CHANGES IN THE ILEAL DISACCHARIDASE ACTIVITIES IN RATS AFTER LONG-TERM ETHANOL FEEDING

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Abstract — Brush border enzymatic activities (maltase, lactase and sucrase) have been determined in the ileal mucosa of rats subjected to a 30% ethanol ingestion for 3 and 5 months. The data were compared with the results obtained with control rats. Mucosal protein content after 3 months of ethanol treatment was similar to that of control rats. Maltase, lactase and sucrase specific activities in ileal mucosa were significantly decreased in ethanol-fed animals as compared to control rats. After 5 months of ethanol consumption, the protein content was decreased in ethanol-fed rats. However, no differences were found between specific activities of maltase, lactase and sucrase of ethanol-fed with respect to control rats. It is suggested that prolonged exposure of rats to ethanol results in adaptive responses to the effects of shorter periods of exposure on intestinal mucosal function.

INTRODUCTION

The disaccharidases are integral carbohydrates of the small intestinal brush border membrane (BBM), responsible for the hydrolysis of carbohydrates which must occur prior to monosaccharide absorption (Lorenzsonn et al., 1987). The most important disaccharidases in mammals are lactase, sucrase and maltase. Disaccharidase activities appear to be broadly correlated to dietary habits (Vonk and Western, 1985). Food is essential for the maintenance of normal mucosal integrity and has a direct effect on brush border enzyme activity in the rat (Yamada et al., 1981; Holt and Yeh, 1992). Thus, the activities of specific enzymes are reduced when their substrates are rare or absent from an animal's diet (Zarling and Mobarhan, 1987; Rivera-Sagredo et al., 1992). On the other hand, other studies suggest that carbohydrate intake increases disaccharidase activities (Shinohara et al., 1986; Samulitis-Dos Santos et al., 1992). Furthermore, there are other factors that can influence disaccharidase activities, such as age and obesity (Flores et al., 1990), a decrease of luminal proteases (Zarling and Mobarhan, 1987) and hormones (Raul et al., 1984).

The distribution of disaccharidase activities among vertebrates is extremely heterogeneous (Martinez del Rio and Stevens, 1989). Moreover, these activities were higher in the proximal and middle small intestine segment and decreased distally (Martinez del Rio and Stevens, 1988; Flores et al., 1990).

A limited number of studies have been directed toward determining the effect of chronic ethanol ingestion on intestinal disaccharidase activities, and a few studies with the effect of high levels of ethanol ingestion. Exposure of the intestinal mucosa to ethanol causes morphological injuries and modifies enzyme activities (Raul et al., 1982). Reduced intestinal sucrase and lactase activities have been reported in alcoholic men (Perlow et al., 1977). Furthermore, other studies (Baraona et al., 1974) showed that rats ingesting ethanol (36% of total calories) have depressed activities of jejunal lactase and sucrase. Leichter (1987) found that chronic alcohol consumption (about 28% of total calories ingested) by adult rats has no effect on the activities of intestinal lactase, sucrase and maltase when compared with control rats. On the other hand, other authors found that low concentrations of ethanol (1–3%) elevated specific activities of lactase, sucrase, and maltase (Nano et al., 1990). Thus, conflicting results concerning the effect of alcohol ingestion on disaccharidase activities in the intestinal mucosa have been reported. Moreover, a great part of...
these studies involved a short experimental time-period and were made in jejunum. The ileum, by contrast, is an area where there are no acute effects of ethanol. The purpose of the present work was therefore to examine the effects of long-term exposure of rats to high levels of ethanol (about 40% of total calories ingested) on the disaccharidase activities in the ileum.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing around 300 g were randomized into four groups. Two groups were submitted to an ethanol treatment through drinking water by a method previously described (Hajjar et al., 1981), modified by our group. In one series of experiments, ethanol was administered gradually, in drinking water, as a 5% (v/v) solution during the first week, a 10% solution during the second week, 15% (3rd week), 20% (4th week) and finally 30% during the subsequent 3 months (group containing 20 animals) or 5 months (group containing 9 animals). Chow diet and ethanol solution were provided ad libitum. The other two groups received water and food ad libitum for 3 and 5 months respectively (15 and 9 rats respectively).

Rats were housed in stainless-steel cages (in groups of five animals/cage) in a well-ventilated room maintained around 22°C and were fed on a standard pellet diet. The average intakes of ethanol, food, water and body weight were recorded every week until the end of the experiment. Each measurement was recorded at 09.00 to avoid changes due to circadian rhythms. Caloric evaluation was calculated as previously described by Veale and Myers (1968).

Animal care complied with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals (NIH Publication no. 80-23 Revised and D.O. no. 1,358, 18-12-86 p.1 86/609/CEE).

Sample preparation

At the end of the experimental period, the rats were starved for 24 h, then anaesthetized with intraperitoneal sodium pentobarbital (4 mg/100 g body weight). Ileal loops (about 15 cm) were removed and rinsed with ice-cold saline solution. The intestinal segments were opened longitudinally on an ice-glass plate and the mucosa was then scraped off with a piece of glass slide in a uniform fashion. The scrapings were immediately dropped into liquid nitrogen and frozen at -80°C until analysed for disaccharidase activities and protein content.

Analysis of samples and calculation of results

Disaccharidase activities were determined by the method of Dahlqvist (1970, 1984). Briefly, the principle of this method is the following: an intestinal homogenate is incubated with the appropriate disaccharide. The disaccharidase activity is then interrupted by the addition of Tris, and the glucose liberated is measured with a glucose oxidase reagent.

The mean weight of the mucosa was approximately 200 mg. Tissue dispersion was carried out by a motor-driven Teflon homogenizer under ice-cold conditions. This mucosal homogenate was diluted to a final volume of 25 ml.

For analysis, the samples were diluted 1:100 (maltase), 1:4 (sucrase) and 1:1 (lactase), and mixed and incubated with substrate–buffer solution at 37°C for 60 min. The reaction was stopped by adding Tris–glucose oxidase reagent, mixed and incubated at 37°C again for 1 h for development of colour. The blanks were a mixture of diluted mucosal homogenate, Tris–glucose oxidase reagent and substrate–buffer solution, in the order mentioned, and incubated at 37°C for 1 h. Standards were a series of glucose solutions containing 7.5–60 μg of glucose each per 200 μl. Samples, blanks and standards were all read at 450 nm.

The amount of glucose formed (μg) (sample minus blank) was determined from the standard curve of glucose. Thus, the activities can be calculated as follows:

$$\text{Units/ml of homogenate} = \frac{\mu g \text{ glucose} \times F}{180 \times 60 \times N}$$

where $F =$ dilution factor of the homogenate (12 500 for the maltose, 1000 for the sucrose and 250 for the lactose); $180 =$ molecular weight of the glucose; $60 =$ incubation time in min; $N =$ number of molecules of glucose liberated by hydrolysis in each sugar ($n = 1$ for sucrose and lactose, $n = 2$ for maltose). (One unit of enzymatic activity hydrolyses 1 μmol of disaccharide/min at 37°C.)
Table 1. Effects of chronic ethanol consumption on rat body weight and food, carbohydrate (CHO), fluid and calorie intake

<table>
<thead>
<tr>
<th>Time</th>
<th>Control (g)</th>
<th>Ethanol (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i-3</td>
<td>f-3</td>
</tr>
<tr>
<td>Body weight</td>
<td>284 ± 12</td>
<td>380 ± 8*</td>
</tr>
<tr>
<td>Food intake</td>
<td>25 ± 1</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>CHO intake</td>
<td>15 ± 1</td>
<td>9± 1**</td>
</tr>
<tr>
<td>Fluid intake</td>
<td>36 ± 6</td>
<td>39 ± 1</td>
</tr>
<tr>
<td>Total calories</td>
<td>73 ± 2</td>
<td>73 ± 2</td>
</tr>
<tr>
<td>as food</td>
<td>73 ± 2</td>
<td>73 ± 2</td>
</tr>
<tr>
<td>% calories as ethanol</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Average body weight (g), food intake (g/rat/day), carbohydrate intake (g/rat/day), fluid intake (ml/rat/day), total caloric consumption (i.e. chow diet plus drinking solution) (calories/rat/day) and percentages of the total caloric intake obtained from ethanol (% calories) were determined in control and ethanol-fed rats after 3 and 5 months of treatment. Results are expressed as means ± SEM.

*Data are presented both as initial (i-) and final (f-), respectively, before and after the treatment durations given.

*P < 0.001; **P < 0.01 (compared to the respective controls)

Table 2. Protein content in control rats and ethanol-fed animals after 3 and 5 months of ethanol treatment

<table>
<thead>
<tr>
<th>Time</th>
<th>Control (mg/ml of homogenate)</th>
<th>Ethanol (mg/ml of homogenate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 months</td>
<td>5 months</td>
</tr>
<tr>
<td>Control rats</td>
<td>14.2 ± 1 (15)</td>
<td>21.1 ± 1 (9)</td>
</tr>
<tr>
<td>Ethanol rats</td>
<td>14.5 ± 1 (20)</td>
<td>17.9 ± 1* (9)</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SEM for the number of rats/group as indicated in parentheses.

*P < 0.02: values are significantly different from the respective control rats.

**RESULTS**

**Average body weight, food, fluid and carbohydrate intake and caloric consumption of the rats**

Table 1 shows the body weights of ethanol-fed rats; they were lower, compared to control animals in both groups (3 and 5 months). Thus, after 3 months of ethanol treatment, the body weights were 380 ± 28 vs 487 ± 15 g in control rats (P < 0.02). The body weights obtained at the end of the experiment were 423 ± 15 g in 5-month-ethanol-fed vs 564 ± 9 g in control rats (P < 0.01).

**Protein content**

The total protein content (mg/ml) of the mucosa from the ileum (Table 2) of 3-month-ethanol-fed rats showed no statistically significant
Table 3. Effect of ethanol on disaccharidase activities in ileal segment of control and ethanol-fed animals after 3 months of ethanol ingestion

<table>
<thead>
<tr>
<th></th>
<th>Specific activities (U/g protein)</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Malate</td>
<td>Lactate</td>
<td>Sucrase</td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td>231 ± 24</td>
<td>6 ± 1</td>
<td>20 ± 2</td>
<td></td>
</tr>
<tr>
<td>(15)</td>
<td>(12)</td>
<td>(15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol rats</td>
<td>157 ± 12*</td>
<td>4 ± 0.3*</td>
<td>14 ± 2*</td>
<td></td>
</tr>
<tr>
<td>(20)</td>
<td>(18)</td>
<td>(18)</td>
<td></td>
<td></td>
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</tbody>
</table>

Values are means ± SEM for the number of rats/group indicated in parentheses. *P<0.001: values are significantly different from the respective control rats.

Table 3 shows the data for specific activity (U/g of protein) of ileum brush border disaccharidases of 3-month-ethanol-fed, and control, rats. After 3 months of treatment, the ileum disaccharidase specific activity of maltase, sucrase and lactase was decreased compared to control rats (P < 0.001 for maltase and P < 0.05 for lactase and sucrase). When the results were expressed per g of mucosa (U/g), the values for maltase, lactase and sucrase after 3 months of ethanol treatment were also significantly decreased (P < 0.001 for maltase; P < 0.01 for lactase and sucrase) (data not shown). At 5 months of ethanol ingestion, no modifications in the ileum disaccharidase activities (U/g protein) with respect to control rats were found. However, when expressed as Units per g of mucosa, only maltase and lactase activities were significantly lower in the 5-month-ethanol-fed animals (P < 0.05) (data not shown).

DISCUSSION

Our study has attempted to determine the effect of long-term (3–5 months) high levels of chronic ethanol ingestion on intestinal disaccharidase activities.

Rats treated with ethanol showed a significantly smaller body weight, in comparison with control rats, despite a similar total calorie ingestion. Weight gain is significantly lower when there is an isocaloric substitution of ethanol for carbohydrates, around 50% of total energy in a balanced diet (Pirola and Lieber, 1972), suggesting increased energy requirements by the body during alcohol consumption, caused by an increase in oxygen consumption (Pirola and Lieber, 1976). Substitution of ethanol for carbohydrate increases the metabolic rate and thermogenesis of humans and rodents (Stock and Stuart, 1974).

Other studies confirmed that alcoholic calories are additive, at least in the diets of light drinkers. However, in the diets of moderate and heavy drinkers, alcoholic calories replace other sources of energy (Gruchow et al., 1985). This is consistent with our data on total caloric consumption for both groups of ethanol-fed rats. In addition, intake of non-alcoholic energy decreased as alcohol intake increased, and it was estimated that 15–41% (around 40% in our study) of the alcoholic calories replaced non-alcoholic energy. Therefore, there is a decrease in the percentage of energy derived from protein, fat and carbohydrate, this being consistent with our data which show a decrease in the carbohydrate intake in both groups of ethanol-fed rats as the nutritional quality of the diet declines (empty calories) (Sherlock, 1984; Hillers and Massey, 1985).

The results of our study showed that the specific activities of ileal lactase, sucrase and maltase are significantly smaller in rats ingesting 40% of their total calories as ethanol for 3 months. These results are consistent with those of Baraona et al. (1974), who reported a decrease in the disaccharidase activities in rats ingesting 36% of the total calories as ethanol. Similar results were obtained in other experiments (Dinda et al., 1979) that showed lowered activities of brush border enzymes such as lactase, sucrase and maltase, after alcohol intake in both healthy volunteers and alcoholic patients.

The protein content of ileal mucosa from 3-month-ethanol-fed rats and their controls was similar. Thus, the data of smaller disaccharidase activities in ethanol-fed rats (with a similar protein content to controls) may indicate a possible reduction in the disaccharidase content of the mucosa, possibly caused by a smaller intake of carbohydrates; disaccharidase activities are known to be decreased when their substrates are reduced in the animal’s diet (Zarling and Mobar-
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han 1987; Rivera-Sagredo et al., 1992; Holt and Yeh, 1992). On the other hand, morphometric studies (Persson et al., 1990) have shown that villus height and the mucosal surface area are reduced in alcoholic as compared to non-alcoholic patients. This hypoplasia is probably also responsible for the decrease of disaccharidase activities.

Previous data covering such a long experimental period (5 months of chronic ethanol consumption) are not available. In our study, we found that there were no differences in lactase, sucrase and maltase activities between control and 5-month-ethanol-fed rats, although the total protein content of the ileal mucosa was smaller in ethanol-fed rats. However, when the results were expressed per g of mucosa, a significant decrease in ethanol-fed rats was found (data not shown). These 5-month data could be explained by an adaptation to a more significant decrease in intestinal proteins after a longer period of undernutrition and/or an adaptation of the disaccharidases at the ethanol ingestion after a long time-period. The adaptive changes in enzymatic activity of the small intestine depends on various exogenous factors (McCarthy et al., 1980).

On the other hand, this supposition is consistent with the great adaptability of the mucosa under different conditions, as has been demonstrated by our group (Carreras et al., 1988) and others (Riby and Kretchmer 1984; Schuman et al., 1990; Debnam and Chowrimootoo, 1992).

REFERENCES


