Study the Effect of Naturally Low Temperature in Animals Experiment

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Abstract. In modern conditions of Arctic development, the problem of hypothermia of humans and animals is becoming fundamental and applicable nature. Studying deep hypothermia effect and body recovery after it on experimental models of animals is relevant for world science. The article presents some aspects of the naturally low temperatures affect (-40° C and below) on the domestic pig’s body under the conditions of Yakutia (experimental research). To perform this task, we simulated the conditions of natural deep hypothermia, in which we studied the mechanisms of entering an anabiosis state and got the data of experimental animals’ brain and heart bioelectrical activity. The experiment was conducted according to the ethical standards of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes No. 123 of 18 March 1986, Strasbourg, and the order of the Ministry of Health of the Russian Federation No. 199n of 01.04.2016 “on approval of the regulations of good laboratory practice”. Permission of the bioethical commission is available.

1. Introduction

I. M. Sechenov (Russian physiologist and educator) wrote: “Organism without an external environment supporting its existence is impossible, therefore the scientific definition of an organism should include the environment”. One factor of the external environment affecting the body is the low temperature. Russia is the coldest country in the world, because of its geographical location, and Yakutia is one of the coldest regions of Russia. In the Far North of Russia, winter lasts nine months of the year, and almost no summer. The average temperature in Oymyakon village is 50 °C [6].

Hypothermia is a state of the body or its parts in which temperature is lower than required to maintain normal metabolism and functioning. The state of hypothermia can be natural or artificial. Research in this area covers areas related to the body functioning in life-threatening conditions of heat transfer to the environment, body cooling in a state of general anesthetic, etc. It is known that strong cooling is a trauma for the body. In conditions of low temperatures in Yakutia, the issue of hypothermia remains relevant, since the mortality rate remains high [1, 2, 7].

In modern conditions of Arctic development, the problem of humans' and animals' hypothermia is becoming fundamental and applicable nature. Study the effect and regeneration of the body after deep hypothermia on experimental models is relevant [1, 2, 6].
In veterinary medicine, the hypothermia and the problem of body regeneration remain open. Over the past 15 - 20 years, many countries have conducted extensive researches and published scientific works confirming the idea of body regeneration. In modern conditions of the Arctic development, the problem of humans and animals hypothermia is becoming an application discipline [1, 2, 3, 6].

The purpose of the research is to study domestic pig brain electroencephalography (EEG) change during freezing and the hypothermia mechanism.

To perform this task, we modeled the conditions of natural deep hypothermia on the domestic pig at a temperature of -40 °C and below in Yakutia, in which we studied the mechanisms of entering an anabiosis state and got data on the brain and heart bioelectric activity.

In the research, we studied the pig’s brain electroencephalography (EEG) and cardiovascular system electrocardiography (ECG) under the deep hypothermia with the aim to detect the EEG and ECG power spectrum changes when decreasing a body temperature which can shed light on the mechanisms of brain and heart functions temperature dependence.

2. Material and methods
The research was conducted in the Faculty of Veterinary Medicine of the Yakut State Agricultural Academy. In the experiments clinically healthy pigs at the age of 2-3 months, with 15 to 20 kg body weight got from the Khatass pig-breeding-farm were used. Neuroplegia was performed at the beginning of the experiment in order to limit the motion of animals (neuroplegic - Xyla 0.2% 0.5 ml and Droperidol 0.5 ml). Then ethyl alcohol in 5-6 ml/kg dose of live weight was used to simulate the alcoholic intoxication state, and the animals were placed in outside at -40 °C... - 43 °C temperature.

The experiment was conducted according to the ethical standards of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes No. 123 of 18 March 1986, Strasbourg, and the order of the Ministry of Health of the Russian Federation No. 199n of 01.04.2016 “On approval of the regulations of good laboratory practice”. Permission of the bioethical commission are available. Recording electrodes were connected to a bioelectrical amplifier, then it was connected to a computer, and recorded on a hard disk. The EEG was registered during the experiment until the signals stopped and an isoelectric amplitude appeared. The ECG was performed using the Poly-spectrum 8/V devices.

3. Results
Meanwhile, thermometry of environment, body surface temperature, and extremities were performed. Lower leg thermometry was measured at 1 and 2.5-3 cm depth, in the hip area at 3 cm depth. Thermometry in the liver, abdomen, lungs, mediastinum, and brain (between the hemispheres).

The obtained data were compared with data of electroencephalography and electrocardiography. The EEG indicators naturally change as the body temperature decreases – the oscillation frequency, their amplitude gradually decreases and, finally, the EEG becomes almost isoelectric (flat) at + 18-20°C body temperature. The ECG decelerates sharply and it went into cardiac arrest at +20-21⁰C temperature. Meanwhile, the in esophageal temperature was +18-20°C and the temperature inside the brain, between the hemispheres was +14-15⁰C. A gradual decrease in brain temperature occurs due to the temperature of the circulating blood, until the cardiac arrest. Also, the decrease in brain temperature occurs due to the external temperature influence directly through the skull bones. Later, after 4-6 hours in the cold, the pig's in esophageal temperature was +17-18°C, the rectal temperature +16-17°C, and the intracerebral temperature 11-12°C. Apparently, the decrease in brain temperature was caused by the direct action of cold through the skull. At the same time, the ECG indications showed the gradual deceleration of atrioventricular conduction and bradycardia followed by arrhythmia and cardiac arrest. The isoelectric line occurred immediately after the cardiac attack.

Figures 2 and 3 show the pig brain EEG at different body temperatures in the "cooling-warming" cycle. B/amplitude beta diffuse activity dominates. It is seen that as far as the body temperature decreases, the EEG indicators naturally change – the oscillation frequency, their amplitude gradually decreases and, finally, the EEG becomes almost isoelectric (flat) at +18-20°C body temperature.
We performed selective antegrade cerebral perfusion technique using a heart-lung apparatus (HLA) with a perfusion rate of 8ml/min body weight with the perfusate gradual heating (maintaining a temperature gradient of less than 5 °C) in order to obtain bioelectric indicators. An aortic cannula was
inserted into an aortic root, and a venous cannula was inserted into a vena cava cranialis after sternotomy for perfusion. Heparinization was performed at a rate of 3 mg/kg. The perfusate was heated using the HLA with maintaining a temperature gradient of less than 5 °C. In experiment No.1, the perfusion was started an hour later after the cardiac attack, and in experiment No.1 after 12 hours. Against the background of perfusion and gradual heating, we did not record the brain's electrical activity. When warming an animal after the deep hypothermia, the EEG was changed insignificantly, but the reliable indicators of electrical activity recovery have not been detected.

As a result of the experiments, it can be found that the decrease in internal temperature depends on the ambient temperature. At the same time, according to the ECG indications (figures 4 and 5), there was a gradual deceleration of the atrioventricular conduction and bradycardia, followed by arrhythmia and cardiac arrest. The isoelectric line occurred immediately after the cardiac attack [4].

The experiments with animals provided an opportunity to establish a number of factors that complicate the brain's vital function regeneration. These include the prolonged exposure of naturally low temperatures and the inability to establish a time when you can "start" the reverse mechanism of brain work regeneration. It is needed a new technical search for revival [1, 2, 5, 6].

Experimental researches on this problem will be continued. The experimental materials show that the brain mechanisms in cold injury have not yet been clarified, and there are challenges ahead for further study of this problem.

4. References
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