EFFECT OF CLOFIBRATE AND ADAPTATION TO LOW TEMPERATURES ON LIVER ENZYME SYSTEMS FOR LIPID CATABOLISM IN RATS

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Besides mitochondria the peroxisomes of mammals play an active role in lipid catabolism. Enzyme systems of β-oxidation of long-chain fatty acids [10] and of the side (aliphatic) chain of cholesterol [13] are found in these subcellular structures. Hypolipidemic agents (clofibrate, bezalip, acetylsalicylic acid, etc.) which lower the serum concentrations of triglycerides and cholesterol, increase the number of peroxisomes and activate enzyme systems for lipid metabolism in cells of the liver and various other organs [7]. Certain physiological procedures and, in particular, increasing the lipid content of the diet [8] and feeding newborn rats on maternal milk [9], have a similar effect. There are thus grounds for suggesting that the mechanisms which regulate peroxisomal metabolism are adaptive in character and control the level of lipid processing in mammals.

Adaptation of warm-blooded animals to low temperatures is known to lead to a reorganization of cell metabolism, aimed at intensifying the use of high-calorie lipids to meet the increased energy requirements of the body [4]. During adaptation of rats to cold, an increase in the number of microperoxisomes is observed in the cells of their brown adipose tissue and both mitochondrial and peroxisomal enzyme system for β-oxidation of fatty acids are activated [12]. An increase in the number of peroxisomes has also been reported in rat liver cells during hypothermia [14], although the enzyme composition of these organelles was not studied.

The aim of the present investigation was to compare the effects of a physiological factor, namely adaptation to low temperatures, and a pharmacological agent with hypolipidemic action, namely clofibrate, on enzyme systems for lipid catabolism in mitochondria and peroxisomes of rat liver cells.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 200-250 g were kept on the animal house diet. The animals were adapted to low temperatures by the method described previously [5]. The temperature of the climatic chamber in which rats of the experimental group were kept was lowered successively by 5°C every week, starting from an initial temperature of 5°C. The process of adaptation ended at −20°C. Animals of the control group were kept at 20°C. At each stage of the experiment five or six rats of the experimental and control groups were killed, the liver was removed, and pieces of tissue were kept at the temperature of liquid nitrogen. To investigate the biochemical parameters in the course of the experiment material was obtained from animals acclimatized to temperatures of 0, −5, −10, and −20°C. None of the animals died in the course of the experiments, whereas in the group of rats kept at −20°C without preliminary adaptation, the mortality exceeded 80% during the first five days. The morphological and physiological changes observed (an increase in the number of reticulocytes and erythrocytes and in the hematocrit index), during cold adaptation of the rats, were similar to those which develop in man under arctic conditions [4].

Another group of rats was used to study the hepatotropic action of clofibrate. The hypolipidemic drug was injected intraperitoneally in a dose of 400 mg/kg body weight daily for 10 days. Homogenization of the liver tissue and differential centrifugation of the homogenate

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TABLE 1. Effect of Adaptation to Low Temperatures and of Clofibrate on Activity of Rat Liver Enzymes (M ± m, n = 5-6)

Enzyme	Specific activity, nanomoles substrate/min/mg protein of homogenate				
	control 1	−5 °C	−20 °C	control 2	clofibrate
Catalase, relative units	0,259±0,019	0,209±0,009	0,264±0,020	0,297±0,015	0,294±0,011
Carnitine acetyltransferase	1,63±0,14	1,78±0,32	2,19±0,38	1,19±0,36	65,15±3,97**
Cyanide-resistant β-oxidation system	0,62±0,07	0,54±0,04	0,52±0,06	0,51±0,07	2,07±0,23*
3-Hydroxyacyl-CoA dehydrogenase	365,1±23,4	313,4±6,4	309,6±41,6	247,8±21,0	453,8±21,0**
β-Ketothiolase	52,4±3,7	49,9±3,9	40,9±6,7	31,2±5,1	244,5±59,3*
Glycerol-3-phosphate dehydrogenase (NAD)	41,1±4,4	46,0±4,2	39,7±5,0	44,0±6,2	61,5±9,1
L-α-Hydroxyacid oxidase	2,09±0,17	2,42±0,16	2,21±0,21	2,67±0,8	1,48±0,10
MDH	7,9±1,2	6,2±0,8	11,6±2,6	24,6±2,5	83,4±8,4**
LDH	1550±120	1540±130	1270±90	1670±120	2660±190*

Legend. Control 1) control group in series of experiments with adaptation of animals to cold; −5° and −20°C) groups of animals acclimatized to those temperatures. Control 2) control group in series of experiments to study effect of clofibrate. *$P \leqslant 0.01$, **$P \leqslant 0.001$.

were carried out as described previously [6]. Catalase and carnitine acetyltransferase activity was measured at 25°C [3]. To study activity of the cyanide-resistant peroxisomal enzyme system for β-oxidation of fatty acids (PESO) the substrate was octanoyl-CoA; the substrate for investigation of 3-hydroxyacyl-CoA dehydrogenase and β-ketothiolase activity was acetoacetyl-CoA [10]. Activity of glycerol-3-phosphate dehydrogenase (NAD), of NADP-dependent malate dehydrogenase (MDH), lactate dehydrogenase (LDH), and also of L-α-hydroxyacid oxidase (sodium glycolate as substrate) was determined as described previously [6]. The velocity of the reactions catalyzed by the above-mentioned enzymes was measured at 37°C. Activity of all enzymes studied (except catalase) was expressed in nanomoles/min/mg protein of the homogenate. Catalase activity was expressed in relative units [6]. Electrophoresis of proteins of the "nonnuclear" homogenate was carried out as described in [15], using 10% polyacrylamide gels containing sodium dodecylsulfate. The protein concentration was determined by the method in [11].

EXPERIMENTAL RESULTS

The hypolipidemic drug clofibrate has a marked hepatotropic action. Under its influence the relative weight of the liver increased (experiment 6.6 ± 0.9%, control 4.3 ± 0.3%, $P \leqslant 0.001$), and the protein concentration in the "nonnuclear" tissue homogenate also increased (experiment 169 ± 37 mg/g, control 146 ± 27 mg/g, $P \leqslant 0.05$, n = 20). An increase in the number of peroxisomes [2] and a change in the activity of several enzymes mainly involved in reactions of lipid metabolism were observed in the hepatocytes. As Table 1 shows, after administration of clofibrate the value of the most sensitive indicator of its effect on liver tissue, namely activity of the enzyme carnitine acetyltransferase, increased by more than 30 times. Activation of the cyanide-resistant enzyme system for β-oxidation of fatty acids, located in the matrix of the peroxisomes, also took place. There was a parallel increase in the activity of two other components of the oxidation chain, namely 3-hydroxyacyl-CoA dehydrogenase and β-ketothiolase. In our experiments to study the activity of these enzymes acetoacetyl-CoA was used as the substrate. This compound takes part in reactions not only by the peroxisomal, but also by the mitochondrial pathway of β-oxidation. The data given in Table 1 thus reflect the combined velocities of the 3-hydroxyacyl-CoA dehydrogenase and β-ketothiolase reactions, taking place in both subcellular structures; the increase in the velocity of the reaction with acetoacetyl-CoA is evidence of activation primarily of the mitochondrial process of β-oxidation [10].

Under the influence of clofibrate an increase in activity not only of enzymes of lipid metabolism was observed, but also of NADP-dependent MDH (decarboxylating) and LDH. The specific activity of L-α-hydroxyacid oxidase, located in peroxisomes, was reduced whereas activity of the most characteristic enzyme of these organelles, namely catalase, was indistinguishable from values obtained in control tests (Table 1). Changes in the enzyme composition of

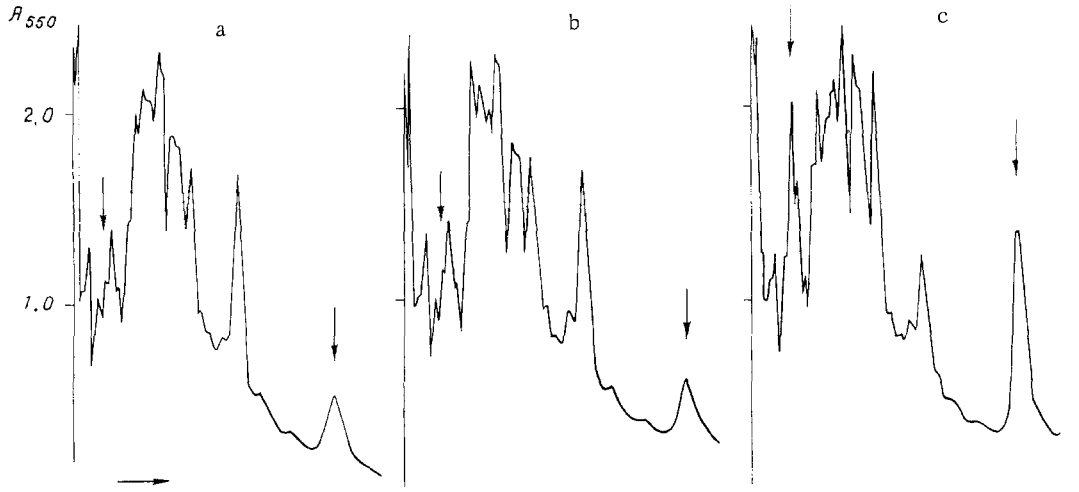

Fig. 1. Electrophoretic polypeptide spectrum of protein of nonnuclear rat liver homogenate. Ordinate, absorption at 550 nm (in optical density units). On left — beginning of gel; a) control sample; b) adaptation to cold (—20°C); c) injection of clofibrate. Vertical arrows indicate zones of polypeptides with mol. wt. 80,000 and 12,000 daltons. Gels stained with Coomassie Bright Blue R = 250.

the hepatocytes were mainly due to the action of the hypolipidemic drug on mechanisms regulating the rate of synthesis of the molecules of the above enzymes [7-9]. Under the influence of clofibrate characteristic changes also were observed in the polypeptide spectrum of proteins in the "nonnuclear" rat liver homogenate (Fig. 1). In particular, there was an increase in the relative content of polypeptides with molecular weights of 80,000 and 12,000 daltons. The polypeptide with molecular weight of 80,000 is a component of the peroxisomal matrix and it evidently consists of subunits of two enzymes of the β-oxidation chain: acyl-CoA oxidase and 3-hydroxyacyl-CoA dehydrogenase. The polypeptide with molecular weight of 12,000 corresponds to the Z protein of hepatocyte cytosol, which is considered to participate in the intracellular transport of sparingly soluble long-chain fatty acids [3]. On the whole, the results obtained in the experiments with clofibrate are evidence of activation of enzyme systems of catabolism of the liver lipids following its administration.

As Table 1 shows, keeping the rats under conditions of hypothermia was not accompanied by any change in activity of the peroxisomal and mitochondrial systems for β-oxidation of fatty acids. This conclusion is confirmed by the results of an investigation of the activity of catalase and carnitine acetyl-transferase in the course of the experiment — during acclimatization to temperatures of 0, —5, —10, and —20°C. Activity of the other enzymes tested, namely L-α-hydroxyacid oxidase, MDH, and LDH, likewise was the same as in the control (Table 1). During adaptation to cold no change was found in the relative weight of the liver and there was no increase in the protein concentration in the homogenate. Electrophoresis showed no significant differences in the polypeptide spectra of protein of the "nonuclear" homogenate of the control and experimental (hypothermia) samples (Fig. 1). In particular, the increase in the relative content of polypeptides with molecular weights of 80,000 and 12,000 daltons, characteristic of the effect of clofibrate, could not be found.

Exposure to low temperatures, other conditions being the same, thus did not lead to any increase in the rate of oxidative degradation of lipids in the liver cells. This fact indicates a difference in the adaptation mechanisms of regulation of lipid metabolism in hepatocytes and in cells of brown adipose tissue, the chief function of which is thermogenesis [12]. It also contradicts the previous observation according to which the number of peroxisomes in hepatocytes increases during hypothermia [14]. We found no change of this kind previously [2], in full agreement with the biochemical data given in the present paper. It must be pointed out, however, that the results of morphological observations require further experimental verification with the use of more specific histochemical methods of detecting catalase or peroxisomal oxidase activity at the electron-microscopic level, which would make it possible to eliminate any possibility of mistaken identification of other subcellular components as peroxisomes [1].

Oxidative reactions in peroxisomes are not coupled with ATP production. This is the basis for the view that they participate in processes of intracellular heat production which, in this particular case, was determined by direct dissipation of the energy formed during oxidation of peroxisomal substrates, primarily lipids [7]. Another probable function of peroxisomes is regulation of the intracellular metabolism of fatty acids and cholesterol, especially when their intake in the diet is increased. The number of peroxisomes in the liver and activity of enzymes of the β-oxidation chain in them increase considerably under these circumstances and the conditions are right for the acceleration of catabolism of high-energy lipids in mammals [8]. The level of peroxisomal metabolism thus depends essentially on dietary factors. At the same time, we know that in persons living in the far north energy metabolism switches from the carbohydrate type to the fat type, and this is accompanied by a change in the composition of the diet, in which lipids and proteins predominate but carbohydrates are appreciably reduced [4]. These facts, allowing for the absence of a direct effect of hypothermia on lipid metabolism in the hepatocytes, suggest that a definite role in mammalian adaptation to low temperatures is played by the alimentary factor — an increase in the lipid content of the diet, whose action is effected through activation of enzyme systems for lipid catabolism in the peroxisomes and mitochondria of the liver and various other organs.

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