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Function and regulation of taurine transport at the inner blood–retinal barrier

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Abstract

In the retina, taurine exerts a number of neuroprotective functions as an osmolyte and antioxidant. The purpose of the present study was to elucidate the taurine transport system(s) at the inner blood–retinal barrier (BRB). [^{3}H]Taurine transport at the inner BRB was characterized using *in vivo* integration plot analysis and a conditionally immortalized rat retinal capillary endothelial cell line (TR-iBRB2 cells). The expression of the taurine transporter (TauT) was demonstrated by RT-PCR and immunoblot analyses. The apparent influx permeability clearance of [^{3}H]taurine in the rat retina was found to be 259 μL/(min•g retina), supporting carrier-mediated influx transport of taurine at the BRB. [^{3}H]Taurine uptake by TR-iBRB2 cells was Na^+-, Cl^-- and concentration-dependent with a K_m of 22.2 μM and inhibited by TauT inhibitors, such as β-alanine and hypotaurine. RT-PCR and immunoblot analyses demonstrated that TauT is expressed in TR-iBRB2 and primary cultured human retinal endothelial cells. The uptake of [^{3}H]taurine and the expression of TauT mRNA in TR-iBRB2 cells increased under hypertonic conditions but decreased following pretreatment with excess taurine. In conclusion, TauT most likely mediates taurine transport and regulate taurine transport at the inner BRB.

Keywords: Inner blood–retinal barrier; Taurine; Transporter; Retina; Endothelial cells

Introduction

Taurine (2-aminoethanesulfonic acid) is the most abundant free amino acid in the retina (12 μmol/g retina ≈ 12 mM in rats) and accounts for more than 50% of the free amino acid content in the rat retina (Pasantes-Morales et al., 1972). The taurine concentration in the retina is about 100 times greater than that in the serum (100–300 μM) (Dawson et al., 1999; Törnquist and Alm, 1986). Although cysteine sulfinic acid decarboxylase, a rate-limiting enzyme for taurine biosynthesis, is expressed in the retina (Lin et al., 1985), its activity in the rat retina is low in comparison with the abundance of retinal taurine (Heinamaki, 1988). Although abnormal electroretinograms and visual disturbances have been found in patients undergoing long-term parenteral nutrition lacking taurine, these abnormalities returned to normal following administration of taurine intravenously (Geggel et al., 1985; Vinton et al., 1990). This evidence prompts the hypothesis that the blood-to-retina transport system(s) of taurine plays a key role in maintaining the taurine concentration in the retina by supplying taurine.

The nutrient supply to the retina from the circulating blood is regulated by the blood–retinal barrier (BRB), which is composed of retinal capillary endothelial cells (inner BRB) and retinal pigment epithelial cells (RPE, outer BRB) (Cunha-Vaz et al., 1966; Hosoya and Tomi, 2005). The transport of β-

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amino acids, such as taurine and β-alanine, are known to be mediated by an Na^+- and Cl^--dependent taurine transporter (TauT/Slc6a6) (Smith et al., 1992). TauT knockout mice are reported to exhibit a loss of vision due to severe retinal degeneration, in addition to having low taurine concentrations in a variety of tissues and reduced fertility (Heller-Stilb et al., 2002), suggesting that TauT is critical for normal retinal development and function. Using the retinal uptake index method, Törnquist and Alm (1986) demonstrated [^{3}H]taurine uptake from the circulating blood to the retina across the BRB and its inhibition by an excess of taurine and β-alanine, suggesting that the BRB supports taurine transport process(es). Although immunohistochemical study revealed that the outer BRB expresses TauT (Bridges et al., 2001), our knowledge of the taurine transport mechanism at the inner BRB is still incomplete. The inner two thirds of the human retina are known to be nourished by a direct blood supply through the inner BRB (Harris et al., 2001). Moreover, the inner BRB equips several amino acid transport systems which were determined by using isolated retinal capillaries (Betz and Goldstein, 1980; Hjelle et al., 1978; Tomi and Hosoya, 2004). Therefore, it is important to investigate the molecular mechanism of taurine transport at the inner BRB as well as the outer BRB in order to clarify the whole system supplying taurine to the retina.

The purpose of the present study was, firstly, to clarify the transport mechanism of taurine at the inner BRB and, secondly, to investigate the regulation of taurine transport by hypertonicity and taurine pretreatment. The characteristics and functions of taurine transport at the inner BRB were examined by *in vivo* integration plot analysis and using a conditionally immortalized rat retinal capillary endothelial cell line (TR-iBRB2).

Materials and methods

Animals

Male Wistar rats, weighing 250–300 g, were purchased from SLC (Shizuoka, Japan). Rats were maintained on 12-h light/12-h dark cycles with food and water provided ad libitum and used to measure taurine transport near the middle of the light phase of their light–dark cycle in order to minimize the effect of light exposure on taurine uptake by the retina (Hillenkamp et al., 2006). The investigations using rats described in this report conformed to the provisions of the Animal Care Committee, Toyama Medical and Pharmaceutical University (currently University of Toyama) (#2003-48) and the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research.

Blood-to-retina [^{3}H]taurine transport studies

The [2-^{3}H(N)]taurine ([^{3}H]taurine, 30.3 Ci/mmol, PerkinElmer Life Science, Boston, MA, USA) transport from the circulating blood to the retina was measured as described previously (Hosoya et al., 2004). Briefly, rats were anesthetized with an intramuscular injection of ketamine–xylazine (1.22 mg xylazine and 125 mg ketamine/kg) and then [^{3}H]taurine (3.6 μCi/head) was injected into the femoral vein. After collection of blood samples, rats were decapitated, and the retinas and cerebrum were removed. All samples were dissolved in 2 N NaOH and subsequently neutralized. The radioactivity was measured in a liquid scintillation counter (LS6500; Beckman-Coulter, Fullerton, CA, USA). The apparent tissue-to-plasma concentration ratio (V_d) was used as an index of the tissue distribution characteristics of [^{3}H]taurine. This ratio [$V_d(t)$] (mL/g tissue) was defined as the amount of [^{3}H] per gram tissue divided by that per milliliter plasma, calculated over the time period (t) of the experiment. The apparent blood-to-plasma concentration ratio (R_B) was also measured to investigate [^{3}H]taurine uptake into blood cells. The apparent tissue uptake clearance of [^{3}H]taurine ($K_{\text{in, tissue}}$) [μL/(min • g tissue)] was determined by integration plot analysis. In brief, the $K_{\text{in, tissue}}$ can be described by the following equation:

$$V_d(t) = K_{\text{in, tissue}} \times \text{AUC}(t)/C_p(t) + V_i,$$

where AUC(t) (dpm • min/mL), $C_p(t)$ (dpm/mL) and V_i (mL/g tissue) represent the area under the plasma concentration time curve of [^{3}H]taurine from time 0 to t, the plasma [^{3}H]taurine concentration at time t and the rapidly equilibrated tissue distribution volume of [^{3}H]taurine, respectively.

Cell culture

TR-iBRB2 cells possess endothelial markers and facilitative glucose transporter 1 (GLUT1), P-glycoprotein, creatine transporter (CRT) and L-type amino acid transporter 1 (LAT1) (Hosoya et al., 2001; Nakashima et al., 2004; Tomi et al., 2005), which are expressed at the inner BRB *in vivo*. Thus, TR-iBRB2 cells maintain certain *in vivo* functions and are a suitable *in vitro* model for the inner BRB. Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Moregate, Bulimba, Australia) was used as the culture medium for TR-iBRB2 cells. The permissive temperature for TR-iBRB2 cell culture is 33°C due to the presence of temperature-sensitive SV 40 large T-antigen (Hosoya et al., 2001). Under hypertonic and taurine-pretreatment conditions, TR-iBRB2 cells were cultured in the presence of 100 mM sucrose and 10 mM taurine, respectively, for 24 h. Primary cultured human retinal endothelial cells were obtained from Dainippon Pharmaceutical (Osaka, Japan) and cultured in endothelial cell basal medium containing growth supplement (Cell Applications, San Diego, CA, USA) at 37°C. All cells were seeded onto rat tail collagen type I-coated tissue culture plates (BD Biosciences, Bedford, MA, USA) and cultured in a humidified atmosphere of 5% CO_2/air.

[^{3}H]Taurine uptake by TR-iBRB2 cells

The [^{3}H]taurine uptake by TR-iBRB2 cells was measured according to a previous report (Hosoya et al., 2004). Briefly, TR-iBRB2 cells (1×10^5 cells/cm^2) were cultured at 33°C on rat tail collagen type I-coated 24-well plates (BD Biosciences) and washed with 1 mL extracellular fluid (ECF) buffer consisting of 122 mM NaCl, 25 mM $NaHCO_3$, 3 mM KCl, 1.4 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.4 mM K_2HPO_4, 10 mM D-glucose and 10 mM HEPES (pH 7.4) at 37 or 4°C. Uptake was initiated by applying 200 μL ECF buffer containing 0.1 μCi [^{3}H]taurine (17 nM) at 37 or 4°C in the presence or absence of inhibitors. Na^+- or Cl^--free conditions were obtained by replacement with equimolar choline or gluconate, respectively. After a predetermined period, uptake was terminated by removing the solution, and cells were washed with 1 mL ice-cold ECF buffer. The cells were then solubilized in 1 N NaOH and subsequently neutralized. An aliquot was taken for measurement of radioactivity and protein content using a liquid scintillation counter and a DC protein assay kit (Bio-rad, Hercules, CA, USA), respectively, with bovine serum albumin as a standard. The uptake of [^{3}H]taurine by TR-iBRB2 cells was expressed as the cell-to-medium ratio (μL /mg protein) as follows:

$$\text{Cell–to–medium ratio} = ([^3\text{H}]\ \text{dpm in the cell per mg protein})/([^3\text{H}]\ \text{dpm in the medium per}\ \mu\text{L}).$$

The D-[1-^{14}C]mannitol (56 mCi/mmol, American Radiolabeled Chemicals, St. Louis, MO, USA) uptake study was performed to estimate the volume of adhering water. The resulting cell-to-medium ratio was 0.1–0.2 μL/mg protein and much less than that of [^{3}H]taurine. Therefore, adhering water was ignored when calculating the cell-to-medium ratio.

For kinetic studies, the Michaelis–Menten constant (K_m) and maximum rate (J_{max}) of taurine uptake were calculated from following equation using the nonlinear least square regression analysis program, MULTI (Yamaoka et al., 1981).

$$J = J_{max} \times [S]/(K_m + [S]),$$

where J and $[S]$ are the uptake rate of taurine at 5 min and the concentration of taurine, respectively.

RT-PCR analysis

Total cellular RNA was prepared using an Rneasy Kit (Qiagen, Hilden, Germany). Single-strand cDNA was made from total RNA by reverse transcription (RT) using oligo dT primer. The polymerase chain reaction (PCR) was performed with rat TauT (Slc6a6, GenBank accession number NM_017206) specific primers through 40 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 1 min. The sequences of specific primers of rat TauT were as follows: the sense sequence was 5′-CTA CGC GTC CAT CGT CAT CGT GTC C-3′ and the antisense sequence was 5′-AAG TGA AGT TGG CGG CGC TAA GGG A-3′. The PCR products were separated by electrophoresis on an agarose gel in the presence of ethidium bromide and visualized under ultraviolet light. The PCR products of the expected length were then cloned into a plasmid vector using p-GEM-T Easy Vector System I (Promega, Madison, WI, USA) and amplified in *Escherichia coli*. Several clones were then sequenced from both directions using a DNA sequencer (ABI PRISM 310; Applied Biosystems, Foster City, CA, USA).

Quantitative real-time PCR

Quantitative real-time PCR was performed using an ABI PRISM 7700 sequence detector system (Applied Biosystems) with 2×SYBR Green PCR Master Mix (Applied Biosystems) according to the manufacturer's protocol. To quantify the amount of specific mRNA in the samples, a standard curve was generated for each run using the plasmid (pGEM-T Easy Vector; Promega) containing the gene of interest. This enabled standardization of the initial mRNA content of cells relative to the amount of β-actin. The PCR was performed using rat TauT or β-actin-specific primers and the cycling parameters are those given above. The sequences of the specific primers of rat β-actin (GenBank accession number NM_031144) were as follows: sense, 5′-TCA TGA AGT GTG ACG TTG ACA TCC GT-3′ and antisense, 5′-CCT AGA AGC ATT TGC GGT GCA CGA TG-3′.

Immunoblot analysis

Proteins were obtained by dissolving cells in sample buffer consisting of 5% sodium dodecyl sulfate (SDS), 250 mM Tris–HCl (pH 6.8), 10% glycerol, 6% 2-mercaptoethanol, 0.01% bromophenol blue and 1% protease inhibitor cocktail (Sigma, St. Louis, MO, USA), followed by heating for 10 min at 95°C and centrifugation for 10 min at 4°C and 9,000×*g*. Supernatants were separated and used as whole cell extracts. The protein (40 μg) was electrophoresed on a 10% SDS–polyacrylamide gel and, subsequently, electrotransferred to a polyvinylidene difluoride membrane. The membranes were incubated with rabbit polyclonal anti-TauT antibody (1:1000; Chemicon, Temecula, CA, USA) as the primary antibody in the presence of blocking agent solution (Block Ace; Dainippon Pharmaceutical) and with HRP conjugate anti-rabbit IgG (Chemicon) as the secondary antibody. The bands were visualized with an enhanced chemiluminescence kit (Amersham Biosciences, Buckinghamshire, UK).

Data analysis

Unless otherwise indicated, all data represent means±SEM. An unpaired, two-tailed Student's *t*-test was used to determine the significance of differences between two groups. Statistical significance of differences among means of several groups was determined by one-way analysis of variance followed by the modified Fisher's least-squares difference method.

Results

Blood-to-retina transport of taurine across the BRB

The *in vivo* blood-to-retina influx transport of taurine from the circulating blood to the retina through the BRB was evaluated and compared with the brain by means of integration plot analysis after intravenous administration of [^{3}H]taurine to rats. The $K_{in,\ retina}$ of [^{3}H]taurine was determined to be 259±39 μL/(min·g retina) (mean±SD) from the slope representing the apparent influx permeability clearance across the BRB (Fig. 1A). The $K_{in,\ brain}$ of [^{3}H]taurine was 9.07±0.77 μL/(min·g brain) (mean±SD) (Fig. 1B), which is 28.6-fold less than that of the retina. The *y* intercept of the integration plot (V_i) in the retina and brain, which is usually comparable with the vascular volume of each tissue, was 100 μL/g retina and 11.3 μL/g brain, respectively. To examine whether blood cells in vessels contribute to concentrative uptake in the retina, the apparent concentration ratio between blood and plasma (R_B) was measured. The R_B of [^{3}H]taurine was slightly increased with a slope of 9.52×10^{-3} min^{-1} (data not shown). By multiplying the slope of the R_B increment and the vascular volume in the tissue, the apparent uptake clearance into retinal and cerebral blood found to be 0.952 μL/(min·g retina) and 0.108 μL/(min·g brain), respectively, and small enough to ignore. These results indicate that taurine is transported from the blood to the retina across the BRB.

Functional expression of TauT in TR-iBRB2 cells

Since the *in vivo* retinal uptake study represents taurine influx transport across both the inner and outer BRB, [^{3}H]taurine uptake at a concentration of 17 nM was performed using TR-iBRB2 cells as an *in vitro* model of the inner BRB in order to determine the kinetic parameters of taurine and characterize taurine transport at the inner BRB. The time course of [^{3}H]taurine uptake by TR-iBRB2 cells is shown in Fig. 2. [^{3}H]Taurine uptake increased linearly for 10 min and the initial uptake rate was 17.0 μL/(min·mg protein). This supports an apparently concentrative behavior because the cell-to-medium ratio of [^{3}H]taurine uptake at 10 min was higher than the cell volume of approximately 3 μL/mg protein, estimated by [^{3}H]3-*O*-methyl-D-glucose uptake through the facilitative glucose transporter, GLUT1, in TR-iBRB2 cells (Hosoya et al., 2001). In contrast, [^{3}H]taurine uptake was reduced by more than 90% at 4°C and in the absence of either Na^+ or Cl^-, suggesting that taurine uptake by TR-iBRB2 cells takes place in a temperature-, Na^+-, and Cl^--dependent manner.

Fig. 3 shows the concentration-dependent uptake of taurine by TR-iBRB2 cells. The intracellular taurine uptake was saturable, and nonlinear least-squares regression analysis revealed that the K_m and J_{max} were 22.2±2.0 μM and 546±12 pmol/(min·mg protein) (mean±SD), respectively.

The effect of amino acids and an organic anion at a concentration of 1 mM on [^{3}H]taurine uptake by TR-iBRB2 cells is summarized in Table 1. β-Amino acids, such as taurine, β-alanine and hypotaurine, markedly inhibited [^{3}H]taurine uptake by more than 90%. γ-Aminobutyric acid (GABA), a γ-amino acid, inhibited [^{3}H]taurine uptake by 55%. In contrast, L-alanine and glycine had a weaker effect and L-leucine and probenecid had no significant effect on [^{3}H]taurine uptake. These types of inhibition are consistent with the presence of TauT in TR-iBRB2 cells.

RT-PCR and immunoblot analyses were performed to examine the expression of TauT in TR-iBRB2 cells and primary cultured human retinal endothelial cells. Rat retina was used as a

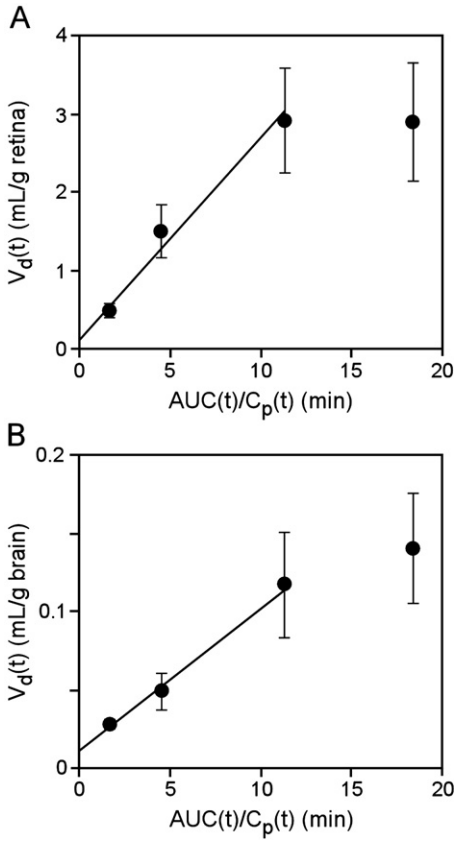

Fig. 1. Integration plot of the initial uptake of [^{3}H]taurine by the retina (A) and brain (B) after intravenous administration. [^{3}H]Taurine (3.6 μCi/head) was injected into the femoral vein. Each point represents the mean±SEM (n=3–4).

positive control. The bands corresponding to the expected 206 bp for TauT were amplified from rat retina and TR-iBRB2 cells (Fig. 4A). The nucleotide sequence of the band of TR-iBRB2 cells was absolutely identical to rat TauT. The expression of TauT protein was also detected in the rat retina, TR-iBRB2 cells and primary cultured human retinal endothelial cells at 70 kDa, which is in agreement with the reported value (Kang et al., 2002) (Fig. 4B).

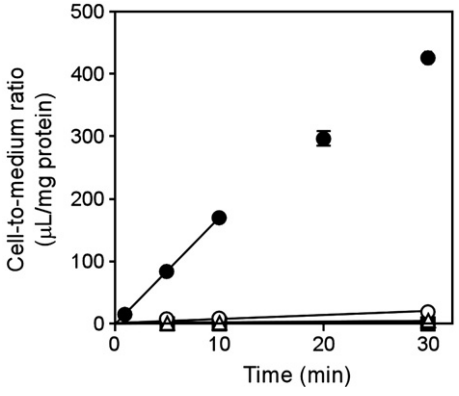

Fig. 2. Time course of [^{3}H]taurine uptake by TR-iBRB2 cells. The [^{3}H]taurine (17 nM) uptake was examined under the following conditions: closed circle, control (at 37°C in the presence of Na^+ and Cl^-); open circle, at 4°C in the presence of Na^+ and Cl^-; closed square, Na^+-free at 37°C; open triangle, Cl^--free at 37°C. Each point represents the mean±SEM (n=4).

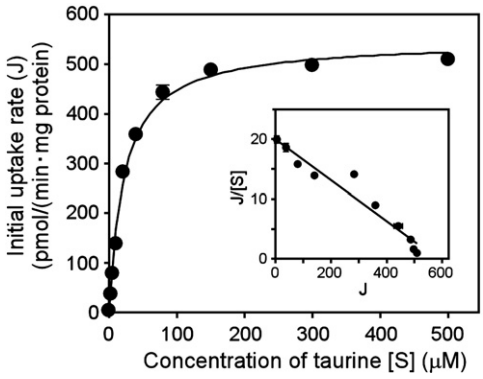

Fig. 3. Concentration dependence of taurine uptake by TR-iBRB2 cells. The [^{3}H]taurine (17 nM) uptake took place for 5 min at 37°C. Each point represents the mean±SEM (n=4). Data were subjected to Michaelis–Menten and Eadie–Scatchard analyses (inset). The K_m is 22.2±2.0 μM and J_{max} is 546±12 pmol/(min•mg protein) (mean±SD).

Regulation of taurine transport activity

To clarify the regulatory mechanism of taurine transport at the inner BRB, [^{3}H]taurine uptake activity and the expression level of TauT mRNA were examined in TR-iBRB2 cells under hypertonic and 10 mM taurine pretreatment conditions (Fig. 5). By exposing TR-iBRB2 cells to hypertonic medium (370 mOsm/kg) for 24 h, [^{3}H]taurine uptake and TauT mRNA expression were increased by 170% and 370%, respectively, in comparison with isotonic medium (270 mOsm/kg). [^{3}H]Taurine uptake was reduced by 56% following pretreatment with 10 mM taurine for 24 h. The expression of TauT mRNA following pretreatment with 10 mM taurine for 24 h was also reduced by 56%, although this was not significantly different from the control.

Discussion

The present study demonstrates that [^{3}H]taurine is transported against a concentration gradient from the circulating blood (100–300 μM in rat serum) (Dawson et al., 1999; Törnquist and Alm, 1986) to the retina (12 μmol/g retina ≈ 12 mM in rats) (Pasantes-Morales et al., 1972) across the inner and outer BRB (Fig. 1A). The apparent influx clearance ($K_{in,\ retina}$) of

Table 1
Effect of several inhibitors on [^{3}H]taurine uptake by TR-iBRB2 cells

Inhibitors	Percentage of control
Control	100±3
Taurine	2.45±0.08**
β-Alanine	8.29±0.56**
Hypotaurine	1.95±0.12**
γ-Aminobutyric acid	44.6±3.0**
L-Alanine	68.2±2.5**
Glycine	84.9±3.9*
L-Leucine	95.2±2.9
Probenecid	93.9±3.5

[^{3}H]Taurine uptake (17 nM) was performed in the absence (control) or presence of 1- mM inhibitors for 5 min. Each value represents the mean±SEM (n=4). *p<0.01, **p<0.001, significantly different from the control.

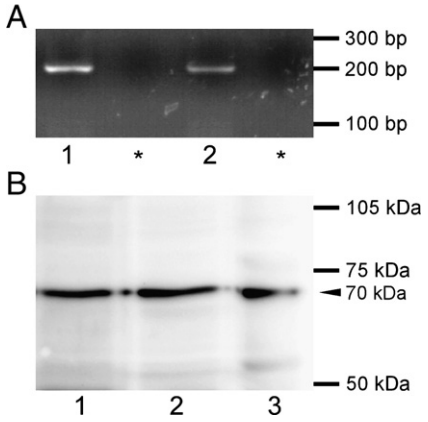

Fig. 4. RT-PCR (A) and immunoblot (B) analyses of taurine transporter (TauT). (A) Lane 1: rat retina; lane 2: TR-iBRB2 cells; *in the absence of reverse transcriptase for the respective left-hand lane. Rat retina was used as a positive control. (B) Lane 1: rat retina; lane 2: TR-iBRB2 cells; lane 3: primary cultured human retinal endothelial cells. Rat retina was used as a positive control.

259 μL/(min·g retina) is far greater than that of [^{14}C]sucrose and [^{3}H]D-mannitol [0.26 and 0.75 μL/(min·g retina), respectively] used as a nonpermeable paracellular marker (Lightman et al., 1987), suggesting that taurine is transported via some carrier-mediated process rather than passive diffusion. This result is in good agreement with an earlier report that estimates the retinal uptake index to be 39% (Törnquist and Alm, 1986). Since the retinal uptake index method estimated it by replacing the blood with a buffer containing [^{3}H]taurine, we performed the *in vivo* integration analysis method to estimate the transport ability by injecting [^{3}H]taurine into the blood. This method makes clear that taurine is still transported even in the presence of 100–300 μM taurine in the blood.

The estimated $K_{in,\ retina}$ of [^{3}H]taurine (259 μL/(min·g retina); Fig. 1A) is almost 30 times greater than the corresponding value across the BBB (9.07 μL/(min·g brain); Fig. 1B). The present result is comparable with an earlier study showing that the uptake index for taurine, which expresses the fractional tissue uptake of taurine as a percentage of that of a diffusible reference substrate, was 39% and 3.6% in the retina and brain, respectively (Törnquist and Alm, 1986). This is partially due to the differences in the surface area of BRB and BBB since the retinal uptake of essential molecules is mediated by RPE (outer BRB) as well as retinal capillary endothelial cells (inner BRB), whereas the brain uptake is exclusively mediated by brain capillary endothelial cells (BBB). However, this reason is not a sufficient explanation since the K_{in} difference of [^{14}C]sucrose and [^{3}H]D-mannitol between the retina and brain is not as great as that of taurine (Ennis and Betz, 1986; Lightman et al., 1987). Another possible reason is that the expression and/or activity of taurine transporter at the inner and outer BRB are greater than that at the BBB. The high transport ability of taurine at the BRB would make a major contribution to maintaining the high concentration of taurine in the rat retina (12 μmol/g retina) (Pasantes-Morales et al., 1972), which is greater than that in the brain (6.6 μmol/g brain) (Levi et al., 1967).

TauT-mediated taurine transport at the inner BRB is supported by the functional expression of TauT in TR-iBRB2 cells used as an *in vitro* inner BRB model. TauT is known to be an Na^+- and Cl^--dependent transporter with a preference for β-amino acids like taurine and β-alanine (Smith et al., 1992). The uptake of [^{3}H]taurine by TR-iBRB2 cells took place in an Na^+-, Cl^--, and concentration-dependent manner (Figs. 2 and 3). The corresponding K_m of 22.2 μM (Fig. 3) is in good agreement with that obtained for taurine uptake by rat TauT expressed COS cells (K_m=43 μM) (Smith et al., 1992), and similar to that at the outer BRB (6.5–8.9 μM) (Bridges et al., 2001; El-Sherbeny et al., 2004). These K_m values at the inner and outer BRB are 4- to 46-fold lower than the serum concentration (100–300 μM) in the rat (Dawson et al., 1999; Törnquist and Alm, 1986). This suggests that the blood-to-retina transport of taurine is more than 80% saturated by endogenous plasma taurine and that the taurine transport system at the BRB plays a role in continuously supplying taurine from the circulating blood to the retina at the constant rate of J_{max}. Indeed, [^{3}H]taurine is transported from blood to the retina across the BRB even in the presence of 100–300 μM taurine in the blood (Fig. 1A). Typical TauT substrates, such as β-alanine and hypotaurine, strongly inhibited [^{3}H]taurine uptake by TR-iBRB2 cells, while α-amino acids, such as L-alanine, glycine and L-leucine, had a weaker effect. GABA, a poor inhibitor of TauT (Smith et al., 1992), partially inhibited [^{3}H]taurine uptake (Table 1). These forms of inhibition in TR-iBRB2 cells are consistent with a previous *in vivo* retinal uptake study which showed that taurine uptake into the retina is inhibited by an excess of taurine and β-alanine (Törnquist and Alm, 1986). RT-PCR and immunoblot analyses revealed that TauT mRNA and protein are expressed in rat retina and TR-iBRB2 cells (Fig. 4). Furthermore, the expression of TauT protein in primary cultured human retinal endothelial cells (Fig. 4B; lane 3) implies that TauT plays a role in the influx transport

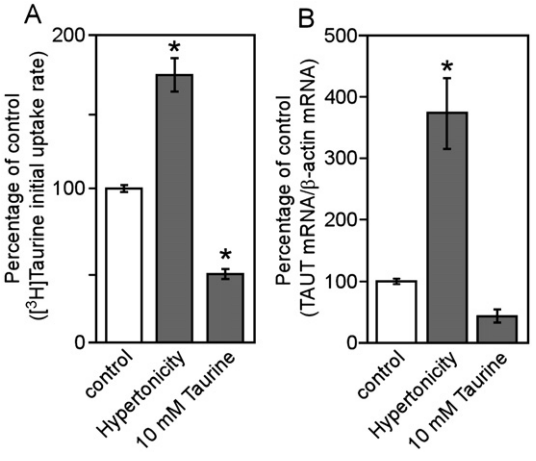

Fig. 5. Regulation of taurine transport in TR-iBRB2 cells. [^{3}H]Taurine uptake activity (A) and the expression level of TauT mRNA (B) were examined in TR-iBRB2 cells following culture under hypertonic and 10 mM taurine pretreatment conditions. (A) [^{3}H]Taurine (17 nM) uptake took place for 5 min at 37°C. (B) The TauT mRNA expression level was determined by quantitative real-time PCR analysis and normalized by the β-actin mRNA expression (TauT/β-actin). Normalized mRNA expression levels were plotted as a relative ratio to the control. Each column represents the mean±SEM (n=3–20). *p<0.001, significantly different from the control.

of taurine in the retinal endothelial cells of rats as well as humans.

In the retina, a variety of protective functions have been ascribed to taurine. The cell volume is regulated by the gain or loss of osmotically active solutes, primarily inorganic ions such as Na^+, K^+, Cl^- (Adorante and Miller, 1990; Bialek and Miller, 1994) or small organic molecules as organic osmolytes. However, electrolytes can harm cells or disrupt metabolic processes when large shifts in their concentrations occur. Even smaller changes in cellular inorganic ion levels can alter resting membrane potential, the rates of enzymatically catalyzed reactions and membrane solute transport that is coupled to ion gradients (Strange, 2004). Therefore, small organic molecules secondary play an important role in cell volume homeostasis since they are compatible or nonperturbing. Especially, retinal taurine is an ideal organic osmolyte because of its high concentration in the retina (Pasantes-Morales et al., 1999). Some retinal diseases, such as ischemia and reperfusion, diabetic retinopathy, macular edema and neurodegeneration, are associated with fluctuations in cell volume (Pasantes-Morales et al., 1999). The present study shows that the uptake of [^{3}H]taurine as well as the expression of TauT mRNA in TR-iBRB2 cells increases under hypertonic conditions (Fig. 5). This result is consistent with previous studies in several types of cells including bovine aortic endothelial cells and rat brain capillary endothelial cells (El-Sherbeny et al., 2004; Kang et al., 2002; Qian et al., 2000; Shioda et al., 2002) and shows that TR-iBRB2 cells sustain a tonicity-responsive element (TonE)/TonE-binding protein (TonEBP) pathway, which regulates osmosensitive transcription of the TauT gene (Ito et al., 2004). This upregulation of taurine transport could markedly affect the achievement of osmotic equilibrium by accumulating taurine in the endothelial cells.

The present study also demonstrates that [^{3}H]taurine uptake, as well as TauT mRNA expression in TR-iBRB2 cells, is reduced following preincubation with 10 mM taurine (Fig. 5), indicating substrate-induced transcriptional suppression of TauT. This result is in good agreement with a previous study in rat brain capillary endothelial cells (Kang et al., 2002) and suggests that the expression of TauT at the inner BRB is normally suppressed to some degree by a high concentration of taurine in the retina but is induced when there is insufficient retinal taurine. Therefore, this regulation appears to play an important role in maintaining the constant milieu of taurine in the retina.

In conclusion, TauT most likely mediates taurine transport from the circulating blood to the retina and regulates taurine transport in retinal capillary endothelial cells. Taurine is physiologically important in the retina, since taurine depletion in the retina reduces cell volume regulation and antioxidant activity, leading to retinal dysfunction (Hansen, 2001). Taurine supplementation is reported to attenuate the upregulation of vascular endothelial growth factor expression in the streptozotocin-induced diabetic rat retina (Obrosova et al., 2001). Therefore, the physiological role of TauT at the inner BRB is to maintain the taurine concentration in the retina. Moreover, taurine is a nonessential amino acid in humans but is considered to be an essential amino acid during fetal growth and lactation (Hansen, 2001), suggesting that TauT at the inner BRB plays an especially important role in supplying taurine to the retina during this period. These findings provide important information to increase our understanding of the physiological role of the inner BRB and the mechanism(s) governing the supply of taurine to the neural retina.

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