period and could lead to reduction of the adaptive powers of these organs vis-a-vis the sensitizing action of the allergen.

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CHANGES IN SERUM LIPIDS AND LIPOPROTEINS OF RATS AFTER OVARIECTOMY AND ADMINISTRATION OF THE METHYL ESTER OF 6-OXY-D-HOMO-8-ISOESTRONE

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KEY WORDS: ovariectomy; lipoproteins; cholesterol; estrogen analogs

During estrogen replacement therapy an increase in the frequency of nonlethal venous thromboembolism and myocardial infarction is observed, and is associated with elevation of the blood triglyceride (TG) level [2]. It was accordingly decided to look for analogs of steroid hormones that possess sufficient estrogenic activity but, at the same time, have no adverse action on lipid metabolism [4]. Under these circumstances a model of hypercholesterolemia induced by ovariectomy is often used [13].

The aim of this investigation was to study lipid metabolism in ovariectomized rats and the possibility of correcting it by estradiol and an isoestrone analog.

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EXPERIMENTAL METHOD

The methyl ester of 6-hydroxy-D-homo-8-isoestrone (MEI) was synthesized by the method of Torgov and Ananchenko [5], at the Department of Natural Compounds of the Chemical Faculty of St. Petersburg State University. Experiments were carried out on female albino rats receiving the standard laboratory diet. Hyperlipidemia was induced by ovariectomy, performed on 4-week-old animals. Rats of the control group underwent a mock operation. The substances for testing were injected 45 days after the operation over a period of 10 days intramuscularly in 0.1 ml of sunflower oil: estradiol in a dose of 0.01 mg/kg and MEI in a dose of 0.1 mg/kg daily. The control group were given 0.1 ml of sunflower oil. The animals were decapitated after deprivation of food for 14-18 h. Lipids were extracted from the blood serum [6] and concentrations of total cholesterol (ChS), α-ChS [7], and TG [11] were determined. To study the lipoprotein (LP) of the blood serum it was subjected to ultracentrifugation in a density gradient [14]. The results were subjected to statistical analysis [1].

From the results of analytical ultracentrifugation of the total serum LP fraction, the main classes and subfractions of LP were obtained [3]. The distribution of LP by flotation rates $F_{1.20}^0$ and S_f was obtained from the results of mathematical correction of schlieren profiles [8], recorded after 8, 14, and 52 min of analytical ultracentrifugation of the total LP fraction. High-speed flotation was carried out on a "Beckman" model E centrifuge with a standard optical system at 26°C and at 52,000 rpm. Before ultracentrifugation all the samples were dialyzed for 18-40 h at 4°C against a solution containing 0.196 M NaCl, 4.017 M NaBr, and 0.01% EDTA (d_0 = 1.300 g/ml).

The total LP fraction with hydrated density d < 1.250 g/ml was isolated by preparative ultracentrifugation for 40 h in a three-step gradient, in a "Beckman" 40 rotor at 4°C and 36,000 rpm. The gradient enabling isolation of the total LP fraction for the needs of analytical ultracentrifugation by one-step ultracentrifugation was formed by mixing blood serum and a solution of 0.196 M NaCl, 6.506 M NaBr, d_0 = 1.485 g/ml in a centrifuge tube in the ratio (v/v) of 1:1.25, by layering from above, up to a volume of 9.0 ml, a solution of 0.196 M NaCl, 3.273 M NaBr, d_0 = 1.250 g/ml and 0.5 ml of physiological saline. Correction of the schlieren profiles, determination of the distribution function of LP by flotation rates $F_{1.20}^0$ and S_f, their graphic representation, and variations in the effects of the test substances, were carried out by HP9845 computer system, using original program packages.

EXPERIMENTAL RESULTS

In the first series of experiments the time course of the change in ChS and TG levels of the rats from the 37th through the 76th days after ovariectomy was studied. A significant increase was found in the serum ChS concentration within the interval from the 42nd through the 54th day and on the 70th day after the operation (Fig. 1). The results of a normalized distribution by flotation rates of the total serum LP fraction of the rats in the 8th week after the operation are given in Fig. 2. Clearly, after ovariectomy, "abnormal" LP particles, with flotation rates below those of low-density lipoproteins (LDL) but higher than those of high-density lipoproteins (HDL), appeared in the blood serum.

The total serum ChS rose in this case from 1.72 ± 0.15 in animals of the control group to 2.09 ± 0.07 mmoles/liter in ovariectomized rats ($p < 0.05$). The TG level in the control and ovariectomized rats showed no significant differences. Determination of α-ChS in the subsequent series of experiments revealed a fall after ovariectomy. At the same time, the results of analytical ultracentrifugation show that the HDL concentration not only did not fall but actually had a tendency to rise. It will be clear from Fig. 2 that this took place mainly on account of "abnormal" HDL, whose flotation rate is close to the flotation rate of nascent HDL, present in the blood of newborn infants, and also of patients with heredity lecithin-cholesterol acyltransferase (LCAT) insufficiency [9]. HDL of this kind, rich in ChS, have been described in the literature [10] as HDLc. They can be detected within the density range from 1.063 to 1.090 g/ml and they are precipitated by heparin and $MgCl_2$. Both HDLc and LDL have been shown to bind equally with the surface receptors of cells, leading to ChS accumulation in the intercellular space [10]. On the other hand, it has been shown that estradiol causes an increase in serum LCAT activity [12]. In this connection, the appearance of "abnormal" HDL, which we found, can be explained as the result of a fall of estrogen levels of ovariectomy.

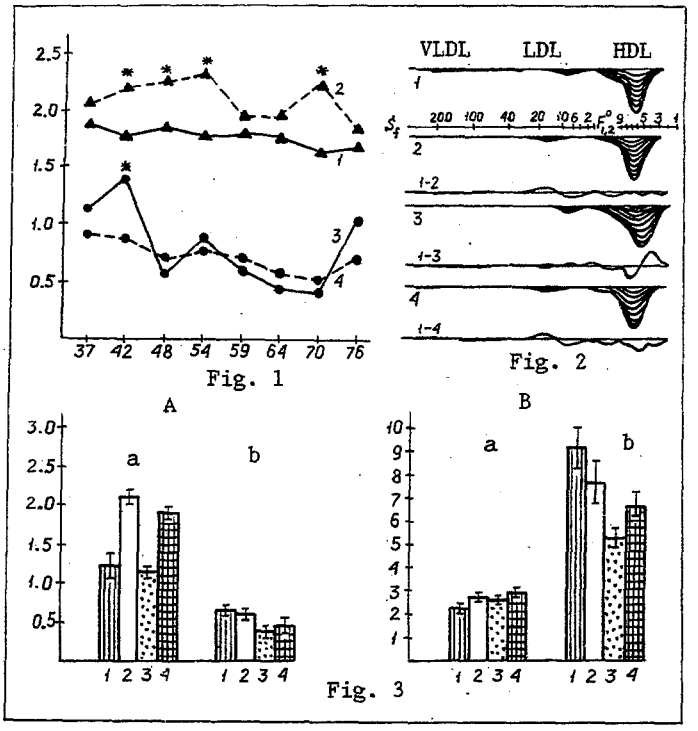

Fig. 1

Fig. 2

Fig. 3

Fig. 1. Effect of ovariectomy on blood serum lipids in rats: 1) ChS content in intact rats; 2) ChS content in ovariectomized rats; 3) TG content in intact rats; 4) TG content in ovariectomized rats. *$p < 0.05$.

Fig. 2. Distribution of lipoproteins by flotation rate in three-step density gradient: 1) spectrum of LP of ovariectomized rats; 2) spectrum of LP of intact rats; 3) spectrum of LP of ovariectomized rats receiving estradiol; 4) spectrum of LP of ovariectomized rats receiving MEI. VLDL) very low density lipoproteins.

Fig. 3. Effect of MEI and estradiol on ChS and TG content in blood serum and liver of ovariectomized rats. A) changes in ChS (a) and TG (b) content in blood serum of rats: 1) intact; 2) ovariectomized; 3) ovariectomized and receiving MEI; 4) ovariectomized and receiving estradiol. B) change in content of ChS (a) and TG (b) in liver of rats: 1) intact; 2) ovariectomized; 3) ovariectomized and receiving MEI; 4) ovariectomized and receiving estradiol.

In the next series of experiments the effect of MEI on the ChS and TG content in the blood serum and liver of the experimental animals was studied. MEI in a dose of 0.1 mg/kg led to normalization of the serum LP spectrum, disturbed after ovariectomy (Fig. 2). The total ChS level under these circumstances fell from 2.09 ± 0.07 in the ovariectomized rats to 1.68 ± 0.06 mmole/liter in animals receiving MEI ($p< 0.01$). The TG level also fell from 0.59 ± 0.03 to 0.41 ± 0.04 mmole/liter ($p < 0.01$) (Fig. 3). The ChS and TG content in the liver of these groups of animals did not differ significantly. Incidentally, estradiol in a dose of 0.01 mg/kg did not lead to normalization of the serum LP spectrum of the ovariectomized rats (Fig. 2). The ChS content in the liver under these conditions increased from 2.68 ± 0.08 in ovariectomized animals to 2.98 ± 0.1 mg/kg ($p < 0.05$) in rats receiving estradiol.

It can be concluded that MEI, given to ovariectomized rats, leads to normalization of their serum LP spectrum, which is not observed when estradiol is given. MEI also possesses a marked hypocholesterolemia action. It can be tentatively suggested that the estrone derivative studied is a promising candidate for possible replacement therapy after ovariectomy, especially to correct disturbances of lipid metabolism.

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AGE DIFFERENCES IN HORMONAL REGULATION OF Na^+,K^+-ATPASE ACTIVITY IN THE RAT RENAL CORTEX

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One of the chief regulators of sodium transport in the renal tubules is aldosterone. The effect of aldosterone is due to regulation of expression of aldosterone-dependent genes as a result of interaction between hormone–receptor complexes and the nucleus of the target cell; aldosterone is known to bind with receptors of two types: high-affinity mineralocorticoid and low-affinity glucocorticoid [12]. The most important of the proteins induced by aldosterone is the enzyme Na^+,K^+-ATPase, responsible for active transport of Na^+ and K^+ ions through the basolateral membrane of the epithelial cells of the nephron. Na,K-ATPase is a tetramer composed of two types of subunits, α and β. In mammals born blind (including rats), definitive morphological and functional formation of the kidneys is complete in the postnatal period, and at an early age the epithelium of the nephron is insensitive to the regulatory action of aldosterone. The ability of rat kidneys to change Na^+ reabsorption in response to aldosterone is manifested only at the end of the period of weaning, i.e., after the 20th-25th day of postnatal life [1, 13]. It was shown previously that mineralocorticoid receptors are present in the cytosol of the renal cortex of 10-day-old rats [2, 4]. However, nothing has previously been published on the localization of the mineralocorticoid receptors along the nephron in early postnatal ontogeny. In adult animals the main target for the action of aldosterone is known to be

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