It is thus clear that the increase in brain ACE activity after intraventricular injection of adrenalin and dibutyryl-cyclic AMP is the result of inductive synthesis of the enzyme. The effects of adrenalin and of cyclic AMP are similar in direction and are mediated through β-adrenergic receptors. The potentiation of ACE activity after blocking of the α receptors can be explained by elimination of their inhibitory effect on the β-adrenergic receptors.

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A MULTICOMPONENT SYSTEM OF ESTRADIOL-BINDING PROTEINS IN RAT LIVER CYTOSOL AND ITS DEPENDENCE ON SEX STEROIDS

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The marked sex differences in the ratio between the hormonal capacity of estradiol-binding components with Stokes' radii (**a**) of 7.0 and 2.5 nm observed in sexually mature animals are somewhat reduced but do not disappear completely after gonadectomy. Prolonged administration of estradiol (50 μg, 8 days) to gonadectomized rats leads to depression of the estradiol-binding activity of all components of liver cytosol of females and males. Injection of testosterone propionate (2 mg, 8 days) into gonadectomized animals leads to selective stimulation of a special estrogen-binding protein with **a** = 2.5 nm, normally characteristic of males alone, in both males and females. It is postulated that sex differences in the system of estradiol-binding proteins of the rat liver cytosol are due to sexual differentiation of the system in the early stages of development, on the one hand, and to the active regulatory influence of androgens and estrogens in the late stages of development, on the other hand.

KEY WORDS: estrogens; estradiol-binding proteins; liver; sex steroids.

The liver of fishes, amphibians, and birds, under the influence of estrogens, can produce the egg proteins lipovitellin and phosphitin and can thus play a direct part in the reproductive process [7, 10]. In mammals, the effects of estrogens on the reproductive function mediated through the liver are not so clearly defined and they

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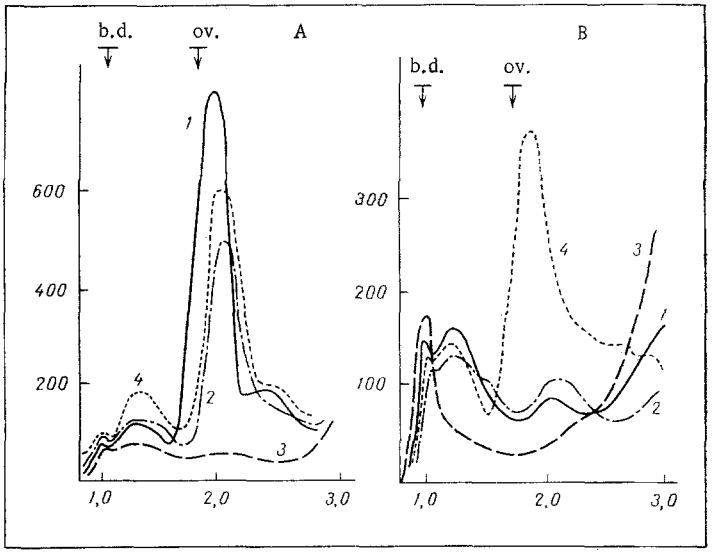

Fig. 1. Elution of complexes of estradiol-^{3}H with liver cytosol proteins of male (A) and female (B) sexually mature rats from Sepharose-6B columns: 1) intact rats; 2) gonadectomized rats; 3) gonadectomized rats receiving estradiol; 4) gonadectomized rats receiving testosterone propionate. Arrows indicate maxima of evolution of blue dextran (b.d.) and ovalbumin (ov.). Abscissa, ratio between elution volume (V_e) and dead volume of column (V_0); ordinate, radioactivity (in cpm/ml/mg protein), measured in 25-30 aliquots of fractions of eluate.

are more complex and varied [8, 12]. At the same time, the very fact that the liver cells of mammals are sensitive to estrogens, irrespective of the character of the hormonal effects, is very interesting in connection with the search for models with which to study the mechanisms of action of steroid hormones. Protein components capable of binding estradiol specifically have been found in the cytoplasm and nuclei of rat liver cells [3, 5]. Significant sex differences were discovered by the writers previously in the system of estrogen-binding proteins in the cytosol of rat liver cells [3].

The object of this investigation was to discover the factors determining these differences.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature female and male rats of a mixed population. The following groups of animals were used: intact, gonadectomized (males, 2-3 weeks before the beginning of the experiment; females, 4-5 weeks before), gonadectomized and receiving 50 μg estradiol or 2 mg testosterone propionate in 0.4 ml propylene glycol intramuscularly daily for 8-9 days. The animals were killed 2 and 1 day, respectively, after the last injection of estradiol and testosterone propionate. The liver was perfused with cold 0.9% NaCl, minced, and washed two or three times with 10 mM Tris–HCl buffer (pH 7.5 at 17°C), containing 10 mM KCl and 1 mM EDTA. The tissue was homogenized at 4°C in the same buffer, with the addition of 6 mM dithiothreitol. All subsequent procedures were carried out at 0-4°C. The supernatant fraction (cytosol) was obtained by centrifugation of the homogenate for 1 h on a Spinco (model E, rotor "I") centrifuge at 140,000g. The cytosol, containing 20-30 mg protein/ml, was incubated with 1-2 ng estradiol-17β-6,7-^{3}H (specific activity 41 Ci/mmole; Radiochemical Centre, Amersham, England). The samples were then applied to Sepharose-6B (Pharmacia, Sweden) columns, calibrated against standard proteins and equilibrated with 10 mM Tris-HCl–10 mM KCl–1 mM EDTA buffer (pH 7.5, 17°C), and eluted with the same buffer. The conditions of incubation of the samples with the hormone and of elution from the columns were described previously [3]. In individual experiments, in order to study the kinetics of dissociation of the hormone–protein complexes, after incubation with estradiol-^{3}H the samples were further incubated with an excess of unlabeled estradiol (400 ng) at 4°C for 45 min. The quantity of radioactivity in the fractions of eluate was determined by the method described earlier [4]. The protein concentration in the samples was determined by Lowry's method [11].

TABLE 1. Effect of Gonadectomy and Injection of Testosterone Propionate into Animals on Distribution of Bound Estradiol-^{3}H between Fractions of Estrogen-Binding Proteins with Stokes' Radii of 7.0 and 2.5 nm in Rat Liver Cytosol (M ± m)

Sex	Group	Estradiol-^{3}H, bound with protein fraction with **a** = 2.5 nm, %
Females	Intact	36,7±1.4 (8)
	Ovariectomized	51,4±1,7 (7)
	Ovariectomized, receiving testosterone	74,4±6,0 (3)
Males	Intact	90,7±1,8 (5)
	Castrated	81,8±2,5 (4)
	Castrated, receiving testosterone	93,5±1.9 (3)

Legend: 1) Total quantity of radioactivity in protein fractions with **a** = 7.0 and 2.5 nm taken as 100%; 2) number of determinations in parentheses.

EXPERIMENTAL RESULTS

Systems of specific proteins binding estradiol in the liver cytosol of male and female rats show some well-defined sex differences: 1) Besides estrogen-binding components with Stokes' radii (**a**) of about 7.0 and 2.5 nm, which are present in both male and female liver, the male liver also contains an additional estradiol-binding component with a value of **a** below 2.0 nm (Fig. 1); 2) the ratio between the hormonal capacity of the estradiol-binding components with values of **a** of 7.0 and 2.5 nm differs sharply in females and males (Table 1); 3) the estradiol-binding protein of males with **a** about 2.5 nm differs qualitatively from the female component with the same value of **a** and also from the other estradiol-binding components of the liver cytosol of males and females.

The results given in Table 2 show that only with this protein does estradiol-^{3}H form labile complexes capable of dissociating virtually completely during incubation for 45 min with unlabeled estradiol.*

Ovariectomy on females had no significant effect on the total content of estradiol-binding components of the liver cytosol (Fig. 1), but the ratio between the hormonal capacity of components with **a** = 7.0 and 2.5 nm was shifted a little toward the latter (Table 1). Castration of the males gave the opposite effect: The hormonal capacity of the component with **a** ~ 2.5 nm fell significantly, whereas that of the component with **a** ~ 7.0 nm was not noticeably changed (Fig. 1). As a result the ratio between the hormonal capacity of components with **a** ~ 7.0 and 2.5 nm was shifted in the characteristic direction for females (Table 1). However, it must be emphasized that although gonadectomy on the rats reduces the difference between females and males as regards the hormonal capacity of the estradiol-binding components of the liver, it does not completely abolish all the sex differences mentioned above. Evidently it is not estrogens alone that determine sex differences in the hormonal capacity of the estradiol-binding components of the liver. Injection of estradiol into gonadectomized animals led to a sharp decrease in the hormonal capacity of all components of the liver cytosol in both females and males. The inhibitory effect of the estrogen was evidently not the result of competition between unlabeled estradiol, introduced in vivo, and estradiol-^{3}H for the binding sites on the proteins during incubation of the cytosol in vitro: In the present experiments 2 days elapsed between the time of the last injection of the hormone and sacrifice of the animals, a time evidently sufficient for virtually complete removal of estrogens from the body [9].

In a certain respect testosterone has the opposite action to estradiol on the system of estradiol-binding proteins of the liver. Prolonged administration of testosterone to gonadectomized animals led to a sharp increase in the hormonal capacity of the cytoplasmic component with **a** ~ 2.5 nm in the liver of the females and

*The estradiol-binding protein of the liver cytosol of males with **a** ~ 2.5 nm will subsequently be described as SEBP (special estrogen-binding protein).

TABLE 2. Kinetic Analysis of Various Fractions of Estrogen-Binding Proteins of Liver Cytosol of Intact Rats and Rats Receiving Testosterone Propionate

Sex	Group	Bound estradiol-^{3}H, cpm					
		7.0-nm fraction		2.5-nm fraction		5-nm fraction	
		1	2	1	2	1	2
Males	Intact	18 195	15 755	76 945	7 588	34 279	34 358
Females	Intact	37 824	31 220	20 001	21 383	—	—
	Ovariectomized, receiving testosterone	33 938	43 390	140 546	19 579	—	—

Legend. 1) Control; 2) after incubation with estradiol-^{3}H, samples were additionally incubated with an excess of unlabeled estradiol.

to a small increase in the capacity of this component in the liver of the males (Fig. 1). The capacity of components with **a** ~ 7.0 nm, however, did not change substantially in either males or females after administration of the androgen. As a result the ratio of the hormonal capacity of components with **a** ~ 7.0 and 2.5 nm was shifted in the direction characteristic of males (Table 1). Investigation of the kinetics of dissociation of the estradiol-^{3}H–protein complexes showed (Table 2) that testosterone stimulates the appearance of the estradiol-binding component characteristic of males, i.e., of SEBP, in females. As regards the regulation of the estradiol-binding component with a value of **a** below 2.0 nm, no definite results have yet been obtained because of incomplete separation on gel filtration of a complex of estradiol-^{3}H with that protein and unbound estradiol-^{3}H.

Four protein components capable of binding estradiol specifically were thus found in the soluble fraction of rat liver cells. The assortment of these components in the system of estrogen-binding proteins and their relative hormonal capacity depend on sex. The results of the study of the effect of gonadectomy on the estradiol-binding proteins of the liver indicate that the sex steroid level is not the only factor determining the various sex differences discovered. It is tempting to suppose that systems of estrogen-binding proteins in the liver are formed in the early postnatal period of life as a result of "imprinting" by androgens, as takes place in the case of the sex differentiation of the functions of the hypothalamus and the system of enzymes of steroid metabolism in the liver [1, 10]. The determinacy of the type of system of estrogen-binding proteins does not by any means exclude, however, an active role of estrogens and androgens in the regulation of the content of estradiol-binding proteins in sexually mature animals. Estrogens act on these liver proteins unselectively, depressing their ability to interact with estradiol in both females and males. Evidence now accumulated on the properties of estrogen-binding liver proteins [3, 6] suggests that these proteins are responsible for the reception of the estrogenic signal by the liver cells. In the light of this interpretation the inhibitory action of estradiol on the hormonal capacity of the receptors of this hormone is physiologically rational. During mass action of estrogens on the liver cells the sensitivity of the hormone-receiving system is lowered because of a decrease in the hormonal capacity of the receptors by the negative feedback principle. This response of the estradiol receptors to estrogens is evidently common to several systems which have been studied [2]. By contrast with estrogens, androgens stimulate binding activity in the liver of gonadectomized animals. Under these circumstances stimulation is selective and aimed at the estradiol-binding component, which is characteristic of the male liver only (SEBP). The sensitivity of this protein to the action of androgens is yet another of its unusual properties, the physiological significance of which will be made clear only as a result of further investigations.

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