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Effects of consecutive high-dose alcohol administration on the utilization of sulfur-containing amino acids by rats

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Abstract

In this study, we attempted to evaluate changes in sulfur-containing amino acid (SCAA) metabolism after short-term high-dose alcohol ingestion. At the beginning of the study, six animals were sacrificed as the baseline group and then other animals in the experiment were consecutively gavaged with alcohol (30%, 3 g/kg) for 7 days. Animals ($n=6$ each) were subsequently sacrificed at the time points of Days 1 (Group E1), 3 (Group E3) and 7 (Group E7). Blood samples and selected tissues were collected at each time interval. SCAA, pyridoxal phosphate (PLP) and glutathione (GSH) levels were analyzed. Results showed that taurine levels of tissues (brain, liver, heart and kidneys) all declined after the ethanol intervention and continued to decrease in selected tissues except the brain during the experiment. Furthermore, the trends of plasma taurine and PLP contents were highly correlated ($r=.98$, $P=.045$). A similar utilization pattern of plasma taurine and PLP indicated that transsulfuration preferred taurine production to GSH synthesis. The trend of plasma taurine levels being positively correlated with PLP levels reveals that dramatic transsulfuration occurred to meet the urgent demand for taurine by brain cells. In conclusion, we reported that continual alcohol ingestion alters SCAA utilization, especially by depletion of taurine and hypotaurine and by elevation of *S*-adenosyl homocysteine in the selected organs.

Keywords: Sulfur-containing amino acids; Taurine; Ethanol; Pyridoxal phosphate; Transsulfuration

1. Introduction

Transmethylation and transsulfuration are two important biosynthetic pathways of sulfur-containing amino acids (SCAAs), which involve their further intermediates such as methionine, *S*-adenosyl methionine (SAM), *S*-adenosyl homocysteine (SAH) and homocysteine. Both SAM and SAH serve as methyl donors for critical biomaterial formation (i.e., taurine, choline and some long-chain fatty acids) [1]. In these reactions, vitamin B_6 helps move the transsulfuration process forward to cysteine whereas vitamin B_{12} and folates serve as cofactors involved in the formation of methionine to homocysteine via remethylation. Cysteine transforms to either taurine or glutathione (GSH) depending on the demands of various organs [2,3]. Furthermore, cysteine converts to taurine by cysteine sulfinic acid decarboxylase and a series of transsulfuration in mammals; pyridoxal phosphate (PLP) plays a critical role in these bioprocesses.

In mammals, both alcohol and SCAA metabolism occur mainly in the liver [2]. Data from a study using a single dose of an ethanol (3 g/kg ip) injection in rats suggested that hepatic levels of methionine and SAH increased within 8 h [4]. Furthermore, in patients with alcoholic hepatitis, the plasma level of SAM, methionine and cysteine dramatically decreased, accompanied by abnormal hepatic gene expression of methionine [5]. The liver plays a central role in SCAA metabolism, whereas the effects of ethanol ingestion are widely distributed across various tissues. In a cell culture experiment, after alcohol treatment, the uptake of cysteine and the release of glutamine increased whereas the release of methionine was inhibited in rat astrocytes [6]. Such descriptions seem to imply that different organs such as the liver and brain may show different physical properties of alcohol. Changes in amino acid metabolism are interpreted as a response to oxidative stress induced by ethanol. However, the correlation between alcohol and SCAA levels in the brain still remains unknown.

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As an end product of SCAA metabolism, taurine is a unique amino acid that is not utilized through the common pathway of protein synthesis. Taurine is regarded as a conditionally essential amino acid that is needed and travels in a free or peptide form in numerous tissues [7,8]. Taurine is considered to have protective actions against alcohol-induced fatty liver and hepatitis [9,10]. Furthermore, taurine also acts as an osmotic regulator in the kidneys and maintains their tonicity [11,12]. With the exception of glycine, taurine is the most abundant amino acid that acts as a neuroactive agent in the brain and that protects neurons during the performance of their regular functions. Recent studies have revealed that ethanol increases taurine efflux in the nucleus accumbens and ventral striatum [13]. Acamprosate, a calcium derivative of taurine, reduces the increase in excitatory amino acid glutamate observed during repeated ethanol withdrawal [14]. This might imply that acamprosate possesses a protective mechanism against neurotoxicity by reducing excitatory amino acids. From a chemical structure point of view, taurine may play a decisive and protective role in brain neurotransmission, especially in alcohol addiction and withdrawal complications. However, the mechanism of SCAAs such as taurine and methionine in the brain still needs more work. Thus, in this study, we evaluated the effect of the acute administration of consecutive high doses of alcohol on the metabolism of SCAAs in rats.

2. Material and methods

2.1. Animal protocol

Twenty-four 12-week-old Sprague–Dawley rats (National Science Council, Taipei, Taiwan) weighing between 250 and 300 g at the start of the experiment were used. They were housed in a temperature-controlled (22–24°C) and humidity-controlled (60%) room on a 12-h light/dark (lights on from 08:00 to 20:00 h) cycle for 7 days. They received rodent chow (Labdiet #5001) and water ad libitum.

2.2. Experimental design

Some of the animals ($n=6$) were sham gavaged and sacrificed immediately as the baseline group (Group B). The other rats were gavaged with a certain amount of 30° alcohol (3 g ethanol/g BW) consecutively for 7 days, and blood samples were drawn 2 h after alcohol ingestion and on Days 1, 3 and 7 (Groups E1, E3 and E7, respectively). Six animals were sacrificed on Days 1, 3 and 7, respectively. Organs (including the brain, liver, heart and kidneys) were collected for SCAA and vitamin B_6 analyses.

2.3. Analytical methods

2.3.1. Analysis of SAM and SAH

SAM and SAH were analyzed using the method described by She et al. [15]. All procedures of sample preparations were carried out at 4°C. Two hundred microliters of plasma or tissue homogenates was deproteinized with an equal volume of 0.4 M of perchloric acid, and the mixture was centrifuged at 10,000×g and 4°C for 20 min. The supernatants were filtered and then directly applied onto an HPLC analytical system. The equipment for the SAM/SAH analysis consisted of a model Hitachi L-7100 pump equipped with an L-7200 autosampler, an L-7455 Photo Diode Array detector and an Insil ODS-2 column (5 μm, 4×250 mm) (GL Sciences, USA). Conditions for SAM/SAH analysis were set at a 1-ml/min flow rate and 254-nm detection. The mobile phase consisted of 40 mM $NH_4H_2PO_4$, 8 mM 1-heptanesulfonic acid sodium salt and 18% (by volume) methanol (pH 3.0, adjusted with hydrochloric acid).

2.3.2. Taurine and other SCAAs

All procedures of taurine and other SCAA analyses were similar to those of SAM/SAH analysis with some modifications [16,17]. Methanol extracts of plasma and tissue homogenates (1/1, by volume) were taken for HPLC analysis. The HPLC apparatus consisted of a model L-7100 Hitachi pump equipped with an L-6200 UV detector. A Hypersil amino acid column was used. The mobile phase consisted of an isocratic eluent A (0.03 M of sodium acetate with 0.25% tetrahydrofuran, by volume) and eluent B (80% acetonitrile with 20% 0.1-M sodium acetate, by volume) at a 1-ml/min flow rate and 254-nm detection.

2.3.3. GSH analysis

A commercial kit (BIOXYTECH GSH-400) was used to determine the GSH level of all samples. Metaphosphoric acid (33–37%) was used to resuspend the erythrocyte lysate (4:1, by volume). The centrifuged (3000×g at 4°C for 10 min) supernatant was collected and reacted with 50 ml of reagent A (4-chloro-1-methyl-7-trifluromethyl-quinolinium methylsulfate) to produce thioesters. Chromophoric thione was produced by adding reagent B (30% NaOH), with a 400-nm detection.

2.3.4. PLP analysis

The analysis of PLP was carried out with minor modifications as described by Bisp et al. [18]. Plasma and tissue homogenates were deproteinized with 1 N perchloric acid. The supernatants were then directly injected into the HPLC for PLP analysis. The HPLC apparatus consisted of a model L-7100 Hitachi pump equipped with an L-6200 fluorescence detector. A C_{18} Hypersil amino acid column was used with a mobile phase of 0.03 M of sodium phosphate under a flow rate of 1 ml/min.

2.4. Statistical analysis

Values are presented as mean±S.D. All data were analyzed using the SAS system. ANOVA with least significant difference test was performed to analyze differences among different ethanol durations. The acceptable

Table 1
Selected SCAA profiles in organs

	Methionine	SAM	SAH	Cysteine	Taurine	Hypotaurine
	ng/g tissue				μg/g tissue	
Liver						
B	94.5±66.0	13.1±5.3[a]	2.45±0.23[a]	56.5±17.0[a]	6.23±0.93[a]	1.99±0.84[a]
E1	93.0±31.9	30.3±14.8[b]	8.58±5.73[b]	45.4±15.4[a]	6.52±1.15[a]	1.87±1.24[a]
E3	58.0±19.9	63.0±26.6[c]	16.5±9.0[b]	72.0±41.2[a]	4.52±0.55[b]	0.72±0.10[b]
E7	111.0±55.3	62.6±11.3[c]	15.4±5.3[b]	165.8±23.8[b]	3.78±1.24[b]	0.25±0.12[b]
Brain						
B	100.4±36.6[a]	5.70±2.17[bc]	4.70±2.93[a]	103.9±39.6	6.11±0.71[a]	0.58±0.16[a]
E1	29.9±7.2[b]	2.65±0.77[a]	5.98±2.36[a]	74.6±10.4	3.32±1.13[b]	0.34±0.18[b]
E3	30.6±13.9[b]	5.82±0.44[b]	16.4±3.6[b]	90.9±18.3	3.58±0.36[b]	0.59±0.25[a]
E7	22.6±3.7[b]	8.11±2.18[c]	16.7±2.7[b]	91.5±4.7	3.89±1.09[b]	0.75±0.29[c]
Heart						
B	103.2±45.0[a]	7.98±1.56[a]	10.5±4.7[a]	292.9±96.2[ab]	19.1±0.2[a]	4.19±0.15[a]
E1	208.8±42.0[b]	2.96±1.30[b]	14.7±3.3[a]	216.5±57.6[a]	16.1±1.3[b]	2.75±0.63[b]
E3	211.4±40.2[c]	3.98±2.17[b]	26.3±3.9[b]	344.3±131.1[b]	15.3±1.4[b]	1.73±0.46[c]
E7	120.9±28.9[ac]	3.37±1.38[b]	24.3±5.7[b]	229.9±63.2[a]	12.3±0.7[c]	2.11±0.62[c]
Kidney						
B	185.8±49.7[a]	19.9±5.8[b]	3.05±2.33[ab]	235.3±75.6[a]	5.75±1.12[a]	1.31±0.11[a]
E1	280.6±22.7[b]	18.4±2.3[b]	3.51±0.96[a]	560.8±136.4[b]	3.79±0.88[b]	1.02±0.07[b]
E3	165.2±41.3[a]	35.9±5.2[a]	8.21±1.39[c]	466.0±191.9[bc]	2.55±0.17[c]	0.66±0.13[c]
E7	143.8±42.9[a]	32.3±7.4[a]	7.71±2.89[b]	378.1±12.1[c]	2.27±0.04[c]	0.44±0.03[c]

Values are presented as mean±S.D. $n=6$ at each time interval. Values in the same column and row with different superscript letters significantly differ at $P<.05$ by one-way ANOVA with least significant difference. Superscripted letters indicate a significant difference between time courses.

level of significance was established at $P<.05$ except when otherwise indicated.

3. Results

3.1. Changes in SCAA levels in tissues with consecutive alcohol administration

Data on SCAAs showed that alcohol exerted minor effects in various tissues in the early stage (Table 1). Interestingly, the concentration of taurine in brain tissue showed a remarkable decrease during alcohol ingestion and was maintained at 1.5-fold-lowering changes until the end of the experiment; a similar status of methionine was also observed during alcohol ingestion (Groups E1, E3 and E7; $P<.01$). The status of SAH showed a significant increase in brain tissue on Days 3 (Group E3, $P<.05$) and 7 (Group E7, $P<.05$) after subsequent alcohol ingestion (Table 1).

In the liver, concentration changes in SCAAs such as methionine and SAM were imperceptible on Days 1 (Group E1) and 3 (Group E3); however, SAM presented a significant increase on Day 7 (Group E7, $P<.05$). Surprisingly, the level of SAH increased on Day 1 (Group E1, $P<.05$) and remained at a higher level to the end of the experiment (Group E3, $P<.05$; Group E7, $P<.05$). Taurine and hypotaurine showed dramatic decreases after alcohol administration (Group E3, $P<.05$), especially on Day 7 (Group E7, $P<.001$). The level of cysteine statistically increased on Day 7 (Group E7, $P<.05$). Hepatic GSH showed a slight increase on Day 7 (Group E7, $P<.05$).

Similarly, the level of taurine and hypotaurine in cardiac and renal tissues had obviously declined by the end of the experiment (Group E7, $P<.05$). The cysteine level was slightly increased in the kidneys (Group E7, $P<.05$), whereas it had decreased in the heart (Group E7, $P=.09$). The cardiac level of SAH increased slightly, as well as in the kidneys; however, contrarily, the level of SAM significantly decreased (Groups E1, E3 and E7; $P=.005$, .009 and .006, respectively) and remained at a low level after the ethanol intervention. Methionine was elevated on Day 1 but then recovered on Day 3 and showed a similar status in the kidneys.

Table 2
Selected SCAA profiles in plasma

Plasma	Homocysteine	Methionine	Cysteine	GSH	Taurine	Hypotaurine
	nmol/L	μmol/L				
B	1.78±1.16	15.64±6.69[a]	13.48±4.81[a]	56.33±11.77[a]	12.56±9.17[a]	7.54±4.92[a]
E1	1.81±0.83	8.58±3.57[b]	9.24±3.57[b]	43.22±11.52[b]	30.63±4.14[b]	27.57±15.27[b]
E3	1.33±0.93	27.86±10.16[a]	7.41±2.59[b]	65.76±15.14[a]	18.71±9.54[a]	11.68±6.30[b]
E7	1.49±0.81	7.36±3.23[b]	15.55±3.66[a]	113.51±4.28[b]	29.16±1.92[b]	4.53±4.67[a]

Values are presented as mean±S.D. $n=6$ at each time interval. Values in the same column and row with different superscript letters significantly differ at $P<.05$ by one-way ANOVA with least significant difference. Superscripted letters indicate a significant difference between time courses.

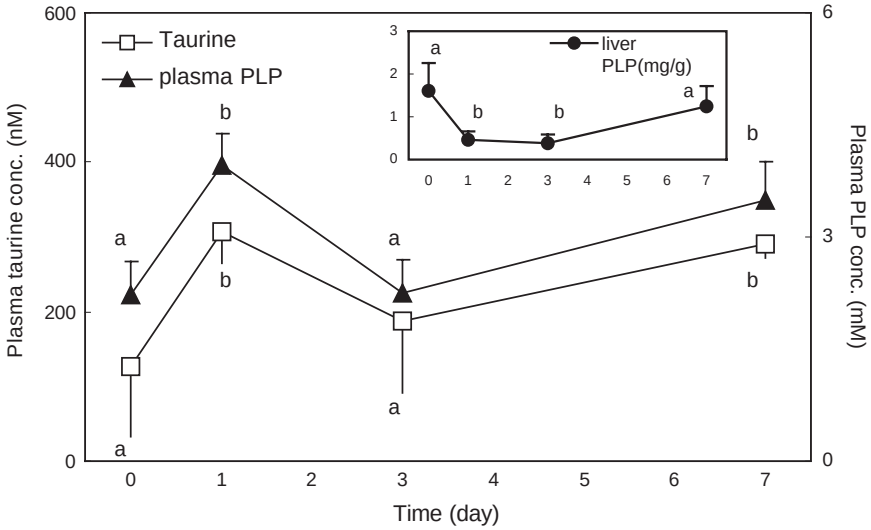

Fig. 1. Changes in plasfsma taurine and PLP levels with continual alcohol administration for 7 days. Different letters in each category indicate a statistical difference between days ($P<.05$).

3.2. Concentrations of plasma SCAA, GSH and pyridoxal-5′-phosphate levels with alcohol administration

The SCAAs of plasma showed no regulatory changes (Table 2). Plasma taurine merely increased after ethanol ingestion and retained this tendency to the end of the study (Group E7). Hypotaurine was also elevated on Day 3 (Group E3) but had returned to normal on Day 7 (Group E7). Cysteine immediately decreased with the ethanol intervention; however, it recovered as did hypotaurine on Day 7 ($P<.05$). At the same time, GSH was continually being released under the stress of ethanol ($P<.05$) and showed a time-dependent response (Group E1 vs. Group E3, $P<.05$; Group E1 vs. Group E7, $P<.001$; Group E3 vs. Group E7, $P<.001$). Surprisingly, during alcohol administration, plasma taurine was highly correspondent to the PLP level ($r=.98$, $P=.045$) (Fig. 1). The level of hepatic PLP was significantly lower on Days 1 (Group E1, $P<.05$) and 3 (Group E3, $P<.05$).

4. Discussion

Acute and chronic ethanol administration altering SCAAs metabolism has raised great interests [2,19]. Such reports suggested that acute alcohol ingestion increases hepatic utilization of cysteine for synthesis of taurine rather than GSH, which indicates that taurine might attenuate ethanol-induced oxidative damage. It was concluded that taurine might have a specific function in dealing with the impact of ethanol. Meanwhile, exposure time is also a key factor that needs to be considered. In the brain, acute ethanol administration causes different SCAA metabolic patterns with long-term addiction to alcoholism. It was reported that an acute single-dose alcohol administration stimulates taurine release in neurons whereas long-term addiction lowers taurine levels [20,21]. Presumably, SCAA alterations may diversify during alcohol withdrawal in alcoholic patients [22]. Results implied that alcohol consumption might cause different responses by taurine, thus leading to different SCAA metabolic reactions.

Data from selected organs showed that the taurine depletion ratio in the brain was the highest among selected tissues (45.46±17.57%). Furthermore, all selected tissues except the liver presented a lowering effect of taurine levels immediately after ethanol ingestion for 2 h. After 3 days of alcohol administration, taurine levels in the heart, liver and kidney tissues had continued to decrease; however, the taurine levels of brain tissues remained consistent. It is reasonable to suggest that hepatic taurine begins to collapse and be released, causing fluctuations in peripheral taurine levels. These phenomena imply that profound alterations in hepatic taurine might play a prospective role (e.g., neuroprotection) in preventing cephalic taurine from further alcoholic stress. Depleted cephalic taurine revealed the exertion of neuronal ethanol detoxification [23], which can be explained by taurine's affiliation with the $GABA_A$ receptor or the NMDA receptor in brain cells [24], the same site to which both ethanol and acetaldehyde bind [25]. Based on the concrete evidences, the brain seems to be the most sensitive organ that is susceptible to ethanol invasion. The taurine-lowering effects could not completely be recovered with subsequent ethanol challenge. Results disclose that taurine is the primary SCAA that thoroughly reflects ethanol intake and that challenge would first impact the brain. Increased depletion of taurine and its precursor, hypotaurine, revealed an urgent demand for taurine production by means of transsulfuration in the brain. Thus, compared with transmethylation, the bioprocess of transsulfuration of SCAAs should receive more consideration as a major energy source in patients with alcoholism.

That alcohol catabolism generates excess amounts of NADH and results in the acceleration in remethylation of

homocysteine to methionine in the liver has been discussed. According to our data, methionine after ethanol ingestion was observed to be increased in the liver but decreased in the brain. The SAM level did increase with methionine in the liver but remarkably decreased in the brain. These phenomena seemed to imply that SAM may not get enough supply from methionine and thus showed various situations in selected organs, especially depletion in the brain. We also calculated the SAM/SAH ratio, which represents the transmethylation of such amino acids' biosynthesis. The brain showed a significant higher ratio on Day 7; however, the ratio of other selected organs remained in lower levels on Day 7. Furthermore, the SAM/SAH ratio decreased in selected organs (liver, kidney and heart) on Days 1 to 7; however, the ratio of the brain remarkably elevated on Day 7. It seems to imply that the transmethylation in selected organs may serve an alternative role, especially in various time intervals. Thus, from the redox point of view, alcohol-induced NADH contribution on the transmethylation or transsulfuration of SCAA in different organs needs further evaluation.

The liver is the major organ that produces and stores taurine. Kim et al. [4] reported that an intraperitoneal injection of a single dose of 40% ethanol (3 g/kg) caused higher taurine levels in the liver after 4 h and maintained for 24 h; however, a decrease was shown after 2 days and recovery occurred after Day 5. In our study, results revealed that hepatic taurine production increased after ethanol absorption and allotment to exposed tissues. With a high dose of alcohol ingestion, the liver might not have a chance to restore taurine, so it continues to decrease. In contrast, plasma taurine levels were elevated as the hepatic taurine continued to rise. This trend did not seem to stop until alcohol ingestion ceased. A similar trend of an SCAA level imbalance was observed in the brain, heart and kidneys during ethanol treatment. Because taurine comprises up to 50% of the total free amino acid pool in the heart and because it has a protective function [26], the continually decreasing levels in the heart and kidneys suggest that specific SCAAs in those tissues may have high priorities for protecting the brain against stress from alcohol.

As the initial material of transsulfuration, cysteine is involved in taurine bioformation and might be influenced by GSH synthesis in the liver. Hepatic and plasma GSH levels were both elevated with increased ethanol ingestion. The increase in cysteine levels did not improve the reduction in taurine; instead, the level of GSH was slightly increased. Numerous studies [27,28] have mentioned that alcohol metabolism represents typical oxidative damage that causes interference with the antioxidant enzyme system. Twenty-eight days of ethanol intake (3 g/kg) decreased GSH levels [19] while acute ethanol administration stimulated the antioxidative enzyme system [27]. Not surprisingly, in our study, 7-day consecutive ethanol gavage stimulated plasma and hepatic GSH assimilation. These phenomena illustrate that tissue GSH (e.g., hepatic GSH) might be released against lipid peroxidation induced by ethanol and its metabolites, moreover increasing the plasma GSH with subsequent administration.

It is noteworthy that plasma taurine presented changes similar to those of plasma PLP during the experiment. Interestingly, hepatic PLP declined in the early stage of alcohol ingestion. The following physical descriptions may provide possible interpretations for this result. First of all, alcohol inhibits dietary vitamin B_6 absorption [29] and accelerates hepatic PLP release for utilization. Furthermore, acetaldehyde has been shown to deplete vitamin B_6 in chronic alcoholics. It also binds with sulfhydryl groups of membrane proteins, altering membrane Ca^{2+} channels [29]. Second, the released vitamin B_6 in the periphery may be used for the bioformation (e.g., transsulfuration) of cysteine [30]. Thus, the findings indicated that with consecutive alcohol ingestion, the transsulfuration cofactor, PLP, shows a metabolic pattern similar to that of taurine with a high correlation ($r=.98$, $P=.045$) but not to that of GSH. These results revealed that the purpose of cysteine transsulfuration favors taurine synthesis to GSH formation. Unfortunately, the present study did not have solid data to explain how taurine relates to vitamin B_6 under alcohol ingestion. Whether taurine directly or indirectly influences brain alcohol metabolism still remains unclear. In conclusion, we reported that continual alcohol administration dramatically alters SCAA utilization, especially by depleting selected tissue methionine, cysteine and taurine in rats.

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References

[1] Roque ME, Giusto NM. Phosphatidylethanolamine *N*-methyltransferase activity in isolated rod outer segments from bovine retina. Exp Eye Res 1995;60:631–43.

[2] Jung YS, Kwak HE, Choi KH, Kim YC. Effect of acute ethanol administration on *S*-amino acid metabolism: increased utilization of cysteine for synthesis of taurine rather than glutathione. Adv Exp Med Biol 2003;526:245–52.

[3] Schuller-Levis GB, Park E. Taurine: new implications for an old amino acid. FEMS Microbiol Lett 2003;226:195–202.

[4] Kim SK, Seo JM, Jung YS, Kwak HE, Kim YC. Alterations in hepatic metabolism of sulfur-containing amino acids induced by ethanol in rats. Amino Acids 2003;24:103–10.

[5] Lee TD, Sadda MR, Mendler MH, Bottiglieri T, Kanel G, Mato JM, et al. Abnormal hepatic methionine and glutathione metabolism in patients with alcoholic hepatitis. Alcohol Clin Exp Res 2004;28:173–81.

[6] Fonseca LL, Alves PM, Carrondo MJ, Santos H. Effect of ethanol on the metabolism of primary astrocytes studied by (13)C- and (31)P-NMR spectroscopy. J Neurosci Res 2001;66:803–11.

[7] Aragon CM, Trudeau LE, Amit Z. Effect of taurine on ethanol-induced changes in open-field locomotor activity. Psychopharmacology (Berl) 1992;107:337–40.

[8] Aragon CM, Amit Z. Taurine and ethanol-induced conditioned taste aversion. Pharmacol Biochem Behav 1993;44:263–6.
[9] Kerai MD, Waterfield CJ, Kenyon SH, Asker DS, Timbrell JA. The effect of taurine depletion by beta-alanine treatment on the susceptibility to ethanol-induced hepatic dysfunction in rats. Alcohol Alcohol 2001;36:29–38.
[10] Razvodovskii Iu E, Doroshenko EM, Prokopchik NI, Smirnov V, Ostrovskii S. Hepatoprotective effects of amino acids with branched hydrocarbon chains and taurine in experimental subchronic alcohol intoxication and ethanol withdrawal. Biomed Khim 2004;50:64–72.
[11] Handler JS, Kwon HM. Regulation of renal cell organic osmolyte transport by tonicity. Am J Physiol 1993;265:C1449-55.
[12] Grunewald RW, Oppermann M, Schettler V, Fiedler GM, Jehle PM, Schuettert JB. Polarized function of thick ascending limbs of Henle cells in osmoregulation. Kidney Int 2001;60:2290–8.
[13] Smith A, Watson CJ, Frantz KJ, Eppler B, Kennedy RT, Peris J. Differential increase in taurine levels by low-dose ethanol in the dorsal and ventral striatum revealed by microdialysis with on-line capillary electrophoresis. Alcohol Clin Exp Res 2004;28:1028–38.
[14] Dahchour A, De Witte P, Bolo N, Nedelec JF, Muzet M, Durbin P, et al. Central effects of acamprosate: Part 1. Acamprosate blocks the glutamate increase in the nucleus accumbens microdialysate in ethanol withdrawn rats. Psychiatry Res 1998;82:107–14.
[15] She QB, Nagao I, Hayakawa T, Tsuge H. A simple HPLC method for the determination of *S*-adenosylmethionine and *S*-adenosylhomocysteine in rat tissues: the effect of vitamin B_6 deficiency on these concentrations in rat liver. Biochem Biophys Res Commun 1994;205:1748–54.
[16] Hirschberger LL, De La Rosa J, Stipanuk MH. Determination of cysteinesulfinate, hypotaurine and taurine in physiological samples by reversed-phase high-performance liquid chromatography. J Chromatogr 1985;343:303–13.
[17] Tcherkas YV, Kartsova LA, Krasnova IN. Analysis of amino acids in human serum by isocratic reversed-phase high-performance liquid chromatography with electrochemical detection. J Chromatogr A 2001;913:303–8.
[18] Bisp MR, Bor MV, Heinsvig EM, Kall MA, Nexo E. Determination of vitamin B_6 vitamers and pyridoxic acid in plasma: development and evaluation of a high-performance liquid chromatographic assay. Anal Biochem 2002;305:82–9.
[19] Pushpakiran G, Mahalakshmi K, Anuradha CV. Taurine restores ethanol-induced depletion of antioxidants and attenuates oxidative stress in rat tissues. Amino Acids 2004;27:91–6.
[20] Olive MF. Interactions between taurine and ethanol in the central nervous system. Amino Acids 2002;23:345–57.
[21] Sakurai T, Miki T, Li HP, Miyatake A, Satriotomo I, Takeuchi Y. Colocalization of taurine and glial fibrillary acidic protein immunoreactivity in mouse hippocampus induced by short-term ethanol exposure. Brain Res 2003;959:160–4.
[22] Dahchour A, De Witte P. Excitatory and inhibitory amino acid changes during repeated episodes of ethanol withdrawal: an in vivo microdialysis study. Eur J Pharmacol 2003;459:171–8.
[23] Zimmerman U, Spring K, Koller G, Holsboer F, Soyka M. Hypothalamic-pituitary-adrenal system regulation in recently detoxified alcoholics is not altered by one week of treatment with acamprosate. Pharmacopsychiatry 2004;37:98–102.
[24] Ferraz AC, Anselmo-Franci JA, Perosa SR, de Castro-Neto EF, Bellissimo MI, de Oliveira BH, et al. Amino acid and monoamine alterations in the cerebral cortex and hippocampus of mice submitted to ricinine-induced seizures. Pharmacol Biochem Behav 2002;72:779–86.
[25] Timbrell JA, Seabra V, Waterfield CJ. The in vivo and in vitro protective properties of taurine. Gen Pharmacol 1995;26:453–62.
[26] Jacobsen JG, Smith LH. Biochemistry and physiology of taurine and taurine derivatives. Physiol Rev 1968;48:424–511.
[27] Dutta P, Seirafi J, Halpin D, Pinto J, Rivlin R. Acute ethanol exposure alters hepatic glutathione metabolism in riboflavin deficiency. Alcohol 1995;12:43–7.
[28] Moss M, Guidot DM, Wong-Lambertina M, Ten Hoor T, Perez RL, Brown LA. The effects of chronic alcohol abuse on pulmonary glutathione homeostasis. Am J Respir Crit Care Med 2000;161:414–9.
[29] Vasdev S, Wadhawan S, Ford CA, Parai S, Longerich L, Gadag V. Dietary vitamin B_6 supplementation prevents ethanol-induced hypertension in rats. Nutr Metab Cardiovasc Dis 1999;9:55–63.
[30] Lin GW. Effect of ethanol and vitamin B_6 deficiency on pyridoxal 5-phosphate levels and fetal growth in rat. Alcohol Clin Exp Res 1989;13:236–9.