MOLECULAR PHARMACOLOGY, 14, 77-85

Taurine: Sodium-Dependent, High-Affinity Transport into Rat Brain Synaptosomes

ROBERT E. HRUSKA,[1] ANTE PADJEN,[2] RUBIN BRESSLER, AND HENRY I. YAMAMURA[3]

Departments of Pharmacology and Internal Medicine, University of Arizona College of Medicine, Tucson, Arizona 85724

(Received January 18, 1977)
(Accepted August 17, 1977)

SUMMARY

HRUSKA, ROBERT E., PADJEN, ANTE, BRESSLER, RUBIN & YAMAMURA, HENRY I. (1978) Taurine: sodium-dependent, high-affinity transport into rat brain synaptosomes. *Mol. Pharmacol.*, 14, 77-85.

Sodium-dependent transport of taurine into rat brain synaptosomes was studied using [^{3}H]taurine of high specific activity (2.8 Ci/mmole). At 2.8 μM [^{3}H]taurine, 57% of the total radioactive accumulation was directly proportional to the sodium ion concentration. The sodium-dependent, high-affinity transport was a linear function of added protein and incubation length, and was maximal between pH 6.6 and 8.6. Kinetic analyses indicated a high-affinity apparent K_m value of 4.76 μM and an apparent V_{max} value of 5.35 nmoles/g of protein per minute. After correction of the high-affinity transport for that portion contributed by the low-affinity system, the true kinetic constants of the high-affinity system were calculated to be 3.20 μM and 2.96 nmoles/g of protein per minute. Similarly, the true kinetic constants of the low-affinity system were calculated to be 3340 μM and 699 nmoles/g of protein per minute. The Hill plot for both the high- and low-affinity transport systems had a slope of about 1, which suggested a 1:1 interaction between taurine and its transport molecule. The sodium-dependent, high-affinity transport of [^{3}H]taurine was decreased by the removal of potassium or chloride ions, and was absent from lysed synaptosomes or when the assay was performed at 2°. The omission of glucose or the addition of dinitrophenol slightly reduced transport. Ouabain inhibited transport in a time- and dose-dependent manner. The Arrhenius plot of [^{3}H]taurine transport revealed an energy of activation (E_a) of 15.6 kcal/mole and an energy quotient (Q_{10}) of 2.34, each of which indicated an active process. The regional distribution of uptake showed that the midbrain, thalamus, and olfactory bulbs had the highest velocity of transport, while the cerebral cortex, spinal cord, and cerebellum had the lowest velocity of transport. Several structural analogues were inhibitors of taurine transport, and analyses of structure-activity relationships revealed that the uptake site was very specific. Only molecules with free anionic and cationic groups were potent inhibitors. These data are consistent with the hypothesis that taurine is a neurotransmitter or neuromodulator in the brain, and we have investigated some of the molecular characteristics of this transport.

This investigation was supported by Grants MH-27257, MH-26967, and HL-13636 from the United States Public Health Service.

[1] Recipient of Postdoctoral Research Fellowship NS-05585 from the National Institute of Neurological and Communicative Disorders and Stroke.

[2] Present address, Department of Pharmacology, McGill University, Montreal, Quebec, Canada.

[3] Recipient of Research Scientist Career Development Award MH-00095 from the National Institute of Mental Health.

0026-895X/78/0141-0077$02.00/0
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INTRODUCTION

Taurine is present at high concentrations in the mammalian brain (1). Recent behavioral, electrophysiological, and biochemical evidence suggests that taurine functions as an inhibitory neurotransmitter or neuromodulator in the central nervous system.

In behavioral experiments the administration of taurine decreases the number and severity of experimentally induced seizures in a variety of animal models (2). Taurine also depresses hypothalamic functions, since it can decrease temperature, conditioned drinking, and conditioned eating, without affecting other types of behavior (3, 4).

Electrophysiological experiments have demonstrated that the iontophoretic injection of taurine depresses the spontaneous firing rates of neurons in the spinal cord (5), brain stem (6), and cerebral cortex (7), and hyperpolarizes neurons in the spinal cord (8). Taurine has been postulated to be a neurotransmitter or neuromodulator in the frog spinal cord, since the application of taurine produces depolarization in the dorsal roots (9–11). On the lobster axon, taurine increases membrane permeability to potassium and chloride ions and therefore decreases excitability of the neuronal membrane (12).

Biochemically, taurine fulfills a number of criteria for a neurotransmitter. It is present in a unique population of synaptosomes (13), and electrical or chemical stimulation increases its release from brain slices (14–16). Since taurine is metabolized very slowly (17–20), it seems reasonable that the released material be removed by a transport system. Recently several investigators have examined the transport of taurine in brain slices (15, 21, 22) and synaptosomal fractions (23–28). However, a specific, high-affinity transport system was not always demonstrated (22), and others have reported variations in transport kinetics, with K_m values ranging from 20 μM (23) to 60 μM (21). Also, these reports did not identify the transport system as a specific, high-affinity uptake process. All previously reported studies utilized material of low specific activity (e.g., [^{35}S]taurine at 0.047 Ci/mmole or [^{14}C]taurine at 0.002 Ci/mmole). In preliminary investigations, performed with [^{3}H]taurine of high specific activity (2.8 Ci/mmole), we demonstrated a high-affinity transport system (29, 30). We now report the demonstration of sodium-dependent transport of [^{3}H]taurine and characterize the specific effects of ions, temperature, and analogues of taurine on the high-affinity transport system.

MATERIALS AND METHODS

Preparation of [^{3}H]taurine. [^{3}H]Taurine was prepared by catalytic tritium exchange by New England Nuclear and was purified before use (31). Briefly, the tritiated product was partially purified by passage through an ion-exchange column containing AG1-X8(Cl^-) anion-exchange resin layered over AG50-X8(H^+)cation-exchange resin. This procedure yielded [^{3}H]taurine of 80% purity. Final purification was achieved by thin-layer chromatography on cellulose plates, using acetone–formic acid–water (16:3:9) as the solvent. Isotopic dilution analyses revealed that the [^{3}H]taurine was 98–100% pure. The specific activity was 2.8 Ci/mmole.

Preparation of rat brain synaptosomes. Male Sprague-Dawley rats (200–300 g) were killed by decapitation. The brains were removed rapidly and placed in ice-cold 0.32 M sucrose. After weighing, the brains were homogenized in 9 volumes of 0.32 M sucrose in a Potter-Elvehjem glass homogenizer fitted with a Teflon pestle. The homogenate was centrifuged at 1000 $\times$ *g* for 10 min. The supernatant fluid was removed and centrifuged again at 17,500 $\times$ *g* for 20 min. The final supernatant was discarded, and the pellet was resuspended in the original volume of 0.32 M sucrose. Aliquots of the tissue suspension (crude synaptosomal fraction) were used in subsequent experiments. The synaptosomal fraction was prepared daily and used within 6 hr after preparation. Protein contents of the tissue suspensions were determined by the method of Lowry *et al.* (32).

Incubation of synaptosomal fraction. The normal incubation medium was Krebs' phosphate-buffered medium (pre-

pared daily), which contained 122 mM NaCl, 4.9 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 15.8 mM Na_2PO_4 buffer (pH 7.4), and 11 mM glucose. The incubation medium (total incubation volume, 1.0 ml) was placed in 1.5 ml polypropylene microfuge tubes, which were kept at 2° in an ice bath. After the tissue suspension (0.2–0.3 mg of protein) was added, the microfuge tubes were first incubated for 10 min in a water bath maintained at 37°. The final incubation at 37° was initiated by the addition of [^{3}H]taurine. After 4 min the accumulation of [^{3}H]taurine was terminated by centrifugation of the tubes for 30 sec in a Beckman Microfuge B. The supernatant was carefully aspirated, and the pellets were surface-rinsed twice with ice-cold 0.9% NaCl. The tips of the microfuge tubes, containing the pellets, were removed and placed in scintillation vials. Eight milliliters of a scintillation mixture composed of Triton X-100 (1 liter), toluene (2 liters), and Omnifluor (16 g) were added to each vial. The radioactivity was extracted from the tissue for at least 12 hr before determination by liquid scintillation spectrometry (Nuclear-Chicago Isocap 300). The counting efficiency of the instrument was 45–50%.

The transport of [^{3}H]taurine in the absence of sodium ion was measured in a duplicate set of tubes. In the sodium-free incubation medium, NaCl was replaced with lithium chloride (122 mM), and sodium phosphate buffer was replaced with Tris-phosphate buffer (15.8 mM, pH 7.4, at 37°). All experimental values were determined in triplicate.

In appropriate experiments, various compounds were added to the incubation media prior to the addition of the tissue.

Metabolism of [^{3}H]taurine. When 98–100% pure [^{3}H]taurine (1.8 μM) was incubated with the synaptosomal tissue preparation, isotopic dilution analyses revealed that 98–100% of the accumulated radioactivity within the synaptosomes was authentic taurine, indicating that there was no metabolism of taurine. In some experiments [^{3}H]taurine of only 80% radiochemical purity was used. In this case isotopic dilution analyses revealed that 85% of the accumulated radioactivity was taurine. After corrections were made to measure only radiolabeled taurine, the results did not differ from those of experiments in which 98–100% pure [^{3}H]taurine was used.

Data analyses. Sodium-dependent transport of [^{3}H]taurine was obtained by subtracting the transport in the absence of sodium from the transport in the presence of 154 mM sodium. All data were analyzed with the aid of a Wang 720C programmable calculator. Linear regression analyses were performed by the least-squares method. Estimation of true kinetic constants was performed by the method of Spears *et al.* (33).

Drugs. Guanidotaurine was synthesized by the method of Thoai and Robin (34). *N*-Acetyltaurine was a kind gift from Dr. Klaus Brendel. All other drugs and compounds were obtained from commercial sources in the purest form available.

RESULTS

Determination of sodium-dependent transport of [^{3}H]taurine. The velocity of the high-affinity transport of [^{3}H]taurine was a linear function of the concentration of sodium ions in the incubation medium (Fig. 1). Of the total radioactive accumulation, 57% was dependent on the presence

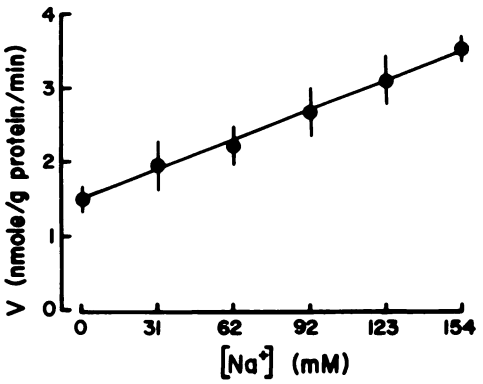

FIG. 1. *Linearity of transport of [^{3}H]taurine (2.8 μM) as a function of sodium ion concentration*

On the ordinate is the velocity (*V*) of [^{3}H]taurine transport. Sodium concentration was varied by replacing NaCl with LiCl, and the sodium phosphate buffer with a Tris-phosphate buffer. Each point is the mean ± standard error of three experiments performed in triplicate.

of sodium ion. Between sodium concentrations of 31 and 123 mM, either sodium phosphate or Tris-phosphate buffer was used. The velocity of transport obtained with either buffer was the same, and the results illustrated in Fig. 1 are averages from experiments in which both buffers had been used. The sodium-dependent transport in Fig. 1 was 2 nmoles/g of protein per minute. In this report, only the sodium-dependent transport of [^{3}H]taurine was studied.

The sodium-dependent transport of [^{3}H]taurine by rat brain synaptosomes was linear with time for 4 min and was also proportional to the amount of synaptosomal protein present in the incubation medium. The transport appeared to be optimal at pH 7.4, with nearly maximal transport in the pH range 6.6–8.6.

Kinetics of [^{3}H]taurine transport. The transport of [^{3}H]taurine by synaptosomes was analyzed in terms of two saturable, sodium-dependent uptake processes. As illustrated by the Lineweaver-Burk plot in Fig. 2, taurine concentrations ranging from 1.4 to 10 μM exhibited sodium-dependent transport, with an apparent high-affinity K_m value of 4.76 μM and an apparent V_{max} value of 5.35 nmoles/g of protein per minute. [^{3}H]Taurine concentrations as low as 0.05 μM did not reveal a transport system with a higher affinity. At concentrations above 20 μM, a lower-affinity system, with an apparent K_m value of 384 μM and an apparent V_{max} value of 116 nmoles/g of protein per minute, appeared (Fig. 2, inset). Interestingly, when the transport of [^{3}H]taurine was examined in the high-affinity range (1–3 μM), a significant proportion of the total transport (25–30%) was provided by the low-affinity transport system. Therefore, in order to separate the two transport systems and to obtain the true transport constants, the contributions of the high- and low-affinity systems must be subtracted from each other. By using the method of Spears *et al.* (33) and performing 10 iterations on each transport system, the true transport constants were calculated to be 3.20 μM and 2.96 nmoles/g of protein per minute for the high-affinity system, and 3340 μM and 699 nmoles/g of protein per minute for the low-affinity

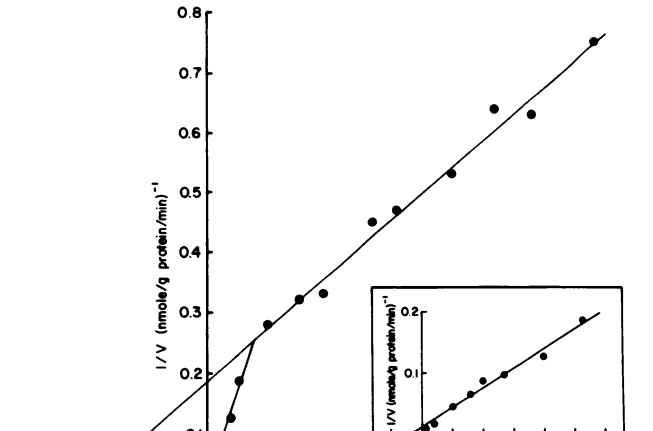

FIG. 2. *Lineweaver-Burk plot of [^{3}H]taurine transport*

The sodium-dependent transport was best fitted by two lines. The high-affinity transport system has an apparent K_m value of 4.76 μM and V_{max} value of 5.35 nmoles/g of protein per minute, while the low-affinity transport system (see inset) has respective values of 384 μM and 116 nmoles/g of protein per minute. The true kinetic constants (see the text) are: for the high-affinity system, 3.20 μM and 2.96 nmoles/g of protein per minute; for the low-affinity system, 3340 μM and 699 nmoles/g of protein per minute. Each point is the mean of eight experiments performed in triplicate.

system. Besides using a Hanes-Woolf plot as recommended by Spears *et al.* (33), we also estimated the true transport constants by using Lineweaver-Burk and Eadie-Hofstee plots. The average values obtained from all three plots differed by less than 30% from the true transport constants reported above.

Hill plot of [³H]taurine transport. When the kinetic data were analyzed by the use of a Hill plot (Fig. 3), the slopes of both the high- and low-affinity lines were approximately equal to 1. This was true whether the apparent K_m and V_{max} values (Fig. 3) or the true transport values were used.

Effect of various incubation conditions and inhibitors on high-affinity transport of [³H]taurine. The effects of various incubation conditions on the transport of [³H]-taurine (2.8 μM) are summarized in Table 1. As stated previously, the transport of [³H]taurine was dependent on the sodium ion concentration in the incubation medium, and was reduced by the same extent when NaCl was replaced by either LiCl, choline chloride, or sucrose. The sodium-dependent transport was reduced markedly by the total removal of potassium ions or by the removal of 93% of the chloride ions.

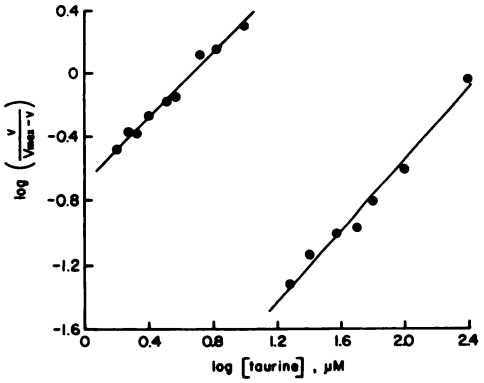

FIG. 3. *Hill plot of transport of [³H]taurine*
The data in Fig. 2 were transformed by using the apparent uncorrected velocities (V) and V_{max} values (V_{max} values of 5.35 and 116 nmoles/g of protein per minute). Similar results are obtained if true velocities and V_{max} values are used. The high-affinity transport system (upper left) has a Hill slope of 1.005, and the low-affinity transport system (lower right) has a Hill slope of 1.097.

TABLE 1
Effect of various incubation conditions on [³H]taurine transport
The transport of [³H]taurine (2.8 μM) was measured for 4 min at 37° under various conditions. The sodium-dependent transport was determined by replacing NaCl with LiCl, and sodium phosphate with Tris-phosphate, and is defined as 0% transport. Each value is the mean ± standard error of four experiments performed in triplicate.

Incubation condition	Sodium-dependent transport
	% control
Control	100 ± 1
LiCl replaced NaCl	0 ± 1
Choline Cl replaced NaCl	0 ± 2
Sucrose replaced NaCl	11 ± 3
KCl omitted	36 ± 6
NaBr replaced NaCl	27 ± 1
Glucose omitted	65 ± 4
$CaCl_2$ omitted	98 ± 9
$MgSO_4$ omitted	98 ± 4
2° replaced 37°	4 ± 2
Lysed synaptosomes[a]	13 ± 1
Dinitrophenol (100 μM)	73 ± 11
Ouabain (1 μM)	63 ± 8
Ouabain (100 μM)	40 ± 9
Ouabain (1 mM)	30 ± 5
Ouabain (1 μM)[b]	44 ± 6

[a] Synaptosomes were lysed by freezing, thawing, and homogenizing with a Polytron instrument.

[b] The preliminary incubation time was 20 min instead of 10 min.

The omission of glucose reduced transport to 68% of control. However, the lack of either calcium or magnesium ions did not alter the transport of [³H]taurine. Incubation of [³H]taurine with intact synaptosomes at 2° or with lysed synaptosomes at 37° eliminated the transport.

Several types of inhibitors also decreased [³H]taurine transport (Table 1). A metabolic uncoupler, dinitrophenol, inhibited transport moderately at 100 μM. Ouabain, a (Na^+ + K^+)-ATPase inhibitor, produced considerable inhibition of transport that was dependent on both the ouabain concentration and the length of preliminary incubation. Increasing the ouabain concentration from 1 μM to 1 mM decreased [³H]taurine transport from 63% to 30% of control. Lengthening the preliminary incubation with ouabain (1 μM) from 10 to 20 min decreased transport from 63% to 44% of control.

The possibility that [^{3}H]taurine transport was part of an exchange process was also examined. After the accumulation of [^{3}H]taurine (2.8 μM) had taken place during the normal incubation procedure, the final pellet was resuspended in ice-cold buffer and a second incubation was performed. Reincubation with 0.1–10 μM unlabeled taurine resulted in a maximum loss of 8% of the radioactivity from the crude synaptosomal fraction.

Similarly, the possibility that taurine analogues or drugs might promote taurine release was examined. After the accumulation of [^{3}H]taurine had taken place, a second incubation was performed in the presence of 100 μM β-alanine, β-guanidinopropionic acid, hypotaurine, guanidotaurine, or chlorpromazine. Under these conditions there was a maximal loss of 11% of the radioactivity.

Effect of incubation temperature on [^{3}H]taurine transport. The Arrhenius plot in Fig. 4 illustrates a linear relationship between the velocity of sodium-dependent, high-affinity [^{3}H]taurine transport and the temperature of incubation from 23° to 37°. As calculated from the regression line, the temperature quotient (Q_{10}) was 2.34 and the energy of activation (E_a) was 15.6 kcal/mole.

Regional variation in [^{3}H]taurine transport. Sodium-dependent, high-affinity transport varied among brain regions (Table 2). The highest transport velocities were found in the midbrain, thalamus, and olfactory bulbs; the lowest velocities were found in the cerebral cortex, cervical spinal cord, and cerebellum. There was a 5-fold greater velocity in the midbrain than in the cerebellum.

Inhibition of [^{3}H]taurine transport by structural analogues of taurine and other compounds. A total of 45 structural analogues and other compounds were examined for their ability to alter the sodium-dependent, high-affinity transport of [^{3}H]taurine (Table 3). Four close structural analogues of taurine (hypotaurine, β-guanidinopropionic acid, β-alanine, and guanidotaurine) were potent inhibitors of transport. Other minor structural modifications of the taurine molecule produced analogues that were moderate to weak inhibitors. The lower homologues of taurine, sulfamic acid and aminomethanesulfonic acid, were weak inhibitors of [^{3}H]taurine transport, while higher homologues produced little inhibition. Analogues in which either the acidic or basic

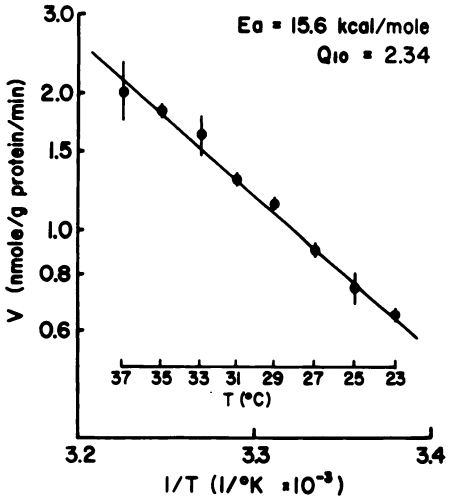

FIG. 4. *Arrhenius plot of transport of [^{3}H]taurine*
On the ordinate is the velocity (V) of [^{3}H]taurine transport. The concentration of [^{3}H]taurine was 2.8 μM. The temperature quotient (Q_{10}) and energy of activation (E_a) were determined from the linear regression line. Each point is the mean ± standard error of four experiments performed in triplicate.

TABLE 2
Regional variation of [^{3}H]taurine transport
The sodium-dependent transport of [^{3}H]taurine (2.8 μM) was measured in 10 regions of the rat brain. Regions were dissected according to the method of Glowinski and Iversen (35). Each value is the mean ± standard error of three experiments performed in triplicate.

Region	Velocity
	nmoles/g protein/min
Midbrain	2.84 ± 0.28
Thalamus	2.76 ± 0.49
Olfactory bulbs	2.28 ± 0.21
Hypothalamus	2.06 ± 0.35
Pons-medulla oblongata	1.88 ± 0.31
Corpus striatum	1.58 ± 0.37
Hippocampus	1.43 ± 0.05
Cerebral cortex	1.30 ± 0.49
Cervical spinal cord	0.77 ± 0.34
Cerebellum	0.58 ± 0.08

TABLE 3

Inhibition of [³H]taurine transport by structural analogues of taurine and by other drugs

The sodium-dependent transport of [³H]taurine (1 μM) was measured after a 15-min preliminary incubation with the indicated compound. Each value is the mean ± standard error of three experiments performed in triplicate.

Compound (100 μM)[a]	Sodium-dependent transport
	% control
Analogues	
Hypotaurine	19 ± 5
β-Guanidinopropionic acid	19 ± 3
β-Alanine	31 ± 6
Guanidotaurine	35 ± 6
γ-Amino-β-hydroxybutyric acid	59 ± 1
γ-Aminobutyric acid	62 ± 1
Aminooxyacetic acid	62 ± 6
Aminomethanesulfonic acid	65 ± 17
Sulfamic acid	66 ± 7
Cysteinesulfinic acid	75 ± 2
Drugs	
Chlorpromazine	1 ± 1
Imipramine	53 ± 1
Bicuculline	61 ± 2

[a] Compounds with little effect on transport (80–95% of control) were *N*-acetyltaurine, isethionic acid, δ-aminovaleric acid, α-aminoisobutyric acid, β-alanylleucine, pantoyltaurine, β-mercaptoethylamine, ε-aminocaproic acid, imidazoleacetic acid, strychnine, and 2-aminocyclohexanesulfonic acid. Compounds with no effect on transport (100% of control) were 3-aminopropanesulfonic acid, 2-aminoethyl hydrogen sulfate, 2-aminoethylphosphonic acid, β-alanylglycine, β-alanylalanine, β-alanyltyrosine, β-alanyl methyl ester, carnosine, alanyl-β-alanine, *N*-benzoyl-β-alanine, hexobarbital, cysteine, alanine, leucine, glycine, histidine, serine, choline, aspartic acid, metrazole, oxotremorine, and picrotoxin.

group was absent (e.g., isethionic acid) or attached to another group (e.g., *N*-acetyltaurine or the dipeptides) did not inhibit transport. Other compounds that resemble taurine even less, such as some amino acids, produced no inhibition of transport.

Of the drugs tested, chlorpromazine was the most potent inhibitor. Imipramine and bicuculline produced a modest amount of inhibition, while hexobarbital was ineffective. Drugs capable of producing convulsions (strychnine, metrazole, oxotremorine, and picrotoxin) had either very little or no effect on transport.

The inhibition constants (K_i) for potent inhibitors of [³H]taurine transport were also calculated (Table 4). The K_i values ranged from 5 to 22 times the true K_m value.

DISCUSSION

The major finding of this report is that a high-affinity transport system for taurine exists in rat brain, and that the transport is sodium-dependent, energy-requiring, and specific. In order to assess the transport carefully at very low concentrations, we used [³H]taurine of high specific activity. We also examined only the sodium-dependent transport, since the transport of most putative neurotransmitters has been found to be sodium-dependent. The sodium-dependent transport of [³H]taurine was best described by two separate transport systems (Fig. 2). The transport in the high-affinity range (1–3 μM) has a low-affinity component contributing up to 30% of the total sodium-dependent transport. We corrected for this overlap in the analyses of the kinetic data by mathematical approximation of the true kinetic constants (33). The true K_m and V_{max} values for the sodium-dependent transport were 3.20 μM and 2.96 nmoles/g of protein per minute. These values were remarkably similar to those we reported previously for temperature-dependent transport (29): K_m = 0.9 μM and V_{max} =

TABLE 4

Inhibition constants (K_i) for the five most potent inhibitors of [³H]taurine transport

Sodium-dependent transport of [³H]taurine (1 μM) was measured after the addition of the indicated compound at 10–100 μM. The IC_{50} value was obtained from a semilogarithmic plot by linear regression analyses of four experiments. The K_i value (mean ± standard error) was calculated from the IC_{50} value by the equation $K_i = IC_{50}/(1 + [S]/K_m)$, where $[S]$ = 1 μM and K_m = 3 μM.

Compound	K_i
	μM
Chlorpromazine	16.2 ± 2.1
Hypotaurine	31.4 ± 4.6
β-Guanidinopropionic acid	45.8 ± 2.3
Guanidotaurine	63.5 ± 1.2
β-Alanine	66.8 ± 1.8

1.1 nmoles/g of protein per minute. In further experiments we examined the removal of very low concentrations of [^{3}H]taurine (in the high-affinity transport range) by a predominantly high-affinity transport system.

The energy requirement of the transport system was measured from an Arrhenius plot (Fig. 4). We calculated that the energy of activation (E_a) or temperature quotient (Q_{10}) was sufficient to suggest that the transport was an active, energy-requiring process. This was in contrast to the poor inhibition produced by dinitrophenol (Table 1). In accord with previous reports (15, 21, 23), our results also suggested that taurine transport was insensitive to dinitrophenol.

The transport site is specific, since structure-activity relationship analyses revealed that only very close analogues of taurine produced inhibition of transport (Table 3). The important criteria for determination of transport inhibition appeared to be the electronegative density at the anionic site, the electropositive density at the cationic site, and the distance between the two sites. The most potent inhibitors have two methylene groups with high charge densities at the anionic and cationic sites. Increasing the distance between the two sites markedly decreases the inhibitory potency. Any substitution or addition that decreases either the electropositive or electronegative densities results in less potent analogues. Some analogues smaller in size than taurine may block transport by combining independently with either the electropositive or electronegative transport site. A number of analogues similar in structure to taurine, such as 2-aminoethyl hydrogen sulfate, were expected to be inhibitors of transport, but were ineffective at the concentration we used.

Several drugs were tested for their ability to inhibit taurine transport. Chlorpromazine was the most potent inhibitor tested, while imipramine produced modest inhibition. Bicuculline, a γ-aminobutyric acid antagonist, was as effective as γ-aminobutyric acid in inhibiting taurine transport. Several convulsant drugs were tested, since strychnine has been reported to block the inhibitory effect of taurine in electrophysiological experiments (6). Strychnine, metrazole, oxotremorine, and picrotoxin did not inhibit transport. This indicated that convulsant drugs do not affect taurine transport; they may still interact at a postsynaptic taurine receptor site.

The inhibition constants (K_i) indicated that the drugs examined were not very powerful inhibitors of taurine transport (Table 4), since all K_i values were larger than the K_m value for transport.

We further characterized the sodium-dependent, high-affinity transport of [^{3}H]taurine. In order to determine the relationship between taurine and its transport site, we constructed a Hill plot (Fig. 3) from the Lineweaver-Burk plot. For both the high- and low-affinity transport sites, the slopes obtained from the Hill plots were about 1, which suggested a 1:1 relationship between taurine and its transport molecule. The transport was also dependent on monovalent ions (sodium, potassium, and chloride), but was unaffected by removal of divalent cations (calcium or magnesium). Ouabain, an inhibitor of ($Na^+ + K^+$)-ATPase, decreased taurine transport. The amount of inhibition was dependent on the ouabain concentration and length of incubation. This suggested that the sodium-dependent transport of taurine is coupled to ($Na^+ + K^+$)-ATPase or to the ion gradients produced by this enzyme.

Transport was dependent on intact synaptosomes, since conditions that lyse synaptosomes eliminated transport. However, since the synaptosomal fraction may be contaminated by other subcellular components, such as glia, part of the total transport may be contributed by an easily lysed contaminant of the synaptosomal fraction.

The small regional variation in transport (Table 2) indicates that taurine may be a neuroactive substance in all areas, and that we should not exclude any area of the brain from our investigations. The 5-fold variation in velocity suggested that there was not a large difference in the number of regional transport sites, al-

though Lombardini (25) has reported a regional variation in the kinetic constants.

In summary, our evidence is consistent with the possibility that taurine is a neurotransmitter or neuromodulator in the central nervous system, since a high-affinity transport system exists and the transport is sodium-dependent, energy-requiring, and specific.

ACKNOWLEDGMENTS

We would like to thank Dr. Klaus Brendel of the University of Arizona for his kind gift of *N*-acetyltaurine. We would also like to thank David Chapman and Thomas McManus for expert technical assistance.

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