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THE INFLUENCE OF VESSEL POLYPEPTIDE COMPLEX ON THE VIABILITY OF RATS UNDER COLD STRESS

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The study of the mechanisms of preserving human viability in deep hypothermia, as well as the resilience of elderly people with hypothermia, is an urgent problem of physiology and medicine. The aim of the work is to study the effectiveness of the use of the vessels polypeptide complex (VPC) to stimulate the functions of the respiratory and cardiovascular systems in violation of life stability in the model of experimental hypothermia. Wistar's line rats were divided into an experimental group (injection of VPC) and a control group (injection of saline solution). VPC was administered intraperitoneal to rats at a dosage of 15.6 mg/kg of animal body weight 1 hour before the animals were immersed in a bath with cold water. The breath rate, heart rate, rectal temperature, esophageal temperature, and the degree of saturation of arterial hemoglobin with oxygen were evaluated. The heart rate in the rectal temperature range of 30-16 ° C was significantly higher after the use of VPC in comparison with the control. The breath rate began to decrease at a rectal temperature of 27.7 ± 0.3 oC during the cooling process in rats, which obtained VPC. A decrease in breath rate in the control group was observed, starting with a higher rectal temperature - 30.4 ± 0.4 oC . The threshold for stopping breathing after the use of VPS was 11.6 ± 0.3 oC for rectal temperature and 14.1 ± 0.3 oC for esophageal temperature. In the control, these indicators were equal, respectively, 16.0 ± 0.3 oC and 19.1 ± 0.3 oC, which is 4-5 oC higher. The cooling time of rats to respiratory arrest increased by 2 times after the use of VPC in comparison with the control. It can be assumed that VPC slows down the process of cold respiratory depression and cardiac activity. The obtained results indicate the ability of the VPC to maintain the functions of the respiratory and cardiovascular systems for a long time in case of a violation of life stability in the model of experimental hypothermia. This indicates the prospects for further investigation of the VPC geroprotective properties and its ability to increase the cold resistance of the body in hypothermia.

Keywords: resilience, cold stress, breathing, vessel polypeptide complex, geroprotection.

Introduction. Prolonged exposure to cold can lead to hypothermia, when thermoregulatory mechanisms fail to maintain normal body temperature. At a body temperature below 35 ºC, the most important functions of the body are disturbed. Especially dangerous is deep hypothermia, when breathing stops and the heart stops, which leads to the risk of death. However, moderate hypothermia is used in medicine in the treatment of patients with brain and heart lesions [4, 11]. It is known that the regulation of body temperature is disturbed in the elderly, which reduces their vitality (resilience). One of the external factors leading to a decrease in vitality is stress caused by hypothermia. In the elderly, the risk of hypothermia is especially high, since people in this group often suffer from chronic diseases and have reduced immunity. To maintain the functional activity of the body in people over 60 years of age under the influence of adverse factors of the internal or external environment, it is important to mobilize the resources of individual viability [2].

The mechanisms leading to cell death under various damaging effects are similar. These are excessive generation of oxygen free radicals, activation of lipid peroxidation, release of glutamate, disruption of the cell membrane potential, and an increase in the intracellular concentration of calcium ions [1, 6, 11]. Along with this, hibernating animals are an example of natural adaptations to a significant decrease in body temperature, which is observed during hibernation, while their cellular structures are not damaged [12]. The development of methods for increasing cold resistance, maintaining breathing and heart function at low body temperatures will allow a person to return to life even after a cardiac arrest during deep cooling.

The polypeptide complex of calf vessels (VPC) has angioprotective properties, normalizes lipid peroxidation, restores the density of microvessels in the cerebral cortex of old animals, and reduces the area of necrosis in a model of acute myocardial infarction in rats [5]. It has been established that VPC has an antiarrhythmic effect, softens the effect of catecholamines on the walls of blood vessels under extreme conditions [7]. The purpose of the work is to study the effectiveness of the use of VPC to stimulate the functions of the respiratory and cardiovascular systems in violation of vitality in the model of experimental hypothermia.

Material and methods. The experiments were performed on 13 white male Wistar rats weighing 300-320 g. I.P. Pavlov Academy of Sciences, supported by the program of bioresource collections of the Federal Agency of Scientific Organizations of Russia. Animals were anesthetized with urethane (125 mg/100 g of body weight, intraperitoneally), local anesthesia was applied (novocaine, 2%). The rats were kept under standard vivarium conditions with natural light and free access to water and food. The studies were carried out in accordance with the

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European Convention for the Protection of Animals Used for Scientific Purposes.

Cooling was carried out in a water bath at a temperature of 9-10 ºC. The animals were fixed on the platform so that the upper back and head were above the water surface. Using copper-constantan thermocouples, the temperature in the rectum at a depth of 4.5 cm (Tr) and in the esophagus (Tes) was recorded. The thermocouple in the esophagus actually measured the temperature of the heart. A sensor for recording the breath rate (BR) was fixed around the chest of the rat. Heart rate (HR) was determined from the electrocardiogram in the second lead. The degree of oxygen saturation of hemoglobin in arterial blood (SpO2) was determined using a veterinary pulse oximeter BP-12C (Biocare), the sensor was attached to the front paw. Rats of the experimental group (n=5) 1 hour before immersion in cold water were injected intraperitoneally with VPC at a dosage of 15.6 mg/kg of animal body weight. The control group of rats (n=8) was similarly injected with 1 ml of saline. The data were recorded using an external E14-140-M module (L-Card, Russia) and the PowerGraph program. The reliability of the results obtained was assessed using Student's t-test using the Statistica 6.0 software package. Experimental data are presented as mean ± error of mean (M ± m). Differences were considered significant at $p<0.05$.

Research and discussion. Before immersion in cold water, the rectal temperature in rats was 35.8±0.3°C, the temperature in the esophagus was 36.4±0.2°C, HR was 441±8 beats/min, BR was 118±6 cycles/min, SpO2 was 98±1%. The cooling time of the control animals to stop breathing was 60±7 min, which corresponded to the previously obtained data [1]. In animals that received VPC, this time increased by 2 times to 125±9 min. The curves reflecting the dependence of the temperature in the esophagus on the rectal temperature during cooling did not differ in the control and experimental groups (Fig. 1). The rate of decrease in Tr and Tes in the first 30 min of cooling did not differ significantly in control (Tr 0.32±0.02 °C/min, Tes 0.28±0.02 °C/min) and experimental rats (Tr 0.35±0.01 °C/min, Tes 0.30±0.02 °C/min). min). During the next 30 minutes, both indicators decreased to 0.1±0.001 °C/min in the control group and to 0.12±0.01 °C/min in the experimental group (p≥0.05). In the experimental group, cooling to respiratory arrest continued, and in the next 60 minutes the rate of decrease in Tr and Tes was 0.07±0.01 °C/min.

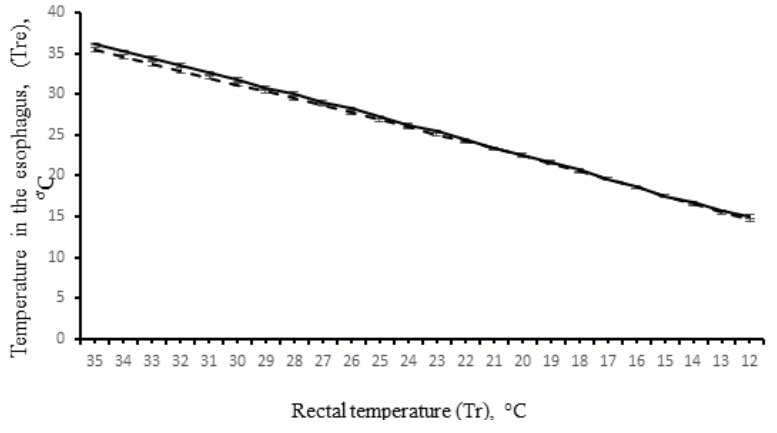

Fig. 1. Temperature dynamic in the esophagus (Tes) depending on the rectal temperature (Tr) when rats are cooled in water: dotted line - control, solid line - the use of VPC

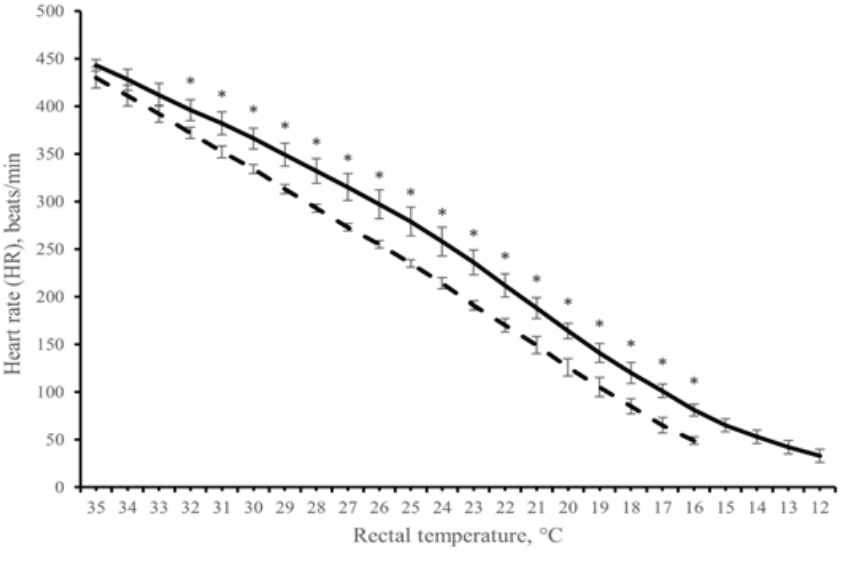

Fig. 2. The effect of VPC on the heart rate (HR) depending on the rectal temperature (Tr) when cooling rats in water: dotted line - control, solid line - the use of PCR.
* - $p < 0.05$ - compared to the corresponding indicator in the control.

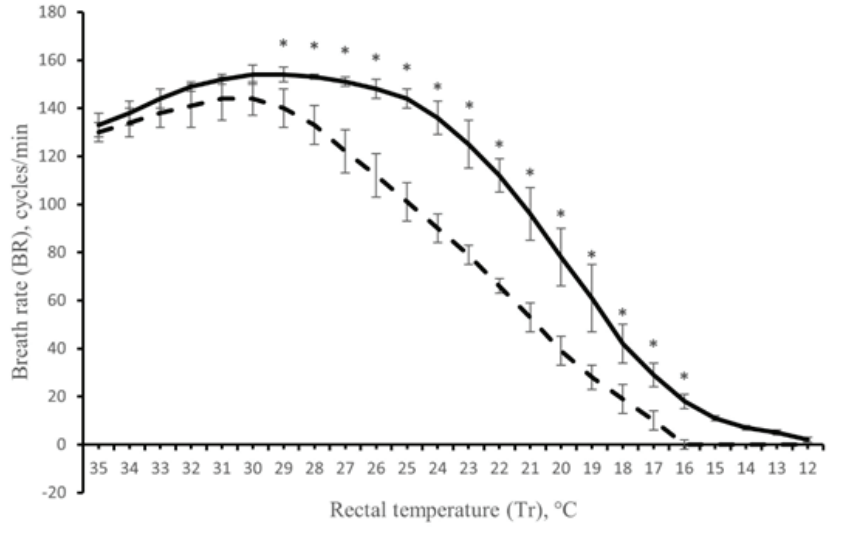

Fig. 3. The effect of VPC on the respiratory rate (BR) depending on the change in rectal temperature (Tr) when cooling rats in water: dotted line - control, solid line - the use of VPC.
* - $p < 0.05$- compared to the corresponding indicator in the control

The rate of decrease in HR in the control in the first 30 min of cooling was 9.8±3.4 beats/min, in the next 30 min it was 2.9±0.4 beats/min. In experimental rats, in the first 30 min, the rate of decrease HR was 9.5±2.2 (p≥0.05), in the next 30 min - 2.7±0.5 beats/min, which also did not differ from the control (p≥0.05), and in the last 60 min - 0.7 ±0.03 beats/min. It was noted that the HR at Tr 31-16 ºC in the experimental animals was significantly higher than in the control (Fig. 2).

In rats that were injected with VPC, the BR did not decrease to Tr 27.7±0.3ºC. At the same time, in control rats, a decrease in BR was observed, starting from Tr 30.4±0.4ºC (Fig. 3). The decrease in BR in the control and in the experimental group occurred at the same rate: in the first 30 min of cooling, 3.1±0.5 cycles/min, in the next 30 min, 2.7±0.4 cycles/min. In the experimental group of rats, within 60 minutes before stopping breathing, the BR decreased very slowly - 0.2±0.02 cycles/min.

Respiratory arrest in control animals was observed at Tp 16.0±0.3 ºC, Tes 19.1±0.3 °C, HR - 20±3 beats/min. In the experimental group of rats, respiration stopped at lower temperatures: Tr - 11.6±0.3 ºC, Tes - 14.1±0.3 °C, HR - 25±4 beats/min (p<0.05). Thus, after the application of VPC, the temperature threshold for respiratory arrest decreased by approximately 4-5ºC. In the experimental group of rats, during the last 60 min of cooling to respiratory arrest, the rate of decrease in heart rate and respiratory rate decreased corresponding to a significant slowdown in the cooling process. At this stage of the experiment, the HR decreased gradually from 70 to 26 beats/min. The BR was low, at first at the level of 11 - 8 cycles/min, and approximately 30 minutes before the respiratory arrest, the respiratory rate decreased to 5 - 3 cycles/min, then breathing stopped. In contrast, in the control, as noted above, respiratory arrest occurred at higher values of Tr and Tes, and the decrease in BR and HR before respiratory arrest occurred quickly. The level of blood oxygen saturation in all rats was kept at the level of SpO2 85-96% to Tr 18-19°C. Then, in control animals, this indicator decreased, in accordance with the decrease in respiration. In animals treated with VPC, with the rare breathing, SpO2 was at the level of 90-92%.

The data obtained complement the previously identified protective effect of VPC on the indicators of the functional activity of the cardiovascular system in models of age-associated pathology [7]. The mechanism of the protective effect of VPC on the cells of the organs of the cardiovascular and respiratory systems in violation of vitality during experimental hypothermia may be due to the tripeptide KED that is part of it [3]. This tripeptide can penetrate into the cytoplasm and nucleus of vascular endotheliocytes and specifically interact with the CACC nucleotides sequence in DNA, regulating the expression of the gene encoding the Ki67 proliferatropic protein [9]. By the same mechanism, or by interacting with the nucleosome (the complex of DNA and histone proteins) [8], the KED peptide can regulate gene expression and protein synthesis of apoptosis (p53), proliferation (VEGF), and adhesion (E-selectin) of endotheliocytes during development. cold stress of cells, pathology of the cardiovascular system and aging [10]. It is likely that by using VPC, which slows down the development of pathological damage in cells during hypothermia, it is possible to slow down the cessation of breathing and increase the time reserve for therapeutic measures, when saving people with deep hypothermia, as well as when hypothermia occurs in people of older age groups.

Conclusion. The results obtained indicate the ability of VPC to maintain the functions of the respiratory and cardiovascular systems for a long time in case of violation of viability in the model of experimental hypothermia. These data expand the possibilities for further research into the geroprotective properties of VPC and its ability to increase the body's resistance to hypothermia.

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