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Brain Research 692 (1995) 57–65

BRAIN RESEARCH

Research report

Taurine transport at the blood-brain barrier: an in vivo brain perfusion study

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Accepted 2 May 1995

Abstract

Taurine transport into six brain regions of equithesin-anesthetized rats was studied by the in situ brain perfusion technique. This technique gives both accurate measurements of cerebrovascular amino acid transport and allows complete control of the perfusate amino acid composition. Final wash procedure showed that taurine efflux occurred rapidly from endothelial cells. The taurine influx into endothelial cells was sodium and chloride dependent suggesting that the sodium and chloride gradients are the principal source of energy for taurine transport into endothelial cells. Taurine transport could be fitted by a model with saturable components. The kinetic constants in the parietal cortex were 1.4×10^{-4} μmol/s/g for the apparent V_{max} and 0.078 mM for the apparent K_m. Competition experiments in the presence of sodium ions showed that [^{14}C]taurine uptake was strongly inhibited by the structural analogs of taurine, hypotaurine and β-alanine.

Keywords: Blood-brain barrier; Taurine; Transport; Brain perfusion technique; Permeability; Endothelial cell

1. Introduction

The high concentration of taurine in brain suggests that it is important as an osmotically active substance involved in cell volume regulation [38], perhaps as a neuromodulator [24,27], an antioxidant [39] and as a membrane stabilizer [26]. Taurine is synthesized in the mammalian body, mainly in the liver and brain, from the sulfur-containing amino acids methionine and cysteine [15]. There is also evidence that dietary taurine can be incorporated into rat brain, indicating that taurine is transported across the blood-brain barrier (BBB) [14].

The cerebral endothelium, in addition to restricting the exchanges of ions, proteins and hydrophilic non-electrolytes, has specific transport systems for the transfer of essential nutrients from plasma to brain. No saturable transport system for taurine has been found by the intravenous technique although studies using the carotid injection technique in immature animals have shown a small decrease in the transport of radioactive taurine when cold taurine was added to the injection medium [18].

Tayarani et al. [36] showed a sodium-dependent, high affinity uptake of taurine into isolated microvessels with a K_m of 0.028 mM. But this in vitro study did not indicate whether the carrier was on the luminal or abluminal membrane of the endothelial cells. This low K_m value obtained by Tayarani et al. suggests that the transport system might be saturated with taurine in intravenous studies, and thus would appear not to exist [17]. The brain perfusion technique [35] offers two major advantages over the other in vivo methods: the brain uptake index [25] and the intravenous technique [23]. The perfusion technique is 100-times more sensitive than the Oldendorf method, and it allows virtually complete control of solute concentrations in brain capillaries.

We have used this technique to determine whether there is a saturable transport system for taurine at the luminal membrane of brain capillaries, and if it exists, to try to measure the kinetic constants, test its sodium and chloride dependency and examine the effects of the taurine analogs, GABA, β-alanine and hypotaurine, on taurine influx. The results indicate that a high affinity, sodium and chloride dependent carrier for taurine is present at the luminal side of the endothelial cells, and that it is shared by β-alanine and hypotaurine.

2. Materials and methods

Male Sprague-Dawley rats weighing 300–400 g were obtained from Iffa Credo (69210, L'Arbresle, France).

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SSDI 0006-8993(95)00648-6

They were kept in a room controlled for temperature (21°C) and light (12:12 h L/D) and given free access to standard chow (UAR, 91360, Epinay sur Orge, France) and water until just before surgery. [1,2-^{14}C]taurine (specific activity, 92.1 mCi/mmol) was obtained from New England Nuclear (Dupont de Nemours, France). Radiochemical purity of [1,2-^{14}C]taurine was verified by thin layer chromatography on silica gel with two solvent systems: n-butanol/acid acetic/water (50:8:20) and n-propanol/ammonium hydroxide (70/130). [6,6′(n)-^{3}H]sucrose (specific activity, 10.2 Ci/mmol) was obtained from Amersham (France).

GABA, β-alanine and hypotaurine were purchased from Sigma (38297 St Quentin Fallavier, France). All experiments were conducted in accordance with the Ministry of Agriculture and Forests authorisation no. 04050.

2.1. Perfusion procedures

Surgical procedure

Rats were anesthetized with an intraperitoneal injection of 300 μl/100 g body weight of Equithesin (4.25% w/v chloral hydrate, 2.12% w/v $MgSO_4$, 16.2% v/v pentobarbital, 42.8% v/v propylene glycol and 11.5% v/v ethanol 90°C). The influx of taurine into the brain was then measured by the in situ brain perfusion technique with some modifications [30]. This procedure does not disrupt the BBB [33]. The right common carotid artery (rCCA) was exposed and the right occipital, pterygopalatine and superior thyroid arteries were coagulated and cut. Two tight ligatures were placed one on the heart sided pole of the rCCA, and the other on the external carotid close to the rCCA bifurcation. Following surgery, the body temperature was maintained between 37°C and 38°C using a rectal thermistor connected to a temperature monitor.

Blood to brain transfer of taurine

In the first set of experiments, taurine influx across the cerebral endothelium was measured. A perfusion catheter (PE 50) filled with heparin (100 UI/ml) was inserted into the rCCA for perfusion of the internal carotid. This catheter was connected to a syringe containing 0.5 μCi/ml [^{14}C]taurine, 2.5 μCi/ml [^{3}H]sucrose dissolved in HCO_3^--buffered physiological saline (in mM: 128 NaCl, 24 $NaHCO_3$, 4.2 KCl, 2.4 NaH_2PO_4, 1.5 $CaCl_2$, 0.9 $MgSO_4$ and 9 D-glucose). The perfusion fluid was filtered, gassed with 95% air, 5% CO_2 and warmed to 37°C. The pH of the solution was 7.4. The syringe containing the perfusion fluid was placed in an infusion pump (Infors AG Basels) and attached to the carotid catheter. The thorax of the rat was opened, the heart was cut, and the perfusion was started at a flow rate of 5 ml/min.

The second set of experiments consisted of looking for sodium and chloride dependency as well as saturability of taurine influx. So perfusions were performed with sodium-free and chloride-free buffers. In low sodium experiments, the first four components of the saline were replaced by 140 mM choline chloride, 4 mM KOH and 11 mM HEPES (pH = 7.4) and the fluid was gassed with 100% air rather than 95% air + 5% CO_2. In perfusion with chloride-free buffer, KCl, NaCl and $CaCl_2$ were replaced by isoosmolar amounts of KNO_3, $NaNO_3$ and $Ca(NO_3)_2$ respectively.

The taurine influx was also measured during perfusion with plasma. On the day of the experiment, whole blood was collected from the abdominal artery of heparinized donor rats and centrifuged for 20 min at 3000 × *g* at 4°C. The resulting plasma was filtered, oxygenated with 95% air, 5% CO_2 and warmed to 37°C. [^{14}C]Taurine and [^{3}H]sucrose were added to plasma at the same concentrations used in the saline perfusion experiments.

The concentration dependence of taurine influx was determined by perfusion with fluid containing [^{14}C]taurine, [^{3}H]sucrose and unlabelled taurine (0–0.5 μmol/ml).

The taurine analogs, β-alanine, hypotaurine and GABA, were added to the saline perfusate at 0.2 mM to determine their effects on taurine transport. The perfusion times were 10 to 60 s for the first experiments and 10 s for all subsequent experiments, with correction for the time required for the perfusate to reach the cerebral hemisphere (5 s) [35].

2.2. Wash procedure

The carotid catheter was connected to a four-way junction which allowed connections to be made to two syringe pumps (A and B, Infors Basels and Infu 362, respectively) containing the perfusate with the radiotracer (syringe A) and without radiotracer (syringe B). The washout solution with the same composition as that of the buffer containing tracer was filtered and its pH was checked to be 7.4. After the carotid cannulation was completed and the appropriate connections were made, syringe A was discharged at a rate of 5 ml/min for 10 to 60 s. Syringe A was switched off and syringe B was switched on simultaneously. After 35 s (actually 30 s of washing) the rat was decapitated.

2.3. Processing of samples

At the end of perfusion, the rat was decapitated and the brain removed and placed on ice-chilled filter paper moistened with saline. The surface blood vessels and meninges were carefully removed, and the brain was dissected into eight different right hemisphere regions. Samples of perfusion fluids (buffer, sodium-free, chloride-free buffers and plasma) were also collected in triplicate (20 μl) to determine perfusate tracer concentration. Tissues and perfusate samples were placed in tared vials that were reweighed. Samples were digested for 2 h in 1 ml of soluene (Soluene 350, Packard) at 56°C, cooled and mixed with a liquid scintillation cocktail (Toluene scintillator, Packard Rungis, France). The ^{14}C and ^{3}H were then counted using a dual

labelled counting program on a scintillation spectrophotometer (Intertechnic SL 3000, Kontro, France).

2.4. Tracer distribution in brain compartments

The distribution of [^{14}C]taurine between brain microvascular and parenchymal compartments was assessed using the capillary depletion method of Triguero [37]. Rats were perfused with buffer perfusate containing [^{14}C]taurine and [^{3}H]sucrose. At the end of perfusion, the right cerebral hemisphere was removed, cleaned of meninges and choroid plexuses, weighed and homogenized in 3.5 ml of physiological buffer (pH 7.4, 10 mM HEPES, 141 mM NaCl, 4 mM KCl, 2.8 mM $CaCl_2$, 1 mM $MgSO_4$, 1 mM NaH_2PO_4 and 10 mM D-glucose). After 15 strokes, 4 ml of a chilled 40% neutral dextran solution (MW 70,000) was added to obtain a final concentration of 20%. All homogenizations were performed at 4°C in less than 1 min.

After taking an aliquot of homogenate, the solution was then centrifuged at $5400 \times g$ for 15 min to obtain a vascular pellet and a supernatant that was capillary free. Tracer levels were determined for pellet, homogenate and supernatant.

2.5. Calculations

Taurine uptake into the brain was expressed as an apparent distribution volume: V_d (ml/g) defined as:

$$V_d = q^*_{tis}/C^*_{pf} \quad (1)$$

where q^*_{tis} (dpm/g) is the quantity of tracer per gram wet weight of tissue, and C^*_{pf} (dpm/ml) is the tracer concentration in the perfusate or plasma.

Vascular radioactivity was subtracted from all tissue samples as:

$$q^*_{tis} = q^*_{tot} - V_v C^*_{pf} \quad (2)$$

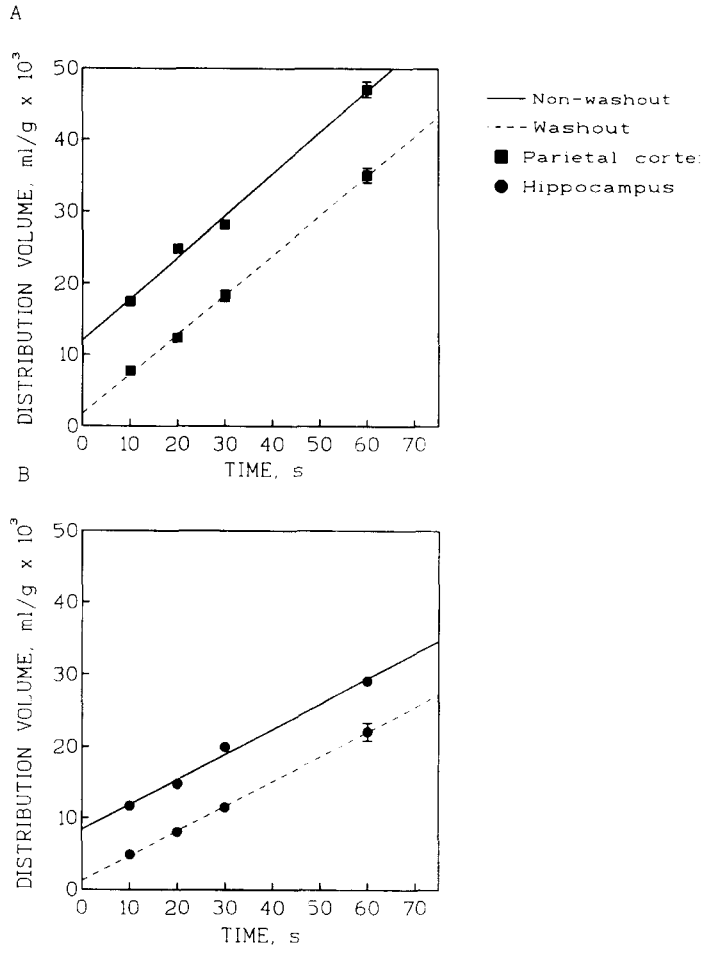

Fig. 1. [^{14}C]Taurine uptake into the right parietal cortex and hippocampus during perfusion with buffer with or without final washout procedure. Uptake is expressed as the q^*_{tis}/C^*_{pf} ratio. Data are means ± SEM (bars) for 4–6 rats. The line is the least squares fit of the equation $q^*_{tis}/C^*_{pf} = K_{in}T + V_i$ to the data, where K_{in} is a transfer coefficient and V_i is a rapidly equilibrating space separate from that accessible to [^{3}H]sucrose. Best-fit values are $K_{in} = 5.82 \pm 0.21 \times 10^{-4}$ ml/s/g and $V_i = 11.82 \pm 0.72 \times 10^{-3}$ ml/g for the parietal cortex (A) and $K_{in} = 3.52 \pm 0.19 \times 10^{-4}$ ml/s/g and $V_i = 8.3 \pm 0.63 \times 10^{-3}$ ml/g for the hippocampus (B) for the perfusion without final wash procedure. K_{in} obtained from perfusion procedures without final wash were not significantly different from those obtained with final wash for both cerebral regions but Y-intercept values (V_i) were close to zero ($P > 0.05$).

where q^*_{tot} (dpm/g) is the total quantity of tracer in the tissue sample (vascular + extravascular).

V_v (ml/g) was measured in each animal using [^{3}H]sucrose, which does not measurably cross the blood-brain barrier during short perfusions.

The blood-brain barrier transfer (K_{in}) was calculated from:

$$K_{in} = \left[q^*_{tot} - V_v C^*_{pf} \right] / T C^*_{pf} \quad (3)$$

where T was time in seconds.

The influx (J_{in}) for taurine was calculated as:

$$J_{in} = K_{in} C_{pf} \quad (4)$$

where C_{pf} is the total concentration of taurine in the perfusate and K_{in} (ml/s/g) is the taurine transfer coefficient calculated according to Eq.3. The concentration dependence of taurine uptake was analysed using three models:

The first model had one saturable Michaelis-Menten component:

$$J_{in} = V_{max} C_{pf} / (K_m + C_{pf}) \quad (5)$$

where K_m is the half saturation concentration (mM) and V_{max} (μmol/s/g) is the maximal influx rate.

The second model contained a non-saturable diffusion constant (K_d), as well as a saturable Michaelis-Menten component:

$$J_{in} = \left[V_{max} C_{pf} / (K_m + C_{pf}) \right] + K_d C_{pf} \quad (6)$$

The third model had two saturable components.

$$J_{in} = \left[V_{max1} C_{pf} / K_{m1} + C_{pf} \right] + \left[V_{max2} C_{pf} / K_{m2} + C_{pf} \right] \quad (7)$$

The 32 observations (J_{in}) for each brain region were divided arbitrarily into four sets of data, so that each curve had one observation for each of the eight concentrations. Best fit parameters were obtained from a weighted non-linear regression analysis of each data set, and the means and S.E.M. were calculated for $n = 4$. Weighting factors ($WT = \text{S.D.}^{-2}$) were calculated as J_{in}^{-2} [1]. Comparison between fits was assessed by the Fischer F test.

2.6. Statistical analysis

One-way analysis of variance and the Bonferroni multiple-comparison test were used to compare the individual means. Dunnett's test was used for comparisons with control. In all cases, the criterion for statistical significance was $P < 0.05$.

3. Results

The blood-to-brain distributions of [^{14}C]taurine (V_d) in the rat hippocampus and parietal cortex are shown in Fig. 1. If uptake is linear, K_{in} can be estimated from a plot of V_d against time according to the formula:

$$q^*_{tis}/C^*_{pf} = K_{in} T + V_i \quad (8)$$

Such a linear uptake has often been observed, and V_i is called a rapidly equilibrating space [2] if it is significantly different from zero. There is a linear relationship between V_d and T from 10 to 60 s with a slope (K_{in}) of 3.52×10^{-4} ml/s/g for the hippocampus and 5.82×10^{-4} ml/s/g for the parietal cortex. The ordinate intercepts V_i, significantly different from zero, were 8.3×10^{-3} ml/g for the hippocampus and 11.8×10^{-3} ml/g for the parietal cortex.

Estimates of K_{in} and V_i for various brain regions from multiple time/graphic analysis of the [^{14}C]taurine data are given in Table 1. The problem lies in identifying what represents the rapidly equilibrating space. It could be taurine bound to the luminal membrane or taurine trapped in endothelial cells and/or followed by efflux of taurine

Table 1
Capillary transfer constants (K_{in}) and rapidly reversible volumes for [^{14}C]taurine (V_i) for various brain regions of the rat

Brain region	K_{in} (ml/s/g×10^4)		V_i (ml/g×10^3)	
	Non-washout	Washout	Non-washout	Washout
Olfactory bulbs	5.45±0.41	5.46±0.45	10.05±1.38	0.86±1.66
Hypothalamus	5.79±0.64	6.81±0.11	11.16±2.13	−1.77±3.8
Frontal cortex	4.76±0.2	4.97±0.24	10.84±0.67	1.74±0.89
Occipital cortex	5.21±0.24	4.87±0.25	10.63±0.82	0.85±0.91
Parietal cortex	5.82±0.21	5.52±0.18	11.82±0.72	1.82±0.66
Striatum	4.5 ±0.26	4.03±0.15	10.23±0.87	1.83±0.56
Hippocampus	3.52±0.19	3.45±0.2	8.34±0.63	1.27±0.7
Thalamus	4.94±0.21	5.04±0.27	11.03±0.69	2.41±0.99

Values are means±S.E.M. for $n = 16$–19. The perfusion rate was 5 ml/min with perfusion times of 10–60 s. K_{in} was obtained by fitting Eq. (8) ($q^*_{tis}/C^*_{pf} = K_{in}T + V_i$) to the uptake data after correction for residual intravascular tracer using [^{3}H]sucrose. Calculated V_i values differ significantly from zero ($P < 0.05$) with the non-washout procedures while they do not differ from zero with the washout.

Table 2
Distribution of [^{14}C]taurine and [^{3}H]sucrose in brain homogenate supernatant and pellet fractions following 10 s brain perfusion with physiological saline (Distribution volume, ml/g×10^3 of brain)

Tracer	Brain homogenate	Supernatant	Pellet
[^{14}C]Taurine	17.91±0.44	15.36±0.48	1.18 ±0.02
[^{3}H]Sucrose	6.05±0.26	5.42±0.08	0.061±0.005
[^{14}C]Taurine (corrected)	11.86±0.42	9.94±0.47	1.12 ±0.02
	(100%)	(84%)	(9.5%)

Values are means±S.E.M. ($n = 6$). Brains were perfused for 10 s at 5 ml/min with buffer perfusate containing [^{14}C]taurine and [^{3}H]sucrose. At the end of perfusion, brains were removed and tracer distributions were determined using the capillary depletion method of Triguero et al. [37]. Values are expressed as distribution volumes (q^*_{tis}/C^*_{pf} = (total dpm per compartment/brain tissue weight)/(dpm/ml perfusate). [^{14}C]Taurine (corrected) indicates values that were adjusted for residual intravascular tracer as [^{14}C]taurine (ml/g×10^3)−[^{3}H]sucrose (ml/g×10^3).

Table 3
Apparent transfer coefficients (K_{app}, ml/s/g$\times 10^4$) for [^{14}C]taurine into brain from saline perfusate

Brain region	K_{app}
Olfactory bulb	16.32 ± 1.05 [a]
Hypothalamus	17.39 ± 1.45 [a]
Frontal cortex	14.80 ± 0.64
Occipital cortex	16.90 ± 0.69 [a]
Parietal cortex	16.17 ± 0.83 [a]
Striatum	14.88 ± 0.98
Hippocampus	11.31 ± 0.6
Thalamus	17.18 ± 0.89 [a]

Values are means $\pm$ S.E.M. for 4–6 animals. The perfusion rate was 5 ml/min. Perfusion time was 10 seconds. K_{app} was calculated from Eq. (3): $K_{in} = [q^*_{tot} - V_v C^*_{pf}] / TC^*_{pf} = K_{app}$.
[a] Differs significantly from the mean value for hippocampus, by Bonferroni adjustment.

back into the blood. To choose between these three possibilities, two experiments were performed: first, we studied the uptake of [^{14}C]taurine as a function of time with the final wash method. The intercept at zero time was very close to the origin, $1.27 \pm 0.7 \times 10^{-3}$ ml/g into hippocampus and $1.82 \pm 0.66 \times 10^{-3}$ ml/g into parietal cortex, indicating that the final wash procedure removed any [^{14}C]taurine within the microvessels or bound to the luminal membrane of its endothelium (Fig. 1). In the second experiments, distribution of [^{14}C]taurine in the brain capillary and parenchymal compartments was measured after perfusion using the capillary depletion method of Triguero et al. [37]. By this method, 84% of the brain [^{14}C]taurine was associated with the parenchymal fraction and 9.5% with the vascular pellet (Table 2). The results suggest that [^{14}C]taurine have crossed the BBB and that efflux occurred from endothelial cells.

Table 3 shows that the transfer coefficient calculated

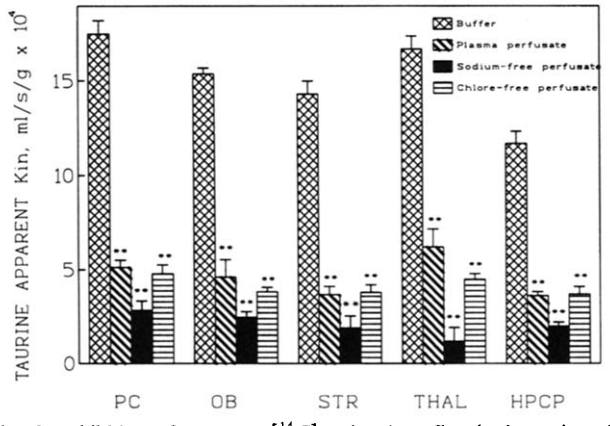

Fig. 2. Inhibition of apparent [^{14}C]taurine into five brain regions by plasma, sodium-free and chloride-free perfusates. Values are apparent transfer coefficients and are the means $\pm$ S.E.M. for 4–6 animals. Values for saline significantly exceeded those for other fluids (** $P < 0.001$). PC, parietal cortex; OB, olfactory bulb; STR, striatum; HPCP, hippocampus; THAL, thalamus.

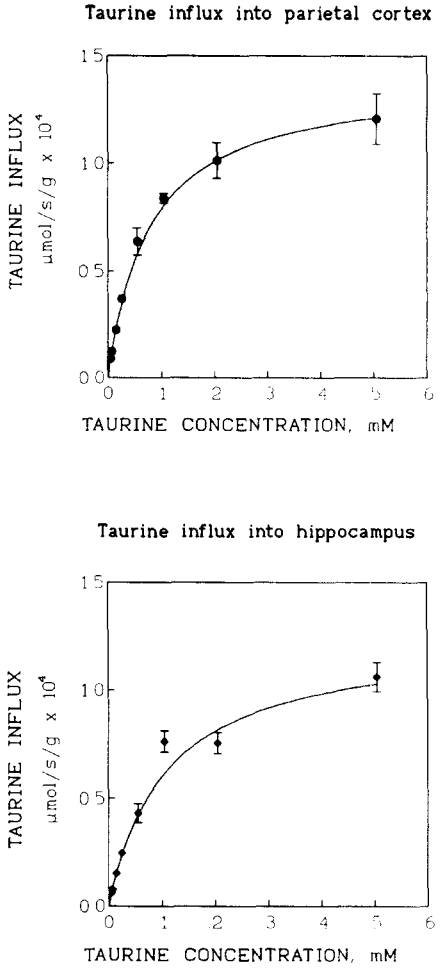

Fig. 3. One-directional apparent taurine influxes into the parietal cortex and hippocampus endothelial cells as a function of taurine concentration in buffer perfusion fluid. Data are means $\pm$ S.E.M. (bars) values for four rats. The curves represent saturable influx as predicted by Eq. (5), where $V_{max} = 1.37 \times 10^{-4}$ μmol/s/g and K_m 0.081 mM for the parietal cortex and $V_{max} = 1.25 \times 10^{-4}$ μmol/s/g and $K_m = 0.109$ mM for the hippocampus.

according to Eq. (3) which is probably only an apparent influx from 0 to 10 s, is much higher than at later times. As we wanted to study taurine initial transport at the luminal membrane of capillaries and not its blood to brain transfer, for all the following experiments, we chose 10 s for calculating K_{in} according to equation 3, and this calculated K_{in} was termed apparent K_{in} (K_{app}). Apparent K_{in} would represent the initial transfer of taurine into the endothelial cell: it was calculated according to Eq. (3), with the single point method. This method uses the plasma volume to correct the total brain radioactivity remaining in the vascular space and underestimate the V_d because backflux was neglected. In our study in the Eq. (3), the luminal membrane was the rate limiting step and K_{app} is the coefficient transfer across the luminal membrane: probably this K_{app} is much higher but it is impossible to deal with a shorter time.

K_{in} calculated according to Eq. (8) represents the net

Table 4
Apparent V_{max} and K_m values for cerebrovascular saturable transport of [^{14}C]taurine from saline perfusate

Brain region	Apparent V_{max} (μmol/s/g$\times 10^4$)	Apparent K_m (mM)
Olfactory bulbs	1.82 ± 0.17	0.115 ± 0.014
Hypothalamus	2.83 ± 0.19 [a]	0.174 ± 0.011 [a]
Frontal cortex	1.3 ± 0.1	0.082 ± 0.0005
Occipital cortex	1.37 ± 0.07	0.081 ± 0.006
Parietal cortex	1.4 ± 0.13	0.078 ± 0.009
Striatum	1.26 ± 0.06	0.092 ± 0.007
Hippocampus	1.25 ± 0.063	0.109 ± 0.009
Thalamus	1.4 ± 0.009	0.082 ± 0.003

[a] Differs significantly from the mean value for the parietal cortex by the Bonferroni test. Values are means ± S.E.M. for 4 determinations. The 32 K_{in} observations for each brain region were divided arbitrarily into four data sets. Best-fit values for V_{max} and K_m were obtained by fitting to the equation $J_{in} = [V_{max}C_{pf}/(K_m + C_{pf})]$.

Table 5
Inhibition of apparent [^{14}C]taurine transport into rat brain from saline perfusate by GABA, β-alanine, hypotaurine and taurine

	Percent inhibition			
	GABA (0.2 mM)	β-alanine (0.2 mM)	Hypotaurine (0.2 mM)	Taurine (0.2 mM)
Olfactory bulb	5	56.7	74.3	69.6
Hypothalamus	17.6	54.9	69.2	60
Frontal cortex	13.1	58.6	82.4	71.2
Occipital cortex	21.7	59.3	82.6	72.9
Parietal cortex	11.9	52.3	83.2	70.7
Striatum	20.4	59.8	84.7	72.3
Hippocampus	11.8	60.9	81	67.5
Thalamus	19.7	56.9	80.7	70.6

Perfusate consisted of HCO_3^--buffered physiological saline containing 0.5 μCi/ml [^{14}C]taurine and 0.2 mM taurine or taurine analogs.

transfer of radiotracer in the brain and in this case, luminal and abluminal membranes are the rate limiting steps.

The sucrose vascular volumes given by the three perfusates were measured and found similar to published values: 0.7–1.2% [35].

The effect on taurine uptake of removing sodium and chloride which permitted to distinguish between binding and influx, and perfusion with plasma and addition of several concentrations of cold taurine to the injectate were studied. Taurine uptake both into parietal cortex and hippocampus decreased by 84% at 10 s in sodium-free perfusate and by 69% and 64% respectively in chloride-free perfusate (Fig. 2). The K_{app} in plasma was 4.4×10^{-4} ml/s/g in the parietal cortex and 3.2×10^{-4} ml/s/g in the hippocampus (Fig. 2). Perfusion with plasma reduced taurine uptake into parietal cortex and hippocampus by 73%.

The concentration dependence of taurine influx into the brain from a saline perfusate is shown in Fig. 3. It is adequately. described by a single saturable transport component. The influx data were fitted to the Michaelis-Menten equation to obtain estimates of saturable apparent V_{max} (1.4×10^{-4} μmol/s/g) and K_m (0.078 mM) in the parietal cortex (Table 4). There were no significant regional differences in either V_{max} or K_m, except for hypothalamus, where they were significantly higher ($P < 0.05$), probably because it contains a small area with no BBB (median eminence). Models with a non-saturable component (K_d, Eq. (6)) or two saturable components (K_{m1}, K_{m2} and V_{max1}, V_{max2}, Eq. (7)) did not significantly improve the fit, as indicated by the F test ($P > 0.05$).

Three amino acids structurally analogous to taurine and taurine itself, all at 0.2 mM, compete with the [^{14}C]taurine uptake by the endothelial cells (Fig. 4). Hypotaurine and taurine strongly inhibited the uptake of taurine (by 83.25% and 70% respectively), whereas β-alanine decreased the K_{app} by 54.3% and GABA by 11.94% (Table 5).

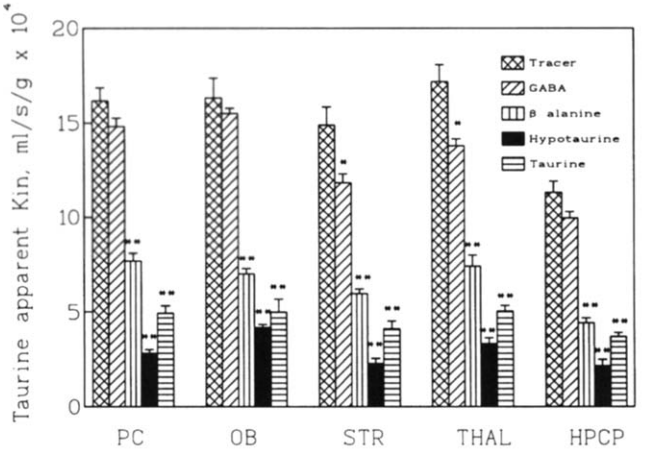

Fig. 4. Inhibition of apparent [^{14}C]taurine uptake by competing amino acids. Amino acids were added to saline perfusate at a concentration of 0.2 mM. Each bar represents the mean ± S.E.M. for 4–6 perfusions.(* $P < 0.05$, ** $P < 0.001$).

4. Discussion

No blood-brain transport system has yet been described for taurine. We therefore used the in situ brain perfusion technique [35] with rapid perfusion of brain vessels with a buffer containing only the substance studied, to examine its uptake. The study of blood-brain transport over time indicated that the blood-brain influx was linear from 10 to 60 s but that the extrapolated line did not pass through the origin at time 0 , as it does for amino acids like phenylalanine [20]. A similar phenomenon has been-described for the blood-brain transport of several substances [2,4] and the space above zero has been called the rapidly equilibrating space because it rapidly and reversibly exchanges with plasma. This space could represent binding to the endothelial cell membrane, or taurine influx into the endothelial cell followed by efflux into blood. Final wash procedures and capillary depletion technique indicated that efflux probably occurred and this explains why taurine vascular volume was perhaps underestimated when there was no washout. This efflux was constant as the two plots were strictly parallel for the two procedures. Taurine influx from 0 to 10 s was thus much higher than at later times: at 10 s, we calculated an apparent K_{in} which represented not the net flux of taurine from blood to brain but the flux of this substance into the endothelial cell and brain. Probably the real coefficient transfer across the luminal membrane is much higher but it is impossible to deal with a shorter time to avoid efflux. We were interested in the initial entry of taurine into the endothelial cell at its luminal membrane and not its net entry into brain as given by the slope of the plot (Fig. 1).

We therefore looked for a transport system at the luminal membrane of endothelial cells and showed that the transport process is saturable, sodium and chloride dependent. The linear part of the influx thus perhaps reflects transport across the abluminal membrane.

As the labelled taurine uptake was lower with both plasma perfusate and cold taurine containing perfusate, there may be a saturable carrier for taurine at the luminal side of brain capillaries and it should be possible to measure its apparent K_m and V_m.

The importance of a sodium and chloride gradients for the transport of taurine into the endothelial cells was demonstrated by replacing the sodium and chloride ions by choline and nitrate respectively. This considerably reduced taurine uptake, indicating that the system depends upon the presence of sodium and chloride in the plasma. The energy required for this transport may thus be provided by the electrochemical potential created by the sodium and chloride gradients across the luminal membrane. This absolute sodium dependency of taurine is in accordance with earlier studies in cultured neuronal or glial cells and isolated brain microvessels [8,9,32,36]. Chloride requirement for taurine uptake has been demonstrated in several tissues, astrocytes [10], brain synaptosomes [19], placental brush-border membrane [21] and kidney [5]. Even an inwardly directed chloride gradient could not energize uptake in the absence of sodium, suggesting a functional interaction among sodium, chloride and substrate at the carrier site. These functional properties are similar to those described for taurine transport in other tissues and suggest structural similarities in these transport proteins. Taurine enters the brain via a sodium and chloride dependent carrier from blood to the endothelial cell.

Non-linear regression analysis (NLR) showed that these cells possess a single saturable influx system and was used to calculate the apparent kinetic constants shown in table 4. The luminal side of the endothelial cell possesses one sodium and chloride-dependent influx system for taurine. The system in the parietal cortex has a high affinity and a low capacity. It is difficult to compare these V_{max} and K_m with the kinetic constants of neutral amino acids at the BBB [34] as we estimated only their apparent values. Two influx systems have been found in other vertebrate cells [13] while invertebrate cells [29] and isolated brain microvessels [36] seem to have only one. Only a carrier transport has been found for taurine and not diffusion as for other amino acids; however, it was difficult to judge all the characteristics of transport when radiotracer measurements were made some time after transport had begun and when efflux had already started.

The results reflect the influx of zwitterionic molecules, since taurine is mainly zwitterionic at pH 7.4 (pK_a 1.5 and pK_b 8.82). The greatest inhibitory effect was obtained by zwitterionic substances in which the charged groups are separated by two methylene groups (hypotaurine and taurine). The importance of the distance between the two charged groups is reflected in the inhibitory effect of substances in which the carbon backbone is lengthened by a methylene group: GABA had little inhibitory effect. Hypotaurine, with a sulfur-containing tetrahedral group, was a significantly better inhibitor than β-alanine, which has a planar carboxyl group. Similar results have been obtained in the rat for the renal brush border membrane [31], hepatocytes [22], isolated brain microvessels [36] and cultured cerebellar granule cells and astrocytes [9]. The inhibition of taurine uptake by hypotaurine was greater than that by taurine; there is good evidence that there is one common carrier system for these two amino acids [16]. β-Alanine, which also inhibited the uptake of labeled taurine, seems to share the same uptake pathway as taurine, whereas GABA, which is much less inhibitory, may be taken up by a different system which overlaps that of taurine.

In addition to this transport system, part of the taurine in the brain can be synthesized in situ [12,28]. The perfusion method, unlike the intravenous injection method, is not subject to errors in measured influx due to tracer metabolism in tissues other than the brain. Once taurine has gained access to brain, its rate of metabolism is very slow [11], the major metabolite in the brain being acid

isethionic: it was demonstrated that only 6% of total radioactivity in the brain was in form of isethionic acid 15 min after an intraperitoneal injection of radioactive taurine [6]. As we checked the radiochemical purity of taurine in perfusate, it is [^{14}C]taurine that is gaining access to brain and not a metabolite.

Once taurine has crossed the endothelial cells, a very efficient carrier-mediated β amino acid specific transport-system, located in the plasma membrane of neurons [3] could transfer the taurine into neurons and help to maintain the steep taurine concentration gradient. The high concentration of taurine in neurons seems to be crucial for the regulation of cell volume and osmolarity [7].

Acknowledgements

We thank Dr. M. Debray (Laboratory of Biomathematics, Faculty of Pharmacy, Paris V) for statistical help and advice. We thank Dr. Q.R. Smith (Laboratory of Neurosciences, national institute on Aging, National Institutes of health, Bethesda, USA) for the gift of the four-way junction apparatus to perform the final wash method.

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