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The effect of free radical stress correction on corticoid signaling in the kidney of rat with different resistance to hypoxia after systemic circulation arrest

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Abstract

Aim. To assess the influence of the pathogenetic action of the succinate-containing drug on corticosteroid regulation in the kidney of rats with different resistance to hypoxia during recovery after systemic circulation arrest.

Methods. The object of the study was male non-inbred white rats weighing 200–220 g. A week after testing for resistance to hypoxia, a 5-minute systemic circulation arrest was simulated by intrathoracic clamping of the vascular bundle of the heart, followed by resuscitation. In the postresuscitation period, the experimental rats were once daily injected with a solution containing inosine + nicotinamide + riboflavin + succinic acid, and the control rats — 0.9% Sodium Chloride solution. The observation period was 35 days. We studied the content of corticosterone, aldosterone in blood plasma, gluco- and mineralocorticoid receptors, carbonylated proteins, dityrosine, and products that react with thiobarbituric acid in homogenates of the kidneys. Statistical data were presented as mean and standard deviation M±σ. Nonparametric Kruskal–Wallis (N) and Mann–Whitney (U) tests followed by Dunn test, Spearman correlation analysis were used. Differences were considered statistically significant at $p \leq 0.05$.

Results. The use of succinate-containing preparation reduced the intensity of free radical processes in both groups of animals. Against this background, in low-resistance rats, on the 1st day, the concentration of glucocorticoid receptors statistically significantly increased to 117% ($p < 0.05$), and then was comparable to the control; the greatest statistically significant changes in the level of mineralocorticoid receptors occurred on the 1st day (increase by 25%, $p < 0.001$) and at 21–35th days (decrease by 22–30%, $p < 0.001$). In highly resistant rats, the correction led to a shift in the maximum content of glucocorticoid receptors from the last day (134% of the control level, $p < 0.01$ without therapy) to the 1st (123%, $p < 0.05$ with succinate-containing therapy) and maintaining the receptors level comparable to the initial, in the future. The level of mineralocorticoid receptors in highly resistant rats was lower than in low resistant rats, both in the group without correction and with correction.

Conclusion. Correction of the course of the postresuscitation period with a succinate-containing drug in animals with a low resistance to hypoxia against the background of a decrease in the intensity of carbonyl stress and restoration of feedback mechanisms causes stabilization of glucocorticoid receptors level and a decrease in mineralocorticoid receptors to control values by the end of the experiment; in organisms highly resistant to hypoxia, against the background of correction, the activity of lipid peroxidation decreases and the level of both types of receptors are restored.

Keywords: glucocorticoid receptors, mineralocorticoid receptors, kidney, hypoxia, postresuscitation state, succinate, resistance to hypoxia.

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Background. Despite the achievements in medicine in recent years, the mortality and disability of patients with a history of a terminal state and who underwent resuscitation procedures remain high, given that systemic complications develop after successful resuscitation in more than 80% of patients, and 80% of them die in the next six months as a result of the development of functional inability of various organs [1].

The activation of free radical oxidation is one of the most important pathophysiological mechanisms affecting the recovery processes after exposure

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to systemic hypoxia and subsequent reperfusion. During reperfusion, oxygen is transformed from a vital component of physiological reactions of oxidative metabolism into a participant in pathological oxidative reactions [2,3]. In the first minutes after pathogenic exposure, severe tissue hypoxia occurs with a disorder of the electron transport function of the respiratory chain [4] and the activation of free radical oxidation not only of lipids but also carbohydrates and proteins, which is accompanied by the accumulation of carbonyl compounds with high reactivity and pronounced toxic properties [5]. The dynamics of the oxidative stress occurrence has significant differences in animals with different phenotypes of resistance to hypoxia, and it determines numerous significant aspects of the course of postresuscitation complications [6,7].

The kidney is extremely sensitive to hemodynamic compromise and ischemia, which makes it one of the main target organs in the disorder of the systemic circulation. Severe circulatory disorders arising during the period of ischemia–reperfusion entail the disorder of the regulation of metabolic processes and their normal course [8].

Even G.Selye revealed that under the stress of any etiology under the influence of corticosteroid hormones, a nonspecific restructuring of physiological and metabolic processes occurs, which is required to ensure the coordinated functioning of all body systems and adaptation to the pathogenic factor. In the kidneys, which are the main target organs for aldosterone, in addition to the glucocorticoid receptors (GRs) that are ubiquitously expressed traditionally, a maximum number of mineralocorticoid receptors (MRs), which are structurally similar molecules, exist; MRs bind glucocorticoids and mineralocorticoids with equal affinity [9].

The selectivity of MRs in the kidneys is provided by the second isoform of 11β-hydroxysteroid dehydrogenase, which converts active forms of glucocorticoids (cortisol in humans and corticosterone in rodents) into inactive metabolites, preventing MR hyperactivation [10]. Under the conditions of oxidative stress, the effects of corticosteroid hormones can be significantly modified due to changes in the sensitivity of receptors in the target tissues with impaired hormone–receptor interaction and the disorders of hormone biotransformation processes, which can have serious maladaptive consequences [9]. Through MR signaling, aldosterone plays a key role in the pathogenesis of ischemia–reperfusion kidney damage, as manifested in the development of progressing renal dysfunction, proteinuria, glomerulosclerosis, tubulointerstitial fibrosis, etc. [11].

The activation of MR by aldosterone alone is not sufficient for the implementation of its negative effects, given that this phenomenon requires a certain pathological environment. Such an environment can arise under the conditions of increased oxidative stress and acidosis [12]. Consequently, the stable resources of antioxidant protection and antihypoxic cytoprotection are the most important factors in the correction of metabolic disorders during the rehabilitation period after the stagnation of circulation [2]

From this point of view, our attention was drawn to a Russian combined antihypoxic drug, in which the antihypoxic effect of succinic acid is supplemented by other components, in particular, riboflavin, which can increase the activity of succinate dehydrogenase, inosine, and nicotinamide. The positive metabolotropic, antihypoxic, and antioxidant effects of this drug combination [13] enables its application in the complex therapy of pathological conditions, whose components of pathogenesis can be hypoxia and oxidative stress, such as during cardiac surgery, in postoperative rehabilitation, pathology of newborns, etc. [14].

This drug is most widely used in the treatment and rehabilitation of patients with hypoxic and ischemic injuries of the central nervous system, namely, ischemic stroke, toxic, hypoxic and discirculatory encephalopathy, and craniocerebral injury; the protocols for using the drug are continually being refined and expanded [13,15].

The positive metabolotropic effect of this succinate-containing drug in the correction of postischemic dysfunction of other organs has been studied to a considerably lesser extent. Thus, the possibility of its use for the correction of renal dysfunction, in which the pathogenesis involving the disorder of corticosteroids, especially mineralocorticoid, signaling plays a significant role, has not been discussed [11]. Limiting the free radical and hypoxic damage will probably reduce the maladaptive consequences for this vital organ by altering corticoid signaling.

The work aimed to evaluate the influence of the pathogenetically directed action of a succinate-containing drug on corticoid signaling in the kidneys of rats with different resistances to hypoxia in terms of the dynamics of recovery after arrest of the systemic circulation.

Materials and Methods of Research. Before the start of the main experiments, the resistance of rats to acute hypobaric hypoxia was determined using a semi-quantitative assessment of the inhibition and restoration of motor activity and postural reflexes during a pressure chamber “rise” to a “height” of 9000 m [7,16]. The animals assigned

to two types of extreme resistance (highly resistant (HR) and non-resistant (NR)) to hypoxia were used in the experiment.

One week after testing under ether anesthesia, a 5 min arrest of systemic circulation was simulated by intrathoracic clamping of the vascular bundle of the heart in accordance with the work of V.G. Korpachev [17]. The resuscitation measures included external cardiac massage and artificial pulmonary ventilation. In the control group of rats after ether anesthesia, circulatory arrest was not reproduced. After 1, 3, 5, 7, 14, 21, and 35 days, the animals were sacrificed under ether anesthesia by decapitation.

The contents of corticosterone and aldosterone in the blood plasma were studied by the immunoradiometric method using the standard test systems made by Immunotech (France), following the manufacturer's protocol. The contents of GRs and MRs in tissue homogenates were determined on a StatFox 2100 analyzer by enzyme-linked immunosorbent assay using a standard kit (China) made by Cloud-Clone Corp (USA) for rat tissues, strictly in accordance with the manufacturer's recommendations. The content of carbonylated proteins in liver homogenates was determined by their reaction with 2,4-dinitrophenylhydrazine followed by spectrophotometric registration of the interaction products, namely, dinitrophenylhydrazones [6, 18], iron-dependent formation of bityrosine by the method of E.E. Dubinina [6, 18], and product reaction with thiobarbituric acid, using a set of reagents TBK-AGAT (AGAT-MED, Moscow).

Study design. A series of experiments was performed on 300 sexually mature male non-inbred white rats weighing 200–220 g. When determining the sample size, the 3R concept and generally accepted experimental approaches were used [19].

Statistical calculations included the indicators of the following animal groups:

–a group of intact rats that were HR to hypoxia: not subjected to clinical death modeling and received ether anesthesia (n = 10);

–a comparison group of rats that were HR to hypoxia: underwent arrest of systemic circulation and received isotonic sodium chloride solution (n = 70);

–an experimental group of rats that were HR to hypoxia: underwent arrest of systemic circulation and received the succinate-containing drug (n = 70);

–a group of intact rats that were NR to hypoxia (n = 10);

–a comparison group of rats NR to hypoxia: underwent arrest of systemic circulation and received isotonic sodium chloride solution (n = 70);

–an experimental group of rats NR to hypoxia: underwent arrest of systemic circulation and received the succinate-containing drug (n = 70).

The Russian drug Cytoflavin, which has well-known antihypoxic and antioxidant effects, was selected to correct the indices of free radical oxidation and antioxidant protection and to prove the leading role of free radical processes in the pathogenesis of post-hypoxic disorders. Exactly 1 ml solution for intravenous administration contained 100 mg succinic acid, 20 mg inosine, 10 mg nicotinamide, 2 mg riboflavin mononucleotide, and 165 mg excipient methyl glucamine.

The solution was administered intravenously once daily for 10 days into the tail veins of rats using an infusion pump at a dose of 8.5 mg per 100 g animal body weight in 0.1 ml 0.9% sodium chloride solution. When selecting a dosage regimen, we were guided by the method of interspecies dose transfer, considering the relationship between the mass and surface area of the human body and laboratory animals, as recommended by the "Guidelines for conducting preclinical studies of drugs" [19]. A conversion factor of 5.9 was used for the rats. The calculation was performed based on the content of the main active substance of the drug, that is, succinic acid. With a rat body weight of 200–220 g, the infusion volume was 0.27–0.3 ml. The drug was first administered immediately after the end of resuscitation measures.

After stagnation of circulation and resuscitation, the control animals, which were selected preliminary based on their "resistance to hypoxia," received the injection of 0.3 ml 0.9% sodium chloride solution at the indicated time.

Conditions. The research was conducted in accordance with the ethical principles established by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The basic rules for the maintenance and care of experimental animals corresponded to the standards defined in the order of the Ministry of Health of Russia No. 199n dated 04/01/2016 "On approval of the Rules of Good Laboratory Practice." The animals were kept under the same conditions, with 6–7 individuals in each cage, natural illumination, air temperatures of 20 °C–22 °C, relative air humidities of 55%–65%, and free access to water and compound feed PK-120-1 (Laboratorsnab). The experiments began at 10:00. Immediately before the experiments, the animals were examined carefully (the condition of the skin and the neurological status were assessed), and temperature measurement and weighing were performed.

Ethical review. The study was approved by the local ethics committee of the Bashkir State Medical University of the Ministry of Health of Russia (protocol of 02/04/2014).

Table 1. Hormonal panel of rat blood plasma in the recovery period after resuscitation without and after correction with a succinate-containing drug, M ± σ.

Day	Groups	Corticosterone, nmol/L		Aldosterone, pg/ml	
		HR	NR	HR	NR
	intact	186.76±27.83	196.90±29.11. p_1=0.8206	331.13±72.22	315.76±84.18. p_1=0.4494
1	comparison	267.90±23.37. p=0.0002	232.35±54.62 p=0.0423. p_1=0.0413	401.23±76.52. p=0.0411	466.13±115.63. p=0.0023. p_1=0.0233
	correction	283.82±43.63. p=0.0005. p_2=0.4057	297.30±67.46. p=0.0015. p_1=0.4057. p_2=0.0587	399.27±76.14. p=0.0384. p_2=0.5450	436.18±108.21. p=0.0126. p_1=0.1508. p_2=0.1988
3	comparison	162.92±23.57. p=0.0343	334.37±43.18. p=0.0002. p_1=0.0002	204.60±91.78. p=0.0111	453.26±83.05. p=0.0025. p_1=0.0003
	correction	203.07±34.67. p=0.1987. p_2=0.0156	166.94±33.11. p=0.0479. p_1=0.03431. p_2=0.0002	349.86±156.94. p=0.4493. p_2=0.0411	424.58±77.79. p=0.0101. p_1=0.0431. p_2=0.4497
5	comparison	175.26±24.37. p=0.2736	154.23±29.10. p=0.0126. p_1=0.1509	189.06±40.42. p=0.0102	399.19±43.03. p=0.0032. p_1=0.0002
	correction	211.74±29.66. p=0.0477. p_2=0.0156	177.81±10.83. p=0.1124. p_1=0.0082. p_2=0.0424	334.76±71.57. p=0.2566. p_2=0.0012	345.37±56.25. p=0.3642. p_1=0.5967. p_2=0.0191
7	comparison	185.46±28.65. p=0.8206	154.16±24.40. p=0.0126. p_1=0.0156	233.69±71.79. p=0.0193	395.60±65.87. p=0.0342. p_1=0.0007
	correction	191.14±29.72. p=0.8206. p_2=0.8154	176.79±29.84. p=0.1736. p_1=0.3258. p_2=0.1509	294.22±90.64. p=0.4053. p_2=0.1506	388.65±87.31. p=0.0587. p_1=0.0283. p_2=0.7623
14	comparison	145.82±30.83. p=0.0041	127.75±27.44. p=0.0004. p_1=0.1988	269.80±78.07. p=0.0819	402.50±65.55. p=0.0101. p_1=0.0032
	correction	220.88±34.57. p=0.0413. p_2=0.0009	215.96±39.37. p=0.1509. p_1=0.8798. p_2=0.0008	327.12±94.66. p=0.2896. p_2=0.0818	337.94±56.27. p=0.4054. p_1=0.3253. p_2=0.0126
21	comparison	129.50±26.02. p=0.0003	189.05±27.00. p=0.8206. p_1=0.0009	223.43±41.16. p=0.0032	466.82±95.92. p=0.0025. p_1=0.0005
	correction	205.75±26.89. p=0.1988. p_2=0.0003	188.33±37.18. p=0.8206. p_1=0.3257. p_2=0.8004	317.68±58.52. p=0.4055. p_2=0.0015	307.85±33.18. p=0.2897. p_1=0.7055. p_2=0.0032
35	comparison	139.52±27.36. p=0.0009	223.36±28.79. p=0.0494. p_1=0.0002	308.43±29.25. p=0.8123	473.63±54.79. p=0.0036. p_1=0.0002
	correction	199.98±29.72. p=0.2265. p_2=0.0015	214.93±41.39. p=0.3643. p_1=0.6501. p_2=0.4963	323.36±31.39. p=0.3256. p_2=0.2899	321.14±65.99. p=0.5965. p_1=0.1736. p_2=0.0002

Note: *p*—probability of the null hypothesis of the existence of differences with the control, Kruskal–Wallis test, and Dunn's test; p_1_differences between the HR and NR groups; p_2—differences between the groups with and without correction, Mann–Whitney test; HR—highly resistant to hypoxia; NR—not resistant to hypoxia.

Statistical analysis. Statistical processing of the results was conducted using the Statistica 8.0 software package. The data are presented as mean values and mean-square deviations M±σ. The nonparametric Kruskal–Wallis (H), Mann–Whitney (U), and Dunn's test were used to determine the differences between the groups. A nonparametric analysis was used with the calculation of Spearman's rank correlation coefficients to identify the statistical relationships between the intensity of characteristics. Differences were considered statistically significant at $p \leq 0.05$.

Results. On day 1 of the postresuscitation period under the conditions of stress growth of plasma concentrations of corticosterone and aldosterone, the expected rapid change in the levels of receptors did not occur in any of the experimental groups (Tables 1 and 2). In animals with high resistance to hypoxia, the GRs remained at the control levels, whereas the MRs decreased. This situation can arise as a result of transient activation of protein carbonylation, which is relatively compensated by the induction of the activity of catalase and reduced glutathione pool, which we described earlier [20].

Table 2. Levels of corticosteroid receptors in the kidneys of rats with different resistances to hypoxia in the postresuscitation period without and after correction with a succinate-containing drug, M ± σ.

Day	Groups	GR. μg/g of protein		MR. ng/g of protein	
		HR	NR	HR	NR
	intact	22.92±4.36	26.51±4.09. p_1=0.0696	175.42±10.55	168.65±8.58. p_1=0.1508
1	comparison	23.20±5.80. p=0.8206	19.67±5.82. p=0.0102. p_1=0.1987	148.32±11.32. p=0.0283	163.83±10.61. p=0.3258. p_1=0.0156
	correction	28.14±5.35. p=0.0432. p_2=0.0821	30.94±4.77. p=0.0493. p_1=0.2264. p_2=0.0015	198.73±15.17. p=0.0025. p_2=0.0002	205.46±13.30. p=0.0002. p_1=0.2567. p_2=0.0002
3	comparison	17.78±4.10. p=0.0102	22.85±5.85. p=0.1306. p_1=0.0343	89.55±10.60. p <0.0001	165.18±10.98. p=0.5967. p_1=0.0002
	correction	26.37±8.13. p=0.4496. p_2=0.0126	27.56±8.16. p=0.9397. p_1=0.7054. p_2=0.1987	150.43±17.81. p=0.0019. p_2=0.0002	205.77±13.68. p=0.0002. p_1=0.0002. p_2=0.0191
5	comparison	26.07±7.67. p=0.4497	21.39±5.15. p=0.0284. p_1=0.2568	119.21±10.44. p=0.0001	226.64±11.99. p <0.0001. p_1=0.0002
	correction	27.45±6.33. p=0.0587. p_2=0.4497	25.14±6.44. p=0.4452. p_1=0.3643. p_2=0.2568	145.48±12.74. p=0.0003. p_2=0.0002	210.57±11.14. p=0.0002. p_1=0.0002. p_2=0.0191
7	comparison	22.32±5.84. p=0.4963	19.02±6.85. p=0.0191. p_1=0.4057	120.12±9.28. p=0.0001	216.99±12.48. p=0.0007. p_1=0.0002
	correction	25.52±7.51. p=0.5454. p_2=0.2568	27.81±8.70. p=0.6452. p_1=0.6502. p_2=0.0284	183.35±14.17. p=0.1735. p_2=0.0002	197.98±11.39. p=0.0002. p_1=0.0283. p_2=0.0041
14	comparison	27.45±6.01. p=0.0412	20.86±5.79. p=0.0413. p_1=0.0233	154.27±11.09. p=0.0019	191.48±12.26. p=0.0012. p_1=0.0002
	correction	27.63±7.23. p=0.1502. p_2=0.9397	26.52±9.55. p=1.0000. p_1=0.7054. p_2=0.0254	172.42±12.40. p=0.6500. p_2=0.0041	207.61±13.30. p=0.0002. p_1=0.0002. p_2=0.0192
21	comparison	29.61±6.90. p=0.0126	15.32±3.68. p=0.0003. p_1=0.0004	161.28±11.33. p=0.0191	199.75±15.58. p=0.0015. p_1=0.0003
	correction	24.13±6.90. p=0.9397. p_2=0.0233	28.55±8.98. p=0.7623. p_1=0.2568. p_2=0.0009	175.60±12.33. p=0.7623. p_2=0.0191	165.32±12.90. p=0.6501. p_1=0.0821. p_2=0.0007
35	comparison	30.60±7.93. p=0.0156	24.03±5.26. p=0.1988. p_1=0.0494	171.80±10.80. p=0.4495	210.68±15.64. p=0.0086. p_1=0.0002
	correction	23.47±5.47. p=0.9271. p_2=0.0045	27.24±6.54. p=0.7054. p_1=0.1988. p_2=0.2798	167.66±10.54 p=0.1123. p_2=0.2899	160.63±11.92. p=0.1124. p_1=0.1988. p_2=0.0009

Note: *p*—probability of the null hypothesis of the existence of differences with the control, the Kruskal–Wallis and Dunn's tests; p_1_differences between the HR and NR groups; p_2—differences between groups with and without correction, Mann–Whitney test; GR—glucocorticoid receptor; MR—mineralocorticoid receptor; HR—highly resistant to hypoxia; NR—not resistant to hypoxia.

During this period, the calculation of the Spearman correlation coefficients revealed statistically significant negative correlations between the concentration of corticosterone and the level of GR (R = −0.58, p = 0.0351) and between the concentration of aldosterone and the level of MR (R = −0.67, p = 0.0267), indicating an attenuation of the hormone–receptor interaction and limitation of signal transduction to regulate the control of the activation of adaptive response [7].

From day 3 to day 7, the level of receptors became adequate for the hormonal regulatory signal, which correlated with the unidirectional dynamics of the levels of GRs, MRs, and hormones. However, starting from day 14 of the study, the level of GRs became statistically significantly higher than the control and continually increased until the end of the study period.

The concentration of corticosterone was significantly lower than the baseline values. The MR con-

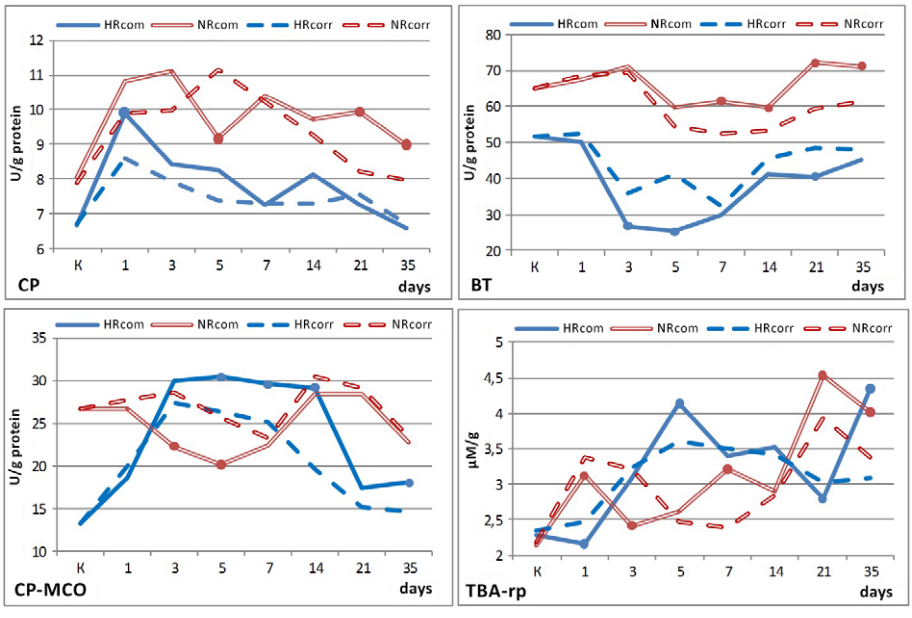


Fig. 1. Indices of free radical oxidation in the kidney tissue of rats with different resistances to hypoxia in the dynamics of the recovery period before and after correction with a succinate-containing drug. HRcom—a comparison group of rats HR to hypoxia which underwent arrest of systemic circulation and received isotonic sodium chloride solution; NRcom—a comparison group of rats that were NR to hypoxia which underwent an arrest of systemic circulation and received isotonic sodium chloride solution; HRcorr—an experimental group of rats that were HR to hypoxia and which underwent an arrest of systemic circulation and received a succinate-containing preparation; NRcorr—an experimental group of rats that are NR to hypoxia which underwent an arrest of systemic circulation and received a succinate-containing preparation; CP—carbonylated proteins; CP-MCO—metal-catalyzed oxidation of proteins; BT—bityrosine; TBA-rp—products that reacted with thiobarbituric acid; the round marker ● marks the statistical significance of differences at $p < 0.05$ in each control interval between the groups of the same name without correction and after correction; the Mann–Whitney test.

tent corresponded to the aldosterone level. Possibly, the insufficient activity of the 11HSD2 enzyme, which converts active corticosterone into an inactive metabolite [10], provided an increase in the pool of corticosterone in the renal tissue with the activation of GR and mobilization of adaptive mechanisms, most of which are anti-inflammatory [21].

In the group of rats NR to hypoxia, on the first 3 days of the postresuscitation period, in the presence of increased concentrations of corticosterone and aldosterone, a decrease in the level of receptors was noted, especially GRs. This result may be a consequence of the higher intensity of free radical oxidation (oxidative modification of proteins and lipid peroxidation) than in HR rats and the inhibition of the antioxidant defense described by us earlier [20].

Starting from day 5, the conjugation of the level of receptors and the plasma concentration of hormones were restored, with a high level of aldosterone corresponding to a high content of MRs and a low concentration of corticosterone corresponding to a reduced content of GRs. This situation persisted until the end of the experiment.

Thus, in the animals that were HR to hypoxia, a short-term period of relative functional incompetence of the hormone–receptor interaction, with the limitation of the possibility of effective structural and functional rearrangement of physiological/metabolic systems for the implementation of a complex of adaptation reactions to changed internal conditions, occurred on day 1. Most of the postresuscitation period (starting from the day 5) was characterized with the increased GR activity and decreased MR activity.

In animals with low resistance to hypoxia, a similar state lasted until the end of day 3. A significant prevalence of the MR level with a statistically significant excess of the control values during the repression of GRs was recorded during most of the postresuscitation period.

The combined succinate-containing drug, which exhibits a set of characteristics that have beneficial effects on cell metabolism and energy production [22], caused the natural modification of the processes of free radical oxidation in both groups of animals (Fig. 1). In HR rats, this condition was manifested by a decrease in the intensity of lipid peroxidation, and in NR animals, it was manifested by a decrease in the intensity of carbonyl stress. These changes in NR rats were expressed functionally by the restoration of the regulatory mechanisms of the negative feedback and the shift in the peak of corticosteronemia on day 1 of the postresuscitation period, which corresponds

to the generally accepted concept of stress activation of the hypothalamic–pituitary–adrenal system (Table 2). The tendency toward the normalization of aldosterone levels was beneficial in the restoration of the circulating blood volume and blood circulation in both groups of animals.

Another significant result of the therapy is the normalization of the MR level in the NR animals in the presence of the hormonal balance optimization. This effect was most pronounced in the late post-hypoxic period (Table 2).

Discussion. As noted above, the enhancement of mineralocorticoid signaling plays a key role in the mechanism of progressive renal dysfunction in ischemia–reperfusion injuries [11], and this effect requires a pathological environment in the form of oxidative stress intensification [12].

Aldosterone stimulates dose dependently, through a MR-induced increase in the production of reactive oxygen species by mitochondria, the formation of cytosolic proteins NLRP3 and ASC, is involved in the activation of caspases 1 and 5 and proinflammatory interleukins-1β and -18, and causes damage to the tubular epithelium [23]. The blockade of MR with spironolactone before or after ischemia prevents the acute functional and structural damage caused by ischemia–reperfusion and reduces the oxidative stress in renal transplant recipients. In rats, fineronone, a non-steroidal MR antagonist, can prevent the acute renal injury caused by ischemia–reperfusion and progressive deterioration of renal function and structure [24].

The GR activation suppresses the inflammatory infiltration by attenuation of the signaling of the phosphatidylinositol-3 kinase/AKT pathway, which is essential in the regulation of adaptive immune responses [25]. The administration of dexamethasone to mice before an episode of ischemia provides protection from ischemia–reperfusion injury, which is manifested in the improvement of renal function and a decrease in the intensity of pathomorphological disorders [8]. If the "aldosterone-MR" complex under conditions of oxidative stress damages glomerular podocytes, causing impairment of the glomerular filter and proteinuria [26], then glucocorticoids, through GR, regulate the transactivation and transrepression of genes in podocytes, which affect their morphological and cytoskeletal aspects, mobility, apoptosis, and survival; prevent protein leakage across the glomerular barrier membrane, affecting the synthesis, transport, and post-translational modifications of the components of slit diaphragm and podocyte intercellular junctions [27].

In the range of various approaches to the correction of hypoxia, researchers focus on the optimization of the tricarboxylic acid cycle, whose physiological functioning is the most important condition for the normal vital functioning of the body [15, 22]. Circulatory hypoxia, which increases progressively during the arrest of systemic circulation, causes a disorder of the body energy balance with the addition of tissue hypoxia; given the decrease in oxygen tension in the mitochondria, the activity of NAD oxidases[1] of the tricarboxylic acid cycle and the phosphorylation associated with mitochondrial oxidation are inhibited [4].

In hypoxia, the FAD-dependent[2] II enzyme ensemble of the mitochondrial respiratory chain is inhibited later than the NAD-dependent oxidases because the oxidation of succinic acid in the respiratory chain of the Krebs cycle is independent of the NAD-dependent enzymes. This link in the tricarboxylic acid cycle can maintain energy production for a certain time in the presence of the oxidation substrate in mitochondria (succinic acid) [2, 4].

However, in critical conditions, the energy deficit becomes nonrenewable by any means of compensation because the problem of hypoxia cannot be solved without the supply of an energy substrate. In addition, the introduction of succinate for the normal functioning of the Krebs cycle in any critical condition can become akin to "prosthetics" of energy deficit [2]. The exogenous succinate bypasses section I of the respiratory chain and supports the electronic transport of section II, providing membrane potential and the synthesis of adenosine triphosphate. This strategy offers a potential future for the treatment of metabolic decompensation arising from mitochondrial dysfunction during hypoxia [28].

The decrease in the intensity of free radical processes with the introduction of a combination of inosine + nicotinamide + riboflavin + succinic acid, as noted above, enables the discovery of the maximum protective properties of glucocorticoids, most of which are due to the transpressive function of GRs, which involves suppressing the genes of the inflammatory response and increasing the transcription of components of anti-inflammatory genes [21]. For this reason, further normalization or a slight increase in the level of GRs, which is characteristic of individuals with an initially low resistance to hypoxia persisting throughout the postresuscitation period, can be regarded as a significant sanogenetic factor contributing to a fast and complete recovery. The normalization of the MR level in the NR animals during optimization of hormonal balance is an important factor in the full recovery of functions and a decrease in the risk of

[1] NAD — nicotinamide-adenine dinucleotide.

[2] FAD — flavine adenine dinucleotide.

developing late profibrotic complications and functional renal failure [29].

Thus, in the early stages of the rehabilitation period, a distinct effect of the succinate-containing drug manifested itself in the restoration of the content of corticosteroid receptors and hormone–receptor interaction. In the late postresuscitation period, this condition was reflected as a decrease in the overwhelming prevalence of MRs in the group of rats NR to hypoxia and that of GRs in rats HR to hypoxia. The reduction of the imbalance between the levels of GRs and MRs and restoration of their ratio are several of the conditions for the full restoration of organ functions [30]. A common characteristic of the changes that occurred in this study was the convergence of the values of the studied parameters of animals with different resistances to hypoxia. A pronounced positive therapeutic effect was achieved in rats NR to hypoxia.

CONCLUSIONS

1. Rats with low resistance to hypoxia were characterized by high intensity of free radical oxidation in the kidneys, as manifested by the development of carbonyl stress associated with a reduction of GRs during the first 3 days of the postresuscitation period (up to 74%, $p < 0.01$) and a significant increase by the end of the monitoring of the MR of (up to 134% of the control level, $p < 0.001$). An increase in mineralocorticoid signaling in the presence of high aldosterone levels and significant intensity of oxidative stress makes the kidney vulnerable to remodeling and fibrosis factors, thereby impairing long-term prognosis.

2. A high degree of resistance to hypoxia was distinguished by a relatively low intensity of free radical oxidation, characterized mainly by an increase in lipid peroxidation during a 35-day monitoring and a transient activation of protein carbonylation on day 1. In the early recovery period, this condition was accompanied by mainly a decrease in the level of MRs (up to 52%, $p < 0.001$) and a significant increase in the concentration of GRs in the late period (up to 136%, $p < 0.05$).

3. Correction of the course of the postresuscitation period using a combination of inosine + nicotinamide + riboflavin + succinic acid resulted in animals that were NR to hypoxia by the end of the experiment, with the normalization of indicators of oxidative modification of proteins, association with the restoration of negative feedback mechanisms, stabilization of the level of GRs, and a decrease in MR-to-control values. In organisms HR to hypoxia, during correction, the activity of lipid peroxidation decreased, and the level of both types of receptors was restored. An increase in glucocorticoid signaling is an essential sanogenetic mechanism that promotes the implementation of the anti-inflammatory function of glucocorticoids.

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