Activity of 11β-Hydroxysteroid Dehydrogenase in Tissues of Hypertensive NISAG Rats

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11β-Hydroxysteroid dehydrogenase activity in the kidneys of NISAG rats (rat strain with hereditary stress-induced arterial hypertension) was 1.5-fold higher than in WAG rats. An inverse relationship was observed in the liver of these animals. After stress exposure 11β-hydroxysteroid dehydrogenase activity remained unchanged in the kidneys of NISAG and WAG rats, but significantly increased in the liver of NISAG rats. Functional activity of 11β-hydroxysteroid dehydrogenase probably reflects the hypertensive state of NISAG rats.

Key Words: *11β-hydroxysteroid dehydrogenase; stress-induced arterial hypertension*

Until recent times the effects of glucocorticoids on target organs were believed to depend on the concentration of hormones in the blood (integral criterion) and receptor density in target organs. However, prereceptor metabolism of hormones by tissue-specific enzymes is an additional important mechanism for regulation of corticosteroid activity. 11β-Hydroxysteroid dehydrogenase (11β-HSD) is one of the enzymes for glucocorticoid metabolism. 11β-HSD catalyzes interconversion of cortisol and cortisone in humans, as well as transformation of corticosterone and 11-dehydrocorticosterone in rats. There are several isoforms of this enzyme. Isoform 1 (11β-HSD1) is a bidirectional enzyme and catalyzes both the dehydrogenase and reductase reactions. It should be emphasized that 11β-HSD1 *in vivo* catalyzes primarily the reduction of cortisone and 11-dehydrocorticosterone into cortisol and corticosterone, respectively. This enzyme isoform is colocalized with glucocorticoid receptors and found in the liver, adipose tissue, central nervous system, and other tissues. Changes in activity of the enzyme isoform are associated with obesity, type 2 diabetes mellitus, cerebral dysfunction, and polycystic ovaries [4,11].

Isoform 2 (11β-HSD2) is colocalized with receptors for mineralocorticoids and found in target organs for aldosterone (kidneys, intestine, sweat glands, vessels, *etc*.). 11β-HSD2 catalyzes oxidation and converts cortisol and corticosterone into relatively inactive cortisone and 11-dehydrocorticosterone, respectively. It prevents binding of glucocorticoids to receptors for mineralocorticoids. Enzyme dysfunction in the kidneys is followed by the development and progression of hypertension and syndrome of apparent mineralocorticoid excess (Na^+ retention, hypokalemia, and normal levels of plasma renin and angiotensin II) [9,12].

Thus, 11β-HSD is a key enzyme determining the effect of glucocorticoids in target tissues. Changes in activity of this enzyme are associated with a variety of diseases. Here we studied 11β-HSD activity in rats with hereditary stress-induced arterial hypertension (NISAG rats) and normotensive WAG rats.

MATERIALS AND METHODS

Experiments were performed on 5-6-month-old male WAG rats (n=6) and NISAG rats (n=6).The animals

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were kept in a vivarium of the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Sciences). To produce stress the animals of each strain (n=6) were placed in tight wire cylindrical cages for 2 h. The rats were decapitated immediately after stress. The blood and tissues were rapidly sampled.

The renal cortex and liver samples (40-50 mg) were weighted on a torsion balance, placed in a cool glass homogenizer, minced with 1 ml 20 mM Tris-HCl buffer containing 5 mM $Mg(CH_3COO)_2$, 30 mM KCl, 250 mM sucrose, and 0.5% Triton X-100 (pH 8.3), thoroughly ground, and centrifuged at 4°C and 10,000 rpm for 15 min. The supernatant was used in further studies.

We measured activity of 11β-HSD. The tissue homogenate (enzyme source) was incubated in the presence of the cofactor and substrate (corticosterone), which underwent oxidation to 11-dehydrocorticosterone. The substrate and reaction product were separated chromatographically. Enzyme activity was estimated from the concentration of 11-dehydrocorticosterone (reaction product). Each homogenate was incubated 2 times. The supernatant (10-100 μl) was incubated with 0.2-30.0 μM corticosterone and 1.5 mM NADP in 0.1 M sodium phosphate buffer (pH 8.5) for 15-60 min. The reaction was stopped by adding 100 μl acetonitrile. Cortisone acetate (10 μl, 0.25 mM) served as an internal standard. The incubation mixture was centrifuged. The supernatant (10 μl) was analyzed by means of microcolumn high-performance liquid chromatography on a Milikhrom-1 chromatograph (Nauchpribor). The column (62×2 mm) was packed with Silasorb C_{18} sorbent (5 μ). The gradient of acetonitrile in water (30-45%) served as an eluant. Detection was performed at a wavelength of 240 nm. Enzyme activity was expressed in nmol 11-dehydrocorticosterone formed in 1 g tissue over 1 min ($nmol \times min^{-1} \times g^{-1}$). The concentrations of corticosterone and 11-dehydrocorticosterone in blood plasma were measured as described elsewhere [2].

The results were analyzed using Statistica software. The data are expressed as $M \pm m$. The significance of differences was evaluated by Student's t test.

RESULTS

Plasma corticosterone concentration in intact NISAG rats was 1.4-fold lower than in WAG rats (46±13 and 66±15 ng/ml, respectively). Stress exposure significantly increased plasma corticosterone concentration in WAG and Wistar rats (159±33 and 250±39 ng/ml, respectively). It should be emphasized that plasma corticosterone concentration in stressed NISAG rats was higher than in WAG rats (p<0.05). No interstrain differences were revealed in 11-dehydrocorticosterone concentration under normal conditions and after stress. 11-Dehydrocorticosterone concentration in intact WAG and NISAG rats was 20±8 and 17±4 ng/ml, respectively. Blood 11-dehydrocorticosterone concentration in stressed WAG and NISAG rats was 24±5 and 21±2 ng/ml, respectively. Our results are consistent with published data [5].

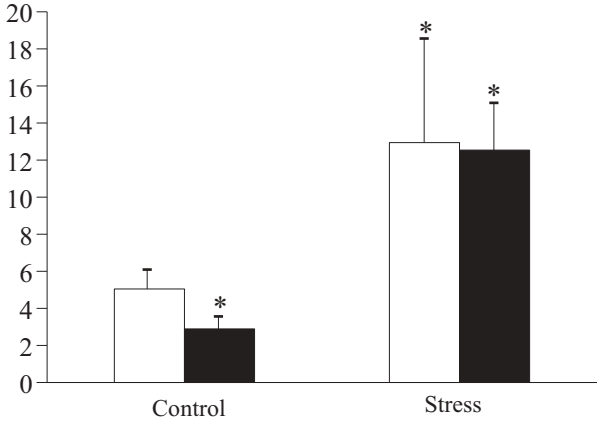

Fig. 1. Corticosterone/11-dehydrocorticosterone ratio in blood plasma. Here and in Fig. 2: light bars, WAG rats; dark bars, NISAG rats. *p<0.05 compared to control rats.

The corticosterone/11-dehydrocorticosterone ratio in blood plasma from intact WAG rats was 5.05±1.05, which is consistent with published data [8]. The corticosterone/11-dehydrocorticosterone ratio in NISAG rats was much lower than in WAG rats (2.90±0.63, Fig. 1). The glucocorticoid/metabolite ratio reflects functional activity of the glucocorticoid system [6]. It was reported that 11β-HSD in the kidneys determines the balance between these hormones in the blood [5,12]. These data are consistent with the results of 11β-HSD assay in rat kidneys. 11β-HSD activity in the kidneys of intact NISAG rats was 1.5-fold higher than in WAG rats (Fig. 2, *a*). After stress 11β-HSD activity slightly increased in WAG rats, but remained unchanged in NISAG rats. 11β-HSD activity in stressed NISAG rats was higher than in WAG rats. NISAG rats are characterized by low concentration of aldosterone in the blood [3]. Since 11β-HSD2 is colocalized with receptors for mineralocorticoids, it can be hypothesized that high activity of this enzyme in the kidneys provides the selectivity of receptors for mineralocorticoids in the kidneys and determines the effect of aldosterone. 11β-HSD activity in NISAG rats remained high. Stress was followed by a 5-fold increase in blood corticosterone concentration, but had no effect on enzyme activity. The corticoste-

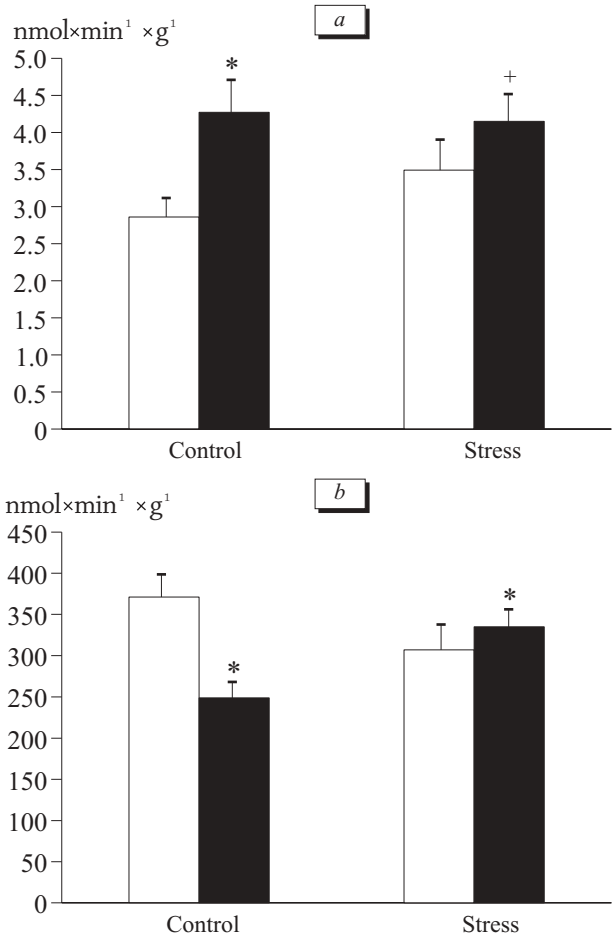

Fig. 2. 11β-HSD activity in the kidneys (*a*) and liver (*b*). $^{+}p<0.05$ compared to stressed rats.

rone/11-dehydrocorticosterone ratio in blood samples from NISAG and WAG rats significantly increased after stress (Fig. 1).

11β-HSD activity in the liver of NISAG rats was 1.5-fold lower than in WAG rats (Fig. 2, *b*). This enzyme isoform in the liver plays a role in activation of key enzymes for gluconeogenesis (glucose-6-phosphatase and phosphoenolpyruvate carboxykinase) [7]. NISAG rats are characterized by exhaustion of the insular apparatus, decrease in glucose tolerance, and predisposition to diabetes mellitus [1]. Our results illustrate specific features of NISAG rats. After stress 11β-HSD activity remained practically unchanged in WAG rats, but significantly increased in NISAG rats.

Our experiments demonstrate significant interstrain differences in 11β-HSD activity in the kidneys and liver of intact and stressed NISAG and WAG rats. Functional activity of the enzyme probably reflects the hypertensive state of NISAG rats.

REFERENCES

1. A. L. Markel', G. M. Dymshits, M. D. Shmerling, and G. S. Yakobson, *Byull. Sib. Otd. Ros. Akad. Med. Nauk*, No. 2, 35-40 (2002).
2. O. P. Cherkasova and V. I. Fedorov, *Probl. Endokrinol.*, **47**, No. 1, 37-39 (2001).
3. G. S. Yakobson, M. D. Shmerling, I. I. Buzueva, *et al.*, *Byull. Sib. Otd. Ros. Akad. Med. Nauk*, No. 2, 164-170 (2004).
4. P. S. Brereton, R. R. Driel, F. B. H. Suhaimi, *et al.*, *Endocrinology*, **142**, No. 4, 1644-1651 (2001).
5. M. Homma, T. Onodera, M. Hirabatake, *et al.*, *J. Pharm. Pharmacol.*, **50**, 1139-1145 (1998).
6. M. Homma, A. Tanaka, K. Hino, *et al.*, *Metab.*, **50**, No. 7, 801-804 (2001).
7. Y. Kotelevtsev, M. C. Holmes, A. Burchell, *et al.*, *Proc. Natl. Acad. Sci.* USA, **94**, 14,924-14,929 (1997).
8. D. E. V. Livingstone, G. C. Jones, K. Smith, *et al.*, *Endocrinology*, **141**, No. 2, 560-563 (2000).
9. A. Odermatt, P. Arnold, and F. J. Frey, *J. Biol. Chem.*, **276**, No. 30, 28,484-28,492 (2001).
10. M. C. Riddle and P. A. Mcdaniel, *J. Clin. Endocrinol. Metab.*, **78**, No. 4, 830-834 (1994).
11. J. R. Seckl and B. R. Walker, *Endocrinology*, **142**, No. 4, 1371-1376 (2001).
12. P. C. White, T. Mune, and A. K. Agarwal, *Endocrine Rev.*, **18**, No. 1, 135-156 (1997).