PROGRAMMING ACTION OF ANDROGENS ON FORMATION OF SEXUAL DIMORPHISM OF THE CORTICOSTEROID-BINDING GLOBULIN LEVEL IN RATS

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Corticosteroid-binding globulin (CBG) is a plasma protein produced by hepatocytes that specifically binds corticosteroids and progestins circulating in the blood stream. Although the physiological role of this protein has not yet been completely explained, CBG evidently plays an important part in the regulation of activity and metabolism of the steroids which it binds [5]. The CBG level in rats exhibits sexual differentiation [1, 4]: its level in females is about 2.5 times higher than in males. This fact enables the CBG level to be used as a model object for the study of principles governing the formation of sexual dimorphism of liver functions. The study of this problem would shed light on the biological importance of the existence of a definite sex-specific level of integration of adaptive and reproductive processes, which is performed by CBG at the blood stream level, by binding corticosteroids and progestins circulating in the blood. Previously [1] the writers showed that a lower concentration of CBG in male rats is due, on the one hand, to its irreversible negative programming by androgens in the prepubertal period, and on the other hand, to the presence of a negative regulatory effect of endogenous androgens in sexually mature males [1]. However, it is not yet clear in what period sensitivity of the CBG level to the determining effect of androgens first appears, and when it disappears. Likewise the problem of the boundaries of the period of formation of the sex-dependent CBG level in physiological rather than model conditions likewise remains unexplained; the possibility that the negative programming effect of androgens on the CBG content may be mediated indirectly through estrogens likewise has not been investigated. The aim of the present investigation was to study these problems.

EXPERIMENTAL METHOD

Experiments were carried out on male and female noninbred albino rats. Adult animals undergoing gonadectomy at the age of 1, 7, 14, 28, and 35 days were used (Fig. 1). The gonadectomized animals received the following hormones: testosterone propionate (TP) 1.25 mg daily from the 1st through the 3rd days of life, and 300 μg daily for 5 days between the ages of 8 and 33 days (Fig. 1), diethylstilbestrol (DS), a synthetic estrogen, in a dose of 100 μg from the 29th through the 33rd days. The hormones were dissolved in olive oil or propylene-glycol and injected subcutaneously. Animals of the control group received the same volume of solvent, by a corresponding schedule. The serum CBG concentration of the rats was determined by a radioligand method, on reaching the age of 10-12 weeks, based on binding with labeled ^{3}H-cortisol [1]. The experimental results were subjected to statistical analysis and the significance of differences between parameters was determined by Student's t test.

EXPERIMENTAL RESULTS

Determination of the serum CBG level in the blood serum of adult rats gonadectomized at different times of ontogeny showed that its level in males gonadectomized on the 1st day, was 30-40% higher between the 7th and 21st days or on the 28th day of life than in males castrated at the age of maturity ($p < 0.01$), and did not differ from its level in intact females ($p > 0.1$; Fig 2). Thus removal of the source of endogenous androgens until the 28th day of life leads to complete feminization of the

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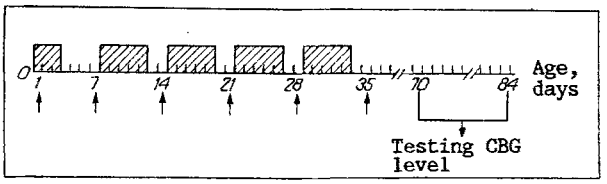

Fig. 1. Diagram showing details of experiments with gonadectomy and subsequent administration of sex hormones. Arrows indicate days of performance of gonadectomy or mock operation. Shading indicates days of injection of solvent or steroid.

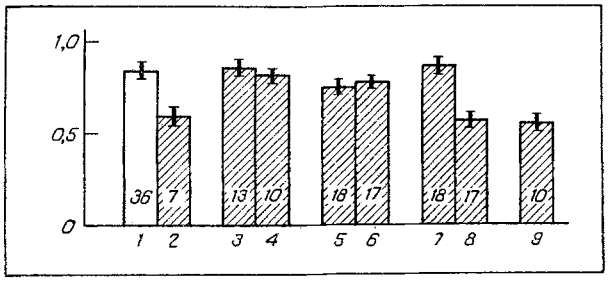

Fig. 2. CBG concentration in adult rats gonadectomized and androgenized at different periods of ontogeny (M ± m). Abscissa: 1) intact females; 2) males castrated at puberty 10-12 weeks after operation; 3, 5, 7, 9) males castrated: 3) on 1st day of life, 5) on 7th, 14th, or 21st days (combined group); 7) on 28th day; 9) on 35th day; 4, 6, 8) males castrated and receiving TP: 4) 1.25 mg from 1st through 3rd days of life; 6) 300 μg from 8th through 12th and from 15th through 19th days or from 22nd through 26th days (combined group); 8) 300 μg from 29th through 33rd days. Animals of groups 3, 5, 7, and 9 received solvent in accordance with the same schedule. Numbers inside columns indicate number of animals in groups. Statistical significance of differences: p_{2-3}, p_{2-5}, $p_{2-7} < 0.01$; p_{1-5}, $p_{1-7} > 0.1$; $p_{2-9} > 0.1$; $p_{1-9} < 0.01$; p_{3-4}, $p_{5-6} > 0.1$; $p_{7-8} < 0.01$; $p_{2-8} > 0.1$; ordinate, CBG level (in mg/ml $\cdot 10^{-3}$). Here and in Fig. 3, unshaded columns indicate females, shaded columns indicate males.

CBG levels in males. Meanwhile the CBG level in adult males castrated on the 35th day of life did not differ from that in males gonadectomized at the age of maturity ($p > 0.1$) and was about 40% lower than in intact females ($p < 0.01$; Fig. 2). This indicates that gonadectomy after the 35th day of life does not prevent the formation of the male phenotype for the level of this protein in males. It follows from the data given above that for irreversible masculinization of the CBG level in males the presence of endogenous androgens is essential until the 35th day of life; under physiological conditions, the formation of the male phenotype for CBG concentration is complete by the 35th day of life.

To study when sensitivity to the effect of androgens, determining the CBG level appears the synthetic androgen TP was injected into males gonadectomized on different days of life (Fig. 1). The results of this series of experiments (Fig. 2) show that the CBG concentration in males castrated on different days of life until the 21st day, and receiving TP after the operation until the 26th day of life, does not differ from that in gonadectomized animals, receiving the solvent alone at the same times ($p > 0.1$). In other words, injection even of large doses of androgens before the 26th day of life does not masculinize the level of this protein. Injection of TP, however, from the 29th through the 33rd days of life into males castrated on the 28th day, however, has the effect that the CBG level in these animals was 30-40% below that in the control rats receiving the solvent ($p < 0.01$), and did not differ from the content of this protein in males castrated at puberty ($p > 0.1$). Thus, injection of the exogenous androgen from the 29th through the 33rd days of life irreversibly masculinizes the CBG level in rats.

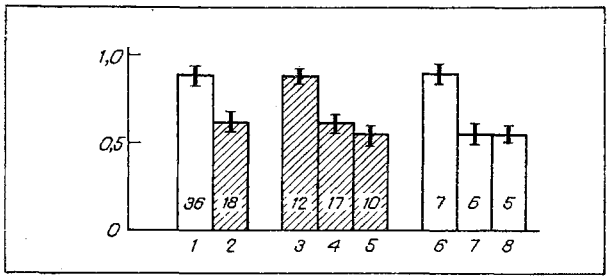

Fig. 3. Effect of injection of TP and DS during a sensitive period of ontogeny on CBG level in adult rats (M ± m). Abscissa: 1) intact females; 2) males castrated in adult life; 3, 6) rats gonadectomized on 28th day of life and receiving solvent from 29th through 33rd days; 4, 7) the same animals, receiving 300 μg TP from 29th through 33rd days; 5, 8) the same animals receiving 100 μg DS from 29th through 33rd days. Numbers within columns indicate number of animals in group. Statistical significance of differences: $p_{5-8} > 0.1$; p_{4-5}, $p_{7-8} > 0.1$; p_{3-4}; p_{3-5}, p_{6-7}, $p_{6-8} < 0.01$; ordinate, CBG level (in ng/ml $\cdot 10^{-3}$).

It can be concluded from an examination of these data that sensitivity to the action of androgens, determining the CBG level, first appears in males on about the 28th day of life. On the other hand, considering data in [1] on the possibility of manifestation of only reversible regulatory effects of androgens on the CBG level in mature females and males, it can be concluded that sensitivity to the programming action of these hormones disappears with the onset of puberty. Under physiological conditions the formation of the male phenotype of the CBG level in males ends before the 35th day of life, whereas sensitivity to androgens does not appear until after the 28th day. Thus negative programming of the CBG level by androgens in males evidently takes place within a sufficiently limited time interval, namely from the 29th through the 35th days of life, as is shown by the range of the sensitive (critical) period of sensitivity of the programmed structures to the action of sex hormones, which we found. Some sexually differentiated brain functions are known to be subjected to irreversible programming in males, both under the influence of aromatized androgens and directly under the influence of estrogens, whereas certain other sex-dependent features are determined under the influence of androgens only [3]. To explain these data the hypothesis of aromatization of androgens has been put forward, according to which testosterone can be converted into estradiol under the influence of an aromatase present in brain structures, and it can exert its own action through estrogenic receptors. In this connection it was interesting to study whether estrogens can have a negative programming effect on the CBG concentration in rats.

To solve this problem we determined the CBG level in rats gonadectomized on the 28th day of life and receiving DS, a synthetic estrogen, not bound with α-fetoprotein or with the estrogen-binding protein of immature rat blood plasma, during the next 5 days. Animals gonadectomized during the same period of life and receiving either TP or the solvent served as the control. The CBG level in these animals was determined when they reached the age of 10-12 weeks.

The results of this series of experiments show that the CBG level in males and females undergoing prepubertal gonadectomy and receiving DS in the sensitive period of ontogeny, do not differ in relation to sex ($p > 0.1$; Fig. 3). The CBG level in animals receiving TP did not differ from that in estrogenized rats ($p > 0.1$). Under these circumstances the CBG level in gonadectomized females and males receiving the solvent was about 30-40% higher than in estrogenized or androgenized rats ($p < 0.001$). The results show that estrogens, if injected into prepubertally gonadectomized animals, have a negative determinant effect on the CBG level similar to that of testosterone, an aromatizing androgen.

Data on the possibility of artificial negative imprinting of the CBG level under the influence of exogenous estrogens indicate that testosterone, possibly under physiological conditions, has a determinant action on the feature studied, being converted into estradiol.

In view of data in the literature on the presence of an aromatize in the brain and its very low activity in the liver [2, 3], it can be postulated that the most probable tissue level of programming of the CBG level under the influence of aromatized androgens is in certain brain structures that are involved in the regulation of activity of hepatic functions. A special investigation is being undertaken for a more detailed study of this problem.

The fact that the programming of the CBG level by androgens in male rats occurs in the prepubertal period, characterized by a quite high degree of differentiation, is a new fact that indicates adaptability of highly organized structures and which broadens our ideas on general and special principles governing the formation of sexual dimorphism of various features. Moreover, this fact may also be of practical importance, for the possibility of irreversible actions of sex hormones on various functions of the body must be taken into account when sex hormone therapy is given prepubertally in man. Under these circumstances the administration of large doses both of androgens and of estrogens may lead to serious and irreversible changes not only of sex-dependent functions of the brain, but also of the liver.

LITERATURE CITED

1. R. M. Kurabekova, G. D. Mataradze, and V.B. Rozen, Probl. Éndokrinol., **34**, No. 6, 66 (1988).
2. V. B. Rozen, Principles of Endocrinology [in Russian], Moscow (1984).
3. C. Bardin and J. E. Catterall, Science, **211**, No. 5, 1285 (1981).
4. R. R. Gala and U. Westphal, Endocrinology, **77**, No. 5, 847 (1985).
5. U. Westphal, Steroid-Protein Interaction, Berlin (1971).

VITAMIN E DEPRESSES THE NEUROCYTOTOXIC ACTION OF KAINIC ACID IN CEREBELLAR GRANULE-CELL CULTURES

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The study of the mechanisms of action of neurocytotoxins — excitatory amino acids interacting with the glutamate receptor (glutamate, N-methyl-D-aspartate, quisqualic, ibotenic, kainic, and quinolinic acids) — is developing extremely rapidly at the present time. Interest in this problem is largely determined by the fact that the action of these neurotoxins on brain cells is regarded as the most adequate model with which to study the pathogenesis of various neurodegenerative diseases (Parkinson's and Alzheimer's diseases, Huntington's chorea, epilepsy, and also cerebral ischemia) [5, 11, 12]. In this connection analysis of the mechanism of action of these neurotoxins and their antagonists is essential if optimal pharmacological approaches are to be found to the prevention and treatment of neurodegenerative diseases of the human CNS.

The mechanism of action of the above-mentioned neurotoxins is known to be a complex cascade process. It begins with interaction of the neurotoxin with the glutamate receptor and activation of the Ca^{2+}-channel associated with the receptor, and it ends with activation of proteolytic and lipolytic Ca^{2+}-dependent enzymes as a result of entry of Ca^{2+} (calcium death) [4, 14]. The intermediate stages of this cascade mechanism have received less study. Data obtained recently point to the involvement of active forms of oxygen in the realization of the neurotoxic action of kainic acid on neurons in culture [6]. Since membrane lipids constitute one of the principal oxidation substrates during the action of active forms of oxygen [13], and since the state of the lipid bilayer of the outer cytoplasmic membrane determines the low intracellular Ca^{2+} concentration, it was natural to suggest that destruction of membrane lipids may be an important step in damage to neurons under the influence of kainic acid. Since vitamin E is a universal stabilizer of neuron membranes [2, 3], it was decided to study the effect of α-tocopherol on the neurotoxic action of kainic acid, using monolayer cultures of cerebellar granule cells.

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