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INDUCTION OF LIVER MALATE DEHYDROGENASE (DECARBOXYLATING) AND LACTATE DEHYDROGENASE ACTION BY CLOFIBRATE

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Numerous investigations have shown that the velocity of ethanol oxidation in liver cells largely depends on the ratio between the concentration of oxidized and reduced forms of pyridine nucleotides (NAD and NADP) in the cytoplasm, which is kept at a certain level through the participation of NAD- and NADP-dependent dehydrogenases. Changes in the activity of these enzymes may have a significant effect on the metabolism of alcohol, especially if it is consumed excessively [2, 10]. The hypolipidemic agent clofibrate can accelerate ethanol oxidation [3, 5] and also modify the steady-state intracellular concentration of various metabolites which are substrates of cytoplasmic dehydrogenases – lactate, pyruvate, glycerol-3-phosphate, etc. [3, 5, 6, 13]. The mechanism of these effects of clofibrate has not been explained. Clofibrate is known to cause marked proliferation of peroxisomes and of the smooth endoplasmic reticulum. Selective induction or repression of the synthesis of catalase, D-amino acid oxidase, and carnitine-acetyltransferase also is observed at the same time [4, 7].

The goal of this research was the discovery of the possible role of certain cytoplasmic dehydrogenases in accelerating the metabolism of ethanol under the action of clofibrate.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-250 g were used. Clofibrate was injected intraperitoneally in a dose of 250 or 800 mg/kg body weight daily. Animals of the control group received injections of physiological saline. The animals were deprived of food for 24 h before sacrifice. Perfusion of the liver, homogenization, preparation of "nuclear-free" homogenate and its differential centrifugation, and also the enzyme control for the composition of the subcellular fractions were carried out as described previously [1, 11]. The effect of clofibrate on cell metabolism was assessed by determining activity of the peroxisomal enzymes catalase, L-α-hydroxy-acid oxidase [1], and carnitine-acetyltransferase [4]. Activity of sorbitol dehydrogenase (SDH) [12], lactate dehydrogenase (LDH), NAD-dependent glycerol-3-phosphate dehydrogenase [1], NAD-dependent malate dehydrogenase (MDH-NAD), and NADP-dependent malate dehydrogenase (decarboxylating; MDH-NADP) [9] was determined. Catalase and carnitine-acetyltransferase activity were determined at 25°C, that of the other enzymes at 37°C, using a Gilford model 250 spectrophotometer. The LDH isozyme spectrum was studied by electrophoresis in

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TABLE 1. Effect of Clofibrate on Enzyme Activity of Peroxisomes and Cytosol (M ± m)

Enzyme	Enzyme activity			
	units/g tissue		units/mg protein	
	control	clofibrate	control	clofibrate
Catalase	(9) 39,2±5,9	46,8±11,8	0,298±0,045	0,299±0,058
L-α-hydroxy-acid oxidase	(12) 1,012±0,155	0,477±0,042†	0,0077±0,0004	0,0031±0,0002†
Carnitine-acetyltransferase	(5) 0,25±0,07	6,57±1,21†	0,002±0,001	0,042±0,012†
LDH	(12) 212,5±52,8	482,8±105,1†	1,59±0,26	3,10±0,54†
MDH-NAD	(5) 1296,3±323,1	1228,2±155,0	8,34±1,90	6,58±1,45
MDH-NADP	(9) 1,40±0,30	8,24±4,45†	0,0086±0,0018	0,0432±0,0210†
SDH	(5) 24,3±10,7	19,6±2,7	0,13±0,03	0,10±0,01
Glycerol-3-phosphate dehydrogenase	(5) 12,1±2,6	18,4±2,8*	0,069±0,007	0,088±0,007
Protein, mg/g tissue	(20) 146±27	169±37*	—	—

Legend. Activity of all enzymes except catalase given in millimoles substrate/min; catalase activity given in units suggested by Leighton et al. [7]; number of animals given in parentheses; clofibrate injected in a dose of 250 mg/kg daily for 16 days; *$P < 0.05$, †$P < 0.001$.

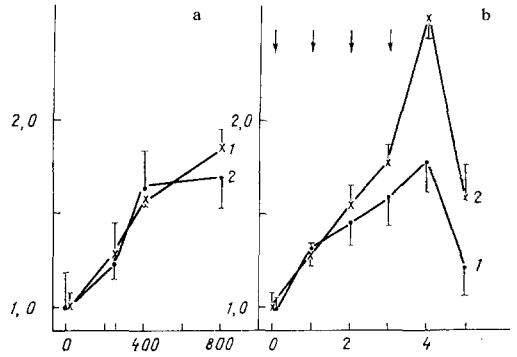

Fig. 1. Dependence of LDH (1) and MDH-NADP (2) activity on dose (a) and duration (b) of administration of clofibrate. Abscissa: a) dose of clofibrate (in mg/kg, daily for 3 days), b) duration of injection of clofibrate (days on which injections were given are indicated by arrows, dose of clofibrate 800 mg/kg); ordinate: ratio of specific enzyme activity in experimental animals to activity in control animals. Number of animals in each group 3-5.

polyacrylamide gels. When the effect of inhibitors of protein synthesis on the induction of enzyme activity was studied clofibrate was injected daily in a dose of 800 mg/kg for 2 days. Cyclohexamide (1.5 mg/kg) or puromycin (5 mg/kg) was given at the same time. The effect of clofibrate and clofibric acid *in vitro* on the above-mentioned enzymes was determined after incubation of these substances with liver homogenate or cytosol fraction at 37°C for 15 min (final concentration in the mixture 10^{-4} or 10^{-3} M). Clofibric acid was obtained by alkaline fibriolyisis of clofibrate. Protein was determined by Lowry's method [8].

EXPERIMENTAL RESULTS

During prolonged administration of clofibrate to the rats a significant ($P < 0.001$) increase was observed in the ratio of the weight of the liver to the total body weight by 38 and 54% on the 10th and 16th days respectively after the beginning of injection of the hypolipidemic agents. The protein concentration also was increased in the "nuclear-free" liver homogenate, and there was a selective change in activity of the peroxisomal enzymes (Table 1). With the dose of clofibrate used no activation of catalase was found. Meanwhile an increase in the dose of the drug to 800 mg/kg for 4 days led to an increase in the specific catalase activity by 20-25% ($P < 0.001$). Total and specific activity of L-α-hydroxy-acid oxidase in the liver homogenate from animals of the experimental group were reduced by 53 and 60% (by 2.1 and 2.5 times) respectively, whereas carnitine-acetyltransferase activity was increased by more than 20 times. The use of this method of administration of clofibrate thus reveals some characteristic manifestation of its hepatotropic action: marked hepatomegaly, an increase in the protein concentration of the homogenate, and changes in peroxisomal enzyme activity. As Table 1 shows, parallel with

the above changes, after administration of clofibrate there was also an increase in the total and specific LDH and MDH-NADP activity. Activity of glycerol-3-phosphate dehydrogenase was increased by a much lesser degree, and MDH-NAD activity was unchanged. Administration of clofibrate also led to a tendency for SDH activity to fall, confirming previous observations by other workers [12]. The clofibrate-dependent increase in LDH and MDH-NADP activity was evidently due to induction of their synthesis. This conclusion is supported by the absence of effect of clofibrate and its pharmacologically active derivative clofibric acid on the above-mentioned enzymes *in vitro* and also by inhibition of the activation process by inhibitors of protein synthesis (cycloheximide and puromycin). The level of LDH and MDH-NADP activation depended on the dose of clofibrate given and reached a maximum with a dose of 800 mg/kg (Fig. 1a). A further increase in the dose of the drug was not accompanied by any increase in enzyme activity. Investigation of dependence of LDH and MDH-NADP activation on the duration of administration of clofibrate showed that the most significant changes were observed 4 days after its first injection (Fig. 1b). After administration of clofibrate had ceased LDH and MDH-NADP activity fell sharply, and after 2 days it was only very slightly higher than in the control. A single dose of clofibrate led to an increase in LDH activity after a latent period of 18-22 h, and the effect reached a maximum after 30 h (by 58%). Electrophoretic fractionation of the LDH isozymes led to the appearance of four bands in the gels corresponding to M_4- and MH_3-isozymes. Clofibrate had no effect on the relative content of each isozyme separately, and consequently it did not change the LDH isozyme spectrum of the hepatocytes. Differential centrifugation of the homogenate showed that more than 95% of LDH and MDH-NADP activity in both the experimental and the control samples was located in the soluble fraction of the cell – no redistribution of enzyme activity between cytosol and subcellular structures was observed under the influence of clofibrate.

The fact that clofibrate induces liver LDH and MDH-NADP activity shows that this hypolipidemic agent not only acts on the enzyme content in peroxisomes, but that it can also selectively modify the enzyme composition of other cell compartments, including the cytosol. The frequently observed and clofibrate-dependent change in the redox state of the cytosol [5, 13] and also in the steady-state concentrations of various metabolites of glycolysis and of the tricarboxylic acid cycle in liver cells [3, 6, 14] may also perhaps be connected with an increase in the activity of these two dehydrogenases. In turn, these factors may contribute to the more rapid metabolism of ethyl alcohol during chronic administration of clofibrate.

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