3. R. J. Ericson and V. F. Baker, Proc. Soc. Exp. Biol. (N.Y.), 122, 88 (1966).
4. R. A. Gorski, Endocrinology, 82, 1001 (1968).
5. S. Mennin and R. Gorski, Endocrinology, 96, 486 (1975).
6. J. J. Peluso, R. W. Steger, and E. S. Hafer, Biol. Reprod., 16, 212 (1977).
7. J. Ramaley, Fed. Proc., 39, 2355 (1980).
8. H. E. Swanson and J. J. van der Werfften Bosch, Acta Endocrinol. (Copenhagen), 45, 1 (1964).
9. Y. Takeo, K. Shirama, K. Shimizo, et al., Endocrinol. Jpn., 22, 453 (1975).
10. P. M. Wise and P. Camp, Endocrinology, 114, 92 (1984).

CONTROL OF THE LEVEL OF UNUSUAL ESTROGEN-BINDING PROTEIN IN RAT LIVER BY SEX STEROIDS AND THE PITUITARY

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Unusual estroggn-binding protein (UEBP) is one component of a system of intracellular proteins of the rat liver that specifically bind sex steroids. It has been suggested that UEBP is responsible for the accumulation of biologically active sex steroids in the hepatocytes, by inhibiting their metabolism, and that it regulates the rate of their binding with the corresponding receptors [4, 8, 10, 13]. Meanwhile UEBP is a sex-dependent liver protein: it is found in large quantities (6-10 picomoles/mg cytosol protein) in the liver of male rats only, and its content in female rats is at a low (basal) level [3, 5, 6, 13]. The high UEBP concentration in the liver of sexually mature males is due to pre- or neonatal imprinting of its level by androgens (AN). Primary injection of AN into female rats in various stages of ontogeny also induces determination of a high UEBP level [6, 13].

It is not yet clear whether AN has a significant role also in the regulation of the already induced level of this protein, or what relations exist between AN and estrogens in regulation of the UEBP concentration.

A number of facts point to a role of the pituitary in the formation of sex differences in metabolic activity of many systems of the liver [1, 9, 11]. The pituitary also is essential for primary AN dependent determination of the UEBP level [6].

The aim of this investigation was to study the role of sex steroids and the pituitary in regulation of the UEBP level in the rat liver.

EXPERIMENTAL METHOD

The following groups of male rats of a mixed population were used: immature (30-40 g), prepubertal (80-90 g), mature (150-200 g), mature and castrated 15-20 days before the experiment or hypophysectomized [6] 20-25 days before the experiment, with the testes intact or removed, and also mature female rats, ovariectomized 15-20 days before the experiment. This last group was used after the following procedures: 1-3 days after induction of the UEBP level in these animals with testosterone propionate (TP), according to the scheme used previously [6], the animals were hypophysectomized, and the UEBP level was determined 20-25 days after the last operation. Nonhypophysectomized females, used at the same times after injection of TP, served as the control. Completeness of removal of the glands from rats of all groups was verified by methods described previously [6].

Hormones were injected intramuscularly in 0.4 ml of propylene-glycol per animal: TP in a dose of 3 mg daily for 3 days, estradiol (E_2) in a dose of 10 μg, once only or daily for 6

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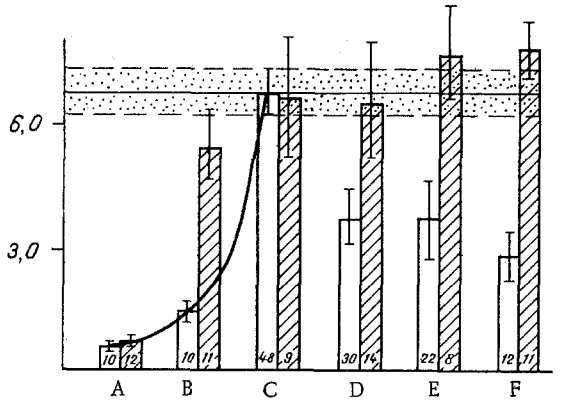

Fig. 1

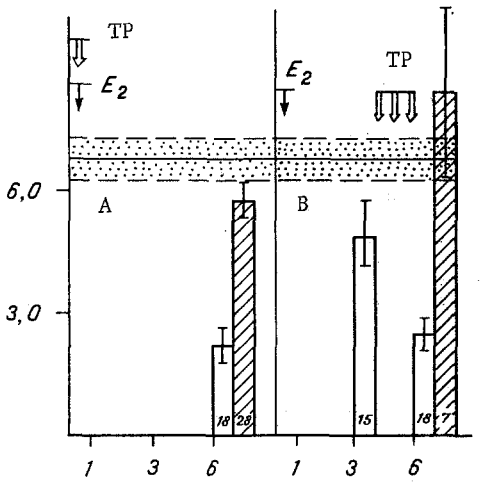

Fig. 2

Fig. 1. Action of AN on UEBP concentration in liver of male rats with different endocrine status. Ordinate, UEBP concentration (in picomoles/mg protein, M ± m) in liver of male rats 1 day after daily injection of 3 mg TP for 3 days (shaded columns) and in liver of male rats not receiving hormone (unshaded columns). A) Immature rats, B) prepubertal, C) mature, D) castrated, E) hypophysectomized, F) castrated and hypophysectomized males. Numbers inside columns indicate number of experiments. Strip bounded by broken lines shows limits of UEBP concentration (M ± m) in liver of mature males.

Fig. 2. Action of AN on effectiveness of inhibitory influence of E_2 on UEBP concentration in liver of mature male rats. Abscissa, time after injection of E_2 (in days); ordinate, UEBP concentration (in picomoles/mg protein, M ± m) in liver of mature males after injection of E_2 along (unshaded columns) and of E_2 and TP (shaded columns). A) Simultaneous injection of single doses of E_2 (10 μg) and TP (3 mg), UEBP level determined 6 days later; B) consecutive injection of a single dose of E_2 (10 μg) and daily injections of 3 mg TP (for 3 days, starting 3 days after injection of E_2), UEBP level determined before 1st injection of TP and 1 day after last injection of TP. Remainder of legend as to Fig. 1.

days. In some experiments a single injection of E_2 (10 μg) and TP (3 mg) was given simultaneously or consecutively (a single injection of 10 μg E_2, 3 days later injections of 3 mg TP daily for 3 days). The times of testing the UEBP level after the end of hormone administration are indicated below.

The concentration of E_2-binding sites of UEBP in the liver cytosol (N_{uebp}) was determined by measuring binding of a "minimal" addition of 2,4,6,7-^{3}H-E_2, with specific radioactivity of 98-100 Ci/mmole (from Amersham Corporation, England), by the method developed previously [6].

The remaining procedures were carried out as described previously [6].

EXPERIMENTAL RESULTS

Data on the effect of AN on the UEBP level in the liver of male rats of the different age groups are given in Fig. 1. The results showed that the concentration of this protein rises during sexual maturation. AN raised the UEBP level only in the prepubertal period (Fig. 1B). Absence of sensitivity to the controlling action of AN in the liver of the immature males (Fig. 1A) can evidently be explained by the as yet inadequate maturity of the AN-receptor apparatus of the hepatocytes in rats of this age. In the mature males, injection of AN could no longer raise the UEBP level (Fig. 1C), evidently because its optimal concentration had been reached due to the action of endogenous AN. Lowering the concentration of endogenous AN by castration or hypophysectomy of the animals led to a decrease in the UEBP concentration ($P < 0.01$). Injection of AN into mature males against this background became effective and compensated fully for the changes in the UEBP concentration due to depression of activity of the pituitary–gonads system (Fig. 1D, F)

TABLE 1. Role of Pituitary in Regulation of UEBP Level and in Realization of Influence of Sex Steroids on Concentration of this Protein (M ± m)

Group of animals	Ovariectomized females with TP-induced UEBP level			
	initial level	after hypophysectomy (alone)		
		with no additional treatment	and after injection of TP (3 mg, daily for 3 days)	and after injection of E_2 (10 μg, daily for 6 days)
Mature males	6,83±0,49 (48)	3,64±1,09 (22) $P_1<0,01$	7,67±1,30 (8) $P_2<0,01$	3,44±0,77 (8) $P_2>0,1$
Castrated males	3,74±0,69 (30)	2,75±0,62 (12) $P_1>0,1$	7,77±0,68 (11) $P_2<0,01$	—
Ovariectomized females with TP-induced UEBP level	0,67±0,24 (6)	0,46±0,13 (7) $P_1>0,1$	—	—

Legend. UEBP levels determined 1 day after end of injection of hormones. Number of determinations shown in parentheses. P_1) Level of significance of differences compared with initial UEBP level, P_2) compared with UEBP level in animals hypophysectomized only.

The results of investigations of relations between E_2 and AN during regulation of the UEBP level are given in Fig. 2. A single injection of E_2 into mature males caused a marked fall in the UEBP level after 6 days. This effect of E_2, as the experiments showed, was independent of the presence of the testes and, consequently, it can be realized directly, irrespective of the AN concentration. Although they have no direct stimulating effect on the UEBP level in mature males, AN prevents both the onset (if injected simultaneously with E_2) and the development (in the case of successive injections of E_2 and TP) of the inhibitory action of E_2 on the UEBP level in animals of this group.

Two types of regulatory influence on the UEBP level are thus characteristic of AN. On the one hand, they have a stimulating effect not only in situations when the initial UEBP concentration is low. The cause of a low UEBP level could be a deficiency of endogenous AN, associated with the prepubertal period, castration, or hypophysectomy, or the inhibitory action of E_2. In all these cases the controlling action of AN is aimed at restoring and maintaining the optimal UEBP level for mature males. On the other hand, although they have no direct effect on the UEBP level, AN prevent realization of the inhibitory effect of E_2, and they thus stabilize the normal UEBP concentration in the liver of mature males.

Data on the effect of hypophysectomy on the UEBP level in the liver of the different groups of rats are given in Table 1. Hypophysectomy on mature males leads to a fall in the UEBP concentration to the level characteristic of castrated animals ($P > 0.1$). Meanwhile removal of the pituitary from castrated males caused no significant fall in the UEBP level. The inhibitory effect of hypophysectomy on the UEBP level in mature males is evidently due, not to the direct action of any pituitary factors, but to the absence of the stimulating action of gonadotropins on AN secretion by the testes. Evidence of this is given by the ability of AN to restore the original UEBP level both after castration and after hypophysectomy (Table 1; Fig. 1). The masculinizing action of hypophysectomy on certain metabolic reactions taking place in the liver of female rats has been shown in a number of investigations [1, 2, 9, 11]. Removal of the pituitary in female rats with a TP-induced UEBP level caused no masculinizing effect whatsoever and on distinct changes in the UEBP concentration compared with the control (Table 1). Pituitary factors evidently play no direct part in the regulation of an already induced UEBP level, although they contribute to its original determination [6].

Investigation of the role of the pituitary in realization of the action of sex steroids showed that its presence is not essential for realization of the stimulatin action of AN on the UE3P level. Meanwhile the pituitary is essential for realization of the inhibitory effect of D_2 on the concentration of this protein (Table 1). In this case any permissive role of the pituitary for E_2 in the regulation of the level of receptors for E_2 in the liver by pituitary hormones is probably ruled out, at least in part [2, 7, 12].

It can be concluded from these data that AN not only are primary determinants of the UEBP level, but also perform an essential regulatory role, aimed at maintaining the optimal concentration of this protein. Their stimulating action, which is effected independently of any direct effect of pituitary factors, is realized only if the UEBP concentration deviates

from normal. AN also modulates the effectiveness of the inhibitory action of E_2. The regulatory effect of E_2 consists essentially of lowering the optimal level of UEBP; realization of the inhibitory action of E_2, moreover, depends on the presence of the pituitary and on the AN level in the animal. The presence of comparable quantities of E_2 and AN in rats of both sexes [2] is evidence of the existence of a fine mechanism of combined regulation of the UEBP concentration under natural conditions that reflects changes in the absolute E_2 or AN levels or in the ratio between them.

LITERATURE CITED

1. A. G. Reznikov, Sex Hormones and Differentiation of the Brain [in Russian], Kiev (1982).
2. V. B. Rozen, Vestn. Akad. Med. Nauk SSSR, No. 2, 80 (1983).
3. A. N. Smirnov, I. T. Nikolov, and V. B. Rozen, Probl. Éndokrinol., No. 5, 66 (1979).
4. A. N. Smirnov, O. V. Smirnova, and V. B. Rozen, Biokhimiya, No. 3, 560 (1977).
5. O. V. Smirnova, E. A. Kizim, A. N. Smirnov, et al., Byull. Éksp. Biol. Med., No. 10, 480 (1980).
6. O. V. Smirnova, M. L. Miroshnichenko, T. A. Shchelkunova, et al., Probl. Éndokrinol., No. 5, 65 (1983).
7. P. Beers and N. Rosner, J. Steroid Biochem., 8, 251 (1977).
8. R. Dickson, R. Aten, and E. Eisenfeld, Endocrinology, 103, 1636 (1978).
9. J.-Å. Gustaffson and Å. Stenberg, Endocrinology, 95, 891 (1974).
10. M. L. Miroshnichenko, O. V. Smirnova, A. N. Smirnov, et al., J. Steroid Biochem., 18, 403 (1983).
11. A. Mode and G. Norstedt, J. Endocrinol., 95, 181 (1982).
12. G. Norstedt, O. Wrang, and J.-Å. Gustaffson, Endocrinology, 108, 1190 (1981).
13. T. Sloop, J. Clark, R. Rumbaugh, et al., Endocrinology, 112, 1639 (1983).