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INFLUENCE OF ETHANOL AND ACETALDEHYDE ON THE ACTIVITY AND RELEASE OF CATHEPSIN A FROM LIVER LYSOSOMES

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Ethanol and acetaldehyde inhibited the activity of cathepsin A. *In vitro* ethanol did not release cathepsin A from liver lysosomes whereas acetaldehyde of high concentration did. Intoxication of rats with ethanol and acetaldehyde evoked a transient increase of free and bound cathepsin A.

Free and bound activity of cathepsin D and E is increased in acute and chronic intoxication with ethanol [1, 2]. Ethanol also releases acid phosphatase from lysosomes [3].

The purpose of this work is to examine the influence of ethanol and acetaldehyde on the activity and release of cathepsin A from liver lysosomes to the rat *in vitro* and *in vivo*.

MATERIALS AND METHODS

A 10% homogenate of rat liver in 0.25 M sucrose with or without 0.2% Triton X-100 was used in the experiments *in vitro*. Inhibition of cathepsin A activity by ethanol and acetaldehyde was measured at pH 5.0 [4]. Ethanol (0.1 ml) or acetaldehyde (0.1 ml) of different concentrations (in the control acetate buffer, pH 5.0) and 0.1 ml of Cbz-L-glutamyl-L-tyrosine (12.5 mmol) was added to 0.3 ml of the homogenate containing sucrose and Triton X-100. After 2 h incubation at 37°C the reaction was stopped by adding 1.25 ml of 5% trichloroacetic acid, and free tyrosine was determined.

The release of cathepsin A from lysosomes by ethanol or acetaldehyde was examined at pH 7.0; 0.1 ml of ethanol or acetaldehyde of different

concentration (in the control 0.25 M sucrose or 0.2% Triton X-100) was added to 0.4 ml of the homogenate in 0.25 M sucrose and mixtures were incubated at 37°C. Aliquots were brought to pH 5.0 at 30 min intervals, centrifuged [5] and cathepsin A activity was measured in supernatant.

During 4 weeks of the experiments *in vivo* the rats received intragastrically either ethanol (0.6 g/100 g body weight per day) or acetaldehyde (25 mg/100 g body weight per day) or 0.15 M NaCl (controls). The liver was taken directly after the intoxication or 7 days after the intoxication had been stoped. A 10% homogenate was then prepared in 0.25 M sucrose with or without 0.2% Triton X-100, brought to pH 5.0 and centrifuged. Activity of cathepsin A and protein content [6] were determined in the cytosol and in the full homogenate.

The results are mean values of 3 experiments carried out *in vitro*. The experiments *in vivo* were carried out in groups of 8 rats. For statistical analysis Student's *t* test was used.

RESULTS AND DISCUSSION

Ethanol and acetaldehyde at the concentration of 400 mM and 100 mM, respectively, slightly inhibited the activity of cathepsin A (Table 1). The weaker inhibitory effect of ethanol as compared with acetaldehyde was related to low chemical reactivity of the alcoholic group and marked reactivity of the aldehyde group.

Ethanol did not release, and high concentrations of acetaldehyde partly released cathepsin A from lysosomes *in vitro* (Table 2). The release of cathepsin A from lysosomes began immediately after acetaldehyde addition and was increased after 30 min of incubation; however, after 60 min the enzyme release decreased, probably because of inactivation by acetaldehyde.

The activity of cathepsin A was markedly increased in cytosol and slightly increased in the full liver homogenate of animals intoxicated with ethanol or acetaldehyde (Table 3). This points to impairment of lysosomes. These changes partly disappeared 7 days after intoxication with ethanol and acetaldehyde had

Table 1

Effect of ethanol and acetaldehyde on the activity of cathepsin A in the liver

Examined compound	Concentration (mM)					
	0	6.25	25.0	100.0	400.0	1600.0
	tyrosine (μM/ml per 2 h)					
Ethanol	0.86	0.86	0.84	0.83	0.75	0.63
Acetaldehyde	0.85	0.86	0.83	0.79	0.67	0.51

Table 2

Effect of ethanol and acetaldehyde on the release of cathepsin A from the liver lysosomes of the rat, determined in vitro

Examined compound	Time of incubation (min)	Concentration (mM)						0.2% Triton X-100
		0	6.25	25.0	100.0	400.0	1600.0	
		tyrosine (μM/ml per 2 h)						
Ethanol	0	0.09	0.10	0.10	0.10	0.11	0.11	0.88
	30	0.10	0.11	0.11	0.11	0.11	0.12	0.87
	60	0.10	0.12	0.12	0.12	0.13	0.15	0.86
Acetaldehyde	0	0.10	0.10	0.10	0.11	0.12	0.22	0.87
	30	0.10	0.11	0.12	0.12	0.36	0.46	0.87
	60	0.10	0.11	0.12	0.13	0.24	0.32	0.85

Table 3

Activity of cathepsin A and concentration of protein in the liver of rats intoxicated with ethanol or acetaldehyde

Rats intoxicated with	Cathepsin A (Tyr. μM/ml per 2 h)		Protein (mg/ml)	
	Cytosol	Full homogenate	Cytosol	Full homogenate
Ethanol	0.21±0.023*	1.12±0.050	2.46±0.38	5.98±0.52
After 7 days without ethanol	0.15±0.012	1.09±0.035	3.02±0.64	6.15±0.33
Acetaldehyde	0.22±0.02*	1.16±0.061	2.54±0.39	6.02±0.48
After 7 days without acetaldehyde	0.17±0.014	1.10±0.036	2.62±0.42	6.08±0.56
Controls	0.12±0.011	1.07±0.042	2.67±0.60	6.17±1.53

* Statistically significant difference in comparison with the controls ($P<0.05$).

been stopped. Contents of protein in cytosol and full homogenate remained unchanged in all the examined groups of animals. The fact that cathepsin A was not released by ethanol from the liver lysosomes *in vitro* although it was released when the rats were intoxicated with this compound shows that impairment of the lysosomal membranes was due to acetaldehyde resulting from oxidation of ethanol. This was confirmed by cathepsin A release *in vitro*

from the liver lysosomes by acetaldehyde. The concentration of acetaldehyde evoking the release of cathepsin A from liver lysosomes *in vitro* was markedly higher than the concentration of acetaldehyde found in the liver of rats intoxicated with ethanol. However, it should be taken into account that the time of acetaldehyde action *in vivo* was much longer.

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