Functional State of Adrenocortical System in Rats with Manifest Alloxan-Induced Diabetes Mellitus

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In the adrenals of male rats with manifest form of alloxan-induced diabetes mellitus (blood glucose >20 mmol/liter), the content of progesterone was higher by 1.6 times, deoxycorticosterone by 2.5 times, corticosterone by 3.3 times, and 11-dehydrocorticosterone by 1.8 times than in the adrenals of control animals. Increased concentration of corticosterone and 11-dehydrocorticosterone were also found in the serum of rats with alloxan diabetes, but the difference between the experimental and control groups by these parameters was less pronounced compared to parameters in the adrenal glands.

Key Words: *alloxan-induced diabetes; adrenal glands; blood; corticosteroids*

Clinical studies showed that non-compensated diabetes mellitus (DM) is associated with increased plasma concentrations of ACTH and cortisol, changed diurnal rhythm of the blood content of this glucocorticoid hormone, and enhanced urinary excretion of free cortisol and its metabolites [1,11]. Hypertrophy of the cortex of the adrenal glands (AG), increased secretion of glucocorticoid hormones and their elevated blood contents, and abnormal reaction of the pituitary-adrenal system to various stress factors were also noted in experimental DM [9,10]. It was hypothesized that metabolic disturbances typical of DM can modulate functional state of the adrenocortical system, which in turn can aggravate DM symptoms [1,3]. Hence, evaluation of the function of the adrenocortical system in DM is of principal importance. The mechanisms responsible for elevation of blood content of glucocorticoid hormones in DM, changes in their synthesis in AG cortex and metabolism in peripheral tissues, and involvement of other corticosteroids remain still poorly understood.

Here we studied functional state of the adrenocortical system in rats with DM by measuring the content of the major corticosteroids in AG and blood by HPLC method allowing detection of the synthesis and metabolism of steroid hormones [2,8].

MATERIALS AND METHODS

The study was performed on 30 male Wistar mice obtained from Nursery of Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences (Novosibirsk). For DM modeling, alloxan trihydrate (LaChema) was injected to experimental animals (n=18) in a dose of 17 mg per 100 g body weight. Controls received 0.9% aqueous solution of NaCl according to the same scheme.

The decrease in the number of pancreatic islets and insulin secretion after alloxan injection led to the development of acute DM (death of experimental animals within 3-5 days of the disease) or chronic DM of various severity [5]. The severity of the disease was evaluated by the content of glucose and immunoreactive insulin in the blood taken from the caudal vein on day 10 after alloxan injection.

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The concentration of glucose was measured by the enzymatic method using Fluitest GLU kits (Biocon), the content of immunoreactive insulin in blood serum was radioimmunoassayed using Rio-INS-PG-^{125}J kits (KHOPIBOKH). In the experimental group, 9 male mice had manifest form of severe noncompensated DM, which was seen from high blood glucose (23.64±1.34 *vs*. 6.24±0.20 mmol/liter in the control; $p<0.01$) and lower serum content of immunoreactive insulin (70.2±11.1 *vs*. 106.8±11.4 pmol/liter in the control; $p<0.05$). These animals were used for further studies of the functional state of the adrenocortical system.

Corticosteroid hormones in AG and blood plasma were measured by HPLC [8]. Blood plasma (0.5 ml) was extracted with 4 ml hexane, the organic fraction was discarded, while the aqueous phase was mixed with 9 ml chloroform, extracted for 5 min, the organic fraction was evaporated to dryness, dissolved in 24 μl eluent and analyzed by HPLC. AG were separated from the adipose tissue, weighed on a torsion balance VT-500, each AG was transferred into individual glass homogenizer placed into an ice bath, and thoroughly homogenized in 2.5 ml cold acetone; 8 μl homogenate was taken for the analysis. The content of steroids was determined by microcolumn HPLC on a Milkhrom-1 chromatograph (Nauchpribor) equipped with an analytic column (62×2 mm, Silasorb C_{18}, 5 μ). Acetonitrile gradient in water (from 30 to 45%) was used as the eluent; the substances were detected at 240 nm.

The concentration of progesterone in AG homogenates was measured by EIA using Steroid IFA-Progesteron kits (Alkor BIO).

Activity of tyrosine aminotransferase (EC 2.6.1.5) in the liver was measured by the formation of p-hydroxyphenylpyruvate (in the reaction of amino group transfer from L-tyrosine to α-ketoglutaric acid) followed by its conversion into p-hydroxybenzaldehyde in alkaline medium. Optical density was measured at λ=331 nm [4].

The data were processed statistically using Mann—Whitney test. The differences were significant at $p<0.05$.

RESULTS

Rats with DM had increased serum content of the major highly reactive glucocorticoid hormone corticosterone (by 2.5 times compared to control animals) and low active 11-dehydrocorticosterone (11-DHC) formed from corticosterone in the reaction catalyzed by 11β-hydroxysteroid dehydrogenase [6]. The corticosterone to 11-DHC ratio reflecting the level of hormonal glucocorticoid activity [7] increased by 2.1 times in DM (Table 1).

In rats with DM, the weight of AG and their mass index (per 100 g body weight) significantly surpassed the corresponding values in the control group: (50.1± 2.9 *vs*. 41.8±1.9 mg, $p<0.05$; 20.0±1.1 *vs.* 12.5±0.6 mg/100 g, $p<0.05$). Hence, both the absolute and relative weights of AG were increased in rats with manifest form of noncompensated DM.

The content of progesterone (precursor in the synthesis of gluco- and mineralcorticoids) in AG of rats with DM was 1.6-fold higher that in controls; the contents of deoxycorticosterone (intermediate product of corticosteroid synthesis from progesterone) and corticosterone were higher by 2.5 and 3.3 times compared to the control. These findings suggest that synthesis of corticosterone in AG increases in DM due to activation of all stages of steroidogenesis.

The content of low-active form of corticosterone, 11-DHC increased by 1.8 times, while the ratio of corticosterone to 11-DHC increased by 2.6 times in rats with DM compared to controls. These changes also attest to accumulation of highly active glucocorticoid corticosterone in AG, which agrees with the data on blood hormone concentrations (Table 1).

Increased activity of tyrosine aminotransferase in the liver (0.82±0.03 and 1.58±0.11 μmol p-hydroxyphenylpyruvate/h×mg tissue in controls and rats with DM, respectively; $p<0.01$) also attested to the increase in blood concentration of physiologically active hormone. Increased activity of tyrosine aminotransferase in the liver under the action of glucocorticoids is a well-known example of hormonal induction of enzyme activity [4].

However, DM is associated with the increase in the synthesis of not only gluco-, but also mineralcorticoids, which was seen from increased content of aldosterone (by 2.3 times compared to the control value, Table 2).

Thus, activity of steroidogenesis in both zona fasciculata and zona glomeruloza of AG was increased in animals with DM with predominant accumulation of highly active hormone corticosterone in AG and blood. The increase in the content of

TABLE 1. Content of Corticosteroids (ng/ml) in Blood Serum from Rats with Manifest DM (*M*±*m*)

Group	11-DHC	Corticosterone	Corticosterone/11-DHC
Control	8.25±1.80	29.30±8.08	4.13±0.68
Experimental	12.67±2.92	73.2±13.2*	8.88±2.95

Note. Here and in Table 2: *$p<0.05$ compared to the control.

TABLE 2. Content of Steroid Hormones (ng/mg issue) in AG of Rats with Manifest DM (*M*±*m*)

Group	Progesterone	Deoxycortico-sterone	Corticosterone	Aldosterone	11-DHC	Corticosterone/ 11-DHC
Control	1.92±0.55	0.60±0.11	3.61±1.49	0.48±0.11	1.18±0.24	2.08±0.63
Experimental	3.01±0.89	1.47±0.26	11.95±3.05	1.10±0.33	2.02±0.45	5.50±1.02

low active form of corticosterone 11-DHC reflects increased production of this steroid in AG, rather than activation of glucocorticoid metabolism. The fact that in rats with DM the level of immunoreactive insulin in blood serum decreased by only 1.5 times, while blood glucose content increased more than 3-fold attests to a considerable contribution of increased synthesis of contrainsulin hormone corticosterone in AG and its increased release into the blood.

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