Thyroid Hormones as Regulators of Binding Capacity of Corticosteroid-Binding Globulin in Rats Subjected to Acute Immobilization Stress

L. I. Nadol'nik, N. V. Emel'yanov, and V. V. Vinogradov

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Decreased binding capacity of corticosteroid-binding protein in rats subjected to prolonged immobilization stress is related to reduction in the contents of triiodothyronine and thyroxin, rather than to a continuous increase in blood corticosterone concentration.

Key Words: *glucocorticoids; corticosteroid-binding globulin; thyroid hormones; regulation*

Our previous experiments and published data show that binding capacity of corticosteroid-binding protein (CBP) significantly decreases in acute stress [1,8,14] due to marked inhibitory effects of glucocorticoids [5,13]. However, recent studies demonstrated that adrenalectomy [10] and administration of glucocorticoid receptor blockers [5] do not prevent the decrease in CBP level during acute immobilization stress (AIS) probably due to the effects of some adrenal factors (but not glucocorticoids) on CBP [6]. It was shown that dexamethasone does not suppress the production of CBP mRNA by cultured fetal rat hepatocytes, but induces the inhibitory effect of interleukin-6 [6].

These data indicate that it is necessary to reconsider the notion of hormonal regulation of CBP biological activity in acute stress. Since triiodothyronine (T_3) stimulates the production of specific CBP in the liver [7], while thyroidectomy reduces its plasma concentration [4], it seems interesting to study not only glucocorticoid, but also thyroid functions under conditions of AIS and to analyze their contribution in the regulation of the main parameters of complex formation with CBP.

MATERIALS AND METHODS

Experiments were performed on outbred albino male rats weighing 160-180 g. AIS was modeled by fixation of animals with a soft ligature in the supine position for 1-72 h.

Plasma concentrations of thyroxin (T_4) and T_3 were measured by radioimmunoassay using RIA-T_4-ST and RIA-T_3-ST kits (Beloris, Minsk).

The content of corticosterone was assayed by high-performance liquid chromatography on a Milikhrom chromatograph using hexane:methanol:chloroform mixture (7:1:1) as a mobile phase [15]. The binding 6,7-$^3H_2$-hydrocortisone (Izotop) with plasma CBP was determined in 0.005 M Tris-buffer containing 1.5 mM EDTA, 10 mM dithiothreitol, and 0.25 M sucrose (pH 7.5). Endogenous steroids were removed with 10% Norit A charcoal coated with 0.1% dextran (37°C, 30 min). Nonspecific binding was analyzed by adding a 1260-fold excess of unlabeled hydrocortisone. The association constant and the concentration of binding sites were calculated in Scatchard coordinates.

RESULTS

Plasma corticosterone content in rats increased by 4.98 times 1 h after the start of AIS (Fig. 1), remained high over 2 days of immobilization, and decreased by the end of day 3, which indicated considerable activation of the pituitary-adrenal system. Functional activity of the thyroid system also changed. Plasma T_4 after 24-h AIS decreased by 2.87 times compared to the control (Fig. 1). The concentration of metabolically active T_3 increased by the 2nd hour of observations,

Laboratory of Biochemistry of Endocrine Glands, Institute of Biochemistry, Belarussian Academy of Sciences, Grodno

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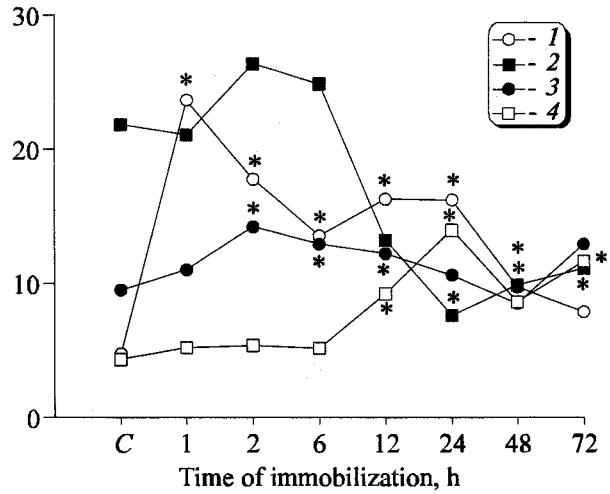

Fig. 1. Plasma concentrations of corticosterone (μM×10, *1*), thyroxin (nM, *2*), and triiodothyronine (nM×10, *3*) and T_3/T_4 ratio in control rats (*C*) and after immobilization stress. *$p<0.05$ compared to the control.

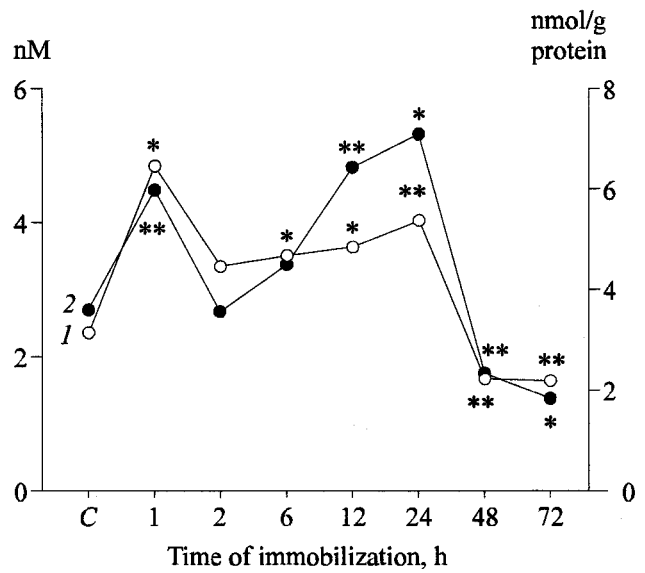

Fig. 2. Absolute (nM, *1*) and relative (nmol/g protein, *2*) concentrations of ^{3}H-hydrocortisone-binding sites during acute immobilization stress. *$p<0.01$ and **$p<0.05$ compared to the control (*C*).

remained high for 12 h, and then decreased to the control level.

The binding capacity of specific CBP was elevated 1 h after the start of AIS due to a 2.06-fold increase in the number of binding sites on the protein (Fig. 2). The increased number of binding sites at the early stages of stress is probably related to the elevation of CBP concentration, because CBP molecule has only 1 high-affinity binding center for glucocorticoids [3]. It was shown that under rest conditions, 68% binding sites on CBP are not occupied by steroids [9], and free CBP undergo polymerization [12] followed by inactivation of binding sites. Taking these facts into account, this rapid response is probably associated with CBP depolymerization [11] at high concentration of plasma corticosterone. However, increased binding capacity of CBP 6, 12, and 24 h after the start of AIS was probably related to the stimulatory effect of T_3 [7], whose concentration was also elevated. The number of binding sites on CBP remained high over 24 h of observations, although the concentration of corticosterone inhibiting CBP [13] was also elevated at this period. The affinity constant reflecting the glucocorticoid binding capacity of CBP remained unchanged (14×10^7 M^{-1}) in AIS. Hence, its complex-forming properties are presented.

The binding capacity of CBP decreased by 1.4 times after 48-h AIS and remained lowered by the end of day 3. This was preceded by a considerable decrease in plasma T_4 concentration 12 and 24 h after the start of AIS (by 1.65 and 2.87 times, respectively, compared to the control). The content of T_3 also returned to the control level 24 h after the start of AIS. The T_3/T_4 ratio increased 12 and 24 h after the start of AIS by 1.46 and 2.19 times, respectively (Fig. 1), which indicated considerable changes in thyroid function. Plasma corticosterone concentration decreased only by the end of day 3. Reduced binding capacity of CBP after 48-h AIS was probably related to a decrease in T_3 concentration 24 h after the start of AIS, since $T_3$was shown to stimulate CBP biosynthesis in the liver [7]. Our previous studies demonstrated that plasma CBP concentration increased (against the background of elevated T_4 content) on day 7 after adrenalectomy and reducing plasma corticosterone level [2]. This fact confirms the role of thyroid hormones in the regulation of CBP activity.

Our findings indicate that thyroid hormone regulate the activity of CBP in AIS. These results explain previous data showing that the blockade of glucocorticoid receptors and adrenalectomy do not prevent the decrease in CBP concentration during acute stress [5, 10] characterized by reduced content of CBP-stimulating thyroid hormones.

Hence, not only the adrenal cortex but also thyroid gland are markedly activated at the early stages of AIS. This activation is accompanied by a considerable increase in the binding capacity of CBP and reflects the adaptive reaction of the body. Decreased binding capacity of CBP during prolonged immobilization is related to the reduction in T_3 and T_4 levels, rather than to continuous increase in blood corticosterone concentration.

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