EFFECT OF SOME ENDOCRINE FACTORS ON THE CONTENT OF A SPECIFIC ESTROGEN-BINDING PROTEIN IN RAT LIVER

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Receptors of steroid hormones are evidently an irreplaceable component of the system for realization of the hormonal signal in the cell. Data so far obtained suggest that the receptor population, even within the same tissue, may be heterogeneous. Heterogeneity of receptor proteins, it is suggested, is one cause of the diversity of the cell response to a hormone [1, 7].

A specific estrogen-binding protein (SEBP), which differs significantly in some of its properties from classical estrogen receptors [3], was isolated and characterized previously in our laboratory. One of the particular features of this protein, of interest at the present time to many investigators [8, 9], is the high dependence of its concentration on the sex steroid level [6].

As a result of the development of a method of differential quantitative determination of SEBP among other liver proteins specifically binding estradiol, it was possible in the present investigation to approach the study of the role of androgens in the induction and regulation of the content of this protein in liver cells, a matter of great importance in connection with determination of the biological role of SEBP.

EXPERIMENTAL METHOD

Rats of a mixed population of the following groups were used: sexually mature males and females; sexually mature males and females androgenized on the 1st-3rd day of life by cutaneous injection of 1.25 mg testosterone propionate (TP); gonadectomized males (32-56 days before the experiment) and females (30-40 days before the experiment); gonadectomized females receiving 0.01-3.0 mg TP in 0.4 ml propylene glycol or the corresponding volume of the solvent alone, intramuscularly for 1-7 days, and used in the experiments 1 day after the last injection.

A cytosol of the liver was obtained as described previously [6], by using 10 mM Tris-HCl buffer, pH 7.5, containing 1.5 mM EDTA, 10 mM KCl, and 6 mM dithiothreitol for homogenization. Estradiol (E_2)-binding sites of SEBP were estimated quantitatively by ion-exchange adsorption on DEAE-cellulose DE-52 under equilibrium conditions [2]. Estradiol-2,4,6,7-^{3}H with a specific activity of 98 Ci/mmole was from the Radiochemical Centre, Amersham, England.

For differential measurement of SEBP mixed with other E_2-binding liver proteins we used: 1) a variant of a method based on differences in stability of complexes of SEBP and other proteins with E_2 [2]; 2) a variant developed in the course of the present investigation, making use of the extremely low affinity for hexestrol that is characteristic of SEBP alone. Gel filtration of the liver cytosol on Sepharose 6B columns also was used to reveal SEBP [4, 5].

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TABLE 1. Concentration of E_2-Binding Sites of SEBP (N_s) in Liver Cytosol from Rats of Different Sexes and Ages and after Certain Experimental Procedures (M ± m)

Sex of rats	Group of rats	N_s	
		× 10^{12} mole/mg protein	× 10^{-5} sites/cell
Males	Sexually mature	11,1±1,1 (23)	9,2±0,9
	Sexually immature	0 (4)	0
	Sexually mature, neonatally androgenized	8,2±1,0 (5)	8,7±1,0
	Sexually mature	0 (15)	0
Females	Ovariectomized	0 (6)	0
	Sexually mature, neonatally androgenized	0 (5)	0

Note: Here and in Table 2, number of determinations given in parentheses.

TABLE 2. Effect of Dose and Duration of Administration of TP on SEBP Content (N_s) in Liver Cytosol and on Relative Weight of Uterus of Ovariectomized Rats (M ± m)

Dose of TP, mg	Duration of administration of TP, days	N_s, × 10^{12} mole/mg protein	Weight of uterus, g/100 g body weight
0*	3	0 (4)	39,8±2,6 (10)
0,03	3	0 (3)	40,9±6,5 (6)
0,1	3	2,6±0,8 (3)	—
0,3	3	6,5±1,3 (4)	40,1±8,5 (8)
3,0	3	6,6±0,9 (15)	96,5±4,3 (30)
3,0	1	0 (4)	66,4±6,9 (8)
3,0	3	6,6±0,9 (15)	96,5±4,3 (30)
3,0	7	5,4±0,3 (3)	171,5±2,5 (6)

*Injection of propylene glycol.

EXPERIMENTAL RESULTS

The results given in Table 1 show that the concentration of SEBP, determined by the ion-exchange adsorption method, in the liver cytosol of sexually mature males was 11.1 ± 1.1•10^{-12} mole/mg protein. Neither in the sexually mature females nor in ovariectomized animals could any determinable quantities of SEBP be found, in agreement with preliminary data obtained by gel filtration [6]. The reason for the absence of detectable quantities of SEBP in the liver of the female rats was evidently not the inhibitory effect of estrogens on the content of this protein, demonstrated previously [6], but some other factor.

It can be postulated on the basis of the existence of sexual dimorphism in the SEBP content in the liver that the presence of high concentrations of SEBP in the adult male liver is the result of the inducing action of androgens. In that case it would be possible, in principle, that either the appearance of SEBP, like sexual differentiation of a number of systems of the body, is determined by early prenatal or neonatal androgenization of the animals, or induction of SEBP, like the development ofmany secondary sexual characteristics, is due to a considerable rise in the androgen concentration in the period of puberty.

Our experiments showed that neonatal androgenization, leading to various disturbances in the reproductive system of the adult females (permanent estrus, closed vagina, underdevelopment of the uterus), cannot induce the appearance of detectable quantities of SEBP in adult animals (Table 1). No SEBP was found in these animals by the gel filtration method likewise. Neonatal administration of androgens also had no significant effect on the SEBP

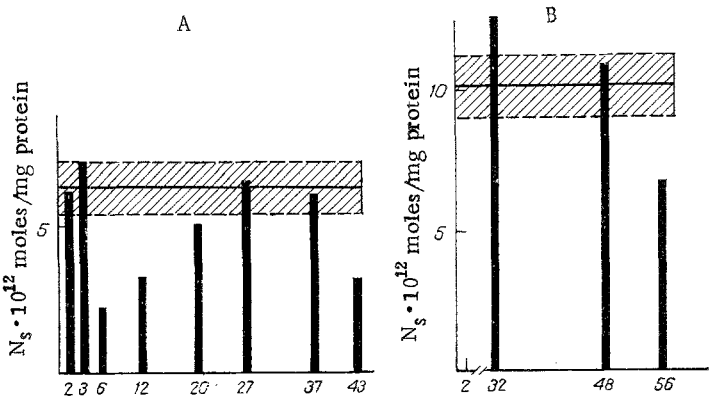

Fig. 1. Effect of termination of androgenic stimulation on content of E_2-binding sites of SEBP (N_S). A) In liver of ovariectomized female rats previously receiving TP (3 mg, 3 days); B) in liver of sexually mature male rats after castration. Horizontal line in shaded sector denotes SEBP content (M ± m). A) In liver of ovariectomized females 1 day after end of TP administration; B) in liver of sexually mature male rats. Vertical columns show values of N_S obtained in one or two determinations. Abscissa: A) days after end of TP administration; B) days after castration of males.

level in adult males (Table 1). In sexually immature males, despite spontaneous prenatal androgenization, no SEBP likewise could be found either by ion-exchange adsorption (Table 1) or by gel filtration. The decisive factor for the appearance of SEBP in males is thus, probably, an increase in the androgen concentration during puberty.

Ovariectomized rats in which SEBP was initially absent are evidently the most convenient object with which to study the conditions for SEBP induction by androgens. The writers showed previously that, in principle, SEBP can be induced by androgens in such animals [6]. It follows from the present investigation (Tables 1 and 2) that TP can induce the formation of SEBP in an amount comparable with the level of this protein in normal males. If TP was given for 3 days, SEBP was observed to be formed in response to a dose of as little as 0.1 mg, and the maximal response developed to a dose of 0.3 mg. A further increase in the quantity of androgen injected did not give any further increase in the SEBP concentration (Table 2). Meanwhile, a single injection of TP, even of the largest dose (3 mg), did not induce SEBP formation. For this to happen the preparation had to be given in the course of at least 3 days. The SEBP level did not rise if the duration of the injections was increased to 7 days (Table 2).

In order to assess the sensitivity of the process of SEBP induction to the action of androgens it was considered interesting to compare this effect with the uterotrophic action of the androgen. When TP was injected for 3 days a uterotrophic effect was observed only if the dose given was 30 times greater than that required to stimulate SEBP formation. Meanwhile, unlike SEBP induction, the action of the androgen on the uterus could be detected not only in response to repeated injection, but also to a single injection of large doses of TP (Table 2). During administration of TP definite differences were thus observed in the response of the uterus and induction of SEBP. The process of SEBP induction is evidently more sensitive to the androgen than the uterus, but it requires longer exposure to the steroid.

The experiments showed that yet another distinguishing feature of SEBP is the duration of its existence in the liver cells after termination of androgenic stimulation. SEBP was found in the liver of the ovariectomized females for at least 43 days after the end of TP administration, in a quantity very close to that found immediately after injections of the hormone ceased (Fig. 1A). Investigation of the SEBP content in the liver of adult males after castration also showed that its level was virtually the same as that in normal animals even 56 days after gonadectomy (Fig. 1B). One possible explanation of this fact is that androgens, while responsible for triggering systems leading to the formation of new SEBP, do not play a decisive role in the maintenance of the already established ability of liver cells to form this protein in the course of a long period of time.

To sum up it can be concluded that induction of SEBP is an androgen-dependent process, and accordingly this protein may be interesting not only as a possible mediator for one of the pathways of action of estrogens on the liver, but also as a marker of the action of androgens on this organ.

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REGENERATION OF THE LIVER WITH EXPERIMENTAL CIRRHOSIS AFTER QUADRUPLE RESECTION

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Many investigations demonstrating the ability of the cirrhotic liver to undergo regeneration have now been published. Several workers [2-7, 9, 11] have noted that resection of the cirrhotic liver favors reversibility of the pathological changes. The study of the compensatory and regenerative powers of the cirrhotic liver after multiple repeated resections is of great interest.

The object of this investigation was to study the course of regeneration of the cirrhotic liver after four resections.

EXPERIMENTAL METHOD

Experiments were carried out on 250 albino rats weighing 160-180 g. Cirrhosis of the liver was produced by subcutaneous injection of carbon tetrachloride (0.2 ml in an oily solution) into each animal four times a week for 5 months. Resections of the liver were carried out at intervals of 45 days. The animals were killed on the 2nd, 3rd, 7th, and 45th days after each operation. Rats with experimental cirrhosis not undergoing resection and intact animals of the same age were sacrificed at the same time. At the first operation the left lateral lobe, weighing 5.11 g (43.3% of the weight of the liver in rats with experimental cirrhosis) was removed. At the second operation the left and right lobules of the central lobe, weighing 3.27 g (42% of the liver after a single resection) were removed. At the third operation the right lateral lobe of the liver, weighing 2.76 g (34.2% of the twice resected liver) was removed. At the fourth operation the caudate lobe of the liver, weighing 1.94 g (24.9% of the thrice resected liver) was removed. The total weight of liver tissue removed in the course of the four operations was equivalent to 144.4% of the weight of the liver of the control rats with cirrhosis.

Pieces of liver taken during the operations and at sacrifice were fixed in neutral 10% formalin and Carnoy's fluid and embedded in paraffin wax. Sections were stained with hematoxylin and eosin, with Water blue, by Van Gieson's method, with Sudan III, and by Mc-

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