RESPONSE OF THE SPECIFIC CORTISOL TRANSPORT SYSTEM TO HEMOBLASTOSIS

N. D. Goncharova, N. P. Goncharov, and V. N. Lebedev*

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The considerable successful experience of the use of glucocorticoid hormones for the treatment of malignant diseases of the blood system is evidence of the possible pathogenetic role of these compounds in the development of hemoblastosis. Investigation of the pattern of function of the steroid-producing glands in hemoblastoses is accordingly a very topical problem.

Previous investigations showed the presence of marked disturbances of the synthesis and metabolism of glucocorticoid hormones [2, 3]. However, for a more complete understanding of the glucocorticoid function it is also important to study specific cortisol transport. In the modern view, the bulk (97-99%) of the cortisol circulates in the blood in the form of a physiologically inactive complex with specific corticosteroid-binding globulin (CBG). Only the free form of the hormone, not bound with a complex-forming protein, is considered to be biologically active [8, 12].

The aim of this investigation was to study interaction of cortisol with CBG (transcortin) in blood plasma from healthy donors and from patients with hemoblastosis.

EXPERIMENTAL METHOD

A group of 17 men and 10 women aged 50 ± 5 and 42 ± 6 years respectively, suffering from hemoblastosis for 1-6 months, in the acute period of the disease before the beginning of treatment, was investigated. Seven patients had chronic lymphatic leukemia, 6 had chronic myeloid leukemia, 8 had acute leukemias, and 6 had lymphogranulomatosis. The control group consisted of 14 clinically healthy men and 17 women of the corresponding ages.

Parameters of specific cortisol transport, i.e., the binding ability of CBG, namely binding capacity, concentration, and also an affinity constant of CBG for cortisol, namely the association constant (K_a) of the CBG–cortisol complex, were estimated by saturation analysis [1] in peripheral blood plasma taken from the cubital vein at 9-10 a.m.

The plasma was freed from endogenous steroids by adsorption on activated charcoal, diluted with phosphate buffer, pH 7.4 (0.1 M with the addition of 1 g gelatin, 9 g sodium chloride, and 1 g sodium azide to 1 liter of solution) 50 or 100 times, and mixed with a buffered solution of ^{3}H-cortisol (1,2,2,6-^{3}H-cortisol, specific activity 95.3 Ci/mmole, from New England Nuclear, USA) so that activity in 0.5 ml of the prepared mixture was 4-6,000 cpm, and samples of 0.5 ml were introduced into test tubes each containing 100 µl of buffered solutions of nonradioactive cortisol in different concentrations (from 0.125 to 1,000 ng/0.1 ml). Quadruplicates were used for each dilution. The contents of the tubes were shaken and incubated for 10 min at 37°C and for 60 min on an ice bath (4°C). To separate free and bound forms of the steroid, 0.5 ml of a 0.4% suspension of activated charcoal was added to each sample, which was then centrifuged for 5 min at 2000 g.

The level of radioactivity in the supernatant was measured with an SL-30 liquid scintillation counter (from Intertechnique, France) with counting efficiency relative to tritium of

*Deceased.

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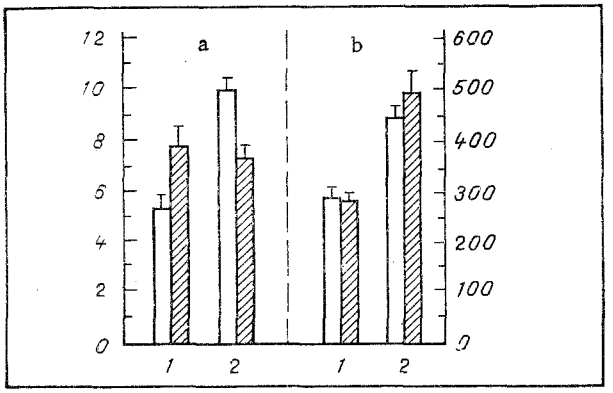

Fig. 1

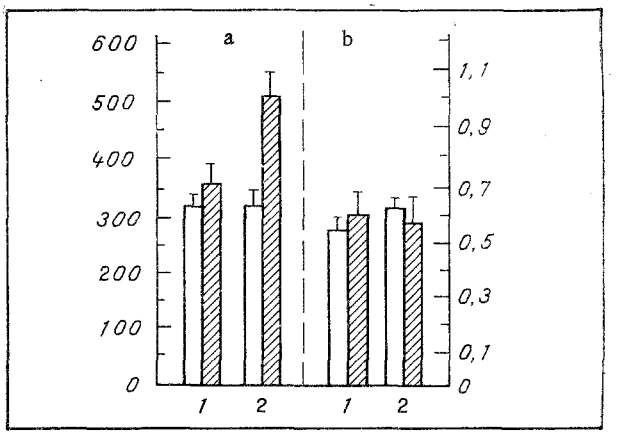

Fig. 2

Fig. 1. K_a for CBG–cortisol complex (1) and binding capacity of CBG (2) in peripheral blood plasma from normal subjects and patients with hemoblastosis (M ± m). Ordinate: on left, K_a (in $M^{-1} \times 10^8$); on right, binding capacity (in μg%). Here and in Fig. 2: a) men, b) women; unshaded columns – healthy subjects, shaded – patients.

Fig. 2. Total cortisol concentration (1) and index of free cortisol fraction (2) in peripheral blood plasma from normal subjects and patients with hemoblastosis (M ± m). ordinate: on left, total cortisol concentration (in nM); on right, index of free cortisol fraction.

50%. A solution of 2,5-diphenyloxazole (PPO) and 1,4-di-(5-phenyl-2-oxazolyl)benzene (POPOP) in toluene (5 g PPO and 0.5 g POPOP to 1 liter toluene) was used as the scintillation mixture.

Nonspecific binding was calculated after addition of 1,000 ng of nonradioactive steroid (it did not exceed 4%). Levels of binding, corrected for nonspecific binding, in per cent of labeled cortisol in samples with different additions of hormone were used to calculate the specifically bound cortisol concentration (in nanomoles/liter) for each addition. A Scatchard plot was constructed on the basis of the results. The graph was used to calculate the maximal binding capacity of CBG and K_a for the CBG–cortisol complex. The Scatchard plots were constructed and the parameters of specific cortisol transport calculated on an "Iskra" computer (USSR) using specially written programs. In all cases the coefficient of correlation for the Scatchard curves exceeded 0.95 (for all 10 points).

The reproducibility of the method, estimated by means of the coefficient of variation of values of the test parameters in control plasma, obtained over a period of 12 months, was 11.8% for the CBG concentration and 10.1% for K_a.

The total (free and bound with CBG) cortisol concentration was determined by the competitive binding method [11]. To assess the form of the hormone not bound with CBG, the index of the free fraction of the compound was calculated as the ratio of the molar concentration of the total cortisol fraction and CBG. The basic assumption was that the CBG molecule contains a single steroid binding site [12] and, under conditions of saturation with the ligand, CBG and cortisol interact in equimolar quantities. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

High affinity of the complex-forming protein for cortisol (K_a about 10^8 M^{-1}) and a relatively low capacity (about 10^{-7} U) were observed for healthy subjects, in agreement with the characteristics of the human CBG given in the literature [8, 10, 12].

For instance, the binding capacity of CBG averaged 450 ± 18 nanomoles/liter in women and 503 ± 26 nanomoles/liter in men, and the values of K_a were (5.8 ± 0.4) × 10^8 and (5.5 ± 0.5) × 10^8 M^{-1} respectively (Fig. 1). Sex differences in the value of binding capacity of transcortin and its affinity constant for cortisol were not statistically significant ($P > 0.05$). Only a tendency for higher values of CBG concentration to occur in male plasma could be detected.

Significant sex differences likewise were not found in the level of total cortisol circulating in the blood stream, and also in the index of the free fraction of the hormone

(Fig. 2). The cortisol concentration in women averaged 274 ± 19 nM, and in men 320 ± 21 nM; the corresponding indices of free cortisol were 0.63 ± 0.03 and 0.65 ± 0.04.

In patients with hemoblastoses (1) K_a for the transcortin–cortisol complex as a whole was similar (10^8 M^{-1}) to that for healthy subjects, and together with the high value of the coefficients of correlation, indicating the straight-line character of the Scatchard plots, this is evidence of the absence of specific corticosteroid-binding proteins different from transcortin in the plasma of hemoblastosis patients.

At the same time, it will be noted that in male patients K_a for the cortisol–transcortin complex in some cases reached 10^9 M^{-1} and, for the group as a whole, it exceeded statistically significantly the analogous parameter in the corresponding control ($P < 0.01$) and in the women investigated ($P < 0.01$). The increase in K_a for the hormone–protein complex studied was evidently connected with definite reorientation of the reactive group in the active centers of the CBG molecules, capable of changing the affinity of transcortin for cortisol.

The appearance of sex differences in the parameters of affinity of CBG for cortisol in patients with hemoblastoses correlated with the character of the change in their CBG binding capacity. An increase in K_a for the hormone–protein complex in men was accompanied by a statistically significant decrease in the binding capacity of transcortin compared with that in the corresponding control group ($P < 0.001$) and in the group of women patients ($P < 0.01$). By contrast with men, the binding capacity of CBG in women with hemoblastosis was virtually unchanged.

Weakening of the binding power of transcortin indicates a fall in the plasma CBG level, which may be due to a fall in the intensity of synthesis of the complex-forming protein or an increase in the rate of its breakdown. Since both these processes take place in the liver [12, 13], and the liver is the organ most frequently and most severely damaged in hemoblastosis [4, 5], in our view a disturbance of the processes of regulation of enzyme systems catalyzing both the biosynthesis and the catabolism of CBG cannot be ruled out.

The decrease in the binding capacity of transcortin in turn caused a marked increase in the index of the free cortisol fraction in the plasma of men patients (1.02 ± 0.09 compared with 0.65 ± 0.04 in the control, $P < 0.001$) and it was the main cause of the statistically significant sex differences in the index of the free forms of the hormone ($P < 0.01$). In men this index was almost twice as high as in women.

The increase in the index of the free cortisol fraction, not bound with specific transport protein, reflects a rise in the level of the biologically active form of the hormone, capable of interacting directly with the target cells and leading to realization of the hormonal effect, in the circulating blood.

Sex differences in the binding capacity of CBG and the index of the free cortisol fraction discovered in patients with malignant diseases of the blood system are evidently due to the greater resistance of the transcortin–cortisol system in women, possibly on account of their higher estrogen level than in men. Femal sex hormones are known to stimulate CBG biosynthesis [10]. Elevation of the level of the free biologically active cortisol fraction is perhaps a reflection of the higher reactivity of the male organism to the pathological process.

The investigations thus demonstrate marked changed in the character of specific cortisol transport in patients with neoplastic diseases of the blood system. Together with disturbances affecting synthesis and metabolism of glucocorticoid hormones discovered previously, these changes are evidence of damage to all stages of the glucocorticoid function of the adrenals by the hemoblastosis process.

Considering the marked regulatory action of glucocorticoid hormones on hematopoiesis and immunopoiesis [6, 7, 9] it can be tentatively suggested that changes in the hormonal function of the adrenal glands have a substantial influence on the immunologic parameters and processes of proliferation and differentiation in hematopietic tissue and facilitate progression of leukemias.

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EFFECT OF SEX HORMONES ON 1,2-DIMETHYLHYDRAZINE METABOLISM IN THE CBA MOUSE KIDNEY

G. Yu. Chemeris and A. F. Karamysheva

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The mechanisms responsible for the manifestation of sexual dimorphism in carcinogenesis have not been adequately studied. Convenient models for their investigation are sarcoma of the renal capsule and adenomas of the kidneys, which can be induced with high frequency in male CBA mice by 1,2-dimethylhydrazine (DMH) [2] but which appear only infrequently in females under the same conditions [1]. Preliminary castration of males sharply reduces the frequency of formation of these tumors [3], while testosterone propionate (TP) restores their frequency to its initial level, but only if it is administered simultaneously with DMH [4]. DMH is a carcinogen with indirect action, requiring metabolic activation in the recipient organism; one cause of differences in the sensitivity of male and female CBA mice to the action of this carcinogen may therefore be a difference in the activity of its metabolism in the target organ.

This paper describes the study of DMH metabolism in the kidneys and liver of CBA mice.

EXPERIMENTAL METHOD

Male and female CBA mice aged 2-3 months were obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR. The males were castrated 2 weeks before the experiment. TP was injected subcutaneously in olive oil in a sessional dose of 0.5 mg per mouse 24 h before the experiment. To determine incorporation of ^{14}C-DMH into DNA in the mouse liver and kidneys, unlabeled DMH·2HCl was added to the ^{14}C-DMH.2HCl (specific radioactivity 8.4 mCi/mmole, from New England Nuclear, USA) until the specific radioactivity was 3.5 mCi/mmole (the pH was adjusted to physiological with dry Na_2CO_3), and the product was injected subcutaneously in a dose of 15 mg/kg body weight (calculated as base). The ^{14}C-DMH was generously provided by the International Cancer Research Agency (Lyon, France).

The microsomal fraction was isolated by the method in [7]. To determine the demethylating activity of the microsomes, the substrate (10 mM) was incubated for 5 min at 37°C in a mixture containing 3 mM NADPH (NADH), 1 mg/ml microsomal protein, 16 mM $MgCl_2$, and 0.1M phosphate buffer, pH 7.4. The reaction was stopped by the addition of 0.5 ml of 20% TCA. After precipitation of the protein by centrifugation for 10 min at 3500 g the formaldehyde concentration in the supernatant was determined by the color reaction described in [9]. To isolate DNA the separate organs were frozen in liquid nitrogen and kept at −20°C for not

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