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SEX DIFFERENCES IN ESTROGEN RECEPTOR ACCUMULATION IN LIVER CELL NUCLEI AND INCREASE OF BLOOD PLASMA ANGIOTENSINOGEN CONCENTRATION AFTER INJECTION OF LOW DOSES OF SYNTHETIC ESTROGENS IN RATS

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The writers previously showed the existence of sexual differentiation of the number of estrogen receptors (ER) in rat liver cytosol and described an endocrine mechanism of its formation and maintenance, based on the negative regulatory action of androgens and the direct regulatory stimulating effect of pituitary somatotrophic hormone [4]. The aim of the present investigation was to study the role of sex differences in the ER level in hepatocytes in the realization of the direct effects of estrogens in the liver of male and female rats. The experimental model for the study of this problem consisted of angiotensinogen (AG). In this decision we were guided by the following considerations. First, it can be taken to be convincingly proved that estrogens have a direct stimulating effect on its production, and that ER of hepatocytes play a key role in the realization of this effect [1, 5, 7, 9, 11]. Second, the plasma AG level in rats is not initially sexually differentiated [1, 5]; this state of affairs facilitates the recordings of differences in the degree of the stimulating action of estrogens on this parameter (if such differences are in fact observed in male and female rats). To stimulate AG production by the liver we used the synthetic estrogens hexestrol (HE) and ethinylestradiol (EE_2), in doses (0.5 and 1 μg) corresponding to physiological concentrations of the natural hormone. Synthetic estrogens, not readily metabolized, were used with the aim of eliminating the "contribution" of enzymes responsible for metabolism of sex steroids and, in particular, of estrogen-binding protein, in the development of the specific reaction.

The question of the presence of sexual differentiation of the reaction of AG production in response to estrogens is interesting because female sex steroids are used therapeutically not only in women, but also in men. It has been shown, for instance, that the use of synthetic estrogens in prostatic carcinoma in men leads to a change in the plasma AG level [6]. It has been known for quite a long time that estrogens play a role in the system maintaining the immune response, and that they have a protective role against radiation damage [8, 10]. The question of the use of estrogens to modulate immunogenetic processes in various immunodeficiency states and in radiation leukopenia is likely to prove highly topical at the present time [12].

EXPERIMENTAL METHOD

Experiments were carried out on male and female noninbred rats. The gonads and pituitary gland were removed 3 weeks before the beginning of the experiment. HE and EE_2 (Sigma, USA) for injection were dissolved in propylene-glycol and injected

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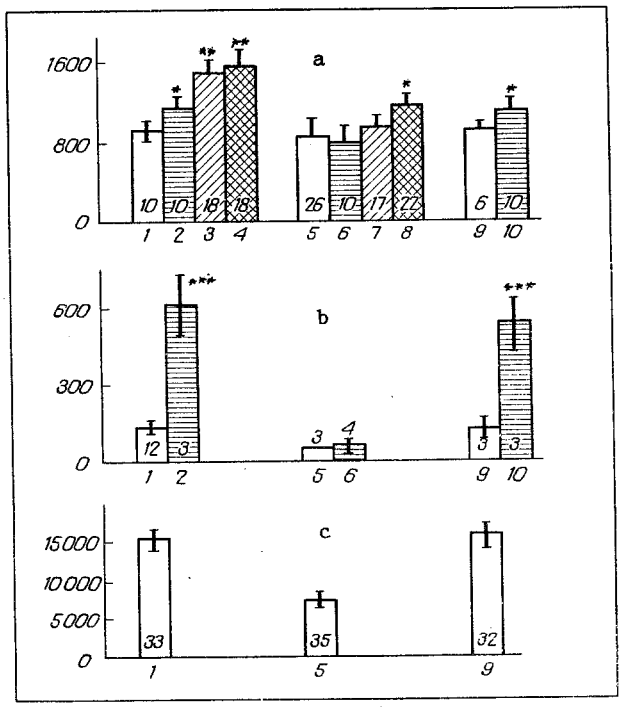

Fig. 1. Initial content of ER in rat liver cytosol and effect of injection of low doses of synthetic estrogens on plasma AG level and on ER content in suspension of rat cell liver nuclei. Abscissa: 1-4) ovariectomized females; 5-8) intact males; 9, 10) gonadectomized males; 1, 5, 9) control animals; 2, 6, 10) after injection of 0.5 μg HE; 3, 7) after injection of 0.5 μg EE_2; 4, 8) after injection of 1 μg EE_2. Ordinate: a) Plasma AG concentration (in ng/ml); b) ER concentration in liver cell nuclei (in bonds per cell); c) ER concentration in liver cytosol (in bonds per cell). Numbers in columns denote number of experiments.

subcutaneously in doses of 0.5 or 1 μg in 0.2 ml. The control animals received an injection of the solvent. The ER concentration in the liver cytosol was determined by the ligand exchange method at a low temperature (0-4°C) with the use of sodium thiocyanate [2]. ER in the nuclear fraction of the liver cells were determined 1 h after injection of the estrogens, when the highest level of accumulation of estrogen–receptor complexes (ERC) was found in the nucleus, by the method described previously [3]. Blood samples for determination of AG were taken 24 h after injection of the estrogens, for it is at that time that the AG level in the blood reaches its maximum [1]. The method of obtaining plasma and details of determination of AG by radioimmunoassay using the SB-REN-2 kit ("Sorin," Italy, France) were described previously [1]. The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Determination of the plasma AG level of rats 24 h after receiving an injection of 0.5 μg HE showed that the concentration of the renin substrate was significantly increased by 20-30% in gonadectomized males and females, whereas the plasma AG level of intact males remained unchanged (Fig. 1a). A similar picture also was observed after injection of 0.5 μg of EE_2. With an increase in the dose of injected hormone (1 μg EE_2) the plasma AG level of the intact males was significantly increased, but the sex differences in the degree of elevation of the plasma renin-substrate concentration still remained. Sex differences in the response of the AG level to injection of estrogens were evidently manifested more clearly when low, threshold doses were used, and, as we showed previously [1, 5], they could disappear if above physiological doses were used.

Comparison of the results given above with the original ER content in the cytosol and with the level of accumulation of ERC in the liver cell nuclei of these animals gave the following results. In the liver of intact males, where the cytosol ER level was only half as high as in females, the content of nuclear ER was extremely low, and showed a small increase 1 h after injection of 0.5 μg HE, although this was not significant (Fig. 1b, c). In females and gonadectomized males, with an initially higher level of cytosol and nuclear receptors, the ERC concentration in the nuclei rose four-fivefold after injection of HE.

The decisive importance of the initial ER content in the liver cell, determining the sensitivity of the test liver fraction to threshold doses of estrogens, was confirmed by the experiments with estrogenization of hypophysectomized female rats. For instance, the original ER concentration in the liver cytosol fell after hypophysectomy from 116 ± 5 (n = 33) to 22 ± 3 (n = 12) fmoles/mg cytosol protein, and injection of 0.5 μg HE was ineffective. The plasma AG level of the estrogenized females after hypophysectomy was 669 ± 18 ng/ml (n = 10), which did not differ from the control values (633 ± 30 ng/ml).

The existence of sexual dimorphism in ER level may thus in some cases evidently determine the differential reaction of metabolic processes in the male and female rat liver in response to estrogens.

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