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SEX DIFFERENCES IN NUMBER OF ESTROGEN RECEPTORS IN RAT LIVER CYTOSOL

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Many functions of the liver, which occupies a central place in metabolism and in the maintenance of homeostasis, exhibit sexual differentiation [4]. In particular, sexual dimorphism is observed in the activity of many of the enzymes of steroid metabolism, the content of corticosteroid-binding plasma globulin, and intracellular steroid-binding proteins (the unusual estrogen-binding protein UEBP) and androgen receptors in rats [2, 7, 10, 11, 15]. The latter, which determine differences in sensitivity of the liver in males and females to the action of androgens and estrogens, are themselves targets for regulatory and programming influences of the sex hormones.

As regards estrogen receptors (ER) of the liver, the question of the presence or absence of sex differences in their number and properties still remains unclear. Taking into account data on the role of ER in the realization of the direct effect of estrogens on projection of angiotensinogen, UEBP, and $\alpha_{2\mu}$-globulin by hepatocytes [3, 13, 15], the solution of this problem may be of fundamental importance for our understanding of the causes of the possible differences in their action on liver function in males and females. Data in the literature on this question are very contradictory [8, 9]. These contradictions are evidently connected with the inappropriateness of the methods used to determine ER by some workers in the rat liver. The point is that besides classical ER, UEBP with high binding capacity also are present in male rat liver cytosol [5]. The presence of UEBP adds significantly to the difficulty of precise quantitative determination of ER in the male rat liver.

The method which we suggested previously for determining ER in rat liver cytosol, with the use of sodium thiocyanate [1], has proved to be capable of the differential determination only of ER and not of UEBP. By using the method in [1] we demonstrated the existence of sex differences in the number of ER in the rat liver and we also studied the effect of gonads and the pituitary on this feature.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats. Males and females underwent gonadectomy at different stages of ontogeny. Animals undergoing mock operations at the same stages of ontogeny served as the control. Animals undergoing gonadectomy at different stages of ontogeny were used in the experiments on reaching the age of 12 weeks, whereas sexually mature animals were used 2-3 weeks after gonadectomy. Rats subjected to hypophysectomy were used in the experiments 3 weeks

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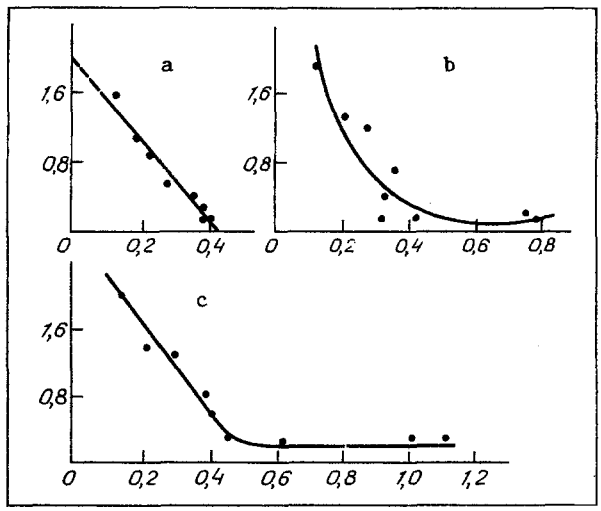

Fig. 1. Scatchard plot of specific binding of $^3HE_2$ in liver cytosol of male rats. a) In presence of NaSCN, b, c) without addition of NaSCN. Here and in Fig. 2: B_S) specifically bound, U) free $^3HE_2$. The following were used to determine nonspecific binding: a, b) excess of unlabeled diethylstilbestrol, c) excess of unlabeled estradiol. For a: concentration of ER 47 fmoles/mg cytosol protein. $K_g = 5 \cdot 10^8\ M^{-1}$. Abscissa, B_S (in nM); ordinate, ratio B_S/U.

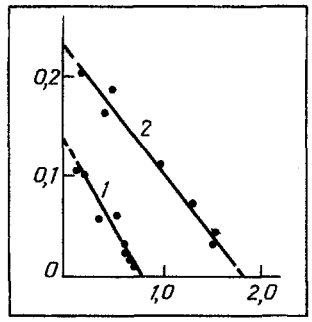

Fig. 2. Scatchard plot for specific binding of $^3HE_2$ in cytosol fraction obtained by salting out with ammonium sulfate at 30% saturation. 1) Males, 2) females. Value of K_g was 1.2 and $2 \cdot 10^8\ M^{-1}$, concentration of ER 89 and 237 fmoles/mg protein of salted out fraction for 1 and 2, respectively.

after the operation. The ER level in the liver cytosol of the rats was determined as described previously [1]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

To determine ER in the liver cytosol of male rats we used the method of low-temperature ligand exchange, with the aid of sodium thiocyanate (NaSCN), on the assumption of differences in interaction of estrogens with UEBP and ER. First, complexes of UEBP with estradiol are very labile and, in addition, UEBP virtually do not interact with synthetic estrogens [5]. For that reason the presence of a chaotropic salt (NaSCN) in the incubation medium, with the property of intensifying dissociation of ligands from their complexes with protein [14], and also the use of an excess of unlabeled synthetic estrogen (diethylstilbestrol) to determine nonspecific binding, enabled us to differentiate specific binding of labeled estradiol ($^3HE_2$) with ER in the liver cytosol of males and binding with UEBP completely (Fig. 1). Thus when the graphs A, B, and C in Fig. 1 are compared it will be seen that the

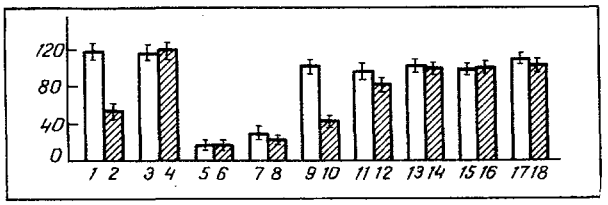

Fig. 3. Effect of sexual maturation, hypophysectomy, and gonadectomy, performed at different stages of ontogeny, on ER level in rat liver cytosol. Abscissa: 1, 2) mature rats, 3, 4) rats undergoing gonadectomy when sexually mature, 5, 6) sexually immature, 7, 8) after hypophysectomy, 9, 10) rats undergoing mock fonadectomy neonatally, 11, 12) neonatally, 13, 14) 1 week after birth, 15, 16) 2 weeks after birth, 17, 18) 4 weeks after birth. $n_1 = 23$, $n_{2,3} = 34$, $n_{4,11,14} = 19$, $n_{5,16,17} = 13$, $n_6 = 25$, $n_{7,8} = 8$, $n_{9,18} = 7$, $n_{10,15} = 15$, $n_{12} = 17$, $n_{13} = 21$. Ordinate, ER level in liver cytosol (in fmoles/mg cytosol protein); unshaded columns denote females, shaded columns — males. Statistical significance of differences: $p_{1\text{-}2} < 0.001$, $p_{1\text{-}3} > 0.1$, $p_{1\text{-}5} < 0.001$, $p_{1\text{-}7} < 0.001$, $p_{1\text{-}9} > 0.1$, $p_{1\text{-}13} > 0.1$, $p_{1\text{-}15} > 0.1$, $p_{1\text{-}17} > 0.1$, $p_{2\text{-}4} < 0.001$, $p_{2\text{-}6} < 0.001$, $p_{2\text{-}8} < 0.05$, $p_{2\text{-}10} > 0.1$, $p_{2\text{-}14} < 0.001$, $p_{2\text{-}16} < 0.001$, $p_{2\text{-}18} < 0.001$, $p_{9\text{-}11} > 0.1$, $p_{10\text{-}12} < 0.001$, $p_{11\text{-}12} > 0.1$.

Scatchard plot is a straight line only in the presence of NaSCN and an excess of diethylstilbestrol, evidence that $^3HE_2$ interacts with one type of binding sites. Under these circumstances the equilibrium association constant (K_g) was $(4.8 \pm 1.9) \cdot 10^8\ M^{-1}$ and corresponded to that in the liver cytosol of female rats when measured under analogous conditions [1]. Specific binding of $^3HE_2$ was completely suppressed by an excess of unlabeled hexestrol, which is a characteristic feature of ER but not of UEBP. By using this method and with one saturating addition of $^3HE_2$, we found that the ER level in the liver cytosol of females ($n = 33$) was twice as high as in the liver cytosol of males ($n = 34$): 116 ± 5 and 55 ± 4 fmoles/mg cytosol protein.

To obtain more convincing proof of sex differences in the ER content in rat liver we used the method of physical separation of ER and UEBP, by salting out the cytosol with ammonium sulfate at 30% saturation. UEBP is known to be salted out by ammonium sulfate only at 55% saturation [5]. Scatchard plot analysis of specific binding of $^3HE_2$ in the salted out fraction of liver cytosol from male and female rats showed that the equilibrium K_g of estradiol with ER is independent of sex, whereas the concentration of binding sites is 2.7 times higher in females than in males (Fig. 2). Determination of the ER concentration by saturating addition in the cytosol fraction obtained at 30% saturation of ammonium sulfate confirmed the presence of sex differences in the ER level in the rat liver cytosol: 108 ± 15 and 233 ± 34 fmoles/mg protein of the salted out fraction in males ($n = 7$) and females ($n = 7$), respectively.

It must be emphasized that differences in the ER level in the rat liver appeared only in animals on reaching sexual maturity. In immature rats the ER level in the cytosol was low and amounted to 15.3 ± 3.0 fmoles/mg cytosol protein in females ($n = 25$) and 15.26 ± 2.6 in males ($n = 25$) (Fig. 3), i.e., no sex differences are present.

These results point indirectly to participation of sex hormones in the formation of the sex dependence of ER in the rat liver. We accordingly studied the liver ER level in rats after gonadectomy, performed at different stages of ontogeny. It will be clear from Fig. 3 that orchidectomy at sexual maturity or prepubertally and neonatally, leads to elevation of the ER level in males up to the characteristic value for females. These data most probably indicate a negative effect of androgens, secreted by male gonads, on the ER level in the rat liver. The effect of androgens is evidently regulatory in character, for gonadectomy performed on males whether during the first day of life or during sexual maturity, gives the same result. Thus unlike various other sex-differentiated liver functions which have been studied from this point of view, such as production of UEBP, transcortin, and $\alpha_{2\mu}$-globulin, and activity of certain enzymes of steroid and xenobiotic metabolism [2, 10, 11, 15], in the case of ER of the liver, under real physiological conditions androgens are unable to give rise to programming (irreversible) effects certain critical stages of ontogeny.

Female sex hormones secreted by the ovaries probably do not play an essential role in the regulation of the ER content in the female rat liver at any of the stages of ontogeny which were studied, for removal of the ovaries at any of the periods specified above does not affect the ER concentration in the female rat liver (Fig. 3).

A decisive role in regulation of the ER level in the rat liver is played by the pituitary, for its removal gives rise to a sudden fall of the ER level in the female liver and a significant decrease in the ER concentration in males after 3 weeks. Sex differences in the ER content in the liver disappear under these circumstances. Considering that functional castration of the males, developing against the background of prolonged absence of the pituitary, ought to have produced the opposite results, namely an increase in the ER content in the liver (Fig. 3), the possibility cannot be ruled out that pituitary secretes a certain factor (or factors) regulating the ER level positively in both female and male rats.

It must be emphasized that the present [3] and other writers [6, 12] have demonstrated a stimulating role of somatotrophic hormone in relation to ER in the liver of female and male rats.

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GROWTH OF AXONS IN ORGANOTYPICAL SPINAL CORD CULTURE

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KEY WORDS: culture; axons; growth bulb; glial cells

Growth of axons and selective establishment of synaptic contacts are important stages in the recovery of function of various parts of the nervous system. Sprouting of axons has been described in the central and peripheral nervous system in adult animals. Particular attention is currently being paid to the study of growth of axons in organotypical and dissociated cultures [4, 15, 7]. The mechanism of growth of axons and formation of selective contacts is contained in the membrane receptors of the cone of growth. The study of the structural organization of the cone of growth has shown that this part of the axon has a complex organization, and the microtubules which constitute its main components are not only responsible for the mechanical structure of the lengthening neurite [6, 9, 11], but are also involved in the function of transporting material to the growing end of the nerve fiber

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