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EFFECT OF 17α-ETHINYLESTRADIOL ON ACTIVITY OF RAT LIVER ENZYMES FOR SYNTHESIS AND HYDROLYSIS OF CHOLESTEROL ESTERS

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Administration of estrogens is known to lower the concentration of cholesterol esters (ChE) in the blood vessel wall and may delay the development of atherosclerosis [9]. At the same time, we know that under the influence of esterogens redistribution of concentrations of free cholesterol (FCh) and ChE takes place in rats between the blood and liver [3], as a result of intensification of receptor-dependent uptake of low-density lipoproteins by the hepatocytes [11]. However, the mechanisms of the intracellular redistribution of FCh and ChE in the liver under these conditions have not been adequately studied.

The aim of this investigation was to study the effects of 17α-ethinylestradiol (ETE) on activity of enzymes involved in synthesis and hydrolysis of ChE by the liver, and levels of FCh and bile acids (BA) in the bile in rats.

EXPERIMENTAL METHOD

Experiments were carried out on 60 male Wistar rats weighing 180-200 g, divided into two groups: 1) 30 animals received ETE (from Gedeon Richter, Hungary) in a dose of 25 μg/100 g body weight daily for 3 days intraperitoneally; 2) physiological saline was injected at the same time and by the same method into 30 animals (control). Twenty four hours before decapitation 10 animals from groups 1 and 2 were given an intraperitoneal injection of ^{14}C-cholesterol (^{14}C-ChS) (from Izotop, Leningrad) in a dose of 100 μCi/100 g, incorporated with lipofundin-20 (Finland). The animals were decapitated after deprivation of food for 16 h and the liver was removed and washed with a cold solution of 0.25 M sucrose, 1 mM EDTA, 5 mM Tris-HCl, pH 7.4, to remove blood, and homogenized in buffer of the same composition in the ratio of 1:5 (w/v). Individual liver fractions were obtained by differential centrifugation [14]. The bile duct was cannulated [3] in 6 experimental and 6 control animals under pentobarbital anesthesia before sacrifice, and bile was collected for 30 min.

Activity of lysosomal [5] and cytoplasmic [10] cholesterol esterases (ChEases; EC 3.1.1.13) was determined by using cholesterol [1-^{14}C]-oleate as the substrate. Activity of acyl-CoA-cholesterol-O-acyltransferse (ACAT, EC 2.3.1.26) was determined [12] in the supernatant

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TABLE 1. Effect of ETE on ChS and ChE Levels in Rat Liver Fractions (M ± m)

Liver fraction	Concentration of lipids, μg/mg protein				Specific radioactivity, cpm/mg protein			
	ChS		ChE		^{14}C-ChS		^{14}C-ChE	
	control (14)	experiment (14)	control (15)	experiment (15)	control (8)	experiment (9)	control (8)	experiment (9)
Homogenate	2,34±0,11	4,2±0,35	0,61±0,05	1,7±0,31	887±77	1932±151	86,8±9,6	176,9±20
Light mitochondria	3,97±0,34	8,2±0,77	0,05±0,006	0,25±0,035	—	—	—	—
Supernatant (12,000g)	2,52±0,21	4,36±0,41	1,32±0,1	3,0±0,3	937±67	2088±155	143±15	319±19

Legend. Significance of differences between control and experiment in all cases at the $P < 0.01$ level. Here and in Table 2, number of animals given in parentheses.

TABLE 2. Effect of ETE on Activity of ACAT, Cytoplasmic and Lysosomal ChEases, Acid Phosphatase, and β-D-Galactosidase of Rat Liver (M ± m)

Enzyme	Control	Experiment	P
ACAT	3,32±0,13 (18)	1,74±0,1 (17)	<0,001
ChEase:			
cytoplasmic	2,22±0,17 (12)	1,99±0,18 (14)	>0,05
lysosomal	2,65±0,13 (18)	3,2±0,15 (18)	<0,01
Acid phosphatase	67,5±1,86 (21)	82,38±1,96 (20)	<0,01
β-D-Galactosidase	1,59±0,04 (20)	1,77±0,05 (12)	<0,05

Legend. ACAT activity shown in percentage esterification of FCh/h/mg protein, ChEase activity in nanomoles cholesterol oleate hydrolyzed/min/mg protein, ChEase activity in nanomoles cholesterol oleate hydrolyzed/min/mg protein, and acid phosphatase and β-D-galactosidase activity in nanomoles nitrophenol/min/mg protein.

after centrifugation of the liver homogenate at 12,000g for 30 min. The radioactive substances used for determination of enzyme activity were obtained from Amersham Corporation (England). The nonsedimented and specific activity of lysosomal acid phosphatase and β-D-galactosidase was determined [6] by the use of p-nitrophenyl phosphate and p-nitrophenyl-β-galactoside (from Serva, West Germany) as the substrate.

Lipids were extracted by the method in [8] and fractionated by thin-layer chromatography on silica-gel H (Ferak, West Germany) in two systems of solvents: hexane—diethylether—acetic acid (60:40:1) and hexane—diethylether—acetic acid (90:10:1). Amounts of FCh and ChE, after extraction with chloroform, were determined spectrophorometrically [15]. FCh and BA were extracted from bile [3] and their concentration determined spectrophotometrically [1]. To construct a calibration curve, FCh (from Sigma, USA) and sodium deoxycholate (from Serva) were used. Radioactivity of products of the enzymic reactions and radioactivity of FCh and ChE in the liver fractions and bile were determined in dioxan scintillator with high counting efficiency [2] on a Mark III liquid scintillation counter (USA) by the STD-2 program. Protein was determined by Lowry's method [13]. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Administration of ETE for 3 days caused lowering of the levels of FCh (22.6 ± 1.6 mg/dl in the control, 9.8 ± 0.7 mg/dl in the experiment; $n = 39$, $P < 0.001$) and Che (37.1 ± 2.0 and 12.7 ± 0.8 mg/dl respectively; $n = 39$, $P < 0.001$) in the blood serum of the experimental animals. Similar changes in specific radioactivity of FCh (153.9 ± 8.3 cpm/μl in the control and 105.7 ± 7.7 cpm/μl in the experiment; $n = 16$, $P < 0.01$) and the ChE (300.1 ± 18.4 and 118.1 ± 20.0 cpm/μl respectively; $n = 16$, $P < 0.05$) were observed in the serum of rats receiving ^{14}C-FCh. Meanwhile, in the liver homogenate, the FCh concentration on average was doubled, the ChE concentration trebled, and specific radioactivity of FCh and ChE was significantly higher than in the control (Table 1).

On the basis of the view that there exist lysosomal and cytoplasmic intracellular "pools" of ChS, we studied concentrations of FCh and ChE in the fraction of "light" mitochondria and

TABLE 3. Concentrations of ChS and Total BA in Bile under the Influence of ETE (M ± m)

Group of animals (n = 6)	ChS concentration, μg/h/100 g body weight	BA concentration, μg/100 g body weight	Specific radioactivity in bile, cpm/100 μl bile	
			^{14}C-ChS	^{14}C-BA
Control	60,2±1,84	3,98±0,21	6392±298	61 270±5 343
Experimental	74,2±2,6	5,29±0,22	9127±425	77 890±3 445
P	<0,02	<0,01	<0,01	<0,05

supernatant of the liver (12,000g, 30 min). Injection of the hormone caused a twofold increase in the FCh concentration and a fivefold increase in the ChE concentration of the fraction of "light" mitochondria, with a more moderate increase in these parameters in the liver supernatant (Table 1). The marked rise in the FCh concentration in the fraction of "light" mitochondria was accompanied by stabilization of the lysosomal membranes, as shown by a decrease in the percentage of a nonsedimentated activity of acid phosphatase (7.32 ± 0.61 in the control, 5.6 ± 0.62 in the experiment; n = 40, $P < 0.05$) and β-D-galactosidase (6.12 ± 0.18 and 3.61 ± 0.12%, respectively, n = 44, $P < 0.05$) of the total activity of these enzymes.

Considering that the ratio of the ChE and FCh concentrations in the tissues is largely determined by the functional activity of intracellular hydrolysis and synthesis of ChE, an attempt was made to compare changes in these parameters with activity of lysosomal and cytoplasmic ChEases and of microsomal ACAT. A paradoxical decrease in ACAT activity was found under these circumstances in the liver of the experimental animals, against the background of a raised level of FCh (Table 2), an activator of this enzyme [4, 12]. These results agree with data in the literature on direct inhibition of ChE synthesis by ETE in hepatocytes in culture [7]. Addition of the estrogen under the present experimental conditions also caused a significant increase in activity of acid ChEase, acid phosphatase, and β-D-galactosidase, calculated per milligram of protein (Table 2); this effect is probably connected with activation of the lysosomal apparatus of the liver when uptake of blood lipoproteins by the hepatocytes is intensified. Activity of cytoplasmic ChEase showed no significant change.

Meanwhile the increase in ChS concentration in the liver under the influence of ETE was accompanied by an increase in the concentration of FCh by 23% and total BA by 33% in the bile of the experimental animals (Table 3), in agreement with data in the literature on an increase in the BA "pool" under the influence of estrogens [3].

The influence of ETE on cholesterol metabolism is thus based on a redistribution of FCh and ChE between the blood and liver. Accumulation of FCh in the liver under these conditions stimulates bile formation. Depression of ChE synthesis in the liver as the result of direct inhibition of ACAT by ETE may contribute to the fall in the ChS content in the body. These mechanisms probably lie at the basis of the positive effect of estrogens in atherosclerosis.

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