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Activity of 11β-Hydroxysteroid Dehydrogenase in the Adrenal Glands, Liver, and Kidneys of Rats with Experimental Diabetes

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We studied activity of the key enzyme of the pre-receptor metabolism of glucocorticoid hormones, 11β-hydroxysteroid dehydrogenase, in rat adrenal glands, renal cortex and liver in the course of development of alloxan diabetes (9, 20, and 28 day). The enzyme activity was increased 3-4 fold in the adrenal glands throughout the experiment. At the same time, according to the adrenal gland level of corticosterone, its precursor 11-deoxycorticosterone and reversible metabolite 11-dehydrocorticosterone, activity of the second isoform of the enzyme dominated at the early stages of diabetes, and that of the first isoform, at later stages. In long-term diabetes (28 days), along with reduced synthesis of corticosterone and production of 11-dehydrocorticosterone in the adrenal glands, the extra-adrenal formation of corticosterone was activated as indicated by enhanced activity of the first isoform in the liver and that of the second isoform in the kidneys. These changes in activity of the enzyme isoforms promote local formation of corticosterone from its reversible metabolite in the liver and persisting hyperglycemia in diabetes.

Key Words: *11β-hydroxysteroid dehydrogenase; alloxan diabetes; corticosterone; 11-dehydrocorticosterone; high performance liquid chromatography*

One of the mechanisms of the regulation of the levels of glucocorticoid hormones in the blood and tissues involves the reactions of mutual transformation of the physiologically active hormone and its reversible metabolite via the key enzyme of the pre-receptor metabolism of glucocorticoid hormones, 11β-hydroxysteroid dehydrogenase (11β-HSD). This enzyme is active in the tissues of many organs and in the adrenal gland [4,12]. The first isoform of the enzyme is associated with glucocorticoid receptors and catalyzes reduction of inactive cortisone in humans (in rats, 11-dehydrocorticosterone) to cortisol (in rats, corticosterone) [13]. For example, the contribution of the first isoform of 11β-HSD to the extra-adrenal synthesis of cortisol from cortisone amounts up to 30% of its synthesis in adrenal gland of a healthy person [3]. The highest activity of the first isoform of the enzyme was found in the liver [6,13]. Increase in activity of the first isoform of 11β-HSD in the liver promotes the local increase in the amount of active glucocorticoids in the hepatocytes and enhances gluconeogenesis [13].

The second isoform of the enzyme is co-localized with mineralocorticoid receptors, it converts active glucocorticoid hormones to inactive metabolites in the aldosterone target tissues (kidney, salivary glands, intestines, *etc*.), thereby providing a selectivity of these receptors [10,13]. Both isoforms of the enzyme are active in the adrenal glands [12]. It is currently unknown, what mechanisms coordinate the synthesis of glucocorticoid hormones in the adrenal glands and their local formation in tissues [8].

Changes in activity of the first and second isoform of 11β-HSD in the tissues of rats with experimental diabetes were not previously examined. However, this issue is extremely important considering the role of glucocorticoid hormones in activation of gluconeoge-

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nesis in the liver and the development of hyperglycemia during insulin deficiency.

Here we studied activity of 11β-HSD and its isoforms in rat adrenal gland, renal cortex, and liver tissue in the dynamics of the alloxan diabetes.

MATERIALS AND METHODS

The work was carried out on adult male Wistar rats. Experiments were carried out in compliance with the principles of humanity as set out in the Directives of the European Community and the Declaration of Helsinki. Rats received a standard vivarium diet with free access to food and water. Alloxan diabetes was induced by a single intraperitoneal injection of alloxan hydrate dissolved in citrate buffer at 170 mg/kg body weight after 18-hour fast. The control group received the same volume of citrate buffer. Rats were withdrawn from the experiment by decapitation on day 9, 20, and 28 after the administration of alloxan or citrate buffer. Serum, liver, and kidney tissues were stored at -20°C until analysis.

Plasma glucose levels were determined by enzymatic method using Fluitest GLU reagent (Analyticon Biotechnologies AG). Concentration of corticosteroid hormones in the blood serum and homogenates of the adrenal glands as well as activity of 11β-HSD in homogenates of the renal cortex and the liver were evaluated by using an earlier developed by us technique by means of microcolumn HPLC [2,5]. Activity of 11β-HSD in the adrenal glands was determined by the ratio of corticosterone to 11-dehydrocorticosterone in the adrenal glands.

The data were statistically processed using Statistica 6.0 soft (StatSoft). Results were presented as $M\pm m$, where M, sample mean; m, standard error of mean. The differences between group means were assessed using a nonparametric Mann–Whitney test. The null hypothesis was accepted at the 5% level of significance.

RESULTS

Serum glucose was significantly increased on day 9 after alloxan administration (6.0±0.3 mmol/liter in the controls, 28.3±4.2 mmol/liter in the rats with alloxan diabetes, $p<0.01$), which remained elevated throughout the experiment (27.2±3.0 and 27.3±2.8 mmol/liter in the experimental group 20 and 28 days after alloxan administration, respectively).

Previously, we have shown that absolute insulin deficiency and hyperglycemia that develop during alloxan diabetes cause a complex of metabolic and osmotic disorders activating the adrenocortical system [1,11]. The results obtained in this study indicated activation of corticosteroid synthesis, especially on experimental day 9 (Table 1). Increased level of adrenal 11-deoxycorticosterone, a precursor in the synthesis of corticosterone, corticosterone, and its reversible metabolite 11-dehydrocorticosterone is against the background of reduced content of the common early precursor, progesterone was indicative of pronounced activation of the late pathway of corticosteroid biosynthesis. The results suggest that activity of 11β-HSD increased 4-fold in comparison with the controls at this stage of the experiment is due to activation of a second isoform of the enzyme catalyzing the conversion of 11-dehydrocorticosterone from corticosterone under conditions of its increased synthesis.

The adrenal level of progesterone returned to the previous level in 20 days. In this case, downward trend in the content of 11-deoxycorticosterone and corticosterone was observed. The level of 11-dehydrocorticosterone reduced 2 times on day 20 in comparison with day 9, but at the same time, activity of 11β-HSD remained high (Table 1), which enables a conclusion about the increased activity of the first isoform catalyzing the formation of active hormone from its reversible metabolite.

TABLE 1. Content of Corticosteroids and Activity of 11β-HSD in the Adrenal Glands in the Course of Experimental Diabetes ($M\pm m$)

Parameter	Control group (n=19)	Experimental group (days after alloxan treatment)		
		9 (n=7)	20 (n=12)	28 (n=9)
Progesterone, ng/g tissue	0.41±0.09	0.12±0.02*	0.70±0.18°	0.51±0.18°
11-Deoxycorticosterone, ng/g tissue	0.75±0.15	2.19±0.93*	1.60±0.24*	0.81±0.21+
Corticosterone, ng/g tissue	5.03±1.17	25.69±3.30*	22.68±6.38*	15.01±3.81*
11-Dehydrocorticosterone, ng/g tissue	1.91±0.31	4.02±0.72*	2.07±0.27°	1.31±0.41°+
Activity of 11β-HSD, arb. units	2.5±0.5	10.1±3.4*	10.6±2.7*	7.7±2.4*

Note. Here and in Table 2: $p<0.05$ in comparison with *control group, °9 days, +20 days.

TABLE 2. Plasma Levels of Corticosteroids and Activity of 11β-HSD in Kidneys and Liver in the Course of Experimental Diabetes (*M*±*m*)

Parameter	Control group (*n*=19)	Experimental group (days after alloxan treatment)		
		9 (*n*=7)	20 (*n*=12)	28 (*n*=9)
Corticosterone, ng/ml	56.1±8.4	116.1±28.8*	197.2±35.2*	62.1±11.8+
11-Dehydrocorticosterone, ng/ml	8.8±1.4	13.8±1.8	5.6±0.5*o	14.3±2.2+
Activity of 11β-HSD in the kidneys, nmol×min^{-1}×g^{-1}	8.6±1.1	8.9±1.4	6.7±0.4	10.1±0.6o
Activity of 11β-HSD in the liver, nmol×min^{-1}×g^{-1}	241.1±13.5	200.0±38.4	266.5±21.4	308.0±27.0*

Activity of corticosterone synthesis from its precursors dropped to the initial level within 28 days of diabetes as evidenced by the levels of progesterone and 11-deoxycorticosterone in the adrenal glands not differing from those in the controls. However, in this case, its formation from 11-dehydrocorticosterone due to high activity of the first isoform of 11β-HSD remained elevated. The observed decrease in 11-dehydrocorticosterone relative to baseline values indicates the diminishing of the possibilities of this mechanism to form corticosterone in the adrenal glands in the long-term alloxan diabetes.

Enhanced synthesis of corticosterone in the adrenal glands after 9 and 20 day of diabetes was accompanied by elevating its blood level, but after 28 days, when the synthesis of this hormone in the adrenal glands was decreased, blood corticosterone did not differ from the initial value (Table 2). Blood 11-dehydrocorticosterone increased after 9 days of diabetes onset, when its adrenal level was heightened; it reduced within 20 days, when the first isoform of 11β-HSD was activated in the adrenal glands. However, on day 28, when the adrenal level of 11-dehydrocorticosterone decreased below the initial level, we recorded an increase in its blood level suggesting enhanced activity of the extra-adrenal formation of 11-dehydrocorticosterone. Indeed, activity of the second isoform of 11β-HSD in the renal cortex was increased at this stage of diabetes. In this case, activity of the first isoform of 11β-HSD in the liver was also increased.

After 28 days of diabetes onset, the rats still showed severe hyperglycemia. Previously, we have shown persistent metabolic disorders persisted severity of which has not changed with respect to the early stages of the experiment in rats with long-term alloxan diabetes [1]. Results obtained in this study suggest that during long-term alloxan diabetes associated with the reduction in the initially enhanced synthesis of glucocorticoid hormones in the adrenal glands, the mechanisms of their extra-adrenal formation were triggered associated with activation of the isoforms of 11β-HSD in the kidneys and liver. This leads to increased local formation of corticosterone directly in the liver and maintains the high activity of gluconeogenesis reactions, which contributes to the persistence of metabolic disorders characteristic of diabetes.

The obtained data on the role of 11β-HSD in the pathogenesis of metabolic disorders during experimental diabetes are consistent with the data that the inhibitors of the first isoform of this enzyme are currently regarded as potential therapeutic agents for the treatment of diabetes [7], obesity, and the metabolic syndrome [9].

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