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COMBINED METABOLIC EFFECTS OF ETHANOL AND THIAMINE DEFICIENCY IN THE CENTRAL NERVOUS SYSTEM*

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Abstract—The acute effects of ethanol on central nervous system (CNS) metabolism in the presence of thiamine deficiency were investigated in mice. The objective was to determine whether ethanol produced metabolic changes in thiamine-deficient mice different from those seen in controls. Thiamine deficiency was induced by a combination of feeding a thiamine-deficient diet and injecting pyrithiamine daily for 9 days. The metabolic effects of an acute dose of ethanol (4 g/kg, intraperitoneally [i.p.]) were determined by killing the mice 15 min after the injection. The metabolites that were studied included adenosine triphosphate, γ-aminobutyric acid (GABA) glucose, 6-phospho-gluconate, pyruvate, lactate, α-oxoglutarate, glucose 6-phosphate and glutamate. Thiamine-deficient mice not treated with ethanol showed elevated levels of most of the above metabolites compared to controls; smaller increases were observed in the cerebellum than in thalamus-hypothalamus and medulla. However, GABA and glutamate levels were significantly decreased in thalamus-hypothalamus in thiamine-deficient mice. The changes induced by ethanol in metabolite levels of both control and thiamine-deficient mice were largely similar; thus the extent of the initially altered levels were still maintained. Differences in the responses to ethanol were seen in 6-phospho-gluconate and glutamate, which were decreased by ethanol in thiamine-deficient mice but unchanged in controls. It is concluded that acute ethanol treatment does not greatly alter the CNS metabolic states in thiamine-deficient mice.

Thiamine deficiency is a common finding in those chronically ingesting large quantities of alcohol, and severe thiamine depletion may affect the nervous system by causing peripheral neuropathy and the Wernicke–Korsakoff syndromes [1, 2]. Freund noted that, since Korsakoff's syndrome is associated with both chronic alcohol consumption and malnutrition, it has been impossible to decide *a priori* which of the two conditions is the cause of Korsakoff's syndrome [3]. He also emphasized that damage from malnutrition and direct ethanol toxicity are not mutually exclusive.

It was not until the last decade that some of the metabolic consequences *in vivo* of thiamine deficiency were determined [1, 4–9]. These include measurements of the activities of the enzymes which require thiamine pyrophosphate as a coenzyme, and levels of the metabolites involved in energy metabolism, glycolysis, pentose phosphate pathway and in the Krebs cycle [1, 4–12]. Decreased amino acid levels [5, 8, 9] and altered catecholamine metabolism [13] have been observed in the brains of thiamine-deficient rats. The biochemical changes induced by ethanol in the central nervous system (CNS) have been reviewed by a number of workers [14–16]. However, the combined effects of acute or chronic ethanol administration and thiamine deficiency on cerebral metabolism have not been investigated. French found that thiamine deficiency decreased the activities of β-hydroxybutyric and lactic dehydrogenases in the rat liver, and that these depressed levels were unaffected by chronic ethanol ingestion [17].

This paper addresses the question of the nature of the acute effects of ethanol on CNS metabolism in the presence of thiamine deficiency in mice. It was hypothesized that, because of the altered metabolic state in thiamine-deficient mice, the ethanol-induced metabolic changes in these animals may be grossly different from those seen in controls.

METHODS

Male C57BL/6J mice (23–25 days old) were purchased from the Jackson Laboratories, Bar Harbor, ME. They were housed singly on a 12-hr light–dark cycle in a controlled environmental room (22–23°) and received Teklad mouse diet (Teklad Mills, Winfield, Iowa) and tap water *ad lib.* for 7 days. Then the food was changed to a thiamine-free diet (ICN Corp., Cleveland, Ohio). The animals were divided into two groups, A and B. Group A mice were given daily i.p. injections of 50 μg pyrithiamine (Sigma Chem. Co., St Louis, Mo.) plus 1 μg thiamine–HCl for 9 days; group B mice (controls) were given 64 μg thiamine–HCl daily by the same route [5, 7]. It has been shown that thiamine deficiency can be rapidly and reproducibly developed by the use of pyrithiamine [5, 18]. The advantages of the combined use of pyrithiamine and a thiamine-deficient diet as compared to the use of just the diet alone have been discussed by Seltzer and McDougal [7]. On day 10, half of the animals from each group were given an injection of ethanol (i.p., 4 g/kg, 20% w/v solution) and the remaining half of each group received 0.9% NaCl. The mice were sacrificed 15 min after the injection by dropping into liquid N_2 for 2 min. They were wrapped in foil and stored in a stoppered jar at −80° until used. The

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choice of killing the mice 15 min after the injection was partly based on our previous work with acute effects of ethanol [19, 20] and partly based on our pilot study showing that the metabolic effects at 1.5 hr were qualitatively similar to those at 15 min. It was assumed that between 15 min and 1.5 hr the magnitudes of the metabolic changes would not be greatly different from those observed at 15 min.

Three regions were dissected from the frozen mouse brains at −25° in a Harris M40P cryostat: the cerebellum (mainly vermis), thalamus-hypothalamus and medulla (pons not included). These samples were weighed at the same temperature in a Roller Smith precision balance; they ranged from 8 to 15 mg. Neutralized perchloric acid extracts were then prepared by the method of Lowry and Passonneau [21].

The following metabolites were measured by the methods of Lowry and Passonneau [21]: adenosine triphosphate (ATP), glucose, 6-phospho-gluconate (6-P-G), pyruvate, lactate, glucose 6-phosphate (G-6-P), α-oxoglutarate (αKG) and glutamate; gamma aminobutyric acid (GABA) was determined by a modification of the method of Scott and Jacoby [19, 22, 23]. Chemicals and enzymes used in these analyses were purchased from Sigma Chemical Co. and Boehringer Mannheim Biochemicals, Indianapolis, Indiana.

Statistical evaluations of the data were performed by utilizing Student's *t*-test and analysis of variance (BMD P2V program).

RESULTS

Symptoms of thiamine deficiency [18] were observed in the mice that had been injected with pyrithiamine for 9 days. These include weakness, ataxia and walking in circles. The effects of thiamine deficiency and acute administration of ethanol on the contents of selected metabolites in the cerebellum, thalamus-hypothalamus and medulla are summarized in Tables 1, 2 and 3 respectively. With the exceptions of ATP and GABA, saline-treated thiamine-deficient mice had increased levels of most of the metabolites in the various brain regions, the cerebellum being the least affected. This is in general agreement with the results reported by Holowach *et al.* [5].

ATP. The cerebellum had a higher content of this metabolite than the medulla and thalamus-hypothalamus. Control and thiamine-deficient mice had similar ATP content and ethanol did not alter these values.

GABA. As previously reported [19], the thalamus-hypothalamus contained more GABA than cerebellum and medulla. Both saline- and ethanol-treated thiamine-deficient mice had a significantly lower GABA level in thalamus-hypothalamus than controls. It has been shown in rats that brain GABA levels were significantly reduced in pyrithiamine-treated rats [9]. Ethanol-induced elevations of GABA were observed in all three brain regions, confirming our previously reported results [19]. Results of an analysis of variance indicate no statistically significant interaction between these treatment groups.

Glucose. Thiamine-deficient animals had substantially elevated glucose content (200–300 per cent) in cerebellum, thalamus-hypothalamus and medulla in comparison with controls. After ethanol treatment, glucose levels increased in all groups, the thiamine-deficient group still having significantly higher glucose. Results of an analysis of variance revealed a significant interaction ($P < 0.005$) only in the cerebellum. It is seen from Table 1 that the glucose content was less affected by ethanol in the thiamine-deficient group than in the controls.

6-P-G. Higher amounts of this metabolite were seen in the thiamine-deficient group, the increases being larger in thalamus-hypothalamus and medulla (4-fold) than in cerebellum (2-fold). Ethanol did not affect 6-P-G in control mice or in the cerebellum of thiamine-deficient mice. However, in thalamus-hypothalamus and medulla of the latter group, significant decreases (ca. 30 per cent) were observed. Therefore, ethanol caused a significantly different change ($P < 0.001$, by analysis of variance) in these two regions in thaimine-deficient mice compared with controls.

Pyruvate. Like 6-P-G, increases of pyruvate seen

Table 1. Contents of metabolites in cerebellum of control and thiamine-deficient mice*

	Control		Thiamine deficient	
	Saline	EtOH	Saline	EtOH
ATP	2.83 ± 0.06	2.67 ± 0.19	2.80 ± 0.09	2.78 ± 0.07
GABA	1.33 ± 0.05	1.63 ± 0.04†	1.26 ± 0.03	1.44 ± 0.05‡
Glucose	2.50 ± 0.16	6.78 ± 0.71†	8.16 ± 0.69†	9.75 ± 0.28‡§
6-P-G	0.0112 ± 0.007	0.0143 ± 0.002	0.0223 ± 0.002†	0.0218 ± 0.002§
Pyruvate	0.304 ± 0.034	0.309 ± 0.057	0.799 ± 0.119†	0.535 ± 0.053§
Lactate	2.58 ± 0.18	1.93 ± 0.25	1.97 ± 0.14†	1.96 ± 0.14
αKG	0.066 ± 0.010	0.0649 ± 0.016	0.239 ± 0.016†	0.253 ± 0.032§
G-6-P	0.135 ± 0.022	0.101 ± 0.009	0.121 ± 0.009	0.126 ± 0.010
Glutamate	11.57 ± 0.27	10.26 ± 0.64	11.06 ± 0.27	9.46 ± 0.28‡

* The results represent the means ± S. E. M., expressed in m-moles/kg wet wt, of eight to ten animals in each treatment group.

† Significantly different from saline controls, $P \leqslant 0.01$.

‡ Significantly different from saline-treated thiamine-deficient groups, $P \leqslant 0.01$.

§ Significantly different from ethanol-treated controls, $P \leqslant 0.01$.

Table 2. Contents of metabolites in thalamus-hypothalamus of control and thiamine-deficient mice*

	Control		Thiamine deficient	
	Saline	EtOH	Saline	EtOH
ATP	2.09 ± 0.10	2.26 ± 0.11	2.27 ± 0.09	2.32 ± 0.11
GABA	4.44 ± 0.11	4.99 ± 0.09†	3.67 ± 0.13†	4.35 ± 0.11‡§
Glucose	1.76 ± 0.13	4.64 ± 0.48†	6.58 ± 0.51†	8.25 ± 0.24‡§
6-P-G	0.0088 ± 0.0006	0.0109 ± 0.0008	0.0451 ± 0.0030†	0.0316 ± 0.0028†§
Pyruvate	0.246 ± 0.016	0.218 ± 0.019	1.99 ± 0.14†	1.73 ± 0.34§
Lactate	3.95 ± 0.53	3.99 ± 0.24	5.79 ± 0.33†	5.75 ± 0.21§
αKG	0.0294 ± 0.003	0.0392 ± 0.003	1.14 ± 0.08†	1.04 ± 0.13§
G-6-P	0.0852 ± 0.010	0.0852 ± 0.012	0.102 ± 0.004	0.0983 ± 0.006
Glutamate	9.26 ± 0.10	10.10 ± 0.95	8.65 ± 0.19†	7.03 ± 0.18‡§

* The results represent the means ± S. E. M., expressed in m-moles/kg wet wt, of eight to ten animals in each treatment group.
† Significantly different from saline controls, $P \leqslant 0.01$.
‡ Significantly different from saline-treated thiamine-deficient groups, $P \leqslant 0.01$.
§ Significantly different from ethanol-treated controls, $P \leqslant 0.01$.

in thiamine-deficient mice were less in the cerebellum (2.5-fold) than in thalamus-hypothalamus (8-fold) and medulla (6-fold). Ethanol failed to cause any significant change in the level of this metabolite in either group.

Lactate. Thiamine-deficient mice had a significantly lower content of lactate in the cerebellum, but higher contents in thalamus-hypothalamus and medulla compared to controls. After ethanol there was no longer a significant difference in the cerebellum between the control and thiamine-deficient groups. Contents in thalamus-hypothalamus and medulla of both groups were unaffected by ethanol.

αKG. Levels of αKG observed in saline controls were comparable to those reported by Collins *et al.* [6] but slightly lower than those reported by Holowach *et al.* [5] for whole brains of Swiss Webster mice. This metabolite was the most affected by thiamine deficiency [5], with 4-fold, 40-fold and 50-fold increases in cerebellum, thalamus-hypothalamus and medulla respectively. No further changes were observed after ethanol administration.

G-6-P. The only significant difference between thiamine-deficient and control mice is the increase observed in the former group in the medulla (Table 3). Ethanol did not alter the content of this metabolite in either group.

Glutamate. No significant difference was seen in the content of glutamate in saline-treated control and thiamine-deficient mice in cerebellum and medulla. However, the lower level in thalamus-hypothalamus of thiamine-deficient mice was significantly different from controls (Table 2). Significant decreases of glutamate levels in whole brains of pyrithiamine-treated mice [5] and rats [9] have been reported. Further, we did not observe any change in this metabolite after ethanol in the thiamine-treated animals (Tables 1–3), in agreement with our previously reported results [17]. However, significant decreases were observed in cerebellum (14 per cent), thalamus-hypothalamus (20 per cent) and medulla (12 per cent) after ethanol treatment in the thiamine-deficient mice. Results of analysis of variance show significant interactions in thalamus-hypothalamus ($P < 0.005$) and medulla ($P < 0.02$).

Table 3. Contents of metabolites in medulla of control and thiamine-deficient mice*

	Control		Thiamine deficient	
	Saline	EtOH	Saline	EtOH
ATP	2.07 ± 0.17	2.25 ± 0.11	2.22 ± 0.11	2.33 ± 0.11
GABA	1.77 ± 0.09	2.16 ± 0.07†	1.59 ± 0.05	1.96 ± 0.08‡
Glucose	2.29 ± 0.12	4.61 ± 0.16†	7.14 ± 0.40†	8.94 ± 0.21‡§
6-P-G	0.0105 ± 0.0009	0.0103 ± 0.0008	0.0474 ± 0.0032†	0.0319 ± 0.0021†§
Pyruvate	0.307 ± 0.044	0.301 ± 0.031	1.85 ± 0.14†	2.07 ± 0.10§
Lactate	3.71 ± 0.48	4.22 ± 0.19	5.78 ± 0.41†	5.90 ± 0.44§
αKG	0.0247 ± 0.002	0.0302 ± 0.004	1.27 ± 0.10†	1.24 ± 0.08§
G-6-P	0.0673 ± 0.007	0.0759 ± 0.004	0.104 ± 0.007†	0.0972 ± 0.006§
Glutamate	8.66 ± 0.22	8.59 ± 0.11	7.54 ± 0.13	6.61 ± 0.23‡§

* The results represent the means ± S. E. M., expressed in m-moles/kg wet wt, of eight to ten animals in each treatment group.
† Significantly different from saline controls, $P \leqslant 0.01$.
‡ Significantly different from saline-treated thiamine-deficient groups, $P \leqslant 0.01$.
§ Significantly different from ethanol-treated controls, $P \leqslant 0.01$.

DISCUSSION

It has been reported that drastic biochemical changes usually accompany the development of severe thiamine deficiency [1–12]. In this report we have described our investigation on the acute effects of ethanol on CNS metabolism in thiamine-deficient mice. This is based on the hypothesis that ethanol might produce biochemical changes in these animals which are different from those observed in animals that are not thiamine deficient. Among the metabolites examined are those involved in glycolysis (ATP, glucose, glucose 6-P, pyruvate and lactate), pentose phosphate pathway (6-P-G) and in the Krebs cycle (α-oxoglutarate); in addition, two putative neurotransmitters (glutamate and GABA) were also studied. We chose to measure metabolite levels rather than enzyme activities because the results of Holowach *et al.* [5] indicate that metabolite levels may be much more sensitive indicators of enzyme deficit than the activities, as measured *in vitro* of the enzymes themselves.

Our results indicate that, of the metabolites examined, saline-treated thiamine-deficient mice had altered metabolite concentrations; exceptions are ATP and glutamate (cerebellum and medulla only). These findings are in general agreement with the results reported by other workers [5, 6, 9, 11, 12]. The cerebellum was less affected than the thalamus-hypothalamus and medulla, as has been observed by Collins *et al.* [6] as well as Seltzer and McDougal [7].

The acute administration of ethanol did not produce any significant changes in ATP, pyruvate, lactate, αKG and G-6-P in both control and thiamine-deficient mice. Levels of glucose and GABA were increased by ethanol in both groups. The magnitudes of the initially elevated metabolic levels in the thiamine-deficient mice (saline-treated) were maintained in the cases of pyruvate, lactate, αKG, G-6-P and GABA.

An exception to the latter metabolite is that GABA in the thalamus-hypothalamus of the thiamine-deficient mice remained significantly lower than those in controls. Our results show that the effects of ethanol on the contents of glutamate (in thalamus-hypothalamus and medulla), glucose (in cerebellum) and 6-P-G (in thalamus-hypothalamus and medulla) were different in the thiamine-deficient group compared to controls. More detailed investigations are required to explain these differences. Nevertheless, the magnitudes of these differences do not appear to be substantial enough to have an important influence on the overall metabolic state. One must bear in mind that, in this study, only one time period after an acute dose of ethanol was examined. Of more interest and importance will be the investigation of the combined effects of chronic ethanol administration concomitant with the gradual development of thiamine deficiency. This study is in progress in our laboratory.

Decreased levels of GABA have been observed in mice [24, 25] during alcohol withdrawal. These data and others [26] suggest that GABA may be involved in the genesis of the hyperexcitable state in alcohol withdrawal. It is possible that the lower content of GABA in thalamus-hypothalamus observed in thiamine-deficient mice may be maintained after chronic ethanol treatment and during ethanol withdrawal, thereby having a greater influence on the severity of some of the withdrawal signs. This possibility is being tested.

It is concluded that, with the exceptions noted, acute ethanol treatment does not greatly alter the CNS metabolic states in thiamine-deficient mice. The accumulation of a series of minor differences over a longer treatment period, such as in the case of chronic ethanol administration together with a gradual development of thiamine deficiency, might have an important influence on the regulation of CNS metabolism.

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