CLOFIBRATE-ACTIVATED ALDEHYDE DEHYDROGENASE OF RAT LIVER PEROXISOMES

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Aldehyde dehydrogenase — ALDH (EC 1.2.1.3) — of mammalian liver plays a leading role in the metabolism of acetaldehyde, the most toxic oxidation product of ethyl alcohol. This enzyme exists in several iso-forms, which differ in their kinetic and molecular properties, and also in their intracellular localization [6, 12]. The view predominantly held previously in the literature was that the chief sites for oxidation of acetaldehyde are the mitochondria, where two varieties of ALDH are found: matrix enzyme with low K_m for short-chain aliphatic aldehydes, and enzyme of the intermembranous space, with high K_m [10]. More recently, however, information has been obtained of the important role of extramitochondrial pathways of acetaldehyde oxidation, especially when ethanol consumption is increased. It has been suggested that enzymes of the cytoplasmic reticulum and cytosol participate in this process [6, 9].

Besides mitochondrial and microsomal oxidative systems mammalian cells are also known to contain a peroxisomal oxidative enzyme system. The important biological function of peroxisomes is the utilization of the excess quantity of energy-rich compounds, mainly through their oxidative degradation [2]. The list of these compounds includes acetaldehyde and ethanol. In view of these considerations it may be expected that peroxisomes play an active role in the oxidation not only of ethanol (by the catalase pathway [2]), but also of acetaldehyde.

The object of this investigation was to test this hypothesis experimentally. Enzymic pathways of acetaldehyde metabolism were studied under normal conditions, during prolonged ethanol consumption by animals, and also after administration of clofibrate, a hypolipemic agent which stimulates proliferation of peroxisomes.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-250 g were used. In the experiments to study the effect of ethanol on ALDH activity the animals were kept on a semiliquid balanced diet of equal calorific value for 5-6 weeks. Rats of the experimental group received 10% (of the total volume of food) ethanol. Ethanol also was added to the drinking water (final concentration 10%). The total quantity of ethanol consumed was 18-20 g/kg body weight daily. Clofibrate was injected intraperitoneally in a dose of 400 mg/kg daily for 10 days. As was shown previously, administration of the drug by this method leads to most intensive proliferation of peroxisomes [1]. The animals were deprived of food for 24 h before sacrifice. Perfusion of the liver, preparation of the homogenate and its differential centrifugation, and the enzyme control of the composition of the fragments were carried out as described previously [3]. Purified fractions of mitochondria and peroxisomes were isolated by isodensity centrifugation of the λ-fraction in a two-step sucrose gradient [3], but hypotonic treatment of the particles was not carried out. In some experiments a multistep gradient was used to separate peroxisomes and mitochondria. Enzymic assessment of the purity of the fractions was undertaken by determining the activity of marker enzymes of peroxisomes — catalase (EC 1.11.1.6) and urate oxidase (EC 1.7.3.3); of mitochondria — glutamate dehydrogenase (EC 1.4.1.2); of lysosomes — acid phosphatase (EC 3.1.3.2); and of the cytoplasmic reticulum — glucose-6-phosphatase (EC 3.1.3.9) [3]. ALDH activity in the whole homogenate and in the

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TABLE 1. Effect of Clofibrate and Ethanol on ALDH Activity in Rat Liver Homogenate (M ± m)

Enzyme	Specific activity			
	control	ethanol	control	clofibrate
ALDH (total activity)	10,80±0,98	10,50±0,87	9,15±0,61	13,10±1,54*
Isozyme I	2,06±0,48	1,73±0,42	2,41±0,18	2,05±0,28
Isozyme II	8,81±0,65	8,80±0,57	6,70±0,58	11,00±1,30*

Legend. Enzyme activity expressed in nanomoles NADH formed per minute per milligram protein; ALDH activity was determined at 20°C; each group contained 6-8 animals; *$P < 0.05$.

subcellular fractions was determined at 37°C by continuous spectrophotometric recording (at 340 nm) of the accumulation of NADH formed in the course of the reaction. Acetaldehyde in a concentration of 5 mM (total enzyme activity) or of 0.05 mM (activity of ALDH with low K_m: isozyme I) was used as the substrate. Activity of ALDH with high K_m (isozyme II) was calculated as the difference between values obtained with the two substrate concentrations [12]. Clofibric acid was obtained by alkaline hydrolysis of clofibrate. Protein was determined by Lowry's method [7].

EXPERIMENTAL RESULTS

Feeding on a diet containing ethanol or administration of clofibrate to the rats was accompanied by an increase in the relative weight of the liver by 38.3% ($P < 0.01$) and 27.7% ($P < 0.01$) respectively. Meanwhile the protein content per gram wet weight of tissue decreased in the first case by 16.5% ($P < 0.05$) and increased in the second case by 27.1% ($P < 0.05$). These changes can be explained by differences in the hepatotropic action of clofibrate and ethanol. Under the influence of clofibrate intensive proliferation of the liver cells took place, with a parallel growth of their smooth endoplasmic reticulum and an increase in the number and size of the peroxisomes [2], whereas ethanol led to fatty degeneration of the liver [4]. The specific ALDH activity in liver homogenate from rats consuming ethanol was the same as in the control (Table 1). After chronic administration of clofibrate total enzyme activity was increased by 43.2% and activity of isozyme II (ALDH with high K_m) increased by 65.7%, whereas activity of isozyme I was unchanged (Table 1). Neither clofibrate itself nor its pharmacologically active derivative clofibric acid had any effect on ALDH activity. An increase in ALDH activity under the influence of clofibrate was observed previously [5]. After differential centrifugation of the homogenate, isozyme I in the control and experimental (administration of clofibrate) samples was located mainly in the nucleo-mitochondrial fraction (55.7 and 68.4% respectively; each group consisted of four animals), whereas not more than 25% of the total activity of the enzyme in the particles was found in the fraction of microsomes and the λ-fraction, enriched with peroxisomes. Similar results were obtained when the intracellular localization of mitochondrial glutamate dehydrogenase was determined. Isozyme II was characterized by a more uniform distribution among fractions of subcellular structures. The relative content of the enzyme in the λ-fraction and mitochondrial fraction was significantly increased by clofibrate (8.2 and 14.6% in the control, 13.8 and 33.8% respectively in the experimental series). The increase observed in these fractions is evidence in support of localization of the isozyme in the peroxisomes.

During centrifugation of the λ-fraction (animals of the experimental group) in a multistep sucrose concentration gradient, 26.5% of the total ALDH activity was found in the region with density 1.22-1.26 g/cm^3, containing peroxisomes, in all samples of the gradient, and the rest of the enzyme was located in fractions rich in mitochondria and microsomes. Meanwhile, as regards specific ALDH activity, this was significantly higher in the peroxisomes than in the mitochondrial fraction. The results obtained during isodensity centrifugation of the λ-fraction in a two-step sucrose concentration gradient are given in Table 2. Specific catalase and urate oxidase activity in the peroxisomal fraction was increased in the control samples by 29.0 and 26.1 times respectively compared with activity in the homogenate. The enzyme control of the purity of the isolated fraction revealed insignificant contamination with mitochondria and microsomes, and its content of peroxisomal protein was 75-80% [3]. Administration of clofibrate not only led to an increase in the number of peroxisomes in the liver cells, but also affected the enzyme composition of these organelles, as a result

TABLE 2. Effect of Clofibrate on Specific Enzyme Activity in Subcellular Fractions (M ± m)

Enzyme	Group of animals	Specific activity			
		homogenate	mitochondria	peroxisomes	microsomes
ALDH (total activity)	Experimental	58,2±6,4	28,2±5,8	37,7±5,8	364,7±122,0
	Control	34,6±4,9	39,1±2,6	30,6±5,8	190,9±42,2
Catalase (relative units)	Experimental	0,179±0,015	0,204±0,031	1,44±0,05	0,146±0,023
	Control	0,115±0,008	0,872±0,276	3,35±0,43	0,058±0,004
Urate oxidase	Experimental	11,3±2,3	17,5±3,0	86,7±4,6	9,6±2,9
	Control	7,2±0,8	50,1±16,5	188,3±28,4	12,9±4,1
Glutamate dehydrogenase	Experimental	1,06±0,59	1,78±0,53	0,09±0,01	0,09±0,02
	Control	1,07±0,12	2,71±0,53	0,14±0,02	0,52±0,05
Glucose-6-phosphate	Experimental	48,0±6,2	41,9±7,7	2,9±1,8	84,8±21,2
	Control	42,8±9,6	63,8±30,5	7,9±2,0	98,1±20,8
Acid phosphatase	Experimental	60,0±6,4	138,5±44,1	49,7±4,5	73,3±8,5
	Control	64,3±9,4	127,5±16,7	104,3±35,4	81,2±13,5

Legend. Enzyme activity expressed in nanomoles substrate or reaction product per minute per milligram protein of fraction; aldehyde dehydrogenase activity was determined at 37°C; number of experiments 4-6. Catalase activity expressed in relative units [8], glutamate dehydrogenase activity in millimoles/min/mg protein.

of which the relative number of catalase and urate oxidase molecules in them was reduced [2]. This evidently explains the decrease in specific activity of these enzymes in the peroxisomal fraction of the experimental group, along with the increased yield of peroxisomal protein (Table 2). It should be pointed out, however, that total catalase and urate oxidase activity (yield of enzyme) was virtually unchanged in the peroxisome fraction.

Comparative analysis of activity of ALDH and marker enzymes of subcellular structures showed that ALDH was present in all fractions studied, including peroxisomes. The specific activity of the enzyme in the peroxisomal fraction was comparable with activity in the fractions of mitochondria and microsomes. Total ALDH activity in peroxisomes isolated from liver homogenate of rats of the experimental group was more than twice as high as in the control, evidence of an increase in the content of the enzyme in this fraction under the influence of clofibrate. Activity of the different ALDH isozymes in purified fractions of organelles was not studied separately in this series of experiments, but the preliminary results show that the enzyme of the peroxisomes had definite similarity in its properties with isozyme II of ALDH.

While the experiments described above were in progress an investigation was published which confirms the conclusion that ALDH is present in peroxisomes of normal rat liver [11]. The workers cited studied the intracellular localization of ALDH both biochemically and cytochemically. They found that the peroxisomal enzyme is active not only against acetaldehyde as substrate (they used final concentrations of acetaldehyde of 5 and 0.05 mM), but also against benzaldehyde and propionic aldehyde and that, in addition, it can reduce $NADP^+$.

The presence of clofibrate-inducible ALDH, active against acetaldehyde, in peroxisomes is further evidence of the role of these organelles in ethanol metabolism. In the light of the data given above there are grounds for regarding the catalase pathway of alcohol metabolism as a peroxisomal enzyme system, including not only catalase but also ALDH, and capable of implementing the complete two-stage oxidation of ethanol to acetic acid.

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CHANGES IN ACID PHOSPHATASE IN THE GASTRIC MUCOSA DURING ULCER FORMATION

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Much information has accumulated in the recent literature on a possible role of proteolytic enzymes in the mechanisms of ulcer formation in the stomach and duodenum [1-3, 5, 8-11, 13]. Besides characteristic changes in the spectrum of proteolytic enzymes exhibiting activity at pH values from 1.0 to 5.0 in the gastric mucosa during the period of ulcer formation, others active also at pH values from 6.5 to 7.0 may also appear. It has been suggested that the source of this activity is the intracellular lysosomal enzymes, which exert their lytic action on the surrounding autologous substrate as a result of liberation of these enzymes from intracellular lysosomes [3].

This paper describes a further study of this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 192 albino rats of both sexes weighing 160-200 g. An acetate model of gastric and duodenal ulcer was used [12]. An ulcer was produced on the anterior wall of the forestomach, the glandular part of the body of the stomach, the antrum, the duodenum, and the terminal portion of the ileum. The animals were killed 10, 60, and 90 min after ulcer formation. Washings from the region of the ulcer and from an area of the intact mucosa of similar size, the gastric or intestinal contents, and tissue from the floor of the ulcer, the edges of the ulcer, and intact areas of mucosa were investigated. A piece of mucosa weighing 100 mg was excised and homogenized in 2.0 ml of distilled water. Acid phosphatase, a marker of liberation of lysosomal enzymes [7], was determined in the material thus obtained, and the proteolytic activity of extracts of the mucosa at pH 6.5-7.0 was determined by the method described previously [6].

EXPERIMENTAL RESULTS

No acid phosphatase was found in the gastric contents of intact (control) rats, but comparison of data obtained 10, 60, and 90 min after the formation of an experimental ulcer in the body of the stomach showed that the acid phosphatase concentration in the gastric contents reached a maximum after 60 min (Table 1). The maximal content of acid phosphatase in washings from the zone of the ulcer also was found after the same interval of 60 min. These observations evidently indicate that this is the time when destructive processes take place most intensively in the tissues of the gastric mucosa after application of acetic acid to the serous membrane.

In view of these results, the next tests were carried out at hourly intervals after formation of the ulcer. Acid phosphatase, which was absent in the control, was found to appear in the gastric contents after formation of an ulcer in the forestomach, while at the same time its level fell sharply in the tissue of the mucosa in the zone of the ulcer (1.0 ± 0.2 μmole p-nitrophenol/g dry weight of tissue compared with 6.4 ± 0.6 in the control — Table 1). Ulcer formation in the antral portion was accompanied by the appearance of considerable quantities of acid phosphatase in washings from the zone of the ulcer (0.165 μmole p-nitrophenol/ml compared with 0). The acid phosphatase content also was significantly in-

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