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ORIGINAL ARTICLE

Taurine in plasma and CSF: a study in healthy male volunteers

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Abstract In order to explore the interrelationship between plasma and cerebrospinal fluid taurine concentrations, three consecutive 6-ml fractions of cerebrospinal fluid were drawn from 30 healthy male volunteers in the early morning after 8 h in the fasting condition. Repeated plasma samples were drawn over 24 h the day before lumbar puncture. Taurine in plasma and cerebrospinal fluid was determined by high performance liquid chromatography. The subjects were categorized as extensive or poor metabolizers with respect to the cytochrome P450 2D6 genotype. The taurine cerebrospinal fluid/plasma ratio at 8 a.m. was negatively influenced by the plasma taurine concentration at 4 p.m. the previous day. It was also negatively influenced by body mass index and positively by the intraspinal pressure. Three poor metabolizers of cytochrome P450 2D6 had higher plasma taurine areas under the curve than 27 extensive metabolizers. Hypothetically, cytochrome P450 2D6 influences the transport of taurine across the blood–brain barrier.

Keywords Taurine · Cerebrospinal fluid · Plasma · Cytochrome P450 2D6 · Genotype

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Introduction

Taurine (2-aminoethanesulphonic acid) is the most abundant free amino acid in several tissues and in the cellular components of blood (Schuller-Levis and Park 2003). In the human foetus, taurine is an essential amino acid as the foetal biosynthetic capacity is almost negligible (Aerts and Van Assche 2002). Otherwise, the nutritional requirements for taurine are met both by dietary sources and biosynthesis from cysteine and methionine (Aerts and Van Assche 2002; Atmaca 2004) preferentially in the liver (Hansen 2001), but also to a small extent in the brain (Huxtable 1989). Taurine is not incorporated into proteins (Aerts and Van Assche 2002).

Taurine plays an essential role in neural development by acting as an inhibitory and neuroprotective neurotransmitter and neuromodulator (Huxtable 1992; Bianchi et al. 2006). Its physiological functions include bile and xenobiotic conjugation, regulation of neuronal excitability, membrane protection, antioxidation, detoxification and osmoregulation (Schuller-Levis and Park 2003; Atmaca 2004). Taurine also participates in thermoregulation, alteration of sleep duration and suppression of eating and drinking (Huxtable 1992).

There is evidence of a pathophysiological role of taurine. An aberrant plasma taurine level has been found in patients with acute polymorphic psychosis (Fekkes et al. 1994) and in schizophrenia (Bjerkenstedt et al. 1985). Furthermore, taurine in lymphocytes was increased before but was normalized after mirtazapine treatment of depressed patients. Interestingly, a correlation was found between the severity of depression and the taurine concentration in lymphocytes before treatment (Lima et al. 2003).

Some studies have addressed the role of taurine in the cerebrospinal fluid (CSF). Unmedicated schizophrenic patients were found to have a lower CSF taurine level than

healthy controls (Bjerkenstedt et al. 1985). An altered CSF taurine function in pathological male gamblers has been reported in two studies (Nordin and Eklundh 1996; Nordin and Sjödin 2006).

The clinical findings in schizophrenia, depression and pathological gambling justify focusing on the exchange of taurine between the peripheral and central compartments. Given that the regulation of brain taurine has been attributed to the flux into and out of the brain at the blood–brain and blood–CSF barriers (Keep and Xiang 1996), along with the observation that taurine in the CSF seems to be influenced by the cytochrome P450 (CYP) 2D6-phenotype (Nordin et al. 2003), the taurine exchange between plasma and the CSF appears to be complex. A correlation between taurine in plasma and the CSF has been questioned (McGale et al. 1977; Hagenfeldt et al. 1984).

The aim of the present study was to examine the CSF disposition of taurine in relation to plasma levels with special reference to the *CYP2D6* genotype. The study design is exploratory and hypothesis-generating.

Materials and methods

A total of 36 healthy male volunteers were recruited among medical students, hospital staff members and their relatives. They were all subjected to a medical check-up including laboratory tests (electrolytes, blood, kidney, liver and thyroid) and a physical examination. The volunteers had to have been medication-free for at least 1 month and free from any form of substance abuse. Smoking was allowed, however.

The volunteers were subjected to a semistructured interview using the structured clinical interview of DSM-IV Axis I disorders (SCID-I) (First et al. 1997a). The interview was directed towards affective disorders, anxiety disorders and drug abuse. The volunteers also completed the SCID-II questionnaire for personality disorders (First et al. 1997b). A total of 30 medication-free and physically healthy volunteers were considered to be eligible for the study with respect to the clinical interview and SCID results.

The study comprised 2 days. On day 1, blood samples were drawn at 8.00 a.m., 12 noon, 4.00 p.m. and 8.00 p.m. On the next day (day 2), a blood sample was drawn and the lumbar puncture was performed at 8.00 a.m. All blood samples were centrifuged in a Sigma 203 centrifuge at 3,500 rpm (1,438*g*) for 10 min. Plasma was separated immediately and frozen at −70°C until analysed.

Lumbar puncture was performed after a minimum of 8 h in the fasting state. There were no restrictions concerning posture or rest during the preceding 8 h. At about 8 a.m. a disposable needle (BD Whitacre Needle 0.7 × 90 mm) was inserted at the L 4–5 level with the subject in the right decubitus position. For convenience, a pillow was placed under the subject's head. The intraspinal pressure was measured using a disposable spinal fluid manometer (Optidynamic®, Mediplast) before and after the collection of three 6-ml CSF fractions. The CSF was allowed to drip into a plastic test-tube. The CSF collection time was recorded using a stopwatch. The 6-ml fractions of CSF were protected from light and centrifuged in a Sigma 203 centrifuge at 3,500 rpm (1,438*g*) for 10 min within 30 min after the puncture. Each 6-ml sample was divided into three 2-ml aliquots, which were placed in a freezer (−70°C) until analysed. The neuroaxis distance (the length of the spine from the external occipital protuberance to the site of puncture) was measured in the lying position.

Using high performance liquid chromatography (HPLC), taurine was separated along with other amino acids (data not reported here) using a Biochrome 30 amino acid analyser. The amino acids were detected by spectrophotometry. The EZ Chrom Elite program was used for the final determination of concentrations (Jeppsson and Karlsson 1972; Ekberg et al. 1974; Brattström et al. 1988).

Blood samples for *CYP2D6* genotyping were collected in EDTA vacutainer tubes and kept frozen at −20°C until analysed. Genomic DNA was isolated from whole blood using the QIAamp® DNA Blood Mini Kit (QIAGEN Ltd). The *CYP2D6* alleles **3*, **4*, **6*, **7* and **8* were analysed using TaqMan® Pre-Developed Assay Reagents for allelic discrimination (primers, probes, positive controls: part numbers 4312554, 4312555, 4312556, 4312557 and 4312558) and the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The *CYP2D6*5* allele (total deletion of the gene) was detected by long PCR followed by 1% agarose gel electrophoresis (Hersberger et al. 2000). The *CYP2D6* gene duplication, which usually confers ultra rapid metabolism, was analysed using long PCR as described (Steijns and van der Weide 1998). When neither the *CYP2D6* variants **3*, **4*, **5*, **6*, **7*, **8* nor gene duplications were detected, the allele was classified as functional *CYP2D6*1*. Three subjects carrying two defective alleles (two with **4*4* and one with **4*5* genotype) were classified as poor metabolizers (PMs), and 27 with one (**1*4* or **1*5*, $n = 10$) or two (**1*1*, $n = 17$) **1* alleles as extensive metabolizers (EMs). No subject carrying gene duplication was found among the subjects studied.

The Statistica 8 (StatSoft, Tulsa, OK, USA) programs were employed. Using the linear trapezoidal method, the area under the plasma concentration versus time curve (AUC) for taurine was calculated from 8.00 a.m. to 8.00 p.m. on day one ($AUC_{0–12\ h}$), from 8.00 p.m. on day 1 to 8.00 a.m. on day 2 ($AUC_{12–24\ h}$), and from 8.00 a.m. on day 1 to 8.00 a.m. on day 2 ($AUC_{0–24\ h}$). A perpendicular

line was drawn from each concentration down to the time axis giving a trapezoid area defined by the product of concentrations and the time interval (Roland and Tozer 1980).

The study was approved by the Regional Ethical Review Board in Linköping. All volunteers received verbal and written information and gave their written informed consent. The principles embodied in the Declaration of Helsinki were adhered to.

Results

Basal data on volunteers are presented in Table 1. Plasma and CSF concentrations of taurine are given in Table 2.

On using a within-subject analysis of variance (ANOVA), an overall difference was found between all five plasma concentrations of taurine ($F_{4:29} = 2.64$; $P = 0.0137$). Repeated post hoc comparisons using the paired t test yielded a difference between the plasma concentrations at 4.00 p.m. and 8.00 p.m. on day 1 ($df = 29$; $t = 3.16$; $P = 0.0037$). No other differences were found. There was no difference between the three CSF fractions ($F_{2:29} = 0.87$; NS). Accordingly, the mean CSF taurine level was used in the subsequent calculations.

In repeated multiple regression analyses employing best subset regression technique (Hill and Lewicki 2006), the mean taurine CSF concentration, the mean CSF taurine level per minute of collection time (an estimate of the mass flow) and the CSF/plasma ratio (8.00 a.m., day 2) were used in turn as the dependent variable. Age, body mass index (BMI), atmospheric pressure, neuroaxis distance, CSF collection time, intraspinal pressure, $AUC_{0–24\ h}$ and (when the CSF/plasma ratio served as dependent variable) $AUC_{0–12\ h}$ served as covariates. No influence of the independent variables was found.

Table 1 Basal data on 30 healthy male volunteers (mean ± SD)

Age (years)	25.4 ± 7.2
Height (cm)	182.3 ± 7.5
Body weight (kg)	79.3 ± 9.8
Body mass index (kg/m^2)	23.9 ± 2.9
Intraspinal pressure (cm H_2O)	18.6 ± 4.1
Atmospheric pressure (hPa)	1006.9 ± 9.3
Neuraxis distance (cm)	60.4 ± 4.3
CSF collection time (min)	
0–6 ml	5.2 ± 2.4
7–12 ml	6.1 ± 2.5
13–18 ml	6.9 ± 3.6
0–18 ml	18.3 ± 8.0

Table 2 Taurine in plasma and CSF in 30 healthy male subjects (mean ± SD)

Plasma (μmol/l)	
Day 1	
08.00 a.m.	73.0 ± 23.7
12.00 noon	78.4 ± 27.6
04.00 p.m.	71.2 ± 22.4
08.00 p.m.	86.5 ± 30.1
Day 2	
08.00 a.m.	77.4 ± 22.2
Mean (days 1–2)	77.3 ± 17.9
Area under the curve (AUC) (μmol/l h)	
$AUC_{0–24\ h}$	1245.1 ± 297.8
$AUC_{0–12\ h}$	917.2 ± 227.2
$AUC_{12–24\ h}$	327.8 ± 83.5
CSF (μmol/l)	
Fraction 1–6 ml	8.2 ± 3.1
Fraction 7–12 ml	7.7 ± 3.0
Fraction 13–18 ml	8.0 ± 2.8
Mean 18 ml	8.0 ± 2.7

On replacing the AUCs with taurine concentrations in plasma at 4.00 p.m. and 8.00 p.m. on day 1 (the only two plasma levels that differed) and also including plasma taurine at 8.00 a.m. on day 2, no effects were found, neither on the mean CSF level nor the CSF level per minute of collection time. However, plasma taurine at 4.00 p.m. on day 1, intraspinal pressure and BMI had an impact on the CSF/plasma ratio (Table 3).

The *CYP2D6* genotype influenced neither the $AUC_{0–24\ h}$ or $AUC_{12–24\ h}$ nor the mean CSF level of taurine. We found, however, that the three PMs had a higher $AUC_{0–12\ h}$ than the 27 EMs (mean ± SD 1590.6 ± 304.1 vs. 1206.7 ± 276.6 μmol/l h; Mann–Whitney U test; $Z = 2.18$; $P = 0.0294$).

Discussion

The observation of a difference between plasma concentrations of taurine at 4.00 p.m. and 8.00 p.m. on day 1 might indicate a diurnal rhythm, which contrasts with a previous study in which amino acids in plasma, except for taurine, threonine, glutamate, alanine, tyrosine and total tryptophan displayed a rhythmicity (Riggio et al. 1989). Whether there is an interaction between plasma taurine and melatonin function is an intriguing question considering that both compounds have a role in the regulation of biorhythms (Durlach et al. 2002).

As illustrated in Table 3, the taurine CSF/plasma ratio at 8.00 a.m. on day 2 was negatively influenced by plasma taurine at 4.00 p.m. the previous day. Owing to little

Table 3 Regression equation for the taurine CSF/plasma ratio$_{08.00\ a.m.\ day2}$ after multiple regression using best subset regression technique. Independent variables are taurine in plasma $_{04.00\ p.m.\ day1}$, taurine in plasma $_{08.00\ p.m\ day1}$, age, body mass index (BMI), atmospheric pressure, CSF collection time, neuraxis distance (ND), CSF storage time and intraspinal pressure (IP)

Dependent variable	Independent variable	
Taurine CSF/plasma$_{08.00\ a.m.\ day2}$	$0.26 - \text{Plasma}_{04.00\ p.m.\ day1} \times \text{E-3} + \text{IP} \times 5\text{E-3} - \text{BMI} \times 6\text{E-3}$	R-squared = 0.27; $F_{3:26} = 3.21$; $P = 0.0396$

biosynthesis and degradation of taurine in the brain (Huxtable 1989), the regulation of brain taurine has been attributed to the flux in and out of the brain at the blood–brain and blood–CSF barriers (Keep and Xiang 1996). The influx of taurine (and β-alanine) is mediated by a Na^+-dependent transporter at the blood–brain barrier (Ohtsuki 2004). An efflux transporter has, however, also been described (Lee and Kang 2004). In an animal experiment, 47% of the injected [^{3}H] taurine was eliminated from the brain within 40 min, but the net transport of taurine was found to occur from the blood to the brain (Lee and Kang 2004). This might be in line with our observation that the 4.00 p.m. level of taurine influences the CSF/plasma ratio the next morning.

Lee and Kang (2004) suggest that the taurine transporter is responsible for both the taurine influx and efflux from the brain. Another hypothesis is that P-glycoprotein, a transporter involved in the efflux of xenobiotics from brain into the blood (Uhr et al. 2004) plays a role in the elimination of taurine from the brain. If this is the case, there might be room for an influence of CYP2D6. In an animal experiment, an overlap in substrate specificity between P-glycoprotein and CYP2D6 was reported for some drugs, but not for other ones (Uhr et al. 2004). Whether such an overlap is applicable to the passage of taurine from CSF to blood (and/or the opposite direction) is not known. Taking the possibility of a bidirectional transport of taurine into account, the concept of a slow passage of taurine between blood and CSF (Oja et al. 1976) might have to be modified. The taurine CSF/plasma ratio also seems to be negatively influenced by BMI and positively by intraspinal pressure, factors that have to be taken into account in future research on CSF taurine.

An interesting finding is that during the daytime the taurine $AUC_{0-12\ h}$ is higher in the three PMs than in the 27 EMs. This observation is, at least in part, in line with a previous report of a negative correlation between taurine in CSF and the CYP2D6 phenotype during (but not prior to) cholesterol lowering with simvastatin (Nordin et al. 2003). This finding makes it justifiable to launch the hypothesis that the taurine transport from blood to CSF or in the opposite direction is influenced by *CYP2D6* genotype. In rodents, the *CYP2D1* gene (the corresponding gene to *CYP2D6* in humans) is constitutively expressed in both neuronal and glial cells (Norris et al. 1996) while in human brain, expression of CYP2D6 has been demonstrated so far only in neuronal cells (Siegle et al. 2001). In vitro studies have shown that tryptamine is an endogenous substrate for CYP2D6 (Martinez et al. 1997), which also catalyses the metabolism of tyramine to dopamine (Hiroi et al. 1998). Therefore, a physiological role for CYP2D6 in the regulation of serotonergic and dopaminergic neurotransmission has been hypothesized. Whether CYP2D6 is expressed at and might contribute to the transport across the blood–brain and/or the blood–CSF barrier has, not to our knowledge, been assessed so far.

Owing to the small number of PMs, our finding pertaining to $AUC_{0-12\ h}$ has to be interpreted with caution. Obviously, the passage of taurine across the blood–brain and brain–CSF barriers needs to be further explored with due attention paid to the role of the *CYP2D6* genotype as well as to that of P-glycoprotein. A larger series is warranted in future studies.

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