



<https://doi.org/10.15407/cryo36.02.128>

UDC:612.825:615.832.9+576.371]:616.12-008.331.1-092.9

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EFFECT OF CRANIOCEREBRAL HYPOTHERMIA AND CELL THERAPY ON AUTONOMIC NERVOUS SYSTEM STATE IN RATS

This study investigated the effects of rhythmic craniocerebral hypothermia (rCCH), administration of cryopreserved nucleated cord blood cells (NCBCs), and their combined application on autonomic regulation of heart rate and arterial blood pressure (BP) parameters in 12-month-old spontaneously hypertensive rats (SHRs) with arterial hypertension (AH). A relationship was established between AH and autonomic nervous system imbalance. The SHRs demonstrated a shift in heart rate variability (HRV) indices toward enhanced sympathetic activity, which may indicate reduced neurohumoral regulation and signs of overstrain and asthenization within central regulatory structures. On day 7 after the combined application of rCCH and NCBCs, the adaptive compensatory capacity of the organism increased significantly. Although these effects diminished by day 30, they remained higher compared to the use of each method separately. In addition, unlike the independent application of rCCH or NCBCs, their combined use resulted in a reduction of both systolic and diastolic BP.

Key words: craniocerebral hypothermia, cryopreserved nucleated cord blood cells, heart rate variability, arterial hypertension, rats.

Arterial hypertension (AH) is a major risk factor for the development of cardiovascular diseases (CVD). According to WHO statistics, CVD affects 10–20% of the adult population and accounts for 5.8% of all deaths. The World Hypertension League emphasizes that more than 1.3 billion people worldwide suffer from AH and its consequences [9, 12].

Alongside heredity and endocrine metabolic imbalance, dysfunction of the central nervous system (CNS) and autonomic nervous system (ANS) plays an important role in the development of AH [6, 7].

Persistently elevated arterial blood pressure (BP) activates adaptive compensatory mechanisms aimed at maintaining vital functions. However, under conditions of chronically high BP — *i. e.*, prolonged hemodynamic load — these mechanisms

become exhausted, leading to target organ damage and the development of complications [6, 9].

When pathological processes progress and stable structural or functional alterations form in organs and systems, it becomes essential to assess the degree of functional strain in the adaptive compensatory and regulatory responses of the CNS and cardiovascular system (CVS), which are responsible for maintaining homeostasis. Interchangeability and mutual compensation within these functional systems during disease progression and therapeutic interventions help create conditions necessary for achieving an adaptive outcome. Under chronic AH, the functional state of the CNS and CVS becomes particularly significant.

In experimental models, AH is associated with a broad spectrum of psychoemotional, somatoauto-

Reference: Lomakin II, Mamontov VV, Kudokotseva OV. Effect of craniocerebral hypothermia and cell therapy on autonomic nervous system state in rats. *Probl Cryobiol Cryomed.* 2026; 36(2): 128–35. <https://doi.org/10.15407/cryo36.02.128>

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nomic, vascular, and endocrine disturbances. The dominance of cortical, hypothalamic, and limbic structures in CNS functioning influences the direction of neurotransmitter processes, neuroendocrine responses, and the formation of regulatory reactions in AH, thereby determining the choice of effective therapeutic approaches.

According to previous studies conducted at the Institute for Problems of Cryobiology and Cryomedicine of the NAS of Ukraine, a promising and therapeutically effective direction in cryomedicine is the use of various types of cold exposure. Such interventions enable targeted modulation of histohematic barrier permeability, correction of autonomic regulation of peripheral processes, and active influence on neurotransmitter systems [2, 3, 11]. Following the application of rhythmic craniocerebral hypothermia (rCCH), which acts as a sensory and systematically applied stimulus, specific changes occur within the thermoregulatory systems of the brain. It has been shown that under rhythmic cold exposure, the functioning of central neurotransmitter systems involved in adaptive activation, thermoregulatory responses, and general mechanisms of homeostasis maintenance is supported by physiologically adequate (selective) permeability of the blood–brain barriers to biologically active substances (BAS) essential for these processes, ensuring their entry into brain cells [1–3].

Administration of cord blood cells (CBCs), a promising therapeutic tool in regenerative medicine [13, 14], can influence a persistent pathological state in three key ways: reducing the activity of a stable pathological compensatory hyperactive state of functional systems; modifying neurotransmitter systems and promoting their adaptive reorganization through activation of the organism's internal reserves; providing targeted biological regulation, with mechanisms similar to those naturally employed by the body for adaptation and protection.

The cardiovascular system is a complex functional system whose regulation is ensured by both intrinsic local autoregulatory mechanisms and external regulatory mechanisms. The latter include the CNS, autonomic regulation, and the endocrine system (the controlling regulatory link), as well as the vascular and visceral systems (the coordinating link).

The activity of the cardiovascular system (CVS) is directed toward ensuring the necessary level of functioning of all body systems to maintain overall homeostasis [4].

Changes in CVS functioning are observed at all stages of pathology development. The dynamics of the informational indicators can be traced through the assessment of the autonomic component within the general chain of regulatory influences [4, 16].

Reflecting the activity of cortical systems and cortico-subcortical interactions within the CNS, the structural-functional organization of the autonomic nervous system (ANS) determines the integration of multiple physiological functions through behavioral responses and its influence on peripheral organs and systems, thereby shaping the direction of optimal adaptive reactions.

Regulatory influence of the ANS on the circulatory system is mediated through the sympathetic and parasympathetic divisions. A close relationship exists between alterations in autonomic regulation of heart rate and the development of cardiovascular diseases. ANS imbalance is a key mechanism in the formation of arterial hypertension (AH) and its complications.

One of the hopeful directions in cardiology is the study of autonomic regulation of the CVS and its changes during treatment, using mathematical analysis of heart rate variability (HRV).

Heart rate variability, as a method for assessing autonomic modulation of cardiac function, is widely used for clinical evaluation of different components of neurohumoral regulation in patients with AH and for determining the effectiveness of therapeutic interventions. The high sensitivity of this methodological approach allows detection of changes in the functional state of the organism as a whole — changes that cannot be identified by other laboratory or instrumental methods [16].

The aim of the study was to investigate the effects of rhythmic craniocerebral hypothermia and cell therapy on autonomic regulation of heart rate and arterial blood pressure parameters in rats with arterial hypertension.

MATERIALS AND METHODS

The study was performed in sexually mature male rats aged 12–13 months. Experiments were done in white outbred rats (normotensive control) and spontaneously hypertensive rats (SHRs), in accordance with the Law of Ukraine "On the Protection of Animals Against Cruelty", and with the requirements of the Bioethics Committee of the Institute for Problems of Cryobiology and Cryomedicine of the NAS of Ukraine (Protocol No. 5 dated

16.11.2021). All procedures complied with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

The SHR_s were selected as a model of chronic arterial hypertension [8, 10]. Animals were divided into eight groups ($n = 5$ per group): 1 — normotensive control (N); 2 — control group of spontaneously hypertensive rats (SHR_s); 3 and 4 — SHR_s on days 7 and 30 after rhythmic craniocerebral hypothermia (SHR_s + rCCH); 5 and 6 — SHR_s on days 7 and 30 after administration of cryopreserved nucleated cord blood cells (NCBCs) (SHR + NCBCs); 7 and 8 — SHR_s on days 7 and 30 after combined application of both interventions (SHR_s + rCCH + NCBCs).

Rhythmic craniocerebral hypothermia was performed once using the "Rimidy1" device ("VostokV", Ukraine) [1].

Cryopreserved human NCBC suspensions were obtained from the cryobank of the Institute for Problems of Cryobiology and Cryomedicine. After thawing, NCBCs were administered once intraperitoneally at a dose of $(3-5) \times 10^7$ viable cells per kilogram of body weight. For the combined treatment protocol, the cell suspension was administered the day after rCCH.

Arterial blood pressure was measured indirectly using medical tonometry and the "Poly—Spectrum" electrocardiograph ("Poly—Spectrum", Ukraine), with specialized compression cuffs "Microlife BP BEO4" (Microlife, Switzerland) and pulse sensors placed on the tail vessels of the animals.

The HRV analysis is based on detecting and measuring the time intervals between RR intervals on the ECG, constructing dynamic series of cardio-intervals (cardiointervalograms), and subsequently analyzing the resulting numerical sequences. Mathematically, the method is performed using Fourier transformation with the construction of a spectrogram and calculation of the spectral area within defined frequency ranges [5, 15, 16]. A variation curve (distribution curve of cardio-intervals or histogram) is constructed, and its main characteristics are determined: mode (Mo) — indicates the state of the humoral regulatory channel of heart rhythm, corresponding to the range of the most frequent RR interval values; amplitude of the mode (AMo) — reflects sympathetic activity and represents the number of cardio-intervals corresponding to the modal value or its range. The regulatory adequacy

index (RAI) is calculated to demonstrate the correspondence between sympathetic ANS activity and the functional state of the sinoatrial node ($RAI = AMo/Mo$).

Spectral analysis allows quantitative assessment of different frequency components of heart rhythm oscillations and provides a clear graphical representation of the relationships among rhythm components, reflecting the activity of specific regulatory mechanisms [16]. The high frequency (HF) component of the HRV spectrum characterizes the activity of the parasympathetic (vagal) cardioinhibitory center of the medulla oblongata. Changes in the activity of the vasomotor center of the medulla are reflected in the low frequency (LF) component. The very low frequency (VLF) component serves as an index of humoral metabolic regulatory processes. VLF oscillations are closely associated with psychoemotional tension. According to recommendations for spectral HRV analysis in rats [5, 16], the following parameters were identified and analyzed:

TP (ms^2) — total power of the heart rhythm spectrum (0.015—3.0 Hz);

HF (ms^2) — power of high frequency oscillations (0.8—3.0 Hz);

LF (ms^2) — power of low frequency oscillations (0.05—0.79 Hz);

VLF (ms^2) — power of very low frequency oscillations (0.015—0.04 Hz).

The autonomic balance index (ABI) was determined to assess the ratio between sympathetic and parasympathetic activity of the ANS. It was calculated using variational pulsometry as the ratio of AMo to the variation range (the difference between the maximum and minimum RR-intervals). The centralization index (CI) was calculated as $(HF + LF)/VLF$.

All measurements in the experiment were performed on days 7 and 30 after the last rCCH exposure or NCBC administration.

Morphometric data were statistically processed using the Microsoft Excel software (Microsoft, USA). The significance of differences was evaluated using the Mann–Whitney U test. Differences were considered statistically significant at $p < 0.05$. Numerical data are presented as the arithmetic mean (M) and the standard error of the mean (m).

RESULTS AND DISCUSSION

We demonstrated that in 12-month-old spontaneously hypertensive SHR_s, HRV parameters shifted

toward enhanced sympathetic autonomic regulation. This was confirmed by significant changes in Mo and AMo values (Table 1). A significant increase in the regulatory adequacy index and CI was likely due to enhanced influence of suprasegmental regulatory levels on heart rhythm, reflecting cerebral ergotropic effects on lower peripheral regulatory levels (Table 1).

These findings may indicate a reduction in neurohumoral regulatory capacity, as well as signs of overstrain and asthenization within the central regulatory structures of the CNS.

The rCCH allows controlled cooling with stabilization of body temperature at the required level. By acting on a relatively small area (in this case, adjacent cortical structures), it produces a rapid decrease in the activity of neural centers responsible for thermoregulation and autonomic functions. When analyzing the responses of functional systems to rCCH, it is necessary to consider the specific features of brain function under conditions of chronic cerebral hypoperfusion associated with arterial hypertension, which affects the formation and activity of central regulatory mechanisms of stress responses.

In SHR rats, an unfavorable consequence of rCCH was the predominance of excitatory regulatory circuits. This was accompanied by reduced stability of regulatory and regulated systems, disruption of autonomic balance and regulatory adequacy, increased strain on subcortical regulatory centers, inhibition of the autonomic contour of heart rate regulation, and reduced compensatory capacity of the organism.

On day 7 after rCCH, hypertensive rats demonstrated a significant decrease in the regulatory adequacy index and AMo, indicating not only reduced mobilizing influence of the sympathetic regulatory branch but also an insufficient or low level of centralized control of heart rhythm. By day 30 after rCCH, sympathetic influences remained reduced, while the autonomic balance index increased, reflecting improved vagosympathetic equilibrium, and the centralization index significantly decreased (Table 1).

According to spectral HRV analysis, SHR rats exhibited a progressive decrease in total power (TP) on days 7 and 30 after rCCH, primarily due to reductions in both sympathetic and parasympathetic components of the ANS (Figure). The attenuation of previously compensated disturbances in efferent vagal activity, together with reduced efferent sympathetic activity 30 days after rCCH, indicated exhaustion of adaptive compensatory capacities in spontaneously hypertensive animals because of the development and progression of cardiovascular pathology.

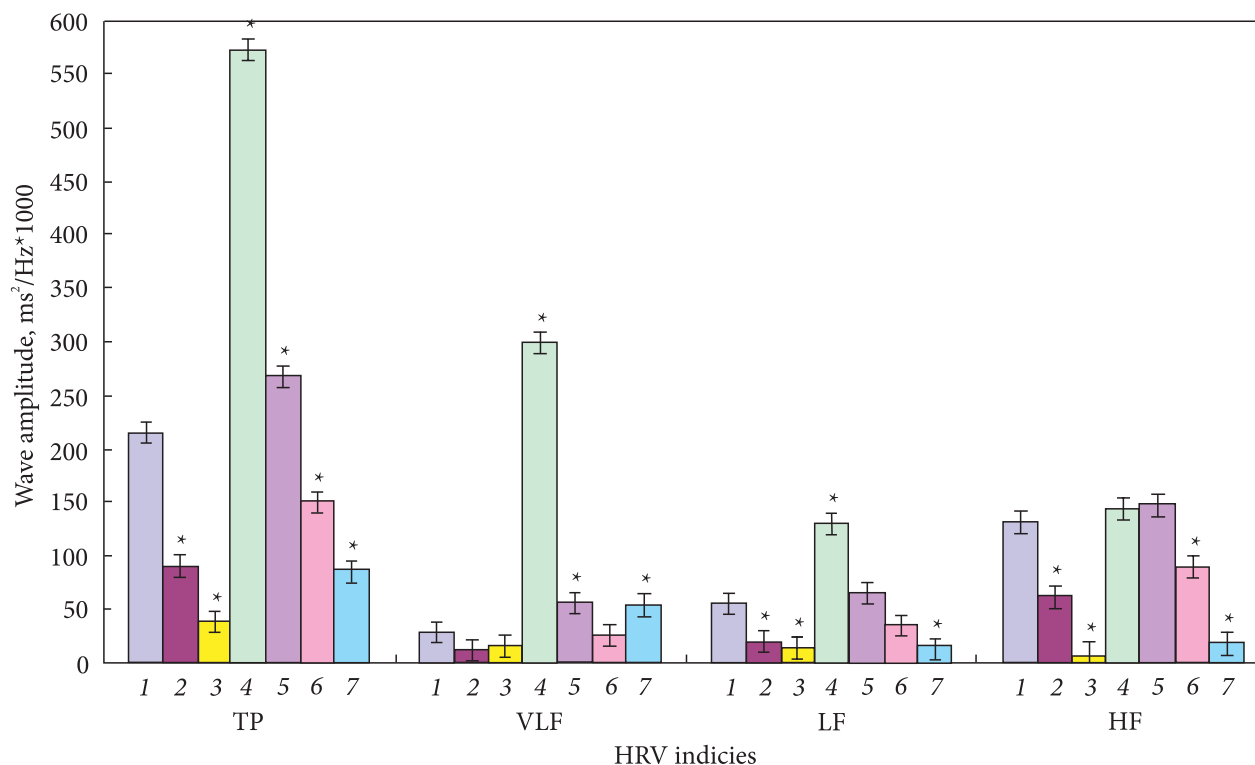
On days 7 and 30 after administration of NCBCs to spontaneously hypertensive rats, significant changes were observed in the variational pulsometry indices ABI and CI (Table 1). At the same time, on day 7 after NCBC administration, the regulatory adequacy index, AMo, and Mo did not change significantly. A nearly sevenfold reduction in CI, together with an increase in ABI, indicates that vagal activity was under tonic inhibitory influence from afferent cardiac sympathetic activity.

Spectral HRV analysis in SHR rats on day 7 after NCBC administration revealed a sharp increase

Table 1. Indices of variational pulsometry in SHRs on days 7 and 30 after rCCH, NCBC administration, and their combined application

Group	Indices of variational pulsometry				
	AMo	Mo	RAI	ABI	CI
1	88.4 ± 2.4 #	0.17 ± 0.01 #	514 ± 21 #	0.42 ± 0.08	0.2 ± 0.02 #
2	94.8 ± 2.2 *	0.127 ± 0.03 *	746 ± 16 *	0.421 ± 0.07	6.51 ± 0.11 *
3	52.3 ± 0.33 *, #	0.131 ± 0.02 *	399 ± 21 *, #	0.311 ± 0.07 *, #	7.26 ± 0.11 *, #
4	52.2 ± 2.4 *, #	0.118 ± 0.036 *	442 ± 26 *, #	1.55 ± 0.07 *, #	1.28 ± 0.13 *, #
5	96.7 ± 4.3 *	0.13 ± 0.01 *	773 ± 46 *	0.90 ± 0.03 *, #	0.93 ± 0.02 *, #
6	86.5 ± 4.1 #	0.12 ± 0.03 *	739 ± 26 *	0.69 ± 0.06 *, #	0.59 ± 0.01 *, #
7	95.7 ± 2.1 *	0.12 ± 0.03 *	790 ± 30 *	0.44 ± 0.02	3.78 ± 0.02 *, #
8	77.3 ± 2.2 *, #	0.15 ± 0.01 #	533 ± 53 *, #	0.39 ± 0.06 #	4.83 ± 0.03 *, #

* Significant differences compared with Group 1; # significant differences compared with Group 2; $p < 0.05$.



Spectral HRV indices in spontaneously hypertensive rats: 1 — control; 2 — day 7 after rCCH; 3 — day 30 after rCCH; 4 — day 7 after NCBC administration; 5 — day 30 after NCBC administration; 6 — day 7 after combined rCCH + NCBC treatment; 7 — day 30 after combined rCCH + NCBC treatment; * — differences are significant relative to the control group, $p < 0.05$

(more than 2.5-fold) in total power (TP), resulting from enhanced tone of both autonomic centers and the humoral-metabolic regulatory branch (Figure). Activation of the trophotropic and ergotropic functions of the ANS occurred, manifested as directed changes in cardiac function.

Table 2. Arterial blood pressure indices in SHR after rCCH, NCBC administration, and their combined application

Group	Arterial blood pressure indices, mmHg	
	SBP	DBP
1	124.3 ± 4.7 [#]	86.4 ± 2.4 [#]
2	221.1 ± 6.4 [*]	129.2 ± 4.2 [*]
3	200.5 ± 5.1 ^{*,#}	90.0 ± 3.1 [#]
4	215.0 ± 5.1 [*]	115.0 ± 2.6 ^{*,#}
5	210.0 ± 3.9 ^{*,#}	100.1 ± 3.0 ^{*,#}
6	215.1 ± 5.3 [*]	95.0 ± 1.9 ^{*,#}
7	195.3 ± 2.2 ^{*,#}	95.0 ± 1.1 ^{*,#}
8	200.0 ± 3.4 ^{*,#}	95.1 ± 2.6 ^{*,#}

* Significant differences compared with Group 1; # significant differences compared with Group 2; $p < 0.05$.

Innervation of different regions of the heart is heterogeneous and asymmetric, determined by its anatomical and physiological organization. For example, stimulation of the right vagus nerve produces a more pronounced negative chronotropic effect (slowing of heart rate), whereas stimulation of the left vagus nerve results in a negative dromotropic effect (slowing of atrioventricular conduction). A similar asymmetry is present in the sympathetic innervation of the heart. Sympathetic nerves on the right side predominantly innervate the anterior ventricular surface and, to a greater extent, the sinoatrial node, while those on the left side innervate the posterior ventricular surface and the atrioventricular node.

The presence of asymmetry in sympathetic and parasympathetic innervation may determine specific features of cardiac function during stress and following exposure to biologically active substances (BAS). In our study, the effect of NCBC administration was most clearly reflected in the slowest regulatory system — the humoral-metabolic regulatory branch. In this case, NCBC administration caused a sharp increase (12-fold) in the relative contribution of VLF waves (Figure).

On day 30 after NCBC administration, a decrease in TP was observed, primarily due to reduced ANS tone (Figure). Thus, the reduction in autonomic sympatho-parasympathetic influences and central regulatory activity was accompanied by predominance of the humoral-metabolic regulatory component.

In the SHR rats on day 7 after combined rCCH and NCBC treatment, the dynamics of variational pulsometry indices were like those observed after NCBC administration alone (Table 1). At the same time, the pattern of changes after the combined intervention differed markedly from that observed after rCCH as an independent therapeutic method. Spectral HRV analysis showed that the total power of neurohumoral regulation decreased compared with the control group due to reduced sympathetic ANS activity. On the spectrogram, this was confirmed by a 20% reduction in the relative contribution of LF waves (Figure).

The obtained experimental data suggest that in spontaneously hypertensive rats, on day 7 after the combined application of rCCH and NCBC administration, adaptive compensatory processes increased, accompanied by balanced vagosympathetic influences and humoral-metabolic regulatory factors.

In SHR rats on day 30 after the combined use of rCCH and NCBCs, a reduction in sympathotonic influences on the probabilistic component of sinoatrial node function was observed. Compared with the control group, Mo significantly increased, while AMo, RAI (by 28.6%), and ABI (by 7.4%) decreased (Table 1). The reduction in total power of neurohumoral regulation (more than 30% compared with day 7) occurred due to decreased tone of the parasympathetic division of the ANS (Figure).

Thus, on day 7 after the combined application of rCCH and NCBCs, spontaneously hypertensive rats exhibited a marked increase in adaptive compensatory capacity, which declined by day 30 but remained higher than with either method used independently.

Blood pressure indices reflect the general trend of autonomic regulatory activity, manifested as a decrease in BP. The HRV findings were consistent with the dynamics of BP changes (Table 2). On day 7 after rCCH alone or NCBC administration alone, SHR rats showed a significant reduction in BP compared with untreated hypertensive controls, al-

though BP values did not reach those of normotensive rats. By day 30, systolic BP in SHR rats (unlike diastolic BP) did not differ significantly from control values (Table 2). Under combined rCCH + + NCBC treatment, in contrast to the independent application of these methods, a significant reduction in both systolic and diastolic BP was observed at both time points (Table 2).

Thus, our findings demonstrate that AH and ANS are closely interconnected; therefore, any disturbances in CNS function may lead to vasomotor dysfunction and a range of associated cardiovascular disorders. It should also be emphasized that HRV analysis has substantial clinical value in AH. HRV assessment enables evaluation of autonomic tone and its components, as well as individualized selection of therapy aimed at achieving the most favorable sympathovagal balance.

CONCLUSIONS

In sexually mature 12-month-old SHR rats, disturbances in autonomic balance were observed, manifested as low synchronization of regulatory components and increased sympathetic regulatory activity.

Experimental HRV data indicate the presence of overstrain and asthenization in CNS regulatory structures in hypertensive animals, which over time may contribute to the development of cardiovascular diseases and premature aging of functional systems.

On day 7 after the combined application of rCCH and NCBCs, spontaneously hypertensive rats exhibited a marked increase in adaptive compensatory capacity, which declined by day 30 but remained higher than with either method used independently.

In SHR rats characterized by high baseline BP values (221.1/129.2 mmHg), combined rCCH + + NCBC treatment resulted in a notable reduction in both systolic and diastolic BP on days 7 and 30 (to 195.3/95.0 and 200.0/95.1 mmHg, respectively). In contrast, when each method was applied independently, significant BP reduction was observed only on day 7.

The regulatory influence of the ANS on AH is mediated through the sympathetic and parasympathetic divisions of the CNS; therefore, any disturbances in their function may lead to vasomotor abnormalities and a range of associated cardiovascular disorders.

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Received 12.01.2026

Accepted for publication 18.05.2026

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ВПЛИВ КРАНІОЦЕРЕБРАЛЬНОЇ ГІПОТЕРМІЇ ТА КЛІТИННОЇ ТЕРАПІЇ НА СТАН ВЕГЕТАТИВНОЇ НЕРВОВОЇ СИСТЕМИ ЩУРІВ

У роботі досліджували вплив ритмічної краніоцеребральної гіпотермії (рКЦГ), введення кріоконсервованих ядровмісних клітин кордової крові (кЯВК КК) та їх поєднаного використання на стан вегетативної регуляції серцевого ритму та показники артеріального тиску (АТ) 12-місячних спонтанно гіпертензивних щурів (SHR) з артеріальною гіпертензією (АГ). Встановлено взаємозв'язок між АГ та дисбалансом вегетативної нервової системи. У щурів SHR відзначалось зміщення показників варіабельності серцевого ритму (ВСР) в бік посилення симпатичного контуру вегетативної регуляції, що може свідчити про зниження рівня нейрогуморальної регуляції та наявність явищ перенапруження і астенізації в регуляторних відділах ЦНС. На 7-му добу після поєднаного застосування рКЦГ і кЯВК КК значно підвищувалися адаптаційно-компенсаторні можливості організму, які знижувалися до 30-ї доби, однак зберігалися на більш високому рівні порівняно з окремим використанням цих методів. Крім того, на тлі поєднаного використання рКЦГ і кЯВК КК, на відміну від їх самостійного застосування, спостерігалось зниження показників систолічного і діастолічного АТ.

Ключові слова: краніоцеребральна гіпотермія, кріоконсервовані ядровмісні клітини кордової крові, варіабельність серцевого ритму, артеріальна гіпертензія, щури.