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Alterations in hepatic metabolism of sulfur-containing amino acids induced by ethanol in rats

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Summary. Alterations in hepatic metabolism of S-amino acids were monitored over one week in male rats treated with a single dose of ethanol (3 g/kg, ip). Methionine and S-adenosylhomocysteine concentrations were increased rapidly, but S-adenosylmethionine, cysteine, and glutathione (GSH) decreased following ethanol administration. Activities of methionine adenosyltransferase, cystathionine γ-lyase and cystathionine β-synthase were all inhibited. γ-Glutamylcysteine synthetase activity was increased from t = 8 hr, but GSH level did not return to control for 24 hr. Hepatic hypotaurine and taurine levels were elevated immediately, but reduced below control in 18 hr. Changes in serum and urinary taurine levels were consistent with results observed in liver. Cysteine dioxygenase activity was increased rapidly, but declined from t = 24 hr. The results show that a single dose of ethanol induces profound changes in hepatic S-amino acid metabolism, some of which persist for several days. Ethanol not only inhibits the cysteine synthesis but suppresses the cysteine availability further by enhancing its irreversible catabolism to taurine, which would play a significant role in the depletion of hepatic GSH.

Keywords: Ethanol – Transsulfuration pathway – Cysteine – Glutathione – Taurine – Hypotaurine

Abbreviations: CβS, cystathionine β-synthase; CDC, cysteine sulfinate decarboxylase; CDO, cysteine dioxygenase; CγL, cystathionine γ-lyase; GCS, γ-Glutamylcysteine synthetase; GSH, glutathione; MAT, methionine adenosyltransferase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine.

Introduction

In mammals the liver plays a central role in S-amino acid metabolism (Mato et al., 1997). S-Amino acid metabolism occurs primarily by the transsulfuration pathway (Fig. 1), which results in the transfer of sulfur from methionine to serine to form cysteine. Other than diet the only known source of cysteine for mammals is this synthetic pathway. The first step in methionine metabolism is formation of S-adenosylmethionine (SAM) that is catalyzed by methionine adenosyltransferase (MAT). SAM serves as a methyl donor for biological methylation reactions and the co-product of transmethylation, S-adenosylhomocysteine (SAH), is hydrolyzed to yield homocysteine which stands at an intersection of two competitive metabolic pathways: remethylation to methionine and transsulfuration to cystathionine (Cooper, 1983; Finkelstein and Martin, 2000; Stipanuk, 1986). Transsulfuration of homocysteine to cysteine via cystathionine is mediated by consecutive actions of cystathionine β-synthase (CβS) and cystathionine γ-lyase (CγL). Cysteine is irreversibly metabolized in liver to yield either taurine, inorganic sulfate, or glutathione (GSH). Cysteine dioxygenase (CDO) catalyzes the oxidation of this amino acid to cysteine sulfinate that is mainly converted to taurine via hypotaurine by the activity of cysteine sulfinate decarboxylase (CDC) (Griffith, 1983). Synthesis of GSH is mediated by γ-glutamylcysteine synthetase (GCS) and GSH synthetase, consecutively.

It has been known that alcohol consumption is associated with impaired S-amino acid metabolism. Patients with cirrhosis of different causes, including alcohol, often have hypermethioninemia and delayed plasma clearance of methionine (Horowitz et al., 1981). Changes in SAM level have been variable. Despite a 50% fall in MAT activity, cirrhotic patients did not have a lower hepatic SAM level (Cabrero et al., 1988). In rats fed the Lieber-DeCarli ethanol liquid diet for 4 weeks hepatic SAM was not changed (Barak

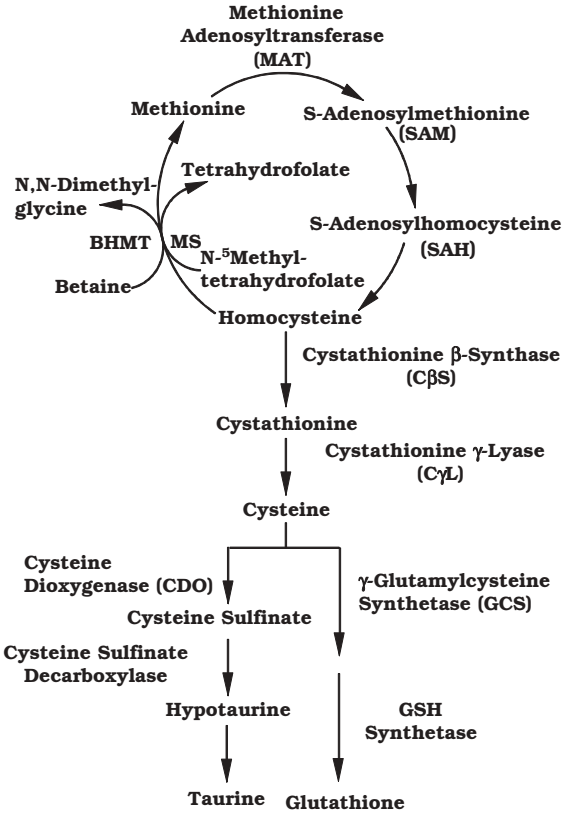

Fig. 1. Simplified metabolic pathway of S-amino acids in liver

et al., 1987), but Trimble et al. (1993) observed a reduction in the level of SAM in rat liver after 3 weeks of ethanol feeding. Also baboons fed ethanol chronically exhibited a lower hepatic SAM level (Lieber et al., 1990). The reason for this discrepancy is unclear. The effects of an acute ethanol intake on the hepatic transsulfuration reactions have been mostly unknown except that the hepatic cysteine concentration was either unaltered (Choi et al., 2000; Lauterburg et al., 1984) or slightly decreased following a reduction in GSH (Speisky et al., 1985).

Reduction of GSH levels in liver by a single or repeated treatment of ethanol has been well documented both in experimental animals and in human (Feo et al., 1986; Guerri and Grisolia, 1980; Lu et al., 1999; Macdonald et al., 1977; Vendemiale et al., 1989). Various mechanisms have been proposed to explain the ethanol-induced inhibition of hepatic GSH. Since ethanol administration is associated with lipid peroxidative damage, it has been suggested that increased lipoperoxidation or binding to acetaldehyde would be responsible for the depletion of hepatic GSH (Kera et al., 1985; Videla et al., 1980; Vina et al., 1980). It has been also suggested that ethanol reduce the GSH levels by increasing its efflux from the liver and/or by inhibiting the biosynthesis (Fernandez-Checa et al., 1987; Speisky et al., 1985). Recently we examined the time-course of changes in hepatic GSH following an acute oral dose of ethanol, and observed that the hepatic GSH efflux played quantitatively the most important role in the reduction of GSH, which was further aggravated by a transient decrease in synthesis and increased consumption associated with its metabolism (Choi et al., 2000).

In the present study we examined the effects of acute ethanol administration on the metabolism of S-amino acids in rats by determining the metabolic intermediates and products, and the enzyme activities involved in the transsulfuration reactions. Especially it was of interest to understand the dynamics of cysteine, the essential substrate for the synthesis of GSH that is the final product in the metabolic pathway with diverse biological functions in animals.

Materials and methods

Animals and treatments

Adult male Sprague-Dawley rats (Animal Breeding Center, Seoul National University), weighing 250–300 g, were used throughout the study. The use of animals was in compliance with the guidelines established by the Animal Care Committee of this institute. Rats were housed in temperature (22 ± 2°C) and humidity (55 ± 5%) controlled rooms with a 12 hr light/dark cycle (Light: 0700–1900, dark: 1900–0700) at least for one week prior to use. Non-purified laboratory rat chow (Purina-Korea, Seoul, Korea) and tap water were allowed *ad libitum* until sacrifice. Ethanol diluted with physiological saline (40% v/v) was administered intraperitoneally to rats at a dose of 3 g/kg body weight at 0830. Control rats received an equivalent volume of saline. For the measurement of urinary taurine excretion rats were transferred to individual metabolic cages (Techniplast Gazzada S.a.r.l., Buguggiate, Italy) immediately after ethanol treatment.

Chemicals

Drugs and chemicals such as NADPH, GSSG reductase, amino acid standards, cystathionine, S-adenosylmethionine iodide salt, S-adenosylhomocysteine, GSH, taurine, hypotaurine, cysteine sulfinate and *O*-phthalaldehyde were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethanol was obtained from Merck (Darmstadt, Germany). γ-Glutamylcysteine was purchased from Kohjin Co. (Tokyo, Japan). All other chemicals and solvents used in this study were of reagent grade or better.

Determination of S-amino acid metabolites

The liver was homogenized in a four-fold volume of cold 1 M perchloric acid. After denatured protein was removed by centrifu-

gation, the supernatant was obtained. Total GSH concentration was determined using an enzymatic recycling method of Griffith (1980) or a HPLC separation/fluorometric detection method of Neuschwander-Tetri and Roll (1989). A HPLC system equipped with a fluorescence detector (FP-920; Jasco Co., Tokyo, Japan) and a 3.5 μm Symmetry C18 column (4.6 $\times$ 75 mm) (Waters Co., Milford, MA, USA) was used. Cysteine levels were estimated by the acid-ninhydrin method (Gaitonde, 1967). The method of She et al. (1994) was employed to determine SAM and SAH concentrations. The supernatant was directly applied to a HPLC system with an UV-975 detector (Jasco Co.) and a TSK-GEL ODS-80TM column (4.6 $\times$ 250 mm) (Tosoh Co., Tokyo, Japan).

The liver was homogenized in a five-fold volume of cold methanol for analysis of amino acids, hypotaurine and taurine. Urine or serum was diluted with a three- to five-volume of methanol. Free amino acids, hypotaurine and taurine were derivatized with *O*-phthalaldehyde/2-mercaptoethanol and quantified using a HPLC system with a fluorescence detector and a 3.5 μm Kromasil C18 column (4.6 $\times$ 100 mm) (Eka Chemicals, Bohus, Sweden). Free amino acids were separated by using the method of Rajendra (1987). The method of Ide (1997) was used to separate hypotaurine and taurine.

Enzyme assays

The liver was homogenized in a three-fold volume of ice-cold buffer consisting of 0.154 M KCl/50 mM Tris-HCl and 1 mM EDTA (pH 7.4). All subsequent steps were performed at 0–4°C. The homogenate was centrifuged at 10,000 g for 20 min. The supernatant fraction was further centrifuged at 104,000 g for 60 min. The 104,000 g supernatant fraction (cytosol) was used to determine the enzyme activities. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

The activity of MAT was estimated by quantifying the SAM and SAH production as described above. Reaction mixtures consisted of 80 mM Tris-HCl/50 mM KCl (pH 7.4), 5 mM ATP, 40 mM $MgCl_2$, 0.1 mM methionine and 1 mg protein of enzyme solution in a final volume of 1 ml. After preincubation for 3 min methionine was added to initiate the enzyme reaction. The incubation carried at 37°C for 30 min was terminated by adding 0.5 ml ice-cold 10% perchloric acid.

The CβS activity was determined by the method of Kashiwamata and Greenberg (1970). The reaction mixture (final volume 1.0 ml) contained 0.1 M Tris buffer (pH 8.3), 0.1 M D,L-homocysteine, 0.1 M L-serine, 0.12 mM pyridoxal 5-phosphate, 0.5 mM $CuSO_4$ and 1 mg protein of enzyme solution. The incubation continued for 45 min at 37°C after preincubation for 5 min without D,L-homocysteine. At the end of incubation 0.1 ml 50% trichloroacetic acid was added to terminate the reaction. For quantification of cystathionine formed during the reaction 1 ml ninhydrin reagent (1 g ninhydrin in the mixture of 100 ml glacial acetic acid and one-third volume of glacial phosphoric acid) was added. The color development was measured at 455 nm.

A modification of the method of Matsuo and Greenberg (1957) was used to determine the CγL activity. The reaction mixture (final volume 1.0 ml) included 32 mM D,L-homoserine, 0.05 mM pyridoxal 5-phosphate, 7.5 mM 2-mercaptoethanol, 7.0 mM EDTA, 0.1 M potassium phosphate buffer (pH 7.5) and 1 mg protein of enzyme solution. The incubation was initiated by addition of 0.1 ml 320 mM D,L-homoserine and carried at 37°C for 30 min. The reaction was stopped by adding 2 ml 10% trichloroacetic acid. The amount of α-ketobutyrate formed during the incubation was quantified after addition of 2,4-dinitrophenylhydrazone in alkaline solution at 510 nm.

The GCS activity was determined using the method of Sekura and Meister (1977). The reaction mixture (final volume 0.5 ml) containing 0.1 M tris-HCl/0.15 M KCl (pH 8.2), 10 mM ATP, 10 mM L-glutamate, 20 mM $MgCl_2$, 2 mM EDTA, 5 mM cysteine and 250 μg protein of enzyme solution was incubated, after preincubation for 3 min without cysteine, at 37°C for 15 min. Formation of γ-glutamylcysteine was quantified by *O*-phthalaldehyde derivatization according to a HPLC separation/fluorometric detection method of Yan and Huxtable (1995). A 3.5 μm Symmetry C18 column (4.6 $\times$ 75 mm) was used for the HPLC system.

The method of Bagley et al. (1995) was used to measure the CDO activity. Protein (1 mg) was incubated for 16 min at 37°C in a final volume of 1 ml containing 0.5 mM ferrous ammonium sulfate, 5 mM hydroxylamine HCl, 2 mM NAD^+ and 5 mM cysteine. The reaction was terminated by adding 2.5 ml ice-cold methanol. The amount of cysteine sulfinate formed during the reaction was quantified by a HPLC system equipped with a fluorescence detector after *O*-phthalaldehyde/2-mercaptoethanol derivatization. Separation was achieved using a 3.5 μm Kromasil C18 column (4.6 $\times$ 100 mm).

The activity of CDC was estimated by measuring the formation of hypotaurine. Protein (100 μg) was added to a test tube, made up to 0.1 ml of 0.1 M potassium phosphate buffer (pH 7.4) containing 5 mM dithiothretol and 0.2 mM pyridoxal 5-phosphate. The mixture was preincubated at 37°C for 3 min. The enzyme reaction, triggered by adding 50 μl 20 mM cysteine sulfinate, continued for 30 min. Addition of 0.6 ml ice-cold methanol stopped the reaction. Hypotaurine was quantified using the method described above.

Data analysis

All results expressed as the mean $\pm$ SEM were analyzed by a two-tailed Student's *t*-test. The acceptable level of significance was established at $P < 0.05$ except when otherwise indicated.

Results

The changes in hepatic concentrations of metabolic intermediates and products in the transsulfuration pathway were determined in rats over one week following acute ethanol administration (Table 1). Increase in the methionine concentration was evident from t = 4 hr following the treatment. However, the SAM level was decreased significantly from t = 6 hr, which was not recovered at least for 48 hr. In contrast, SAH was elevated for 24 hr after ethanol treatment. Consequently the ratio of SAM/SAH was markedly reduced from t = 6 to 48 hr; the maximal decrease shown at t = 8 hr. The hepatic cysteine level was also reduced from t = 6 to 48 hr.

The hepatic GSH concentration was reduced significantly, reaching approximately one half of control at t = 8 hr following ethanol treatment. The reduction in hepatic GSH persisted at least for 24 hr. On the other hand, the hepatic hypotaurine and taurine levels were rapidly increased by ethanol, reaching a peak concentration of more than three times greater than control. Elevation of hypotaurine was reversed at t = 18 hr.

Table 1. Effect of ethanol on concentrations of S-containing amino acids and the metabolites

Time	Group	Methionine	SAM	SAH	Cysteine	GSH	Hypotaurine	Taurine	Taurine
		nmole/g liver				μmole/g liver			μmole/ml serum
2 h	Control	64 ± 2	108 ± 4	25 ± 2	106 ± 4	6.70 ± 0.16	0.20 ± 0.02	2.45 ± 0.43	0.36 ± 0.02
	Ethanol	58 ± 2	96 ± 4	24 ± 1	97 ± 3	6.20 ± 0.17	0.20 ± 0.05	3.30 ± 0.52	0.40 ± 0.03
4 h	Control	57 ± 6	107 ± 5	24 ± 2	103 ± 2	6.30 ± 0.17	0.16 ± 0.01	3.40 ± 0.33	0.38 ± 0.02
	Ethanol	83 ± 5*	110 ± 5	27 ± 3	104 ± 4	4.70 ± 0.13***	0.46 ± 0.04**	5.06 ± 0.25**	0.85 ± 0.13*
6 h	Control	59 ± 7	111 ± 4	25 ± 1	117 ± 6	6.18 ± 0.38	0.17 ± 0.01	2.04 ± 0.32	0.33 ± 0.02
	Ethanol	79 ± 3*	96 ± 2**	26 ± 2	85 ± 3**	4.04 ± 0.25**	0.46 ± 0.06**	6.92 ± 0.76**	0.92 ± 0.05***
8 h	Control	66 ± 3	101 ± 5	22 ± 1	121 ± 8	5.87 ± 0.21	0.19 ± 0.02	2.57 ± 0.61	0.40 ± 0.04
	Ethanol	98 ± 12*	85 ± 7*	45 ± 3***	100 ± 2*	2.96 ± 0.34***	0.90 ± 0.16**	5.55 ± 0.41**	1.32 ± 0.09***
18 h	Control	N/D	111 ± 2	26 ± 2	108 ± 5	6.88 ± 0.21	0.17 ± 0.02	2.43 ± 0.67	N/D
	Ethanol	N/D	96 ± 4**	33 ± 3**	82 ± 3**	5.41 ± 0.21**	0.11 ± 0.01**	2.04 ± 0.58	N/D
24 h	Control	62 ± 4	108 ± 5	25 ± 2	106 ± 5	6.68 ± 0.19	0.20 ± 0.02	3.25 ± 0.77	0.44 ± 0.05
	Ethanol	56 ± 7	92 ± 3*	37 ± 1**	93 ± 7	5.65 ± 0.22*	0.12 ± 0.02*	2.92 ± 0.63	0.41 ± 0.04
48 h	Control	64 ± 5	99 ± 4	23 ± 1	108 ± 3	7.19 ± 0.18	0.22 ± 0.02	2.21 ± 0.42	0.40 ± 0.03
	Ethanol	72 ± 6	76 ± 4**	23 ± 2	86 ± 6**	6.94 ± 0.76	0.10 ± 0.02**	1.09 ± 0.13*	0.25 ± 0.01**
120 h	Control	N/D	98 ± 4	23 ± 1	113 ± 4	6.64 ± 0.32	0.18 ± 0.02	2.58 ± 0.54	0.29 ± 0.03
	Ethanol	N/D	99 ± 2	25 ± 1	123 ± 4	7.10 ± 0.12	0.19 ± 0.01	1.01 ± 0.21*	0.25 ± 0.01
168 h	Control	N/D	107 ± 3	24 ± 1	110 ± 3	7.17 ± 0.14	0.20 ± 0.01	2.28 ± 0.47	N/D
	Ethanol	N/D	105 ± 6	26 ± 1	121 ± 1*	7.56 ± 0.28	0.21 ± 0.05	2.69 ± 0.34	N/D

Each value represents the mean ± SEM for more than four rats
*,**,*** Significantly different from the control at $P < 0.05$, 0.01, 0.001, respectively (Student's *t*-test)

The changes of hepatic hypotaurine preceded those of taurine. The hepatic taurine level was decreased below control from t = 48 hr. The changes in taurine concentration in serum seemed to parallel those of liver. The serum taurine level was increased from t = 4 to 8 hr after ethanol treatment and decreased at t = 48 hr.

Corresponding changes in the activities of enzymes involved in the transsulfuration reactions were observed (Table 2). The activity of MAT was reduced from t = 4 to 8 hr after ethanol treatment. Ethanol also decreased the activities of the two enzymes critical for the synthesis of cysteine. Ethanol administered 8 hr before inhibited the activity of CβS that mediates the synthesis of cystathionine from homocysteine. The activity of CγL that releases cysteine from cystathionine was decreased significantly from t = 2 to 6 hr.

The activities of hepatic enzymes mediating the metabolic conversion of cysteine to GSH, hypotaurine or taurine were also significantly altered. Increase in the activity of GCS, the rate-limiting enzyme in the synthesis of GSH from cysteine, was observed from t = 8 hr after ethanol treatment. The activity of CDO was rapidly elevated from t = 4 hr following the treatment but prolonged reduction was shown from t = 24 to 120 hr. The activity of CDC that mediates the transformation of cysteine sulfinate to hypotaurine was also increased transiently.

The excretion of taurine through urine was measured (Table 3). The urine volume of rats treated with ethanol was reduced to a level approximately 45% of control for the first 24 hr. However, the total amount of urinary excretion of taurine in ethanol-treated rats was increased greater than two times for the same period. The excretion of taurine through urine was reduced from t = 24 hr, which was consistent with the changes in hepatic taurine level.

Discussion

The present study examined the alterations in S-amino acid metabolism induced by acute ethanol administration in rats. The results show that a single dose of ethanol causes profound changes in the metabolism of S-amino acids in liver. First, ethanol administration led to a rapid increase in the hepatic methionine concentration accompanied with a decrement in the SAM and cysteine concentrations indicating that the transsulfuration reactions from methionine to cysteine were inhibited. Secondly ethanol induced time-dependent changes in the hepatic concentration of the final products in the metabolic reactions, GSH and taurine. Hepatic GSH concentration was decreased significantly below control for the first 24 hr, but taurine and its immediate precursor, hypotaurine, were

Table 2. Effect of ethanol on activities of MAT, CβS, CγL, GCS, CDO and CDC in liver

Time	Group	MAT	CβS	CγL	GCS	CDO	CDC
		product formed (nmole)/min/mg protein					
2 h	Control	0.110 ± 0.002	17.7 ± 0.5	63.6 ± 3.5	7.3 ± 0.3	0.55 ± 0.05	14.9 ± 1.1
	Ethanol	0.103 ± 0.006	16.2 ± 0.9	48.9 ± 1.5**	6.7 ± 0.2	0.53 ± 0.12	17.2 ± 1.3
4 h	Control	0.116 ± 0.006	19.9 ± 0.9	65.0 ± 4.2	7.3 ± 0.5	0.52 ± 0.05	16.0 ± 1.3
	Ethanol	0.093 ± 0.003*	21.8 ± 1.7	50.7 ± 2.6*	7.5 ± 0.7	0.70 ± 0.03*	15.2 ± 1.6
6 h	Control	0.118 ± 0.007	19.6 ± 0.9	68.8 ± 3.4	7.5 ± 0.4	0.55 ± 0.05	13.4 ± 1.0
	Ethanol	0.094 ± 0.001**	21.5 ± 0.8	60.6 ± 1.3*	7.5 ± 0.3	0.89 ± 0.10*	17.1 ± 1.1*
8 h	Control	0.109 ± 0.006	18.9 ± 0.7	72.2 ± 2.8	6.2 ± 0.7	0.49 ± 0.03	15.1 ± 1.0
	Ethanol	0.084 ± 0.006*	15.9 ± 1.3*	67.0 ± 7.7	9.1 ± 1.3*	0.57 ± 0.16	14.3 ± 4.0
24 h	Control	0.097 ± 0.004	16.7 ± 0.6	66.4 ± 7.0	6.0 ± 0.2	0.54 ± 0.04	13.1 ± 2.3
	Ethanol	0.103 ± 0.003	17.1 ± 0.5	55.3 ± 2.6	8.5 ± 0.3**	0.34 ± 0.04**	12.9 ± 0.9
48 h	Control	0.103 ± 0.003	16.5 ± 1.7	69.3 ± 5.2	5.7 ± 0.3	0.64 ± 0.04	14.9 ± 0.8
	Ethanol	0.113 ± 0.003	16.4 ± 0.4	61.4 ± 4.2	10.6 ± 0.3***	0.25 ± 0.06**	14.1 ± 1.3
120 h	Control	0.121 ± 0.004	16.4 ± 1.2	55.4 ± 3.1	6.8 ± 0.5	0.48 ± 0.02	14.1 ± 0.4
	Ethanol	0.120 ± 0.005	18.2 ± 2.1	60.1 ± 2.1	7.2 ± 0.3	0.32 ± 0.04**	15.3 ± 1.1
168 h	Control	0.108 ± 0.006	18.3 ± 0.7	58.7 ± 2.2	7.0 ± 0.6	0.53 ± 0.05	15.9 ± 0.9
	Ethanol	0.112 ± 0.003	20.3 ± 1.4	64.6 ± 7.7	7.2 ± 0.6	0.62 ± 0.05	12.4 ± 2.1

Each value represents the mean ± SEM for more than four rats
*,**,*** Significantly different from the control at $P < 0.05$, 0.01, 0.001, respectively (Student's *t*-test)

Table 3. Effect of ethanol on urinary excretion of taurine

	Total Amount of Taurine Excretion in Urine (μmole/kg body weight)					
	0–8 h	8–24 h	24–48 h	48–72 h	72–96 h	96–120 h
Control	115.1 ± 14.9	191.4 ± 12.1	305.3 ± 31.7	307.1 ± 51.3	337.6 ± 33.9	346.5 ± 55.1
	(12.2 ± 2.9)	(33.7 ± 3.0)	(39.0 ± 3.0)	(35.0 ± 4.2)	(31.3 ± 3.3)	(29.7 ± 4.3)
Ethanol	217.0 ± 55.7	421.4 ± 82.9*	241.7 ± 49.0	166.0 ± 13.8*	210.0 ± 38.2*	236.4 ± 27.3
	(4.9 ± 0.5*)	(15.5 ± 4.9*)	(23.5 ± 2.8**)	(33.3 ± 2.5)	(28.2 ± 3.3)	(28.3 ± 1.8)

Each value represents the mean ± SEM for four rats. Values in parenthesis indicate the urine volume (ml/kg body weight).
*,** Significantly different from the control at $P < 0.05$, 0.01 (Student's *t*-test)

rapidly elevated to peak levels approximately three to five times greater than those of naïve rats. Increase in hypotaurine was reversed from t = 18 hr as the GSH levels gradually recovered.

In rats given ethanol repeatedly induction of the hepatic MAT and CβS activity was observed (Finkelstein et al., 1974). Recently increases in the mRNA level of both liver specific and non-liver specific MAT forms were demonstrated in rats fed with ethanol (Lu et al., 2000). Trimble et al. (1993) suggested that the increase in methionine catabolism reflect the need of cells to replenish GSH via the transsulfuration pathway to prevent the cell components from ethanol-induced free radical damage. But changes in the S-amino acid metabolism in early stages of alcoholic liver injury remained mostly unknown. In the present study both the MAT activity and SAM generation in liver were depressed rapidly. The activities of CβS and CγL, the enzymes catalyzing irreversible steps in the biosynthesis of cysteine from homocysteine, were also decreased. These results suggest that an acute dose of ethanol lead to inhibition of the cysteine synthesis, which in turn could affect the generation of the final products in the transsulfuration pathway.

The maintenance of hepatic cysteine is a dynamic process. In liver the cysteine concentration is regulated by a balance between the rates of its synthesis in the transsulfuration pathway, hepatic uptake from blood, and metabolism to GSH, inorganic sulfate and taurine (Coloso et al., 1990; Garcia and Stipanuk, 1992). It was suggested that the cysteine availability

would be a major determinant for partitioning of cysteine sulfur to either GSH, taurine or inorganic sulfates in rat liver (Kwon and Stipanuk, 2001; Stipanuk et al., 1992). Low cysteine availability would favor its utilization for the synthesis of GSH; high cysteine availability enhance its catabolism to inorganic sulfate and taurine. An acute ethanol treatment decreased the hepatic cysteine concentration in this study. However, the consequence is contrary to the suggestion made earlier in that the decrease in concentration of this S-amino acid in liver is accompanied with a massive elevation in hypotaurine and taurine concentrations.

Synthesis of GSH in liver is limited mostly by two factors, the availability of cysteine and the activity of GCS (Meister and Anderson, 1983). GCS, which catalyzes a rate limiting step in the synthesis of GSH, is regulated by the feedback inhibition of GSH and also by the availability of its substrate, cysteine (Huang et al., 1993; Richman and Meister, 1975). The GCS activity was elevated from 8 hr after ethanol administration in this study, which could account for the gradual recovery in hepatic GSH. On the other hand, cysteine was decreased rapidly following the treatment indicating that the reduction of cysteine availability played a significant role in the ethanol-induced decrease of the GSH level in liver.

Of particular interest is the remarkable elevation of hypotaurine and taurine synthesis induced by ethanol. Ethanol administered at this dose was cleared from blood linearly at 0.50 mg $\pm$ 0.02 mg/ml/hr, and consequently, disappeared completely in 8 hr in this study (data not shown). Therefore, the increase in hypotaurine and taurine concentrations was significant while ethanol was being actively metabolized. Serum level and urinary excretion of taurine were also elevated indicating that the increase in hepatic hypotaurine and taurine could not be explained by impediment of the transport of these substances across the membranes. The changes in hypotaurine and taurine concentrations appear to be inversely related to the reduction in hepatic GSH, although the later persists for a longer period. Considering the well-known role of GSH in the detoxification of reactive oxygen species and reactive metabolites, the ethanol-induced increase in the hypotaurine and taurine synthesis at the expense of the reduced generation of this tripeptide is paradoxical.

It has been shown that taurine exhibits considerable cytoprotective effects both in vitro and in vivo test systems, which is often attributed to its antioxidant potential (Huxtable, 1992; Timbrell et al., 1995). However, studies have indicated that taurine has minimal direct scavenging activities against oxygen-derived radicals (Aruoma et al., 1988; Mehta and Dawson, 2001). Instead hypotaurine at a concentration of several hundred μM has been shown to be an excellent scavenger of reactive oxygen species including hydroxyl radical, hypochlorous acid, peroxynitrite and singlet oxygen (Aruoma et al., 1988; Shi et al., 1997). Especially Pecci et al. (1999) demonstrated that hypotaurine was oxidized to taurine in the presence of singlet oxygen generated with methylene blue as a photosensitizer.

Ethanol is first biotransformed to acetaldehyde by several enzyme systems mainly located in liver. Acetaldehyde itself or oxygen radicals generated during breakdown of ethanol have been suggested to be responsible for the initiation of lipid peroxidation (Castillo et al., 1992; Muller and Sies, 1982). Accordingly it is suspected that the ethanol-induced lipid peroxidative damage would increase the demand for antioxidant potential, which has been proposed as the mechanism of GSH depletion by some authors (Kera et al., 1985; Videla et al., 1980; Vina et al., 1980). However, the results in this study show that cysteine, the essential substrate for GSH synthesis, is preferentially utilized for synthesis of taurine rather than GSH in liver of rats challenged with a dose of ethanol. This observation suggests a potential role of cysteine catabolism to hypotaurine and taurine in animal tissues under oxidative stress.

In conclusion the present study shows that acute single administration of ethanol results in significant alterations of S-amino acid metabolism, some of which persist for a much longer period beyond disappearance of ethanol from blood. Inhibition of the transsulfuration reactions by ethanol leads to reduction of the SAM and cysteine concentrations in liver. The decrease in cysteine availability for GSH synthesis is further aggravated by the increased utilization of this sulfur amino acid for the production of taurine, which appears to play a significant role in the depletion of hepatic GSH. The significance of the elevation in hypotaurine and taurine synthesis remains to be investigated. Whether this is a mere consequence of ethanol effect or due to an unclarified role of hypotaurine and/or taurine in alcoholic liver injury is being studied in this laboratory.

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