The steady state concentrations of low-molecular-weight solutes in the aqueous reflect a dynamic equilibrium in which substances continuously enter from the plasma or surrounding tissues and leave through the trabeculum and uveoscleral drainage. In addition, the specific concentrations of organic anion may be maintained by active secretion by the ciliary epithelium.[42] The human trabecular meshwork tissue is known to have the capacity to respond to thyroid hormones.[43]

In contrast, in humans, three organic anion transporters have been reported: liver-specific organic anion transporter LST-1[44] and LST-2[45] and human OATP.[46] Compared with rat oatps, the expression of these isolated organic anion transporters is very much organ specific.[47] LST-1 and -2 are exclusively expressed in the liver, whereas OATP is expressed in the brain. In addition, although LST-1 and -2, and OATP transport thyroid hormones into the brain and the liver, no molecules except OATP-E– and mOATP–[48] transporting thyroid hormone have been identified in other tissues. We have recently published work on the novel human organic anion transporter OATP-E, which transports thyroid hormone in various peripheral tissues.[12] Our findings would also be helpful in understanding the delivery of thyroid hormone to ocular tissues in humans.

In conclusion, the expression of oatp-E in rat ocular tissues and its functional characterization suggest that oatp-E may have a role in facilitating the transport of thyroid hormones in the eye. Further study is necessary to characterize fully the distribution of oatp isoforms and to understand the intraocular cycling of thyroid hormone and other substances such as retinal. However, our findings may serve as a guide for the study of this unique transport system in the eye.

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