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HEPATIC ALCOHOL METABOLIZING ENZYMES AFTER PROLONGED ADMINISTRATION OF SEX HORMONES AND ALCOHOL IN FEMALE RATS

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Abstract—To study the effect of sex hormones and alcohol on the hepatic activities of alcohol metabolizing enzymes, estradiol or testosterone were administered for 4 weeks to ovarectomized or sham operated adult female rats pair-fed nutritionally adequate liquid diets containing either alcohol (36% of total calories) or isocalorically replaced carbohydrates. Estradiol increased the hepatic activities of alcohol dehydrogenase and catalase in both ovarectomized and sham operated female rats on the control diet, whereas this enhancing property was virtually lost in animals on the alcohol diet. The hepatic activities of the microsomal ethanol-oxidizing system remained unaffected under these experimental conditions irrespective of the diet used. Testosterone increased the hepatic activities of the microsomal ethanol-oxidizing system and of catalase and decreased the alcohol dehydrogenase activity in female rats on the control diet, but these changes were either not reproducible or markedly reduced in similarly treated female rats fed the alcohol diet. Thus, sex hormones may strikingly influence the hepatic activities of alcohol metabolizing enzymes, but the changes are modulated by prolonged alcohol consumption.

Disturbances of sex hormone production and metabolism are common features after prolonged alcohol consumption both in man [1–4] and experimental animals [5–10]. These changes occur in males [1–6, 8, 10] as well as in females [1–4, 7, 9] and develop within a few weeks [5–10]. It is well established that sex hormones may profoundly influence the hepatic activities of alcohol metabolizing enzymes [11–18] which in turn are involved in the pathogenesis of reversible and irreversible lesions in a variety of organs [4, 19] including the liver [19, 20]. On the other hand, the hepatic activities of alcohol metabolizing enzymes may also be influenced by chronic alcohol consumption [21–23].

It is unknown to what extent the hepatic activities of alcohol metabolizing enzymes are influenced by the combination of prolonged alcohol consumption and the application of sex hormones. The present study was therefore undertaken to evaluate the effect of sex hormones, ovarectomy and alcohol consumption either as single or combined factors on the hepatic activities of the microsomal ethanol-oxidizing system, alcohol dehydrogenase and catalase in female rats.

MATERIALS AND METHODS

Materials. The enzymes and chemicals were obtained from the following sources: estradiol benzoate (Progynon® B oleosum) and testosterone (Testoviron® Depot) from Schering Corp. (Berlin, F.R.G.); rhizinus oil, sesame oil and benzylbenzoate from Caesar & Loretz Corp. (Hilden); ether pro narcosi from Hoechst Corp. (Frankfurt); human albumin from Behring Corp. (Marburg); NAD^+ (grade II), NADH (grade II), $NADP^+$-disodium salt, and isocitric dehydrogenase (grade II) from Boehringer Corp. (Mannheim); ethanol absolute, acetaldehyde, semicarbazide hydrochloride, sodium azide, sodium dithionate, and trichloroacetic acid from Merck Corp. (Darmstadt); disodium-EDTA and DL-sodium isocitrate from Serva Corp. (Heidelberg).

Animals. Mature female Lewis rats with an age of 12–14 weeks were purchased from the Zentralinstitut für Versuchstierzucht (Hannover). The animals were fed Altromin® chow and tap water *ad libitum*.

Operative procedure. When indicated, female rats were ovarectomized under light ether anaesthesia via a medial abdominal incision. After ligation of the uterine hornes on both sides, the ovaries and distal fallopian tubes were *en bloc* removed, and the abdominal wall was subsequently closed in two layers with single stiches. All other animals not ovarectomized were sham operated.

Dietary regimen. The animals were housed after surgery in individual cages and allowed to recuperate for 1 week. They were then pair-fed for 4 weeks nutritionally adequate liquid diets containing either ethanol (36% of total calories) as the alcohol diet or isocalorically replaced carbohydrates as the control diet. The two liquid diets were prepared and administered as described by DeCarli and Lieber [24]. The control diet contained protein (18% of total calories), fat (35%), dextrine-maltose as carbohydrates (47%), vitamins and trace elements.

Treatment by sex hormones. For treatment with estradiol, Progynon® B oleosum was used in a mixture of rhizinus oil and benzylbenzoate (5:4, v/v) to achieve an estradiol concentration of 150 μg/ml.

* Deceased 2 November 1982.

Estradiol in rhizinus oil and benzylbenzoate was administered at a dose of 15 μg/100 g of body weight once a week for 4 weeks by subcutaneous injection, starting at the first day on the liquid diet. Animals not treated with estradiol received the vehicle alone. When indicated, the animals received testosterone as Testoviron® Depot which contains 20 mg/ml of testosterone propionate as a short acting derivative and 55 mg/ml of pure testosterone. Testoviron® Depot was diluted with sesame oil to achieve a testosterone concentration of 25 mg/ml. Testosterone in sesame oil was applied at a dose of 2.5 mg/100 g of body weight once a week for a total of 4 weeks by subcutaneous injection. The hormonal treatment was started at the first day on the liquid diet. Animals not treated with testosterone received the vehicle only.

Biochemical determinations. Twenty-four hours after the last hormonal treatment, the animals were killed, and their livers were perfused *in situ* with ice-cold 0.15 mol/l KCl, excised, chilled and homogenized in 3 vol. of 0.15 mol/l KCl to obtain a 25% homogenate. The hepatic catalatic activity of catalase was assessed in the 25% liver homogenate [25]. To obtain the cytosolic and microsomal fractions of the hepatocytes, the 25% liver homogenate was centrifuged at 10,000 *g* for 30 min. The pellet was discarded, and the supernatant was spun at 105,000 *g* for 30 min. The resulting supernatant corresponds to the cytosolic fraction of the hepatocytes and was used for the determination of alcohol dehydrogenase activity [26, 27]. The resulting pellet was resuspended in 0.15 mol/l KCl and centrifuged again at 105,000 *g* for 30 min. The pellet obtained after the latter procedure was resuspended and consisted of washed microsomes which were used for the determination of the activity of the microsomal ethanol-oxidizing system (MEOS) [27]. Microsomal losses during the preparative procedures were corrected as described previously [22]. Protein determination was performed according to the method of Lowry *et al.* [28] using crystalline human albumin as standard.

Statistical analysis. All measurements were carried out in duplicate. Each individual result was compared to the value of its corresponding control, and the means ± S.E.M. and individual differences were calculated. The significances were assessed by the Student's *t*-test.

RESULTS

Dietary intake and body weight

All experimental groups had a similar total dietary intake with respect to volume of either the control or the alcohol diet (Table 1). Since 1 ml of each of the diets contained 1 kcal, the total caloric intake was also similar. Moreover, total ethanol intake as well as average daily ethanol intake were similar in all experimental groups fed the alcohol diet.

The administration of either the control or the alcohol diet for 4 weeks left the body weights virtually unchanged (Table 2). This was also the case for animals which were treated by ovarectomy, estradiol, or the combination of both, irrespective whether the animals were fed the control or the alcohol diet. The administration of testosterone to

Table 1. Intake of diet and ethanol during 4 weeks of various treatments

Ovarectomy	Estradiol	Testosterone	Total dietary intake (ml)		Average daily dietary intake (ml)		Total ethanol intake (g)		Average daily ethanol intake (g)	
			control diet	alcohol diet	control diet	alcohol diet	control diet	alcohol diet	control diet	alcohol diet
−	−	−	1376 ± 24	1295 ± 64	49 ± 0.9	48 ± 1.1	—	59.1 ± 2.5	—	2.10 ± 0.10
+	−	−	1395 ± 39	1335 ± 47	49 ± 1.4	49 ± 1.6	—	61.3 ± 2.0	—	2.10 ± 0.07
−	+	−	1348 ± 12	1306 ± 36	48 ± 0.4	48 ± 1.0	—	61.1 ± 1.4	—	2.20 ± 0.05
−	−	+	1410 ± 34	1334 ± 60	50 ± 1.2	49 ± 1.5	—	66.9 ± 0.6	—	2.20 ± 0.10
+	+	−	1363 ± 23	1306 ± 56	49 ± 0.8	48 ± 0.9	—	61.7 ± 2.1	—	2.20 ± 0.08
+	−	+	1461 ± 38	1396 ± 61	52 ± 1.3	50 ± 2.0	—	64.3 ± 2.6	—	2.30 ± 0.09

Each experimental group consisted of 8 animals. The data are given as means ± S.E.M.

Table 2. Body weight during 4 weeks of various treatments

			Body weight (g)									
			at start		after 1 week		after 2 weeks		after 3 weeks		after 4 weeks	
Ovarectomy	Estradiol	Testosterone	control diet	alcohol diet	control diet	alcohol diet	control diet	alcohol diet	control diet	alcohol diet	control diet	alcohol diet
−	−	−	195 ± 1.2	207 ± 1.7	198 ± 2.0	196 ± 4.6	203 ± 2.6	198 ± 5.9	205 ± 1.7	194 ± 6.9	195 ± 2.4	204 ± 4.9
+	−	−	202 ± 1.5	204 ± 3.1	197 ± 5.2	203 ± 2.6	212 ± 4.2	206 ± 5.0	221 ± 4.0	206 ± 4.6	207 ± 2.6	211 ± 5.3
−	+	−	185 ± 0.1	212 ± 2.6	191 ± 1.4	194 ± 3.8	196 ± 3.2	196 ± 5.3	193 ± 2.8	197 ± 3.3	192 ± 1.9	200 ± 4.9
−	−	+	177 ± 1.0	202 ± 1.7	182 ± 1.0	197 ± 3.0	211 ± 2.2	207 ± 4.7	213 ± 2.6	218 ± 4.7	216 ± 2.2	214 ± 4.9
+	+	−	194 ± 3.2	209 ± 6.8	200 ± 2.9	192 ± 5.9	200 ± 2.9	194 ± 7.7	197 ± 1.5	191 ± 6.7	197 ± 2.3	197 ± 6.8
+	−	+	184 ± 1.0	206 ± 2.6	190 ± 1.2	204 ± 5.8	215 ± 3.3	212 ± 6.0	221 ± 3.2	216 ± 8.6	223 ± 3.7	219 ± 4.2

Each experimental group consisted of 8 animals. The data are given as means ± S.E.M.

Table 3. Liver weight and protein content after 4 weeks of various treatments

			Liver weight (g)		Liver weight (g/100 g body weight)		Liver protein (mg/g liver)		Liver protein (mg/100 g body weight)	
Ovarectomy	Estradiol	Testosterone	control diet	alcohol diet	control diet	alcohol diet	control diet	alcohol diet	control diet	alcohol diet
−	−	−	6.0 ± 0.1	7.0 ± 0.2	2.9 ± 0.08	3.4 ± 0.07	68.0 ± 2.5	64.0 ± 4.7	207.9 ± 6.3	220.4 ± 16.5
+	−	−	5.2 ± 0.2	7.3 ± 0.2	2.5 ± 0.08	3.5 ± 0.12	66.0 ± 2.3	62.0 ± 3.3	167.7 ± 10.1	209.5 ± 8.2
−	+	−	6.6 ± 0.2	7.6 ± 0.3	3.4 ± 0.07	3.9 ± 0.16	67.7 ± 3.2	55.9 ± 2.5	231.4 ± 13.2	211.4 ± 11.3
−	−	+	6.3 ± 0.2	7.4 ± 0.2	2.9 ± 0.09	3.5 ± 0.14	59.3 ± 1.5	56.3 ± 3.3	171.8 ± 4.0	197.2 ± 13.5
+	+	−	6.8 ± 0.1	7.3 ± 0.3	3.4 ± 0.07	3.7 ± 0.08	60.7 ± 2.4	58.7 ± 1.9	207.9 ± 6.5	216.8 ± 7.2
+	−	+	6.6 ± 0.2	8.0 ± 0.4	3.0 ± 0.05	3.7 ± 0.16	63.3 ± 1.5	64.5 ± 3.8	187.2 ± 4.5	233.9 ± 12.3

Each experimental group consisted of 8 animals. The data are given as means ± S.E.M.

ovarectomized or sham operated animals led to a body weight gain which was much more pronounced in animals fed the control diet compared to those on the alcohol diet.

Liver weight and liver protein

Total liver weight varied somewhat between each of the experimental groups (Table 3). When the liver weights were expressed per 100 g of body weight, the values were higher for the animals fed the alcohol diet compared to those on the control diet, and this difference persisted independently of additional treatments such as ovarectomy, estradiol, testosterone or combinations thereof. However, the most striking difference in body weight gain was observed in ovarectomized animals fed the control diet compared to similarly treated animals on the alcohol diet.

When expressed per 100 g of body weight, the hepatic protein content was higher after alcohol feeding compared to pair-fed controls, even when additionally treated by ovarectomy, testosterone or combinations thereof, whereas the reversed constellation was found after treatment by estradiol alone (Table 3). The lowest hepatic protein content per 100 g of body weight was observed in ovarectomized animals fed the control diet and the highest values in ovarectomized and testosterone supplemented animals fed the alcohol diet.

Microsomal ethanol-oxidizing system (MEOS)

Compared to pair-fed controls, the administration of the alcohol diet for 4 weeks to female rats resulted in a significant increase of the hepatic MEOS activity, and this enhancement persisted even after various treatments such as ovarectomy and the administration of either estradiol or testosterone (Table 4).

In comparison to animals fed the respective liquid diet, ovarectomy had no significant effect on the hepatic MEOS activity in animals fed the control diet but increased the enzymic activity in alcohol-fed rats when expressed per g of liver wet weight or per 100 g of body weight (Table 4). Estradiol administration left the hepatic MEOS activity virtually unchanged in female rats fed either the control or alcohol diet, but it prevented the increase of MEOS activity observed after ovarectomy and prolonged alcohol feeding when the enzymic activity was expressed per g of liver wet weight or per 100 g of body weight. Testosterone application showed a marginal increase of hepatic MEOS activity per 100 g of body weight in the female rats fed the control diet but it was without effect in animals on the alcohol diet. A striking enhancement of hepatic MEOS activity was observed after the combined treatment with ovarectomy and testosterone in female rats fed either the control or the alcohol diet.

Alcohol dehydrogenase (ADH)

Hepatic ADH activity was increased after prolonged alcohol intake, and this enhancement was not abolished by ovarectomy or sex hormone administration (Table 5).

Ovarectomy strikingly reduced the hepatic ADH activity in female rats fed the control diet without altering enzymic activity in alcohol-fed animals

Table 4. Hepatic microsomal ethanol-oxidizing system activity after 4 weeks of various treatments

			Microsomal ethanol-oxidizing system					
			nmoles/min/mg microsomal protein		nmoles/min/g liver		nmoles/min/100 g body weight	
Ovarectomy	Estradiol	Testosterone	control diet	alcohol diet	control diet	alcohol diet	control diet	alcohol diet
−	−	−	7.3 ± 0.4	16.4 ± 1.8****	125.1 ± 13.6	268.0 ± 24.2****	369.6 ± 44.6	921.6 ± 92.2****
+	−	−	9.4 ± 0.9	17.3 ± 0.7****	145.4 ± 19.5	374.6 ± 33.9****	353.9 ± 38.5	1355.3 ± 170.3****
−	+	−	6.1 ± 0.2	15.2 ± 1.3****	120.7 ± 5.9	203.6 ± 18.5***	410.4 ± 13.5	734.0 ± 81.0***
−	−	+	8.3 ± 0.3	17.2 ± 2.1***	157.0 ± 14.0	250.5 ± 29.5**	460.7 ± 52.9	943.0 ± 141.3**
+	+	−	6.1 ± 0.3	17.1 ± 1.3****	135.4 ± 12.0	263.8 ± 29.4***	465.4 ± 45.0	955.8 ± 104.1***
+	−	+	8.6 ± 0.3	20.8 ± 2.3****	169.3 ± 6.7	348.4 ± 50.6***	500.4 ± 19.9	1383.4 ± 238.8***

Each experimental group consisted of 8 animals. The data are given as means ± S.E.M. The statistical symbols refer to the respective group fed the control diet: *not significant, **$P < 0.05$; ***$P < 0.01$; ****$P < 0.001$.

Table 5. Hepatic alcohol dehydrogenase activity after 4 weeks of various treatments

Ovarectomy	Estradiol	Testosterone	Alcohol dehydrogenase					
			nmoles/min/mg cytosolic protein		nmoles/min/g liver		nmoles/min/100 g body weight	
			control diet	alcohol diet	control diet	alcohol diet	control diet	alcohol diet
−	−	−	12.4 ± 3.7	29.2 ± 4.0***	243.9 ± 80.5	494.7 ± 69.0**	708.6 ± 227.1	1718.8 ± 244.8**
+	−	−	6.9 ± 0.6	34.9 ± 5.9****	108.3 ± 14.1	537.0 ± 79.4****	273.0 ± 45.5	1841.3 ± 271.3****
+	+	−	17.6 ± 1.0	30.0 ± 3.3**	342.2 ± 10.9	490.5 ± 57.4**	1166.3 ± 29.8	1937.3 ± 311.7**
−	−	+	3.1 ± 0.5	21.9 ± 4.2***	56.8 ± 8.7	343.8 ± 67.4***	161.6 ± 21.4	1236.0 ± 263.8***
+	+	−	18.7 ± 1.1	29.2 ± 3.9**	340.3 ± 17.1	516.0 ± 70.9**	1171.0 ± 71.3	1966.3 ± 309.8**
+	−	+	2.6 ± 0.4	20.7 ± 3.7****	51.9 ± 8.7	342.8 ± 67.1***	151.7 ± 24.3	1265.4 ± 257.1***

Each experimental group consisted of 8 animals. The data are given as means ± S.E.M. The statistical symbols refer to the respective group fed the control diet: *not significant; **$P < 0.05$; ***$P < 0.01$; ****$P < 0.001$.

(Table 5). The application of estradiol increased the hepatic ADH activities only in female rats fed the control diet but it was without effect in animals on the alcohol diet, and this was also the case for the combined treatment consisting of ovarectomy and estradiol administration. Moreover, after administration of testosterone the hepatic ADH activity was much more reduced in animals fed the control diet compared to those on the alcohol diet, and similar results were obtained when the animals were additionally pretreated by ovarectomy plus testosterone.

Catalase

Prolonged alcohol consumption resulted in a significant rise of the hepatic catalatic activity of catalase in female rats, and this difference also persisted in part after ovarectomy or sex hormone administration (Table 6).

Ovarectomy left the hepatic catalase activities virtually unchanged in alcohol-fed animals and in their pair-fed controls compared to rats fed the respective diet alone (Table 6). Estradiol increased the hepatic catalase activity expressed per 100 g of body weight only in animals fed the control diet but not in those on the alcohol diet, and this was also the case after the combined treatment consisting of ovarectomy plus estradiol administration. Finally, testosterone had a striking enhancing effect on the hepatic catalase activity only in female rats fed the control diet, and this enhancement persisted when ovarectomy was additionally performed. On the other hand, in alcohol-fed female rats testosterone alone had no significant effect on the hepatic catalase activity and some enhancing effect when ovarectomy was additionally performed.

DISCUSSION

The present study clarifies the respective roles of sex hormones as well as of prolonged alcohol consumption for the hepatic activities of alcohol metabolizing enzymes in female rats. In particular, enzymic activities may be influenced by female and male sex hormones as well as by ovarectomy, and the degree of the observed changes may depend upon whether a control or an alcohol diet was administered (Tables 4–6).

In agreement with previous studies [21–23], prolonged alcohol consumption leads to increased hepatic activities of MEOS (Table 4). Compared to controls, prolonged alcohol consumption also resulted in enhanced hepatic ADH activities in the present study (Table 5) as previously reported [14, 29, 30], although also no change [21, 23, 31–34] or even decreased activities have been reported [21, 35, 36]. These differing results may be explained by differences in experimental conditions including strain of animals used and technique of alcohol feeding. This also applies to hepatic catalase activities which were found to be increased after prolonged alcohol consumption in the present study (Table 6) and in another report [37], whereas also no changes have been published [21, 23].

Ovarectomy alone had little if any effect on the hepatic activities of alcohol metabolizing enzymes

Table 6. Hepatic catalase activity after 4 weeks of various treatments

Ovarectomy	Estradiol	Testosterone	Catalase			
			U × 10^3/g liver		U × 10^3/g liver protein	U × 10^3/100 g body weight
			control diet	alcohol diet	control diet	alcohol diet	control diet	alcohol diet
−	−	−	2.3 ± 0.3	4.1 ± 0.8**	35.1 ± 6.3	62.8 ± 12.3**	6.6 ± 0.4	14.1 ± 2.8**
	−	−	2.6 ± 0.2	4.6 ± 0.6***	38.9 ± 2.3	75.3 ± 9.8***	6.5 ± 0.3	15.1 ± 1.9***
−	+	−	2.8 ± 0.2	3.9 ± 0.6**	41.1 ± 3.0	68.4 ± 9.3**	9.4 ± 1.8	14.8 ± 2.3**
−	−	+	5.9 ± 0.4	3.4 ± 0.4****	99.6 ± 5.1	60.3 ± 8.5***	17.2 ± 1.1	11.9 ± 1.3**
+	+	−	3.0 ± 0.2	4.1 ± 0.5**	48.8 ± 4.6	69.4 ± 6.3**	10.3 ± 0.6	15.3 ± 1.6**
+	−	+	5.6 ± 0.4	4.7 ± 0.6*	88.6 ± 5.0	74.0 ± 10.0*	16.6 ± 1.1	17.2 ± 2.3*

Each experimental group consisted of 8 animals. The data are given as means ± S.E.M. The statistical symbols refer to the respective group fed the control diet: *not significant; **$P < 0.05$; ***$P < 0.01$; ****$P < 0.001$.

per 100 g of body weight in adult female Lewis rats on either the control or alcohol diet (Tables 4–6) except for a striking increase of MEOS activity in alcohol-fed animals (Table 4) and a reduction of ADH activity in animals on the control diet (Table 5). Other studies using pre-pubertal female spontaneously hypertensive rats fed a diet without alcohol have shown that ovarectomy had no effect on the hepatic ADH activity [14], and similar data were obtained under these experimental conditions for female Sprague–Dawley rats [18].

Estradiol strikingly enhanced the hepatic activities of ADH (Table 5) and catalase (Table 6) expressed per 100 g of body weight after both ovarectomy or sham operation in female rats on the liquid control diet, whereas MEOS activity remained virtually unchanged under these experimental conditions (Table 4). An increased hepatic ADH activity by estradiol in the absence of treatment by alcohol containing diets was also observed in other studies with female Sprague–Dawley rats [18] but not with pre-pubertal spontaneously hypertensive female rats [14]. Of particular interest was the almost complete ineffectiveness of estradiol in altering hepatic activities of alcohol metabolizing enzymes under conditions of prolonged alcohol feeding as shown in this study (Tables 4–6). These data therefore indicate that female sex hormones such as estradiol may change the hepatic activities of some alcohol metabolizing enzymes in female rats under the control diet, whereas such an effect may be abolished by the administration of alcohol containing diets.

Testosterone was found to enhance hepatic MEOS (Table 4) and catalase (Table 6) activities per 100 g of body weight and to reduce ADH activity (Table 5) in female rats fed the control diet following either ovarectomy or sham operation, confirming other observations on ADH [14]. However, this study clearly shows that the effect of testosterone on the hepatic activities of alcohol metabolizing enzymes was much less pronounced in female rats fed the alcohol diet compared to equally treated animals on the control diet following either ovarectomy or sham operation (Tables 5 and 6) except for MEOS activity which was increased by testosterone plus ovarectomy in alcohol fed rats far above the activity level observed after the alcohol diet alone (Table 4). It therefore appears that the action of testosterone on the hepatic activities of alcohol metabolizing enzymes is strikingly influenced by concomitant alcohol consumption.

In conclusion, the results of this study show that the hepatic levels of alcohol metabolizing enzyme activities are greatly modulated by sex hormones such as estradiol and testosterone in female rats, and the degree and direction of the observed changes are influenced by prolonged alcohol consumption.

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