Original Research

In Vivo and In Vitro Proton NMR Spectroscopic Studies of Thiamine-Deficient Rat Brains

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Thiamine deficiency (TD) in rats produces lesions similar to those found in humans with Wernicke's encephalopathy, an organic mental disorder associated with alcoholism. Male Sprague–Dawley rats ($n = 24$) were deprived of thiamine in a regimen of thiamine-deficient chow and daily intraperitoneal injections of the thiamine antagonist pyrithiamine hydrobromide for 12 days (0.5 mg/kg). In rats with TD, significant changes were observed in the choline peak (reduction and dose-dependent recovery after thiamine replenishment), which was confirmed by the extraction study. Changes were mainly due to the reduction in glycerophosphorylcholine (GPC), suggesting that a reduction in GPC may be relevant to the primary biochemical lesion in TD. These data are compatible with the hypothesis that a decrease in choline compounds is the cause of the biochemical abnormalities that precede neuroanatomic damage characteristic of Wernicke's encephalopathy. J. Magn. Reson. Imaging 2001;13: 163–166. © 2001 Wiley-Liss, Inc.

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THIAMINE (VITAMIN B_1) in its active form, thiamine pyrophosphate (TPP), is involved in the enzymatic reactions that are essential for aerobic glucose metabolism and neurotransmitter production. About 95% of thiamine deficiency (TD) is a frequent complication of alcoholism, resulting from decreased absorption and impaired utilization, or other disorders that interfere with normal ingestion of food, and may result in brain dysfunctions including Wernicke–Korsakoff (WK) syndrome, cerebellar degeneration, or alcoholic dementia (1,2). The clinical manifestations of these include memory and learning deficit and the reduced cerebral volume. In chronic alcoholics, neurobehavioral deficits related to TD and brain glucose metabolism have been shown to recover during abstinence with adequate nutritional intake. However, the mechanism(s) whereby thiamine treatment facilitates recovery of brain functions during detoxification and continued abstinence and mechanism of reversibility with treatment are not fully understood. Reductions in the activities of the thiamine-utilizing enzymes and/or effects on neurotransmission by impaired synthesis of neurotransmitter have been implicated in tissue injury due to TD. TD in alcoholics results from inadequate nutritional intake, poor gastrointestinal absorption of thiamine, or impaired phosphorylation of thiamine, and this could potentiate the neurotoxic effects of alcohol (1,3).

It has been demonstrated that animals treated with the centrally and peripherally acting thiamine antagonist pyrithiamine, interfering with the synthesis of active form TPP from thiamine after absorption, produce lesions of Wernicke's encephalopathy, which is recognized as ataxia, nystagmus, and unconsciousness. At early stage, most of the neurologic symptoms of pyrithiamine-induced Wernicke's encephalopathy can be reversed with thiamine replenishment, without exacerbation to Korsakoff syndrome showing the amnestic psychosis (2,4). Previous work suggests that TD induced by pyrithiamine in rats causes reliable reductions in brain choline-containing compounds, which are reversed in a dose-dependent fashion subsequent to thiamine hydrochloride administration, which is in line with findings in detoxifying alcoholic patients during abstinence (5,6).

The purpose of this work was to examine the changes in brain choline (Cho)-containing compound(s) during pyrithiamine-induced TD in the rat and its reversal with the administration of thiamine hydrochloride, followed by chemical identification of specific choline compounds derived from brain extracts.

MATERIALS AND METHODS

Animal Preparation

Thirty-two male Sprague–Dawley rats weighing 200–250 g were used for the experiments according to a protocol that was approved by the Animal Care Committee at Vanderbilt University Medical Center. The animals were maintained under conditions of constant temperature, humidity, and 12-hour light/dark cycles. After 1 week of stabilization on an ad lib diet, the rats were divided into four groups; normal ($n = 8$), TD ($n = 8$), TD with low-dose thiamine hydrochloride treatment (Sigma-Aldrich, St Louis, MO.; 5 mg/kg; $n = 8$), and TD with high-dose thiamine hydrochloride treatment (100 mg/kg; $n = 8$). TD was induced by feeding the thiamine-deficient diet (Teklad Mills, Madison, WI) in combination with daily injecting the thiamine antagonist pyrithiamine hydrobromide from Monday to Friday (0.5 mg/kg body weight, i.p.) for 12 days. The thiamine

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was administered in doses of either 5 mg/kg or 100 mg/kg, and after 2 hours, magnetic resonance spectroscopy (MRS) acquisitions were initiated. Neurologic status was evaluated daily by observing the spontaneous movement, gait, and righting reflex, and rats were used at presymptomatic stage (i.e., before they developed the evidence of neurologic abnormalities including spontaneous or sensory evoked seizures, typically reached after 12 days).

NMR Experiment

In vivo localized proton MRS experiments were performed using a 4.7-T/440 Spectroscopy Imaging Systems Corporation (SISCO) imaging spectrometer. Localized proton spectra were acquired from a region of interest (ROI; 4 × 4 × 4 mm) inside the brain using a STEAM sequence (TR/TE: 3000/68 ms). A saddle coil (8 cm i.d.) was used as a transmitter, and a surface coil (2.5 cm i.d.), as a receiver. The spectral width was 2 KHz and the number of data points was 4 K; 256 signal averages were acquired. The spectra were processed with a 3-Hz line broadening with zeroth and first order phase correction. Anesthesia was induced with 5% halothane in O_2 (2 L/min) and maintained with 1.25% halothane in O_2 (2 L/min) flowing through the small-animal ventilator (Anesco Inc., Georgetown, KY) housing the transmitter and receiver radiofrequency (RF) coils. Body temperature was kept constant throughout the experiment by using circulating heated water controlled with thermostat. In vivo spectra from normal, TD, and TD with thiamine treatment rats were obtained on day 13.

After in vivo MRS experiments, rats were killed by microwave irradiation (5 kW) focused on the head for a duration of 2 sec while they were still under anesthesia (7). The brain was removed immediately and ground in a mortar containing liquid N_2. Metabolite extraction was done according to a modification of the Blight–Dyer technique (8,9), using a mixture of chloroform/methanol/water at a proportion of 2:2:1 (vol/vol). The mixture was centrifuged at 10,000 g for 20 minutes at 4°C to separate hydrophilic and hydrophobic phases. Hydrophilic phase was dried under a stream of nitrogen gas at room temperature. The dried hydrophilic phase was stored at −80°C until dissolved in 0.5 mL of deuterium oxide for high-resolution NMR study. In vitro spectra were acquired on a DRX500 Bruker spectrometer with TR = 15 sec, NA = 8, 45° pulse width, and TSP (trimethylsilyl propionic acid, Sigma-Aldrich, St. Louis) as an internal standard for the chemical shift and the concentration. Metabolite concentrations were determined by comparing the integrated peak intensity of the compounds of interest with that of TSP peak after correcting for the number of contributing protons and for the tissue weight. The spectral width was 8 KHz, and 0.3-Hz line broadening was applied before Fourier transformation. The resonances were identified from the literature values (10,11). All the NMR spectra were processed using a commercial NUTS (Acorn NMR Inc., Fremont, CA) NMR data-processing program to measure the areas under the curves for quantification.

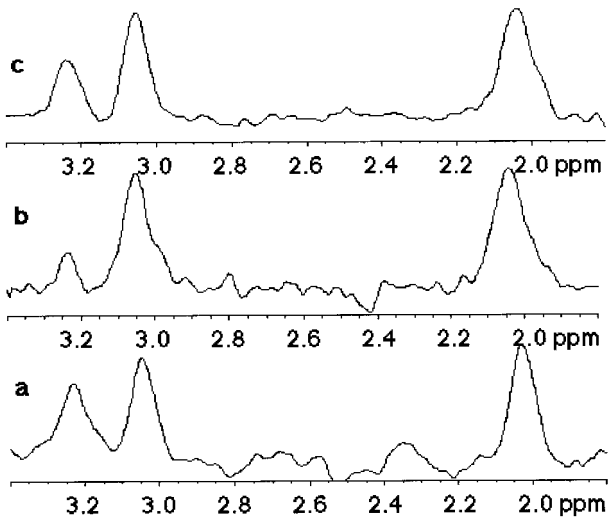

Figure 1. Representative in vivo spectra from **(a)** normal, **(b)** TD, and **(c)** TD with high-dose thiamine treatment (100 mg/kg), demonstrating the effect of TD and thiamine treatment on rat brain.

RESULTS

Figure 1 shows the representative in vivo spectra demonstrating the effect of TD and thiamine treatment on rat brain. As also shown previously (5), it displays the characteristic changes in Cho-containing compounds in rat brains after 12 days of pyrithiamine injection, whereas no significant change occurred in either total creatine (Cr) and phosphocreatine (PCr) or *N*-acetylaspartate (NAA) peaks. Detailed quantitative results of the changes were published before (5). Cho peak from TD rats decreased by more than 40% compared with normal rats at this stage of TD. If TD was continued untreated, rats displayed loss of righting reflex and spontaneous or sensory evoked seizures, and no significant Cho peak was observed from MRS study. Figure 1 also shows an increase/recovery in Cho peak when TD rats were scanned 2 hours after administration of thiamine hydrochloride at the dosage of 100 mg/kg. The in vivo Cho peak at 3.2 ppm is mainly from the trimethyl ammonium group in glycerophosphorylcholine (GPC) and phosphorylcholine (PC) with small contributions from free choline (Cho), phosphorylethanolamine (PE), and glycerophosphorylethanolamine (GPE) (12,13). GPC, PC, and Cho could be easily identified by their peaks at 3.21, 3.23, and 3.24 ppm, respectively, in the brain extract spectra (Fig. 2). The resonances were identified from the literature values (10,11). Figure 2a is the spectrum from one of TD rat, while b and c show zoomed portions of spectra ranging from 2.95 to 3.35 ppm from the normal and TD rats, respectively. Table 1 summarizes the results of high-resolution extract spectra showing the concentration of metabolites in four different groups of rats, demonstrating that GPC was the primary component responsible for the observed decrease in Cho peak from brains of TD rats, which were still able to demonstrate the normal righting reflex. The levels of GPC and PC are lower than published values, but are consistent with literature values using the same extraction method (8,9). This could be due to the different extraction method and/or the extraction efficiency.

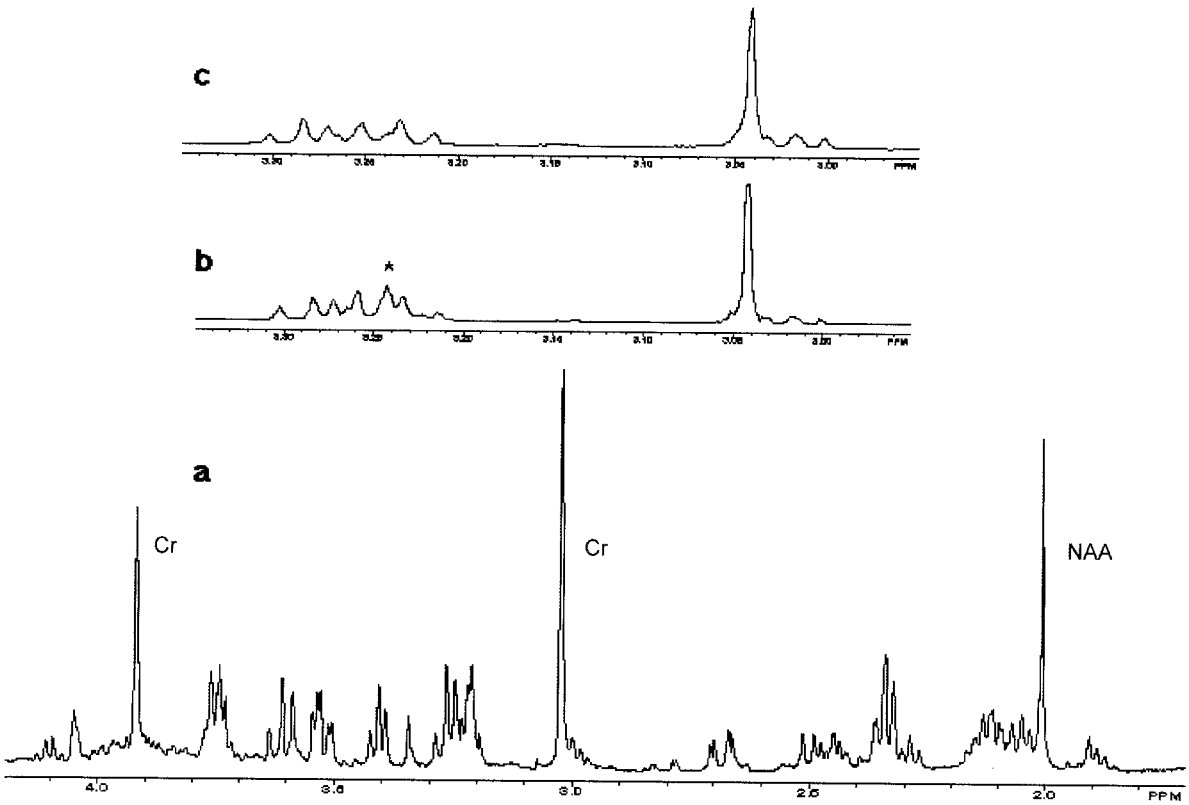

Figure 2. The high-resolution spectra of the brain extract **(a)** from TD rat, **(b)** and **(c)** from zoomed portions of spectra ranging from 2.95 to 3.35 ppm of the normal and TD rat, respectively. Identification of the resonances observed in spectra of brain extract was based on the literature values (10,11).

DISCUSSION

Thiamine is phosphorylated into its active form, TPP. TPP is the cofactor for the three enzymes, pyruvate dehydrogenase (PDH), alpha-ketoglutarate dehydrogenase (α-KDGH), and transketolase (TK). PDH catalyzes the oxidative decarboxylation of pyruvate to acetyl coenzyme A (CoA). If the conversion from pyruvate to acetyl CoA is hampered, pyruvate concentration increases, and this excessive pyruvate is then converted to lactate, leading to the lactic acidosis associated with thiamine deficiency (14,15). α-KDGH catalyzes the oxidative decarboxylation of α-ketoglutarate to succinyl CoA (16). In the thiamine-deficient state, decreased activity of α-KDGH could develop a metabolic block in the citric acid cycle (17,18). Transketolase catalyzes the reactions of the pentose phosphate pathway. TD, a frequent complication of alcoholism, contributes significantly to alcohol-induced brain damage (19,20). Brain has a high level of metabolism for its function, and the oxidation of glucose is the only substantial source for energy production. Therefore in TD, intracellular energy levels of neurons may be severely compromised by the incapacitation of thiamine-dependent enzymes involved in energy production (21–25).

The laboratory rat is a most reliable model of brain damage by TD induced by a thiamin-deficient diet or/and daily injection of the thiamine antagonist pyrithiamine. In this study, in pyrithiamine-induced TD in the rat, we have shown a decrease in brain concentration of GPC and recovery with thiamine replenishment. Main contributions to the in vivo 3.2-ppm resonance peak come from the trimethylammonium groups in PC and GPC. PC and GPC are known to be intermediates in phospholipid synthesis and degradation, respectively (12). A reduced cerebral Cho peak is typical of patients with disturbance of energy metabolism such as hepatic encephalopathy and hydrocephalus (26,27). But the liver disease and brain damage dose not generally show

Table 1
Concentration of Metabolites from Rat Brain Extract

	GPC	PC	Cho	PCr/Cr	NAA
High dose	0.35 (0.10)	0.23 (0.10)	0.04 (0.03)	6.81 (0.71)	5.81 (0.76)
Low dose	0.30 (0.10)*	0.24 (0.06)	0.04 (0.02)	6.17 (0.65)	5.53 (0.60)
TD	0.12 (0.03)*	0.16 (0.07)*	0.08 (0.04)	6.15 (0.84)	5.52 (0.89)
Normal	0.41 (0.09)	0.24 (0.10)	0.05 (0.03)	6.51 (0.77)	5.92 (0.89)

High dose: 100 mg/kg thiamine treatment; low dose, 5 mg/kg thiamine treatment, mean ± SD (mmol/wet kg).
*$P < 0.01$ versus normal one-tailed Student's t test.

correlation (28). Our results, showing a dose-dependent increase/recovery in Cho/NAA without change in Cr/NAA after administration of thiamine hydrochloride treatment, suggest that an increase in Cho-containing compounds may be mechanistically implicated in the recovery/treatment and are compatible with a role for white matter injury in the pathophysiology of alcohol-related brain dysfunction (29). The importance of thiamine replenishment in the recovery process of TD was also noted. The i.p. injection of choline solution to rat exerts minimal effect on the in vivo Cho peak (data not shown), demonstrating that the observed changes were not due to the lack of substrate. Because the GPC can be further broken down to dihydroxyacetone phosphate, an intermediate of glycolysis, it is postulated that TD could facilitate the cleavage of GPC to form glycerol 3-phosphate. These findings suggest that a reduction in GPC may be relevant to the primary biochemical lesion in TD, and is compatible with reduced catabolism of choline metabolites (25,30). Studies also show that GPC is a cerebral osmolyte. Similar findings of reduction of GPC in diseases such as hepatic encephalopathy hydrocephalus, and mild ischemia, in which the impaired energy metabolism leading to disrupted osmoregulation could provide a more concerted pathophysiologic mechanism in TD (26,27,31–33). The exact process and role of TPP in GPC synthesis and recovery is not known, but the recovery from energy impairment by TPP could initiate GPC recovery and potentially lead to membrane repair similar to other energy impairment studies (34). Consequently, these data are compatible with the hypothesis that a decrease in choline compounds in TD is the cause of the biochemical abnormalities that precede neuroanatomic damage characteristic of Wernicke's encephalopathy (4).

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