Journal of Stress Physiology & Biochemistry, Vol. 16, No. 3, 2020, pp. 126-131 ISSN 1997-0838

ORIGINAL ARTICLE

Cerebral Microcirculation during Respiratory Arrest in Deep Experimental Rat Hypothermia

Nadezhda N. Melnikova

[1] *Laboratory of Respiration Physiology, I.P.Pavlov Institute of Physiology of the RAS; 199034, St, Petersburg, Russia*

*E-Mail: ***melnn@mail.ru***

Received July 5, 2020

We investigated the effect of breath stimulation on cerebral microcirculation at deep hypothermic condition of Wistar rats using the method for measuring microcirculation in real time and optical system of LUMAM-1 microscope. After a hypothermic respiratory arrest in the group without the usage of mechanical ventilation, the blood flow velocity decreases sharply and completely stops after 10 minutes. In the group with the usage of mechanical ventilation, the blood flow velocity is increased compared to the rate when breathing stops and for a long time stays at an elevated level. We have found that mechanical lung ventilation significantly improved cerebral blood flow and prolonged heart function.

Key words: cerebral microcirculation, deep hypothermia, mechanical lung ventilation, respiration arrest, rats

Deep hypothermia is a life-threatening condition for the homeothermic organism. Accidental hypothermia is an involuntary reduction in the core body temperature to <35 ℃ and is classified as mild (32-35℃), moderate (28-32℃), and severe or deep hypothermia (<28℃) (Alyabyev, 2008; Murakami, 2019; Romanovsky, 2018). With prolongation of deep hypothermia, the risk of death increases. In moderate to severe hypothermia, a high mortality rate of 12% to 80% is observed (Martin, 2005). The mechanisms leading to death with deep hypothermia are diverse and still not quite clear. Immersion hypothermia, which develops when the body is completely or partially immersed in cold water, is one of the most dangerous types of hypothermia, in which there is a rapid decrease of body temperature and the disorder of the vital organism functions, till the high heat capacity of the water speeds significantly up the process of heat transfer.

The clinical manifestations of hypothermia vary depending on body temperature. At ≤35℃, tremors, tachycardia, tachypnea, and apathy can occur (Frink, 2012; Gong, 2015). At ≤ 32℃, tremors can disappear, and bradycardia, slow breathing, which leads to carbon dioxide retention and acidosis (Datta, 2006) and confusions can appear. At ≤28 ℃, a decrease in blood pressure, apnea and coma can be observed. Patients with severe hypothermia have an increased risk of lethal arrhythmias and experience multiple abnormal electrocardiogram (ECG) results, including atrial fibrillation, elongated QT intervals, T-wave inversion, and Osborne waves (Murakami, 2019).

In conditions of developing hypothermia, there is a progressive decrease in pulmonary ventilation and oxygen consumption. As the body temperature decreases, the affinity of hemoglobin with oxygen increases, blood viscosity increases, the pumping function of the heart and pulmonary ventilation decrease, and the activity of the respiratory centers is inhibited (Konnov, 2015). Achieving a deep degree of hypothermia in rats is accompanied by hyperaggregation and thrombinemia with hypocoagulation (Lycheva, 2017). Deep hypothermia suppresses the vital functions of the body, which ultimately becomes incompatible with life. So, the electroencephalogram in rats becomes isoelectric at a rectal temperature of about 20℃ (Westover, 2015), the heart stops beating at a temperature less than 15℃, and breathing stops at 16-19℃ (Ivanov, 2016; Melnikova, 2016). In the experiments on rats in the mild hypothermia zone, there are practically no reactions of brain microcirculation (Lutsenko, 2008); microvessels of all types, both arterioles and pre- and post-capillary venules, do not change diameter. The author considers that is due to the presence of compensatory mechanisms in the brain that prevent the development of ischemic disorders. With deep hypothermia in pigs, it was shown (Gaasch, 2019) that cerebrovascular reactivity was impaired only with a deep degree of hypothermia, and cerebral autoregulation was lost only at temperatures below 18℃ (Ehrlich, 2002).

The neuroprotective properties of hypothermia, which reduces ischemic brain damage after acute cerebral strokes, are well known now. Experimental data and clinical experience show that induced moderate hypothermia affects metabolism, molecular and cellular mechanisms, contributing to tissue conservation (Frink, 2012). In recent years, there has been a tendency to achieve adequate protection of the brain or spinal cord during surgical procedures, including complete cardiac arrest, the use of deep hypothermia (Westover, 2015), despite the fact that it has significant complications, including an increased risk of bleeding, coagulopathy and infection (Niquet, 2015). Although the optimal temperature and cooling and heating rate remain the subject of active research, some experts recommend focusing on temperatures in the range of deep hypothermia (Huber, 2019).

Hypothermic exposure is accompanied by the development of a response from all organs and systems. However, the cardiovascular system is the key link that ensures the adequate functioning of the body under hypothermia.

The aim of research was to study cerebral blood flow and heart rate while spontaneous respiration stopped under conditions of immersion hypothermia and after transferring the animal to mechanical lung ventilation.

MATERIALS AND METHODS

The study was performed on anesthetized (intravenous, urethane 1250 mg/kg) Wistar rats weighing 280-300 g. Animals were from the Collection of mammals laboratory of different taxonomic affiliations of I.P. Pavlov Institute RAS.

During hypothermia the animal was fixed in a special cage so that the head was above the surface in 8-10^{0}C water. In the control (group I, n = 6), respiratory arrest of the animal was fixed. In the group II (n = 8) after hypothermal respiratory arrest and complete cessation of respiratory movements for 90 s, a mechanical ventilation (MV) for small rodents was used. Respiratory rate was 13 breaths per minute, the inspiratory volume was 1 ml.

To study the microhemocirculation of the brain in the parietal region, a trepanation window of about 1 cm^2 was made, the dura mater was removed, the pial vasculature was observed by vital microscopy using a LUMAM-1 microscope and a TS-6020 PSC color video camera. At the same time, respiratory rate, heart rate (HR) were recorded with the help of the L-791 analog-to-digital converter (L-Card), rectal temperature was recorded by copper-constantan thermocouples.

Blood flow velocity in pial microvessels was measured and analyzed with hypothermic respiratory arrest and MV. In each individual vessel, blood flow velocity was calculated at a 10-fold slowdown in the video sequence using the Pinacle Studio 15 software package. A total of 150 microvenules with a diameter of 12 to 40 μm were examined, and the blood flow velocity in them was measured at the moment of respiratory arrest, after 1, 3, 10, 30 and 60 min, and with almost complete stoppage of blood flow in the venules.

The use of rats in experiments was carried out in accordance with the European Convention for the Protection of Vertebrate Animals and Directives 86/609 / EEC.

Statistical analysis was performed using the nonparametric Wilcoxon test for the associated variants using the Statistica 6.0 software. The critical level of significance in testing statistical hypotheses was equal to 0.05. All experimental data are presented as mean error ± mean (M ± m).

RESULTS AND DISCUSSION

Cooling animals in water of 8-10°C led to a gradual decrease in body and brain temperature, a reduction in respiratory rate and heart rate. In the deep stage of hypothermia in animals, cold paralysis of the respiratory center occurs, which leads to respiratory arrest with a working heart.

Prior to rats cooling the mean arterial pressure was averaged 106.7 ± 6.1 mm Hg. During the immersion the arterial pressure was kept at rather high level practically till the terminal period and only at the lowing to Trect 20^{0}C startes to be reduce. At the moment of the breath arrest mean arterial pressure reached the value 55.8 ± 5.2 mm Hg then sharply fell.

Upon rats cooling the heart rate decreased almost in 10 times: from 392±18 beats per minute to 26±5 beats/min at control and from 398±15 beats/min to 36±8 beats/min at the 2d group. The respiratory arrest occurred 90 min after the start of cooling, while the brain temperature is 21.8 ± 0.7^{0}C and rectum temperature is 19.4 ± 0.5^{0}C or 19.36 ± 1.0^{0}C at control (p> 0.05).

After the animal stopped breathing, an interval of 90 s at which there were no respiratory movements kept, after that MV was connected. It has been found that MV activates the heart from the first minute. After 1 min after MV using, the heart rate increased by 21% (p <0.05), and after 3 min - by 76% (p <0.001) of the parameter with breathing stopped (Tabl. 1). This level of heart rate is kept during further cooling of the rat for more than 20 minutes, after which it begins to decrease slightly. Therefore, for the 30th minute, the heart rate was 48±4 beats/min, for 60 min - 40±2 beats/min. A rapid drop in heart rate occurs 5-8 minutes before the blood flow stops, at a blood flow velocity of 11.7±1.3 μm/s, the heart rate was 19 ± 3 beats/min.

The transfer of animals to mechanical lung ventilation after stopping their own breathing helps to restore heart rate and cerebral blood flow (Fig. 1). After MV using the appearance of rats own respiratory movements was observed. In most of the studied animals, 1 to 11 spontaneous breaths were added, but this process was observed only in the first minutes of connecting the apparatus. Probably, the increased blood circulation of the brain with an increasing in heart rate

for some time activated the work of neurons of the respiratory center. However, after 10-15 minutes, the brain temperature dropped to the limit, when there is complete paralysis of the respiratory center in the brain, its own breathing stopped.

It is important to note that under using MV, rats continued to cool in water. After 30 minutes, the temperature threshold for stopping blood flow in the cerebral vessels was lower than the temperature threshold for respiratory arrest in the rectum by 6-8°C, and in the brain by 5°C.

The temperature threshold for respiratory arrest is quite stable. According to various authors (Ivanov, 2016; Romanovsky, 2018; Murakami, 2019), breathing stops in adult rats at a rectal temperature in the range 13-18^{0}C, T brain – 18-20^{0}C. Spontaneous restoration of breathing in conditions of deep hypothermia is possible only if the animal is removed from the water and heated. In experiments on neonatal rats (Tattersall G.J., 2003), it was shown that during cooling, respiration ceased at a Trect below 10.7 ± 0.24^{0}C, and was restored spontaneously when the Trect reached 13.3 ± 0.38^{0}C. During cooling, the respiratory rate gradually decreased, while the tidal volume increased until the Trect dropped below 15^{0}C, after which it decreased, but not lower than the normothermic level. The authors suggest that failure occurs at the level of the central rhythm generator for breathing and is not due to an inability to sustain the level of motor output.

During respiratory arrest, the linear blood flow velocity in the venules in both groups did not practically differ and amounted to ~ 100 μm/s. In the control, the velocity decreased significantly after 3 minutes, and at 10-15 minutes the blood flow stopped. Figure 2 shows graphs with changes in blood flow velocity during respiratory arrest, using MV until blood flow is completely stopped, and in control, without using MV.

In the second group, at the time of hypothermal respiratory arrest, the blood flow rate was 99.9 ± 2.94 μm/s, the same speed remained at the 1st minute after MV using, but already from the 3rd minute it increased by 18.4% ($p < 0.05$) and for a long time remained at an elevated level. In some experimental animals, the increase in blood flow rate was up to 30%. Half an hour after MV, the blood flow velocity somewhat decreased from the level obtained when the respiratory arrest was stopped. A sharp decrease in blood flow velocity correlated with a reduction and mismatch in heart function.

Mechanical ventilation of the lungs after cold respiratory paralysis ensures long-term maintenance of cerebral blood flow with deep hypothermia due to activation of the heart, when the oxygen supply is restored, it is possible to maintain cerebral blood flow for a long time in values close to those before breathing stopped.

Table 1. Physiological parameters of rat in norm, with hypothermic respiratory arrest and after mechanical ventilation (MV)

Parameters	Experiment stages	Before cooling	Respiratory arrest	After respiratory arrest		
				1 min	3 min	10 min
Rectal temperature, ^{0}C	Control	36,4±0,64	19,36±1,0	18,7±1,3	18,4±1,2	18,2±1,1
	MV	34,0±0,3	19,4±0,54	19,1±0,78	18,9±0,79	18,0±0,9
Breathing rate, breaths/min	control	102±8,8	0	0	0	0
	MV	124±7,0	0	17,3±1,5	16,1±1,8	14,3±0,5
Heart rate, beats/min	control	392±18,4	26±5,0	23,3±3,2	18,3±3,1	13,7±1,6
	MV	398±15,1	36,3±8,3	43,8±6,6	64,0±8,8	64,4±7,1

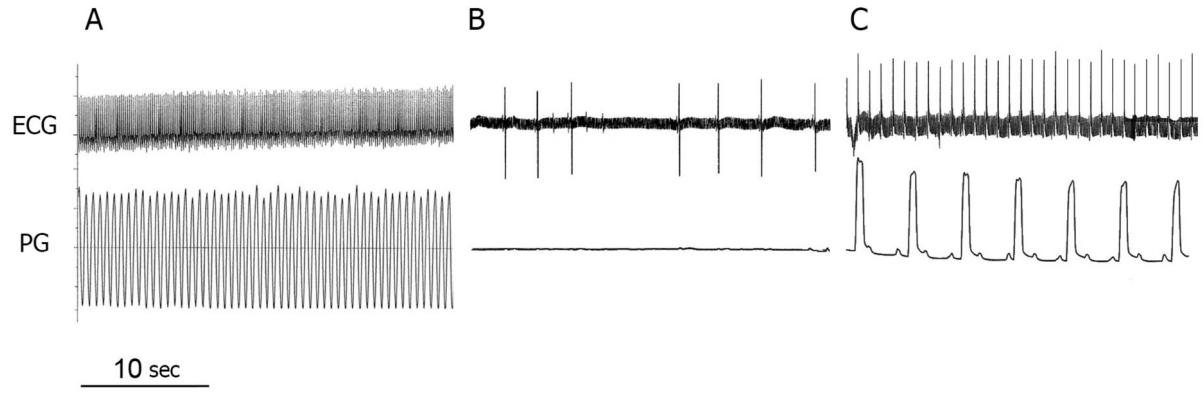

Figure 1. Fragments of the recording of an electrocardiogram (ECG) and pneumogram (PG) in a rat during cooling in water (A), at the moment of cold respiratory arrest (B), and for 10 minutes after using of the mechanical ventilation (C)

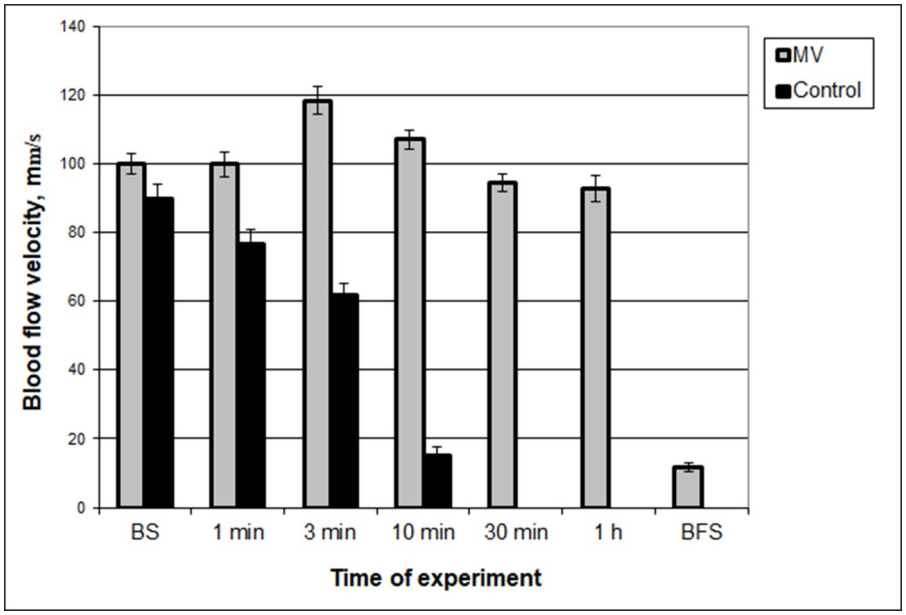

Figure 2. Comparison of changes in the linear blood flow velocity in the pial venules of rats after hypothermic respiratory arrest in experiments with mechanical lung ventilation and without it (control).
MV – mechanical ventilation; BS – breathing stop; BFS – blood flow stop

CONCLUSION

Thus, the use of MV has significantly prolonged heart function (by 1.5-2 hours) and for a long time to maintain cerebral blood flow. With deep hypothermia, causing the cessation of their own breathing, the use of mechanical ventilation significantly increases the time of effective work of the heart, which ensures the maintenance of cerebral blood flow.

ACKNOWLEDGEMENTS

This work was supported by the Program of Fundamental Scientific Research of State Academies for 2013-2020. (GP-14, section 65).

REFERENCES

Alyabyev F.V., Parfiryeva A.M., Chesalov N,P, Shamarin Yu.A., Osipov A.I. (2008) Functional-morphologic changes of the heart in hypothermia. *The Siberian Med J* 23 (1), 68-71.

Datta A., Tipton M. (2006) Respiratory responses to cold water immersion: neural pathways, interactions, and clinical consequences awake and asleep. *J Appl Physiol* 100, 2057-2064.

Erlich M.P., McCullough J.N., Zhang N. (2002) Effect of

hypothermia on cerebral blood flow and metabolism in the pig. *Ann Thorac Surg* 73(1), 191-197.

Frink M., Flohe S., van Griensven M., Mommsen Ph., Hildebrant F. (2012) Facts and fiction: the impact of hypothermia on molecular mechanisms following major challenge. *Mediators of Infammation*. 2012: ID 762840

Gaasch M., Putzer G., Schiefecfer A.J., Martini J., Strapazzon G., Ianosi B., Thome C., Paal P., Brugger H., Mair. P., Helbok R. (2020) Cerebral autoregulation in impaired during deep hypothermia - a porcine multimadal neuromonitoring study. *Ther Hypothermia Temp Manag.* 10(2):122-127.

Gong P., Zhao Sh., Wang J., Yang Zh., Qian J., Wu X., Cahoon J., Tang.W. (2015) Mild hypothermia preserves cerebral cortex microcirculation after resuscitation in a rat model of cardiac arrest. *Resuscitation* 97, 109-114.

Huber Ch., Huber M., Ding Yu. (2019) Evidence and opportunities of hypothermia in acute ischemic stroke: clinical trials of systemic versus selective hypothermia. *Brain Circ* 5(4), 195-202.

Ivanov K.P., Arokina N.K. (2016) Maintenance of the cardiovascular function in a deeply cooled homeothermic organism by physiological methods without external rewarming. *Bull Exp Biol Med*. 160(4): 407-409.

Konnov D.Yu., Rjnnjva T.Yu., Lukyanov S.A., Shapovalov K.G. (2015) Changes on heart phythm and breathing in acute system injury due to cold. *Gen. Reanimatology* 11(3), 16-23.

Lutsenko D.G. (2008) Rat`s brain microcirculation after hypothermic effect. *Problems of cryobiol*. 18 (1), 81-84.

Lycheva N.A., Shakhmatov I.I., Sedov A.V., Makushkina D.A., Vdovin V.M. (2019) Condition of microcirculatory and hemostasis systems in rats after moderate hypothermia. *I.P.Pavlov Russ Med Biol. Herald* 27(2), 160-171.

Martin R.Sh., Kilgo P.D., Miller P.R.(2005) Injury-associated Hypothermia: An Analysis of the 2004 National Trauma Data Bank. *Shock*. 24 (2): 114-118.

Melnikova N.N., Petrova L.A. (2016) Effect of hypothermia-induced respiratory arrest on cerebral circulation in rats. *Bull Exp Biol Med* 160(5), 593-595.

Murakami T., Yoshida T., Kukokochi A., Takamatsu K., Teranishi Yu, Shigeta K., Tamaki S., Morita Sh., Mizuno R., Oya M. (2019) Accidental hypothermia treated by hemodialysis in the acute phase: three case reports and a review of the literature. *Intern Med* 58, 2743-2748.

Niquet J., Gezalian M., Baldwin R., Wasterlain C.G. (2015) Neuroprotective effects of deep hypothermia in refractory status epilepticus. *Ann Clin Transl Neurol*. 2(12), 1105–1115.

Romanovsky A.A. (2018) The thermoregulation system and how it works. *Handb Clin Neurol.*;156:3-43.

Tattrsall G.J., Milson W.K. (2003) Hypothermia-induced respiratory arrest and recovery in neonatal rats. *Rerspir Physiol Neurobiol* 137(1), 29-40.

Westover B., Ching S., Kumaraswamy V.M. (2015) The Human Burst Suppression Electroencephalogram of Deep Hypothermia. *Clin Neurophysiol*. 126(10), 1901–1914.