

BBAMEM 74589

## Sodium-dependent high-affinity uptake of taurine by isolated rat brain capillaries

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(Received 21 April 1989)

Key words: Blood-brain barrier; Taurine; Amino acid transport; Sodium ion dependence; Brain capillary; (Rat)

Transport of taurine has been demonstrated in capillary preparations from adult rat brains using [<sup>3</sup>H]taurine. Taurine transport is mediated by a saturable high-affinity system which is entirely dependent on sodium ions. The apparent maximal influx ( $V_{max}$ ) and half-saturation concentration ( $K_m$ ) corresponded to  $1.06 \cdot 10^{-4}$   $\mu\text{mol}/\text{min}$  per mg protein and 27.5  $\mu\text{M}$ , respectively. Competition experiments in the presence of sodium ion showed that [<sup>3</sup>H]taurine uptake was strongly inhibited by 0.1 mM unlabeled structural analogues of taurine such as  $\beta$ -alanine and hypotaurine as well as unlabeled taurine.  $\gamma$ -Aminobutyric acid (GABA) (0.1 mM) inhibited the uptake of labeled taurine by 30%, whereas isethionic acid, L-methionine, L-2,4-diaminobutyric acid, glycine, L-cysteinesulfonic acid and cystamine did not exhibit any inhibitory effect. The results suggest that the  $\text{Na}^+$  gradient is the principal source of energy for taurine transport into isolated brain capillaries. This transport system may play an active role in the regulation of taurine concentration in the brain extracellular space.

### Introduction

Taurine is one of the most abundant amino acids, occurring in high concentrations in the central nervous system (CNS), particularly in the developing brain [1] and in excitable tissues [2]. Several roles have been proposed for taurine. These include: neurotransmission or neuromodulation [3-5], regulation of  $\text{Na}^+/\text{K}^+$ -ATPase activity in the brain [6], an antioxidant [7] and membrane stabilizer [8].

There is increasing evidence suggesting that alterations in taurine levels may be involved in the pathogenesis of epilepsy. Preliminary experiments in human epileptic subjects and animals have demonstrated that this amino acid has anticonvulsant action [9,10]. Furthermore, experimental taurine depletion lowers seizure thresholds in neonatal rats [11], and administration of taurine has been shown to substantially diminish excitatory-induced seizure activity [12].

Detailed studies of taurine transport in the CNS have been performed in vitro using brain slices [13], cultured

neuronal and glial cells [14-16], synaptosomes [17,18], and membrane vesicles prepared from neuroblastoma and glioma hybrid cell lines [19]. Taurine transport has also been well documented in vivo in rat brain [20,21]. Animals with a genetically determined dysfunction of taurine transport have a markedly increased susceptibility to seizure [22].

The blood-brain barrier, constituted of brain capillaries, maintains homeostasis in the CNS by controlling the penetration into and removal of substances from the brain. There has been no report so far concerning the transport of taurine in isolated brain capillaries. This study was undertaken to determine whether or not a transport mechanism for taurine is present in capillaries isolated from rat brain. A brief report of this work has already been published [23].

### Materials and Methods

2-month-old male and female Sprague-Dawley rats (Iffa Credo, France), maintained on a 12 h light/dark cycle (8 a.m./8 p.m.) with food and water freely available, were used in these experiments. The animals (10-12 rats per experiment) were decapitated and the brains (devoid of cerebella) were quickly removed and placed in an oxygen-saturated buffer containing 145 mM NaCl, 4 mM KCl, 3.2 mM  $\text{CaCl}_2$ , 1.2 mM  $\text{MgCl}_2$ , 15 mM

Abbreviations: CSF, cerebrospinal fluid; GABA,  $\gamma$ -aminobutyric acid, L-DABA, L-2,4-diaminobutyric acid; BSA, bovine serum albumin.

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Hepes, 5 mM D-glucose, and 1% fraction V BSA (buffer A). The pH was adjusted to 7.4 by the addition of NaOH. For the experiments in the absence of sodium, NaCl was replaced by choline chloride (145 mM) and KOH was used to bring the pH to 7.4 in this buffer.

Meninges, choroid plexus, ependyma and the visible superficial vessels were removed from cerebral cortices. Cortical shells were then pooled, weighed, and chopped to a fine mince in 10–15 ml of fresh preparation buffer. Brain microvessels were isolated according to the method of Goldstein et al. [24] with minor modifications. Briefly, cerebral cortices were homogenized in 4-fold (v/w) buffer A in a Teflon and glass homogenizer with 0.25 mm clearance by 20 up-and-down strokes at 400 rpm, with the aid of a Heidolph motor driven homogenizer. The homogenate was diluted to give a 10% (w/v) suspension and then centrifuged at 1000 g for 10 min. To remove myelin and cellular debris, supernatant was discarded and the pellet was resuspended in approx. 12:1 (v/w) in the buffer A without BSA, but with 17.5% dextran ( $M_r$  70000), and was centrifuged for 10 min at 4000 g. The pellet was pooled in 20–30 ml of buffer A and gently teased through a 118  $\mu$ m nylon mesh. The arterioles were left on top of the mesh and the capillaries, red cells and nuclei were collected in the filtrate. This mixture was passed over four 1  $\times$  1 cm glass bead columns (0.25 mm glass beads) which had been suspended on a 40  $\mu$ m nylon mesh and the columns were rinsed with an excess volume of buffer A (150–200 ml) in order to wash off the red cells and nuclei while the microvessels were retained by the beads. The glass beads with adherent microvessels were washed into a beaker and swirled. As the beads sedimented to the bottom, the floating capillary segments in supernatants were decanted and spun at 500  $\times$  g for 5 min. All of the above steps were carried out at 0–4°C. The purity of the capillary preparations was assessed using phase-contrast microscopy. Microvessels consisted of small, branching segments with tubular structures and regular outline. Occasional erythrocytes trapped in the lumen and some small arterioles were seen.

#### *Uptake studies*

The final capillary pellet was resuspended in buffer A, and aliquots of 0.2 ml (approx. 100  $\mu$ g of protein) were incubated at 37°C in a shaker bath. After a 20 min preincubation of the capillary suspensions, uptake was initiated by the addition of 0.05 ml volumes of the buffer A (free of BSA) containing [<sup>3</sup>H]taurine (1  $\mu$ Ci/ml), [<sup>14</sup>C]sucrose (0.1  $\mu$ Ci/ml) and various amounts of non-radioactive taurine to the capillary suspension to give final desired concentrations in the incubation medium. All non-radioactive competitors, when present, were added at the same time as labeled taurine and sucrose. Radioactive sucrose was used as an extracellular marker to estimate the fraction of the total

[<sup>3</sup>H]taurine that was extracellular. The amount of taurine uptake was calculated as the difference in dpm between sucrose and taurine radioactivities using the following relationship; ( $[^3\text{H}]\text{taurine}$ ) – ( $[^{14}\text{C}]\text{sucrose} \times ^3\text{H}/^{14}\text{C}$ ). Since the final concentration of [<sup>3</sup>H]taurine used in these experiments was greater than that of [<sup>14</sup>C]sucrose, the dpm value obtained for [<sup>14</sup>C]sucrose was multiplied by the ratio of <sup>3</sup>H/<sup>14</sup>C in the above equation in order to account for this difference. Uptake of labeled taurine was measured at given intervals up to 15 min in the presence of unlabeled taurine. Uptake was terminated by the addition of 4 ml ice-cold saline (stop solution) to the incubation medium followed immediately by filtration under reduced pressure over nylon filters (40  $\mu$ m pore size). The filters were rapidly washed with 15–20 ml stop solution and transferred to individual 10 ml plastic tubes containing 1 ml distilled water, and then sonicated for 2 min (Sonicator NSE, series M 745). Aliquots were added to vials containing 1 ml solouene 350 (United Technology Packard) and incubated at 50°C (30 min). After incubation and cooling, 10 ml scintillation fluid was added to each vial, and the <sup>14</sup>C and <sup>3</sup>H radioactivities were counted 12 h later using an Intertechnique SL-3000 scintillation counter. Aliquots were also taken for protein determination by the method of Lowry et al. [25] after overnight solubilization in NaOH (0.5 M), using bovine serum albumin as a standard. Nonspecific radioactivity, due to binding of the labeled taurine to the nylon sieve, was also tested by omitting the capillary suspensions in the incubation mixture; it never exceeded 30 dpm.

Kinetic parameters for taurine uptake were measured by increasing the concentration of this amino acid from 2 to 200  $\mu$ M in the presence and absence of sodium ion. In the absence of sodium ion, choline chloride was used as described above.

[1,2-<sup>3</sup>H]taurine (specific activity 1.07 TBq/mmol) and [<sup>14</sup>C]sucrose (specific activity 20 GBq/mmol) were obtained from Amersham International (U.K.). Unlabeled amino acids were Sigma products. Albumin (fraction V) was obtained from Boehringer-Mannheim (F.R.G.).

#### **Results**

The time-course of the uptake of [<sup>3</sup>H]taurine in the presence of unlabeled taurine (0.1 mM) by isolated brain capillaries is shown in Fig. 1. The net uptake of taurine increased with time. The velocity of this uptake, however, declined progressively after 1 min of incubation. Based on these results, an incubation time of 1 min was used to measure the initial rates of taurine uptake as well as the uptake in the presence of inhibitors.

Kinetic parameters for the uptake of [<sup>3</sup>H]taurine were determined at various concentrations of taurine in the presence of NaCl (145 mM) or choline chloride (145

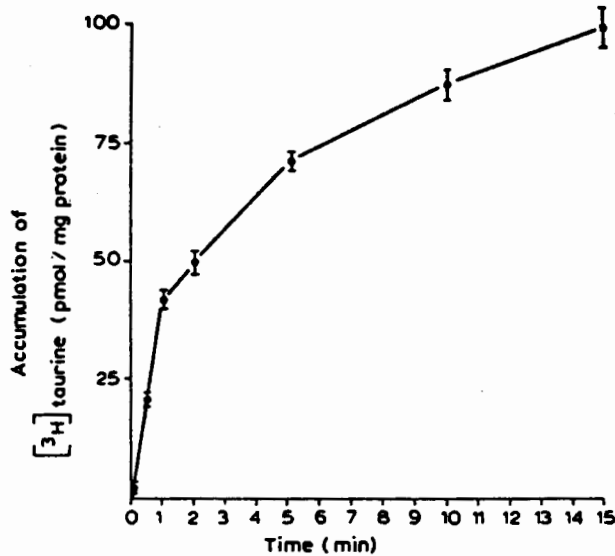


Fig. 1. Time-course of capillary uptake of [ $^3\text{H}$ ]taurine. Uptake assay was carried out for the indicated time intervals in the presence of sodium ion and in the presence of 0.1 mM unlabeled taurine as described in Materials and Methods. Results are the means  $\pm$  S.D. of three determinations.

mM) as described in Materials and Methods. Taurine uptake in the presence of NaCl as a function of taurine concentration followed a hyperbolic curve (Fig. 2, upper curve). When NaCl was replaced by choline chloride, the uptake increased linearly with increasing taurine concentration (Fig. 2, lower curve). The total uptake in the presence of NaCl is the sum of both sodium-ion-mediated and nonsaturable uptake, whereas in the presence of choline chloride the uptake is nonsaturable and is therefore the result of diffusion. The sodium-ion-de-

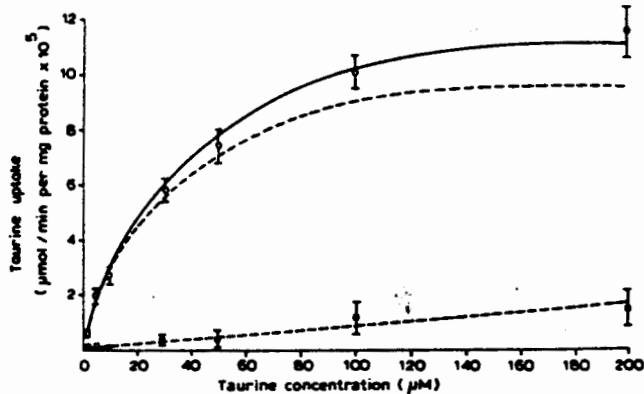


Fig. 2. Taurine uptake by the brain capillaries as a function of taurine concentration (2 to 200  $\mu\text{M}$ ). The curves represent total uptake in the presence of sodium ion (upper curve), in the presence of choline chloride which corresponds to the nonsaturable uptake component in the absence of sodium ion (lower curve), and the saturable uptake which is the difference between total and nonsaturable uptakes (middle curve). Results are the means  $\pm$  S.D. of three determinations.

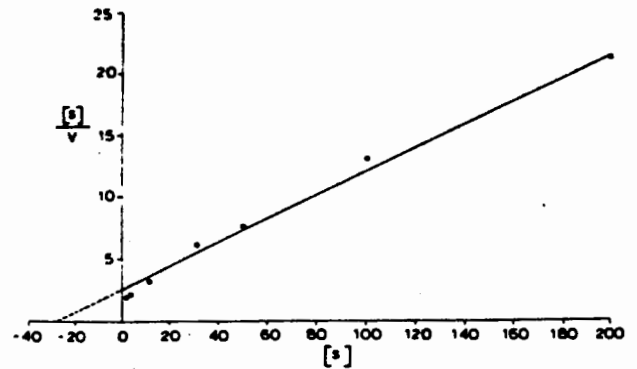


Fig. 3. Hanes plot of the sodium-ion-dependent taurine uptake by isolated brain capillaries. A least-squares fit of the data to a straight line yields a  $K_m$  of 27.5  $\mu\text{M}$  and a  $V_{max}$  of  $1.06 \times 10^{-4}$   $\mu\text{mol}/\text{min}$  per mg of protein, with a correlation coefficient of  $r = 0.995$ .  $[S]$  = taurine concentration ( $\mu\text{M}$ ) and  $V = \mu\text{mol}/\text{min}$  per mg of protein  $\times 10^5$ .

pendent uptake was calculated as the difference between the total uptake and the nonsaturable component (Fig. 2, middle curve). This curve is characteristic of a saturable transport system that follows Michaelis-Menten kinetics. A Hanes plot of the data obtained for the sodium-ion-dependent taurine uptake indicates the presence of a high-affinity transport system with apparent  $K_m$  equal to 27.5  $\mu\text{M}$  and  $V_{max}$  equal to  $1.06 \times 10^{-4}$   $\mu\text{mol}/\text{min}$  per mg protein (Fig. 3).

Nine amino acids, structurally analogous to taurine as well as taurine itself, were studied at a single concentration (0.1 mM) for their ability to inhibit competitively the uptake of [ $^3\text{H}$ ]taurine by isolated brain

TABLE I

Effects of taurine and its structural analogues and ouabain on [ $^3\text{H}$ ]taurine uptake by isolated rat brain capillaries

Brain capillaries were incubated for 20 min in buffer A prior to uptake studies. After preincubation, 0.05 ml of buffer A (without BSA) containing 3.4  $\mu\text{M}$  [ $^3\text{H}$ ]taurine (1  $\mu\text{Ci}/\text{ml}$ ), [ $^{14}\text{C}$ ]sucrose (0.2  $\mu\text{Ci}/\text{ml}$ ) as well as the stated compounds (0.1 mM), were added to capillary suspensions. The uptake was terminated after 1 min of incubation as described in Materials and Methods. No structural analogue was present in the control reaction mixture. Results are the means  $\pm$  S.D. from three determinations. 100% uptake corresponds to 13.2 pmol/min per mg protein.

Compound (0.1 mM)	Uptake $\pm$ S.D. (% of control)
None (control)	100 $\pm$ 6
Taurine	17 $\pm$ 2
Hypotaurine	17 $\pm$ 3
$\beta$ -Alanine	36 $\pm$ 4
GABA	70 $\pm$ 6
L-Methionine	97 $\pm$ 8
L-DABA	97 $\pm$ 7
Isothionic acid	99 $\pm$ 7
Glycine	101 $\pm$ 9
L-Cysteinesulfonic acid	102 $\pm$ 8
Cystamine	102 $\pm$ 9
Ouabain (1 mM)	76 $\pm$ 4

capillaries. Among these compounds, hypotaurine and taurine strongly inhibited the uptake of labeled taurine, whereas  $\beta$ -alanine and GABA inhibited the uptake by 64% and 30%, respectively (Table I). L-Methionine, L-DABA, isethionic acid, glycine, L-cysteinesulfonic acid and cystamine did not have any inhibitory effect on the uptake of labeled taurine. Ouabain, an inhibitor of  $\text{Na}^+/\text{K}^+$ -ATPase, inhibited the uptake of labeled taurine by 24%.

## Discussion

The results reported in this paper show that isolated rat brain capillaries possess a high-affinity transport system for taurine which is saturable and entirely sodium-ion-dependent. The importance of an  $\text{Na}^+$  gradient for the accumulation of taurine in brain capillaries was demonstrated by the replacement of sodium ion by choline, which completely abolished taurine uptake. The total inhibition of transport following the removal of sodium ion indicates that the system is dependent upon the presence of this ion in the external medium. Thus, the energy required for this transport activity may be provided by the electrochemical potential created by an  $\text{Na}^+$  gradient across the membrane of brain capillary endothelial cells. The sodium-ion-dependent, high-affinity uptake of taurine has been described by other investigators studying brain synaptosomes [18], membrane vesicles of neuroblastoma [19], cultured cerebellar granule cells and astrocytes [16], and glioma cells [15]. Our results confirm and extend these data with respect to isolated rat brain capillaries.

The uptake of taurine was only slightly inhibited by ouabain, an inhibitor of  $\text{Na}^+/\text{K}^+$ -ATPase. This relative insensitivity of taurine transport to ouabain may be due to the short incubation period (1 min) used in these experiments; Ouabain may require more time to act. Experiments in which the incubation period with ouabain is extended have shown that ouabain markedly inhibits cerebral capillary uptake of substances such as  $\alpha$ -methylaminoisobutyric acid [26].  $\text{Na}^+/\text{K}^+$ -ATPase, which is most active in the antiluminal membranes of brain capillary endothelial cells [27], is responsible for the active transport from brain to blood of such ions as potassium [28] and of several amino acids. Given that ouabain did exhibit a slight inhibitory effect during the short incubation time employed here, the involvement of  $\text{Na}^+/\text{K}^+$ -ATPase in the active transport of taurine cannot be ruled out.

The sodium-ion-dependent uptake of taurine in brain capillaries followed Michaelis-Menten kinetics when the amino-acid concentration was in the range of 2 to 200  $\mu\text{M}$ . The  $K_m$  of the taurine uptake was on the order of 27.5  $\mu\text{M}$ , which is similar to that found in a number of cells and tissues other than brain capillaries [16,19,29],

and it is of the same order of magnitude as those of the high-affinity transport systems for GABA, glycine, and noradrenaline in regions where these compounds are thought to have a transmitter role [3,15].

The substrate specificity of taurine transport system was determined by the addition of 0.1 mM unlabeled taurine and its structural analogs (Table I). Only amino acids closely related to taurine, such as hypotaurine and  $\beta$ -alanine, appear, *in vitro*, to inhibit the accumulation of labeled taurine. This is consistent with the reported interaction of these amino acids with taurine transport in neuronal [14] and in synaptosomal preparations [18]. By contrast, the  $\alpha$ -amino acids, such as methionine, glycine, L-DABA, and cysteine sulfonic acid, do not seem to compete with taurine uptake by isolated brain capillaries and do not influence taurine accumulation by this tissue. Taurine and hypotaurine inhibited the uptake of radioactive taurine to the same extent. This finding supports the hypothesis that one common carrier system exists for these two amino acids [31]. It should be noted that the presence of unlabeled taurine does not actually inhibit taurine uptake; rather, the uptake system is saturated in proportion to the relative concentrations of unlabeled and labeled taurine. Since, in the incubation medium, the concentration of unlabeled taurine (0.1 mM) was much higher than that of labeled taurine, a great reduction (inhibition) in radio-labeled taurine uptake was observed. This suggests the presence of a saturable process. Furthermore, these results suggest that  $\beta$ -alanine, which inhibited the uptake of labeled taurine by 64%, shares to a large extent the same pathway as taurine, whereas GABA, which was much less inhibitory, may utilize a different system of transport which overlaps that of taurine. It appears, therefore, that the saturable, sodium-ion-dependent system for taurine transport into isolated brain capillaries described in the present work is highly specific for this amino acid. This uptake system resembles those reported for various neurotransmitters [30] insofar as it is both high-affinity and totally sodium-dependent.

Isolated capillaries *in vitro* provide an excellent model for the study of transport system in the brain-to-blood direction. The surface area of the antiluminal membrane in these capillaries greatly exceeds that of the luminal membrane [32]. Furthermore, since in these preparations the lumen is partially collapsed [24,33], it is the antiluminal membrane which is more accessible to the substrate. Therefore, it appears likely that the taurine uptake system we have described here is present on the antiluminal surface.

Despite changes in plasma taurine concentrations, brain taurine levels remain unaltered [21]. The cerebral taurine level appears to be predominantly regulated at the level of endogenous biosynthesis of this amino acid [34]. There is also evidence to suggest that dietary taurine can be incorporated into rat brain, indicating

that movement of taurine across the blood-brain barrier occurs and could contribute to brain taurine levels [35]. Our data suggest that a transport system for taurine on the antiluminal surface of capillary endothelial cells may also participate in regulation of taurine concentration in the brain extracellular space. Abnormal functioning of this system may be one of the factors involved in the pathogenesis of seizures.

#### Acknowledgements

We are grateful to Dr. K. Pepper for reviewing the manuscript. This work was supported by CNIEL.

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