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Thiamine deficiency in utero alters response to ethanol in adulthood

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Abstract. To determine whether prenatal thiamine deficiency, a frequent concomitant of alcoholism, reduces the response to ethanol during adulthood in the rat as does ethanol exposure in utero (Abel et al. 1981), pregnant Sprague-Dawley rats received either control or thiamine deficient diets together with daily injections of the thiamine antagonist pyrithiamine. At 7 months of age, male offspring were exposed to precisely regulated ethanol vapor concentrations in an inhalation chamber for 24 h and blood ethanol concentrations (BECs) and ethanol-induced intoxication were determined. Prenatally thiamine deficient rats and controls were indistinguishable in terms of appearance, body and liver weights, and the ratios of liver to body weight and brain to liver weight. However, total body water was significantly greater, and BECs and behavioral impairment were decreased, in the experimental rats. These findings indicate that prenatal thiamine deprivation is associated with reduced pharmacologic effect of ethanol as a result of increases in its volume of distribution and rate of metabolism.

Key words: Alcoholism – Ethanol metabolism – Ethanol tolerance – Thiamine deficiency

The relationship between maternal alcohol consumption and congenital neurological and physical abnormalities is now well established. A distinct pattern of dysmorphologies and mental retardation, called the fetal-alcohol syndrome (FAS), has been associated with severe maternal alcoholism (Jones et al. 1973; Clarren and Smith 1978; Streissguth et al. 1985). However, subtle abnormalities of attention, behavior, and learning are also frequently observed in children of alcoholic mothers (Shaywitz et al. 1980; Streissguth et al. 1984). The greater the amount of alcohol consumed by the pregnant woman, the greater the likelihood of adverse affects in the offspring (Streissguth et al. 1980, Little et al. 1982).

In the clinical situation, it may be difficult to establish which of several factors associated with alcoholism contribute significantly to ethanol toxicity and the resultant adverse effects on pregnancy outcome (Abel 1984). Much of our knowledge, therefore, concerning the pathophysiologic effects of ethanol abuse during pregnancy has been derived from studies in which ethanol-related birth effects have been reproduced experimentally in animals exposed to ethanol in utero. Abel et al. (1981) have demonstrated that prenatal administration of ethanol to pregnant rats resulted in reduced response of offspring to this and certain other drugs at 6 months of age. Although subtle central nervous system (CNS) alterations following prenatal ethanol exposure (Barnes and Walker 1981; West et al. 1981; Church and Holloway 1984; Riley et al. 1986a, b; Vingan et al. 1986) may be responsible for these changes in pharmacologic responsiveness, the exact mechanism(s) involved remain to be elucidated.

In the adult, chronic consumption of alcoholic beverages leads to the development of tolerance and dependence accompanied by an increased rate of ethanol metabolism and reduced central nervous system (CNS) sensitivity to ethanol (Kalant et al. 1971). In addition, alcoholism is frequently associated with thiamine deficiency due to inadequate nutritional intake, decreased absorption, or impaired utilization (Hoyumpa 1980). It is believed that thiamine deficiency alters intermediary metabolism and thereby contributes to the toxicity of ethanol in various organ systems (Martin et al. 1986). For example, thiamine deficiency has been implicated as a possible contributing factor in intrauterine growth retardation, a prominent characteristic of FAS in which neural development is compromised (Levin et al. 1985; Roecklein et al. 1985). Thiamine insufficiency during gestation may have significant fetal CNS effects because the midline periventricular structures of the brainstem and diencephalon are among the most vulnerable to the pathologic effects of this avitaminosis in the adult (Victor et al. 1971; Hakim and Pappius 1983).

It has been suggested that pathophysiologic changes resulting from thiamine deprivation may contribute to ethanol tolerance observed in chronic alcoholics because a severe previous episode of thiamine deficiency significantly decreased ethanol-induced intoxication and hypothermia as well as the area under the blood ethanol concentration curve in the adult rat (Martin et al. 1985). The present experiment was designed to determine whether prenatal thiamine deprivation in the rat influences the response to ethanol during adulthood in a similar manner as does ethanol exposure in utero.

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Materials and methods

Prenatal thiamine deprivation. Timed-pregnant 10-week-old female Sprague-Dawley rats (Taconic Farms, New York) were housed individually in a room with controlled temperature and a 12/12 light/dark cycle from the 2nd postcoital day. Wire-bottom cages were used to prevent coprophagy and water was provided ad lib. Dams were randomly assigned to receive either control ($n=5$) or thiamine deficient ($n=5$) diets (Bio-Serv Inc., Frenchtown, New Jersey) containing 4.06 and 0.11 mg thiamine per kilogram of diet, respectively. Each day, both groups were injected subcutaneously with 50 μg/100 g body weight of the thiamine antagonist pyrithiamine hydrobromide (Sigma Chemical Co., St Louis, MO) and control dams were pair-fed to those receiving thiamine deficient diet. Pyrithiamine alone has been shown not to affect fetal body weight, placental weight, brain weight, or liver weight (Roecklein et al. 1985), and therefore it was used to accelerate the development of thiamine deficiency in the dietary thiamine-deprived group. When the experimental group demonstrated characteristic signs of thiamine deficiency (ataxia, loss of righting reflex, opisthotonus, and severe weight loss), pyrithiamine injections were stopped. Both groups were then injected subcutaneously with 100 mg/kg body weight of thiamine hydrochloride (Sigma Chemical Co., St. Louis, MO) and were given free access to control diet for the remainder of the gestational period. After birth, control and experimental litters were randomly culled to eight pups and housed with surrogate mothers who had never been thiamine deficient. Offspring were weaned at about 22 days of age, were placed in cages with two to three littermates of the same sex, and had free access to water and to normal diet (Ralston Purina Co, St. Louis, MO) until completion of the study.

Ethanol exposure. At 7 months of age, control ($n=9$) and prenatally thiamine-deprived ($n=5$) male rats (500–660 g) were placed in airtight chambers in which the ethanol concentration in inspired air could be precisely regulated (Karanian et al. 1986). Female rats were not studied due to procedural difficulties. The ethanol vapor concentration was 25 mg/l for 12 h and then was increased to 38 mg/l for the next 12-h period. At the end of the ethanol exposure period, the rats were removed from the chambers and the level of intoxication (0 = normal; 1 = sedated; 2, 3, 4 = increasing degrees of ataxia; 5 = loss of righthing reflex; 6 = coma) of each animal was assessed by an evaluator who was blind to the treatments (Majchrowicz 1975). Blood samples for ethanol determinations (Sigma diagnostics alcohol procedure no. 332–UV, Sigma Chemical Co., St. Louis, MO) were obtained from the tail artery 12 and 24 h after initiating ethanol exposure and at 3 and 6 h after discontinuation of ethanol. After 2 weeks' recovery under usual housing conditions, control and experimental male rats were sacrificed and their livers and brains were weighed.

Total body water. An estimation of total body water (TBW) content in male (controls, $n=5$; experimental, $n=5$) and female (controls, $n=3$; experimental, $n=3$) rats which had not been exposed to ethanol was determined using a previously published method (Impeduglia et al. 1987). Tritiated water (0.4 ml, 50 μCi/ml; New England Nuclear, Boston, MA) was injected IP and 100 μl blood samples were collected from the tail vein of each rat into heparinized microcentrifuge tubes at 1, 2, 3, 4, and 5 h. After centrifugation, 20 μl plasma was transferred into vials for liquid scintillation counting (Beckman Beta Counter LS 2800) using 10 ml liquid of scintillation cocktail (Beckman Instruments, Inc., Fullerton, CA). Two vials containing 1/20 diluted doses were counted and averaged to estimate the total activity injected (disintegrations per minute). The average of values obtained at 2, 3 and 4 h after injection of tritiated water was taken as the equilibrium activity, and the TBW was calculated according to the equation TBW (ml) = dose injected (disintegrations per min)/[equilibrium activity (disintegrations per min)/plasma volume (ml)].

Results

All pregnant animals from both treatment groups delivered live offspring and the number and gender of offspring per litter were not significantly different in the prenatally thiamine deficient and control rats. Offspring from the experimental group [males ($n=10$), 18.8 ± 0.67 g; females ($n=6$), 18.5 ± 1.4 g] weighed less than their respective controls [males ($n=14$), 21.2 ± 0.58 g; females ($n=10$), 20.5 ± 0.4 g] at 1 week of age ($P<0.05$), in agreement with previous observations (Roecklein et al. 1985). At 7 months of age, mean body weight, liver weight, the ratio of liver to body weight, and the ratio of brain to liver weight of prenatally-thiamine deprived and control rats were not significantly different (Table 1). Brain weights of previously thiamine-deficient male rats were significantly decreased compared to the control group. Total body water was significantly greater in the prenatally thiamine-deficient rats of both genders than in the controls.

After 12 h in the chamber with an ethanol vapor concentration of 25 mg/l, mean (±SEM) blood ethanol concentrations (BEC) in the prenatally thiamine deficient males (0.510 ± 0.070 mg/ml) was decreased 2-fold compared to the control group (1.18 ± 0.13 mg/ml) (Fig. 1). After the etha-

Table 1. Effects of thiamine deficiency in utero on adult mean (±SEM) body, liver, and brain weights, body water, and liver/body and brain/liver ratios

	Body weight (g)	Body water (l/kg)	Liver weight (g)	Liver/body ratio (%)	Brain weight (g)	Brain/liver ratio (%)
Males						
Control	620 ±20 (5)	0.668 ±0.074 (5)	23.9 ±1.0 (5)	3.85 ±0.14	2.14 ±0.04 (3)	8.50 0.32
Experimental	577 ±30 (5)	0.765* ±0.017 (5)	23.1 ±2.0 (5)	3.98 ±0.16	1.78* ±0.08 (5)	7.86 ±0.51
Females						
Control	425 ±21 (6)	0.555 ±0.021 (3)	15.7 ±1.3 (6)	3.69 ±0.21	1.97 ±0.08 (3)	11.9 ±1.8
Experimental	360 ±20 (5)	0.633* ±0.016 (3)	13.2 ±1.9 (5)	3.70 ±0.60	1.97 ±0.04 (3)	14.8 ±3.0

* $P<0.05$

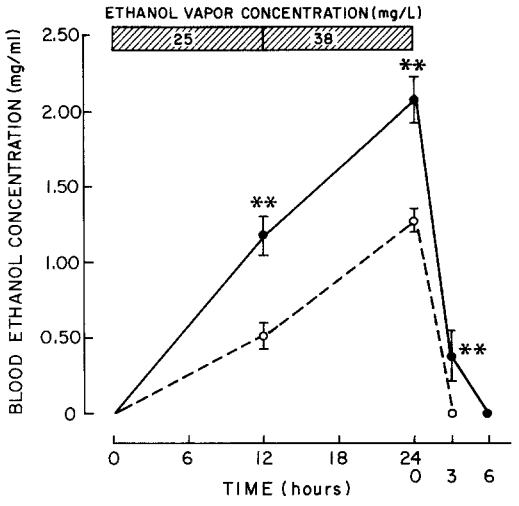

Fig. 1. Blood ethanol concentrations (mg/ml) in prenatally thiamine deficient ($n=5$) and control ($n=9$) adult male rats during and following exposure to ethanol vapor concentrations of 25 and 38 mg/l. ** $P<0.01$. • Control ($n=9$); ○ TD recovered ($n=5$)

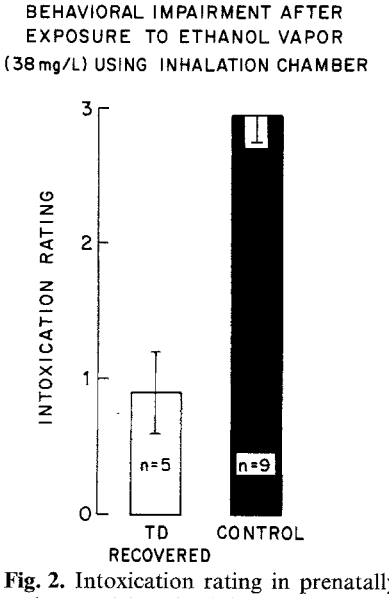

Fig. 2. Intoxication rating in prenatally thiamine deficient ($n=5$) and control ($n=9$) adult male rats after exposure to ethanol vapor concentration of 38 mg/l

nol vapor concentration had been increased to 38 mg/l for a further 12 h, BEC remained significantly lower in thiamine deficiency recovered (1.26 ± 0.08 mg/ml) compared to controls (2.02 ± 0.15 mg/ml). Three hours after the rats were removed from the chamber, ethanol was no longer detected in the blood of previously thiamine-deprived rats. Among the control group, BEC was 0.357 ± 0.167 mg/ml at 3 h and 0 at 6 h following termination of ethanol exposure.

Immediately after removal from the ethanol chamber, prenatally thiamine deficient rats were significantly less intoxicated (0.90 ± 0.32) than the control group (2.95 ± 0.24) ($P<0.01$, Wilcoxon's test) (Fig. 2).

Discussion

A thiamine-deficient diet alone is not sufficient to cause significant reduction of this vitamin in the rat within its short gestation period (Roecklein et al. 1985) and thiamine-deficient female rats do not reproduce (Greenwood et al. 1983). Therefore, the thiamine antagonist pyrithiamine, which alone has been shown not to affect pregnancy outcome (Roecklein et al. 1985), has previously been used in conjunction with dietary thiamine deprivation for investigation of the mechanisms by which nutritional factors may contribute to the intrauterine growth retardation characteristic of FAS (Levin et al. 1985; Roecklein et al. 1985). In the present study, this animal model was used to explore the possible role of thiamine deficiency in more subtle fetal alcohol effects. Our major findings are that thiamine deficiency in utero significantly reduces BECs and also the degree of intoxication observed in adult male rats during exposure to ethanol vapor under the precise control of an automated inhalation chamber.

BECs obtained under the conditions of the ethanol vapor chamber should be determined primarily by the volume of distribution and the rate of ethanol metabolism (Karanian et al. 1986). Prenatally thiamine-deficient rats also showed a 15% increase in total body water in adulthood. The permanent alteration in body composition would result in an increase in the volume of distribution of ethanol (Wiberg et al. 1971), but this alone is insufficient to explain the magnitude of the reductions in BEC and indicates a significant increase in the rate of ethanol elimination after thiamine deficiency in utero.

Although the extrapolation of data from an adult animal model of thiamine deprivation to the situation during gestation should be done with caution, the findings from the present experiment resemble our observations of a permanent acceleration of ethanol metabolism in previously thiamine-deprived adult rats (Martin et al. 1985). We have recently demonstrated that the persistent change in ethanol metabolism following dietary thiamine deprivation in the adult rat was mediated by an increase in liver alcohol dehydrogenase activity, the enzyme which catalyzes the rate-limiting step in oxidation of ethanol (Martin et al., unpublished observation). This change was accompanied by a marked reduction in plasma growth hormone concentrations without significant alterations in thyroxine, testosterone, or estradiol levels, suggesting that a rather specific biochemical lesion in the periventricular region of the hypothalamus may have resulted from previous thiamine deficiency. Together, these findings suggest that neuropathophysiologic changes resulting from thiamine deficiency in utero may contribute to increased ethanol tolerance in adulthood, perhaps through neurohumeral effects on ethanol metabolism.

The importance of these observations rests on the frequent association of thiamine deficiency with chronic ethanol consumption (Hoyumpa 1980). Presumably, thiamine insufficiency in the alcoholic mother during pregnancy would contribute to the reduced pharmacological effect of ethanol observed in adulthood following prenatal exposure to ethanol (Abel et al. 1981). Our findings also suggest that the effect of prenatal ethanol exposure on the volume of distribution of ethanol and its metabolism also need to be evaluated. Finally, it would be important to compare the pharmacologic effects of ethanol in prenatally thiamine-deficient (and also ethanol-exposed) rats and controls at equivalent BECs to determine to what degree pharmacokinetic and/or pharmacodynamic (CNS) alterations may contribute to these findings.

Rats recovered from thiamine deprivation in utero and

control rats were indistinguishable in terms of overall appearance and body and liver weights. However, the significant reduction in brain weight observed in prenatally thiamine-deficient male rats and the increase in total body water in rats of both genders may represent the pathophysiologic basis for long-term behavioral pharmacologic effects. Furthermore, these gross alterations associated with thiamine deprivation in utero share some of the characteristics of fetal alcohol effects. For example, prenatal exposure to ethanol has been shown to increase fetal body water (Abel and Greizerstein 1979). Significant increases in glucose utilization as measured by the deoxyglucose method were observed in the supraoptic nucleus and the neurohypophysis of rats following prenatal ethanol exposure (Vingan et al. 1986). These same periventricular regions of the adult rat brain have been shown to undergo metabolic and histologic changes during thiamine deprivation (Hakim and Pappius 1983). The hypothalamic-neurohypophysial axis is directly involved in osmoregulation via release of vasopressin and possibly in the development and maintenance of tolerance to ethanol (Tabakoff and Hoffmann 1988).

In conclusion, the adult male offspring of rats deprived of thiamine during pregnancy may be expected to have decreased brain concentrations of ethanol after a given dose and diminished acute sensitivity to ethanol due to a significant reduction in total body water. Furthermore, greater amounts of ethanol could presumably be consumed by these animals on a chronic basis as a result of increased rates of ethanol metabolism. Whether these pharmakokinetic changes are accompanied by pharmacodynamic alterations that would also reduce response to ethanol remains to be determined. These findings should only be extrapolated to the human condition of alcoholism with caution. Nevertheless, it is intriguing to speculate how the effects of prenatal thiamine deficiency on the response to ethanol in adulthood may relate to the genetic susceptibility to alcoholism (Impeduglia et al. 1987; Mukherjee et al. 1987).

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