Saturable Disposition of Taurine in the Cerebrospinal Fluid of the Rat

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ABSTRACT

Taurine, a β-amino acid, plays an important role as a neuromodulator and is necessary for the normal development of the brain. Since *de novo* synthesis of taurine in the brain is minimal and *in vivo* studies suggest that taurine does not cross the blood-brain barrier, the blood-cerebrospinal fluid (CSF) barrier is likely to play a role in taurine transport between the central nervous system and the systemic circulation. Therefore, we examined *in vivo* elimination of taurine from the CSF in the rat to characterize *in vivo* kinetics of elimination for taurine from the CSF is consistent with the *in vitro* study. Using a stereotaxic device, cannulaes were placed into the lateral ventricle and the cisterna magna of the rat. Radio-labelled taurine and inulin (a marker of CSF flow) were injected into the lateral ventricle, and the concentrations of the labelled compounds in the CSF were monitored for up to 3 hrs in the cisterna magna. The apparent clearance of taurine from CSF was greater than the estimated CSF flow ($p<0.005$), indicating that there is a clearance process in addition to the CSF flow. Taurine distribution into the choroid plexus was at least 10 fold higher than that found in other brain areas (*e.g.*, cerebellum, olfactory bulb and cortex). When unlabelled taurine was co-administered with radio-labelled taurine, the apparent clearance of the labeled taurine was reduced ($p<0.01$), suggesting a saturable disposition of taurine from CSF. Distribution of taurine into the choroid plexus, cerebellum, olfactory bulb and cortex was similarly diminished, indicating that the saturable uptake of taurine into these tissues is responsible for the non-linear disposition. A pharmacokinetic model involving first order elimination and saturable distribution described these data adequately. The Michaelis-Menten rate constant estimated from *in vivo* elimination study is similar to that obtained in the *in vitro* uptake experiment Collectively, our results demonstrate that taurine is transported in the choroid plexus via a taurine is cleared from the CSF via a saturable process. This process may be functionally relevant to taurine homeostasis in the brain.

INTRODUCTION

Taurine, a β-amino acid, is essential for normal development and function of the cerebellum and visual cortex, as well as the retina[1-4]. Also, taurine has been shown to alter the release of neurotransmitters

such as acetylcholine and norepinephrine [5], and, thus, it is generally accepted to be a neuromodulator [5,6].

Biosynthesis of taurine is minimal in brain[6,7] so that a systemic source is essential. Since taurine is zwitterionic at physiologic pH, it is unlikely that taurine diffuses passively through biological membranes. Therefore, its transport into or out of the brain must involve carrier-mediated process(es) at the blood-brain barrier and/or the blood-cerebrospinal fluid (CSF) barrier. In general, such membrane-associated transport system(s) at the barriers of the central nervous system (CNS) functions to provide a protected environment for the brain by selectively excreting certain substances and reabsorbing others. However, the site and underlying mechanism for taurine's entry into or exit from the CNS is currently unknown.

Recently, Na^+-dependent taurine transporter has been cloned from the human thyroid [8], the human placenta [9] and the mouse brain [10]. Interestingly, Liu *et al.* [10] reported that the brain microvessel, the blood brain barrier, did not contain mRNA for the taurine transporter. This observation suggests that the blood-CSF barrier, rather than the blood-brain barrier, may be an important site for maintaining homeostasis of taurine in the brain. We previously demonstrated that the rabbit choroid plexus contained Na^+-dependent transport system(s) [11]

Based on our recent finding and other biochemical evidence, we hypothesized that the blood-CSF barrier is a site for the transport of taurine via the CSF. Thus, the objectives of this study were to characterize the kinetics of elimination for taurine from the CSF in vivo. We report that taurine cleared from the CSF by a saturable process with similar kinetic characteristics to that found in vitro. Therefore, the saturable clearance of taurine may be physiologically important in maintaining homeostasis of taurine in the CSF and, ultimately, the extracellular fluid of the brain.

MATERIALS AND METHODS

Stereotaxic Surgery

Rats underwent a stereotaxic surgery to implant cannulaes into the lateral ventricle and the cisterna magna [12]. Briefly, the rat was mounted on a stereotaxic device (David Kopf Instrument, Tujunga, CA) under a ketamine and acepromazine anesthesia (80mg/kg and 10mg/kg, respectively). The frontal, parietal and

occipital bones were exposed through a linear midline incision. Using coordinate obtained from a stereotaxic atlas of rat brain [13], the left lateral ventricle was located (1.0mm posterior, 1.6mm lateral, and 3.6mm ventral to the bregma). A burr hole was drilled and a steal guide cannulae (22GA, 4mm below the pedestal, Plastics One, Roanoke, VN) was lowered using an electrode manipulator of the stereotaxic device to pierce the dura and enter the lateral ventricle. The cannulae was secured to two anchoring screws using a dental acrylic cement (Lang Dental, Chicago, IL).

A polyethylene catheter was also placed into the cisterna magna for serial sampling of the CSF. The catheter consisted of PE10 (9.5mm in length) tubing, inserted 3mm inside PE 50 tubing (20mm in length). A stainless steal wire was inserted inside the PE tubings to add rigidity to facilitate piecing the dura. A burr hole was drilled at 3 mm posterior to the lambda and the PE 10 portion of the catheter was inserted caudally. The stainless steal guide was then removed. A microsyringe (Gastight #1802, Hamilton Co., Reno, NV) was connected to the catheter and CSF flow was induced by a gentle pull of the syringe. Upon confirming the CSF flow, the microsyringe was disconnected and the catheter was secured to two anchoring screws by a dental acrylic. The animal was allowed to recover for 1.5hr before the start of the study. The ketamine/acepromazine anesthesia was maintained throughout the study.

Ventriculocisternal Procedure

The objective of this study was to determine elimination kinetics of taurine from the rat CSF. Thus, 5 μl of solution, containing ^{3}H-taurine (40 pmole) and ^{14}C-mannitol (60 μg; *i.e.*, a marker of the CSF flow) in sterile water, was administered into the lateral ventricular cannulae using a microsyringe (Hamilton Co., Reno, NV). Sterile water was used as a vehicle because mock CSF or physiological saline would have produced a hypertonic injection solution (*i.e.*, approximately 700 mOsm). Preliminary experiments showed that taurine elimination kinetic was not significantly affected by vehicles (data not shown).

Taurine dose dependency was studied, 0.3 μmole or 2 μmole of unlabelled taurine was administered along with the radio-labelled compounds. When selectivity of taurine elimination was studied, 2 μmole of α- or β-alanine was added to the injection solution. The CSF sample (5 μl) was collected at time 2, 5, 15, 30, 45, 60, 90, 120, 180 min. Scintillation fluid (5 ml, Cytocint ES) was mixed with the CSF and the radioactivity for [^{14}C] and [^{3}H] in the CSF was then determined by a dual isotope liquid scintillation counting on a Beckmann

Model 1801 liquid scintillation counter (Beckmann Instruments Inc., Fullerton, CA). Counting efficiency of [^{3}H] ranged from 45 to 47 % and of [^{14}C] ranged 92 to 94 %.

The distribution of ^{3}H-taurine into selected parts of brain was determined at the end of the 180 min CSF collection. The rat was decapitated and the brain was immediately removed. Representative samples (approximately 10 mg) from the olfactory bulb, cortex and cerebellum were obtained. Also, the choroid plexi from each lateral ventricle were obtained. The dissection and the isolation procedure was completed in approximately 10 min. Isolated brain tissues were then weighed on pre-weighed aluminum foils and dissolved in 200 μl of 3N NaOH overnight. After the tissue was completely dissolved, 50 μl aliquots of tissue lysate were added to scintillation vial. Then, the lysate was neutralized with 50 μl of 3N HCl solution and the tissue associated radioactivity was determined by a dual isotope liquid scintillation counting. Tissue to media (T/M) ratio, representing the distribution of radiolabelled taurine into the representative brain tissue, was then calculated.

Pharmacokinetic Analysis

Kinetics of taurine disappearance from the CSF were analyzed by a standard pharmacokinetic analysis. A potential pharmacokinetic model was first constructed by assuming first order elimination from the CSF via bulk flow clearance, saturable distribution into the brain tissue, including the choroid plexus, and first order transfer from the deep to the shallow compartment. In this model, we assumed that the saturable rate process is reasonably described by the Michaelis-Menten kinetics. Then, the mass balance was written as shown in Scheme 1, model 1. Definition of pharmacokinetic variable is as follows: V_1, the apparent volume of distribution of the CSF; CL_{bulk}, bulk flow clearance; CL_{21}, transfer clearance from the brain tissue to the CSF; V_{max}, apparent maximal velocity; K_M,the Michaelis-Menten constant; V_2, apparent volume of distribution of the brain tissue. Then, more complex pharmacokinetic models were built on to model 1 (*e.g.*, an additional saturable elimination from the CSF was added in model 2; see Scheme 1). Overall, four potential pharmacokinetic models were constructed and analyzed to determine the model which best describes our results with the least complexity.

Since the equations in Scheme 1 contained non-linear terms, C_{CSF} cannot be analytically integrated

to obtain a concentration vs. time relationship. Therefore, all the data in the taurine dose dependency study were simultaneously fitted to each model by the Runge-Kutta numerical integration method using PCNONLIN (Statistical Consultants Inc., Lexington, KY) running on a 386 personal computer. The sums of squares, the Akieke's information criteria, and the Schwartz criteria were then calculated to determine the most appropriate pharmacokinetic model.

Data Analysis

When taurine uptake into the brain tissue of the rat was examined, a tissue to media ratio (T/M)[14] was calculated by the following equation:

eq 1

To calculate the apparent clearance and the volume of distribution, the moment analysis was used [14]. The areas under the concentration vs. time curve (AUC) and the concentration-time product vs. time curve (AUMC) were calculated by a linear trapezoidal method up to 180 min. Remaining area to infinity for AUC was then estimated by dividing concentration of the last collection time by the terminal slope. Remaining area from the last sampling time to infinity for AUMC was estimated by the following equation:

Eq 2

The apparent clearance was then calculated by dividing dose by AUC. The volume of distribution (Vss) was calculated by the following equation:

eq 3

Materials

[^{3}H] Taurine (21.9 Ci/mmol) and [^{14}C] inulin (2.8 mCi/g) was obtained from Du Pont-New England Nuclear (Boston, MA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). New Zealand White rabbits were purchased from Nitabell Rabbitry (Hayward, CA). Male Sprague-Dawley rats (270-300 g) were purchased from Simonson Inc. (Gilroy, CA). Cytocint ES scintillation fluid was obtained from ICN Biomedical Inc. (Irvine, CA). Acepromazine was obtained from Aveco Co., Inc. (Fort Dodge, Iowa). Ketamine was obtained from Fort Dodge Laboratories, Inc. (Fort Dodge, Iowa).

RESULTS

Taurine Dose-Dependency Study

When 40 pmole of radiolabelled taurine was injected into the lateral ventricle, the concentration of taurine declined in a bi-exponential manner (Fig. 1) while inulin followed an apparent mono-exponential decline (data not shown). The clearances, calculated from the moment parameter, for taurine (40 pmole) and inulin were 73.4±27.7 μl/min and 3.85±1.08 μl/min, respectively ($p<0.005$). When 40 pmole of the labelled taurine was administered with 0.3 μmole of unlabelled taurine, the apparent clearance for the labelled taurine was decreased to 24.7±11.8 μl/min ($p<0.01$). The clearance was further decreased in the presence of 2 μmole of unlabelled taurine (15.5±0.0438 μl/min). This observation suggests that taurine disappeared from the CSF by a clearance process(es) in addition to bulk flow and that the additional clearance process is saturable. The decrease in the clearance was associated with similar decrease in the Vss parameter ($p<0.01$, Table I). The inulin clearance was not affected by the unlabelled taurine co-administration.

The uptake of labelled taurine was also examined in representative brain areas. When expressed as a tissue to media ratio (T/M, in ml/g) at 3hrs, taurine uptake into the choroid plexus was at least 10 fold higher than in other parts of the brain (Fig 2, $p<0.005$). In addition, taurine uptake into the cortex and the choroid plexus decreased in a dose-dependent manner ($p<0.01$, Fig. 2). These observations suggest that the saturable disposition of taurine in the CSF is due to saturable distribution of taurine into the brain.

Pharmacokinetic Analysis

To analyze these observations quantitatively, four potential pharmacokinetic models were constructed (Scheme 1) and a nonlinear regression analysis was conducted via a simultaneous fitting of all three dose (8 observations for each dose, Fig. 1) to each pharmacokinetic model shown in Scheme 1. Therefore, this analysis would allow an estimation of pharmacokinetic parameters encompassing a dose range of 62,500 fold. The Aikeike's information criteria and the Schwarz criteria were calculated to determine a model which best describes our results with the least complexity (Table II). Also, weighted sums of squares was calculated to select a model that results in the least deviation from the observations. Based on all the criteria we used, model 1 best described our results (Table III)

DISCUSSION

Taurine is highly concentrated in the mammalian brain and is known to have important functions in the CNS[1-6]. In particular, it has been suggested that taurine is vital for the normal development of the brain, and deficiency is associated with neurologic dysfunction[1-3]. For example, pediatric patients who develop taurine deficiency while receiving longterm intravenous alimentation have abnormal electroretinograms[15].

In this study, we demonstrated that taurine is eliminated from the CSF by a mechanism in addition to bulk flow and that this additional clearance process decreased with taurine dose (Table I). The decrease in the clearance of taurine was accompanied by a similar decrease in the volume of distribution (Table I) and in the distribution of taurine into specific brain areas (Fig. 2). These observations indicate that the distribution of taurine into the brain is saturable. Therefore, a standard pharmacokinetic analysis was performed to estimate distribution and elimination kinetics of taurine. Nonlinear regression analysis revealed that the taurine distribution into the brain compartment was saturable with estimated K_M of 40 μM. Also, estimated volume of distribution for the brain compartment is 56 ml, indicating the taurine readily distributed into the brain. The volume of taurine distribution into the CSF compartment was estimated to be 0.210 ml, similar to the estimated volume of CSF (*i.e.*, inulin volume of distribution, Table I). As expected, first order clearance of taurine from the CSF was estimated to be 26 μl/min, close to the apparent clearance in medium and high taurine dose (Table I). Overall, the goodness of the fit acceptable considering the dose range of 62,500 fold (Fig. 1).

Taurine distribution into the representative brain areas was dose-dependent (Fig. 2). Among the brain areas examined, the choroid plexus had at least 10 fold higher tissue to media ratio for all doses. In addition, the calculated tissue to media ratio at 3 h for the choroid plexus was over 100, indicating the taurine concentration in the choroid plexus was substantially higher than that of the CSF. In this study, we did not attempt to characterize the release kinetics of taurine into the systemic circulation from the choroid plexus. However, in a preliminary experiment, we attempted to measure the concentration of radio-labelled taurine in the systemic circulation after taurine was administered into the lateral ventricle. We could not detect any

radioactivity in the plasma up to 2 hr after the injection (data not shown), a time when the taurine in the CSF would have reached an equilibrium with the choroid plexus. Taken together, these observations suggest that taurine is actively transported into the choroid plexus, and leaks out from the tissue slowly. Such slow release of taurine from variety of tissue has been reported in an *in vitro* study [16].

The Michaelis-Menten rate constant obtained *in vivo* taurine dose dependency study was similar to that found in our previous uptake experiment [11]. Thus, these data are consistent with the hypothesis that the high affinity transport system for taurine in the choroid plexus plays a role, at least in part, in taurine elimination from the CSF. However, further studies (*e.g.*, *in situ* hybridization study) will be necessary to elucidate clearly the location of the transporter and its physiologic significance.

In conclusion, taurine is cleared from the CSF by a process in addition to bulk flow. The clearance is dose-dependent and accompanied by a reduction in the distribution of taurine into the cerebral cortex and the choroid plexus. This clearance process* may be functionally relevant in maintaining taurine homeostasis in CSF and, ultimately, the extracellular fluids of the brain.

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Table I: Clearance and volume of distribution of taurine and inulin in dose dependency and in inhibitor studies. The zero (*i.e.*, area under the taurine concentration in the CSF *vs.* time) and the first (*i.e.*, area under the product of time and the concentration *vs.* time) moments were calculated by the linear trapezoidal rule. Apparent clearance and volume of distribution were then calculated by the standard pharmacokinetic method. Data are expressed in the means ± S.D. of three runs.

	Taurine		Inulin	
	Apparent Clearance (μl/min)	Vss (ml)	Apparent Clearance (μl/min)	Vd (ml)
Low taurine dose (40pmole taurine)	73.4±27.7	5.23±2.26	3.85±1.08	0.240±0.106
Medium taurine dose (0.3μmole taurine)	24.7±11.8**	1.56±1.22**	5.04±1.64	0.253±0.082
High taurine dose (2μmole taurine)	15.5±0.0437**	0.819±0.161**	7.34±3.36	0.404±0.178
β-alanine coadminstration (40pmole taurine+2μmole β-alanine)	8.85±1.29*	0.414±0.225*	3.97±0.659	0.204±0.0528
α-alanine coadministration (40pmole taurine+2μmole α-alanine)	12.5±1.15*	0.430±0.245*	3.44±0.409	0.162±0.0384

*; statistically different from low taurine dose by $p<0.05$ (t-test)

**; statistically different from low taurine dose by $p<0.01$ (one-way ANOVA and Turkey's multiple comparison)

Table II: Summary of non-linear regression analysis.

	AIC[a]	SC[b]	Sum of residual[c]
Model 1	33.9	43.4	2.11
Model 2	84.1	93.6	17.1
Model 3	50.9	60.3	4.28
Model 4	N.A.[d]	N.A.	N.A.

[a]: Akaike's Information Criteriaon (AIC)

$$= N \cdot \ln(Sum\ of\ residual) + 2 \cdot p$$

where N represents number of observations and p is number of parameters in a model

[b]: Schwartz Criteria (SC)

$$= N \cdot \ln(Sum\ of\ residual) + p \cdot \ln(N)$$

[c]: Sum of residual

$$= \sum_{i=1}^{N} \{(y_{i,obs} - y_{i,cal})^2 / y_{i,obs}\}$$

where $y_{i,obs}$ represents observed concentration and $y_{i,cal}$ is calculated concentration based on a pharmacokinetic model

[d]: N.A., not applicable. A convergence could not be achieved for this pharmacokinetic model and, therefore, AIC, SC and sum of residual could not be calculated.

Table IV: Summary of estimated pharmacokinetic parameters. Pharmacokinetic parameters were estimated based on model 1 (Scheme 1). Data from taurine dose dependency study was used in the non-linear regression analysis. The estimates are represented by estimated parameter ± standard error generated during the fitting procedure.

Pharmacokinetic Parameters	Regression Estimate
V_1 (ml)	0.21 ± 0.051
V_{max} (nmole/min)	4.1 ± 2.2
K_M (μM)	40 ± 25
V_2 (ml)	56± 35
CL_{21} (ml/min)	0.17 ± 0.12
CL_{bulk} (ml/min)	0.026 ± 0.0059

FIGURE LEGEND

Figure 1: Dose dependency of radiolabelled taurine concentration in the CSF vs. time curve. Key: l, 40 pmole labelled taurine dose; ▲, 40 pmole labelled taurine + 0.3 μmole unlabelled taurine dose; n, 40 pmole labelled taurine + 2 μmole unlabelled taurine dose. Solid line was generated by a nonlinear regression fitting of a pharmacokinetic model shown in Scheme 1, model 1 with parameters in Table III. Data are expressed as means of 3 runs ± S.D.

Figure 2: Distribution of radiolabelled taurine in brain areas 3hr after administration. Key: Low TAU, 40 pmole labelled taurine dose; Med TAU, 40 pmole labelled taurine + 0.3 μmole unlabelled taurine dose; High TAU, 40 pmole labelled taurine + 2 μmole unlabelled taurine dose; Panel A, taurine distribution in the olfactory bulb; Panel B, taurine distribution in the choroid plexus; Panel C, taurine distribution in the cerebellum; Panel D, taurine distribution in the cortex. Data are expressed as means of 3 runs ± S.D. *; statistically different from Low TAU by $p<0.05$ (t-test). **; statistically different from Low Tau by $p<0.01$ (one-way ANOVA, followed by Turkey's multiple comparison test).

$$T/M = \frac{dpm\,[^3H]\,taurine\ in\ brain\ tissue \Big/ g\ of\ brain\ tissue}{dpm\,[^3H]\,taurine\ in\ CSF\ at\ 3\ hr \Big/ ml\ of\ CSF} - \frac{dpm\,[^{14}C]\,inulin\ in\ brain\ tissue \Big/ g\ of\ brain\ tissue}{dpm\,[^{14}C]\,inulin\ in\ CSF\ at\ 3\ hr \Big/ ml\ of\ CSF}$$

$$\int_{3hr}^{\infty} t \cdot C_{CSF} dt = \frac{(t \cdot C_{CSF})_{3hr}}{terminal\ slope} + \frac{C_{CSF,\,3hr}}{terminal\ slope^2}$$

$$V_{ss} = \frac{Dose \cdot AUMC}{AUC^2}$$