PHOSPHATIDYLETHANOLAMINE N-METHYLTRANSFERASE ACTIVITY IS INCREASED IN RAT INTESTINAL BRUSH-BORDER MEMBRANE BY CHRONIC ETHANOL INGESTION

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Abstract — Aims: Phosphatidylethanolamine N-methyltransferase (PEMT) catalyses the synthesis of phosphatidylcholine from phosphatidylethanolamine. The aim of this study was to evaluate the effect of chronic ethanol ingestion on PEMT activity in the jejunal brush-border membrane (BBM) of adequately nourished rats. Methods: For this purpose, rats were fed a liquid diet containing ethanol [ethanol-fed group (EFG)] or an isocaloric liquid diet without ethanol [pair-fed group (PFG)] for 4 weeks. Diet ingestion, body weight, nitrogen balance and urinary creatinine excretion were monitored during the experimental period, and serum transferrin levels were determined at the end. BBM was isolated for the determination of PEMT activity. Results: PEMT activity was significantly increased in the jejunal BBM of the EFG. Nutritional parameters, however, did not differ between groups. Conclusions: The increase in PEMT activity may be attributed exclusively to chronic ethanol ingestion, since a major nutritional deficit was excluded.

INTRODUCTION

Phosphatidylcholine is a major phospholipid of many plasma membranes. The synthesis of phosphatidylcholine can occur by two different pathways: the cytidyldiphosphocholine (CDP-choline) pathway (Kennedy and Weiss, 1956), and by two different pathways: the cytidyldiphosphocholine pathway (Kennedy and Weiss, 1956), and the latter by phosphatidyl-

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Materials and Methods

Animals

All animals were treated according to the CIOMS International Guiding Principles for Biomedical Research Involving Animals (1985). Thirty-one male Wistar rats weighing 225 ± 4 g were obtained from a local supplier. The animals were divided into two groups: an ethanol-fed group (EFG, n = 15) and a pair-fed control group (PFG, n = 16). The rats were housed in individual metabolic cages on a 12-h light–dark cycle in a temperature-controlled room.

Experimental design

The animals were pair-fed for 4 weeks receiving a nutritionally adequate Lieber–DeCarli liquid diet as the sole source of food (Dyets Inc., Bethlehem, PA, USA). Diets followed American Institute of Nutrition (AIN) recommendations for adult rodent’s maintenance (Reeves et al., 1993) and contained 1 kcal/ml. The EFG received the liquid diet ad libitum but cornstarch was substituted isocalorically by ethanol to provide 35% of the total calories. The PFG received the isocaloric liquid diet without ethanol in a volume equal to that ingested by rats of the EFG. Liquid diets were renewed twice a day, so the animals consumed ethanol continuously.

On experimental day 29, the rats were killed by decapitation, and blood was collected. The small intestine was rapidly removed, washed with ice-cold saline, and placed on a pre-chilled glass plate. The intestine was divided into three equal segments. The first segment was opened longitudinally along the mesenteric border and the mucosal surface was removed by gently scraping with a microscope slide, placed into preweighed tubes, which were immediately frozen in liquid nitrogen and stored at −80°C for later purification of the BBM.

Nutritional evaluation

Body weight was recorded twice a week and diet ingestion was quantified daily. Nitrogen balance was obtained from the difference between the calculated nitrogen intake and the measured urinary and fecal nitrogen excretion, and was evaluated during a period of three consecutive days, at the first and last weeks of the experiment. Nitrogen was determined according to Fleck and Munro (1965). The 24 h urinary creatinine excretion was evaluated weekly by a modification of the kinetic Jaffé reaction (Larsen, 1972). Serum transferrin was determined in the blood collected at death and expressed as the percentage of serum iron/total iron-binding capacity ratio, using a colorimetric assay kit provided by Labtest (Belo Horizonte, MG, Brazil).

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Isolation of BBM

The method used for isolation of the BBM from rat small intestine mucosa has already been presented in detail (Booth and Kenny, 1974). All work was performed at 0–4°C. Mucosa was homogenized in 4 vol. of ice-cold 300 mM mannitol–12 mM Tris buffer, pH 7.3, and the homogenate was prepared by the Mg<sup>2+</sup> precipitation treatment. The homogenate was centrifuged at 1500 g for 12 min and the supernatant was subsequently centrifuged at 21 000 g for 12 min. The pellet was re-suspended in 8 ml of 50 mM mannitol–2 mM Tris buffer, pH 7.3, and MgCl<sub>2</sub> was added to give a concentration of 10 mM and then stirred gently for 15 min in an ice bath. This suspension was centrifuged at 2200 g for 12 min and the resulting supernatant was subsequently centrifuged at 21 000 g for 30 min. The final pellet was re-suspended in 300 µl of 50 mM mannitol–2 mM Tris buffer, pH 7.3, and aliquots were stored at –80°C. Protein was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Cytochemical method

The efficiency of the preparative method was assessed by a cytochemical method to determine the presence of alkaline phosphatase, a BBM enzyme marker. A sample of the final pellet obtained from the BBM preparation was embedded in synthetic resin (Tissue-Tek, Sakura, USA) and frozen in liquid nitrogen. The blocks obtained were stored at –80°C. Sections of 6 µm were obtained in a cryostat. After fixation in 4% paraformaldehyde buffer, the sections were prepared according to the method of Brenan and Bath (1989). In brief, slides were incubated with a substrate solution containing chloro-iodoxyl phosphate dissolved in dimethylformamide and tetranitro blue tetrazolium for 30 min in a darkened humidified chamber at 37°C, washed in distilled water and mounted with Aquamount. The positive reaction, a dark blue staining, represented the presence of alkaline phosphatase in the sample.

Electron microscopy

Additionally, membrane preparations were examined in a transmission electron microscope, to assess the absence of contaminating structures. A sample of the final pellet of the BBM was fixed with 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4 for 2 h, post-fixed with a 1% buffered osmium tetroxide solution (w/v), dehydrated through graded alcohols and embedded in epoxy resin (EM-Bed; Electron Microscopy Sciences, Fort Washington, PA, USA). Ultrathin sections of about 70 nm thickness were double stained with uranyl acetate and lead citrate and examined under a transmission electron microscope (Carl Zeiss 109) operating at 80 kV.

Table 1. Daily caloric intake (protein, fat, carbohydrate and ethanol) and total weight gain

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein (kcal/day/rat)</th>
<th>Fat (kcal/day/rat)</th>
<th>Carbohydrate (kcal/day/rat)</th>
<th>Ethanol (kcal/day/rat)</th>
<th>Total (kcal/day/rat)</th>
<th>Weight gain (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFG (n = 15)</td>
<td>6.30 ± 0.14 (11%)</td>
<td>5.58 ± 0.13 (10%)</td>
<td>22.40 ± 0.49 (40%)</td>
<td>19.44 ± 0.44 (35%)</td>
<td>55.86 ± 1.27 (100%)</td>
<td>40.95 ± 4.80</td>
</tr>
<tr>
<td>PFG (n = 16)</td>
<td>6.30 ± 0.14 (11%)</td>
<td>5.58 ± 0.13 (10%)</td>
<td>41.84 ± 0.93 (75%)</td>
<td>0 (0%)</td>
<td>55.86 ± 1.27 (100%)</td>
<td>34.64 ± 2.72</td>
</tr>
</tbody>
</table>

EFG, ethanol-fed group; PFG, pair-fed group. There was no significant difference in total weight gain between the two groups.
Cytochemical and electron microscopic analysis

Cytochemical analysis of the final pellet sample from the BBM preparation showed an intense dark blue reaction in practically all tissue indicating the massive presence of alkaline phosphatase, which confirmed the efficacy of the preparative method.

The ultrastructural analysis of the final BBM preparation showed membranes without organelle contaminants (Fig. 1).

Enzymatic study

Figure 2 shows the PEMT activity determined in the jejunal BBM of the EFG and PFG. At 5, 10, 20 and 40 min of incubation, respectively, PEMT activity was significantly increased \((P < 0.01)\) in the EFG \((826 \pm 37, 883 \pm 23, 1002 \pm 47\) and \(1079 \pm 58\) fmol/mg of protein) as compared with the control group \((738 \pm 18, 747 \pm 23, 858 \pm 42\) and \(1009 \pm 46\) fmol/mg of protein).

PEMT activity did not change between 5 and 10 min of incubation within either group. After this period, methylation increased with maximal PEMT activity observed at 40 min \((P < 0.001)\).

Discussion

In the present study, we have observed a significant increase in PEMT activity in the jejunal BBM of rats ingesting ethanol for 4 weeks. However, evaluation of nutritional parameters did not show significant differences between the groups. All animals consumed adequate amounts of calories, protein, fat, carbohydrate, minerals and vitamins, according to AIN measurements (Reeves et al., 1993). Furthermore, rats gained weight significantly during the total experiment period, with no difference between the groups. The nitrogen balance, however, is known to be a more appropriate parameter than weight for the evaluation of the extent of protein catabolism (Trocki et al., 1986). In the present study, nitrogen balance was positive for both groups, indicating efficient nitrogen retention and protein synthesis. Concurrently, body muscle mass and visceral protein, evaluated by the urinary creatinine excretion and serum transferrin levels, respectively, were not affected by ethanol ingestion (Blackburn and Thornton, 1979; Lorenz-Mayer et al., 1980).

The increase in jejunal PEMT activity observed suggests that chronic ethanol consumption might stimulate the synthesis of phosphatidylcholine by the transmethylation pathway, since it may be assumed that phosphatidylcholine is the main product resulting from the enzymatic methylation of phosphatidylethanolamine (Bremer and Greenberg, 1961). A previous study, however, has shown a decrease in the total phospholipid and phosphatidylcholine content in the jejunal BBM of rabbits fed ethanol chronically, which resulted in a lower jejunal BBM phospholipid/cholesterol and choline/amine phospholipid ratio (Keelan et al., 1985). The choline/amine

Table 2. Nitrogen balance and serum transferrin levels

<table>
<thead>
<tr>
<th>Group</th>
<th>First week (g N/period)</th>
<th>Last week (g N/period)</th>
<th>Serum transferrin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFG ((n = 9))</td>
<td>0.33 \pm 0.06</td>
<td>0.35 \pm 0.09</td>
<td>43.18 \pm 2.70</td>
</tr>
<tr>
<td>PFG ((n = 10))</td>
<td>0.46 \pm 0.05</td>
<td>0.31 \pm 0.07</td>
<td>50.48 \pm 5.65</td>
</tr>
</tbody>
</table>

EFG, ethanol-fed group; PFG, pair-fed group.

Table 3. Urinary creatinine excretion

<table>
<thead>
<tr>
<th>Group</th>
<th>First week (g/24 h)</th>
<th>Second week (g/24 h)</th>
<th>Third week (g/24 h)</th>
<th>Fourth week (g/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFG ((n = 9))</td>
<td>11.54 \pm 0.93</td>
<td>9.42 \pm 0.77</td>
<td>10.77 \pm 1.11</td>
<td>9.94 \pm 0.64</td>
</tr>
<tr>
<td>PFG ((n = 10))</td>
<td>12.22 \pm 1.24</td>
<td>9.34 \pm 0.80</td>
<td>9.06 \pm 0.71</td>
<td>10.67 \pm 0.92</td>
</tr>
</tbody>
</table>

EFG, ethanol-fed group; PFG, pair-fed group.

Fig. 1. Ultrastructure of the jejunal brush-border membrane (BBM). The sample was composed of electron dense granular structures, typical of a cellular membrane. Notice the absence of other cellular organelles amongst the BBM. Original magnification \(\times 45000\).
phospholipid ratio was also reported to be decreased in the small intestine of alcoholic subjects (Bender-Braulia et al., 1990).

Our results differ from those of Duce et al. (1988) and Lieber et al. (1994), but are in agreement with those of Uthus et al. (1976) and Carrasco et al. (2002). Duce et al. (1988) showed a marked decrease in PEMT activity in liver biopsies from human patients with alcoholic or post-hepatic cirrhosis. Nevertheless, these authors did not find any change in relative phospholipid composition. Lieber et al. (1994) have also demonstrated a decrease in PEMT activity in liver biopsies from baboons with alcohol-induced fibrosis, prior to the development of cirrhosis. This effect was accompanied by a decrease in the hepatic content of phosphatidylcholine. In contrast, Uthus et al. (1976) and Carrasco et al. (2002) reported that chronic ethanol ingestion markedly increased hepatic PEMT activity.

The precise mechanism by which ethanol stimulated the jejunal BBM transmethylation pathway in our study remains to be determined. In the liver, the two major pathways for the biosynthesis of phosphatidylcholine (the CDP-choline and the transmethylation pathways) seem to be regulated in a coordinated way, i.e., an inhibition of the CDP-choline pathway is accompanied by a stimulation of the transmethylation route (Mato and Alemany, 1983). If a similar regulation occurs in the small intestine, it is possible that the increase in PEMT activity observed in the present study is associated with an inverse effect on the CDP-choline pathway, leading to a deficient synthesis of phosphatidylcholine by the latter pathway.

Another possibility which could explain the elevation of PEMT activity in response to chronic ethanol ingestion is the increase of phosphatidylethanolamine levels, as a consequence of ethanol metabolism within the intestinal cell. The availability of phosphatidylethanolamine has already been demonstrated to regulate the PEMT activity (Ridgway et al., 1989; Cui et al., 1993). Indeed, Carrasco et al. (1996) reported that the levels of phosphatidylethanolamine were elevated in the hepatocytes after their incubation in the presence of ethanol and radiolabelled ethanolamine. Furthermore, in a previous study, we have shown that chronic ethanol ingestion significantly increased phosphatidylethanolamine levels (62%) in the mucosa of human small intestine (Bender-Braulia et al., 1990). Recently, Carrasco et al. (2002) have clearly demonstrated in rat hepatocytes that ethanol activates the biosynthesis of phosphatidylethanolamine and the methylation pathway of phosphatidylethanolamine to produce phosphatidylcholine, after both short-term and chronic ethanol treatments. In addition, these authors have also reported the inhibitory effect of chronic ethanol intake in the liver phosphatidylcholine synthesis in the CDP-choline pathway.

In conclusion, our results show a significant increase in jejunal PEMT activity due to chronic ethanol intake. Since no protein or caloric malnutrition was detected, we may assume that ethanol independently affects the phospholipid transmethylation pathway in the small intestinal mucosa.

REFERENCES


