Pharmaceutical Research, Vol. 23, No. 4, April 2006 (© 2006)
DOI: 10.1007/s11095-006-9745-3

Research Paper

Functional Expression of Taurine Transporter and its Up-Regulation in Developing Neurons from Mouse Cerebral Cortex

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Received August 30, 2005; accepted December 15, 2005

Purpose. In the present study, we investigate the characteristics of taurine transport in primary cultures of neurons from mouse cerebral cortex to understand the possibility that taurine might attenuate the effects of central nervous system drugs.
Methods. Primary cultured neurons from mouse cerebral cortex were used to determine the transport characteristics of taurine. The expression of taurine transporter (TAUT) in mouse neurons was determined by use of reverse transcriptase–polymerase chain reaction and Western blotting.
Results. *In vitro* transport of taurine in mouse cerebrocortical neurons at day 9 was Na^+-dependent and saturable with a Michaelis–Menten constant (K_t) of 10.6 ± 4.1 μM and a maximum velocity (V_{max}) of 6.68 ± 0.85 nmol/mg protein/10 min. Na^+- and Cl^- activation kinetics revealed that the Na^+-to-Cl^--to-taurine stoichiometry was 2:1:1. Na^+-dependent [^{3}H]-taurine transport was competitively inhibited by β-alanine with an inhibitory constant (K_i) of 47.4 ± 6.5 μM. γ-Aminobutyric acid also inhibited Na^+-dependent [^{3}H]-taurine transport with relatively low affinity (K_i = 273 ± 71 μM). TAUT mRNA was detected in mouse primary cultured neurons, and TAUT protein was also expressed at ~70 kDa. Na^+-dependent taurine transport activity was increased with developing neurons and corresponded with the increasing mRNA and protein level of TAUT.
Conclusions. The present study revealed that Na^+/Cl^--coupled taurine transporter TAUT is responsible for taurine uptake in mouse cerebrocortical neurons, and that the expression of TAUT is increased with developing cerebrocortical neurons.

KEY WORDS: CNS; neurons; RT-PCR; taurine; taurine transport; TAUT; Western blotting.

INTRODUCTION

Taurine (2-aminoethanesulfonic acid) is a β-amino acid that contains a negatively charged sulfonic acid group. Taurine is structurally similar to a number of other endogenous substances, such as glycine and γ-aminobutyric acid (GABA), and its second or third most abundant amino acid within the brain of vertebrates, being present in millimolar concentrations, in regions such as the cerebellum and cerebral cortex. Taurine is involved in a variety of biological phenomena, such as osmoregulation, detoxication, neuroprotection, cell proliferation, and Ca^{2+} channel modulation (1,2).

Taurine is known to be transported by a specific transporter (TAUT), whose sequence homology places it within the gene family of Na^+- and Cl^--dependent neurotransmitter transporter (3,4). TAUT is found in many tissues and has been cloned from various species with remarkable similarity (3–7). In most tissues, TAUT expression is regulated osmotically, a finding in good agreement with the importance of taurine as an organic osmolyte (5,8,9). Furthermore, taurine deficiency is related to certain forms of retinal-related blindness. Of interest in this regard is the evidence that both glucose and insulin enhance retinal taurine uptake by altering the transport capacity of TAUT (10). An interesting potential role for TAUT in central nervous system (CNS) is in ischemia. In hippocampus, Na^+-dependent taurine efflux may exert a neuroprotective function in glutamate-induced excitotoxicity that accompanies ischemia. This efflux is regulated by nitric oxide (NO)-generating compounds, suggesting that the transduction process involves retrograde NO signals from *N*-methyl D-aspartate (NMDA) receptors on postsynaptic neurons onto presynaptic neurons containing TAUT (11).

TAUT is reported to be expressed in both the adult and developing brain (12,13). For its neuronal expression, Kishi *et al.* (14) demonstrated that high-affinity taurine transport system was expressed in developing primary cultured neurons prepared from mouse cerebral cortex. Although their report indicated that primary cultured neurons are expressing taurine transporter, the detailed functional characterization and molecular identification of TAUT expressed in mouse cerebrocortical neurons have not been determined.

In the present study, we described the functional characterization of Na^+- and Cl^--dependent taurine transporter expressed in primary cultures of neurons from mouse cerebral cortex. Reverse transcriptase–polymerase chain reaction (RT-PCR) experiments revealed that intraneuronal

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taurine was fluxed from extracellular space mediated by TAUT, but was not enzymatically synthesized from cysteine. We further demonstrate that the TAUT activity during developing neurons is elevated by increasing TAUT mRNA and protein expression.

MATERIALS AND METHODS

Materials

[1,2-^{3}H]-Taurine (specific radioactivity, 30 Ci/mmol) was obtained from Amersham Biosciences (Piscataway, NJ, USA). Dulbecco's modified Eagle's medium (DMEM), DMEM/F12, streptomycin, carbenicillin, B-27 supplement, and other culture reagents were purchased from GIBCO-Invitrogen (Gaithersburg, MD, USA), and fetal bovine serum (FBS) was obtained from Eqitech-Bio, Inc. (Kerrville, TX, USA). Unlabeled taurine, β-alanine, GABA, and other amino acids were purchased from Sigma (St. Louis, MO, USA) or Wako Pure Chemical Industries Ltd. (Osaka, Japan). Other chemicals were all of guaranteed grade.

Cell Culture

Pregnant ddY mice were obtained from Shimizu Laboratory Supplies, Co. (Kyoto, Japan). All animal procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of Kyoto Pharmaceutical University. Primary neuronal cultures were prepared from the cerebral cortex of fetal mice, as described previously (15,16). Briefly, 15-day-old-fetal mouse was decapitated under ether anesthesia, and their brains were quickly removed. After the cerebral cortex was dissected, the meninges were carefully removed in a Ca^{2+}-free Puck's solution (pH 7.4). Tissues were minced and washed with Ca^{2+}-free Puck's solution, followed by treatment with 0.1% trypsin dissolved in Ca^{2+}-free Puck's solution at 37°C for 5 min under the stream of gas mixture of 95% O_2/5% CO_2. The trypsin digestion was terminated by the addition of ice-cold DMEM supplemented with carbenicillin (0.1 mg/ml), streptomycin (0.1 mg/ml), and 20% FBS, and tissues were triturated with a Pasteur pipette. The dispersed cells were collected after centrifugation at 900 × *g* at 4°C for 2 min. The resultant pellet was then resuspended in DMEM followed by trituration, then the cell suspension was passed through a nylon sieve (mesh size 60 μm) and seeded on a 24-well plate (1×10^5 cells/well). The medium was replaced by fresh serum-free DMEM/F12, which did not contain taurine, supplemented with B-27 supplement (Invitrogen) and antibiotics, and the cells were successively incubated for 2 days. On the third day, cells were exposed to 10 μM cytosine-β-D-arabinofuranoside dissolved in DMEM/F12 for 24 h to prevent the growth of nonneuronal cells. The culture medium was then replaced by fresh serum-free DMEM/F12.

Uptake Measurements

In most cases, the uptake characteristics of taurine in primary cultured neurons were measured at 7–10 days *in vitro* (DIV). After removal of the culture medium, the cells were washed with transport buffer and incubated with fresh transport buffer containing radiolabeled compounds. The composition of the transport buffer was 25 mM HEPES/Tris (pH 7.4), 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5 mM glucose (16–19). Following incubation, the buffer containing radiolabeled compound was aspirated off, and the cells were washed twice with 2 ml of ice-cold transport buffer. The cells were solubilized with 500 μl of 1% sodium dodecyl sulfate (SDS) in 0.2 M NaOH, aliquots (450 μl) of samples were transferred to a counting vial, and the radioactivity associated with the cells was counted by liquid scintillation spectrometry (Model LSC6000, Beckmann). In experiments in which the cation and anion dependence of the uptake process was investigated, NaCl was replaced iso-osmotically with choline chloride, *N*-methyl-D-glucamine (NMDG) chloride, and sodium gluconate. Saturation kinetics was evaluated by nonlinear regression analysis, and the kinetic parameters derived from this method were confirmed by linear regression analysis. The Na^+/Cl^-/taurine stoichiometry was calculated by measuring taurine uptake with varying concentrations of Na^+ or Cl^-.

RT-PCR

Total RNA was isolated form mouse neurons and various tissues using Sepazol RNA I reagent (Nacalai Tesque, Kyoto, Japan) according to the manufacturer's instructions. Reverse transcription was performed with 5 μg of total RNA using Superscript II (Invitrogen, Carlsbad, CA, USA). The primer pairs used in this study were summarized in Table I. PCR was performed according to the following conditions: 94°C for 45 s, 58–65°C for 45 s (see Table I), 72°C for 90 s, repeated for 30 cycles. This was followed by a single additional extension step at 72°C for 7 min. PCR products were separated on a 1% agarose gel and were visualized with ethidium bromide under ultraviolet light.

Immunoblotting

Preparation of crude membrane fraction and Western blot analysis were performed according to previous reports (19,20). In brief, mouse neuronal cultures were homogenized in buffer A consisting of 0.32 M sucrose containing 10 mM Tris–HCl (pH 7.4), 1 mM ethylenediaminetetraacetic acid (EDTA), and 0.5 mM phenylmethylsulfonyl fluoride. The suspension was centrifuged at 1000 × *g* for 10 min, and the resulting supernatant was centrifuged at 32,000 × *g* for 30 min. The pellet was resuspended in buffer A without 0.32 M sucrose by repeated passage through 27-gauge needle. The protein concentration of the membrane suspension was measured using a Bio-Rad protein assay. The membrane fractions were solubilized in 2× SDS–polyacrylamide gel electrophoresis (PAGE) sample buffer (20 mM Tris–HCl, 2 mM EDTA, 10% 2-mercaptoethanol, 2% SDS, 20% glycerol, and 0.2% bromophenol blue, pH 6.8), were separated by 10% SDS-PAGE, and were transferred onto Hybond P membrane (Amersham Biosciences) using semidry electroblotting. The membranes were blocked in Tris-buffered saline containing 5% nonfat milk and 1% bovine serum albumin and were treated with the affinity-purified rabbit anti-rat TAUT antibody (Alphadiagonistics, 1:500 dilution).

Table I. Primers Used in Polymerase Chain Reaction Amplification

Gene	Primer sequence (5′ to 3′)	Annealing (°C)	Product size (bp)	Accession no.
TAUT	Forward: 5′-ATC GAC GCT GGA ACT CAG AT-3′ Reverse: 5′-AGG GGA TAC ACA GCA TGG AG-3′	56	791	L03092
CDO	Forward: 5′-CCT ACG AGA GCA ATC CTG CCG-3′ Reverse: 5′-GCC CCA CTG CTG TTC ACT ACC-3′	65	1033	M35266
CSD	Forward: 5′-CCT GCT TTT CTG GGA CTT GGC ACC-3′ Reverse: 5′-GGC TCC ATG ACC AAC TCA AAT CC-3′	65	643	AJ132661
SNAP-25	Forward: 5′-ATG AAC TGG AGG AGA TGC AG-3′ Reverse: 5′-CGA TTC TGG GTG TCA ATC TC-3′	56	502	NM_011428
ASCT1	Forward: 5′-GTT TGC FAC GGC TTT TGC GAC CTG-3′ Reverse: 5′-GCA TCC CCT TCC ACG TTC ACC ACA-3′	58	399	AB103401
ASCT2	Forward: 5′-TTC TTC AAC TCC TTC AAT GA-3′ Reverse: 5′-ACT AGC CAG TCC ACG GCC AA-3′	58	478	AJ132846
GAPDH	Forward: 5′-CCA TCA CCA TCT TCC AGG AG-3′ Reverse: 5′-CCT GCT TCA CCA CCT TCT TG-3′	58	576	X02231

The immunoreactive proteins were detected on an X-ray film by enhanced chemiluminescence with a horseradish peroxidase-conjugated anti-rabbit IgG antibody (1:1000, New England Biolab).

Data Analysis

Each experiment was determined with duplicate or triplicate monolayers. Results are expressed as mean ± SEM. Statistical significance was determined by Student's t test for unpaired sample for assuming equal variance; $p < 0.05$ was considered significant. Statistical differences among multiple different groups were determined by one-way analysis of variance, and $p < 0.05$ was considered by Dunnett's test using commercial software (Instat, GraphPad software, San Diego, CA, USA).

RESULTS

Functional Characterization of [^{3}H]-Taurine Transport into Mouse Cerebrocortical Neurons

The uptake of [^{3}H]-taurine into mouse cerebrocortical neurons was linear for up to 30 min (data not shown). Thus, all uptake studies were performed with a 10-min incubation period. The involvement of Na^+ and Cl^- in the uptake process was evaluated by measuring the uptake of taurine in mouse neurons in the presence and absence of Na^+ and/or Cl^-. This was performed by iso-osmotically replacing NaCl in the transport buffer with NMDG chloride, choline chloride, LiCl, sodium gluconate, $NaNO_3$, NaSCN, and mannitol (Table II). The uptake of [^{3}H]-taurine was completely abolished when Na^+ was replaced with other cations in the transport buffer. In addition, replacement of NaCl by sodium gluconate completely inhibited [^{3}H]-taurine uptake, whereas replacing Cl^- with NO_3^- or SCN^- reduced the uptake of [^{3}H]-taurine by 12.9 or 32.4% of the control (NaCl-containing buffer), respectively.

Figure 1 shows the Na^+-dependent uptake of taurine into mouse cerebrocortical neurons that occurred via a saturable process. The Eadie–Hofstee plot (Fig. 1, inset) was linear, providing evidence for a single transport system for taurine uptake in mouse neurons. The kinetic parameters were calculated by nonlinear regression. The transport process of taurine was saturable with a K_t of 10.6 ± 4.1 μM and a V_{max} of 6.68 ± 0.85 nmol/mg protein/10 min.

As shown in Table II, the presence of Na^+ and Cl^- is obligatory for taurine transport into mouse cerebrocortical neurons. We therefore investigated the effect of Na^+ and Cl^- on the uptake of taurine by measuring the uptake of [^{3}H]-taurine in mouse neurons in the presence of varying concentrations of extracellular Na^+ or Cl^-. The concentration of NaCl in the transport buffer was varied from 0 to 140 mM. The transport rate of [^{3}H]-taurine was sigmoidally related to the concentration of Na^+ (Fig. 2A), and the Hill coefficient for the activation process for Na^+ was 2.2 ± 0.1 (Fig. 2A, inset). On the other hand, the transport rate of [^{3}H]-taurine was hyperbolically related to the concentration of Cl^- (Fig. 2B), and the Hill coefficient for the activation process for Cl^- was 1.1 ± 0.1 (Fig. 2B, inset). This result indicates that, for every taurine molecule transported, 2 Na^+ and 1 Cl^- ions are cotransported, and that the transport process is elec-

Table II. Ion Dependence of [^{3}H]-Taurine Transport in Mouse Cerebrocortical Neurons

Salt	[^{3}H]-Taurine uptake		p value
	pmol/mg protein/10 min	%	
NaCl	35.5 ± 1.83	100	
NMDG chloride	0.16 ± 0.00	0	<0.001
Choline chloride	0.09 ± 0.00	0	<0.001
LiCl	0.13 ± 0.01	0	<0.001
Sodium gluconate	0.35 ± 0.04	0.01	<0.001
$NaNO_3$	4.58 ± 0.21	12.9	<0.001
NaSCN	11.5 ± 0.06	32.4	<0.001
Mannitol	0.19 ± 0.00	0.01	<0.001

Neurons were incubated with taurine (1 μM) for 10 min either at 37°C in the control buffer (25 mM HEPES/Tris, pH 7.4, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose, and 140 mM NaCl) or in buffer in which NaCl was replaced with 140 mM of various inorganic salts or 280 mm mannitol. The concentration of [^{3}H]-taurine was kept at 30 nM. When the influence of anions was studied, KCl and $CaCl_2$ in the buffer were replaced with potassium gluconate and calcium gluconate, respectively. Results are expressed as mean ± SEM (n = 4).

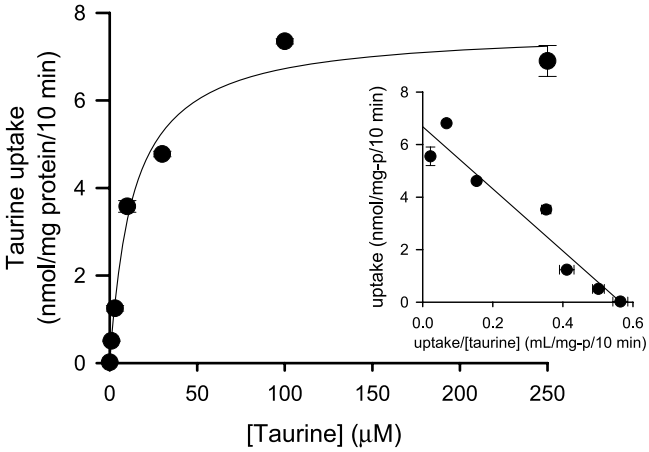

Fig. 1. Saturation kinetics of Na^+-dependent taurine transport in mouse cerebrocortical neurons. Uptake of taurine was measured in mouse neurons with a 10-min incubation in the NaCl- or choline-chloride-containing buffer at pH 7.4 over a taurine concentration range of 1–250 μM. The concentration of [^{3}H]-taurine was kept constant at 30 nM. Na^+-dependent uptake of taurine was obtained by subtracting the uptake in the choline-chloride-containing buffer from the uptake in the NaCl-containing buffer. Each point represents mean ± SEM (n = 4). Inset: Eadie–Hofstee plot.

trogenic. The $K_{0.5}^{Na}$ and $K_{0.5}^{Cl}$ values (i.e., the concentration of Na^+ or Cl^- ion needed for inducing half-maximal transport rate) were 70.0 ± 0.1 and 24.1 ± 6.5 mM, respectively.

Substrate Specificity of Taurine Transport in Mouse Neurons

Cis-inhibitory effects of various compounds on the uptake of [^{3}H]-taurine by mouse neurons are shown in Fig. 3. The uptake of taurine (1 μM) was strongly inhibited by β-amino acids (unlabeled taurine and β-alanine). GABA, a γ-amino acid, partially but significantly inhibited the uptake of [^{3}H]-taurine. No significant inhibition was observed by glycine, proline, betaine, and 1-aminocyclohexane-1 carboxylic acid (ACHC).

We next investigated the ability of β-alanine and GABA to inhibit Na^+-dependent [^{3}H]-taurine transport in mouse cerebrocortical neurons. Both compounds inhibited the Na^+-dependent [^{3}H]-taurine transport in a concentration-dependent manner (data not shown). The K_i values for β-alanine and GABA were 47.4 ± 6.5 and 273 ± 71 μM, respectively.

Culture Day-Dependent [^{3}H]-Taurine Transport Activity in Mouse Cerebrocortical Neurons

We next determined the time-dependent changes in [^{3}H]-taurine transport activity after seeding the mouse neuronal cells. As shown in Fig. 4A, Na^+-dependent [^{3}H]-taurine transport activity in mouse neurons was significantly elevated from 1 to 9 DIV. To confirm the involvement of Na^+/Cl^--dependent taurine transporter (TAUT), we examined the expression of TAUT at both mRNA and protein levels. Figure 4B shows that TAUT mRNA transcript was expressed in mouse cerebrocortical neurons in primary cultures. Semiquantitative RT-PCR revealed that TAUT mRNA was elevated from 1 to 12 DIV as well as SNAP-25 mRNA, which was used as a neuron-specific gene. In addition, similar results were obtained at protein level by Western blotting using anti-TAUT antibody (Fig. 4C).

RT-PCR for the Taurine Biosynthetic Enzymes and the Na^+-Dependent Neutral Amino Acid Transporters

Intracellular taurine accumulation results primarily from the uptake of exogenous taurine mediated by TAUT as well as from endogenous biosynthesis of taurine (21). Two enzymes, cysteine sulfinate decarboxylase (CSD) and cysteine dioxygenase (CDO), were reported to be responsible for the biosynthesis of taurine from L-cysteine (22). The intracellular transport of L-cysteine occurs through several

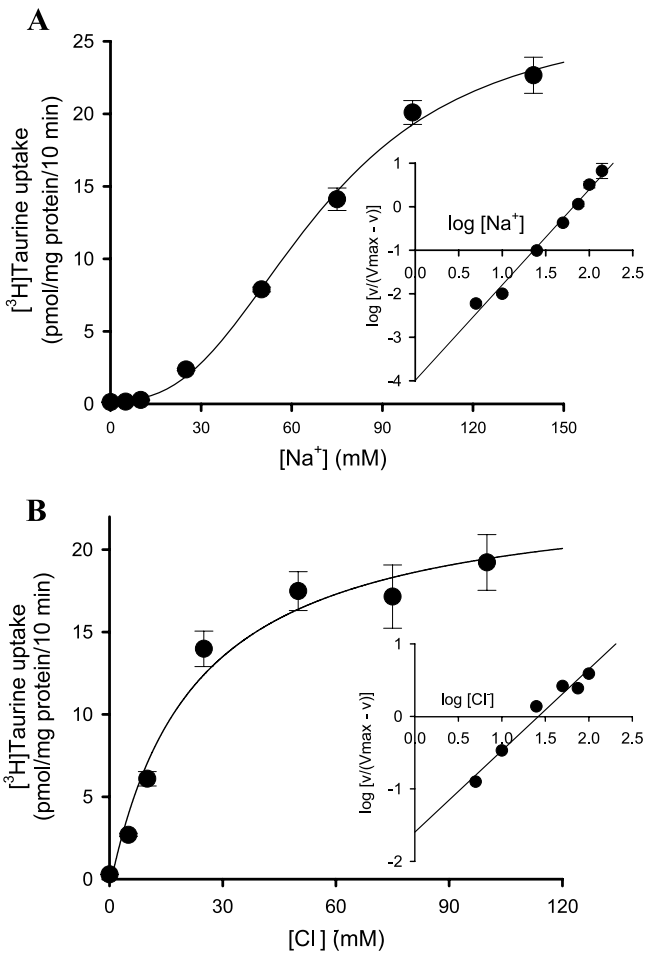

Fig. 2. Effect of Na^+ and Cl^- on the uptake of taurine in mouse cerebrocortical neurons. Uptake of taurine (1 μM) was studied in mouse neurons with a 10-min incubation in the presence of increasing concentration of (A) Na^+ (0–140 mM) and a fixed concentration of Cl^- (140 mM), or (B) a fixed concentration of Na^+ (140 mM) and Cl^- (0–140 mM) in the extracellular medium. The concentration of [^{3}H]-taurine was kept at 30 nM. The osmolality of the medium was kept constant by replacing NaCl with appropriate concentrations of choline chloride (A) or sodium gluconate (B). Each point represents mean ± SEM (n = 4). Insets: Hill plots of the same data. v, uptake rate in nmol/mg protein/10 min; V_m, the maximal uptake rate calculated from the experimental data using the Hill equation.

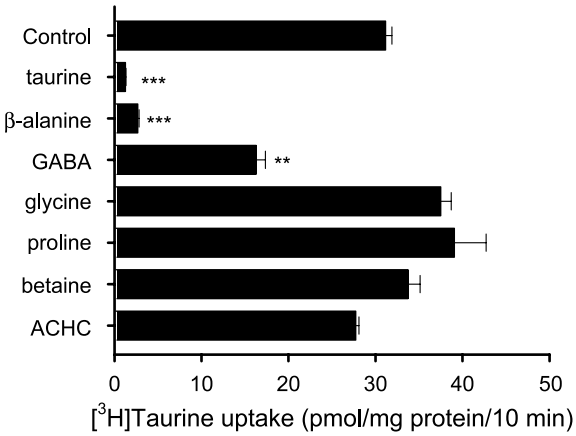

Fig. 3. Substrate specificity of Na^+-dependent taurine uptake in mouse cerebrocortical neurons. Na^+-dependent uptake of taurine uptake (1 μM) was measured in mouse cerebrocortical neurons at 37°C for 10 min in the presence or absence of NaCl (pH 7.4). The concentration of [^{3}H]-taurine was kept at 30 nM. Unlabeled compounds were used at a concentration of 1 mM. Results are expressed as mean ± SE of three to six experiments.

transport systems, including Na^+-dependent transporters, such as neutral amino acid transporter system ASC and system A, and Na^+-independent transporters, such as system L and system asc (23). Yamamoto *et al.* (24) reported that neurons accumulate a high level of L-serine as well as cysteine by using a Na^+-dependent and high-affinity neutral amino acid transporter ASCT1 but not ASCT2. Therefore, we determined the time-dependent changes in the taurine biosynthetic enzymes and Na^+-dependent neutral amino acid transporters after seeding the mouse neuronal cells.

As shown in Fig. 5, neither CSD mRNA nor CDO mRNA was detected in mouse cerebrocortical neurons over 1–12 DIV. CSD, the rate-limiting enzyme in the synthesis of taurine from cysteine (22), was shown to be strictly localized in astrocytes in the brain (25), and actual synthesis of taurine from cysteine was demonstrated in astrocyte primary cultures (26). The present data were in agreement with previous reports (25,26).

Furthermore, ASCT1, but not ASCT2, mRNA was found in mouse cerebrocortical neurons (Fig. 5). These results were consistent with previous report by Yamamoto *et al.* (24). No significant change in ASCT1 mRNA was observed in mouse neurons over 1–12 DIV.

DISCUSSION

The present study represents the first detailed report of the functional characterization of taurine transporter (TAUT) in primary cultures of neurons from mouse cerebral cortex. In 1988, Kishi *et al.* (14) demonstrated that the high-affinity taurine transport system was expressed in developing primary cultured neurons from mouse cerebral cortex. They showed that the taurine uptake in neurons was Na^+-dependent and was partially inhibited by metabolic inhibitors, such as ouabain, 2,4-dinitrophenol, monoiodoacetate, and NaN_3 (14). Furthermore, they reported that β-alanine competitively inhibited taurine uptake by neurons with a K_i value of ~100 μM. As shown in Table II, the [^{3}H]-taurine uptake was attenuated under Na^+- and Cl^--free conditions and was strongly inhibited by unlabeled taurine and β-alanine, a known competitive inhibitor of taurine uptake (Fig. 3),

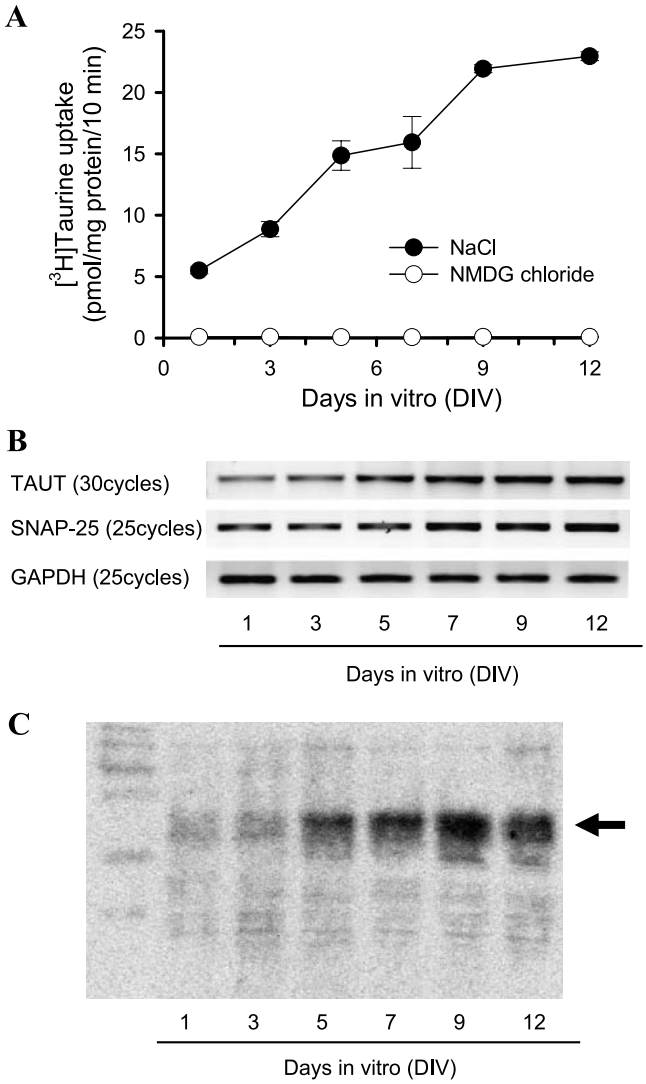

Fig. 4. Culture day-dependent increase of taurine transporter. (A) Culture day dependency of taurine transport in mouse cerebrocortical neurons. The uptake of taurine uptake (1 μM) was measured in mouse cerebrocortical neurons at 37°C for 10 min in the presence (●) or absence (○) of NaCl (pH 7.4). $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* 1 DIV by analysis of variance. (B and C) Expression of TAUT mRNA and protein in mouse cerebrocortical neurons. Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis of mouse TAUT, SNAP-25, and GAPDH mRNA in primary cultures of neurons from mouse cerebral cortex. The total RNA (5.0 μg) isolated from the cells was reverse-transcribed into cDNA and subjected to PCR using TAUT-, SNAP-25-, and GAPDH-specific primers (Table I). The RT-PCR products were analyzed by 1% agarose gel electrophoresis. Western blot analysis of TAUT protein in mouse cerebrocortical neurons. Crude membrane fractions from the cells (50 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (10%) and were then immunoblotted using rabbit anti-TAUT antibody (Alphadiagonistics). Immunoreactive protein was detected by using a commercially available enhanced chemiluminescence Western blotting detection kit.

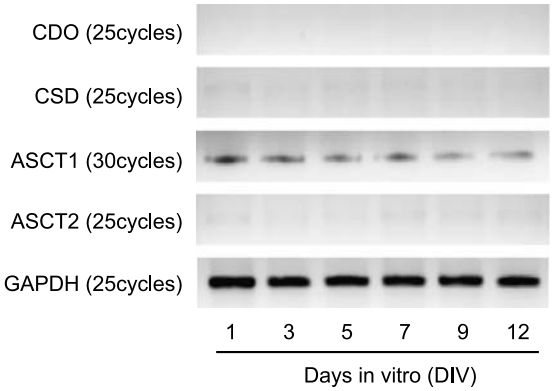

Fig. 5. Expression of taurine biosynthetic enzymes and Na^+-dependent neutral amino acid transporters mRNA in mouse cerebrocortical neurons. RT-PCR analysis of cysteine dioxygenase (CDO), cysteine sulfinate decarboxylase (CSD), ASCT1, and ASCT2 mRNA in primary cultures of neurons from mouse cerebral cortex. The total RNA (5.0 μg) isolated from the cells was reverse-transcribed into cDNA and subjected to PCR using CDO-, CSD-, ASCT1-, ASCT2-, and GAPDH-specific primers (Table I). The RT-PCR products were analyzed by 1% agarose gel electrophoresis. Western blot analysis of TAUT protein in mouse cerebrocortical neurons.

suggesting that Na^+- and Cl^--dependent and β-alanine sensitive transporter (TAUT)-mediated transport is involved in [^{3}H]-taurine uptake by cerebrocortical neurons. Na^+ and Cl^- activation kinetics revealed that transport of one taurine molecule was associated with the transfer of two Na^+ and Cl^- (Fig. 2). Because taurine is considered to be a neutral zwitterion at physiological pH, this stoichiometric relationship indicates that the uptake process is electrogenic with a net transfer of positive charge into the cells. The corresponding K_t value of 10.6 ± 4.1 μM is in good agreement with an apparent K_t of 13.2 and 6 μM for mouse and human TAUT, respectively (4,7).

β-Alanine and GABA inhibited the taurine uptake by mouse neurons in a concentration-dependent manner; their K_i values are 47.4 ± 6.5 and 273 ± 71 μM, respectively. Liu *et al.* (4) demonstrated that the apparent K_t value for β-alanine uptake by mouse TAUT-expressing *Xenopus* oocytes was 56 μM. Vinnakota *et al.* (27) reported that β-alanine and GABA inhibited the taurine transport with IC_{50} value of 52.5 and 501.2 μM, respectively, using the same TAUT expression system. In addition, Kishi *et al.* (14) reported that β-alanine competitively inhibited the taurine transport with K_i value of 100 μM. These results are well consistent with the present results, strongly suggesting that the taurine transporter TAUT is expressed in mouse cerebrocortical neurons. Furthermore, RT-PCR analysis showed that mouse cerebrocortical neurons express TAUT mRNA (Fig. 4B), and TAUT protein was detected at ~70 kDa in mouse neurons by Western blot analysis (Fig. 4C). A band of this size has been also reported in TAUT expressing *Xenopus* oocytes (28). In addition to TAUT, Na^+/Cl^--coupled GABA transporters, GAT2 and GAT3, accept taurine and β-alanine as substrates (3,4). Our preliminary RT-PCR experiments revealed that neither GAT2 nor GAT3 was expressed in mouse cerebrocortical neurons (data not shown). Furthermore, H^+-coupled amino acid transporter PAT1 is reported to be expressed in neurons and to accept taurine and praline with a low affinity (29). As shown in Fig. 4A, however, the [^{3}H]-taurine uptake in the absence of Na^+ was negligible, and praline could not inhibit the [^{3}H]-taurine uptake by neurons (Fig. 3). Taken collectively, this evidence supports that TAUT is functionally expressed in mouse cerebrocortical neurons.

The [^{3}H]-taurine transport activity in mouse neurons was significantly elevated from 1 to 9 DIV (Fig. 4A). In addition to transport activity, semiquantitative RT-PCR and Western blot analyses revealed that TAUT mRNA and protein were up-regulated in a culture day-dependent manner (Fig. 4B and C). At present, it is unknown that TAUT expression is age-dependently elevated. Although taurine is not used for protein synthesis, it is normally present in high concentrations in mouse eggs and oviductal and uterine fluid, and acts as an osmolyte in mouse oocytes and embryos (30) and promotes embryo development in a variety of species, including mouse (31) and humans (32). Therefore, the taurine transport into neurons might be correlated to neuronal development and/or construction of neural network.

Intraneuronal taurine accumulation results primarily from uptake of exogenous taurine through TAUT as well as from endogenous biosynthesis of taurine (1). We therefore investigated the gene expression of the taurine biosynthesis enzymes CDO and CSD by measuring their mRNA levels in mouse cerebrocortical neurons (Fig. 5). Reymond *et al.* (25) reported that these enzymes are strictly localized in astrocytes in the hippocampus and cerebellum. In the present study, neither CDO nor CSD mRNA was detected in mouse cerebrocortical neurons at any age cultures (Fig. 5), indicating that neurons could not synthesize taurine enzymatically. Tsuji and Terasaki, and their coworkers (33–36), demonstrated that taurine is transported from the circulating blood to brain mediated by TAUT. TAUT is expressed in both the luminal and abluminal membranes of blood–brain barrier, which is formed by a complex of tight junctions of brain capillary endothelial cells as well as astrocytes (33–36). Therefore, taurine in brain interstitial fluid might be supplied from blood circulation and/or astrocytes.

Taurine has been shown to inhibit the NMDA receptor and is likely to be the endogenous ligand for glycine receptor in some areas of the brain (11). Thus, pharmacokinetic properties of taurine and its derivatives in brain may affect the signal transduction in neurons. Actually, acamprosate (calcium acetylhomotaurine), which is a taurine derivative and is used as a drug for the treatment of alcoholism (37), reduces alcohol intake and the propensity to relapse during abstinence. At present, its detailed molecular mechanism for "anticraving" effect is not fully understood (37,38). The precise transport characteristics of taurine in neurons, therefore, might provide useful information to clarify the molecular mechanism of anticraving effect of acamprosate.

In conclusion, we demonstrated that the functional TAUT is expressed in mouse cerebrocortical neurons, and that its transport activity is up-regulated during developing neurons. The physiological role of taurine in the CNS is not unclear, mainly because it is present in all of the brain tissues at relatively high concentration. A mouse model with a disrupted gene coding for TAUT ($taut^{-/-}$ mice), reported

recently by Haussinger *et al.* (39,40), exhibits severe retinal degeneration, suggesting that TAUT is critical for normal retinal development and function. In addition, the TAUT gene has been mapped to human chromosome 3p24–p25 (7), a region associated with mental retardation and other neurological defects. In the future, physiological, psychological, and pharmacological roles of TAUT and taurine remain to be seen in taut$^{-/-}$ mice.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research in Priority Areas (16015305, 16047229), Academic Frontier Project "Research on Medicinal Chemistry Based on Molecular Recognition: Aiming of Development of New Drugs to Overcome Difficult Diseases," and the 21st Century Center of Excellent Program "Development of Drug Discovery Frontier Integrated from Tradition to Proteome" from the Ministry of Education, Science, Sports and Culture of Japan and by grant from Takeda Science Foundation.

REFERENCES

1. R. J. Huxtable. Physiological actions of taurine. *Physiol. Rev.* **72**:101–163 (1992).
2. C. E. Wright, H. H. Tallan, and Y. Y. Lin. Taurine: biological update. *Annu. Rev. Biochem.* **55**:427–453 (1996).
3. K. E. Smith, L. A. Borden, C.-H. D. Wang, P. R. Hartig, T. A. Branchek, and R. L. Weinshank. Cloning and expression of a high affinity taurine transporter from rat brain. *Mol. Pharmacol.* **42**:563–569 (1992).
4. Q.-R. Liu, B. Lopez-Corcuera, H. Nelson, S. Mandiyan, and N. Nelson. Cloning and expression of a cDNA encoding the transporter of taurine and β-alanine in mouse brain. *Proc. Natl. Acad. Sci. USA* **89**:12145–12149 (1992).
5. S. Uchida, H. M. Kwon, A. Yamauchi, A. S. Preston, F. Marumo, and J. S. Handler. Molecular cloning of the cDNA for an MDCK cell Na^+- and Cl^--dependent taurine transporter that is regulated by hypotonicity. *Proc. Natl. Acad. Sci. USA* **89**:8230–8234 (1992).
6. S. M. Jhiang, L. Fithian, P. Smanik, J. McGill, Q. Tong, and E. L. Mazzaferri. Cloning of the human taurine transporter and characterization of taurine uptake in thyroid cells. *FEBS Lett.* **318**:139–144 (1993).
7. S. Ramamoorthy, F. H. Leibach, V. B. Mahesh, H. Han, T. Yang-Feng, R. D. Brakely, and V. Ganapathy. Functional characterization and chromosomal localization of a cloned taurine transporter from human placenta. *Biochem. J.* **300**:893–900 (1994).
8. H. Pasantes-Morales and A. Schousboe. Volume regulation in astrocytes: a role for taurine as an osmoeffector. *J. Neurosci. Res.* **20**:503–509 (1988).
9. H. K. Kimelberg, S. K. Goderie, S. Higman, S. Pang, and R. A. Waniewski. Swelling-induced release of glutamate, aspartate, and taurine from astrocytes cultures. *J. Neurosci.* **10**: 1583–1591 (1990).
10. R. Salceda. Insulin-stimulated taurine uptake in rat retina and retinal pigment epithelium. *Neurochem. Int.* **35**:301–306 (1999).
11. P. Saransaari and S. S. Oja. Taurine release modified by nitric oxide-generating compounds in the developing and adult mouse hippocampus. *Neuroscience* **89**:1103–1111 (1999).
12. P. Saransaari and S. S. Oja. Involvement of metabotropic glutamate receptors in taurine release in the adult and developing mouse hippocampus. *Amino Acids* **16**:165–179 (1999).
13. P. Saransaari and S. S. Oja. Characteristics of ischemia-induced taurine release in the developing mouse hippocampus. *Neuroscience* **94**:949–954 (1999).
14. M. Kishi, S. Ohkuma, M. Kimori, and K. Kuriyama. Characteristics of taurine transport system and its developmental pattern in mouse cerebral cortical neurons in primary culture. *Biochim. Biophys. Acta* **939**:615–623 (1988).
15. M. Oka, Y. Itoh, M. Wada, A. Yamamoto, and T. Fujita. A comparison of Ca^{2+} channel blocking mode between gabapentin and verapamil: implication for protection against hypoxic injury in rat cerebrocortical slices. *Br. J. Pharmacol.* **139**:435–443 (2003).
16. M. Wada, S. Miyakawa, A. Shimada, A. Yamamoto, and T. Fujita. Functional linkage of H^+/peptide transporter PEPT2 and Na^+/H^+ exchanger in primary cultures of astrocytes from mouse cerebral cortex. *Brain Res.* **1044**:33–41 (2005).
17. T. Uchiyama, Y. Matsuda, M. Wada, S. Takahashi, and T. Fujita. Functional regulation of Na^+-dependent neutral amino acid transporter ASCT2 by *S*-nitrosothiols and nitric oxide in Caco-2 cells. *FEBS Lett.* **579**:2499–2506 (2005).
18. K. Tanaka, A. Yamamoto, and T. Fujita. Functional characterization and adaptive regulation of Na^+-dependent neutral amino acid transporter SNAT2/ATA2 in normal human astrocytes under amino acid starved condition. *Neurosci. Lett.* **378**:70–75 (2005).
19. T. Fujita, H. Katsukawa, E. Yodoya, M. Wada, A. Shimada, N. Okada, A. Yamamoto, and V. Ganapathy. Transport characteristics of *N*-acetyl-L-aspartate in rat astrocytes: involvement of sodium-coupled high-affinity carboxylate transporter NaC3/NaDC3-mediated transport system. *J. Neurochem.* **93**:706–714 (2005).
20. T. Fujita, T. Kishida, M. Wada, N. Okada, A. Yamamoto, F. H. Leibach, and V. Ganapathy. Functional characterization of brain peptide transporter in rat cerebral cortex: identification of the high-affinity type H^+/peptide transporter PEPT2. *Brain Res.* **997**:52–61 (2004).
21. R. J. Huxtable. Taurine in the central nervous system and the mammalian actions of taurine. *Prog. Neurobiol.* **32**:471–533 (1989).
22. J. De La Rosa and M. H. Stipanuk. Evidence for a rate limiting role of cysteine sulfinate decarboxylase activity in taurine biosynthesis *in vivo*. *Comp. Biochem. Physiol. B.* **81**:565–571 (1985).
23. M. Palacin, R. Estevez, J. Bertran, and A. Zorzano. Molecular biology of mammalian plasma membrane amino acid transporters. *Physiol. Rev.* **78**:969–1054 (1998).
24. T. Yamamoto, T. Nishizaki, S. Furuya, Y. Hirabayashi, K. Takahashi, S. Okuyama, and H. Yamamoto. Characterization of rapid and high-affinity uptake of L-serine in neurons and astrocytes in primary culture. *FEBS Lett.* **548**:69–73 (2003).
25. I. Reymond, K. Almarghini, and M. Tappaz. Immunocytochemical localization of cysteine sulfinate decarboxylase in astrocytes in the cerebellum and hippocampus: a quantitative double immunofluorescence study with glial fibrillary acidic protein and S-100 protein. *Neuroscience* **75**:619–633 (1996).
26. A. Brand, D. Leibfriz, B. Hamprecht, and R. Dringen. Metabolism of cysteine in astroglial cells: synthesis of hypotaurine and taurine. *J. Neurochem.* **71**:827–832 (1998).
27. S. Vinnakota, X. Qian, H. Egal, V. Sarthy, and H. K. Sarkar. Molecular characterization and *in situ* localization of a mouse retinal taurine transporter. *J. Neurochem.* **69**:2238–2250 (1997).
28. X. Han, R. W. Chesney, A. M. Budreau, and D. P. Jones. Regulation of expression of taurine transport in two continuous retinal epithelial cell lines and inhibition of taurine transporter by a site-directed antibody. *Adv. Exp. Med. Biol.* **403**:173–191 (1996).
29. M. Boll, M. Foltz, I. Rubio-Aliaga, G. Kottra, and H. Daniel. Functional characterization of two novel mammalian electrogenic proton-dependent amino acid transporter. *J. Biol. Chem.* **277**:22966–22973 (2002).
30. J. C. Dumoulin, L. C. van Wissen, P. P. Menheere, A. H. Michiels, J. P. Geraedts, and J. L. Evers. Taurine acts as an osmolyte in human and mouse oocytes and embryos. *Biol. Reprod.* **56**: 739–744 (1997).
31. J. C. Dumoulin, J. L. Evers, M. Bras, M. H. Pieters, and J. P. Geraedts. Positive effect of taurine on preimplantation development of mouse embryos *in vitro*. *J. Reprod. Fertil.* **94**:373–380 (1992).

32. F. Devreker, M. Van den Bergh, J. Biramane, R. L. Winston, Y. Englert, and K. Hardy. Effects of taurine on human embryo development *in vitro*. *Hum. Reprod.* **14**:2350–2356 (1992).
33. J. Komura, I. Tamai, M. Senmaru, T. Terasaki, Y. Sai, and A. Tsuji. Sodium and chloride ion-dependent transport of β-alanine across the blood–brain barrier. *J. Neurochem.* **67**:330–335 (1996).
34. J. Komura, I. Tamai, M. Senmaru, T. Terasaki, Y. Sai, and A. Tsuji. Brain-to-blood active transport of β-alanine across the blood–brain barrier. *FEBS Lett.* **400**:131–135 (1997).
35. I. Tamai, M. Senmaru, T. Terasaki, and A. Tsuji. Na^+- and Cl^--dependent transport of taurine at the blood–brain barrier. *Biochem. Pharmacol.* **50**:1783–1793 (1995).
36. Y. S. Kang, S. Ohtsuki, H. Takanaga, M. Tomi, K. Hosoya, and T. Terasaki. Regulation of taurine transport at the blood–brain barrier by tumor necrosis factor-α, taurine and hypertonicity. *J. Neurochem.* **83**:1188–1195 (2002).
37. F. M. Olive. The anti-craving taurine derivative acamprosate. *Adv. Exp. Med. Biol.* **526**:481–484 (2003).
38. A. Smith, C. J. Watson, R. T. Kennedy, and J. Peris. Ethanol-induced taurine efflux. *Adv. Exp. Med. Biol.* **526**:485–492 (2003).
39. B. Heller-Stilb, C. van Roeyen, K. Rascher, H. G. Hartwig, A. Huth, M. W. Seeliger, U. Warskulat, and D. Haussinger. Disruption of the taurine transporter gene (taut) leads to retinal degeneration in mice. *FASEB J.* **16**:231–233 (2002).
40. U. Warskulat, U. Flogel, C. Jacoby, H. G. Hartwig, M. Thewissen, M. W. Merx, A. Molojavyi, B. Heller-Stilb, J. Schrader, and D. Haussinger. Taurine transporter knockout depletes muscle taurine levels and results in severe skeletal muscle impairment but leaves cardiac function uncompromised. *FASEB J.* **18**:577–579 (2005).