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INFLUENCE OF CHRONIC ALCOHOL CONSUMPTION ON HEPATIC HEME AND PORPHYRIN METABOLISM

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Abstract—To study the effect of prolonged alcohol consumption on hepatic heme and porphyrin metabolism, female Wistar rats were fed for 60 days a nutritionally adequate liquid diet containing 36% of total calories as ethanol, whereas the control diet was isocaloric and contained no alcohol. Compared to pair-fed controls, the administration of the alcohol diet resulted in an increased hepatic activity of δ-aminolevulinic acid synthase by 223% (112.3 ± 19.6 nmoles/hr/100 g b.wt. vs. 362.8 ± 42.5; $P < 0.01$), an enhanced urinary excretion of δ-aminolevulinic acid by 101% (64.8 ± 11.8 nmoles/day vs. 130.8 ± 22.4; $P < 0.05$), and an augmented urinary output of total porphyrins by 142% (1.2 ± 0.2 nmoles/day vs. 2.9 ± 0.5; $P < 0.05$). Concomitantly, the hepatic content of cytochrome P-450 was significantly enhanced and that of hepatic catalase activity marginally increased, whereas the hepatic iron content remained unaltered. In summary, the feeding of rats with a liquid alcohol diet for 60 days results in changes of hepatic heme and porphyrin metabolism which are associated and may be causally related with an induction of hepatic hemoproteins and subsequent derepression of hepatic δ-aminolevulinic acid synthase, whereas hepatic iron appears to play no pathogenic role.

Acute alcohol administration has been shown to result in various alterations of the heme and porphyrin metabolism both in man [1, 2] and experimental animals [2–4]. A relationship has also been established between chronic alcohol consumption and chronic hepatic porphyria in man [2, 5, 6]. At present, however, a suitable animal model for studies on the pathogenic mechanism of altered hepatic heme and porphyrin metabolism associated with long-term consumption of alcohol is lacking.

In view of the potential clinical importance of chronic hepatic porphyria along with prolonged alcohol consumption the question was studied whether experimental feeding of an alcohol containing liquid diet to rats for 60 days leads to changes of the hepatic heme and porphyrin metabolism. Moreover, it was of interest whether chronic hepatic porphyria could be reproduced by this experimental animal model.

MATERIALS AND METHODS

Materials. The chemicals were obtained from the following sources: ethanol 99.5%, tris(hydroxymethyl)aminomethane, potassium thiocyanate, nitric acid, potassium dihydrogen phosphate, disodium hydrogen phosphate 2-hydrate, perhydrol, sodium azide, semicarbazide hydrochloride, isocitrate dehydrogenase from pig heart, sodium dithionite, potassium chloride, magnesium chloride 2-hydrate cryst., from Merck Corp., Darmstadt, F.R.G.; glutathione reductase, α-oxoglutarate, glycyl glycine, L-γ-glutamyl-3-carboxy-4-nitro-anilide, lactate dehydrogenase, L-aspartate, malate dehydrogenase, α-ketoglutarate, ADP, L-alanine, triethanolamine, ammonium acetate, *p*-nitrophenyl phosphate, NAD, NADH, NADP (disodium salt 98%), NADPH (tetrasodium salt), isocitric dehydrogenase (grade II), 7-ethoxycoumarin from Boehringer Corp., Mannheim, F.R.G.; DL-isocitric acid Na_2-salt, Na_2-EDTA, cytochrome *c* from horse heart from Serva Corp., Heidelberg, F.R.G.; benzo(α)pyrene from Ferak Corp., Berlin, F.R.G.; 7-hydroxycoumarin from EGA-Chemie Corp., Heidelberg, F.R.G.

Animals. Female Wistar rats (N = 24) were purchased from Zentralinstitut für Versuchstierzucht, Hannover, F.R.G. and fed Altromin chow and tap water *ad libitum* until they reached a body weight of about 190–220 g. They were then housed in individual cages and pair-fed nutritionally adequate liquid diets containing either ethanol (36% of total calories) or isocalorically substituted carbohydrates until sacrifice [7]. The feeding period was either 10 or 60 days. Urine was collected over a period of 24 hr preceding killing. The animals were sacrificed by decapitation, and their blood was collected from the neck vessels to be used for the determination of serum enzyme activities. The livers were perfused with ice-cold 0.15 mol/l KCl through the portal vein, excised and weighed.

Methods. The cytosolic and microsomal fractions were prepared using a 25% liver homogenate as described previously [8]. The microsomal content of cytochrome P-450 [9] and the activities of the microsomal ethanol-oxidizing system [8], 7-ethoxycoumarin deethylase [10], arylhydrocarbon hydroxylase [11] and of the NADPH-cytochrome *c* reductase [12] were determined in the microsomal fraction. Microsomal losses during the preparative procedures were corrected as described previously [13]. The activity of alcohol dehydrogenase was determined in the cytosolic fraction of the hepatocytes [8]. The hepatic content of cytochrome P-450

Table 1. Effect of alcohol feeding for 10 or 60 days on liver weight and protein content.

	10 days treatment		60 days treatment	
	Control	Alcohol	Control	Alcohol
Liver weight (g)	5.6 ± 0.1	5.9 ± 0.2	6.5 ± 0.1	6.5 ± 0.1
(g/100 g b.w.)	3.0 ± 0.1	3.2 ± 0.1	2.8 ± 0.1	2.9 ± 0.1
Hepatic cytosolic protein (mg/g liver)	77.4 ± 0.8	73.5 ± 1.1*	74.3 ± 0.8	73.0 ± 1.1
(g/100 g b.w.)	0.24 ± 0.01	0.23 ± 0.01	0.21 ± 0.01	0.21 ± 0.01
Hepatic microsomal protein (mg/g liver)	20.7 ± 0.9	25.9 ± 1.0†	22.1 ± 0.9	28.6 ± 0.5†
(g/100 g b.w.)	0.06 ± 0.01	0.08 ± 0.01†	0.06 ± 0.01	0.08 ± 0.01†

The data are derived from 6 animals of each experimental group and are expressed per g of liver wet weight or per 100 g of body weight. The results are given as means ($\bar{x}$ ± SEM). The statistical analysis compares the alcohol treatment with the respective control diet.

* $P < 0.05$.

† $P < 0.01$.

[9] and Fe^{3+} [14] as well as the hepatic activity of catalase [15] were assessed in liver homogenates. Serum activities were determined for glutamate oxalacetate transaminase [16], glutamate pyruvate transaminase [17], glutamate dehydrogenase [18], alkaline phosphatase [19] and gamma-glutamyltransferase [20]. The determination of protein was performed according to the method of Lowry *et al.* [21], using crystalline human albumin as standard. The hepatic activity of δ-aminolevulinic acid synthetase was estimated in liver homogenates according to the method of Marver *et al.* [22]. Quantitative excretion of total urinary porphyrins was determined by the method of Doss and Schmidt [23]. Urinary δ-aminolevulinic acid and porphobilinogen were measured according to the method of Doss and Schmidt [24].

Statistical analysis. The results are expressed as means ± SEM, and the significances of the differences were assessed by the Wilcoxon test for unpaired samples.

RESULTS

Caloric intake and body weight

During the feeding period of 10 days the mean daily intake ($\bar{x}$ ± SEM) was 42.8 ± 0.1 ml of the control diet and 40.7 ± 0.5 ml of the alcohol diet, and the corresponding values of the treatment period for 60 days were 44.3 ± 0.1 ml and 44.2 ± 0.1 ml. The total alcohol intake was 18.4 ± 0.3 and 130.7 ± 0.2 g in animals on the alcohol diet for 10 and 60 days, respectively. Body weight gain was similar in all experimental groups treated for either 10 or 60 days.

Liver weight and protein

Compared to pair-fed controls, alcohol feeding to rats for 10 or 60 days resulted in similar liver weights whether expressed as total liver weight or per 100 g of body weight (Table 1). After feeding periods of 10 or 60 days the cytosolic protein content was similar in the experimental groups when the alcohol-fed animals were compared with their respective pair-fed controls. Hepatic microsomal protein content was significantly enhanced following alcohol treatment for either 10 or 60 days when compared to pair-fed controls.

Hepatic δ-aminolevulinic acid synthase

In comparison to pair-fed controls, alcohol consumption in rats resulted in a significant rise of the hepatic activity of δ-aminolevulinic acid synthase, whether the activity was expressed per g of liver protein, per g of liver wet weight or per 100 g of body weight (Table 2). This increase was much more pronounced following short-term feeding of the alcohol diet than after long-term consumption for 60 days.

Urinary excretion of porphyrins

After 10 days on the alcohol diet, the urinary excretion of the porphyrin precursors δ-aminolevulinic acid and of porphobilinogen was increased in the rats, but statistical significance was not achieved (Table 2). Urinary excretion of total porphyrins remained unchanged after 10 days of treatment with the alcohol diet in comparison with pair-fed controls. Following the treatment lasting 60 days with the alcohol diet, urinary excretion of δ-aminolevulinic acid and of porphobilinogen was enhanced, although the level of statistical significance could not be achieved for the latter parameter. Concomitantly, there was a 2.4-fold rise of urinary excretion of total porphyrins.

Hepatic alcohol metabolizing enzymes

Alcohol consumption in rats for 10 days caused significantly increased hepatic activities of the microsomal ethanol-oxidizing system and catalase when the activities were expressed per g of liver wet weight or per 100 g of body weight, whereas the hepatic activity of alcohol dehydrogenase remained virtually unchanged (Table 3). On the other hand, prolonged alcohol intake for 60 days led to a significantly decreased hepatic activity of alcohol dehydrogenase and an enhanced activity of the microsomal ethanol-oxidizing system, whereas the catalase activity only showed a marginal increase.

Table 2. Effect of alcohol feeding for 10 or 60 days on hepatic δ-aminolevulinic acid synthase and urinary excretion of porphyrins. For experimental details see legend to Table 1

	10 days treatment		60 days treatment	
	Control	Alcohol	Control	Alcohol
Hepatic δ-aminolevulinic acid synthase				
(nmoles/hr/g liver)	36.6 ± 6.3	223.3 ± 21.4†	50.0 ± 2.3	125.7 ± 14.7†
(nmoles/hr/100 g b.w.)	141.2 ± 6.4	717.6 ± 66.7†	112.3 ± 19.6	362.8 ± 42.5†
Urinary δ-aminolevulinic acid (nmoles/24 hr)	55.4 ± 25.2	79.0 ± 17.7	64.8 ± 11.8	130.8 ± 22.4
Urinary porphobilinogen (nmoles/24 hr)	32.7 ± 4.4	57.9 ± 7.3	33.1 ± 6.8	44.0 ± 4.1
Urinary total porphyrins (nmoles/24 hr)	1.8 ± 0.5	1.9 ± 0.3	1.2 ± 0.2	2.9 ± 0.5*

* $P < 0.05$.
† $P < 0.01$.

Table 3. Effect of alcohol feeding for 10 or 60 days on the activities of hepatic alcohol metabolizing enzymes. For experimental details see legend to Table 1

	10 days treatment		60 days treatment	
	Control	Alcohol	Control	Alcohol
Alcohol dehydrogenase				
(nmoles NADH/min/mg cytosolic protein)	3.9 ± 0.2	3.6 ± 0.2*	3.0 ± 0.1	2.6 ± 0.1
(nmoles NADH/min/g liver)	295.8 ± 17.5	257.2 ± 13.5	226.2 ± 8.0	194.5 ± 7.5*
(nmoles NADH/min/100 g b.w.)	915.5 ± 42.9	876.9 ± 66.6	641.2 ± 19.1	552.0 ± 18.0†
Microsomal ethanol-oxidizing system				
(nmoles acetaldehyde/min/mg microsomal protein)	8.5 ± 0.3	15.4 ± 0.7†	8.0 ± 0.4	17.6 ± 5.6†
(nmoles acetaldehyde/min/g liver)	352.1 ± 18.9	495.6 ± 52.6*	322.8 ± 17.8	798.9 ± 76.0†
(nmoles acetaldehyde/min/100 g b.w.)	1095.3 ± 42.3	1699.4 ± 202†	949.5 ± 41.1	2010.6 ± 144.3†
Catalase				
($U \times 10^3$/g liver)	1.8 ± 0.2	2.7 ± 0.3*	2.1 ± 0.2	2.8 ± 0.3
($U \times 10^3$/100 g b.w.)	5.6 ± 0.6	7.5 ± 0.6	5.9 ± 0.7	7.9 ± 0.8

* $P < 0.05$.
† $P < 0.01$.

Hepatic microsomal components and enzymes

Hepatic microsomal parameters such as cytochrome P-450 content, 7-ethoxycoumarin deethylase activity and arylhydrocarbon hydroxylase activity were strikingly enhanced in rats following both short-term treatment with alcohol containing diets for 10 days as well as prolonged administration for 60 days (Table 4). Moreover, increased hepatic activities of microsomal NADPH-cytochrome *c* reductase were only observed after long-term treatment with the alcohol diet for 60 days but not following a 10 days lasting application.

Hepatic iron

The hepatic content of iron (Fe^{3+}) was significantly enhanced in rats fed the alcohol diet for 10 days compared to their pair-fed controls, when the content was expressed per g of liver wet weight or per 100 g of body weight (Table 5). After long-term treatment with the alcohol diet for 60 days there were no significant changes.

Serum enzymes

The serum activities of glutamate pyruvate transaminase, alkaline phosphatase and γ-glutamyltransferase were significantly increased after 10 and 60 days of alcohol treatment, whereas no increase or only a marginal enhancement was observed for glutamate oxalacetate transaminase and glutamate dehydrogenase (Table 6).

DISCUSSION

This report shows the influence of prolonged and standardized alcohol feeding on various aspects of hepatic heme and porphyrin metabolism. The animals were fed a nutritionally adequate liquid diet for a total of 60 days in which the alcohol formula contained 36% of total calories as ethanol. In previous experimental studies with alcohol administered to rats for up to 30 months as 5%, 10% or 15% solution as drinking water, urinary porphyrin excretion has been found to remain unaltered [25],

Table 4. Effect of alcohol feeding for 10 or 60 days on hepatic microsomal parameters. For experimental details see legend to Table 1

	10 days treatment		60 days treatment	
	Control	Alcohol	Control	Alcohol
Cytochrome P-450				
(nmoles/mg microsomal protein)	0.4 ± 0.1	0.9 ± 0.1†	0.6 ± 0.1	1.1 ± 0.1†
(nmoles/g liver)	17.7 ± 2.9	24.2 ± 3.1	22.1 ± 1.6	46.6 ± 3.7†
(nmoles/100 g b.w.)	35.3 ± 2.4	70.1 ± 3.2†	39.1 ± 1.0	83.9 ± 1.0†
NADPH-cytochrome *c* reductase				
(nmoles/min/mg microsomal protein)	122.7 ± 7.7	139.2 ± 3.1	115.5 ± 7.5	151.0 ± 11.8*
(nmoles/min/g liver)	5377.5 ± 487.9	4948.0 ± 336.5	4356.0 ± 225.1	6419.2 ± 728.4
(nmoles/min/100 g b.w.)	16391.3 ± 1180.3	16775.8 ± 1669.8	12380.0 ± 647.4	18438.0 ± 2159.7
7-Ethoxycoumarin deethylase				
(nmoles/min/mg microsomal protein)	0.6 ± 0.1	2.1 ± 0.3†	0.8 ± 0.1	1.8 ± 0.2†
(nmoles/min/g liver)	18.9 ± 0.9	54.7 ± 6.0†	13.3 ± 0.7	45.1 ± 4.3†
(nmoles/min/100 g b.w.)	59.0 ± 2.6	168.5 ± 16.1†	45.8 ± 1.6	142.7 ± 12.5†
Arylhydrocarbon hydroxylase				
(nmoles/20 min/mg microsomal protein)	4.3 ± 0.4	6.2 ± 0.7	6.4 ± 0.3	8.1 ± 0.4†
(nmoles/20 min/g liver)	93.3 ± 7.9	148.3 ± 17.9†	149.8 ± 11.4	229.0 ± 15.0†
(nmoles/20 min/100 g b.w.)	326.8 ± 32.9	474.4 ± 44.7	421.1 ± 28.9	658.6 ± 45.8†

* $P < 0.05$.
† $P < 0.01$.

Table 5. Effect of alcohol feeding for 10 or 60 days on the hepatic iron content. For experimental details see legend to Table 1

	10 days treatment		60 days treatment	
	Control	Alcohol	Control	Alcohol
Fe^{3+} (mg/g liver)	0.4 ± 0.1	0.6 ± 0.1*	0.9 ± 0.1	0.8 ± 0.1
(mg/100 g b.w.)	1.4 ± 0.1	2.0 ± 0.1*	2.7 ± 0.3	2.3 ± 0.1

* $P < 0.05$.

whereas the administration of alcohol as part of a nutritionally adequate liquid diet for 24 days has been shown before to cause some increase of urinary coproporphyrin excretion and a reduction of uroporphyrinogen decarboxylase activity in the liver and spleen [26].

In the present experimental study changes of hepatic heme and porphyrin metabolism were observed after 60 but not after 10 days of alcohol feeding as evidenced by a variety of altered parameters including increased urinary excretion of total porphyrins (Table 2). These alterations were associated with a striking rise of urinary δ-aminolevulinic acid excretion, a marginal enhancement of urinary porphobilinogen excretion, and a pronounced increase of hepatic δ-aminolevulinic acid synthase activity. Similar but much more striking changes have been found in patients with chronic hepatic porphyria [2, 5, 27–31]. Human chronic hepatic porphyria may show variable biochemical and clinical expressions according to its particular stage [2, 5, 28–31], and it appears from the present results that feeding of an alcohol containing diet for as long as 60 days is not a suitable model for chronic hepatic porphyria comparable to the human disease.

The primary compound initiating the development of chronic hepatic porphyria appears to be the heme which may be decreased at least transiently in the liver cell as a consequence of alcohol treatment [32]. Hepatocytes contain high levels of mitochondrial, microsomal and cytosolic hemoproteins that exhibit turnover rates ranging from a few hours to about a week [33]. The hepatic hemoproteins cytochrome P-450 and catalase together utilize about two-thirds of the heme produced in the liver, and heme requirements are transiently increased during substrate-mediated induction of cytochrome P-450 [33]. An increased hepatic content of cytochrome P-450 has been found following an alcohol treatment for 10 and 60 days (Table 4) as well as after intermediate periods [34] due to the appearance of a specific ethanol-inducible form of cytochrome P-450 [35]. Concomitantly, there was a marginal increase of hepatic catalase activity (Table 3) and a significantly increased hepatic content of cytochrome b_5 [36]. These data therefore suggest an increased heme requirement in the liver cell for the enhanced synthesis of hemoproteins after prolonged alcohol consumption and explain the augmented hepatic activity of δ-aminolevulinic acid synthase (Table 2), an enzyme which is under negative feedback control by the heme and regulates the heme and porphyrin synthesis [2, 33]. The enhanced activity of the hepatic δ-aminolevulinic acid synthase (Table 2) in turn may

Table 6. Effect of alcohol feeding for 10 or 60 days on the activities of serum enzymes. For experimental details see legend to Table 1

	10 days treatment		60 days treatment	
	Control	Alcohol	Control	Alcohol
Glutamate oxalacetate transaminase (U/l serum)	110.5 ± 10.4	113.3 ± 4.7	110.2 ± 11.0	123.7 ± 13.7
Glutamate pyruvate transaminase (U/l serum)	27.6 ± 2.1	35.7 ± 2.3*	31.6 ± 2.7	50.9 ± 5.8*
Glutamate dehydrogenase (U/l serum)	3.6 ± 0.6	4.5 ± 0.5	2.9 ± 0.4	4.0 ± 0.7
Alkaline phosphatase (U/l serum)	84.4 ± 5.9	121.4 ± 12.6*	62.3 ± 5.2	106.7 ± 11.6†
Gamma-glutamyltransferase (U/l serum)	0.1 ± 0.1	0.9 ± 0.2†	0.1 ± 0.1	0.4 ± 0.1†

* $P < 0.05$.
† $P < 0.01$.

causally be related to the increased urinary excretion of porphyrins found after alcohol treatment for 60 days (Table 2).

Although a variety of other enzymes involved in heme and porphyrin metabolism including δ-aminolevulinic acid dehydratase and uroporphyrinogen synthase are altered in their activities by alcohol [2], these changes appear to play no role for the development of chronic hepatic porphyria and, hence, additional mechanisms have to be incriminated. Among these are the reduction of the hepatic uroporphyrinogen decarboxylase activity in experimental and human chronic hepatic porphyria [2, 28] and following prolonged alcohol consumption [26]. Additional pathogenic factors for the observed changes of hepatic heme and porphyrin metabolism include the presence of liver injury as evidenced by the changes of serum enzyme activities (Table 6). Moreover, the enhancement of the microsomal content of cytochrome P-450 is associated with increased activities of microsomal enzymes (Table 4) including the microsomal ethanol-oxidizing system (Table 4). This leads to an increased production of the toxic acetaldehyde which might cause alterations of the heme and porphyrin metabolism. It is known that the induction of δ-aminolevulinic acid synthase activity by an acute alcohol administration is completely blocked by inhibition of the alcohol metabolism, suggesting that metabolites of alcohol or redox changes are more essential than alcohol itself [4]. The present study also shows that an excess of the hepatic iron content is not essential for the observed changes of hepatic heme and porphyrin metabolism since it was found unaltered after 60 days of alcohol treatment (Table 5).

In conclusion, alcohol feeding for 60 days results in various changes of hepatic heme and porphyrin metabolism. These alterations are less pronounced compared to those found in patients with chronic hepatic porphyria, and it appears that experimental alcohol feeding for 60 days is not a suitable animal model for human chronic hepatic porphyria.

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