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ORIGINAL ARTICLE

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Influence of Slavinorm on Resuscitation of Rats Cardiorespiratory Functions under Deep Hypothermia

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The problem of resuscitating a man whose respiration and heart were arrested under strong cooling is urgent for the reanimation practice. The aim of this work was to study the influence of Slavinorm (a polypeptide complex of the vessels) on the heart work activation and the regeneration of their inherent respiration in the Wistar rats with the help of artificial ventilation of lungs (ALV) and warming the sternum region under deep hypothermia. The animals narcotized with urethan were cooled in water (9,2±0,5ºC) up to the arrest of respiration. The experimental group of rats 1 h before cooling were injected intraperitoneally with Slavinorm (15,6 mg/kg of weight) and the control rats were injected with physiological solution. In control rats the arrest of respiration was observed at the rectal temperature (Tr) 16,0±0,3ºC, in the esophagus (Te) 19,1±0,3ºC. The artificial lung ventilation (ALV) and heating the breast region resulted in the initiation of natural respiration in 29,2±0,4 min from the beginning of ALV at Tr 13,8±0,2ºC, Te 16,1±0,2ºC. The conclusion: Slavinorm increases the resistance of an organism to cold, assists the prolongation of the process of cooling up to the arrest of respiration, decreases the temperature thresholds of the heart activation and resuscitation of natural respiration.

Key words: hypothermia, respiration, rats, heart, Slavinorm – polypeptide vessel complex

Deep hypothermia may result in violation of the main functions of an organism and to its death (Anderson *et al.*, 2016; Kim *et al.*, 2022). In recent years the studies are carried out of the pathological processes occurring in an organism cells at low temperatures, of the protective effects of moderate hypothermia upon medical treatment of a number of traumata, diseases of humans and animals (Gunn, 2017; Shevelev *et al.*, 2019; Sone and Yamaguchi, 2024). The death of an organism during deep hypothermia is associated with the action of cooling on the respiration center resulting in its cold paralysis, which then results in the hypoxic damage of tissues and organs, in the arrest of the heart. After the arrest of the heart the reanimation becomes difficult, but the cases of saving the people even in such situations are described (Mroczek *et al.*, 2020). Under cold stress a number of pathological processes occur in an organism, which result in the destruction of membranes, ionic gradients in the cells (Hendriks *et al.*, 2020; Hirata *et al.*, 2023; Shevelev *et al.*, 2019). The hibernating animals represent a natural model of adaptation to low temperatures, in this period their metabolism, the frequencies of respiration and of heart contraction decrease substantially. Upon coming out of hibernation these animals in several hours restore the normal body temperature and the functions of organism, in this case the organism cells are protected against cold destruction (Giroud *et al.*, 2020; Sone and Yamaguchi, 2024).

With the aim of inducing the states similar to hibernation in nonhibernating animals the studies of the efficiency of various preparations and reanimation methods are going on (Ivanov and Arokina, 2015; Zakharova *et al.*, 2021). Lately we succeeded to show that Slavinorm (PVC – polypeptide complex from the vessels of large-sized cattle) exerts an activating effect on the heart work and respiration during cooling the rats in water (Arokina *et al.*, 2022).

The aim of this work was the study of Slavinorm (polypeptide vessel complex) influence on the activation of the heart work and resuscitation of natural respiration in Wistar rats with the help of artificial lung ventilation (ALV) and warming the breast region during deep hypothermia.

MATERIALS AND METHODS

The experiments were carried out on 15 white male Wistar rats 310±10 g in weight. The animals were obtained from the biocollection “The collection of laboratory mammals of various taxonomy” of I.P. Pavlov Institute of Physiology, RAS. The animals were narcotized with urethane (125 mg/100g of body weight intraperitoneally). The rats were kept in vivarium under natural light with free access to water and food. The investigations were performed in compliance with European convention about the defense of animals used for scientific purposes. Experiments were supported by the Commission for control over the care and use of laboratory animals of I.P. Pavlov Institute of Physiology, RAS (N: 08/02.2021).

The rats were cooled in a bath with cold water (9,2±0,5 ºC). We recorded the temperatures in the rectum (Tr) and esophagus (Te), the frequency of heart contractions (HCF) and of respiration (FR). We determined the saturation of hemoglobin of the arterial blood with oxygen (SpO_2) by a BP-12C (Biocare) pulsoximeter on a front paw. For connecting the apparatus of artificial lung ventilation (ALV) a cannula was inserted into the rats trachea, for anesthetization we used Novocain 2%. The experimental rats (n=5) 1h before submerging into cold water were intraperitoneally inserted with 1 ml of the solution of polypeptide vessel complex – Slavinorm (OOO Samson-Med, cattle vessels polypeptides) 15,6 mg/kg of body weight, the control animals (n=10) were inserted with 1 ml of physiologic solution. The rats from experimental group 5 min after cold paralysis of respiration were connected to the ALV apparatus (respiration frequency 18 cycles per min, the volume of inhalation 1 ml). In 7 min after starting ALV the rats were extracted from water, 1-2 min before a “Deltaderm” salt heater was put on the breast region (temperature 38-40ºC).

The data were recorded and treated with the help of an E14-140-M modulus (L-Card, Russia), the PowerGraph program. The reliability of the differences were estimated with the help of Tukey's parametrical criterium, the Statistica 6.0; InStat 3.02 (“GraphPad

Software Inc." USA) programs. The data are given as the average ± the error of the average was (M ± m), the differences were considered reliable at $p<0.05$.

RESULTS

The starting parameters in the rats of control and experimental groups were the following: Tr35,7±0,2°C, Te 36,5±0,2°C, HCF 446±10 pulsations/min, RF 112±7 cycles/min, SpO_2 99±2%. The time of cooling control animals till the arrest of respiration was 59±5 min, in experimental animals – 124±8 min. The arrest of respiration in control animals was observed at Tr 16,0±0,3 °C, Te 19,1±0,3°C, HCF 20±3 pulsations/min. In the experimental group of rats the respiration was arrested at lower temperatures: Tr 11,7±0,3 °C, Te14,1±0,3 °C, HCF 24,6±3,9 pulsations/min ($p<0.05$). During the last 30 min of cooling the respiration frequency was kept to the level of 5-3 cycles/min, HCF – 45-40 pulsations/min. Figs. 1A and B give the diagrams showing the character of HCF and RF changes in the rats of control and experimental groups at various stages of cooling.

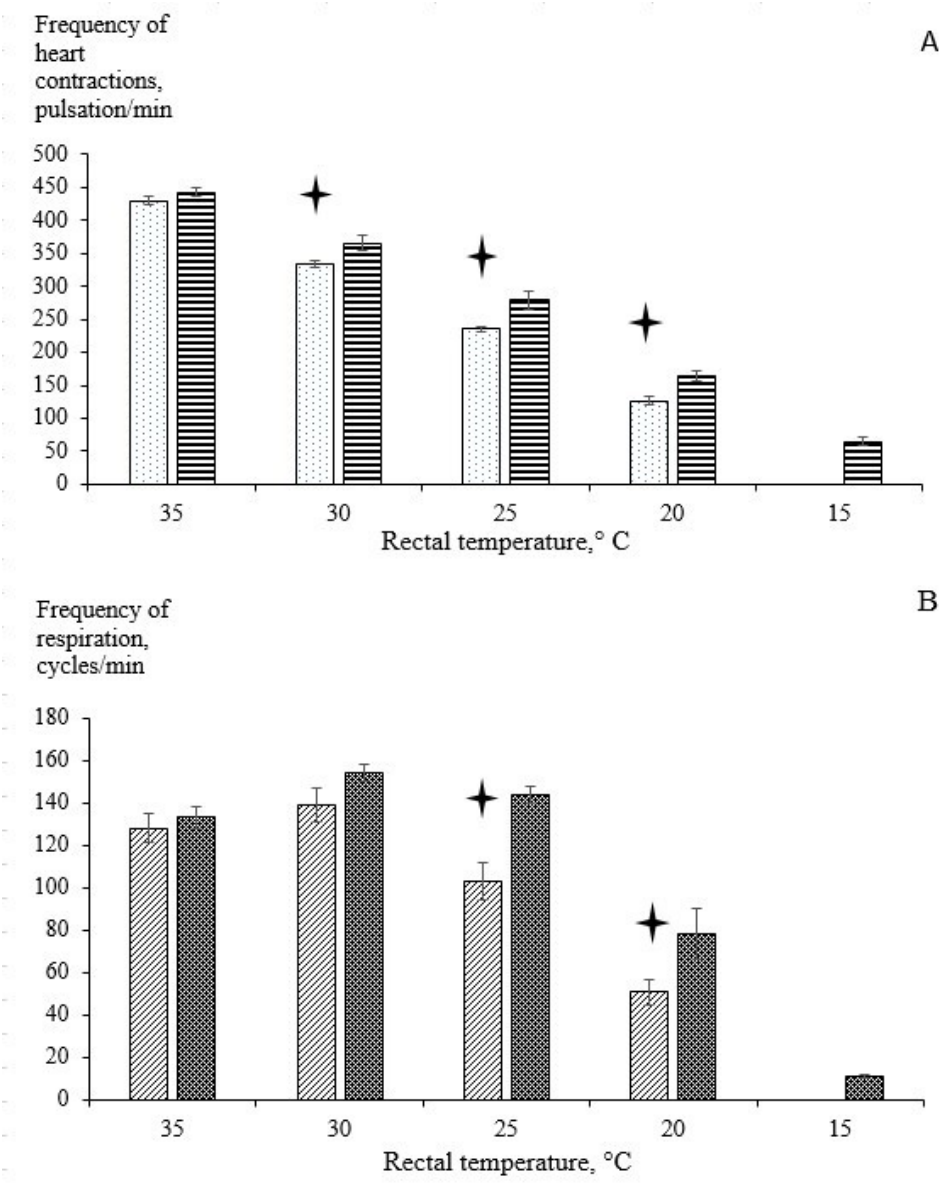

Figure 1. Changes in the frequency of heart contractions (A) and respiration frequency (B) in the rats of the experimental group (insertion of Slavinorm) during the development of deep hypothermia.

A – control – a column with points; the experimental group – a column with horizontal strips. B – control – column with sloping lines; the experimental group – black columns. Asterix – the reliability of the variation from control $p<0.05$.

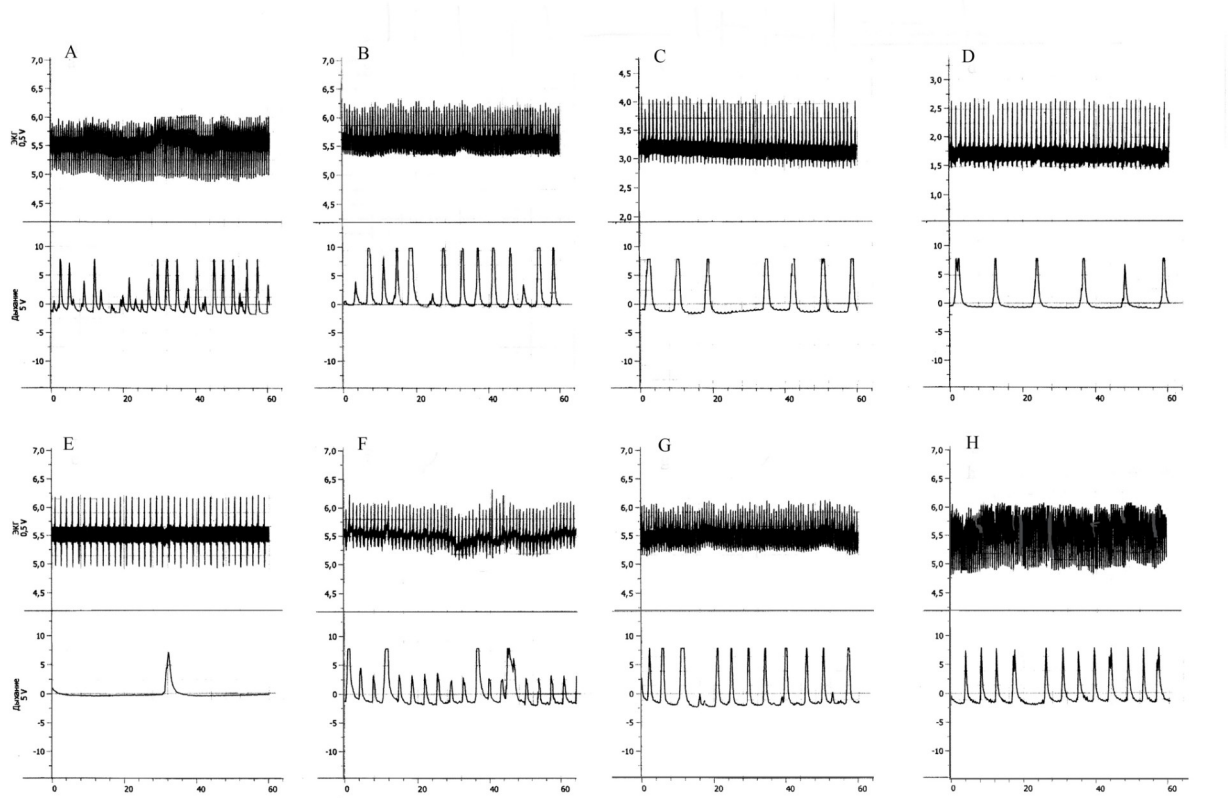

Figure 2. Fragments of recording the ECG and pneumograms at various stages of one of the experiments, where a rat was inserted with Slavinorm before cooling.

a– 70-th min of cooling Tr 16,5°, Te 20°C, RF 25 cycles/min, HCF 100 pulsations/min;
b – 77-th min, Tr 15,9°; Te 19°C; RF 14 cycles/min, HCF 84 pulsations/min;
c – 100-th min, Tr 14,0°, Te 16,7°C, RF 7 cycles/min, HCF 57 pulsation/min;
d – 120-th min, Tr 12,5°, Te 15,0°C, RF 6 cycles/min, HCF 46 pulsations/min;
e – 153-th min, Tr 11,4°, Te 13,7°C, RF 1 cycle/min, HCF 37 pulsations/min;
f – The rat is extracted from water, 15-th min of heating, Tr 13,4°, Te 16,4°C, RF 18 cycles/min, HCF 96 pulsations/min;
g – the ALV is switched off, the rat breathes naturally, Tr 17,6°; Te 19,5°C, RF 12 cycles/min, HCF 96 pulsations/min;
h – 25-th min after switching off ALV, Tr 18,0°; Te 20,0°C, RF 12 cycles/min, HCF 108 pulsations per min.

Table 1. Activation of the heart work and resuscitation of natural respiration in the rats of experimental group (Slavinorm insertion) with the help of ALV and heating the breast region (n=5)

Stages of the experiment	Rectal temperature, °C	Esophagus temperature,°C	Respiration frequency, cycles/min	Heart contraction frequency, pulsations/min
Arrest of respiration	11,7±0,3	14,1±0,3	0	24,6±3,9
Switching on ALV	11,6±0,3	14,0±0,3	18	24±4
Beginning of HCF increase	11,4±0,4	13,8±0,3	18	28,4±3,2
Before heating	11,3±0,3	13,7±0,2	18	30,2±3,8
Start of natural respiration	12,5±0,4	14,6±0,3	22,2±1,0	43,6±3,4
Switching ALV off, the rat breathing naturally	13,8±0,2	16,1±0,2	21,4±0,4	57,2±7,1
1 h after starting ALV	18,2±0,1	19,8±0,3	22,6±0,5	99,0±5,6

For the rats of experimental group ALV was switched on in 5 min after the arrest of respiration, in 7 more min the animals were extracted from water then the heater was installed. The activation of the heart work was observed in 390±25 s fron AVL switching, HCF increased with the rate 1,6±0,2 pulsation/min. An increase in Tr and Te started in 64±6 s after the beginning of heating, the rate of the increase in the Tr was 0,14±0,01°C, and in the Te - 0,15±0,01°C. Against the background of AVL in about 310±24 s from the beginning of the increase in Tr and Te additional respiration movements appeared in the pneumogram. The respiration frequency became 21-22 cycles/min (the frequency of the AVL apparatus + natural respiration), SpO_2 – 86±4%. In 29,2±0,4 min of the AVL apparatus operation it was switched off, the rats breathed independently. The observations went on for 1 hour from the beginning of AVL, at the end of the experiment HCF increased almost to 100 pulsation/min and RF was maintained at the level of 21-23 cycles/min, SpO_2 – 95±3%.

The Table 1 gives the average values of Tr, Te, RF, and HCF, recorded in the rats of the experimental group at various stages of the experiment. Fig. 2 gives fragments of ECG and pneumogram made in one of the experiments, in which the rat was inserted with Slavinorm. It can be seen that with the help of contacting the ALV and heating we were able to activate the heart work and resuscitate the respiration in the rats under deep hypothermia

DISCUSSION

We compared the results obtained in this study with our earlier investigations (“Model 2”, Arokina, 2021). In the experiments carried out by “Model 2” the arrest of respiration occurred at Tr 16,4±0,2°C, Te 18,5±0,4°C, HCF was 26±3 pulsations/min. The threshold of the heart arrest was decreased by 4-5ºC at the expense of artificial lung ventilation after the arrest of natural respiration. Therefore, we succeeded in demonstrating the important role of oxygen supply of an organism for maintaining the heart work. After extracting the rats from water and heating the breast region the resuscitation of natural respiration was observed at Tr 20,5±0,4°C, Te 22,5±0,6°C, HCF was 105±8 pulsations/min.

In the experiments with the rats inserted with Slavinorm, contrastingly, we were able to decrease the cold threshold of the arrest of respiration to Tr 11,7±0,3°, Te 14,1±0,3°C without using ALV, in this case the heart continued working with the frequency 25±4 pulsation/min. The time of cooling the rats till the arrest of respiration increased by a factor of almost two compared to control. When the arrest of respiration was observed in the control rats, RF in the experimental rats was 11,6±0,4 cycles/min, HCF – 76±7 pulsations/min. Later in the experimental animals the heart continued working for about an hour with a rare respiration, this state bears a resemble to a numbness observed in hibernating animals during hibernation.

In the experiments with the insertion of Slavinorm after switching ALV the heart work was activated, the natural respiration at the background of ALV appeared in about 5 min of heating the breast region. And after 30 min from the beginning of ALV, when the body temperature increases to Tr 13,8±0,2°, Te 16,1±0,2°C the rats breathed naturally, HCF was 57±7 pulsations/min. It is important to emphasize, that in these experiments the resuscitation of cardio-respiratory functions in the rats was obtained after a prolonged cooling and keeping the animals for about 1 hour in the hypometabolic state.

The cells of hibernating organisms were shown to survive longer upon cultivating at low temperatures than the cells of nonhybernating organisms, they can maintain the activity of mitochondria at low temperatures (Giroud *et al.*, 2020; Sone and Yamaguchi, 2024). During hybernation the levels of antioxidant enzymes and low-molecular absorbents of active forms of oxygen decrease in the blood and cerebrospinal liquid of hibernating animals (Hirata *et al.*, 2023). It is possible that a man and nonhybernating animals have a potential property to transform into the state of hypometabolism and leave it, and in the state of hypometabolism the cells become tolerant to hypoxia and low temperatures.

The developers of Slavinorm believe that it can influence the expression of genes, the synthesis of apoptosis peptides (Khavinson *et al.*, 2021; Zhurkovich *et al.*, 2020). This preparation seems to be able to

generate the transformations on the cell level, which provide for the maintenance of an organism functions at low temperatures. It is possible that in the rats inserted with Slavinorm before cooling the resistance to cold of the neurons of respiration center increased, the metabolism level decreased, then for maintaining the heart work a rare respiration was enough. Hence the respiration was arrested at lower temperatures than in control, and consequently, the respiration was restored more quickly after the beginning of heating.

CONCLUSION

We can conclude that Slavinorm increases the cold resistance of an organism, promotes the prolongation of the process of cooling up to the arrest of respiration, decrease the temperature thresholds of the heart activation and of resuscitation of natural respiration.

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CONFLICTS OF INTEREST

The author declares that they she has no potential conflicts of interest.

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