of external sensory stimulation, observed when activity of the hippocampal NA-ergic pool is depressed by 6-OHDA; these events probably reflect prolongation of the retrieved memory traces relating to reinforcing stimuli, possibly through a reciprocal increase in the activity of the hippocampal serotoninergic pool [3].

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INDIRECT MECHANISM OF THE POSITIVE ACTION OF ESTROGENS ON CORTICOSTEROID-BINDING GLOBULIN LEVEL IN RATS

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Corticosteroid-binding globulin (CBG) is a plasma protein secreted by the liver. CBG specifically binds corticosteroids and progestins circulating in the blood stream, and it is thus involved in modulation of the effect of these steroids. This function of CBG may evidently be realized differently in female and male rats, for the level of this protein differs in rats of different sexes [3, 9, 11]. The question arises of the role of sex hormones in the maintenance of sexual dimorphism for CBG concentration in rats.

The writers showed previously [3] that the lower CBG level in male rats is due to the negative programing effect of androgens in the prepubertal period and their negative regulatory action in sexually mature animals. Meanwhile, removal of the source of endogenous estrogens in the early period of ontogeny does not affect the CBG level in adult rats, and the same is true of ovariectomy in mature females or administration of physiological doses of estradiol to them.

However, the view continues to be held in the scientific literature that the CBG level in rats is an estrogen-dependent trait [10]. This view is based on data obtained by several workers [6, 9] in the 1960s, which have not subsequently been re-examined. An important role in the creation of this opinion concerning the positive action of estrogens on the CBG level in rats was evidently played by data on the stimulating effect of estrogens in respect to this trait in guinea pigs and man [4, 5, 7]. Analysis of data on estrogenic regulation of the CBG level in rats reveals their contradictory nature, for long-term administration of large doses of estradiol leads to an increase in the CBG concentration only in intact males and does not change its level in castrated males and females. Accordingly it was decided to undertake a careful study of the role of estrogens in the regulation of the CBG level in rats.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats. Gonadectomy and adrenalectomy were performed under ether anesthesia by the usual method; the animals were used 2 weeks after the operation. Estradiol was injected subcutaneously in propylene glycol in a dose of 100 μg per rat for 3 weeks. Dexamethasone, a synthetic corticosteroid, also was injected

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TABLE 1. Effect of Prolonged Estrogenization of Male Rats on Body Weight, Weight of Testes, and Blood Levels of CBG and Androgens (M ± m)

Group of animals	CBG level, ng/ml	Body weight, g	Weight of testes, g	Ratio of weight of testes to body weight	Androgen concentration, pg/ml
Males receiving solvent for 3 weeks (n=8)	458±35	224±12	2,56±0,1	0,01±0,001	2981±412
Males receiving 100 µg of estradiol for 3 weeks (n = 7)	679±19 p<0 001	178±05 p<0,01	1,15±0,13 p<0,001	0,007±0,001 p<0,001	441±17 p<0,001

subcutaneously in propylene glycol in a dose of 1 µg per animal for 3 days. Animals receiving the solvent served as the control.

The serum CBG level of the rats was determined by a variant of the radioligand method, developed by ourselves and based on binding of labeled cortisol by the serum CBG [3]. Serum androgen levels were determined by radioimmunoassay using the kit from "International CIS" (France) [2]. The statistical significance of differences between values of the parameters was determined by Student's t test.

EXPERIMENTAL RESULTS

We showed by the use of our modification of the method that injection of estradiol raised the CBG level of intact males by about 50% ($p < 0.001$) compared with animals receiving the solvent (Fig. 1), whereas the CBG level in gonadectomized females and males, subjected to estrogenization, did not differ from that in the control animals ($p > 0.1$; Fig. 1). Analysis of these data suggests that the positive effect of estrogens in males is due to the fact that their testes were intact. To test this hypothesis we compared the body weight, weight of the testes, and blood androgen levels in estrogenized males and control animals. The results, given in Table 1, show that prolonged estrogenization of intact males leads to reduction of the body weight by about 25% ($p < 0.01$) and in the absolute and relative weight of the testes by 55% ($p < 0.001$) and 30% ($p < 0.001$) respectively, and also to a more than sixfold decrease in the blood androgen concentrations ($p < 0.001$).

It is well known that large doses of sex hormones can considerably depress pituitary gonadotrophic function in males on the negative feedback principle [1]. Evidently in this case injection of estrogens depresses pituitary gonadotrophic function in males, with the result that the secretion of androgens by their testes is reduced. The end result, namely elevation of the CBG concentration, is realized through abolition of the negative effect of endogenous androgens, and not to the true positive effect of the injected estrogens. This conclusion also is confirmed by the fact that reduction of the body weight was observed in estrogenized males as a result of weakening of the anabolic effect of endogenous androgens. The absence of estrogenic stimulation of the CBG level in females and castrated males now becomes clear. These results are evidence of insensitivity of the CBG level in rats to estrogens.

However, considering the stimulating action of estrogens on the blood corticosteroid levels [1], it can be tentatively suggested that the positive effect of estrogens on the CBG level, if such exists, is not exhibited due to the compensatory negative action of the corticosteroids. Removal of the adrenals, the chief source of endogenous corticosteroids, leads to an increase in the CBG concentration by 63% in females ($p < 0.001$) and by 128% ($p < 0.001$) in males compared with intact animals (Fig. 2). To study the contribution of each group of hormones to regulation of the CBG concentration a series of experiments was carried out on adrenalectomized animals.

Injection of estradiol into adrenalectomized females did not lead to any further rise of the CBG level ($p > 0.1$; Fig. 2). Comparison of the CBG level in adrenalectomized females and females with combined removal of the gonads and adrenals shows that the level of this protein was virtually the same in the animals of these groups ($p > 0.1$; Fig. 2). Thus neither endogenous estrogens nor prolonged administration of estradiol after adrenalectomy leads to any further increase in the CBG concentration. At the same time, the possibility cannot be ruled out that in the complete absence of cortisteroids (the principal negative regulators of the CBG level in rats) the greatest possible rise of the CBG level takes place. In this case, the presumptive positive effect of estrogens need not necessarily lead to a further increase in the concentration of this protein. However, injection of dexamethasone into adrenalectomized females and adrenalectomized and gonadectomized females (Fig. 2) shows that

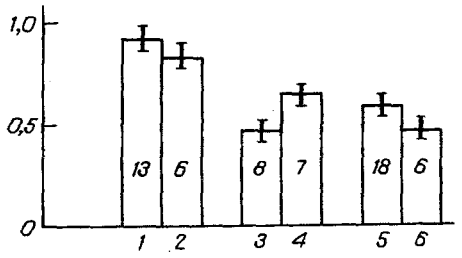

Fig. 1

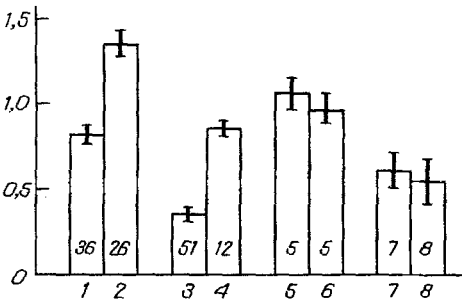

Fig. 2

Fig. 1. Effect of prolonged estrogenization on CBG level in rats (M ± m). Abscissa, groups of animals: 1, 3, 5) ovariectomized females, intact and castrated males, each receiving 0.2 ml of solvent for 3 weeks respectively; 2, 4, 6) the same animals, receiving 100 μg estradiol for 3 weeks; ordinate, CBG level (in ng/ml·10^3). Numbers inside columns indicate number of animals.

Fig. 2. CBG concentration in adrenalectomized rats receiving injections of estradiol and dexamethasone (M ± m). Abscissa, groups of animals; 1, 3) intact females and males respectively; 2, 4) adrenalectomized females and males respectively; 5, 6) adrenalectomized females, receiving for 3 weeks solvent and estradiol respectively; 7) adrenalectomized females, receiving 1 μg dexamethasone for 3 days. 8) adrenal-, gonadectomized females, receiving the same dose of dexamethasone; ordinate, CBG level (in ng/ml·10^3). Numbers inside columns indicate number of animals.

endogenous estrogens do not cause the CBG level to rise, although its initial concentration is relatively low because of the inhibitory action of dexamethasone.

Thus the results show that estrogens have no independent positive action on the CBG level in female and male rats. At the same time, the action of large doses of these hormones is not mediated by adrenal factors.

The importance of examination of the effects of estrogens on various androgen-dependent functions in males from the standpoint of their effect on secretion of endogenous androgens must be emphasized. It will be evident that before true reactivity of the androgen-dependent trait to estrogens can be postulated, the possibility of their indirect action through depression of testicular function must be ruled out.

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