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Taurine concentration in the brain and in the plasma following intraperitoneal injections

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Summary. The effect of different taurine doses (0.050, 0.125, 0.250, 0.500 and 1.000 g/kg) administered intraperitoneally to Wistar rats was studied in both the plasma and the hippocampal microdialysate content.

The samples were analyzed by reverse phased HPLC for the microdialysate samples and by HPLC with ion-exchange post-column derivatization (ninhydrin) for the plasma samples.

In both plasma and microdialysate, we observed a dose dependent increase of taurine concentration. The AUC curves obtained from both microdialysate and plasma samples showed that the increase of taurine concentrations were linear. The mean ratio between AUC's microdialysate and plasma was 1.63 ± 0.21 showing thus an unbalance between plasma and brain taurine content; a mechanism which enhance taurine transfer from the plasma to the brain was assumed.

Keywords: Taurine – Microdialysis – Plasma – Hippocampus – Rat – HPLC

Introduction

Taurine, a sulfonated β amino acid, is nearly ubiquitously distributed in the body and its tissue concentration rates in the μmole per gram wet weight range, while the concentration in the body fluids are lower in the range of 10–100 μM. Musculature contains the majority of the total amount of body's taurine with the highest concentrations found in the brain, particularly in the cerebral cortex and cerebellum as well as in the olfactory bulb, and in the heart as well as the eye (Huxtable, 1992).

Taurine transport has been demonstrated in capillary preparations from rat brains (Tayarani et al., 1989), in primary cultures of bovine brain capillary endothelial cells (BCEC) (Tamai et al., 1995; Tsuji et al., 1996), in intact brains (Benrabh et al., 1995) and in cultured bovine artery endothelial (BAE) cells (Qian et al, 2000). The luminal uptake of taurine involved a single saturable process. The stoechiometry among taurine, Na^+ and Cl^- in the luminal uptake is one taurine molecule associated with two sodium ions and one chloride ion, implying a transporter of net positive charge (Benrabh et al., 1995; Tamai et al., 1995). Furthermore, taking the transport efficiencies in both membranes and physiological concentrations of taurine together, it is expected that *in vivo* net transport of taurine occur in the direction from blood to brain. Taurine is a hydrophilic compound which is poorly absorbed as it does not diffuse readily across membranes although taurine transporters are present on many cell types which is leading to an intracellular: extracellular concentration ratio as high as 600:1, then intracellular taurine concentrations are maintained at a significantly higher concentration than extracellular levels (Della Corte et al., 2002).

Taurine could play a role in a variety of physiological functions (osmoregulation, Ca^{++} modulation), pharmacological actions (neurotransmission, inhibitory neuromodulation) and pathological states.

The purpose of this study was to investigate, in both hippocampal microdialysate extracellular content and peripheral plasma content, the effect of a single ip injection of different taurine doses and consequently to determine the ratio of taurine absorption in the brain and in the body.

Materials and methods

Part I

Male Wistar rats, 200–250 g, 8 in each group, were housed in standard individual plastic cages and maintained in a temperature (22°C) and light controlled environment (light/darkness cycle 12 h/12 h, with lights on at 7.30 am) with food and tap water ad libitum.

Surgery

After anesthetization with chloral hydrate (400 mg/kg ip), the rats were placed in a stereotaxic apparatus. A guide cannula was inserted into the hippocampus CA2 region using standard stereotaxic techniques (A/P −4.3 mm; M/L 4.0 mm D/V −3.0 mm) according to the atlas of Paxinos and Watson (Paxinos and Watson, 1982). The guide cannula was secured to the skull with two steel screws and cranioplastic cement and kept patent with a 26 gauge stainless steel obturator. The dialysis experiments commenced at the earliest 72 h post operation recovery period. The dialysis probes were constructed as described by Robinson and Whishaw (1988). Dialysis tubing extended 3 mm beyond the tip of the probe. The probe was connected to a micro-infusion pump (Infusion syringe pump 22, Harvard apparatus) and continuously perfused at a flow of 1.1 μl/min with Ringers solution containing 145 mM NaCl, 4 mM KCl, 1.3 mM $CaCl_2$, buffered to pH 7.2 with phosphate buffer.

For the microdialysis experiments, groups of rat, eight in each group, received an intraperitoneal injection of taurine, 1.0, 0.5, 0.25, 0.125 or 0.05 g/kg. An appropriate control group was also injected with saline. Microdialysate samples were collected every 20 minutes over a 4 hour period and analyzed for their amino acid content by HPLC with electrochemical detection using pre column derivatization. The derivatizing agent, O-phthaldialdehyde, OPA, 27 mg, was dissolved in 1 ml methanol HPLC grade to which 10 μl mercaptoethanol was added; this solution was diluted with 9 ml 0.1 M sodium tetraborate, pH 9.3, and stored at 4°C in the dark. The working solution was prepared each day by diluting 1 ml of this solution with 3 ml 0.1 M sodium tetraborate. The dialysate sample, 20 μl, was mixed with the derivatization solution, 10 μl, within the automatic sampler after the internal standard homoserine 10^{-5} M had been added. The sample was then injected onto the HPLC system, which consisted of a LDC ConstaMetric 3200 pump delivering 0.80 ml/min of the mobile phase (0.1 M Na_2HPO_4, 0.13 mM EDTA, 27% methanol HPLC grade, 73% water, Millipore grade, pH 6.4) at a pressure of 5300 psi. Separation of the amino acids was achieved by reversed phase column chromatography, (125 × 3 mm, ODS Hypersil 3 μm) (VDS Optilab, Chromatographie Technik, GmbH) and detected coulometrically (ESA Coulochem II; Bedford, Mass. USA) using three electrodes, a guard (0.4 V), preoxidation (−0.4 V) and working (+0.6 V) electrodes (analytical cell ESA5011).

The position and height of taurine peak within the dialysates was compared to a solution of amino acids containing the same amino acids, 5×10^{-6}, and the taurine area quantified by a PC Integration pack (Kontron Instruments).

The mean baseline value was calculated by averaging the concentration of the three perfusate sample values immediately before injection of taurine. The variation of concentrations in each perfusate was then expressed as a percentage of the baseline value. An *in vitro* recovery of the microdialysis probes was performed in order to calculate the real concentration level in the studied brain region from the perfusate sample value.

Histology

Upon completion of experiments, rats were killed and the brain fixed with 10% formalin. Coronal sections through the extent of the cannula tracks were cut (100 μm) with a vibratome (Polaron H 1200, Biorad, Cambridge, MA, USA). Dialysis probe placement was localized according to the atlas of Paxinos & Watson (1982).

Part II

Eight group of 8 male Wistar rats, 200–250 g, were maintained in a temperature (22°C) and light controlled environment (light/darkness cycle 12 h/12 h, with lights on at 7.30 am) with food and tap water ad libitum. Blood samples were collected from the retro-orbital sinus under ether anesthesia using a micropipette at 0, 20, 40, 80, 120 and 240 min after taurine (1.0, 0.5, 0.25, 0.125 or 0.05 g/kg) ip administration. A control group was injected with saline. Samples were placed in microcentrifuge tubes containing sodium fluoride (S-1504, Sigma Aldrich Chemie, Gmbh, Steinheim, Germany) as the anticoagulant. The samples tubes were placed over wet ice until centrifugation at 15°C at 3000 rpm for 10 min to obtain plasma. The plasma samples were then stored immediately at −20°C until HPLC analysis. The red blood cells were discarded.

Plasma samples were treated as following: first Seraprep (Pickering Laboratories, Inc., Mountain View, CA, USA) was added to achieve the dilution factor which gave the best result, second the solution obtained was centrifuged at 15000 rpm during 5 min and third the supernatant was filtered through a 4 mm syringe filter 0.2 μm PVDF (Alltech Associates Inc. Lokeren, Belgium). The purified samples were placed in an autosampler (Kontron Autosampler 560, Kontron Instruments S.p.a, Milano, Italy) and analyzed by high performance liquid chromatography (HPLC) with post-column derivatization. The derivatizing solution was the Pickering's patented TRIONE® ninhydrin reagent (Pickering Laboratories). The TRIONE ninhydrin was kept in the reagent bottle under Helium (Helium N45, Air Liquide SA, Liège, Belgium) pressurized atmosphere (100 kPa) as required by Pickering. The HPLC system consisted of a gradient pump (Bio-Tek P System 526, Bio-Tek Instruments S.R.L., Milano, Italy) delivering 0.3 ml/min of mobile phase (Lithium Eluant Li280, Lithium Eluant Li750 and Lithium Regenerant RG003, Pickering Laboratories) at a pressure of 160 psi. Separation of the amino acids was achieved by phase chromatography (Pickering's Amino Acid Analysis Lithium Cation exchange part number: 0353150) (Pickering Laboratories PCX 5200) and detected by UV/visible detector (570 nm and 440 nm) (Kontron Detector 535).

The concentration of taurine was determined by Data System 450-MT2 rel.4.0 (Kontron Instruments) using external standards prepared each day from commercial standard (Amino acid standard basics A6282, Physiological amino acid neutral A6407, Sigma Aldrich Chemie, Gmbh, Steinheim, Germany) diluted with Pickering's Lithium Diluent Li220 to achieve the correct concentration.

Statistical analysis

The results are presented as mean ± standard error. For the evaluation of statistical differences, the variation of amino acids concentrations obtained from both experiments were analyzed by two-way analysis of variance (ANOVA 2) treatment groups, (i.e. Taurine vs. Saline) x time, with repeated measures on one factor (Time). The ANOVA 2 was followed by the least-significant difference test of multiple comparisons (Fischer LSD protected t-test) to determine statistical difference (which was set at < 0.05), between each time point (GB-STAT, Dynamic Microsystems, Silver Spring, MD, USA).

These experiments were approved by the Belgian governmental agency under the authorized number LA 1220028 as well as the European Communities Council Directive concerning the Use of Laboratory Animals.

Results

In the microdialysis experiment, the ip administration of 1, 0.5, 0.25, 0.125 or 0.05 g/kg of taurine produced a significant taurine hippocampal microdialysate content increase but not for the smaller dose by comparison to the saline one (Fig. 1a) [$F(5;986) = 22.74$; $p < 0.0001$]. This significant increase lasted at least for the first 20 minutes after the taurine ip injection and was dependent on the dose administered. There were a significant effect of the time [$F(17;986) = 39.55$; $p < 0.0001$] as well as a significant interaction between the dose administered and

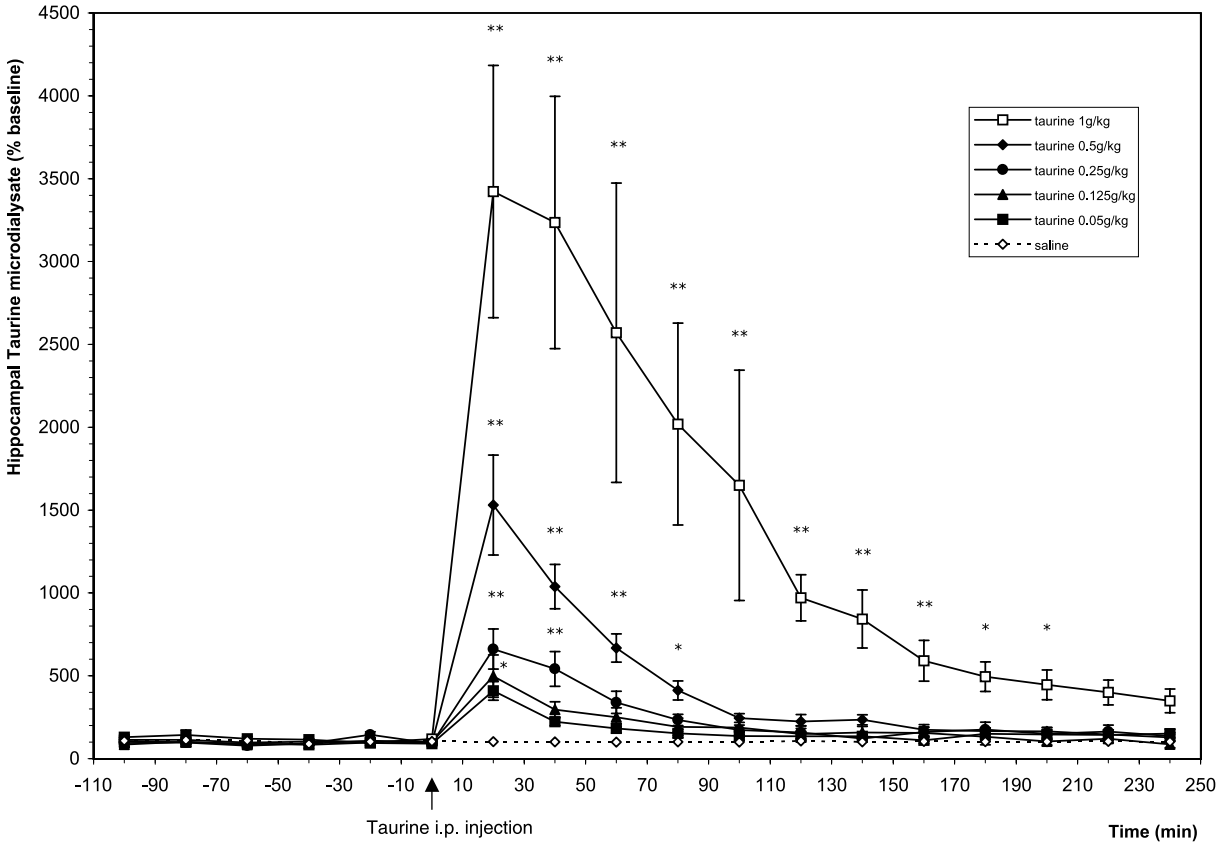

Fig. 1a. Effect of different taurine doses i.p. injection on taurine hippocampal microdialysate contents represented as percentage of baseline value. Significant time points between each doses and saline are represented by $^{*}p<0.05$, $^{**}p<0.01$. Results are presented as mean ± standard error

the time [$F(85;986)=13.36$; $p<0.0001$]. In addition, when we were looking at the data by concentration form (Fig. 1b) in place of percentage of baseline value we also observed this significant increase dependent of the dose [$F(5;812)=20.58$; $p<0.0001$] and a significant effect on time [$F(14;812)=35.56$; $p<0.0001$] as well as a significant interaction between dose and time [$F(70;812)=11.98$; $p<0.0001$]. The dose dependent increase was clearly showed on Fig. 2.

In the plasma study, the same taurine dose administrations produced a significant plasma taurine concentration increase for all doses except the smaller one (Fig. 3) [$F(6;315)=64.35$; $p<0.0001$]. There were a significant effect on the time [$F(5;315)=147.71$; $p<0.0001$] as well as for the interaction between dose and time [$F(30;315)=37$; $p<0.0001$]. This plasma taurine concentration increase was also dose dependent as showed on Fig. 4.

We determine the AUC's (Area Under the plasma Concentration time curve) for each doses on both the plasma and hippocampal microdialysate content (Table 1). The ratio between hippocampal microdialysate and plasma was also calculated for each dose (Table 2). The average of all the ratios gave us a value of 1.63 ± 0.21.

Discussion

In the body, taurine is present in very high concentration in the intracellular compartment by comparison to the extracellular one. To maintain this difference in concentrations, a specific active carrier-mediated transport system dependent on sodium and chloride ions and located on the apical and the basolateral surface of endothelial cell membranes was found; this process was reported saturable (Benrabh et al., 1995; Tamai et al., 1995; Qian et al., 2000). The kinetic system involved for taurine to reach the brain cells is comprised of the following: a- plasma compartment (extracellular); b- luminal uptake by endothelial cells into the cell (intracellular), c- antiluminal release into the brain cerebro-ventricular compartment (extracellular) and d- taurine uptake into brain cells (intracellular). We particularly focused our study on *in vivo* taurine level measurement inside the extracellular

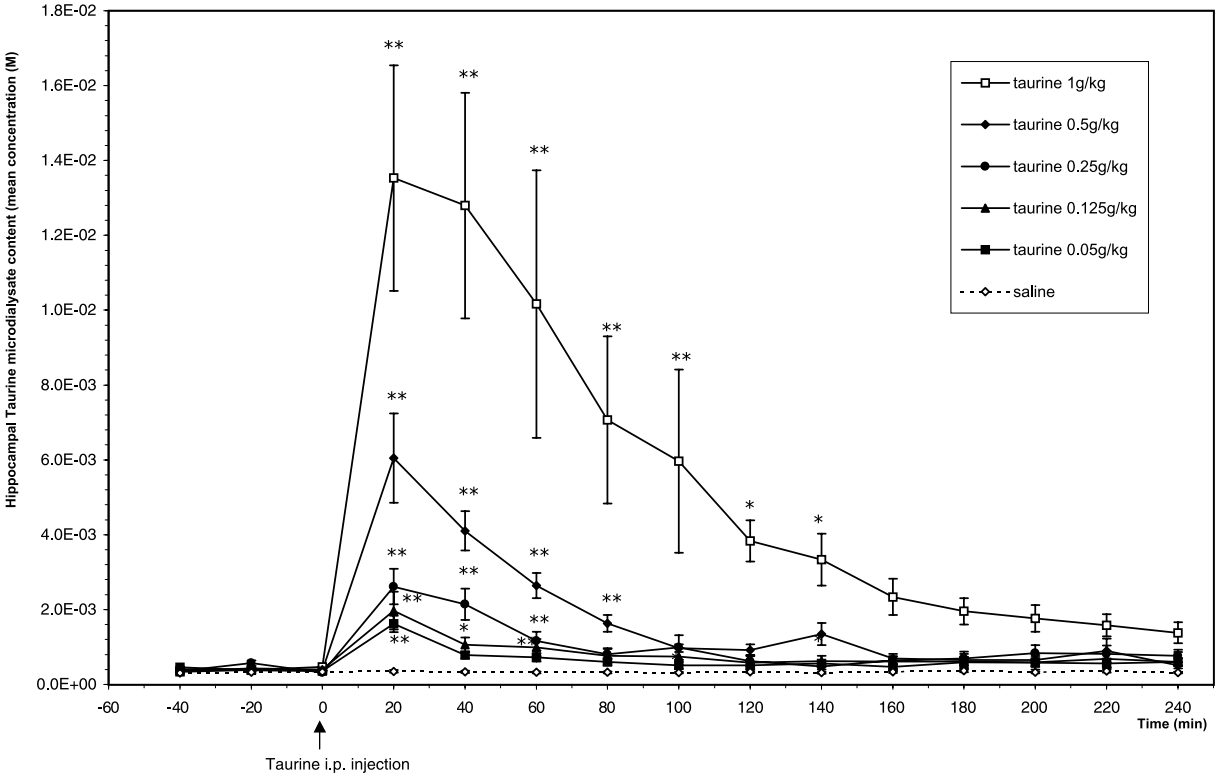

Fig. 1b. Effect of different taurine doses i.p. injection on taurine hippocampal microdialysate content expressed in concentration value. Significant time points between each doses and saline are represented by $^{*}p < 0.05$, $^{**}p < 0.01$. Results are presented as mean ± standard error

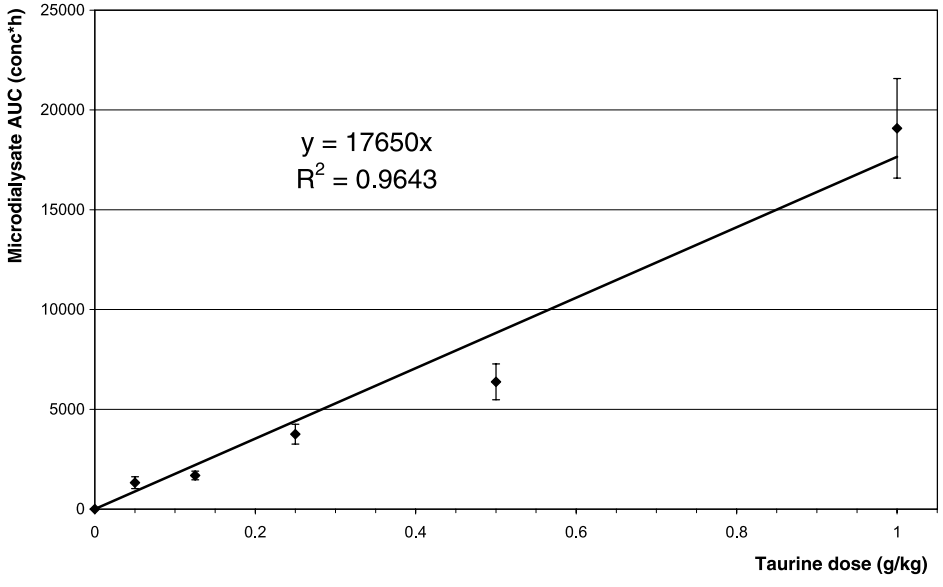

Fig. 2. Microdialysate AUC (taurine baseline level removed). Lines were drawn by linear regression analysis. Results are presented as mean ± standard error

brain content and in the plasma, following i.p. administration of different doses of taurine (0.05, 0.125, 0.250, 0.500 and 1.000 g/kg). For each taurine dose administered (except 0.05 g/kg), we observed a significant taurine increase in plasma content. In a similar experiment, Korang et al. (1996) estimated the pharmacokinetic of 0.8 g/kg of taurine i.p injected in the rat plasma. In the rat plasma, they found that 0.8 g/kg i.p. taurine adminis-

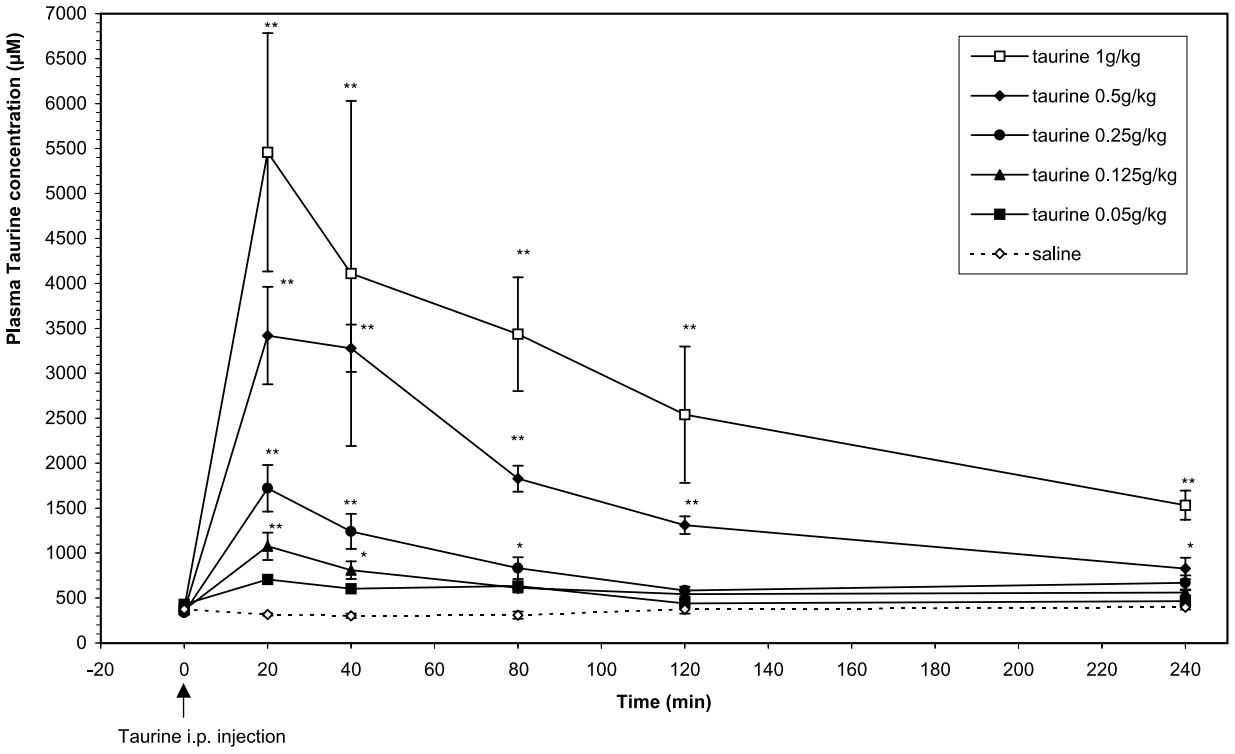

Fig. 3. Taurine plasma concentration. Effect of different taurine doses i.p. injection on plasma taurine contents. Significant time points between each doses and saline are represented by $^{*}p < 0.05$, $^{**}p < 0.01$. Results are presented as mean ± standard error

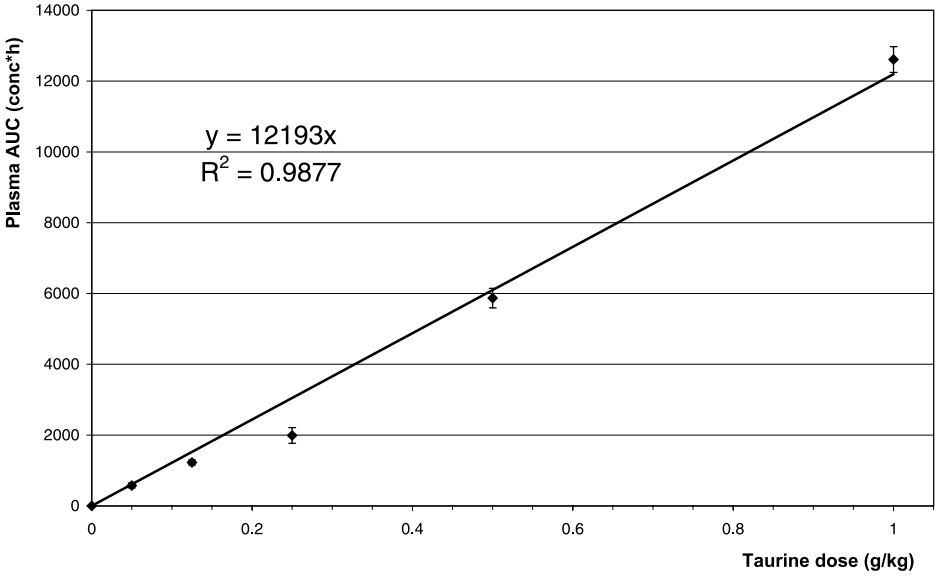

Fig. 4. Plasma AUC (taurine baseline level removed). Lines were drawn by linear regression analysis. Results are presented as mean ± standard error

tration induced a taurine level of 4893 μM, while we obtained 5458 μM following 1 g/kg of taurine i.p. injection. Those two results were thus very similar. Concerning the fate of taurine in the plasma, Korang et al. (1996) observed a progressive decline approaching the baseline value after 4 hours. We also observe the same decline following 0.5 g/kg taurine i.p. administration.

Table 1. Mean plasma and microdialysate AUC

Location in the body	Dose (g/kg)	Mean AUC (conc*h)	Standard error
hippocampal microdialysate	0.050	1325.04	298.46
	0.125	1682.62	214.85
	0.250	3752.92	494.13
	0.500	6378.54	898.36
	1.000	19081.59	2497.52
plasma	0.050	576.50	67.94
	0.125	1226.49	71.78
	0.250	1989.59	223.58
	0.500	5870.11	276.55
	1.000	12610.36	362.86

Table 2. Ratio between microdialysate AUC and plasma AUC

Dose (g)kg	Ratio
0.050	2.30
0.125	1.37
0.250	1.89
0,500	1.09
1.000	1.51
Mean ratio	Standard error
1.63	0.21

When comparing all the doses together, we found a dose dependent increase of both the plasma and the hippocampal microdialysate taurine content. Furthermore, each extracellular concentration of taurine was always higher in the brain than in the plasma. This result remains in accordance with the hypothesis of Tamai (1995) expecting that the *in vivo* net transport of taurine would occur in the direction from blood to brain when taking into account the transport efficiencies in luminal membranes of BCEC's and physiological concentrations together. Moreover, when looking at the ratios between the brain AUC and the plasma AUC, there were always higher than one meaning that the influx was in the direction of the plasma to the brain. Nevertheless, the AUC ratios and thus the flux directions were not equivalent for each doses of taurine. The 0.05 g/kg dose of taurine exhibited a ratio of 2.3 while the 0.5 g/kg showed a ratio of 1.09, thus very near to the balance state between brain and plasma, suggesting that the active transport may act differently depending on the concentration of taurine. Our results showed no saturation of the transport as we recorded a higher extracellular concentration of taurine in the brain content than in the plasma 20 minutes after every taurine doses ip injected. This result seems to be in contradiction with those observed in previous studies, which found the transport process saturable (Qian et al., 2000, Tamai et al, 1995, Benrabh et al., 1995). It may be that the doses we administered were not high enough in order to reach the saturation of the taurine transport.

In summary, our study clearly showed that taurine crosses the blood brain barrier freely but not by passive equilibrium as a higher extracellular taurine concentration was found in the brain indicating the presence of an active transport. Further investigations are needed to determine at which dose saturation of the taurine transport occurred and to determine the level of other amino acids modified with taurine ip administration.

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