THIAMINE DEFICIENCY AS PREDISPOSITION TO, AND CONSEQUENCE OF, INCREASED ALCOHOL CONSUMPTION

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Abstract — It was found that the activity of the marker thiamine-dependent enzyme, transketolase (TK), was decreased (down to 61-79% of control) in blood, liver and brain of inbred rats following a 6-month consumption of 15% ethanol as their only source of drinking fluid. After ethanol withdrawal, the enzyme activity was gradually restored, but did not reach the control values until 1 month following cessation of alcohol consumption. Moreover, in rats preferring ethanol, the decrease of TK activity was more pronounced than in water-prefering rats. Another experiment showed that thiamine deficiency induced by the thiamine antagonist, oxythiamine (200mg/kg), led to a prolonged increase of the preferential intake of ethanol solutions in inbred rats. Significantly lower liver TK activities and thiamine pyrophosphate content were found in Finnish AA line rats as opposed to ANA line rats which had been obtained by selective outbreeding for high and low voluntary alcohol intake, respectively. Significantly lower TK activity was also found in the whole brain (89%), cerebellum (79%) and pons-medulla oblongata (87%) of AA rats as compared to ANA animals. Our own findings and the literature data confirm the hypothesis that thiamine deficiency can be both predisposing to, and a consequence of, increased alcohol consumption.

INTRODUCTION

Chronic ethanol consumption and alcoholism are frequently associated with vitamin B1 or thiamine deficiency (Dastur et al., 1976; Morgan, 1982), due to low dietary intake, decreased absorption and disturbances in storage and metabolism of thiamine (Baker et al., 1975; Abe and Itokawa, 1977; Hoyumpa, 1980; Laforenza et al., 1990). In alcoholic patients with severe thiamine deficiency, some specific complications have been found: Wernicke's encephalopathy, Korsakoff psychosis, polyneuropathy and myopathy (Juntunen et al., 1979; Harper and Kril, 1990; Nixon et al., 1990; Yung et al., 1991; Martin et al., 1993). Histologically diagnosed Wernicke's encephalopathy was revealed in more than half of the cases of consecutive alcohol-related deaths (Naidoo et al., 1991). In alcoholic patients with the diagnosis of Wernicke–Korsakoff syndrome, a dramatic reduction of thiamine phosphates in the blood (Tallaksen et al., 1993) and the thiamine-dependent enzyme transketolase in the autopsied cerebellum (Butterworth et al., 1993) were demonstrated. Early treatment of patients with Wernicke's encephalopathy by high doses of thiamine permits rapid reversal of some cognitive and neurological impairments and decreases the signs of brain atrophy judging from computed tomography data (Meyer et al., 1985). Optimal thiamine supply could reduce or prevent ethanol-induced morphological damage in rat brain (Wenisch et al., 1995). This confirms the thiamine deficient nature of the pathology.

The interactions between alcohol and thiamine deficiency were demonstrated in the experiment where the combined treatment by ethanol and thiamine-deficient diet induced more severe brain damage than the sum of those produced by the two treatments given separately (Phillips, 1987). The progression of the typical neurological deficits due to dietary thiamine deprivation was shown to be accelerated by concomitant ethanol administration (Zimitat et al., 1990). Similar neuroanatomical damage and learning deficits in animals were found both following chronic alcohol consumption and thiamine deficiency induced by pyrithiamine (Jrle and Markowitsch, 1983).

It was shown also that thiamine deficiency,
produced by dietary deprivation of thiamine or by the thiamine antagonist pyrithiamine, increases the voluntary alcohol consumption and tolerance to ethanol in rats (Pekkanen, 1979, 1980; Eriksson et al., 1980). A previous episode of thiamine deficiency has been shown to significantly reduce ethanol-induced hypothermia and severity of intoxication and to accelerate ethanol metabolism in rats (Martin et al., 1985, 1989). Taking into account the above data, we suggested that thiamine deficiency can be both a predisposition to, and consequence of, increased alcohol consumption. The purpose of the present study was to test this hypothesis experimentally.

MATERIALS AND METHODS

Animals and chemicals

Inbred albino rats were obtained from the Rappolovo breeding colony of the Academy of Medical Sciences of Russia. They were maintained in a room with a 12 h light: 12 h dark cycle and a 20–24°C temperature, in groups of six animals in plastic cages; tap water and food were given ad libitum.

AA and ANA rats genetically differing in alcohol consumption were bred and maintained in the animal facilities of the Research Laboratories, Alko Ltd, Helsinki, Finland. Adult males of about 300 g were used. They were housed in groups of four to six animals in stainless-steel wire cages, with constant access to R3 rodent pellet food (Ewos AB, Sodertalje, Sweden) and tap water, in a room illuminated from 06:00 to 18:00 and kept at 22–24°C and 55% humidity. The AA and ANA rats were obtained by selective outbreeding for high and low voluntary alcohol intake respectively (Sinclair et al., 1989). None had ever been treated with ethanol.

Orcinol was obtained from Merck, the barium salt of ribose-5-phosphate, NADH and alcohol dehydrogenase were from Reanal and thiamine diphosphate was from Polfa. Hydroxythiamine was synthesized by the method of Oparin et al. (1987) in the Institute of Biochemistry, Academy of Sciences of Belarus. All other reagents were of analytical grade purity.

Study design

In the first experiment, 180 male inbred rats (initial age 2.5 months, weight 120–160 g) received 15% (v/v) ethanol solution as the sole source of drinking fluid (the mean daily absolute ethanol consumption was ~6 g/kg) for 6 months. In the 5–6th months of the experiment all the animals were tested in the situation of free choice between 15% (v/v) ethanol solution and water (three times during 2 days, at 2 week intervals). Thirty rats with maximal (ethanol-prefering, EP) and minimal (water-prefering, WP) craving for alcohol were selected. Indices of alcohol preference (% of the daily consumption of ethanol solution/total fluid consumption) in those groups were 60.6 ± 2.9 and 3.7 ± 0.26%, respectively. Thirty naive rats taken concurrently (serving as a control group throughout the experiment) received only water as a drinking fluid. Five rats from every group were killed by decapitation at 12 h and at 7 and 30 days after ethanol withdrawal.

In the second experiment, 24 adult male heterogeneous stock rats were tested in the situation of free choice between 5% (v/v) ethanol solution and water (the procedure and the calculations were as in the first experiment) and divided into two groups equal in alcohol preference and consumption (calculated intake of absolute ethanol/kg of body weight/day) levels. The animals were injected subcutaneously by the thiamine antagonist, hydroxythiamine, 50 mg/kg (dissolved in water, pH was adjusted to 6.0 by NaOH), four times, at 3 h intervals. Control rats were injected with the same volume of 0.9% (w/v) NaCl. Three hours following the last injection, rats were placed in individual cages for 17 days where they could freely choose between 5% ethanol solution and water.

The third experiment was undertaken to examine the thiamine status in animals genetically differing in voluntary alcohol consumption. Five rats from AA and ANA lines were compared in terms of their liver and brain transketolase activity and thiamine pyrophosphate level.

Estimation of thiamine status

Samples of tissues from all animals were frozen and stored in liquid nitrogen. For the estimation of thiamine status, the activities of the marker thiamine-dependent enzyme, transketolase (TK; EC 2.2.1.1) were measured spectrophotometrically by the modified method (Bruns et al., 1958; Ostrovsky, 1979) in blood, liver and brain, and
Table 1. Transketolase activity following cessation of chronic alcohol consumption

<table>
<thead>
<tr>
<th>Time following cessation</th>
<th>Transketolase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood</td>
</tr>
<tr>
<td>12 hours</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.26 ± 0.02</td>
</tr>
<tr>
<td>WP</td>
<td>0.17 ± 0.01***</td>
</tr>
<tr>
<td>EP</td>
<td>0.16 ± 0.01***</td>
</tr>
<tr>
<td>7 days</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>WP</td>
<td>0.17 ± 0.01***</td>
</tr>
<tr>
<td>EP</td>
<td>0.15 ± 0.01***</td>
</tr>
<tr>
<td>30 days</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>WP</td>
<td>0.20 ± 0.01***</td>
</tr>
<tr>
<td>EP</td>
<td>0.19 ± 0.02***</td>
</tr>
</tbody>
</table>

The activity assays and the experimental design have been described in the Materials and Methods. Values for transketolase activity (μmol of sedoheptulose-7-phosphate/ml blood or nmol of sedoheptulose-7-phosphate/mg tissue/min) are expressed as means ± SEM for five animals in each group. Each control group included five naive rats of the same age maintained throughout the experiment under the same conditions and receiving water as the only source of drinking fluid. WP = rats preferring water; EP = rats preferring ethanol.

*P < 0.05; **P < 0.01; ***P < 0.001 as compared to control.
†P < 0.05; ††P < 0.01; †††P < 0.001 as compared to WP rats.

The activity was expressed as μmol of sedoheptulose-7-phosphate/ml blood or nmol of sedoheptulose-7-phosphate/mg tissue/min.

The level of thiamine pyrophosphate (TPP) in the tissues was measured by the modified enzymatic method (Ullrich et al., 1966; Ostrovsky, 1979).

For the statistical analysis the values were expressed as means ± SEM and Student’s t-test was used.

RESULTS

Thiamine status following chronic alcohol consumption in inbred rats

Following chronic (6 months) ethanol consumption (15% solution as the sole source of fluid), blood, liver and brain showed a lower TK activity. The enzyme activity recovered slowly, but did not reach the control values until 1 month following cessation of alcohol consumption. More pronounced TK inhibition was demonstrated in EP animals as compared to WP rats (Table 1). During the experiment, the animals consuming alcohol...
Table 2. Transketolase activity and content of thiamine pyrophosphate in the liver of ANA and AA rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>ANA rats</th>
<th>AA rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transketolase</td>
<td>3.41 ± 0.17</td>
<td>2.74 ± 0.16*</td>
</tr>
<tr>
<td>(nmol of sedoheptulose-7-phosphate/mg tissue/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiamine diphosphate</td>
<td>9.75 ± 0.70</td>
<td>5.06 ± 0.70*</td>
</tr>
<tr>
<td>(ng/mg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The biochemical assays and experimental design have been described in the Materials and Methods. Values are expressed as means ± SEM for five rats from each rat line. *P < 0.05 as compared to ANA rats.

Table 3. Transketolase activity in brains of ANA and AA rats

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>ANA rats</th>
<th>AA rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole brain</td>
<td>1.36 ± 0.06</td>
<td>1.21 ± 0.03*</td>
</tr>
<tr>
<td>Telencephalon</td>
<td>1.13 ± 0.02</td>
<td>1.06 ± 0.03</td>
</tr>
<tr>
<td>Diencephalon + mesencephalon</td>
<td>1.23 ± 0.06</td>
<td>1.20 ± 0.03</td>
</tr>
<tr>
<td>Pons + medulla oblongata</td>
<td>1.46 ± 0.05</td>
<td>1.27 ± 0.05*</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.63 ± 0.07</td>
<td>1.30 ± 0.04*</td>
</tr>
</tbody>
</table>

The activity assays and experimental design have been described in Materials and methods. Values (nmol of sedoheptulose-7-phosphate/mg tissue/min) are expressed as means ± SEM for five rats from each rat line. *P < 0.05-0.01 as compared to ANA rats.

Thiamine status in rats genetically differing in alcohol intake

In rats with genetically high voluntary alcohol consumption (AA line) the liver TK activity and TPP content were much lower as compared to the animals with inborn low alcohol consumption (ANA line) (Table 2). A significantly lower TK activity was also found in the whole brain and some of its parts (such as the cerebellum and pons + medulla oblongata) of AA rats versus to ANA animals (Table 3).

DISCUSSION

Among the variety of methods available to produce animal analogues of alcohol addiction, we used the oral, 'voluntary' administration of ethanol solution, since this method requires minimal manipulation of subjects and probably corresponds most closely to the manner in which humans usually consume alcohol (Jrle and Markowitsch, 1983). In these experiments, the loss of weight of animals consuming alcohol was usually observed as compared to naive ones. The chronic consumption of the ethanol solution as the single source of drinking fluid in our experiment inhibited strongly the marker thiamine-dependent enzyme, TK, in rat tissues. Moreover, these changes were slowly reversible and retained in the body for at least 1 month following cessation of the alcohol consumption (ethanol withdrawal). These results are in agreement with the data on lower thiamine and TPP levels in the liver of rats forced to drink ethanol solution (Kiessling and Tilander, 1960) or following 3 weeks of intra-gastric administration of ethanol solution (Abe and Itokawa, 1977). A significant decrease of TK activity and enzyme protein concentration was found in the rat brain, but not in the liver following 10 weeks of liquid alcohol diet (Yung et al., 1991). Since thiamine deficiency can also be seen in rats following alcohol diet (Martin et al., 1989) and in alcoholic patients (Dastur et al., 1976; Morgan, 1982), it can be suggested that chronic alcohol consumption provokes thiamine deficiency both in humans and animals independently of the way of alcohol administration. Apparently the significant reduction of the TPP content in the body is the main cause of the decrease of TK activity (Nixon et al., 1990), although the
possibility of a direct effect of ethanol and, especially of acetaldehyde on the enzyme protein (Abe and Itokawa, 1977; Yung et al., 1991) cannot be ruled out. But it should be noted that thiamine deficiency is not always observed in experiments with pair feeding, where ethanol is substituted by carbohydrates (Shaw et al., 1981). This discrepancy can be explained by the data on high-carbohydrate diet increasing the requirement for thiamine (Gorenshtein et al., 1988), which is probably not a suitable control for the experiments with alcohol-induced thiamine-deficiency. The more severe thiamine deficiency found in EP rats, as compared to WP rats, in our experiment can be both a consequence of their increased alcohol intake and the known characteristics of their inborn metabolism such as poorer thiamine status of inbred rats preferring ethanol as compared to animals preferring water (Ostrovsky et al., 1985).

The results of the second experiment have shown that hydroxythiamine-induced thiamine deficiency increases alcohol preference in rats. Earlier, Pekkanen (1980) did not find this effect following 2 week–daily injections of 2 mg/kg of hydroxythiamine; probably this dosage was too low to compete with vitamin B_{12} for thiamine kinase and induce thiamine deficiency in the body (Zimatkina et al., 1989). We used the total dose of 200 mg/kg for one day, which induced severe thiamine deficiency in rats. The previous investigations also showed that the enhanced voluntary alcohol consumption following thiamine deficiency was caused in animals by dietary deprivation of vitamin B_{12} or by the thiamine antagonist, pyrithiamine (Pekkanen, 1979, 1980; Eriksson et al., 1980). It may be concluded that thiamine deficiency can enhance alcohol preference and voluntary alcohol consumption in rats and can be not only a consequence of, but also predisposing to, increased alcohol consumption. There may be several explanations for this phenomenon. TPP is a coenzyme of a number of key enzymes of carbohydrate metabolism, such as pyruvate dehydrogenase (EC 2.2.4.1), 2-oxoglutarate dehydrogenase (EC 1.2.4.2) and TK. Therefore, thiamine deficiency results in a considerable impairment of energy-yielding metabolism, by the way of the citric acid cycle, glycolysis and the pentose phosphate pathway (Holowack et al., 1968; Collins et al., 1970). This may explain the craving for ethanol as a high-energy compound requiring no thiamine to its metabolism. In addition to the specific coenzyme function in energy metabolism of the animal body, thiamine is directly associated with a regulation of nerve conductivity and neurotransmitter metabolism (Haas, 1988). Consequently, thiamine deficiency can generally interfere with brain functions, animal behaviour and, specifically, with alcohol consumption. It was shown that thiamine deficiency reduces dopamine and noradrenaline turnover in the brain, whereas alcohol consumption increased it (Sjoquist et al., 1988). This may probably be to restore the reward system and induce compensatory craving for ethanol.

Our results have shown poorer thiamine status in tissues of AA rats (with genetically high voluntary alcohol intake) as opposed to the animals with inborn low alcohol intake (ANA line). Among the brain regions, a particular decrease was found in the cerebellum. It correlates with the lower level of TPP in the cerebellum of inbred rats preferring ethanol as compared to rats preferring water (Ostrovsky et al., 1985). These regional differences can be related to the heterogeneity of thiamine metabolism in the brain: the thiamine turnover period among the rat brain regions is also shortest in the cerebellum (Rindi et al., 1984). It can be suggested that the variations in the thiamine status in these animals are related to genetic specificities of their alcohol intake. These data are in good agreement with the observations of Impeduglia et al. (1987) that rat strains with genetically greater propensity to develop thiamine deficiency encephalopathy demonstrated higher tolerance to and preference for ethanol.

In conclusion, our own and literature data demonstrate the close relationships between vitamin B_{12} deficiency and alcohol and confirm the hypothesis that thiamine deficiency can be both predisposing to, and a consequence of, increased alcohol consumption.

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