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Na^+- AND Cl^--DEPENDENT TRANSPORT OF TAURINE AT THE BLOOD-BRAIN BARRIER

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Abstract—The characteristics of carrier-mediated transport of taurine at the blood-brain barrier (BBB) were studied by using primary cultured bovine brain capillary endothelial cells (BCECs), *in situ* brain perfusion and brain capillary depletion methods in rats. The uptake of [^{3}H]taurine by cultured cells showed that the active transporter functions on both the luminal and antiluminal membranes of BCECs. The kinetic parameters for the saturable transport of taurine were estimated to be: for the luminal uptake, the Michaelis constant, K_t, was 12.1 ± 0.5 μM, and the maximum uptake rate, J_{max}, was 4.32 ± 0.05 nmol/30 min/mg protein; for the antiluminal uptake, K_t was 13.6 ± 2.4 μM and J_{max} was 2.81 ± 0.22 nmol/30 min/mg protein. The luminal and antiluminal uptakes of [^{3}H]taurine were each dependent on both Na^+ and Cl^-. Stoichiometric analyses suggest that two Na^+ and one Cl^- are associated with the luminal uptake of one taurine molecule. β-Amino acids such as β-alanine and hypotaurine strongly inhibited the uptake of [^{3}H]taurine, whereas α- and γ-amino acids had little or no effect. Furthermore, by *in situ* brain perfusion and *in vivo* brain capillary depletion methods, the carrier-mediated transport found by *in vitro* experiments was confirmed to function for the translocation of the taurine molecule from the vascular space into the brain. From these results, it was concluded that a Na^+ and Cl^- gradient-dependent transport (uptake) system for taurine exists in both the luminal and the antiluminal membranes of BCECs.

Key words: β-amino acid; blood-brain barrier; cultured brain capillary endothelial cell; carrier-mediated transport; taurine

Taurine is a unique β-amino acid because it has a sulfonic acid moiety instead of a carboxylic acid. Many physiological functions of taurine have been reported in various tissues [1], especially in the central nervous system (CNS) [2, 3], where taurine is present at a high concentration [4]. In the brain, taurine plays important roles as a neurotransmitter, neuromodulator, and membrane stabilizer. There was also a study using isolated capillaries of bovine brain, which demonstrated the existence of a Na^+-dependent high-affinity uptake system for taurine at the antiluminal surface of BCECs‡ [5]. However, the polarity of taurine transport is difficult to establish with isolated capillaries, because the overall uptake may include both luminal and antiluminal components. Primary cultured monolayers of bovine BCECs are useful for studies of the uptake and transendothelial transport of various substances, including nutrients [6–8], drugs [9–13], and proteins (14–16). Furthermore, it is expected that the monolayer of the BCEC remains polarized, because several membrane functions have been reported to localize in a manner that is consistent with BCECs *in vivo* [10, 17]. These features of the cultured cells enable us to study the polarized transport characteristics in luminal and antiluminal membranes. Moreover, it is important to know whether a transport system observed *in vitro* functions under physiological conditions. For this purpose, the *in situ* brain perfusion and brain capillary depletion techniques are useful, because these methods allow a measurement of transport of taurine into brain in the physiological state.

The purpose of the present study was to clarify the transport mechanism of taurine at the BBB by using an *in vitro* experimental system of the primary cultured monolayers of BCECs, *in situ* brain perfusion and brain capillary depletion techniques.

MATERIALS AND METHODS

Materials

[1,2-^{3}H]Taurine (28–32 Ci/mmol) and L-[G-^{3}H]glutamic acid (50 Ci/mmol) were purchased from Amersham International plc (Buckinghamshire, England). L-[ring-2,6-^{3}H(N)]Phenylalanine (49.7 Ci/mmol) and [^{14}C(U)]sucrose (4.03 mCi/mmol) were obtained from New England Nuclear (Boston, MA, U.S.A.). Horse serum was purchased from Gibco (Grand Island, NY), rat tail collagen (type I) from Collaborative Research Inc. (Bedford, MA, U.S.A.), human fibronectin from Boehringer Mannheim GmbH (Mannheim, Germany), and bovine serum albumin (Fraction V) from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other chemicals were commercial products of reagent grade.

Animals

Male Wistar rats (7–8 weeks of age) were purchased from the Sankyo Laboratory Co. Ltd. (Toyama, Japan). They had free access to food and water.

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‡ Abbreviations: BBB, blood-brain barrier; BCECs, brain capillary endothelial cells; DNP, 2,4-dinitrophenol; and GABA, γ-aminobutyric acid.

Isolation and culture of BCECs

Capillary endothelial cells were isolated from cerebral gray matter of bovine brains by a method described previously [8]. The isolated BCECs were stored at −100° in culture medium containing 20% horse serum and 10% dimethyl sulfoxide until used. Prior to seeding, the dishes (4-well multidish, 16 mm diameter, Nunc, Denmark) or Transwell™ polycarbonate membranes (12 mm diameter, 12 μm pore size, Costar, Cambridge, MA) were coated with rat tail collagen, sterilized in UV light, and coated with human fibronectin. Isolated BCECs were cultured at 37° with 95% air and 5% CO_2. Transport experiments were performed when cells had reached confluence (in 10–12 days). These cultured cells were demonstrated to be endothelial cells by an immunostaining method using Factor VIII-related antigen (data not shown).

In vitro *uptake experiments*

Luminal uptake of ^{3}H- or ^{14}C-labeled compounds by cultured BCECs grown on the dishes was measured by a method reported previously [8]. Briefly, cultured cells were first washed three times with 1 mL of incubation solution (141 mM NaCl, 4 mM KCl, 2.8 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM D-glucose, 10 mM HEPES, and 0.1% bovine serum albumin, pH 7.4, 290 mOsmol) at 37°. Uptake was initiated by adding 250 μL of incubation solution containing [^{3}H]taurine (100 nM) and [^{14}C]sucrose onto cells. [^{14}C]Sucrose was used as the extracellular space marker. To measure antiluminal uptake, cultured BCECs were grown on Transwell™ membrane. Cultured BCECs were washed three times with 1 mL of Na^+- and Cl^--free incubation solution (sucrose solution) containing 240 mM sucrose, 4 mM potassium gluconate, 2.8 mM $CaSO_4$, 1 mM $MgSO_4$, 10 mM D-glucose, 10 mM HEPES, and 0.1% bovine serum albumin, pH 7.4, at 37°. Uptake was initiated by adding to the antiluminal side 1.5 mL of incubation solution containing [^{3}H]taurine (20 nM) and [^{14}C]sucrose and to the luminal side 500 μL of sucrose solution. To minimize re-uptake from the luminal side, sucrose solution on the luminal side was replaced with fresh sucrose solution every 5 min. To terminate the transport reaction, cells were washed three times with 1 mL of the ice-cold incubation solution at the designated time. For quantitation of the radioactivity associated with the cells, the washed cells were solubilized with 300 μL of 1 N NaOH at room temperature for 60 min. After neutralization with 60 μL of 5 N HCl, the resultant sample was put into a plastic scintillation vial containing 4 mL of Clear-sol I (Nacalai Tesque Inc., Kyoto, Japan). The radioactivity was then measured by a liquid scintillation counter, LSC-1000 (Aloka Co., Ltd., Tokyo, Japan). Protein content in cultured cells was measured by the method of Lowry *et al.* [18], using bovine serum albumin as a standard. Net uptake was expressed as the cell-to-medium concentration (C/M) ratio (μl/mg protein) or as an uptake rate (nmol/mg protein/30 min or pmol/mg protein/30 min) after correction for extracellularly adsorbed taurine, estimated from the apparent uptake of [^{14}C]sucrose. To estimate the kinetic parameters of [^{3}H]taurine uptake in cultured monolayers of BCEC, the uptake rate (J) was fitted to the following equation (1), by using the nonlinear least-squares regression analysis program MULTI [19]:

$$J = J_{max}\, s/(K_t + s) \quad (1)$$

where J_{max} is the maximum uptake rate for the carrier-mediated process, s is the concentration of substrate, and K_t is the half-saturation concentration (Michaelis constant). To determine the stoichiometry among Na^+, Cl^-, and taurine, the uptake rate (J) was fitted to the following Hill equation (2):

$$J = J_{max}\,[Na^+ \text{ or } Cl^-]^n/(K_t^n + [Na^+ \text{ or } Cl^-]^n) \quad (2)$$

where n is the Hill coefficient. Furthermore, the data were analyzed kinetically by means of a Hill-type plot, and the Hill coefficient was determined. The significance of differences was evaluated by using Student's t test.

In situ *brain perfusion technique*

Brain perfusion was performed by the method of Takasato *et al.* [20]. In brief, rats were anesthetized with intramuscular doses of ketamine hydrochloride (235 mg/kg, Sankyo Co., Ltd., Tokyo, Japan) and xylazine hydrochloride (2.3 mg/kg, Sigma Chemical Co.). After exposure of the right carotid artery, all branches of the internal and external carotid arteries were cut or coagulated. The right external carotid artery was catheterized for infusion to the internal carotid artery with polyethylene tubing (SP-10) filled with sodium heparin (100 IU/mL). The perfusate (bicarbonate-buffered physiological saline, 142 mM NaCl, 28 mM $NaHCO_3$, 4.2 mM KH_2PO_4, 1.7 mM $CaSO_4$, 1.0 mM $MgSO_4$, 6.0 mM D-glucose, pH 7.4) containing [^{3}H]taurine (30 nM) and [^{14}C]sucrose, which was used as the brain intravascular volume marker, was oxygenated for 3 min with 95% O_2 and 5% CO_2 and perfused through the catheter at a rate of 4.98 mL/min by an infusion pump (Harvard Apparatus, South Natick, MA). The perfusate was kept at 37° by a temperature-regulating circulator (HAAKE, Germany). At time zero, the perfusion was started, and the right common carotid artery was ligated right under the catheterized site to prevent mixing with the blood. The perfusion was continued for periods up to 36 sec. Six sec were required for the perfusate to reach the brain. At the end of the perfusion, the rat was decapitated, and the right cerebral hemisphere was dissected from the perfused brain and weighed. The perfused right cerebral hemisphere was solubilized in a glass scintillation vial containing 1.5 mL of Solvable (New England Nuclear, Boston, MA) at 50° for 3 hr. Then 0.1 mL of 30% hydrogen peroxide was added to the vial. It was kept at room temperature for 1 hr, and 10 mL of scintillation fluid was added. After neutralization with 150 μl of 5 N HCl, the radioactivity was measured. *In vivo* brain uptake (μL/min/g brain) was calculated by the use of Eqn (3) after correcting for the intravascularly remaining taurine estimated from the apparent brain uptake of [^{14}C]sucrose:

$$\text{Brain uptake} = -F_{pf}\ln(1\text{-}E/100) \quad (3)$$

where F_{pf} is the flow rate of the perfusate (μL/min/g brain) and E is the extraction of [^{3}H]taurine (%) given as follows:

$$E = q_{br}/(T \cdot F_{pf} \cdot C_{pf}) \cdot 100 \quad (4)$$

where q_{br} is the amount of [^{3}H]taurine in the brain (dpm/g), T is the perfusion time (min), and C_{pf} is the concentration of [^{3}H]taurine in perfusate (dpm/μL).

Capillary depletion study

[^{3}H]Taurine (70 μCi/mL) and [^{14}C]sucrose (14 μCi/mL) were infused retrogradely into the external carotid artery at a rate of 50 μL/min for 10 min in ketamine-anesthetized rats. From the preliminary experiments, it was expected that taurine takes a relatively long time to cross the BBB in a detectable amount. Therefore, in the present study, we employed a brain perfusion technique different from the usually used brain perfusion technique for *in vivo* capillary depletion studies to expose the brain to [^{3}H]taurine for a longer time (10 min) under blood supply. After the infusion was terminated, the brain was isolated and treated using the capillary depletion technique [21]. The capillary and parenchyma fractions solubilized with Solvable and blood were mixed with scintillation fluid and subjected to radioactivity determination. The apparent distribution volumes of taurine and sucrose were obtained by dividing the amount in the brain by their blood concentrations calculated using both infusion rate (50 μL/min) and blood flow of 600 μL/min [22].

HPLC analysis

Metabolism of taurine was evaluated by the HPLC analysis of ^{3}H-labeled compound in the cultured cells after incubation of [^{3}H]taurine with BCECs for 30 min at 37°. Retention time of unchanged taurine was determined by absorbance at 570 nm using post-derivatization of taurine with ninhydrin reaction. The cells were solubilized with 1 N NaOH and then neutralized with 5 N HCl. Then, 100 μL of the resultant solution was applied to the HPLC system. The HPLC condition used was as follows: analytical column, Partsil 10-SCX 10 (Nippon Densi Kagaku Co., Ltd., Tokyo, Japan); mobile phase, 0.15 N sodium citrate adjusted to pH 3.5 with perchloric acid; and flow rate, 0.7 mL/min. The eluents were collected in a plastic scintillation counting vial, and the radioactivity in each fraction (0.35 mL) was determined using a liquid scintillation counter.

RESULTS

Uptake and metabolism of [^{3}H]taurine by cultured cells

Time courses of both luminal and antiluminal uptake of [^{3}H]taurine into cultured monolayers of BCECs are shown in Fig. 1. Values are expressed as cell/medium ratios of [^{3}H]taurine after correction for extracellular space, determined by using [^{14}C]sucrose. As shown in Fig. 2, no metabolism of taurine within the cells was observed; the accumulation of radioactivity can be ascribed to the intact [^{3}H]taurine alone. The data show that the luminal uptake of [^{3}H]taurine was linear over a 180-min incubation period (Fig. 1A). The antiluminal uptake of [^{3}H]taurine was also linear over a 60-min incubation period (Fig. 1B). Based on these results, all subsequent uptake experiments were conducted for 30 min.

Concentration dependence of [^{3}H]taurine uptake

Figure 3 shows the relationship between the initial luminal or antiluminal uptake rate and the concentration of taurine (0.044 to 100 μM for luminal uptake, 2 to 100 μM for antiluminal uptake). Both the luminal and the antiluminal uptake of taurine were saturable. When analyzed by means of an Eadie-Hofstee plot, a single

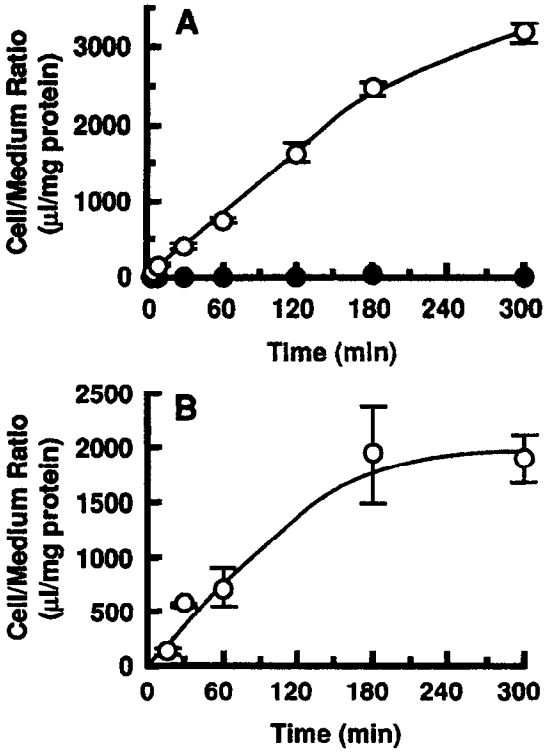

Fig. 1. Time courses of luminal (A) and antiluminal (B) uptake of [^{3}H]taurine by cultured BCEC monolayers. The luminal uptake of 100 nM [^{3}H]taurine was measured at 37° in the presence (○) and the absence (●) of Na^+. In the absence of Na^+, Na^+ was replaced with choline. The antiluminal uptake of 20 nM [^{3}H]taurine was also measured at 37°. Each point is the mean ± SEM of four experiments. The SEM is indicated by bars (smaller than the symbol in most cases).

straight line was obtained in each case ($r^2 = 0.96$ for the luminal uptake, 0.93 for the antiluminal uptake), showing that the uptake occurred by a single transport process. A nonlinear least-squares analysis of these data based on Eqn (1) yielded the following kinetic parameters (values are means ± SD): K_t and J_{max} were 12.1 ± 0.5 μM and 4.32 ± 0.05 nmol/30 min/mg protein, respectively, for the luminal uptake, and 13.6 ± 2.4 μM and 2.81 ± 0.22 nmol/30 min/mg protein, respectively, for the antiluminal uptake.

Effect of temperature and metabolic inhibitors on the uptake of [^{3}H] taurine

The effects of temperature and metabolic inhibitors on both luminal and antiluminal uptake of [^{3}H]taurine are summarized in Table 1. The incubation of [^{3}H]taurine with BCECs at 4° for 30 min resulted in a remarkable decrease in both the uptakes to only 0.5% of the control values, showing a marked dependence on temperature. Addition of 1 mM DNP, an uncoupler of oxidative phosphorylation, or 1 mM potassium cyanide or 10 mM sodium azide, to inhibit the respiratory chain, significantly diminished the luminal uptake of [^{3}H]taurine. The antiluminal uptake of [^{3}H]taurine also was inhibited significantly by DNP. Moreover, 0.5 mM ouabain, an inhibitor of Na^+, K^+-ATPase, reduced both the uptakes of [^{3}H]taurine.

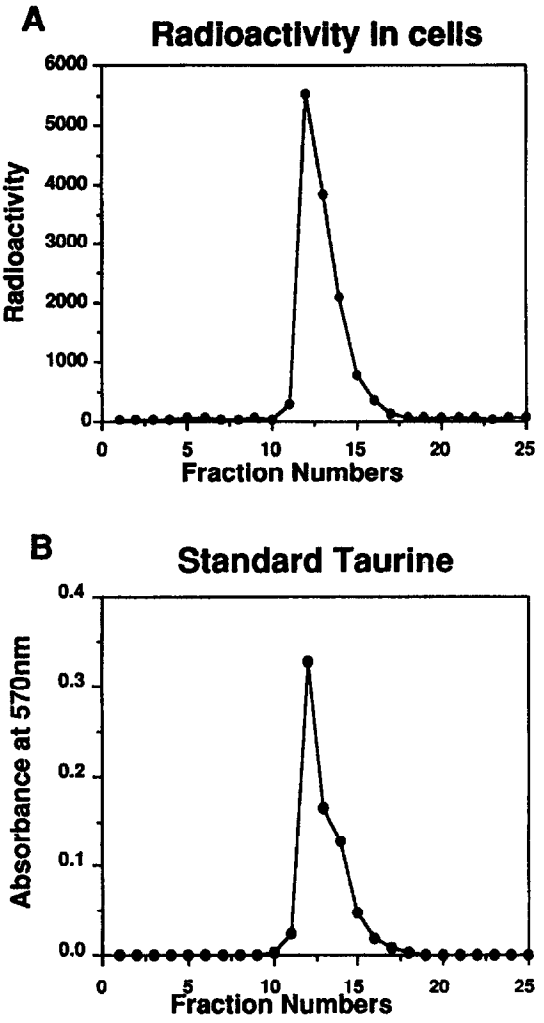

Fig. 2. HPLC chromatograms of radioactivity in cultured cells (A) and standard taurine (B). (A) [^{3}H]Taurine was incubated with cells for 30 min at 37°, and the radioactivity associated with cells was fractionated by HPLC as described in Materials and Methods. (B) Standard taurine was detected by the absorbance at 570 nm, using post-derivatization of taurine with ninhydrin reaction.

Effect of Na^+ *and* Cl^- *on the uptake of* [^{3}H] *taurine*

Time courses of luminal uptake of [^{3}H]taurine, in the presence or absence of sodium ion, into cultured monolayers of BCECs are shown in Fig. 1A. Substituting choline ion for sodium in the incubation solution completely halted the luminal uptake of [^{3}H]taurine, demonstrating a marked sodium dependence. Therefore, the effects of replacement of cations on the luminal and antiluminal uptakes of [^{3}H]taurine were studied, and the results are summarized in Table 2A. The substitution of sodium ion with choline, *N*-methylglucamine, potassium, lithium, or rubidium with the counter anion of chloride completely abolished the luminal uptake of [^{3}H]taurine. The antiluminal uptake also was abolished completely when sodium was replaced with choline or *N*-methylglucamine. In addition, the effects of anions on the taurine uptake were examined in the presence of sodium ion (Table 2B). Substitution of chloride with Br^-, SCN^-, or NO_3^- was moderately inhibitory, whereas SO_4^{2-} or gluconate completely abolished the luminal uptake of [^{3}H]taurine. Among the anions tested, the ability to stimulate the Na^+-dependent taurine uptake was in the following order: $Cl^- > Br^- > SCN^- > NO_3^- \gg SO_4^{2-}$ = gluconate. The antiluminal uptake of [^{3}H]taurine also decreased when chloride was replaced with Br^-, SCN^-, or gluconate, Moreover, the substitution of sodium chloride with sucrose completely abolished both the luminal and antiluminal uptake. These results suggest that sodium and chloride ions are both involved in the transport of taurine.

Effect of Na^+ *and* Cl^- *gradients on luminal uptake of* [^{3}H] *taurine*

To clarify the mechanism of the sodium and chloride requirements in taurine transport, the time courses of luminal uptake of [^{3}H]taurine in the presence and absence of these ion gradients were examined by using ATP-depleted, cultured BCECs prepared by preincubation with a buffer containing NaN_3 in the absence of D-glucose. The results are shown in Fig. 4. In the presence of a Na^+ or a Cl^- gradient, the uptake exhibited a slight overshoot phenomenon. In the presence of both inwardly directed Na^+ and Cl^- gradients, the luminal uptake of [^{3}H]taurine exhibited a marked overshoot phenomenon. In contrast, in the absence of an NaCl gradient or when NaCl was replaced with potassium gluconate, the uptake of [^{3}H]taurine was slow or negligible, respectively.

Stoichiometry of Na^+ *and* Cl^-*-dependent luminal uptake of* [^{3}H] *taurine*

Figure 5 shows the Na^+ and Cl^- concentration dependencies of taurine uptake. Figure 5A represents the relationship between the luminal uptake rate of [^{3}H]taurine and the concentration of Na^+ or Cl^- (0–200 mM). The luminal uptake rate of [^{3}H]taurine increased with increasing concentrations of Na^+ or Cl^-. In the case of Na^+, the curve was sigmoidal, showing that taurine uptake interacts with more than one Na^+. To determine the number of sodium ions interacting with the carrier, the data were analyzed kinetically by means of a Hill-type plot as shown in Fig. 5B. The Hill coefficient (n) was 1.84 ± 0.12 (mean ± SD), suggesting that two Na^+ are associated with transport of one taurine molecule. In the case of Cl^-, the luminal taurine uptake exhibited a simple hyperbolic curve as the Cl^- concentration increased. From the Hill-type plot analysis (Fig. 5B), n was 0.798 ± 0.097, suggesting that one Cl^- is involved in the transport of one taurine molecule.

Effect of structural analogs and various amino acids on the uptake of [^{3}H] *taurine*

The effects of various compounds on luminal and antiluminal uptakes of [^{3}H]taurine are summarized in Table 3. Both of the luminal and antiluminal uptakes of [^{3}H]taurine were strongly inhibited by β-amino acids (*i.e.* hypotaurine, β-alanine, and nipecotic acid). Isethionic acid, a metabolite of taurine, inhibited the luminal uptake, but not the antiluminal uptake. Taurocholate, another metabolite of taurine, did not inhibit the luminal uptake. Glycine and α-alanine showed concentration-dependent inhibition of the luminal uptake of [^{3}H]taurine. They also significantly inhibited the antiluminal uptake of [^{3}H]taurine. The γ-amino acid GABA also reduced both the luminal and antiluminal uptake of

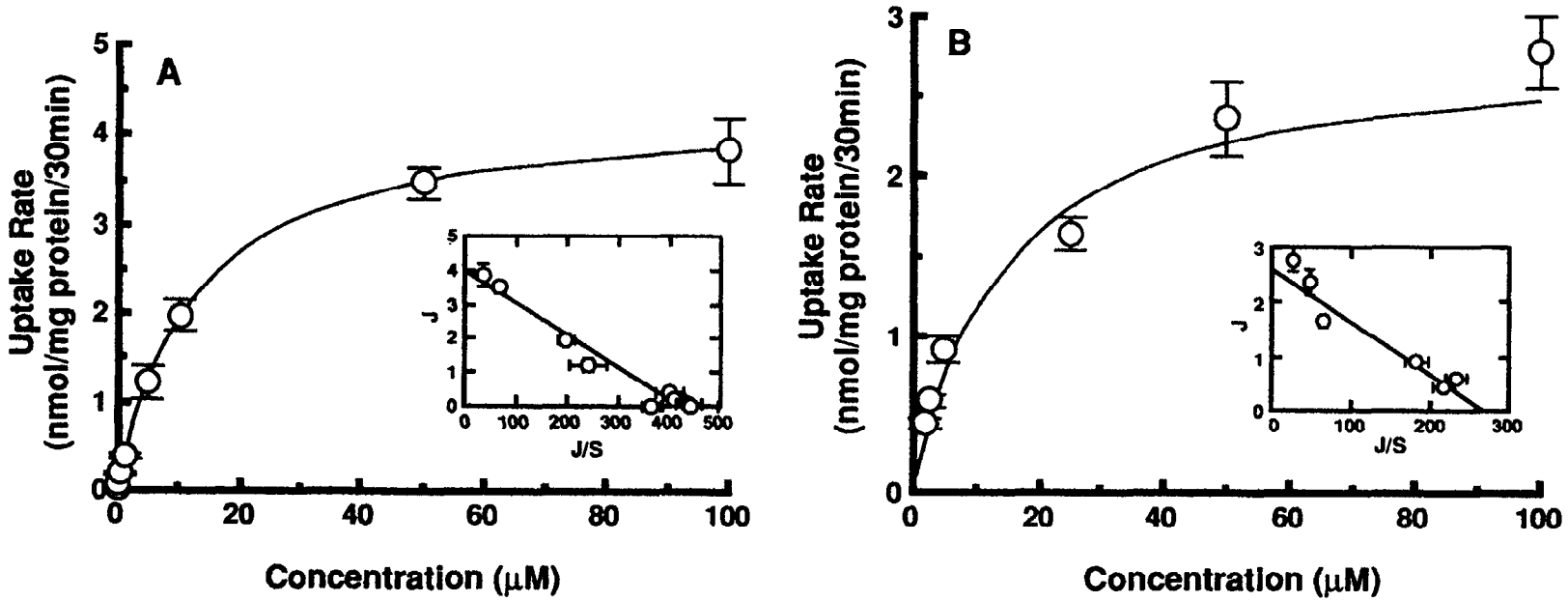

Fig. 3. Concentration dependence of luminal (A) and antiluminal (B) uptake of [^{3}H]taurine by cultured BCEC monolayers. Initial uptakes at various concentrations of taurine (0.044 to 100 μM for luminal uptake or 2 to 100 μM for antiluminal uptake) were measured at 37° for 30 min. Each point is the mean ± SEM of three to four experiments. The SEM is indicated by bars (smaller than the symbol in some cases). The solid line represents the saturable uptake rate generated from Equation 1, using the MULTI-fitted parameters as described in the text.

Table 1. Effects of temperature and metabolic inhibitors on the uptake of [^{3}H]taurine by cultured BCEC monolayers

Condition	Concn (mM)	Uptake of [^{3}H]taurine (% of control) Luminal membrane	Antiluminal membrane
Control		100	100
Low temperature (4°)		0.5 ± 0.2*	0.5 ± 0.5*
Ouabain	0.5	32.4 ± 1.5*	48.2 ± 5.7*
DNP	1	22.6 ± 1.5*	71.1 ± 6.7*
NaN_3	10	43.3 ± 2.2*	ND†
KCN	1	44.2 ± 0.8*	ND

BCECs were preincubated for 20 min at 37° with a metabolic inhibitor in the absence of D-glucose (except in the case of ouabain). The uptake of [^{3}H]taurine (100 nM for the luminal uptake, 30 nM for the antiluminal uptake) was measured at 37° or 4° for 30 min in the presence or absence of a metabolic inhibitor and D-glucose. Control uptakes were 199 μL/30 min/mg protein for the luminal membrane and 468 μL/30 min/mg protein for the antiluminal membrane. Each value is the mean ± SEM of three to four experiments. DNP = 2,4-dinitrophenol.

* $P < 0.05$ vs control.

† ND, not determined.

[^{3}H]taurine. A neutral α-amino acid, L-leucine, and an acidic α-amino acid, aspartate, did not inhibit the uptake of [^{3}H]taurine. Methionine, cysteine, and L-phenylalanine inhibited the antiluminal uptake, whereas they did not affect the luminal uptake. The luminal uptake was enhanced by glutamate but the antiluminal uptake was inhibited.

Effect of organic anions on the uptake of [^{3}H] *taurine*

The effects of organic anions on the uptake of [^{3}H]taurine were studied, and the results are summarized in Table 4. Organic anions (*i.e.* probenecid, BCG, PAH, and DIDS) had no effect on the luminal uptake of [^{3}H]taurine, but except for probenecid they significantly stimulated the antiluminal uptake of [^{3}H]taurine. Salicylic acid and benzylpenicillin slightly inhibited the luminal uptake, but the other drugs did not. The antiluminal uptake was increased significantly by valproic acid and tended to be enhanced by salicylic acid.

Effect of β-alanine or GABA on the uptake of [^{3}H] *taurine*

Figure 6A shows the Lineweaver-Burk plots for the luminal uptake rate of [^{3}H]taurine, demonstrating inhibition of β-alanine or GABA at a concentration of 25 or 250 μM, respectively. Figure 6B shows the Lineweaver-Burk plot for the antiluminal uptake rate of [^{3}H]taurine, revealing the inhibition by β-alanine at a concentration of 50 μM. The result demonstrates that β-alanine competitively inhibited both the luminal and antiluminal uptake of [^{3}H]taurine. The inhibitory constants (K_i) of β-alanine were 28.6 μM for the luminal uptake and 18.9 μM for the antiluminal uptake. GABA also competitively inhibited the luminal uptake of [^{3}H]taurine with a K_i of 470 μM.

Effect of amino acids and ion replacement on the in vivo *brain uptake of* [^{3}H] *taurine*

The *in vivo* brain uptake determined by rat brain perfusion for [^{3}H]taurine and the effects of amino acids and ion replacement on the brain uptake of [^{3}H]taurine are shown in Fig. 7. The uptake of [^{3}H]taurine (30 nM) was 36.6 ± 3.10 μL/min/g brain. It was reduced significantly by unlabeled taurine and β-alanine to the extent of 3.8 ± 1.1 and 11.4 ± 1.6% (N = 4, mean ± SEM) of the control, respectively, but not by L-phenylalanine. Furthermore, the replacement of NaCl with *N*-methylglucamine Cl or sodium gluconate or sucrose almost completely abolished the uptake of [^{3}H]taurine.

Table 2. Effects of Na and Cl ions on the uptake of [^{3}H]taurine by cultured BCEC monolayers

A. Na^+ replacement with cations in the presence of Cl^-

Cation	Uptake of [^{3}H]taurine (% of control) Luminal membrane	Antiluminal membrane
Control	100 ± 13.8	100 ± 2.0
Choline	0	0
K^+	0	ND*
Li^+	0	ND
Rb^+	0	ND
N-Methylglucamine$^+$	0	0
Sucrose	0.2 ± 0.01	0

B. Cl^- replacement with anions in the presence of Na^+

Anion	Uptake of [^{3}H]taurine (% of control) Luminal membrane	Antiluminal membrane
Control	100 ± 13.8	100 ± 2.0
Br^-	48.3 ± 4.0	30.5 ± 5.0
SCN^-	30.7 ± 2.7	8.8 ± 0.6
NO_3^-	9.9 ± 0.3	ND*
SO_4^{2-}	1.2 ± 0.2	ND
Gluconate$^-$	1.3 ± 0.1	0

The uptake of [^{3}H]taurine (100 nM for the luminal uptake, 20 nM for the antiluminal uptake) was measured at 37° for 30 min. "Cation" indicates that Na^+ in the incubation solution was replaced by another cation or sucrose. "Anion" indicates that Cl^- in the incubation solution was replaced by another anion. In the case of SO_4^{2-}, NaCl and KCl were replaced with 71 mM Na_2SO_4 and 2 mM K_2SO_4, respectively. Control uptakes were 229 ± 31.6 μL/30 min/mg protein for the luminal membrane and 706 ± 14.2 μL/30 min/mg protein for the antiluminal membrane. Each value is the mean or mean ± SEM of four experiments.

* ND, not determined.

In vivo *capillary depletion study*

Table 5 shows the apparent distribution volume (μL/g brain) of [^{3}H]taurine and [^{14}C]sucrose in the rat brain parenchyma and capillary fractions. The parenchyma distribution volume for [^{3}H]taurine was about 2.2 times larger than that for [^{14}C]sucrose, suggesting that taurine permeates into brain across the BBB.

DISCUSSION

The cell/medium ratios obtained by the luminal and the antiluminal uptake of [^{3}H]taurine were 3100 and

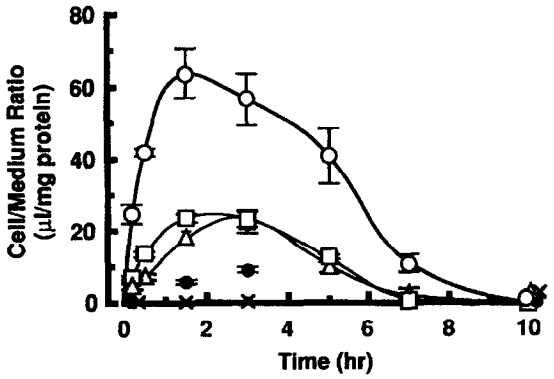

Fig. 4. Effects of Na^+ and Cl^- gradients on the luminal uptake of [^{3}H]taurine by ATP-depleted cultured BCEC monolayers. Preincubation of cells (8 hr) and uptake studies were performed with 10 mM HEPES/KOH buffer, pH 7.4, in the presence of 10 mM NaN_3 and in the absence of D-glucose. To study uptake in the presence of maximum driving forces, cells were preincubated with 141 mM potassium gluconate followed by uptake in the presence of 141 mM NaCl (○). The effect of the Na^+ gradient was studied in the presence of 141 mM NaCl with cells preincubated with 141 mM *N*-methylglucamine Cl (□). The effect of the Cl^- gradient was studied in the presence of 141 mM NaCl with cells preincubated with 141 mM sodium gluconate (△). To produce a situation of no Na^+ or Cl^- gradient, cells were preincubated with 141 mM NaCl, and the uptake was studied in the presence of 141 mM NaCl (●). For the study in the absence of NaCl, cells were preincubated with 141 mM potassium gluconate, and the uptake was studied in the presence of 141 mM potassium gluconate (X). All the uptake media contained 100 nM [^{3}H]taurine. Each point is the mean ± SEM of three to four experiments. The SEM is shown by bars (smaller than the symbol in some cases).

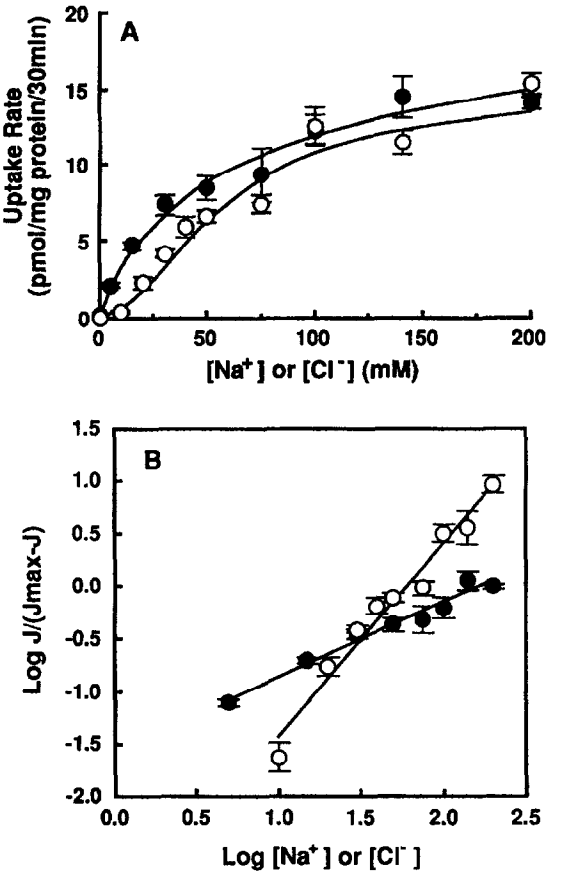

Fig. 5. Na^+ and Cl^- dependences of luminal uptake of [^{3}H]taurine by cultured BCEC monolayers (A). Uptake of [^{3}H]taurine was measured in 10 mM HEPES/KOH buffer, pH 7.4, containing 4 mM KCl, 2.8 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM D-glucose, 10 mM HEPES, and various concentrations of Na^+ obtained by replacement of NaCl with choline chloride to study Na^+ dependence (○) or of Cl^- obtained by replacement of NaCl with sodium gluconate to study Cl^- dependence (●). An initial uptake rate of 100 nM [^{3}H]taurine was measured at 37° for 30 min. Each point is the mean ± SEM of three to four experiments. Panel B shows a Hill plot analysis of the data in panel A. J, initial uptake rate; J_{max}, maximal uptake rate. Lines were drawn by linear regression analysis. The slope gives the Hill coefficient, *n*.

1800 μL/mg protein at 5 hr, respectively (Fig. 1). Because the volume of the endothelial cell was estimated to be approximately 10 μL/mg protein [8] and no metabolism of taurine was observed in the cells (Fig. 2), taurine accumulation in the cells is about 200–300 times more than would be expected from the cell volume. Such an extensive accumulation of [^{3}H]taurine in BCECs was thought to be caused by the specific active transport across both the luminal and antiluminal membranes. Therefore, this study was designed to examine precisely the mechanisms of the luminal and antiluminal membrane transport of taurine.

The most important finding in the present study is that a specific active carrier-mediated transport system that is dependent on sodium and chloride ions functions in the luminal and antiluminal membranes of BCECs. The characteristics of the transporter can be summarized as follows. Significant temperature and metabolic energy dependencies were observed (Table 1). An energy requirement for the accumulation of [^{3}H]taurine was obvious, because DNP, sodium azide, and potassium cyanide significantly reduced the initial uptake rates. Ouabain, an inhibitor of (Na^+, K^+)-ATPase, which localizes in the antiluminal membrane [23], also reduced both the luminal and antiluminal uptake of [^{3}H]taurine. It may act by reducing the sodium gradient and the membrane potential. The luminal and antiluminal uptakes of taurine each involved a single saturable process (Fig. 3). Based on the values of J_{max}/K_t (mL/30 min/mg protein), taurine seems to be transported with similar efficiency in the luminal (0.36) and antiluminal (0.21) membranes, although several different transport features were observed between the two membranes, as discussed later. The K_t values obtained, 12.1 ± 0.5 μM for the luminal uptake and 13.6 ± 2.4 μM for the antiluminal uptake, were reasonable for carrier-mediated entry of taurine into the endothelial cells, because the plasma and brain interstitial fluid concentrations of taurine are about 100 μM [24] and 10 μM [25], respectively. Furthermore, taking the transport efficiencies in both membranes and physiological concentrations of taurine together, it is expected that *in vivo* net transport of taurine occurs in the direction from blood to brain.

As suggested by the effect of ouabain, one of the driving forces for [^{3}H]taurine transport was Na^+, because various other cations were unable to substitute for Na^+ (Table 2A). Cl^- was also required for efficient [^{3}H]taurine transport (Table 2B). The stoichiometry among taurine, Na^+ and Cl^- in the luminal uptake of

Table 3. Effects of amino acids and structural analogues on the uptake of [^{3}H]taurine by cultured BCEC monolayers

Inhibitor	Concn (mM)	Uptake of [^{3}H]taurine (% of control) Luminal membrane	Antiluminal membrane
Control		100	100
Hypotaurine	0.5	1.1 ± 0.1*	1.5 ± 0.3*
Taurocyamine	0.5	11.8 ± 1.1*	ND†
β-Alanine	0.5	5.8 ± 0.2*	8.5 ± 0.5*
α-Alanine	0.5	69.8 ± 3.8*	56.0 ± 3.7*
	5.0	60.5 ± 2.4*	ND
GABA	0.5	67.5 ± 1.5*	25.4 ± 3.0*
Glycine	0.5	82.1 ± 1.5*	76.0 ± 5.5*
	5.0	55.9 ± 1.7*	ND
Nipecotic acid	5.0	30.1 ± 1.1*	38.6 ± 3.7*
Taurocholate	0.5	82.1 ± 7.9	ND
Isethionic acid	5.0	70.7 ± 3.2*	98.7 ± 11.3
Cysteine	5.0	77.0 ± 4.9	10.1 ± 1.5*
Methionine	5.0	81.2 ± 8.6	59.9 ± 5.4*
L-Cystine	0.2	88.7 ± 5.1	101 ± 7.9
L-Cysteic acid	5.0	84.0 ± 3.7	88.6 ± 7.9
L-Phenylalanine	5.0	97.2 ± 9.0	62.2 ± 7.4*
L-Leucine	5.0	83.3 ± 6.4	100 ± 14.5
L-Glutamate	5.0	136 ± 9.4*	67.4 ± 1.8*
L-Aspartate	5.0	112 ± 2.9	86.0 ± 6.7

The uptake of [^{3}H]taurine (100 nM for the luminal uptake, 20 nM for the antiluminal uptake) was measured at 37° for 30 min in the presence of each compound. Control uptakes were: 145 ± 15.0 μL/30 min/mg protein for the luminal membrane and 706 ± 14.2 μL/30 min/mg protein for the antiluminal membrane. Each value is the mean ± SEM of three to four experiments.

* $P < 0.05$ vs control.

† ND, not determined.

Table 4. Effect of organic anions on the uptake of [^{3}H]taurine by cultured BCEC monolayers

Inhibitor	Concn (mM)	Uptake of [^{3}H]taurine (% of control) Luminal membrane	Antiluminal membrane
Control		100 ± 7.7	100 ± 2.8
Probenecid	1	111 ± 7.8	106 ± 12.9
BCG	0.1	104 ± 6.4	134 ± 3.8*
PAH	10	88.9 ± 5.1	142 ± 14.7*
DIDS	0.1	95.1 ± 2.5	157 ± 14.7*
Valproic acid	10	91.3 ± 3.9	126 ± 7.9*
Salicylic acid	10	71.6 ± 5.9*	131 ± 13.5
Sulbenicillin	10	86.7 ± 2.6	ND†
Benzylpenicillin	10	78.0 ± 3.9*	ND
Ampicillin	10	87.8 ± 5.6	ND

The uptake of [^{3}H]taurine (100 nM for the luminal uptake, 20 nM for the antiluminal uptake) was measured at 37° for 30 min in the presence of each compound. Control uptakes were: 213 ± 16.3 μL/30 min/mg protein for the luminal membrane and 567 ± 16.1 μL/30 min/mg protein for the antiluminal membrane. Abbreviations: BCG, bromcresol green; PAH, *p*-aminohippuric acid; and DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid. Each value is the mean ± SEM of three to four experiments.

* $P < 0.05$ vs control.

† ND, not determined.

[^{3}H]taurine (Fig. 5) suggests that one taurine molecule is associated with two sodium ions and one chloride ion, implying a transporter of net positive charge. The result obtained in this study is similar to those reported for taurine transport in rat renal brush-border membrane vesicles [26] and rabbit jejunal brush-border membrane vesicles [27], where the stoichiometries were 1:2:1 and 1:3:1 for taurine, Na^+ and Cl^-, respectively. Furthermore, the same stoichiometry was observed for GABA [28] in synaptic plasma membrane vesicles. Some anions other than Cl^- can partly drive the uptake of taurine. The order in which the anions stimulated taurine uptake was $Cl^- > Br^- > SCN^- > NO_3^- > SO_4^{2-}$ = gluconate, whereas the membrane permeability was in the order of $SCN^- > Cl^- > NO_3^- > SO_4^{2-}$ > gluconate [29, 30]. Therefore, the effect of Cl^- on taurine transport cannot simply be ascribed to the alteration of membrane potential difference, but should involve a specific effect on the taurine transporter. Br^- was the next most preferred anion, and this anion dependence was very similar to that reported in taurine transport in human placental brush-border membrane vesicles [31].

To clarify whether the stimulation of taurine transport by Na^+ and Cl^- is caused by their binding to the carrier protein or whether it is caused by cotransport of the ions with taurine, the effect of gradients of these two ions on the taurine transport was examined by using ATP-depleted BCECs. The use of ATP-depleted cells makes it possible to manipulate intracellular conditions [32]. The uptake of [^{3}H]taurine in the presence of both Na^+ and Cl^- gradients exhibited a significant overshoot phenomenon. In the presence of only a Na^+ or Cl^- gradient, a slight overshoot of uptake was seen (Fig. 4). These results indicate that taurine is co-transported with both Na^+ and Cl^-. Therefore, the energy for the intracellular accumulation of taurine is thought to come from the Na^+ and Cl^- gradient.

The taurine transporter showed specificity similar to those in other tissues (Table 3 and Fig. 6). The taurine transporter in BCECs appears to be highly selective for β-amino acids, which are zwitterionic compounds having positive and negative charges separated by an aliphatic backbone of two carbon atoms. GABA and glycine also possess positive and negative charges separated by three and one carbon atoms, respectively, but they had less effect on taurine transport than β-alanine had. These results suggest that the affinity to the taurine transporter depends on the number of carbon atoms between positive and negative charges. In fact, the K_i value of GABA (470 μM) determined in this study was 17 times larger than that of β-alanine (28.6 μM) (Fig. 6), showing that the affinity of GABA for the taurine transport carrier is lower than that of β-alanine. Nipecotic acid, which is a β-amino acid possessing a bulky structure, also inhibited [^{3}H]taurine uptake, but its inhibitory potency was less than those of aliphatic β-amino acids. These results suggest that aliphatic β-amino acids are likely to be better substrates for the taurine transport system.

It would be interesting to know whether or not the luminal and antiluminal carriers for taurine have identical characteristics. Some differences were observed in the inhibitory effects of cysteine, methionine, and phenylalanine on the uptake of [^{3}H]taurine between the luminal and the antiluminal membranes (Table 3). It is interesting that glutamate slightly stimulated the luminal

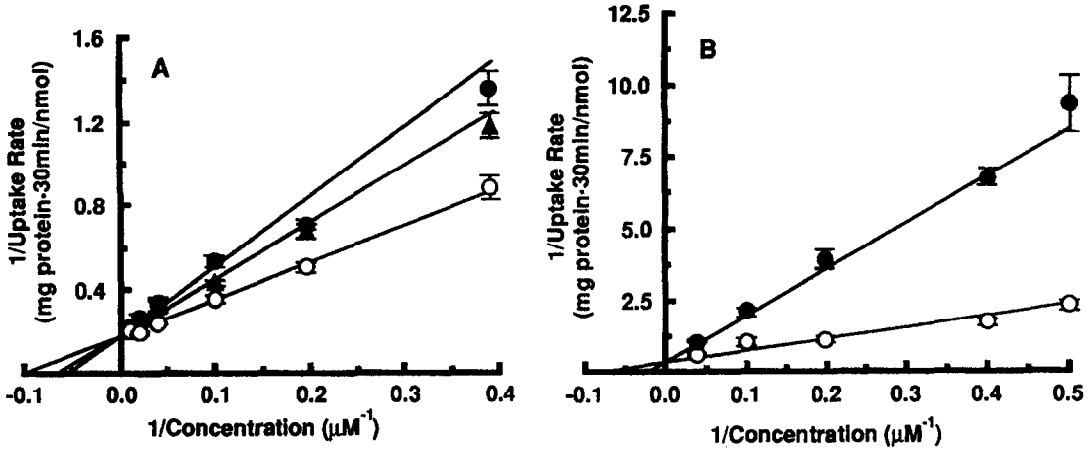

Fig. 6. Lineweaver-Burk plots of luminal (A) and antiluminal (B) uptake rates of [^{3}H]taurine by cultured BCEC monolayers. The initial uptake rate of [^{3}H]taurine (2–100 μM for luminal uptake or 2–25 μM for antiluminal uptake) was measured at 37° for 30 min in the absence (○) and presence of 25 μM (for the luminal uptake) or 50 μM (for the antiluminal uptake) unlabeled β-alanine (●) or 250 μM unlabeled GABA (▲). β-Alanine or GABA was added at the initiation of [^{3}H]taurine uptake. Each point is the mean ± SEM of three to four experiments.

uptake of [^{3}H]taurine, but significantly inhibited the antiluminal uptake. Because the antiluminal uptake of [^{3}H]glutamate was not inhibited by taurine (data not shown), the inhibitory effect by glutamate may be nonspecific. However, these effects suggest that the luminal transport carrier is different from the antiluminal one with respect to substrate specificity.

To demonstrate that the taurine transport system observed in the cultured cells functions physiologically, we utilized an *in situ* brain perfusion technique. The *in vivo* brain uptake of [^{3}H]taurine (37 μL/min/g brain) was much smaller than those of L-phenylalanine (3010 μL/min/g brain, data not shown) and L-leucine (630 μL/min/g brain) [33]. However, uptake of [^{3}H]taurine was inhibited by unlabeled taurine and β-alanine and was not reduced by L-phenylalanine (Fig. 7). These observations suggest that taurine uptake by brain endothelial cells occurs via a carrier-mediated mechanism that is different from the L-system. Furthermore, the uptake of [^{3}H]taurine was abolished completely by replacement of sodium or chloride with *N*-methylglucamine or gluconate, respectively, and by replacement of both sodium and chloride ions with sucrose. In contrast, the brain uptake value of [^{3}H]L-phenylalanine, of which influx into the brain is sodium independent [34], was not inhibited by substitution of sodium and chloride ions for sucrose (data not shown), showing that the sodium and chloride ion dependencies were specific. Therefore, the characteristics of [^{3}H]taurine transport, including substrate specificities and the driving forces, observed *in vivo* are retained in the primary cultured cells.

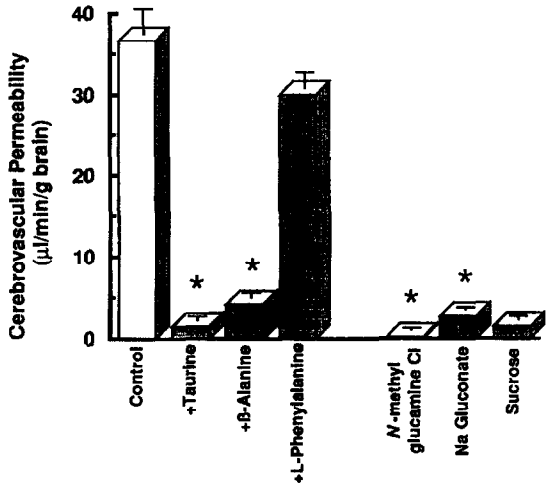

Fig. 7. Effects of amino acids and ion replacement on the *in vivo* brain uptake rate of [^{3}H]taurine in rats. *In vivo* brain uptake rate of [^{3}H]taurine (30 nM) was measured at 37° for 30 sec by perfusing 4.98 mL/min of bicarbonate-buffered physiological saline (pH 7.4) containing 142 mM NaCl, 28 mM $NaHCO_3$, 4.2 mM KH_2PO_4, 1.7 mM $CaSO_4$, 1.0 mM $MgSO_4$, 6.0 mM D-glucose, and an indicated amino acid oxygenated for 3 min with 95% O_2 and 5% CO_2. *N*-methylglucamine Cl means that NaCl and $NaHCO_3$ were replaced with *N*-methylglucamine Cl and $KHCO_3$, respectively. Na gluconate means that NaCl was replaced with sodium gluconate. Sucrose means that NaCl and $NaHCO_3$ were replaced by sucrose and $KHCO_3$, respectively. Each value is the mean ± SEM of four experiments. Key:* significantly different from the control ($P < 0.05$).

Table 5. Apparent distribution volume of [^{3}H]taurine and [^{14}C]sucrose in the rat brain hemisphere at 10 min

Compound	Apparent distribution volume (μL/g brain)	
	Parenchyma	Capillary
[^{3}H]Taurine	18.9 ± 1.04*	0.69 ± 0.191
[^{14}C]Sucrose	8.66 ± 2.33	0.27 ± 0.053

After a 10-min infusion of [^{3}H]taurine (70 μCi/mL) or [^{14}C]sucrose (14 μCi/mL) into the carotid artery at a rate of 50 μL/min, the brain was isolated and treated by the capillary depletion technique. The result was expressed as the apparent distribution volume obtained. Each value is the mean ± SEM of three experiments.

* $P < 0.05$ vs [^{14}C]sucrose.

In conclusion, the results obtained in the present study indicate that carrier-mediated transport of taurine driven by inwardly directed Na^+ and Cl^- gradients occurs in both the luminal and antiluminal membranes of primary cultured BCECs, which is in accordance with the characteristic of taurine transport observed in various tissues. These transport systems observed in *in vitro* primary cultured BCECs were confirmed to function in a physiological condition. Furthermore, the capillary depletion study indicated that taurine may penetrate into the brain from the vascular space across the BBB. From the transport parameters obtained in the present study and physiological conditions, net direction of taurine transport was suggested to be from the blood to the brain.

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REFERENCES

1. Huxtable RJ, Physiological actions of taurine. *Physiol Rev* **72:** 101–163, 1992.
2. Huxtable RJ, Taurine in the central nervous system and the mammalian actions of taurine. *Prog Neurobiol* **32:** 471–533, 1989.
3. Wade JV, Olson JP, Samson FE, Nelson SR and Pazdernik TL, A possible role for taurine in osmoregulation within the brain. *J Neurochem* **51:** 740–745, 1988.
4. Rassin DK, Sturman JA and Gaull GE, Sulfur amino acid metabolism in the developing rhesus monkey brain: Subcellular studies of taurine, cysteinesulfinic acid decarboxylase, γ-aminobutyric acid, and glutamic acid decarboxylase. *J Neurochem* **37:** 740–748, 1981.
5. Tayarani I, Cloëz I, Lefauconnier JM and Bourre JM, Sodium-dependent high-affinity uptake of taurine by isolated rat brain capillaries. *Biochim Biophys Acta* **985:** 168–172, 1989.
6. Audus KL and Borchardt RT, Characteristics of the large neutral amino acid transport system of bovine brain microvessel endothelial cell monolayers. *J Neurochem* **47:** 484–488, 1986.
7. Takakura Y, Kuentzel SL, Raub TJ, Davies A, Baldwin SA and Borchardt RT, Hexose uptake in primary cultures of bovine brain microvessel endothelial cells. I. Basic characteristics and effects of D-glucose and insulin. *Biochim Biophys Acta* **1070:** 1–10, 1991.
8. Terasaki T, Takakuwa S, Moritani S and Tsuji A, Transport of monocarboxylic acids at the blood-brain barrier: Studies with monolayers of primary cultured bovine brain capillary endothelial cells. *J Pharmacol Exp Ther* **258:** 932–937, 1991.
9. Saheki A, Terasaki T, Tamai I and Tsuji A, *In vivo* and *in vitro* blood-brain barrier transport of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. *Pharm Res* **11:** 305–311, 1994.
10. Tsuji A, Terasaki T, Takabatake Y, Tenda Y, Tamai I, Yamashima T, Moritani S, Tsuruo T and Yamashita J, P-Glycoprotein as the drug efflux pump in primary cultured bovine brain capillary endothelial cells. *Life Sci* **51:** 1427–1437, 1992.
11. Tsuji A, Tamai I, Sakata A, Tenda Y and Terasaki T, Restricted transport of cyclosporin A across the blood-brain barrier by a multidrug transporter, P-glycoprotein. *Biochem Pharmacol* **46:** 1096–1099, 1993.
12. Tsuji A, Saheki A, Tamai I and Terasaki T, Transport mechanism of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors at the blood-brain barrier. *J Pharmacol Exp Ther* **267:** 1085–1090, 1993.
13. Van Bree JBMM, Audus KL and Borchardt RT, Carrier-mediated transport of bacrofen across monolayers of bovine brain endothelial cells in primary culture. *Pharm Res* **5:** 369–371, 1988.
14. Raub TJ and Newton CR, Recycling kinetics and transcytosis of transferrin in primary cultures of bovine brain microvessel endothelial cells. *J Cell Physiol* **149:** 141–151, 1991.
15. Smith K and Borchardt RT, Permeability and mechanism of albumin, cationized albumin and glycosylated albumin transcellular transport across monolayers of cultured bovine brain capillary endothelial cells. *Pharm Res* **6:** 466–473, 1989.
16. Terasaki T, Takakuwa S, Saheki A, Moritani S, Shimura T, Tabata S and Tsuji A, Absorptive-mediated endocytosis of an adrenocorticotropic hormone (ACTH) analogue, ebiratide, into the blood-brain barrier: Studies with monolayers of primary cultured bovine brain capillary endothelial cells. *Pharm Res* **9:** 529–534, 1992.
17. Audus KL, Bartel RL, Hidalgo IJ and Borchardt RT, The use of cultured epithelial and endothelial cells for drug transport and metabolism studies. *Pharm Res* **7:** 435–451, 1990.
18. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
19. Yamaoka K, Tanigawara Y, Nakagawa T and Uno T, A pharmacokinetic analysis program (MULTI) for microcomputer. *J Pharmacobiodyn* **4:** 879–885, 1981.
20. Takasato Y, Rapoport SI and Smith QR, An *in situ* brain perfusion technique to study cerebrovascular transport in the rat. *Am J Physiol* **247:** H484–H493, 1984.
21. Triguero D, Buciak J and Pardridge WM, Capillary depletion method for quantification of blood-brain barrier transport of circulating peptides and plasma proteins. *J Neurochem* **54:** 1882–1888, 1990.
22. Pardridge WM and Fierer G, Blood-brain barrier transport of butanol and water relative to *N*-isopropyl-*p*-iodoamphetamine as the internal reference. *J Cereb Blood Flow Metab* **5:** 275–281, 1985.
23. Betz AL, Firth JA and Goldstein GW, Polarity of the blood-brain barrier: Distribution of enzymes between the luminal and antiluminal membranes of brain capillary endothelial cells. *Brain Res* **192:** 17–28, 1980.
24. Törnquist P and Alm A, Carrier-mediated transport of amino acids through the blood-retinal and the blood-brain barriers. *Grafes Arch Clin Exp Ophthalmol* **224:** 21–25, 1986.
25. Tossman U, Wieloch T and Ungerstedt U, γ-Aminobutyric acid and taurine release in the striatum of the rat during hypoglycemic coma, studied by microdialysis. *Neurosci Lett* **62:** 231–235, 1985.
26. Zelikovic I, Stejskal-Lorenz E, Lohstroh P, Budreau A and Chesney RW, Anion dependence of taurine transport by rat renal brush-border membrane vesicles. *Am J Physiol* **256:** F646–F655, 1989.
27. Miyamoto Y, Tiruppathi C, Ganapathy V and Leibach FH, Actie transport of taurine in rabbit jejunal brush-border membrane vesicles. *Am J Physiol* **257:** G65–G72, 1989.
28. Radian R and Kanner BI, Stoichiometry of sodium- and chloride-coupled γ-aminobutyric acid transport by synaptic plasma membrane vesicles isolated from rat brain. *Biochemistry* **22:** 1236–1241, 1983.
29. Benyajati S and Johnson JL, Characteristics of renal transport of taurine in reptilian brush-border membranes. *Am J Physiol* **260:** R879–R888, 1991.
30. Miyamoto Y, Nakamura H, Hoshi T, Ganapathy V and Leibach FH, Uphill transport of β-alanine in intestinal

brush-border membrane vesicles. *Am J Physiol* **259:** G372–G379, 1990.
31. Moyer MS, Insler N and Dumaswala R, The role of chloride in taurine transport across the human placental brush-border membrane. *Biochim Biophys Acta* **1109:** 74–80, 1992.
32. Carter-Su C and Kimmich GA, Membrane potentials and sugar transport by ATP-depleted intestinal cells: Effect of anion gradients. *Am J Physiol* **237:** C67–C74, 1979.
33. Smith QR, Takasato Y, Sweeney DJ and Rapoport SI, Regional cerebrovascular transport of leucine as measured by the *in situ* brain perfusion technique. *J Cereb Blood Flow Metab* **5:** 300–311, 1985.
34. Momma S, Aoyagi M, Rapoport SI and Smith QR, Phenylalanine transport across the blood-brain barrier as studied with the *in situ* brain perfusion technique. *J Neurochem* **48:** 1291–1300, 1987.