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Characteristics of taurine transport system and its developmental pattern in mouse cerebral cortical neurons in primary culture

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Developmental patterns and pharmacological and biochemical properties of taurine transport system were investigated using developing primary cultured neurons prepared from mouse cerebral cortex by trypsin treatment. [^{3}H]Taurine was incorporated into neurons via a high-affinity transport system of which the K_m value as well as the V_{max} value increased during neuronal development in vitro. This transport system was also inhibited by sodium withdrawal from incubation medium and exposures for 15 h to several metabolic inhibitors such as 2,4-dinitrophenol and monoiodoacetate. In addition, [^{3}H]taurine uptake in both neurons cultured for 3 and 14 days was competitively inhibited by β-alanine, guanidinoethanesulfonate and hypotaurine. Cysteic acid and cysteine sulfinic acid, metabolic intermediates produced in the process of taurine biosynthesis in the brain from cysteine, induced significant reductions in [^{3}H]taurine uptake in both types of cultured neurons, while cysteine, isethionic acid, cysteamine and cystamine exhibited no alterations in [^{3}H]taurine transport. Moreover, non-competitive inhibition of [^{3}H]taurine uptake by cysteic acid was observed in both neurons. These results clearly indicate that taurine uptake was mediated by the sodium- and energy-dependent transport system with high affinity in 14-day-old neurons as well as neurons cultured for 3 days and that both the K_m and V_{max} values of this transport system increase during neuronal development in vitro. The results described above suggest that the decrease in taurine content observed in developing brain is unlikely to be due to alteration in the capacity of the taurine transport system during neuronal development.

Introduction

The taurine content in the central nervous system is found to decrease during brain development [1–8], while the activities of cysteine sulfinic acid decarboxylase and cysteine dioxygenase involved in the biosynthesis of taurine from its metabolic precursor, cysteine, show elevations [9–12]. Such a reduction in taurine content during brain development different from the developmental patterns of contents of other neurotransmitters such as acetylcholine and γ-aminobutyric acid (GABA) is assumed to be evidence supporting that taurine has a role as a neuromodulator, but not as a neurotransmitter, in the central neuron system [13]. In addition, the alterations in taurine content as well as in the activities of both cysteine sulfinic acid decarboxylase and cysteine dioxygenase [14] that have been found in primary cultured neurons obtained from mouse cerebral cortex during their development in vitro are similar to those in the developing brain in vivo. Therefore, it is considered that primary cultures of mouse cerebral cortical neurons are appropriate to

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investigate the biochemical mechanisms underlying the decrease in taurine content during the early stage of brain development [14].

In the present study, we attempted to clarify the developmental changes in the activity of the taurine transport system, which is assumed to be one of the causes inducing the reduction of taurine content in developing neurons, using mouse cerebral cortical neurons possessing a taurine biosynthesizing system, and to investigate the biochemical characteristics of taurine transport mechanisms in these neurons.

Materials and Methods

Primary culture of cerebral cortical neurons

Dissociation and primary culture of cerebral cortical neurons were carried out as described previously [14]. Neopallium free of meninges obtained from 15-day-old fetus of STD : ddy strain mouse was treated with trypsin following adequate mincing by scissors. The cells thus obtained were subsequently centrifuged ($900 \times g$, 4°C, 5 min) and passed through nylon mesh (mesh size: 60 μm). After cells were suspended with modified Eagle's minimum essential medium (pH 7.4) [14], an aliquot of cell suspension was seeded on a Corning culture dish (60 mm in diameter) pretreated with poly(L-lysine). The medium was exchanged to modified Eagle's minimum essential medium supplemented with 15% fetal calf serum 15 min after inoculation and cells were incubated at 37°C in humidified 95% air/5% CO_2 for 3 days. Cells were then exposed to 20 μM cytosine arabinoside dissolved in modified Eagle's minimum essential medium containing 15% horse serum for 24 h to suppress the proliferation of astroglial cells concurrently dissociated with neurons. Following the exposure to cytosine arabinoside, cells were continuously cultured in modified Eagle's minimum essential medium supplemented with 15% horse serum, and this medium was exchanged to fresh medium every 4 days. Primary cultured neurons thus prepared had morphological characteristics specific to neurons and immunohistochemical investigations using the antibody to glial fibrillary acidic protein, a specific marker of astroglia, revealed that the contamination by astroglial cells was less than 5% [14].

Measurement of [^{3}H]taurine uptake

The assay of [^{3}H]taurine uptake in mouse cerebral cortical neurons in primary culture was performed as follows. The medium in the culture dishes was discarded by aspiration and the cells were washed three times with ice-cold Krebs-Ringer bicarbonate buffer (buffer A, pH 7.4). After rinsing the dishes, 3 ml of warm (37°C) buffer A was added into a culture dish and the cells were incubated at 37°C. After 20 min of incubation, buffer A was changed by warmed (37°C) fresh buffer A. The reaction of uptake was initiated by the addition of 10 μM [^{3}H]taurine into a culture dish and incubation at 37°C was continued for 15 min. After incubation, the reaction was terminated by the aspiration of medium containing [^{3}H]taurine with consequent washing five times with ice-cold buffer A (total volume: 15 ml). The procedure to terminate the reaction was always carried out within 45 s. Washed cells were then digested with 1 ml of 0.1 M NaOH and scraped off from culture dishes with a rubber policeman. An aliquot of digested cells neutralized with 0.1 M acetic acid was transferred to a scintillation vial containing Triton-toluene scintillator (Triton X-100/toluene (containing 5 g PPO and 0.3 g POPOP per liter), 1 : 2 (v/v)) for measuring radioactivity transported into cells. The net uptake of [^{3}H]taurine was calculated by subtracting inulin space determined using [^{3}H]inulin from total [^{3}H]taurine uptake. In order to examine the effects of metabolic inhibitors such as 2,4-dinitrophenol, ouabain and monoiodoacetate on [^{3}H]taurine uptake, the cells were preincubated and incubated in the presence of each inhibitor at the concentration of 100 μM. For investigating the effects of various sulfur-containing amino acids on [^{3}H]taurine uptake, these amino acids at 100 μM were added into culture dishes immediately before the addition of [^{3}H]taurine. Sodium-free medium was prepared by complete displacement of sodium by equimolar choline to examine the effect of sodium withdrawal on [^{3}H]taurine uptake. In the preliminary experiments, we confirmed that the addition of metabolic inhibitors at the concentration examined in this study showed no effects on [^{3}H]inulin space.

Protein measurement

Protein content in neurons were determined by the method of Lowry et al. using bovine serum albumin as standard [15].

Materials used

[^{3}H]Taurine (740 GBq/mmol) and [^{3}H]inulin (6.253 GBq/g) were obtained from New England Nuclear (Boston, U.S.A.). Fetal calf serum and horse serum were purchased from Flow Laboratories (North Ryde, Australia) and Gibco Laboratories (Chagrin, U.S.A.), respectively. Bovine serum albumin (fraction V, powder) was obtained from Miles Laboratories (Elkhart, U.S.A.). Cytosine arabinoside was a kind gift of Nippon Shinyaku Co., Ltd. (Kyoto, Japan). Other drugs used were locally available and of analytical grade.

Result

Time course of [^{3}H]taurine uptake

Fig. 1 shows the time course of [^{3}H]taurine uptake at 37 °C in neurons cultured for 14 days. [^{3}H]Taurine was transported into neurons with linearity until 20 min after the addition of [^{3}H]taurine. Thereafter, the rate of [^{3}H]taurine transport was gradually decreased and reached a plateau 45 min after the initiation of reaction. Similar pattern of [^{3}H]taurine uptake was also found in neurons cultured for 3 days. Based on these results, the initial rate of [^{3}H]taurine uptake in both neurons cultured for 3 and 14 days were determined during 15 min after the addition of [^{3}H]taurine. In contrast to [^{3}H]taurine uptake at 37 °C, it was observed that only little amount of [^{3}H]taurine was transported into neurons at 4 °C during incubation period indicated in Fig. 1.

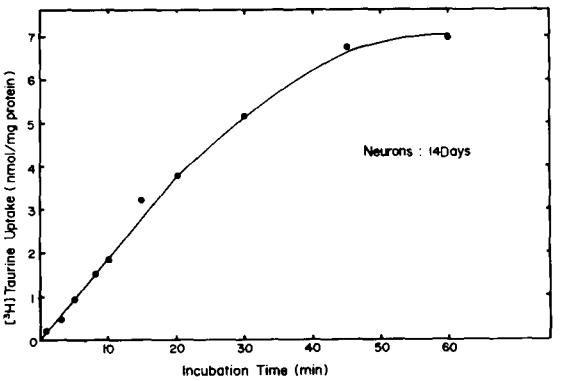


Fig. 1. Time course of [^{3}H]taurine uptake in mouse cerebral cortical neurons 14-day-old in primary culture. Following the discard of culture medium and rinsing the dishes three times with ice-cold Krebs-Ringer bicarbonate buffer (buffer A, pH 7.4), the neurons were incubated with warm (37 °C) buffer A at 37 °C for 20 min. After the preincubation, buffer A was aspirated and fresh warm buffer A was added and the reaction was then initiated by the addition of 10 μM [^{3}H]taurine into the culture dishes. After incubation for the intervals indicated in the figure, the reaction was terminated by rapid aspiration of the medium and consequent washing of the dishes with ice-cold buffer A. The digested cells with 0.1 M NaOH were subjected to measure the radioactivity of [^{3}H]taurine transported into the neurons by liquid scintillation spectrometry following the neutralization of alkaline digested cells by 0.1 M acetic acid. Each value represents the mean of four separate experiments.

Changes in kinetic parameters of [^{3}H]taurine uptake during neuronal development

To determine the characteristics of [^{3}H]taurine transport system in primary cultured neurons, neurons were incubated in the presence of various concentrations of [^{3}H]taurine (2.5–500 μM). In neurons cultured for both 3 and 14 days, the rates of [^{3}H]taurine uptake were found to be increased with the increase in concentration of [^{3}H]taurine in medium. However, the curve of [^{3}H]taurine uptake as a function of extracellular [^{3}H]taurine concentration showed a saturable manner (Fig. 2). In addition, the amount of [^{3}H]taurine transported into neurons cultured for 14 days was larger than that in 3-day-old neurons in culture (Fig. 2). For determining the kinetic parameters for [^{3}H]taurine uptake, data shown in Fig. 2 were analyzed by Lineweaver-Burk plot. It was found that taurine transport systems in both neurons consisted of a single component with high affinity (Fig. 3). The kinetic parameters of [^{3}H]taurine uptake system in neurons cultured for 3, 7 and 14 days calculated from Lineweaver-Burk plot were presented in Table I. The K_m value as well as the V_{max} value of [^{3}H]taurine uptake increased during neuronal growth in vitro. The linearity in kinetic data for taurine uptake was confirmed by various linear transformations described previously [16].

Effects of metabolic inhibitors and sodium withdrawal on [^{3}H]taurine uptake

The effects of various metabolic inhibitors

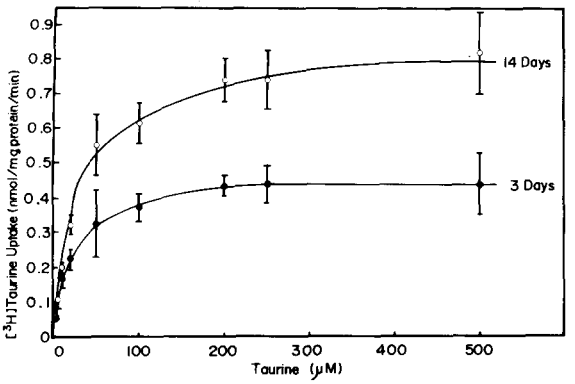


Fig. 2. Rate of [^{3}H]taurine uptake in mouse cerebral cortical neurons in primary culture as a function of the external [^{3}H]taurine concentration. Neurons were incubated at 37 °C for 15 min in the presence of various concentrations of [^{3}H]taurine (2.5 μM–500 μM) following the preincubation at 37 °C for 20 min. Each value represents the mean ± S.E. obtained from three or four separate experiments.

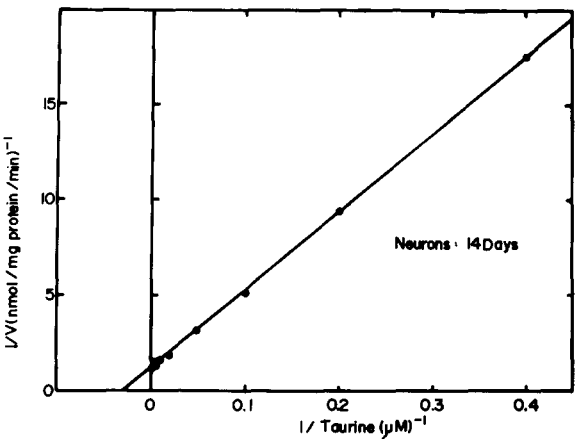


Fig. 3. Lineweaver-Burk plot of [^{3}H]taurine uptake in mouse cerebral cortical neurons cultured for 14 days. Each value obtained from the experiments presented in Fig. 2 represents the mean.

(ouabain, 2,4-dinitrophenol, monoiodoacetate, potassium cyanide and sodium azide) and sodium withdrawal on [^{3}H]taurine uptake in primary cultured neurons maintained for 3 and 14 days were examined (Table II). Ouabain at the concentration of 100 μM exhibited a significant inhibition of [^{3}H]taurine uptake in both types of neurons. In addition, it was found that [^{3}H]taurine uptake was completely abolished by sodium withdrawal from incubation medium. On the other hand, the exposure to 2,4-dinitrophenol, monoiodoacetate and sodium azide at concentrations of 100 μM and 1 mM did not induce any significant alterations in [^{3}H]taurine uptake by primary cultured neurons, although a significant reduction of [^{3}H]taurine transport was found after treatment with 100 μM and 1 mM potassium cyanide (Table II). When neurons cultured for 14 days were, however, maintained with 100 μM 2,4-dinitrophenol, 100 μM sodium azide and 10 μM monoiodoacetate, respectively, for 15 h, the significant decreases in [^{3}H]taurine uptake were observed with no remarkable morphological changes (Table II). Moreover, the rates of Trypan blue exclusion in neurons treated with metabolic inhibitors as well as in neurons with no treatment were almost identical and more than 95% under the conditions described above.

Effects of various amino acids and guanidinoethane sulfonate on [^{3}H]taurine uptake

The effects of various amino acids formed in the taurine biosynthesizing pathway from cysteine as a metabolic precursor, and of β-alanine and guanidinoethanesulfonate, the latter two are well known to be competitive inhibitors for taurine transport system, on [^{3}H]taurine uptake in neurons of both 3- and 14-day-old in culture was also examined (Table III). β-Alanine, guanidinoethanesulfonate and hypotaurine showed remarkable inhibitory actions on [^{3}H]taurine uptake, although other amino acids did not. To examine the inhibitory manner of β-alanine, guanidinoethane-

TABLE I

DEVELOPMENTAL CHANGES IN KINETIC PARAMETERS OF [^{3}H]TAURINE UPTAKE IN MOUSE CEREBRAL CORTICAL NEURONS IN PRIMARY CULTURE

Neurons were incubated with Krebs-Ringer bicarbonate buffer (pH 7.4) in the presence of various concentrations of [^{3}H]taurine (2.5–500 μM) at 37 °C for 15 min following the preincubation at 37 °C for 20 min without [^{3}H]taurine.

Culture	No. of expts.	K_m (μM)	V_{max} (nmol/mg protein/min)
3 days	4	21.2 ± 0.7 [b]	0.485 ± 0.043 [b]
7 days	4	29.5 ± 0.7 [a]	0.879 ± 0.025 [a]
14 days	3	29.2 ± 2.8 [a]	0.764 ± 0.069 [a]

[a] $P < 0.05$, [b] $P < 0.001$.

TABLE II

EFFECTS OF METABOLIC INHIBITORS AND SODIUM WITHDRAWAL ON [^{3}H]TAURINE UPTAKE IN MOUSE CEREBRAL CORTICAL NEURONS IN PRIMARY CULTURE

Neurons were incubated in Krebs-Ringer bicarbonate buffer (buffer A, pH 7.4) in the presence of 10 μM [^{3}H]taurine and metabolic inhibitors at the concentration indicated in table at 37°C for 15 min following the preincubation of neurons with metabolic inhibitors in buffer A at 37°C for 20 min. (a) Neurons were preincubated in modified Eagle's minimum essential medium (14) supplemented 15% horse serum in the presence of metabolic inhibitors at 37°C for 15 h and then subjected to experiments for [^{3}H]taurine uptake. Control values of [^{3}H]taurine uptake in neurons cultured for 3 and 14 days were 0.129 ± 0.007 and 0.248 ± 0.016 nmol/mg protein/min, respectively. Each value represents the mean ± S.E. obtained from 4–7 separate experiments. n.d., not detectable.

Metabolic inhibitors	[^{3}H]Taurine uptake (% of control)	
	3 days	14 days
Control	100.0 ± 5.7	100.0 ± 6.3
Sodium-free	n.d.	n.d.
Ouabain (100 μM)	79.0 ± 2.7 [c]	12.9 ± 1.9 [d]
2,4-Dinitrophenol		
(100 μM)	106.4 ± 3.4	93.1 ± 9.3
(1 mM)	87.3 ± 6.2	87.7 ± 1.9
(100 μM) [a]	–	55.7 ± 7.9 [c]
Monoiodoacetate		
(100 μM)	97.5 ± 2.4	94.4 ± 13.5
(1 mM)	88.4 ± 5.9	89.9 ± 3.2
(10 μM) [a]	–	11.3 ± 1.4 [d]
KCN		
(100 μM)	86.5 ± 2.1 [a]	75.6 ± 8.3
(1 mM)	77.8 ± 1.2 [c]	64.3 ± 10.6 [a]
NaN_3		
(100 μM)	92.2 ± 3.2	90.2 ± 7.7
(1 mM)	97.6 ± 7.3	104.4 ± 6.3
(100 μM) [a]	–	73.7 ± 5.1 [b]

[a] $P < 0.05$, [b] $P < 0.02$, [c] $P < 0.01$, [d] $P < 0.001$ (each one versus the control).

sulfonate and hypotaurine for taurine transport, the data obtained were analyzed using the Lineweaver-Burk plot. As shown in Fig. 4, it became clear that β-alanine competitively inhibited [^{3}H]taurine uptake by neurons cultured for 14 days. Similar competitive inhibition was also found in 3-day-old neurons. K_i values of β-alanine for

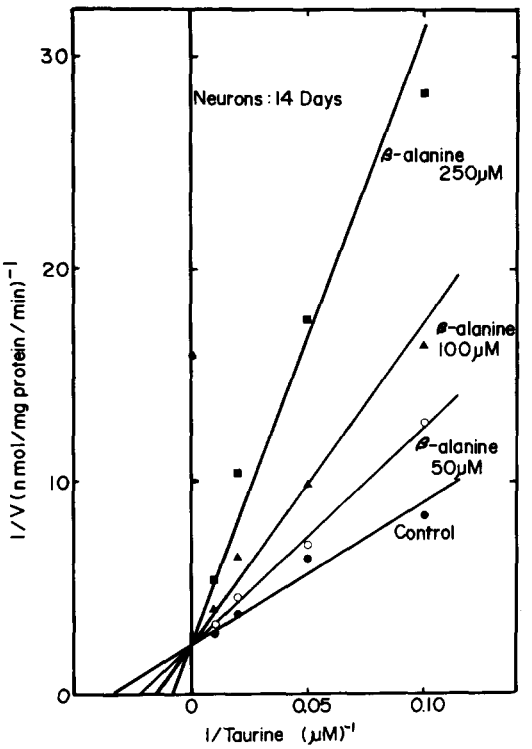


Fig. 4. Effect of β-alanine on [^{3}H]taurine uptake in neurons cultured for 14 days. Neurons were incubated in the presence of various concentrations of β-alanine (50–250 μM) and [^{3}H]taurine (10–100 μM) at 37°C for 15 min. Each value represents the mean of three separate experiments. The S.E. value at each point was less than eight percent of the mean value.

[^{3}H]taurine uptake in 3- and 14-day-old neurons, which were obtained by the analysis using Dixon plot, were 104 and 100 μM, respectively. Similarly, guanidinoethanesulfonate and hypotaurine exhibited competitive inhibition of [^{3}H]taurine uptake by both types of neurons (data not shown).

Several amino acids such as cysteine and isethionic acid, and cystamine and cysteamine (concentration 100 μM) did not induce any significant decreases in [^{3}H]taurine uptake in primary cultured neurons of both 3- and 14-day-old in culture (Table III). In contrast, 100 μM cysteic acid exhibited significant inhibition of [^{3}H]taurine uptake in neurons cultured for both 3 and 14 days. Cysteine sulfinic acid (100 μM) inhibited [^{3}H]taurine uptake in 3-day-old neurons (Table III). To examine the inhibitory manner of cysteic

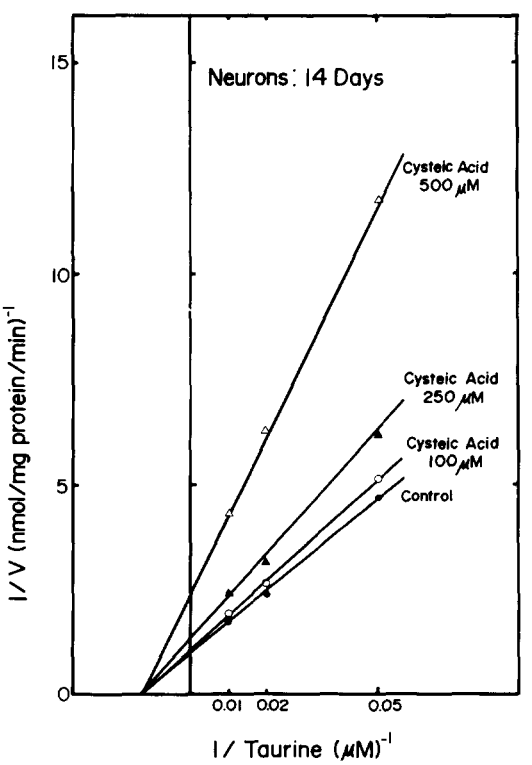
TABLE III

EFFECT OF VARIOUS AMINO ACIDS ON [^{3}H]TAURINE UPTAKE IN MOUSE CEREBRAL CORTICAL NEURONS IN PRIMARY CULTURE

Neurons were incubated in Krebs-Ringer bicarbonate buffer (buffer A, pH 7.4) in the presence of 10 μM [^{3}H]taurine and various amino acids (100 μM) at 37 °C for 15 min after the preincubation in buffer A at 37 °C for 20 min. Control values of [^{3}H]taurine uptake in neurons cultured for 3 and 14 days were 0.131 ± 0.005 and 0.236 ± 0.019 nmol/mg protein/min, respectively. Each value represents the mean ± S.E. obtained from four or five separate experiments.

Amino acids	[^{3}H]Taurine uptake (% of control)	
	3 days	14 days
Control	100.0 ± 4.1	100.0 ± 1.2
β-Alanine	37.4 ± 1.7 [c]	45.6 ± 3.1 [c]
Guanidinoethane sulfonate	32.8 ± 2.7 [c]	47.8 ± 5.0 [c]
Hypotaurine	14.7 ± 0.6 [c]	25.4 ± 1.3 [c]
Cysteine	95.6 ± 1.4	81.8 ± 13.3
Cysteine sulfinic acid	73.9 ± 2.2 [a]	78.1 ± 10.9
Cysteic acid	83.1 ± 2.8 [b]	79.1 ± 7.3 [a]
Cystamine	104.0 ± 2.9	100.2 ± 2.4
Cysteamine	110.9 ± 3.1	96.3 ± 16.7
Isethionic acid	102.9 ± 2.0	91.9 ± 3.7

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ (each one versus control).



acid for [^{3}H]taurine transport in neurons, the data were analyzed using Lineweaver-Burk plots. Fig. 5 shows that the uptake of [^{3}H]taurine into neurons cultured for 14 days as well as that into 3-day-old neurons is clearly suppressed by cysteic acid in a non-competitive manner.

Discussion

It is well known that taurine plays a role as neurotransmitter or neuromodulator in the mammalian central nervous system, although its exact role is controversial [13]. One of the reasons supporting that taurine has neuromodulatory action in the central nervous system is that the content of taurine in the brain shows a steep reduction during the early stage of brain development [1–8] in contrast to the progressive elevation of activities of enzymes involved in taurine biosynthesis [9–12]. Similarly, the contents of taurine, cysteine sulfinic acid and cysteic acid, the latter two amino acids are intermediates produced during taurine biosynthesis from its metabolic precursor, cysteine, remarkably reduced with the concomitant increase in activities of cysteine sulfinic acid decarboxylase and cysteine dioxygenase at the early stage of in vitro growth of cerebral cortical neurons well separated from other types of cells including glial cells [14]. These lines of evidence may be, therefore, lead to the conclusion that primary cultured neurons used in the present study are appropriate for investigating the mechanism of reduction of taurine content during brain growth in vivo.

One of the mechanisms causing the reduction of taurine content at the early stage of brain development assumes to be due to the decrease in taurine transport from blood to brain via the blood-brain barrier. It is well established that taurine penetrates the blood-brain barrier in adult mammals, although the exchange rate of taurine in brain is slower than that in other organs [17–19]. In addition, Sturman and his co-workers [20] have reported that taurine administered to lactating

Fig. 5. Effect of cysteic acid on [^{3}H]taurine uptake in mouse cerebral cortical neurons 14-day-old in primary culture. Neurons were incubated in the presence of cysteic acid (100–500 μM) and [^{3}H]taurine (20–100 μM). Each value represents the mean obtained from four separate experiments.

rats is transferred to their pups via milk and radiolabeled taurine is detected in brains of pups. These data clearly indicate that the blood-brain barrier in the early stage of brain development possesses the capacity to transport taurine from blood to brain. Considering these data, the possibility that a developmental change in capacity to transport taurine into neurons may be one of the factors decreasing the taurine content in the brain is assumed. In this study, therefore, we attempted to examine the developmental patterns of taurine uptake during neuronal growth and the characteristics of taurine transport system using mouse cerebral cortical neurons in primary culture.

The increase in [^{3}H]taurine uptake with increasing temperature in neurons of 14-day-old in culture clearly demonstrates that [^{3}H]taurine uptake in primary cultured neurons is dependent on temperature. The relationship between [^{3}H]taurine uptake in neurons cultured for both 3 and 14 days and the extracellular concentration of [^{3}H]taurine was found to be saturable. In addition, the results obtained in this study indicate that the capacity to transport taurine into neurons increases with increasing the culture period. The analysis of data on [^{3}H[taurine uptake as a function of the extracellular taurine concentration using the Lineweaver-Burk plot clearly revealed that [^{3}H]taurine was transported into both types of neurons via a single transport system with high affinity and the increase in [^{3}H]taurine transport observed during neuronal growth in vitro was attributed to the increases in both the K_m and the V_{max} values. Mandel and his co-workers [21] have reported the developmental pattern of taurine transport in primary cultured neurons. In their report, they observed a decrease in the V_{max} value of taurine transport system with high affinity during neuronal growth in vitro, which is contrary to the data presented in this study, although they did not demonstrate the biochemical characteristics of taurine transport mechanism in primary cultured neurons. Such a difference in developmental pattern of taurine uptake observed in cultured neurons may be due to the difference in methods to dissociate and cultivate neurons, although the exact mechanism is not clear.

A similar transport system of taurine with high affinity observed in primary cultured neurons was also observed in brain slices [22]. On the other hand, several investigators have reported that taurine uptake is mediated via transport mechanisms with both high and low affinities in brain synaptosomes and slices [23–27]. In addition, [^{3}H]taurine uptake was completely abolished by sodium withdrawal and was also significantly suppressed by ouabain, a Na^+/K^+-ATPase inhibitor, in primary cultured neurons as demonstrated in this study, which is essentially in agreement with the reports described previously [21–26]. Based on these data, it is concluded that the [^{3}H]taurine uptake with high affinity in the primary cultured neurons used in the present study is definitely mediated by a sodium-dependent mechanism. That the low-affinity uptake system has not been detected in the neurons used in this study may be due to the fact that the range of the extracellular concentration of taurine which we examined is too low, since the K_m values for low-affinity taurine transport are 1 to 6 mM [23,28].

In order to examine whether the taurine transport system in cultured neurons was energy-dependent or not, neurons were preincubated in the presence of various metabolic inhibitors prior to the uptake study. 2,4-Dinitrophenol, monoiodoacetate, potassium cyanide and sodium azide at the concentration of 100 μM, respectively, did not induce any significant changes in [^{3}H]taurine uptake in neurons cultured for either 3 or 14 days. Following the exposure of 14-day-old neurons in culture to 100 μM 2,4-dinitrophenol, 100 μM sodium azide and 10 μM monoiodoacetate, respectively, for 15 h, a significant reduction in [^{3}H]taurine uptake was found. Furthermore, no morphological changes in neurons and no alterations in the ability to exclude Trypan blue dye after these treatments were observed. Considering these results and the temperature dependency of taurine transport system, it is concluded that taurine is transported into neurons by an energy-dependent mechanism. In brain synaptosomes and slices, a similar transport mechanism with energy-dependency has been reported previously [22–24,26,27]. The difference in effects of metabolic inhibitors on taurine transport in primary cultured neurons according to the difference in exposure period may be due to the low permeability of cultured neurons to these metabolic inhibi-

tors, since a similar tendency was observed in [^{3}H]choline and [^{3}H]GABA transports in cultured neurons (unpublished data). However, the exact mechanism producing such a phenomenon is not exactly explained in the present.

The effects of several inhibitors specific to the taurine transport system on [^{3}H]taurine uptake were examined. [^{3}H]Taurine uptake in neurons cultured for 3 and 14 days was significantly inhibited by β-alanine, hypotaurine and guanidinoethanesulfonate. The inhibitory action of these amino acids on taurine uptake has also been found in brain slices [22–24], brain synaptosomes [26,27] and other tissues such as heart [29] and liver [30,31]. In addition, the analysis using the Lineweaver-Burk plot revealed that these amino acids inhibited taurine transport in cultured neurons in a competitive manner, which indicated that taurine was incorporated into neurons by the β-amino acid transport system. It is noted that the sensitivity of the taurine transport system to these competitive inhibitors is not altered during neuronal growth in vitro, suggesting that the biochemical and pharmacological properties of the taurine transport mechanism may be established in the early stage of neuronal development.

Among the amino acids produced in the process of taurine biosynthesis in the central nervous system, cysteic acid and cysteine sulfinic acid showed remarkable inhibitory actions on [^{3}H] taurine transport in primary cultured neurons. Similar effects of cysteine sulfinic acid on taurine transport have been reported in brain synaptosomes [26,27], in which cysteine also showed a significant reduction of taurine uptake [26]. In contrast, no alteration or slight decrease in taurine transport in cultured neuroblastoma cells and in neuroblastoma × glioma hybrid cells in the presence of either cysteic acid or cysteine sulfinic acid was observed [32,33]. Therefore, it is considered that the mode of inhibition of taurine uptake by these amino acids in primary cultured neurons used in this study resembles that in synaptosomes derived from brain rather than that of cell lines originated from brain tumors possessing biochemical characteristics different from normal neuronal cells. In addition, the analysis using the Lineweaver-Burk plot revealed that [^{3}H]taurine transport found in neurons cultured for 3 and 14 days was inhibited by cysteic acid in a non-competitive manner. One of new findings on taurine transport in primary cultured neurons observed in this study was that cysteic acid exhibited a non-competitive inhibition of taurine transport, which may be one of the regulatory mechanism to maintain the taurine level in neurons. At present, however, the physiological significance of the inhibitory action of cysteic acid on taurine transport remains to be elucidated.

In summary, primary cultured neurons possess a sodium- and energy-dependent high-affinity transport system for taurine similar to that in neurons in vivo, and this transport system shows an increase in the capacity to accumulate taurine from extracellular space during their growth in vitro. Therefore, the decrease in taurine content in cultured neurons observed at the early stage of development in vitro is unlikely to be due to the alterations in transport capacity occurring during neuronal growth in vitro. In addition, the neurons used in this study are considered to be a experimental tool useful to investigate the regulatory mechanism of the taurine transport system in the central nervous system.

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References

1 Agrawal, H.C., Davis, J.M. and Himwich, W.A. (1966) J. Neurochem. 13, 607–615.
2 Agrawal, H.C., Davis, J.M. and Himwich, W.A. (1968) J. Neurochem. 15, 917–923.
3 Agrawal, H.C., Davidson, A.N. and Kaczmarek, L.K. (1971) Biochem. J. 122, 759–763.
4 Lajtha, A. and Toth, J. (1973) Brain Res. 55, 238–241.
5 Cutler, R.W.P. and Dudzinski, D.S. (1974) J. Neurochem. 23, 1005–1009.
6 Sturman, J.A. and Gaull, G.E. (1975) J. Neurochem. 25, 831–835.
7 Sturman, J.A. (1979) J. Neurochem. 32, 811–816.
8 Sturman, J.A., Rassin, D.K., Gaull, G.E. and Cote, L.J. (1980) J. Neurochem. 35, 304–310.
9 Rassin, O.K. and Gaull, G.E. (1975) J. Neurochem. 24, 969–978.

10 Pasantes-Marales, H., Mapes, C., Tapia, R. and Mandel, P. (1976) Brain Res. 107, 575–589.
11 Misra, C.H. and Olney, J.W. (1976) Brain Res. 97, 117–126.
12 Oja, S.S., Karvonen, M.-L. and Lähdesmäki, P. (1973) Brain Res. 55, 173–178.
13 Kuriyama, K. (1980) Fed. Proc. 39, 2680–2684.
14 Ohkuma, S., Tomono, S., Tanaka, Y., Kuriyama, K. and Mukainaka, T. (1986) Int. J. Dev. Neurosci. 4, 383–395.
15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
16 Dowd, J.E. and Riggs, D.S. (1965) J. Biol. Chem. 240, 863–869.
17 Urquhart, N., Perry, T.L., Hansen, S. and Kennedy, J. (1974) J. Neurochem. 22, 871–872.
18 Spaeth, D.G. and Schneider, D.L. (1974) Proc. Soc. Exp. Biol. Med. 147, 855–858.
19 Oja, S.S., Lehtinen, I. and Lähdesmäki, P. (1976) Q. J. Exp. Physiol. 61, 133–143.
20 Sturman, J.A., Rassin, D.K. and Gaull, G.E. (1977) Pediatr. Res. 11, 28–33.
21 Borg, J., Ramaharobandro, N., Mark, J. and Mandel, P. (1980) J. Neurochem. 34, 1113–1122.
22 Kaczmarek, L.K. and Davidson, A.N. (1972) J. Neurochem. 19, 2355–2362.
23 Lähdesmäki, P. and Oja, S.S. (1973) J. Neurochem. 20, 1411–1417.
24 Honegger, C.G., Krepelka, L.M., Steiner, M. and Von Hahn, H.P. (1973) Experientia 29, 1235–1237.
25 Kontro, P. and Oja, S.S. (1978) Neuroscience 3, 761–765.
26 Schmid, R., Sieghart, W. and Karobath, M. (1975) J. Neurochem. 25, 5–9.
27 Hruska, R.E., Padjen, A., Bressler, R. and Yamamura, H.I. (1978) Mol. Pharmacol. 14, 77–85.
28 Lähdesmäki, P., Pasula, M. and Oja, S.S. (1975) J. Neurochem. 25, 675–680.
29 Huxtable, R.J., Chubb, J. and Azari, J. (1980) Fed. Proc. 39, 2685–2690.
30 Ohkuma, S., Tamura, J., Kuriyama, K. and Takino, T. (1981) Japan. J. Pharmacol. 31, 1061–1070.
31 Ohkuma, S., Tamura, J., Kuriyama, K. and Mukainaka, T. (1984) Cell Biochem. Function 2, 71–77.
32 Borg, J., Balcar, V.J., Mark, J. and Mandel, P. (1979) J. Neurochem. 32, 1801–1805.
33 Kurzinger, K. and Hamprecht, B. (1981) J. Neurochem. 37, 956–967.