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# Features of the dynamics of corticosteroid receptors in the myocardium of animals with different resistance to hypoxia in the post resuscitation period

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## Abstract

**Aim**. To characterize the peculiarities of the dynamics of the level of corticosteroid receptors in the myocardium of animals with different resistance to hypoxia in the post-resuscitation period.

**Methods**. Experimental studies were carried out on male non-inbred white rats, divided into 2 groups by hypoxia resistance. A 5-minute arrest of the systemic circulation was modeled under ether anesthesia with intrathoracic clamping of the cardiovascular cluster with subsequent resuscitation. The observation period was 35 days. The content of corticosterone and aldosterone was determined in the blood plasma, the concentration of glucocorticoid and mineralocorticoid receptors in myocard homogenates was determined as well.

**Results**. On days 1 to 3 of the post-resuscitation period in rats highly resistant to hypoxia, the dynamics of plasma corticosterone concentration and the content of corticosteroid receptors was unidirectional. Starting from the 5th day, against the background of a statistically significant decrease in the level of plasma corticosterone, a gradual increase in the density of corticosteroid receptors, mostly glucocorticoid, was observed, most pronounced on the 14th day and remaining until the end of the observation. In animals with low resistance to hypoxia, the dynamics of corticosteroid receptors was characterized by a predominance of mineralocorticoid content in almost all periods of observation. On days 1–3 of post-resuscitation period on the background of high concentrations of corticosteroid hormones, the minimum content of glucocorticoid receptors was noted. A decrease in the mineralocorticoid receptor level was recorded only on the first day, and in all subsequent periods of the experiment, the control indicators were significantly higher by 1.4–1.6 times. Strengthened mineralocorticoid signaling in the myocardium, characteristic of animals with low resistance to hypoxia, may be associated with the development of hypertrophy and fibrosis, inflammation, impaired electrical function. An increase in glucocorticoid receptors, characteristic of animals with a high resistance to hypoxia, has an adaptive effect, limiting the inflammatory response, the potential mechanism may be associated with increased expression of type 11β-hydroxysteroid dehydrogenase.

**Conclusion**. The identified features can have a significant influence on the course of the post-resuscitation period and determine the long-term forecast.

**Keywords**: glucocorticoid receptors, mineralocorticoid receptors, resistance to hypoxia.

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Numerous clinical and experimental studies have established that clinical death and subsequent resuscitation of the body, which are based on two typical interrelated processes, hypoxia and reoxygenation, cause severe dysfunction of vital organs and systems, induce much damage, and generate protective reactions [1]. In any population of non-inbred animals, there are individuals that differ significantly from others in their resistance to hypoxia, in which the functional and metabolic consequences of oxygen deficiency have significant aspects [2] that can affect the individual's survival and course of the postresuscitation period.

Metabolic support of the adaptive and compensatory response to extreme circumstances is implemented through the participation of the hypothalamic-pituitary-adrenal system, among others [3]. Deviations in the functional state of the neuroendocrine system can be caused not only by dysfunction of glucocorticoid secretion and dys-

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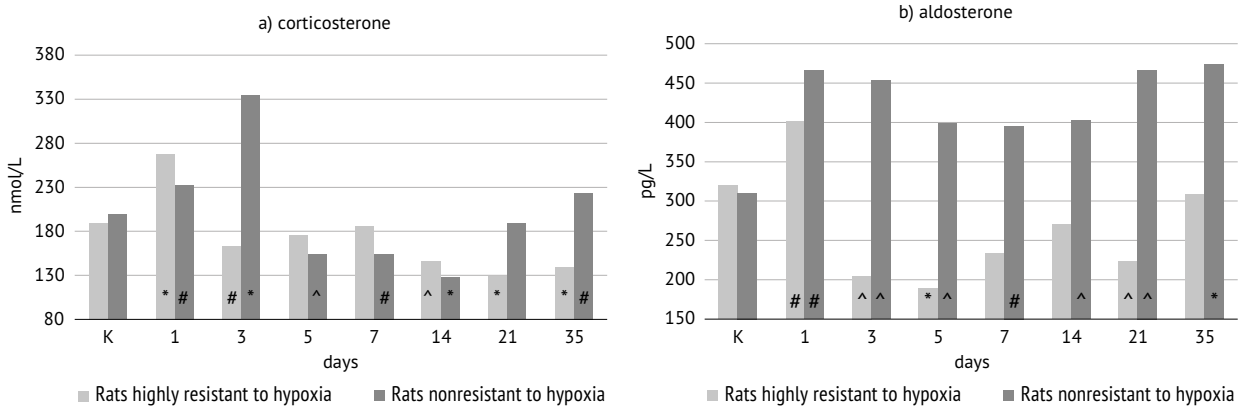


**Fig. 1.** Level of corticosterone (a) and aldosterone (b) in the blood plasma of rats with different resistance levels to hypoxia in the dynamics of the postresuscitation period. Statistical significance of differences as compared with the control group: #$p \leq 0.05$; ^$p \leq 0.01$; *$p \leq 0.001$.

regulation of feedback mechanisms but also by the functional failure of the hormone-receptor interaction, which results in a lack of effective signal transduction to control the creation of the adaptive response, leading to development of maladaptive states and death [3].

Most of the effects of endogenous glucocorticoids are implemented through closely related glucocorticoid (GR) and mineralocorticoid (MR) receptors. The concentrations of endogenous glucocorticoids exceed the concentrations of aldosterone by two to three orders of magnitude; therefore, they serve as a physiological ligand for both MR and GR, with the exception of aldosterone target cells, in which glucocorticoid prereceptor inactivation occurs via type 2 11β-hydroxysteroid dehydrogenase enzyme [4]. This enzyme is almost absent in cardiomyocytes [5]. Consequently, the effects of glucocorticoids in cardiomyocytes are potentially mediated by both GR and MR. Both receptor types can bind and activate many of the same target genes, nevertheless causing different and sometimes opposite effects during the glucocorticoid action [6]. Thus, the balance between the expression of MR and GR plays a significant role in maintaining homeostasis, and elucidation of the corresponding physiological roles of these receptors in the cardiovascular system is important for understanding their pathophysiological significance and for developing new therapeutic approaches for cardiac pathologies.

This study aimed to characterize the aspects of the dynamics of the corticosteroid receptor level in the myocardium of animals with different levels of resistance to hypoxia during the postresuscitative period.

The experimental studies were conducted in accordance with the ethical principles declared by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (adopted in Strasbourg in 18.03.1986). All studies were approved by the local ethics committee of the Bashkir State Medical University.

The experiments were conducted in the morning. The test group, after testing for hypoxia resistance, included 160 healthy, sexually mature outbred white male rats weighing 200 to 220 g. Resistance to acute hypobaric hypoxia was determined using semiquantitative assessment of inhibition and restoration of motor activity as well as postural reflexes in the altitude chamber using a "rise to height" of 9000 m, which is the threshold for rats with a presence of disease [7].

The animals in the experiment were allocated to two groups based on extremes in stability: highly resistant (HR) and nonresistant (NR) to hypoxia. The groups included 70 rats in the experimental group (10 animals for each follow-up period) and 10 control rats. One week after testing under ether anesthesia, a 5-min systemic circulation stop was reproduced by intrathoracic cross-clamping of the vascular bundle of the heart, followed by resuscitation according to the methods of V.G. Korpachev [8]. Clinical death was not modeled in control animals after ether anesthesia. On days 1, 3, 5, 7, 14, 21, and 35, animals under ether anesthesia were decapitated, and blood and tissues were sampled for examination.

To determine the corticosterone and aldosterone levels in blood plasma, standard test systems manufactured by IMMUNOTECH (France) were used. The content of GR and MR in myocardial homogenates was determined by enzyme immunoassay using standard ELISA kits (China) manufactured by Cloud-Clone Corp. (USA) that are intended

**Table 1.** Level of corticosteroid receptors in the myocardium of rats with different resistance levels to hypoxia in the postresuscitative period, Me ($Q_1$–$Q_3$)

| day | Groups of animals for resistance to hypoxia | | | |
|---|---|---|---|---|
| | highly resistant | nonresistant | highly resistant | nonresistant |
| | Glucocorticoid receptors. μg/g protein | | Mineralocorticoid receptors. ng/g protein | |
| control | 35.02 (32.44–37.49) | 32.55 (28.71–34.16) $p_1$=0.2899 | 106.16 (102.34–112.31) | 110.34 (105.34–113.26) $p_1$=0.4962 |
| | n=10, 100% | n=10, 100% | n=10, 100% | n=10, 100% |
| day 1 | 42.99 (34.76–47.51) p=0.0483 | 18.98 (16.59–22.64) p=0.0009. $p_1$=0.0065 | 116.78 (107.09–122.18) p=0.0587 | 76.45 (66.38–84.56) p=0.0002. $p_1$=0.0015 |
| | n=10, 122.8% | n=10, 58.3% | n=10, 110.8% | n=10, 69.3% |
| day 3 | 25.40 (20.16–30.15) p=0.0002 | 15.52 (12.33–19.77) p=0.0002. $p_1$=0.0012 | 36.16 (32.26–44.56) p=0.0002 | 125.97 (115.23–132.26) p=0.0082. $p_1$=0.0002 |
| | n=10, 72.5% | n=10, 49.5% | n=10, 34.1% | n=10, 114.2% |
| day 5 | 39.55 (35.66–42.61) p=0.0821 | 24.14 (20.69–30.48) p=0.0342. $p_1$=0.0025 | 150.26 (140.65–155.32) p=0.0002 | 120.03 (115.66–131.27) p=0.0102. $p_1$=0.0002 |
| | n=10, 112.9% | n=10, 74.2% | n=10, 141.5% | n=10, 109% |
| day 7 | 46.25 (40.13–48.56) p=0.0343 | 40.79 (35.64–45.64) p=0.0005. $p_1$=0.1736 | 111.48 (98.87–12.76) p=0.7623 | 182.68 (174.65–186.56) p=0.0002. $p_1$=0.0002 |
| | n=10, 132.1% | n=10, 125.3% | n=10, 105% | n=10, 166.5% |
| day 14 | 58.52 (52.69–64.15) p=0.0002 | 28.61 (22.59–38.45) p=0.3257. $p_1$=0.0002 | 109.02 (102.31–115.62) p=0.8798 | 150.86 (142.63–158.64) p=0.0002. $p_1$=0.0002 |
| | n=10, 167.1% | n=10, 81.8% | n=10, 103.7% | n=10, 136.7% |
| day 21 | 52.40 (45.69–55.67) p=0.0002 | 26.62 (23.44–32.04) p=0.0587. $p_1$=0.0002 | 118.94 (112.36–128.65) p=0.0126 | 154.68 (145.75–168.45) p=0.0002. $p_1$=0.0002 |
| | n=10, 149.6% | n=10, 93.4% | n=10, 112% | n=10, 140.2% |
| day 35 | 43.77 (39.37–49.09) p=0.0025 | 30.41 (27.60–34.57) p=0.5967. $p_1$=0.0025 | 121.69 (112.13–129.53) p=0.0081 | 160.55 (151.23–175.64) p=0.0002. $p_1$=0.0002 |
| | n=10, 125% | n=10, 93.4% | n=10, 114.6% | n=10, 145.5% |

Note: The statistical significance of differences p — with control, $p_1$ — between groups with different resistances to hypoxia.

for rats, in strict adherence to the manufacturer's protocol.

For statistical analysis of the results, the standard software package Statistica 6.0 was used. Descriptive statistics of the data are presented at the median and quartile range of Me [$Q_1$–$Q_3$]. The statistical significance of the differences was determined using the nonparametric Mann–Whitney test (*U*). Statistical relationships were evaluated using nonparametric correlation analysis, with calculation of the Spearman rank correlation coefficients (*R*). Differences were considered statistically significant at $p \leq 0.05$.

By the end of the first 24 hours after the critical effect, an increase in the concentration of plasma corticosterone, which serves as the main ligand for corticosteroid receptors in the myocardium, and the level of GR were recorded in rats HR to hypoxia (a significant correlation was found between the indicators, $R = 0.78$, $p = 0.0287$; Fig. 1*a*, Table l). A tendency for MR to increase in content was also observed.

Because the effects of glucocorticoids were implemented through GR and MR, it is logical to assume that the level of receptor expression reflects the degree of hormone involvement in the

regulation of metabolic processes in the myocardium, which can withstand environmental challenges. The beneficial effect of glucocorticoids under the action of ischemia-reperfusion is mainly a result of their ability to limit the acute inflammatory response. GR inhibits the activation of inflammatory response genes by inhibiting transcription factors such as activator-1 protein and nuclear factor-κB, on one hand, and increasing the transcription of components of anti-inflammatory genes, on the other hand. This function, called *transpression*, determines most of the anti-inflammatory effects of glucocorticoids [9].

In addition, the cardioprotective effect of glucocorticoids in ischemic-reperfusion injury of the myocardium may be associated with a selective increase in the expression level of L-prostaglandin of D synthase through GR and activation of prostaglandin synthesis with a predominance of prostaglandin $D_2$ in cardiomyocytes, which prevents platelet aggregation, induces endothelial-dependent arterial relaxation, and has several anti-inflammatory and antioxidant effects through the mechanism mediated by peroxisome proliferator-activated receptor–γ [10].

Beginning on day 3 of the experiment, a decrease in corticosterone levels was noted in animals HR to hypoxia, accompanied by a significant decrease in the level of corticosteroid receptors, which generally reflects the physiological nature of the receptor-ligand relationship. However, during the next control period, the content of both receptor types increased. After the jump in content recorded on day 5, the MR level was subsequently maintained at the values close to that of the control group, and the GR content steadily increased, reaching a peak on day 14 (167.1% of the control level, $p = 0.0002$; see Table 1) and remaining significantly higher than the initial indicators until the end of the experiment.

It should be separately noted that there was an increase in the level of receptors with the presence of a reduced concentration of plasma corticosterone (days 14–21), which was comparable with the control level (days 5–7) and statistically significant. However, active glucocorticoids can also be locally regenerated from circulating inert 11-keto-metabolites with enzyme type 1 11β-hydroxysteroid dehydrogenase [5, 11], which usually has low expression and activity in the heart but can quickly change in response to external stimuli [5].

Following the above logic, it can be assumed that the increase in GR level detected from day 7 to day 35, as well as that of MR on day 5, in animals HR to hypoxia is associated with increased expression of type 1 11β-hydroxysteroid dehydrogenase. In particular, the proinflammatory cytokines interleukin-1 and tumor necrosis factor–α can stimulate the activity of this enzyme in the myocardium [12], the massive formation of which occurs during endotoxicosis of the postresuscitation period.

Obviously, the short-term increase in MR levels noted on day 5, which have a high affinity for glucocorticoids, is the result of increased cross-stimulation of receptors as a result not only of the increased concentration of circulating corticosterone in the presence of minimal aldosterone levels, but also of locally synthesized corticosterone. Subsequently, as the concentration of regenerated corticosterone increases, there is an increase in the level of GRs, which have a lower affinity for corticosterone. However, a prolonged increase in enzyme expression can contribute to ventricular remodeling, because glucocorticoids, both endo- and exogenous, inhibit angiogenesis [5]. In addition, it was revealed that type 1 11β-hydroxysteroid dehydrogenase in fibroblasts regulates the release of proinflammatory mediators [11].

In the group of animals NR to hypoxia, an increase in blood corticosterone level by the end of day 1 not only did not lead to an increase in either GR ($R = -0.82$, $p = 0.0233$) or MR ($R = -0.79$, $p = 0.0308$) but also was accompanied by a decrease of 42% ($p = 0.0009$) and 31% ($p = 0.0002$), respectively, which serves as indirect evidence that the hormone-receptor interaction was weakening. The decrease in GR and MR in the early recovery period, which occurred under conditions of severe energy deficiency in animals with low resistance to hypoxia, reflects the maladaptation processes, because glucocorticoid regulation is a necessary condition for the development of adaptive changes [13].

On day 3 of the postresuscitation period, with a high level of corticosterone and aldosterone, minimal levels of GR and elevated levels of MR were recorded, which contribute to the expression of inflammatory cytokines [14]. In the absence of type 2 11β-hydroxysteroid dehydrogenase, which converts glucocorticoid into inactive metabolites, aldosterone cannot compete with corticosterone for binding to MR; therefore, it seems likely that it is corticosterone, and not aldosterone, that occupies MR and affects the proinflammatory response after ischemia effects [15]. Therefore, the cardioprotective effect of glucocorticoids can be neutralized by activation of proinflammatory MR in response to hypoxic stress [9].

However, under normal conditions, the occupation of MR with glucocorticoids in myocardial tissue does not mimic the effects of aldosterone [16]. The stimuli for activation of the glucocorticoid-MR

complex can be an imbalance in redox and development of oxidative stress [17]. Induction of MR by active oxygen metabolites leads to the activation of NADPH oxidase[1] and stimulates further synthesis of these metabolites and tissue damage, thereby forming a vicious circle [18]. In addition, there is evidence that under pathological conditions, including hypoxia, the expression and activity of type 2 11β-hydroxysteroid dehydrogenase (which is usually present in a small amount in the heart, except for the vascular endothelium) increase, which allows aldosterone to compete for binding to MR [11].

Beginning on day 5 of the postresuscitation period in animals NR to hypoxia, the level of corticosterone decreased strongly while the concentration of aldosterone in blood plasma remained high (Fig. 1*b*). This situation persisted until the end of the experiment, allowing the effects of aldosterone to manifest. To date, a number of negative effects of aldosterone on the cardiovascular system have been established, and MR is responsible for its physiological effects. Abnormal activation of the renin-angiotensin-aldosterone system is one of the most important mechanisms in the development of chronic heart failure [16]. Increased MR signaling in the myocardium is associated with development of inflammation, hypertrophy and fibrosis, and ventricular arrhythmias [19].

## CONCLUSIONS

1. In the animals HR to hypoxia in the postresuscitative period, an imbalance in the content of corticosteroid receptors with a predominance of glucocorticoid receptors in the myocardium was recorded, which generally provides an adaptive effect, limiting the inflammatory response. The revealed increase in the level of glucocorticoid receptors might be associated with the increased expression of type 1 11β-hydroxysteroid dehydrogenase.

2. In the animals nonresistant to hypoxia in the postresuscitative period, the balance of corticosteroid receptors was also disturbed in the myocardium. Enhanced signaling through mineralocorticoid receptors in the myocardium can be associated with the development of hypertrophy and fibrosis, inflammation, and impaired electrical function. The identified aspects can have a significant impact on the course of the postresuscitation period and determine the long-term prognosis.

*The authors declare no conflict of interest.*

[1] NADPH — reduced nicotinamide adenine dinucleotide phosphate.

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