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EFFECT OF HYPOTHERMIA ON THE RAT CEREBRAL MICROVASCULAR REACTIONS UNDER CONDITIONS OF HEMODYNAMIC STABILITY AND BLOOD LOSS

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Accidental hypothermia commonly goes with trauma, which is often accompanied by hemorrhage. The research studies the reaction of microcirculation of the cerebral cortex of rats with hemodynamic stability and the blood loss, with continuous exposure to decreasing body temperature till to the hypothermal arrest of the animal's breathing. Video microscopy of cerebral pial arterioles during the cooling of animals in water with a temperature of 12-130C was carried out on anasthezed rats (uretanum, i/ p, 1000 mg/kg). The results were obtained that in the group of animals without hemorrhage, the cooling led to an initial short-term vasodilation and subsequent vasoconstriction up to 10-20% of the norm. In the group of animals with a pre-caused by hemorrhage, the narrowing of the vessels was 20% at the normothermia and intensified during the animal immersion in water on 35% of the initial state of the vessels.

However, the functional state of the animal during blood loss and the subsequent cooling slightly differed from the normovolemic state of the body under hypothermic influence.

Keywords: hypothermia; blood loss; cerebral microarterioles; video microscopy; rats.

Introduction. Overall study of the mechanisms of hypothermal states is one of the current problems of modern medicine. Benefits and complications after using hypothermia with different kinds of surgical interventions (induced hypothermia) and exposure to low environmental temperature on the body (accidental hypothermia) are discussed. The induced hypothermic effect on the body is actively used in clinical practice during heart operations, for the treatment of patients with hypoxic, ischemic damage after brain strokes, in neonatal encephalopathy and spinal cord injury [7, 12, 14]. The study of the body's reactions to the unintentional cold effect and the methods to escape from this state are relevant not only in the conditions of the Far North and Siberia [3, 5, 17].

Accidental or unintentional hypothermia in a homoeothermic organism is classified into the following degrees: mild (32-35℃), moderate (28-32℃), severe (28-20℃) and deep or profound (<20℃) [1, 5, 12, 17, 19]. The symptoms and clinical manifestations in different degrees of hypothermia are shown in Table 1. Accidental hypothermia together with trauma are often accompanied by hemorrhage. Central hypovolemia leads to hypoperfusion of tissues and hypoxia, including the brain. Acute compensatory mechanisms involved in maintaining perfusion pressure and blood flow to vital organs influence an the increase in heart rate frequency and systemic vasoconstriction [9]. Low environmental temperatures may affect the ability of the body's cardiovascular system to suffiently react to hypovolemia, i.e. tolerance to hemorrhage can be intensified or reduced. In some clinical studies [6, 20], it was revealed that the decrease in body temperature below 35 is a factor predisposing to more severe complications and increased mortality. It is reported [15] that up to 66% of patients entering emergency care for serious injury, suffer from accidental undercooling.

Most clinical studies on hypothermia and circulatory disorders [8, 22] are devoted to the positive use of the temperature lowering of the body or certain organs during surgical interventions, i.e. the use of induced hypothermia and different approaches to the recovery of the body after it. In some experimental models of hemorrhagic shock, moderate hypothermia increases the survival rate of animals [11, 14, 18] by reducing the body's oxygen demand and is considered as a possible therapy for traumatic bleeding. It should be noted that the number of experimental studies on the effect of low temperature on hemodynamics in conditions of hypovolemia is small. For example, it was shown [21] that local hy

Table 1

Symptoms and clinical manifestations at different degrees of hypothermic exposure

Symptoms	Hypothermia		
	Mild	Moderate	Deep
	32-35 °C	28-32 °C	<28 °C
Neuro-muscular	ataxia shivering	stiffness of muscles and joints the disappearance of tremors	muscle contraction loss of reflexes and the ability to voluntary movements
Neurological	confusion apathy	limited consciousness	loss of the corneal reflex coma
Cardiovascular	tachycardia increased cardiac output hypertension peripheral vasoconstriction	bradycardia reduction of cardiac output On the ECG: - widening of QRS complex - inversion of the T-wave - QT segment prolongation - wave J (Osborne) Atrial fibrillation	reducing system pressure progressive bradycardia asystole entricular fibrillation
Respiratory	tachypnae shift of HbO2 curve to the left	bradypnae bronchial constriction	lactic acidosis respiratory arrest

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pothermia does not have a harmful effect on dogs during hemorrhage (20% of the blood volume) and improves the perfusion of the microvessels of the stomach and oral cavity and their oxygenation. Another study revealed [10] microvascular changes in the sublingual region, in the intestinal villi and in the renal cortex in hemorrhagic shock in sheep with 340C hypothermia.

However, the effect of systemic hypothermia in severe hemorrhagic shock on cerebral hemodynamics has not been studied. Since the results of the literature analysis are quite contradictory and there are no data on the effect of hypothermia at different stages of its development during hemorrhagic shock, an experimental work was carried out, the purpose of which was to study the cerebral microvascular circulation of the rats subjected to hemorrhagic shock, with a continuous decrease in body temperature up to hypothermic respiratory arrest of the animal.

Materials and Methods. The experiments were carried out on anesthetized (urethane, i/p, 1000 mg/kg) male Wistar rats weighing 300-320 g. The study was conducted on animals from the biological collection "Collection of laboratory mammals of different taxonomic affiliation" of the I.P.Pavlov Institute of Physiology of the Russian Academy of Sciences in the agreement with the basic norms and rules of biomedical ethics (European Community Council Directives 86/609/EEC).

The animals were subjected to the following surgical procedures: to study the pial microvessels, a 7x5mm trepanation hole was made in the parietal bones, the dura mater was removed. A catheter was inserted into the left femoral artery for direct measurement of blood pressure, and a similar catheter was inserted into the right femoral artery for blood withdrawal. During the operations, the rats maintained a rectal temperature of 37-380C with a heating pad. Then the animals were randomly divided into 2 groups: the first (n=6) – the control group, in which the animals were cooled without blood loss, and the second (n=11) – with a preliminary withdrawal of blood (the features are described below).

Rats of both groups were subjected to gradually developing immersion hypothermia in water with a temperature of 12-130C until hypothermic respiratory arrest. During cooling, the animals were fixed in a special device (dental, ear holders, and soft fixation of the limbs) in a shallow bathtub so that the body was immersed in water, and the head was above water. The arterial blood of animals from the 2nd group, was taken at the rate of 2.1 ml per 100 g of animal weight or 35% of the volume of circulating blood, i.e., from a rat with a weight of 300 g, the total blood intake was 6.3 ml. The blood sampling time was ~20 min, the average withdrawal rate was 0.3 ml/ min., while blood pressure was maintained at the level of 40 mm Hg before cooling.

Visualization and monitoring of the pial microvascular circulation was carried out with the help of microscopy system, which included a LUMAM-1 microscope with a contact dark-field lens and an ACUMEN AiP-B84A color video camera. The resulting image was processed on the computer by the Pinnacle Studio software package. The measurements were calibrated using a standard object-micrometer (the division price is 10 microns).

During the experiment, the reactions of pial arteries with an initial diameter of 10 up to 50 microns to progressively increasing hypothermia were studied. The diameter of the microvessels was measured at 50 different areas of the arteries in the control group and at 100 areas in the 2nd group at twelve stages of the experiment: before the start of exposure, at rectal temperature 36, 35, 32, 30, 28, 26, 24, 22, 20, 18 0C and when the animal stops breathing. The ECG, mean blood pressure and respiratory rate were continuously recorded. With a help of the E-154 ADC (L-Card, Russia) analog signals were digitized and recorded.

The STATISTICA 6.0 software package was used for statistical data processing, the reliability of differences within each group was evaluated using the nonparametric Wilcoxon criterion, the nonparametric Mann-Whitney criterion was used to identify the differences between the groups, the level of reliability of differences was $p<0.05$. All experimental data are presented as the average ± error of the average (M±m).

Results and Discussion. The using of immersion hypothermia in these experiments allowed us to observe changes in the work of the cardiovascular and respiratory systems during cooling of the body in a fairly short time up to hypothermic respiratory arrest.

After surgery, before the start of cooling, the rectal temperature (Tr) in rats of both groups did not differ statistically and was 36.7±0.110C in the control group, 37.14±0.150C in the 2nd group before blood withdrawal, 36.7±0.170C after withdrawal. Thus, the cooling of the animals began with almost the same rectal temperature. Immersion of the animals in water led to a decrease in body temperature and, eventually, to hypothermic respiratory arrest. The temperature threshold for rats breath stop is a stable parameter. Respiratory arrest in rats occurs at Tr in the range of 12-190C [16], and spontaneous restoration of respiration in conditions of deep hypothermia is possible only if the animal is removed from the water and warmed up. The stoppage of respiratory movements in the control group was recorded at Tr 13.1±0.330C, in the 2nd group-at 15.12±0.80C ($p<0.05$). The cooling time in the 1st group was 183±2 min, in the 2nd-160±22 min ($p>0.05$). The average rate of immersion cooling of animals in different groups did not significantly differ: in the control - 0.128±0.010C/min, in the group with preliminary blood withdrawal – 0.158±0.0170C/min.

Table 2 shows the main physiological parameters of animals of both groups before cooling and after hypothermic respiratory arrest. It should be noted that the physiological parameters before cooling of group 2 rats after withdrawal 35% of the circulating blood volume significantly differed from the normal parameters.

The changes in physiological parameters during the cooling of animals show

Table 2

Physiological parameters of rats in different series of the experiment during normothermia and after hypothermic respiratory arrest

Parametres	Before the start of exposure		After blood withdrawal	After breath stop	
	control	2-nd group	2-nd group	control	2-nd group
Rectal themperatire, ^{0}C	36.7±0.11	37.14±0.15	36.7±0.17	13.1±0.33	15.12±0.8*
Mean arterial pressure, Hg mm	99.7±2.49	95.5±5.57	38.7±1.65 †††	28.3±1.8	18.2±1.43***
Heart rate, min^{-1}	432±5.8	442.3±8.99	393.8±9.67 ††	36.7±1.8	36.6±7.56
Breath rate, min^{-1}	104±11.7	104.4±7.99	89.7±6.86 †	0	0

* $p<0.05$, * * * $p<0.001$ between the parameters of the control group and the second group after breath stop. † $p<0.05$; † † $p<0.01$; † † † $p<0.001$ between the parameters of group 2 before exposure and after blood withdrawal.

at Figure 1. The cooling of the animals was accompanied by the development of a response from all organs and systems. At the beginning of hypothermia exposure, a slight increase in mean arterial blood pressure (MAP) was observed in the control group rats with normovolemia, probably [1, 2], due to an increase in the level of metabolism and norepinephrine-mediated peripheral vasoconstriction. In this study, it was shown that with a decrease in Tr below 340C, blood pressure remained quite high (at the normal level) and decreased only after 200C. The breath rate (BR) also increased ($p<0.001$) at the beginning of cooling. This is a typical reaction of the external respiration function, which is characterized by an increase in pulmonary ventilation, frequency and depth of breathing [2]. Then, after reaching the degree of moderate hypothermia, a consistent suppression of the functional state of the body was observed, and BR gradually decreased until respiratory arrest. It was reported [4] that when rats are cooled to a rectal temperature of 310 C, HR decreases slightly, but the results of the standard deviation and the coefficient of variation increase. In our work, a significant decrease in HR was recorded already at a temperature of 320C (by 25%). At temperatures in the range of 32-200C, HR decreased by 3 times, and at the time of respiratory arrest it was 36.7±1.8 beats/min. It is shown [18] that cooling is accompanied by a decrease in the impact volume, along with this, oxygen consumption also decreases.

In the experiment blood loss in rats led to a decrease in blood pressure to the level of 40 mm Hg, which was maintained before the start of immersion. After the start of cooling, MAP increased significantly (up to 55-63 mm Hg) and was on such a plateau, decreasing only in a state of deep hypothermia. In this regard, it is necessary to take into account that with hypothermia, the blood pressure indicator may not correspond to the actual volume of circulating blood and be significantly higher. HR in the 2nd group decreased throughout the entire cooling and did not differ statistically compared to the control group, and BR decreased immediately after the start of cooling, and after reaching Tr 280C, a further decrease in BR was comparable to the indicators in the control. According to [18], hypothermia also suppressed HR and increased MAP in hemorrhagic shock. It is assumed that a lower HR in hypothermia reduces the myocardial oxygen demand, and a higher blood pressure can improve tissue perfusion, which leads to a decrease in metabolic acidosis. The study [13] showed that mild hypothermia (330C) after experimental cardiac arrest improves cerebral microcirculatory and reduces the increased coefficient of oxygen extraction by the brain. Perhaps this provides an additional mechanism for protecting the brain during hypothermia.

Polytrauma is characterized by pathogenesis links called the "triad of death": hypothermia, azidosis and coagulopathy, and is a complex vicious circle that is observed in patients with severe trauma and hemorrhagic shock [6, 18].

The importance of body temperature correction and maintaining normothermy is emphasized in the modern protocol for the treatment of patients with polytrauma. However, most experimental studies [8, 18, 23] have shown the benefit of therapeutic hypothermia in hemorrhagic shock. It is proved that moderate hypothermia at a temperature of 33°C decreases coagulation,

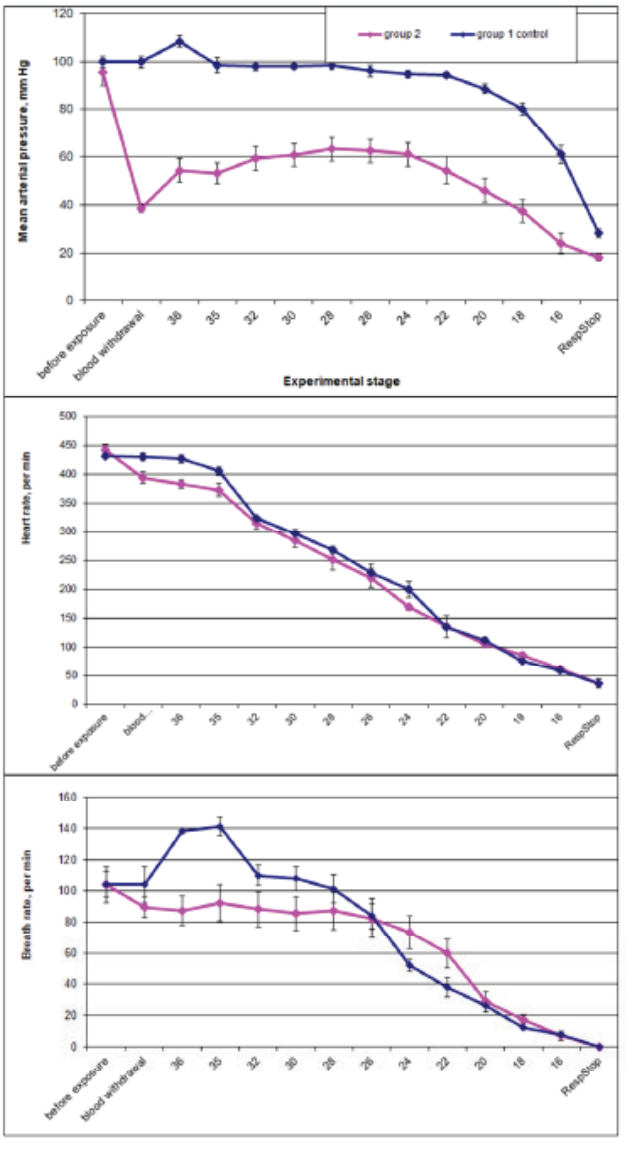

Fig. 1. Blood pressure, heart rate and breath rate in rats during cooling in water 12^{0}C

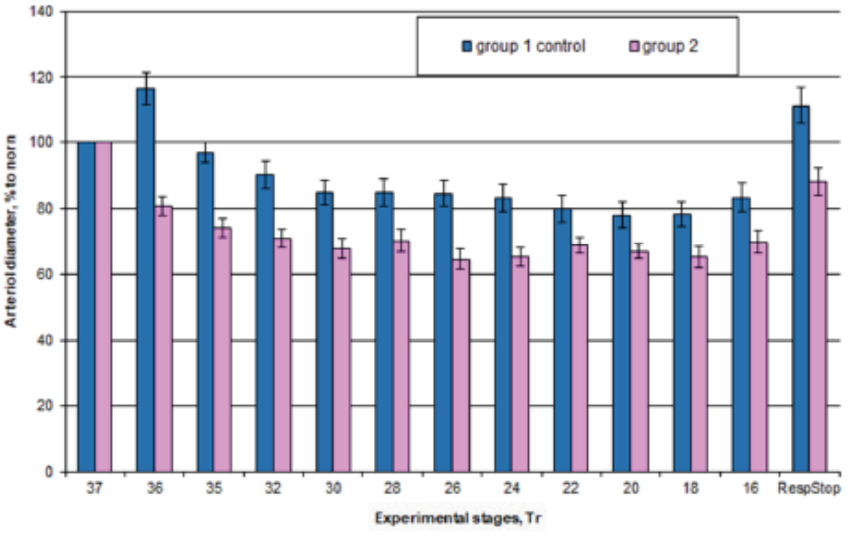

Fig. 2. Changes in the diameter of the pial arteries of rats with developing hypothermia

but, nevertheless, prolongs the survival of rats after hemorrhagic shock and resuscitation. Hypothermia reduces the consequences of secondary brain damage due to several mechanisms, including a decrease in excitotoxicity, oxidative stress, apoptosis, autophagy and inflammation [11, 20]. It was shown [23] that after cooling to Tr 340C for 2 hours after hemorrhagic shock (blood withdrawal of 3 ml/100 g of the animal and subsequent maintenance of blood pressure of 40 mm Hg), the survival of rats improves compared to the same cooling of animals for 12 hours.

Figure 2 shows the change in the diameter of the pial arteries at various stages of the experiment. For 100% (norm) we took the diameter of the cerebral vessels before the start of the effects (in the 1st group-before the immersion cooling, in the 2nd – before blood withdrawal and immersion). In both series of the experiment, about 4-5 minutes passed from the beginning of the animal's immersion in water to reaching the Tr mark of 360C. During this time, the diameter of the vessels in the control significantly increased (by 16.5±4.8%), whereas in the 2nd group, vasoconstriction was observed after blood withdrawal. The diameter of the arteries in group 2 after blood collection was 86.9±3.1% of the norm, and after the start of cooling – 81.0±2.8%. Throughout the experiment, significant statistical differences in the diameter of the arteries in rats between the groups were observed.

It was found that in the control group, after initial vasodilation at a rectal temperature of 360C, subsequent vasoconstriction was observed at a temperature of 350C. At the area of temperature decrease from 30 to 200C, the diameter of the vessels did not significantly change and ranged from 80 to 85% of the norm. In the same temperature range in the group with preliminary blood loss, the diameter of the arterioles ranged from 71 to 65% of the norm. Respiratory arrest is characterized by vasodilation, which is almost comparable to the state of the vessels at the beginning of cooling for both the 1st and 2nd groups.

Conclusion. The study showed changes in vital indicators of the body, such as heart rate, blood pressure and breath rate, during cooling in water 12-130C up to complete hypothermic respiratory arrest. Pre-induced hemorrhage in rats (up to 35% of the circulating blood volume) made worse these physiological parameters to some extent, leading to an increase in the temperature threshold for respiratory arrest. Disorders of cerebral blood flow during hypothermic exposure were assessed by the diameter of the pial vessels change of the rat brain. In hypothermia, after the initial vasodilation by 16%, vasoconstriction by 10-20% followed at the beginning of cooling. When modeling hemorrhagic shock, vasoconstriction during normothermia was 20% and increased during immersion of the animal in water to 35% of the initial state of the vessels. Nevertheless, the functional state of the animal during blood loss and subsequent cooling slightly differed from the normovolemic state of the body under hypothermic exposure.

Thus, the conducted experimental work showed that additional cooling of the body does not make the state of cerebral microcirculation worse in severe blood loss in rats. Hemorrhage with subsequent strong cooling does not lead to significantly greater disorders of cerebral blood flow than in conditions of hypothermia alone. It can be assumed that in extreme conditions, there is no need to focus on warming the body in order to provide emergency care for hemorrhagic shock, since all indicators function at a sufficient level even in conditions of deep hypothermia.

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EVALUATION OF THE MUTAGENIC PROPERTIES OF THE FUROCOUMARIN EXTRACT FROM THE CELL CULTURE OF *CONIUM MACULATUM L.*

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The study presents data on the genotoxicity study of standardized by the amount and ratio of furocoumarins extract from the cell culture of Conium maculatum. The extract containing furocoumarins in the ratio: isopimpinellin - 42.97%, bergapten - 35.18% and xanthotoxin - 15.41%, in experiments demonstrated pronounced antithrombotic, myelo- and hepatoprotective effects under conditions of chemotherapeutic aggression - the introduction of the maximum tolerated dose of cisplatin. The extract is positioned as a promising herbal remedy for the relief of chemotherapeutic complications. The need to assess the genotoxicity of the presented composition and ratio of furocoumarins is primarily determined by ambiguous information on their effect on the genetic apparatus in various test systems. In vivo, the effect of a single and course intragastric administration of a standardized extract of a cell culture of Conium maculatum containing the amount of furocoumarins (isopimpinellin - 42.97%, bergapten - 35.18% and xanthotoxin - 15.41%), at doses of 30 and 150 mg / kg was studied. A cytogenetic analysis of the metaphase plates of the bone marrow of male and female CBA mice was carried out taking into account the number of damaged metaphases, the number of aberrant chromosomes, single fragments of chromosomes and polyploid cells in % per 100 cells. A 1% starch suspension was used as a negative control. Prior to the studies on D. Melanogaster, the dose of the investigated furocoumarin extract was determined by the survival rate of P1 females (wild type), which, at the maximum dose used, should not be less than 50%. The mutagenic activity of the extract was studied by somatic recombination (mosaicism) in D. melanogaster using marker mutations yellow and singed on 1000 females at a dose of 150 mg / kg. Thus, it was determined that the use of a Conium maculatum cell culture extract containing furocoumarins in the ratio: isopimpinellin - 42.97%, bergapten - 35.18%, and xanthotoxin - 15.41%, does not induce genetic damage in CBA mice and D. melanogaster, which is one of the objective criteria for the safety of its use. The results obtained determine the possible prospects for continuing research in terms of developing a new herbal medicinal product.

Keywords: chromosome aberrations, recombination, genotoxicity, furocoumarins.

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A wide range of pharmacological effects of pure furocoumarins and their combinations (antioxidant, anti-inflammatory, antiproliferative, gonadotropic and choleretic effects, the ability to modulate various biochemical pathways, use in dermatology, etc.), draws attention to this group of substances as a potential herbal medicines [7 , 11, 12, 13, 14]. At the same time, their chemical diversity, and more than 50 natural furocoumarins (FC) and a significant number of their combinations are known, when obtained from plant raw materials, complicates the search for the target efficiency of substances [8, 10]. In this regard, it seems promising to obtain furocoumarins standardized in terms of quantity and ratio from cell cultures of *Conium maculatum* (hemlock spotted). It is from the extract from *Conium maculatum* cell cultures containing furocoumarins (isopimpinellin - 42.97%, bergapten - 35.18% and xanthotoxin - 15.41%) that data on pronounced antithrombotic, myelo- and hepatoprotective effects have been obtained, which determines the prospects for its use in conditions of chemotherapeutic aggression [5]. At the same time, it is known that, depending on the chemical structure of the FC molecule, on the number and nature of substituent radicals in the compound, on the arrangement of cyclic systems (angular or linear - the bond of the furan ring with coumarin), as well as on the combinations and concentrations, a number of FCs have genotoxic effects [1, 6, 10, 15]. However, information on genotoxicity is not of a systemic nature, since the results of studies conducted *in vivo* or *in vitro* vary depending on the test systems used, doses and duration of use [7, 15]. The study of the genotoxicity of compounds, including those of plant origin, is the most important preventive measure to identify substances potentially dangerous to humans and their heredity [1].

Based on this, the aim of this work was to study the genotoxicity of the hemlock spotted cell culture extract in vivo in the bone marrow cells of CBA mice and somatic cells of *D. melanogaster*.