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Effects of Intraperitoneal Administration of Mifepristone on Glucocorticoid Status of Experimental Animals

N. A. Pal'chikova, N. V. Kuznetsova, V. G. Selyatitskaya, O. P. Cherkasova, and O. I. Kuz'mina

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We studied the content of corticosterone and its precursors in the adrenal glands, corticosterone in blood serum and daily urine of rats, and activity of first and second isoforms of 11β-hydroxysteroid dehydrogenase in the liver and kidneys of rats after 15 daily intraperitoneal injections of 0.9% NaCl or glucocorticoid receptor blocker mifepristone in 0.9% NaCl. Daily injections of NaCl reduced the levels of pregnenolone, progesterone, and corticosterone in the adrenal glands, increased corticosterone excretion with urine, enhanced activity of the first isoform of 11β-hydroxysteroid dehydrogenase in the liver and reduction in activity of the second isoform of this enzyme in the kidneys. These changes are typical manifestations of chronic stress. Mifepristone restored pregnenolone content in the adrenal glands and increase in corticosterone concentration in the blood. Under these conditions, activity of the first isoform of 11β-hydroxysteroid dehydrogenase in the liver did not change, and a decrease in activity of the second isoform of the enzyme in the kidneys was less pronounced. The results suggest that mifepristone abolished the stress-mediated increase in activity of the first isoform of 11β-hydroxysteroid dehydrogenase in the liver and reduced local production of glucocorticoid hormones and their metabolic effects in hepatocytes.

Key Words: *mifepristone; corticosterone; adrenal glands; prereceptor metabolism, 11β-hydroxysteroid dehydrogenase*

Synthesis of corticosteroids in the adrenal cortex maintains the physiological level of glucocorticoid hormones in the blood and target organs. Necessary concentrations of glucocorticoid hormones in cells (e.g. liver cells) are maintained by local mechanism, in which the key role is played by the first isoform of the key enzyme of prereceptor metabolism of glucocorticoid hormones 11β-hydroxysteroid dehydrogenase (11β-HSD-I) [5]. This enzyme is coupled with glucocorticoid receptors and catalyzes reduction of cortisone non-active in humans (11-dehydrocorticosterone in rats) into cortisol (corticosterone), thus ensuring local increase in active glucocorticoid concentration in hepatocytes and stimulation of gluconeogenesis [7]. Activity of 11β-HSD-I increases during stress, which leads to potentiation of the metabolic effects of glucocorticoid hormones [4]. The second isoform of the enzyme (11β-HSD-II) is co-localized with mineralocorticoid receptors and promotes transformation of active glucocorticoid hormones into non-active metabolites in the aldosterone target organs, primarily in the kidneys [11]. This mechanism maintains certain level of the substrate for 11β-HSD-I.

Mifepristone (MF), a blocker of glucocorticoid receptors, reduces their metabolic effects and is used for the therapy of patients with Cushing syndrome [1]. MF can increase glucocorticoid function of the adrenal glands via the feedback mechanisms [8]. We have previously shown that administration of MF to rats *per os* for 12 days induces hypertrophy of the adrenal glands, stimulates steroidogenesis, elevates corticosterone concentration in the plasma, and increases

Institute of Experimental and Clinical Medicine, Novosibirsk, Russia. ***Address for correspondence:*** labend@centercem.ru. N. A. Pal'chikova

its excretion with urine [2]. However, little is known about changes in 11β-HSD-I and 11β-HSD-II activities in the liver and kidneys after MF administration.

Here we studied the intensity of corticosterone synthesis in the adrenal glands and its production via prereceptor metabolism in the liver of experimental animals against the background of long-term intraperitoneal administration of MF.

MATERIALS AND METHODS

Experiments were performed on mature male Wistar rats. The study was conducted in accordance to the European Convention for Protection of Vertebrate Animals used for Experimental or Other Scientific Purposes (Strasbourg, 1986). The animals were kept in individual cages with free access to water and standard vivarium food.

Three groups of animals were formed: group 1 (n=17) comprised intact controls, group 2 animals (n=11) received daily intraperitoneal injections of 0.9% NaCl (0.4 ml per 100 g body weight), and group 3 rats (n=12) received MF (Sigma) in 0.9% NaCl (1 mg MF per 100 g body weight).

Daily urine for measuring corticosterone excretion was collected from each rat 2 days before sacrifice.

The animals were decapitated at the next day after the 15th injection of the substance. Serum level of corticosterone, corticosteroid concentrations in the adrenal glands, and activities of 11β-HSD-I in the liver and 11β-HSD-II in the adrenal cortex were measured. For measuring corticosteroid content in the adrenal glands, the quarters of this organ were incubated in Krebs–Ringer bicarbonate buffer (pH 7.4) containing glucose and saturated with 95% O_2 and 5% CO_2 for 30 min at 37°C.

Corticosterone concentration in blood serum, urine, and incubation medium was measured by ELLISA using commercial Corticosterone ELISA kits (Enzo Life Sciences). Progesterone and pregnenolone were extracted from the incubation medium with ethyl acetate, the extracts were evaporated, the sediment was dissolved in the buffer in accordance with instructions, and hormone concentrations were measured using Progesterone-IFA (KhEMA) and Pregnenolone-ELISA (DDC) kits, respectively.

Activities of 11β-HSD in homogenates of the renal cortex and liver were measured by an original method [3] by means of microcolumn HPLC. Enzyme activity was expressed in nmol 11-dehydrocorticosterone produced over 1 min in 1 g tissue ($nmol \times min^{-1} \times g^{-1}$).

Statistical analysis of obtained results was performed using Statistica 6.0 software (StatSoft). Kruskal–Wallis test was used for multiple comparisons, Mann–Whitney test was used for paired comparisons. Probability of null hypothesis correctness was taken at 5% of significance.

RESULTS

In our experiments, we used intraperitoneal administration of MF. It is known that invasive administration results in injection-induced stress in animals and an increase in corticosterone level in the blood [9,10].

The levels of corticosterone precursors pregnenolone (early precursor) and progesterone (late precursor) and corticosterone in the adrenal glands decreased in animals of groups 2 and 3 in comparison with the control group (Table 1). This decrease was more pronounced in group 2 than in group 3, where it was insignificant. Serum concentration of corticosterone slightly increased in group 2 rats and by 1.9 times in group 3 rats (in comparison with the control). Urinary excretion of non-metabolized corticosterone increased by 3 times in groups 2 and 3 in comparison with the control, which attested to ongoing enhanced production of corticosterone in the adrenal glands. Reduced contents of corticosterone and its precursors in the

TABLE 1. Evaluation of Glucocorticoid Status in Rats after Administration of NaCl Solution or MF ($M \pm m$)

Parameter	Group 1 (control)	Group 2 (NaCl solution)	Group 3 (MF)
Concentration in incubation medium, ng/mg of tissue			
pregnenolone	0.186±0.049	0.116±0.007	0.179±0.045
progesterone	0.334±0.055	0.125±0.078*	0.145±0.098
corticosterone	64.6±10.3	37.9±8.4*	33.8±4.5*
Serum corticosterone concentration, nmol/liter	71.9±4.8	82.9±7.0	137.9±26.6*+
Urinary excretion of corticosterone (after 13 injections), nmol/day	0.39±0.16	1.21±0.06*	1.28±0.13*

Note. Here and in Table 2: p<0.05 in comparison with *group 1, +group 2.

TABLE 2. Activity of 11β-HSD Isoforms (nmol×min^{-1}×g^{-1}) in Rat Tissues after Administration of NaCl Solution or MF (*M±m*)

Parameter	Group 1 (control)	Group 2 (NaCl solution)	Group 3 (MF)
Activity of 11β-HSD-I in the liver	206.5±18.0	270.9±30.4	203.4±14.6^{+}
Activity of 11β-HSD-II in the kidneys	14.6±0.7	7.6±0.8*	10.1±1.2*
Ratio of liver 11β-HSD-I to renal 11β-HSD-II	13.7±1.8	38.1±4.8*	22.6±2.3*$^{+}$

adrenal glands can be related to active production and rapid release of the hormone into circulation under conditions of chronic injection-induced stress.

These findings agree with the data showing that observed changes in glucocorticoid status correspond to conditions of chronic stress [6]. Under these conditions, MF administration to group 3 rats additionally activated early stages of steroidogenesis in the adrenal glands, which manifested in higher (by 1.5 times) level of pregnenolone in the adrenal glands and corticosterone in blood serum in comparison with group 2.

In group 2 rats, 11β-HSD-I activity in the liver increased and of 11β-HSD-II activity in the kidneys decreased (Table 2). Hence, reduction of 11-dehydrocorticosterone into corticosterone increased and transformation of corticosterone into non-active form decreased. In group 3 rats, 11β-HSD-I activity in the liver did not increase, but 11β-HSD-II activity in the kidneys decreased, but less markedly than in group 2. In general, liver 11β-HSD-I to renal 11β-HSD-II activity ratio increased by 3 times in comparison with the control level in group 2 and less than by 2 times in group 3.

The described changes in activities of 11β-HSD isoforms in group 2 rats also correspond to chronic stress, when another stress exposure does not induce the same rise in corticosterone level as during the initial stages of the stress response, while metabolic effects of the hormone are mediated by its local production in hepatocytes [7,12]. MF administration prevents activation of local production of corticosterone in hepatocytes probably due to MF-induced blockage of glucocorticoid receptors coupled with 11β-HSD-I [5].

Therefore, intraperitoneal administration of MF promotes activation of hormone synthesis in the adrenal glands and increase in its level in the blood additional to this enhancement induced by injection stress, but blocks 11β-HSD-I activity in the liver, which prevents local generation of corticosterone in hepatocytes, and attenuates in the intensity of metabolic effects of the enzyme. These findings suggesting that the effects of MF in Cushing syndrome are mostly mediated by 11β-HSD-I blockage and suppression of local production of glucocorticoid hormones and their metabolic effects in hepatocytes.

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