SPECIFIC ESTROGEN-BINDING PROTEIN OF RAT LIVER AND SEX STEROID METABOLISM

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A soluble protein, specific estrogen-binding protein (SEBP), capable of specifically binding natural estrogens and certain androgens, has been identified in the liver of male rats [1, 2, 5]. The determinant ligands of SEBP include the phenolic 3-hydroxy group, the 17-β-hydroxy or 17-keto-group in estrane derivatives, the 17-β-hydroxy-group and also the 3-keto-group in Δ^4- or 5-α-derivatives of androstane, and the 3-α-hydroxy-group in 5-α-androstan-3-α-17-β-diol [1]. The high lability of complexes of the protein with the ligand ($t_{0.5}$ < 1 min), together with the moderate value of the equilibrium constant of association (10^7-10^8 M^{-1}) and the high concentration of protein binding sites (about 10^{-11} mole/mg cytosol protein) [2, 3] suggest that SEBP can take part in regulation of the dynamics of sex steroids in the liver.

To test this hypothesis, model experiments were conducted to study the effect of a highly purified preparation of SEBP on the intensity of estradiol and testosterone metabolism under the influence of enzymes in liver homogenate from female rats, not containing SEBP [3].

EXPERIMENTAL METHOD

The liver of mature female rats was homogenized (Teflon/glass) in two volumes of 50 mM Tris-HCl buffer, pH 7.5, containing 600 mg% of glucose. The ^{3}H-steroid was preincubated for 15 min at 0-4°C with 0-4 μg of the preparation of SEBP (200 μl), after which 0.4 millimole of $NADH^+$ and NADPH and homogenate of 0.3-30 mg tissue were added. The volume of incubation mixture was made up to 300 μl. Incubation continued for 5-15 min at 0-4°C. Steroids were extracted with 5 ml of ethyl alcohol. The solvent was evaporated under a jet of air. The extract in methanol was applied to Silufol plates (15 × 84 mm). ^{3}H-Steroids and their metabolites were separated in a benzene–ether system (1 : 1). The exception was ^{3}H-estriol, for which a benzene–ether–methanol system (1 : 1 : 0.2) was used. Chromatograms were cut into strips 3 mm wide, placed in scintillation fluid, and their radioactivity measured. The results were expressed as percentages of converted ^{3}H-steroid relative to total radioactivity in the zones of the original and converted steroid. All experiments were repeated two to four times, with similar results.

A standard preparation of partially purified SEBP was obtained from liver cytosol of mature male rats by the method described previously [2]. For subsequent purification, affinity chromatography on estradiol-agarose was used. To prepare the affinity sorbent, the ethyl ester of 17-β-estradiol-17-α-acetic acid was prepared by Reformatsky's reaction between estrone and ethyl bromoacetate. After alkaline hydrolysis of the ester bond, the 17-β-estradiol-17-α-acetic acid was conjugated by the carbodiimide method with hexamethylenediamine-agarose. The concentration of the covalently bound steroid was 6-7 μmoles/g wet weight of gel. Partially purified SEBP (200-300 ml) was passed through the estradiol-agarose column (20 ml) at the rate of 1 ml/min. The column was then washed at the same speed with 1 liter of 10 mM Tris-HCl buffer, pH 7.5, containing 10 mM KCl, 1 mM EDTA, 0.5 mM dithiothreitol (TPED buffer) and 500 mM NaCl. Protein was eluted with a 70 μM solution of specific estriol ligand in the same buffer. After dialysis against TPED buffer, the SEBP was separated from its partial degradation products and accidental contamination by other proteins by ion-exchange chromatography on a DEAE-Toyopearl 650M column (0.8 × 30 cm; from Toyo Soda, Japan). A linear 0-125 mM NaCl gradient in TPED buffer was used for elution. All procedures were carried out at 0-4°C. The resulting SEBP preparation had specific binding activity of about 0.25 mole/mole of ligand, it preserved

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TABLE 1. Effect of SEBP on Metabolic Rate of ^3H-E_2 and ^3H-T by Liver Homogenate from Female Rats

^3H-steroid	Preparation	Dose of preparation µg/ml	Percent of metabolized ^3H-steroid
^3H-E_2*	SEBP	0	84,4
		2,8	53,7
		5,6	49,7
		8,4	52,5
		14,0	47,0
	BSA	25	85,3
^3H-T**	SEBP	0	82,1
		0,5	82,2
		1,0	82,7
		2,0	82,2
		3,0	72,3
		5,0	65,3

Legend. *) Incubation at 0-4°C for 15 min with homogenate (1.7 mg tissue), **)incubation at 0-4°C for 5 min with homogenate (0.3 mg tissue). Results of one of four analogous experiments are given.

its basic hormone-binding properties, and gave one band of a protein, staining with Coomassie, with molecular weight of 31 kilodaltons on electrophoresis in polyacrylamide gel in the presence of sodium dodecylsulfate [6].

Radioactivity in the test samples was measured on a Mark II liquid scintillation counter (Nuclear Chicago, USA), using dioxan scintillator [4]. The following ^3H-steroids, used in the investigation were obtained from Amersham Corporation (England): [2,4,6,7-^3H]-estrone (^3H-E_1, specific radioactivity SR = 80 Ci/mmole); [2,4,6,7-^3H]-estradiol (^3H-E_2, SR 104 Ci/mmole; [2,4,6,7-^3H]-estriol (^3H-E_3, SR 109 Ci/mmole); [1-α, 2-α(n)-^3H]-testosterone (^3H-T, SR 51 Ci/mmole); [2,4,6,7-^3H]androstenedione (^3H-A, SR 97 Ci/mmole). The radiochemical purity of the ^3H-steroids was verified by thin-layer chromatography.

EXPERIMENTAL RESULTS

Before the study of the effect of SEBP on metabolism of the ^3H-steroids under the influence of liver enzymes could be started it was necessary to discover whether SEBP possesses its own enzyme activity relative to the steroids to be tested. Incubation of ^3H-E_1, ^3H-E_2, ^3H-E_3, ^3H-T, and ^3H-A with the SEBP preparation in the presence of 0.4 mM $NADH^+$, NADH, $NADPH^+$, and NADPH at 30°C for 30 min was found not to lead to the appearance of new zones of radioactivity on the chromatogram. It could thus be concluded that SEBP has no oxidoreductase activity of its own with respect to these steroids, and does not contain enzymes with such activity as impurities.

With the conditions of incubation used (low temperature, short exposure), of the ^3H-steroids studied, only ^3H-E_2 and ^3H-T underwent metabolic conversion under the influence of enzymes of liver homogenate. In both cases the main or only product was a less polar compound with mobility close to that of ^3H-E_1 and ^3H-A respectively. Strict identification of the products was not carried out, because this did not affect the interpretation of the results.

Preliminary incubation of ^3H-E_2 and ^3H-T with SEBP had a marked inhibitory effect on the intensity of metabolic conversions of these steroids under the influence of enzymes of liver homogenate. The effect was specific for SEBP, for bovine serum albumin (BSA), which binds steroids nonspecifically, was ineffective under these same conditions. The effect of SEBP on the intensity of conversions of ^3H-E_2 and ^3H-T depended on dose (Table 1). The inhibitory action of the protein was more effective against metabolism of ^3H-E_2 then against metabolism of ^3H-T, and the effect began to be exhibited in the presence of lower concentrations of SEBP (0.2-0.4 µg/ml). This is in agreement with data showing the higher affinity of SEBP for E_2 than for T[1]. The action of SEBP on the metabolic rate of ^3H-E_2 and ^3H-T was evidently reversible. This was shown by the reduction and gradual disappearance of the effect of the protein with an increase in the duration of incubation with the homogenate or an increase in activity of its enzymes. The data described above are evidence that interaction between SEBP and ligands can really affect the dynamics of these steroids in the liver. It can be tentatively suggested that one of the functions of SEBP is to buffer the sharp fluctuations in concentration of sex steroids (for example, during coitus, during diurnal waves of secretion and, possibly, during stress).

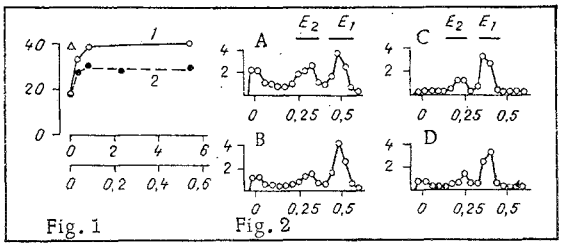

Fig. 1 Fig. 2

Fig. 1. Action of estrone (1, top scale) and estriol (2, bottom scale) on velocity of metabolic conversion of ^3H-E_2 by liver homogenate of female rats in presence of SEBP. Abscissa, concentration of E_1 and E_3 (in μM); ordinate, percentage of metabolized ^3H-E_2. Triangle denotes intensity of metabolism of ^3H-E_2 in presence of SEBP and unlabeled steroids. Incubation (0-4°C, 5 min) with homogenate (0.3 mg tissue). Concentration of SEBP 2 μg/ml.

Fig. 2. Effect of estrone on intensity of metabolism of ^3H-E_2 by liver homogenate of male rats (A, B) and female rats (C, D). Abscissa, electrophoretic mobility (in relative units); ordinate, radioactivity (in cpm · 10^3). A, C) In presence of E_1; B, D) in presence of 0.7 μM E_1. Bold lines denote position of standards on chromatograms: E_2) estradiol, E_1) estrone. Incubation (0-4°C, 5 min) with homogenate (17 mg tissue) in absence of coenzymes.

Another possible function of SEBP is that of intermediary in the effect of some steroids on the dynamics of others. This is shown by the results of the following experiments. On addition of unlabeled E_1 to the reconstituted system (SEBP, homogenate, coenzymes, and ^3H-steroids) the inhibitory action of SEBP on the metabolic rate of the ^3H-steroids was abolished (Fig. 1). In the absence of SEBP, E_1 did not affect the metabolic rate of the ^3H-steroids. Another specific ligand of SEBP, namely E_3, had a similar action. The effect of E_3, however, was incomplete, evidently on account of its partial competition with the ^3H-steroid for binding sites of of the metabolizing enzyme (Fig. 1).

The ability of E_1 to block the effect of SEBP was used to study whether the concentration of endogenous SEBP contained in male liver is really sufficient to exert its effect on the dynamics of the sex steroids. Liver homogenates from male and femals rats were incubated with ^3H-E_2 without the addition of coenzymes, in the presence and in the absence of unlabeled E_1. As might be expected, E_1 did not affect the metabolic rate of ^3H-E_2 by liver homogenate from females (not containing the essential quantities of SEBP), but caused marked acceleration of ^3H-E_2 metabolism by male liver homogenate (Fig. 2). These results indicate that SEBP can really take part in regulation of the dynamics of sex steroids in the liver.

The results of the present investigation are the first experimental confirmation of the hypothesis, put forward previously, that SEBP has a regulatory function in relation to the dynamics of sex steroids in the liver. The use of the effect of competition between steroids for binding sites of SEBP may prove to be a convenient tool in the future for the study of the functions of this protein and in more complex systems, including the organism as a whole.

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