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EFFECT OF ETHANOL ADMINISTRATION ON THE ACTIVITY OF HEPATIC LYSOSOMAL ENZYMES*

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Abstract—The activities of hepatic lysosomal enzymes were determined in rats after the acute administration of ethanol and its feeding for periods of 1 and 6 months. The acute administration of ethanol and its feeding for 1 month did not result in any changes in lysosomal enzymes. By contrast the feeding of ethanol for a period of 6 months resulted in increases in the total activities of β-glucuronidase, β-*N*-acetylglucosaminidase and arylsulfatase A measured in the homogenates, and in the specific activities of these enzymes plus acid phosphatase measured in the lysosomal fraction. In addition, there were increases in the free activities of β-glucuronidase and acid phosphatase measured in the homogenates. Hyaluronidase activity was not changed by ethanol feeding. The increases in lysosomal enzymes were accompanied by histologic changes consisting only of mild fatty infiltration.

Alcohol has been demonstrated to cause liver damage in animals [1, 2] and in man [3]. The pathologic changes caused by alcohol in the liver in animals occur despite intake of an adequate diet, and include the whole spectrum of liver disease [1] observed in alcoholic patients. Tissue injury caused by toxins [4, 5], viruses [6] or ischemia [7] has been associated with disruption of lysosomal membranes, and the release of lysosomal enzymes. Studies of the effect of acute and short-term administration of ethanol on hepatic lysosomal enzymes have yielded varying results with increases [8], no change [8, 9] or decreases [10] in their total activity and increases [8, 9] or decreases [10] in their free activity. In the present study the effect of acute, short-term and prolonged chronic administration of ethanol on the total and free activity of hepatic lysosomal enzymes was investigated.

METHODS

Animals and diets

Male albino rats weighing between 90 and 110 g initially were kept in individual wire cages at a constant temperature. The following acute and chronic experiments were carried out.

Acute experiment. Sixteen rats were fed Purina Chow *ad lib.* Ethanol (4 g/kg of body weight) was administered twice, 8 hr apart, by stomach tube as a 40% solution to one-half the rats. The control rats were given isocaloric amounts of sucrose. The animals were sacrificed 24 hr after the first dose.

Chronic experiments. Thirty-two rats were fed a liquid diet in which either ethanol or sucrose made up 36% of the calories. The final caloric compositions per liter of the diets were: protein 19.5% (47.3 g), fat 39.4% (42.5 g), carbohydrate 5.0% (12.2 g), and either ethanol (50.0 g) or sucrose (87.5 g) 36.1%. Vitamins and minerals were present in adequate amounts.‡ The ethanol-containing diet was fed *ad lib.* to one-half the animals, while the remainder of the animals were pair-fed the sucrose-containing diet. In addition, the rats receiving the ethanol-containing diet were given a weekly acute oral load of ethanol of 4 g/kg of body weight given as a 40% solution by stomach tube, while the control rats received isocaloric amounts of sucrose. The mean intake of the diets was 32 ± 1.5 (S.E.) g/100 g of body weight/day. Animals on the ethanol diet consumed 15.4 ± 0.75 (S.E.) g ethanol/kg of body weight/day. One-half of the animals were sacrificed at 1 month, and the remainder at 6 months after the start of the pair-feeding.

Tissue preparation

The animals were sacrificed by a blow on the head after 14 hr of fasting. The livers were immediately removed, rinsed in cold 0.25 M sucrose and weighed. A small piece was fixed in 10% formaldehyde for histological examination and the remainder homogenized in a Potter–Elvehjem homogenizer in a volume of 0.25 M sucrose solution containing 1×10^3 M EDTA, pH 7.0, equivalent to four times the liver weight. Five ml of the homogenate were saved for the determination of total and free lysosomal enzyme activities, and the remainder subjected to differential centrifugation at 4°, using a Sorvall refrigerated centrifuge, according to Sawant *et al.* [11] to obtain the lysosomal-rich light mitochondrial fraction (FI). The homogenate was centrifuged at 750 *g* for 10 min, and the resulting supernatant was centrifuged at 3,300 *g* for 10 min, and again at 16,300 *g* for 20 min. The resulting precipitate was resuspended in 5 ml of 0.15 N NaCl. Three ml were separated, sonicated (three times for 30 sec), and used for the determination of lysosomal enzymes. The remaining 2 ml were

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‡ Same as the composition used in the high alcohol liquid diet of General Biochemicals, Chagrin Falls, Ohio.

centrifuged at 16,300 *g* for 20 min. The precipitate was separated and washed with 5 ml of 0.15 N NaCl and recentrifuged at 16,300 *g* for 20 min, twice. The final precipitate dissolved in 5 ml of 0.15 M NaCl was sonicated (three times for 30 sec), centrifuged at 27,000 *g* for 20 min and the supernatant recovered for the determination of hyaluronidase activity.

Enzyme assays

The activities of β-glucuronidase, β-*N*-acetylglucosaminidase, acid phosphatase and arylsulfatase A were determined in the homogenates, and in the lysosomes. The determinations in the homogenates were done in the presence and absence of 0.2% Triton X-100 to obtain total and free activities, while the determinations in the lysosomes were done only in the presence of 0.2% Triton X-100. Hyaluronidase activity was assayed only in the lysosomes after repeated washings with 0.15 N NaCl and in the presence of 0.2% Triton X-100.

β-Glucuronidase was assayed by the method of Gianetto and De Duve [12] with phenolphthalein glucuronide as a substrate. β-*N*-acetylglucosaminidase was determined as described by Walker *et al.* [13] using *p*-nitrophenyl *N*-acetyl-β-glucosamine as a substrate. Acid phosphatase was determined according to the method of Appelmans *et al.* [14] with sodium β-glycerophosphate as a substrate. Arylsulfatase A was assayed by the method of Roy [15]. Hyaluronidase activity was assayed by measuring the liberation of *N*-acetylglucosamine end-groups from hyaluronic acid according to the method of Hutterer [16]; the *N*-acetylglucosamine end-groups were measured by the method of Reissig *et al.* [17]. Protein concentration was determined by the method of Lowry *et al.* [18] with bovine serum albumin used as a standard.

Statistical analysis

The results are expressed as means ± S.E.M. The data were analyzed by the Student's *t*-test [19].

RESULTS

Acute experiment

The liver weights and the protein concentration in the lysosomes were similar in the ethanol and control rats. The acute administration of ethanol resulted in no significant changes in the total and free activities of the lysosomal enzymes measured in the liver homogenates (Table 1) or in their specific activity in the lysosomes (Table 2). No changes in liver histology were found by light microscopy after the acute administration of ethanol.

Chronic experiments

In the rats fed ethanol for a period of 1 month, the mean gain in body weight, the final liver weight and the lysosomal protein concentration were not different from the values obtained in the control animals (Table 3). The chronic administration of ethanol in the diet for 1 month resulted also in no significant changes in the total and free activities of the lysosomal enzymes determined in the homogenates (Table 4), or in their specific activity in the lysosomes (Table 5).

The average weight gain of the rats fed ethanol for a period of 6 months was significantly less than that of the control animals (Table 3). The final mean liver weight of the ethanol-fed rats was also less than that of the control animals ($P < 0.01$); however, no significant difference was found when the weights of the livers were expressed as a per cent of body weight. There were no differences in the concentration of protein in the lysosomes.

The total activities of β-glucuronidase, β-*N*-acetylglucosaminidase and arylsulfatase, but not of acid phosphatase, were increased by ethanol feeding for a period of 6 months (Table 4). The free activities of β-glucuronidase and acid phosphatase were also increased. Determination of the activity of the enzymes in the lysosomes revealed increases in the specific activities of β-glucuronidase, β-*N*-acetylglucosaminidase, acid phosphatase and arylsulfatase (Table 5). Hyaluronidase activity was not changed by ethanol feeding.

The livers of the animals fed ethanol for periods of 1 and 6 months were found to have variable amounts of small droplet fat infiltration on examination by light microscopy. No hyaline bodies, necrosis or fibrosis was found. No changes in liver histology were found in the control rats.

Table 1. Effect of acute ethanol administration on the total and free activities of lysosomal enzymes in liver homogenates*

Determination		Lysosomal enzyme activity (μmoles/g liver/min)	
		Control	Ethanol
β-Glucuronidase	T†	0.57 ± 0.035	0.52 ± 0.101
	F	0.12 ± 0.021	0.15 ± 0.012
	F/T (%)	21.6 ± 1.4	27.3 ± 2.3
β-*N*-acetylglucosaminidase	T	3.56 ± 0.250	3.42 ± 0.306
	F	2.94 ± 0.225	2.62 ± 0.305
	F/T (%)	73.0 ± 4.73	73.1 ± 4.23
Acid phosphatase	T	5.35 ± 0.590	3.87 ± 0.483
	F	1.84 ± 0.270	1.47 ± 0.302
	F/T (%)	32.6 ± 1.77	35.6 ± 4.19
Arysulfatase A	T	3.15 ± 0.463	3.21 ± 0.475
	F	1.86 ± 0.377	1.74 ± 0.062
	F/T (%)	56.4 ± 3.55	54.1 ± 5.18

* All values are expressed as means ± S.E.M. The differences in values are not statistically significant.

Table 2. Effect of acute ethanol administration on the specific activity of lysosomal enzymes*

Determination	Lysosomal enzyme activity (nmoles/mg protein/min) Control	Ethanol
β-Glucuronidase	28.7 ± 1.32	32.0 ± 3.31
β-N-acetylglucosaminidase	459.2 ± 75.50	436.7 ± 80.95
Acid phosphatase	459.7 ± 24.6	496.4 ± 24.6
Arylsulfatase A	271.5 ± 54.2	366.5 ± 59.0
Hyaluronidase	76.4 ± 33.9	42.4 ± 11.5

* All values are expressed as means ± S.E.M. of eight animals. The differences in values are not statistically significant.

Table 3. Effect of chronic ethanol feeding on weight gain, liver weight and lysosomal protein concentration*

Determination	One month Control	One month Ethanol	Six months Control	Six months Ethanol
Weight gain (g/day)	1.34 ± 0.020	1.22 ± 0.015	1.20 ± 0.052	0.98 ± 0.030†
Liver weight (g)	5.5 ± 0.25	5.8 ± 0.25	8.4 ± 0.37	6.9 ± 0.21†
(g/100 g body wt)	3.4 ± 0.143	3.6 ± 0.082	3.2 ± 0.13	3.1 ± 0.11
Lysosomal protein (mg/g)	7.1 ± 0.32	6.6 ± 0.51	2.96 ± 0.181	2.94 ± 0.205

* All values are expressed as means ± S.E.M. of eight animals.
† Significant difference from control, $P < 0.01$.

Table 4. Effect of chronic ethanol feeding on the total and free activities of lysosomal enzymes in liver homogenates*

Determination		Lysosomal enzyme activity (μmoles/g liver/min) One month Control	Lysosomal enzyme activity (μmoles/g liver/min) One month Ethanol	Lysosomal enzyme activity (μmoles/g liver/min) Six months Control	Lysosomal enzyme activity (μmoles/g liver/min) Six months Ethanol
β-Glucuronidase	T†	0.57 ± 0.067	0.63 ± 0.045	0.76 ± 0.036	1.14 ± 0.164‡
	F	0.13 ± 0.007	0.14 ± 0.012	0.13 ± 0.014	0.49 ± 0.088§
	F/T (%)	20.4 ± 1.04	22.4 ± 1.03	18.5 ± 2.16	44.7 ± 7.06‖
β-N-acetylglucosaminidase	T	3.48 ± 0.206	3.35 ± 0.285	3.69 ± 0.323	5.30 ± 0.537‡
	F	2.33 ± 0.186	2.38 ± 0.255	3.42 ± 0.523	4.85 ± 0.585
	F/T (%)	68.3 ± 3.25	70.1 ± 4.02	87.4 ± 4.71	86.1 ± 2.34
Acid phosphatase	T	2.67 ± 0.209	3.04 ± 0.206	6.44 ± 0.568	5.92 ± 0.476
	F	1.11 ± 0.155	0.96 ± 0.135	2.72 ± 0.416	4.12 ± 0.352‡
	F/T (%)	43.0 ± 8.83	37.0 ± 4.06	36.4 ± 5.73	70.8 ± 6.25‖
Arylsulfatase A	T	2.64 ± 0.201	2.84 ± 0.166	3.52 ± 0.124	4.64 ± 0.316‖
	F	0.73 ± 0.115	1.19 ± 0.214	1.28 ± 0.184	1.92 ± 0.280
	F/T (%)	26.3 ± 3.44	34.9 ± 5.21	35.6 ± 3.29	39.5 ± 3.71

* All values are expressed as means ± S.E.M. of eight animals.
† T, total; F, free; F/T, free/total.
‡ Significant difference from control, $P < 0.05$.
§ Significant difference from control, $P < 0.001$.
‖ Significant difference from control, $P < 0.01$.

Table 5. Effect of chronic ethanol feeding on the specific activity of lysosomal enzymes*

Determination	Lysosomal enzyme activity (nmoles/mg protein/min) One month Control	Lysosomal enzyme activity (nmoles/mg protein/min) One month Ethanol	Lysosomal enzyme activity (nmoles/mg protein/min) Six months Control	Lysosomal enzyme activity (nmoles/mg protein/min) Six months Ethanol
β-Glucuronidase	29.0 ± 2.58	32.7 ± 2.46	31.5 ± 1.75	51.2 ± 6.68†
β-N-acetylglucosaminidase	465.4 ± 41.62	512.8 ± 63.81	599.9 ± 52.50	1200.5 ± 244.40‡
Acid phosphatase	460.6 ± 28.03	533.8 ± 28.32	536.4 ± 31.61	812.0 ± 65.3†
Arylsulfatase A	259.7 ± 21.95	316.4 ± 23.45	287.8 ± 54.49	540.0 ± 107.8‡
Hyaluronidase	ND§	ND	25.8 ± 4.14	26.2 ± 3.21

* All values are expressed as means ± S.E.M. of eight animals.
† Significant difference from control, $P < 0.01$.
‡ Significantly different from control, $P < 0.05$.
§ ND, not determined.

DISCUSSION

The effect of the acute administration of ethanol on hepatic lysosomal enzymes was studied here for the first time, and found not to result in any changes in lysosomal enzymes. By contrast, the acute administration of carbon tetrachloride, a more powerful toxin which, unlike ethanol, results in the development of hepatocellular necrosis, has been shown to increase the free activities of a number of lysosomal enzymes in liver homogenates [4, 5, 20] and to elevate their activities in the serum [5]. Ischemia of the liver induced by ligation of the vascular pedicle of a lobe has also resulted in an increase in the free activities of a number of lysosomal enzymes [7]; however, there is no evidence that the release of lysosomal enzymes is the cause of the cell injury since evidence of necrosis precedes detectable increases in the free lysosomal enzymes [5]. The feeding of ethanol for a period of 1 month resulted also in no changes in the activity of lysosomal enzymes. Other investigators, with the exception of finding a transient increase in total β-glucuronidase activity in one study after 10 days of ethanol feeding [8], also did not find changes in the total activity of lysosomal enzymes after ethanol feeding for periods up to 80 days [8, 9]. They demonstrated increases, however, in the free activities of β-glucuronidase [8, 9], acid phosphatase [8] and acid deoxyribonuclease [9]. The differences in the effect of short-term ethanol feeding between the above studies and ours could be due to differences in the technique used in homogenizing the liver, in that the animals in the above studies were not pair-fed, or in the age of the animals used. Increases in the free activity of lysosomal enzymes were reported to occur after acute hepatic injury in old but not in young rats, in one study [21]. Our animals, at the start of the feeding of ethanol, weighed 90–110 g, while the animals in the above studies weighed between 200 and 300 g. In another study, ethanol, given by intraperitoneal injections in a dose of 1 g/kg of body weight for 14 days, resulted in decreases in lysosomal protein concentration, and in the total and free activities of β-*N*-acetylglucosaminidase and hyaluronidase [10]. This study, however, cannot be compared with ours because of the different dosage and route of administration of the ethanol.

In contrast to the lack of changes in the activities of lysosomal enzymes after the acute and short-term chronic administration of ethanol, its feeding for a period of 6 months resulted in increases in the total activities of β-glucuronidase, β-*N*-acetylglucosaminidase and arylsulfatase A, and in the specific activity of these enzymes plus acid phosphatase determined in a lysosomal fraction. In addition, there were increases in the free activities of β-glucuronidase and acid phosphatase.

The cause of the increase in the total activity of lysosomal enzymes after ethanol feeding is unknown. Various studies suggest that lysosomal enzymes are synthesized in the endoplasmic reticulum and then transferred to the lysosomes [22, 23]. Some of the lysosomal enzymes such as β-glucuronidase have the characteristic of a dual distribution, a significant portion of the enzyme activity being present in the microsomes [24]. The administration of ethanol has been shown to result in increases in the smooth endoplasmic reticulum and in activity of microsomal enzymes [25, 26]. The administration of phenobarbital, a powerful inducer of microsomal enzymes, has resulted in increases in the volume of the liver occupied by the lysosomes [27], and in increases in the specific activities of lysosomal enzymes in the lysosomes in one study [28], but not in increases in their total activity determined in liver homogenates in another study [29]. However, the prolonged oral administration of phenobarbital was recently demonstrated to increase the total activities of lysosomal enzymes in intestinal homogenates [30]. Nevertheless, it is unlikely that the mechanism for the increase in the activity of lysosomal enzymes, after prolonged ethanol administration, is induction of their synthesis in the microsomes with secondary shift to the lysosomes, since no increase in lysosomal enzymes was found after 1 month of ethanol feeding, at a time when induction of microsomal enzymes by ethanol is at a peak [26].

Malnutrition may be a mechanism for the changes in the enzymes after chronic ethanol feeding, since starvation [31, 32] has been shown to result in increases in the total and free activities of the lysosomal enzymes. In addition, children with kwashiorkor were found to have increases in the size and number of lysosomes in liver biopsies examined by electron microscopy [33], and elevations in the activity of lysosomal enzymes in their sera [34]. Malnutrition is suggested by the slower gain in body weight of the ethanol-fed rats, which occurred despite their pair-feeding with the control animals. Its most likely cause is malabsorption, which has been demonstrated in animals after the administration of ethanol [35, 36] and in chronic alcoholic patients [37].

Lysosomal enzymes may play a role in the degradation of mucopolysaccharides present in the ground substance of collagen. The mucopolysaccharides are degraded to oligosaccharides by mucopolysaccharidases such as hyaluronidase, and the oligosaccharides in turn can be degraded by other lysosomal enzymes such as β-glucuronidase and β-*N*-acetylhexosaminidases [38]. Parallel changes in hepatic hyaluronidase and β-glucuronidase have been described, with decreases in acute liver injury and in irreversible fibrosis, and increases in reversible liver fibrosis [16]. In this study no changes in the activity of hyaluronidase were found after chronic ethanol feeding despite the increases in the activity of β-glucuronidase and of the other lysosomal enzymes measured. However, ethanol feeding also did not result in acute hepatocellular injury or fibrosis, suggesting that changes in hyaluronidase activity occur either by a different mechanism than the changes in the other lysosomal enzymes, or only in association with severe tissue injury.

The increases in total and free lysosmal enzymes, demonstrated after chronic ethanol feeding in this study, may explain the increased activity of lysosomal enzymes found in the serum of chronic alcoholic patients [39]. Increases in the ratio of free to total lysosmal enzymes have been found in liver biopsies of patients with viral hepatitis and active cirrhosis [40], and increases in the serum activities in patients with acute, and chronic hepatitis, and cirrhosis

[41, 42]; however, in this study the increases in lysosomal enzymes were accompanied by histological changes consisting only of mild fatty infiltration of the liver and no evidence of hyaline bodies, necrosis or fibrosis. The same histological findings have been found by other investigators after similar periods of ethanol feeding [43, 44]. More prolonged feeding of ethanol has resulted in the development of increased fatty infiltration and hyaline bodies in rats [44], and necrosis, fibrosis and cirrhosis in monkeys [1].

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