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Journal of Advanced Scientific Research (ISSN: 0976-9595)
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BIOENERGY INDICATORS OF THE HEAD BRAIN IN HYPOTHERMIA

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Abstract: In experiments on white rats, the main energy substrates of the brain - glucose and glycogen, key enzymes and their transformations (hexokinase, amylase, phosphorylase), as well as the main ways of their utilization - respiration, glycolysis and the pentose pathway in the brain at various stages of cooling were studied. The phase structure of the restructuring of metabolic processes at the stages of hypothermia is shown, accompanied by qualitative and quantitative changes in the relationship of the main pathways.

Keywords: brain, hypothermia, glucose, glycogen, hexokinase activity, respiration, glycolysis.

Topicality: The question of metabolism during a decrease in body temperature has long attracted the attention of researchers [1, 4]. However, the issues of brain metabolism, and, in particular, energy metabolism during hypothermia, remain the least studied [3, 5]. Most of the works deal with the dynamics of oxygen consumption by the brain or the content of individual metabolites of energy metabolism [10]. The available materials on brain bioenergetics during hypothermia are highly contradictory. This is mainly due to the fact that the results of studies by different authors often cannot be subjected to comparative analysis, since the method of obtaining hypothermia was different [2, 8]. In addition, the overwhelming majority of works devoted to the study of brain metabolism during a decrease in body temperature can only to a limited extent be used to study cooling, since general physical cooling was combined with the preliminary administration of narcotic and neuroplegic substances [11, 12].

In this work, an attempt was made to highlight the relationship between the main pathways for the transformation of the main energy substrates of the brain - glucose and glycogen - at various stages of cooling of warm-blooded animals.

Materials and methods of research. Studies were carried out on white male rats weighing 170-200 g. Cooling of the body of animals was induced in a bath with cold water and ice, which dramatically change the course of metabolic processes. Observations were carried out in stages: at normal body temperature (control), with its decrease to 33-35^{0}C (stage of excitation) and with a decrease in body temperature of animals to 19-20^{0}C.

In experiments on the brain obtained after decapitation of animals in a cold room at a temperature of 0-30 ° C and immersion in ice, the following were determined: lactate - by colorimetric paraoxyphenyl, glucose - by the Biotest method, total glycogen - according to Kerr, free glycogen (easily extracted from brain tissue water or a weak solution of trichloroacetic acid) according to the method of Bloom et al. The amount of bound glycogen was judged by the difference between total and free glycogen [6, 7].

Glycolysis and enzyme activity were studied in tissue homogenates. The intensity of aerobic glycolysis was judged by the amount of lactic acid formed in an oxygen atmosphere. Anaerobic glycolysis was determined by the polarographic method (Polarograf – LP-9) using a rotating quartz platinum electrode [9]. Amylase activity (EC 3.2.1.1. α-1,4-glucan-4-glucanhydrolase) was determined according to the Biotest, hexokinase (EC 2.7.1.1.ATP: D-hexose-6-phosphotransferase) - according to Leng [13], phosphorylase (EC 2.4.1.1.1,4-glucan: orthophosphate glucosyl transase) according to Corey [14].

The state of the pentose cycle in the brain of chilled rats was judged by the activity of its oxidative enzymes: glycose-6-P-dehydrogenase (D-glucose: NADP-oxidoreductase) and 6-P-gluconate dehydrogenase (6-P-D-gluconate: NADP oxidoreductase /decarboxylating). The activity of these enzymes was measured by the

Journal of Advanced Scientific Research (ISSN: 0976-9595)
Vol.2. Issue 2 page 23
https://doi.org/10.5281/zenodo.6797537

amount of reduced NADP homogenates with the corresponding substrates. The protein content was determined according to Lowry [15].

On mitochondria isolated from the brain, respiration was studied - polarographically, phosphorylation - according to the method of V.P. Skulachev [9].

Research results. The beginning of body cooling is characterized by a very intensive and fairly rapid utilization of glucose and glycogen in the brain (Table 1).

Table 1

Carbohydrate resources and key enzymes of carbohydrate transformations in the brain during hypothermia (M ± m)

Indicators of carbohydrate-energy metabolism	Body temperature, ^{0}C		
	38	33 - 35	19 - 20
Glucose content, mg %	32,8±0,83	21,1±0,99	48,9±2,33
Glycogen concentration, mg %:			
general	75,1±2,1	60,7±1,59	98,3±3,37
free	9,1±0,65	5,1±0,49	13,1±0,8
associated	65,0±1,49	55,7±1,68	83,4±2,57
Hexokinase activity, µg of phosphorylated glucose per 1 hour (mmol/g.h)	279,1±14,7	361,5±16,9	360,8±14,9
Phosphorylase activity (µmol/g.h)	6,2±0,9	10,1±0,7	5,4±0,5
Amylase activity, mg of hydrolyzed starch per 1 mg of tissue in 1 hour	1,04±0,04	1,07±0,04	0,75±0,05

Note: Reliability vs Control (38^0): * - p<0,05,
** - p<0,01, *** - p<0,001.

The amount of the main energy substrates in the brain at a body temperature of 33-350C decreased by 38 and 20%, respectively. On the contrary, in the future, when the temperature continued to progressively fall, reaching 19-200C, and deep inhibition developed in the central nervous system, the content of glucose and glycogen increased significantly, amounting to 142 and 133%. It is interesting to note

that at the stages of cooling, the most accessible and labile form of glycogen, free glycogen, is primarily involved in the exchange.

Thus, in the brain of rats during hypothermia, the phase dynamics of the dynamics of the energy substrates of the brain - glucose and glycogen - emerges. At the beginning of cooling, in the stage of excitation, their level decreases, and with deep cooling - an increase.

The decrease in the glucose content in the brain, observed at a temperature of 33-350C, and its accumulation during deep hypothermia occurs against the background of an increased content of glucose in the blood, which is apparently the result of the mobilization of liver glycogen, the amount of which in it is significantly reduced. During various periods of a decrease in body temperature, the brain is able to vigorously retain glucose during hypothermia, so it can be assumed that the change in the amount of glucose during the stages of cooling is the result of processes developing in the central nervous system under the influence of low temperatures.

The fact of accumulation of carbohydrate resources in the brain under conditions of deep hypothermia is of particular interest. On the one hand, the accumulation of carbohydrates in the nervous tissue during this period is ensured by reducing their use along the Embden-Meyerhof pathway and in the reactions of the hexose monophosphate pathway. On the other hand, the possibility of enhancing the resynthesis of these compounds from other substances in the reactions of gluconeogenesis cannot be ruled out, especially since many researchers believe that during inhibition in the brain, the resynthesis of carbohydrates prevails over their breakdown. A direct confirmation of this is the retention of high activity of phosphatases under conditions of deep supercooling. Phosphatases (diphosphofructose phosphatase and glucose-6-phosphatase), together with phosphopyruvate carboxylase, provide endergonic pathways for the reversal of glycolysis, being the key enzymes of gluconeogenesis. Obviously, the excessive accumulation of carbohydrate reserves during this period provides a significant

increase in heat production during the period of body temperature increase during self-warming of the animal.

Enzymes involved in the mobilization of carbohydrates in the brain play the most important role in the described dynamics. Such enzymes for glucose are hexokinase, and for glycogen - amylase and phosphorylase.

The activity of hexokinase in the brain of chilled animals differs significantly from the activity of controls. And at 33-350C, and at 19-200C, the activity of hexokinase in the brain increases significantly. It is noteworthy that high activity of hexokinase remains in the stage of "cold anesthesia", when a significant accumulation of glucose occurs in the brain. Consequently, during deep hypothermia in the brain, there is no inhibition of trigger mechanisms for the use of glucose, on the contrary, the potential capabilities of the cells of the central nervous system in relation to glucose utilization remain high. At the same time, in other organs at the same body temperature, hexokinase activity is inhibited.

During hypothermia, different relationships of the main pathways of glycogen mobilization are created in the brain. A decrease in body temperature to 33-350C leads to a sharp activation of phosphorylase, while the activity of amylase does not undergo significant changes. A further decrease in body temperature to the stage of inhibition leads to inhibition of the activity of both enzymes, however, the activity of amylase is inhibited to a greater extent than the activity of phosphorylase, which indicates the inhibition of the amylolytic pathway of glycogen breakdown to a greater extent than the phosphorolytic one, and leading to the accumulation of glycogen in the brain tissues in period of deep hypothermia.

As is known, the main pathways for further utilization of carbohydrates are glycolysis, respiration, and the pentose phosphate pathway. Data on their state in the brain of chilled animals are presented in Table 2.

Table 2.

Respiration, glycolysis and the pentose phosphate pathway in the brain during hypothermia (M±m)

Journal of Advanced Scientific Research (ISSN: 0976-9595)
Vol.2. Issue 2 page 26
https://doi.org/10.5281/zenodo.6797537

Way of utilization of carbohydrates	Index	Body temperature, ^{0}C		
		38	33-35	19-20
glycolysis	Lactate content, µmol	3,65±0,085	5,03±0,08	1,64±0,11
	Anaerobic glycolysis (lactate formation), µmol/g/h	72,65±1,54	92,67±1,88	53,4±1,29
	Oxygen uptake by mitochondria, µA/min.	4,27±0,225	3,91±0,152	2,4±0,15
Breath	NF consumption, µA/min	7,59±0,44	4,96±0,34	5,83±0,35
	R/O	1,79±0,069	1,27±0,077	2,44±0,099
	Glucose-6-phosphate dehydrogenase activity, µmol NADP • H2 (µmol/g.h)	1,03±0,022	1,01±0,028	9,69±0,034
Pentose cycle	6-F-gluconate dehydrogenase activity, micromoles NADP • H2 (µmol/g.h)	0,62±0,025	0,65±0,025	0,47±0,03

At the first stage of cooling, both anaerobic and aerobic glycolysis are activated in the brain, which leads to an increase in the content of preformed lactic acid in the brain. The uptake of oxygen by the brain mitochondria at this stage of cooling remains unchanged, while the esterification of inorganic phosphate drops sharply, indicating the uncoupling of oxidation and phosphorylation.

The energy of oxidation, which cannot be accumulated and cannot be captured by macroergic phosphate bonds, is now used as the most important source of animal warmth. Uncoupled respiration cannot successfully compete with glycolysis for ADP and FN during this period. In such an emergency, the body forms high-energy phosphates due to the more ancient mechanism of energy production - glycolysis, which ensures that the brain cells at this moment maintain their functions. As for the pentose pathway of transformation, the activity of its oxidative enzymes at 33-350C remains unchanged.

In the future, when, despite emergency measures, the body temperature continues to fall and the stage of excitation is replaced by the stage of "cold anesthesia", the main pathways for the utilization of carbohydrates - respiration, glycolysis and the pentose pathway - are inhibited in the brain. However, the hexose monophosphate pathway and glycolysis are suppressed in the brain to a much lesser extent than respiration.

It can be thought that suppression of the pentose and glycolytic pathways of carbohydrate utilization to a lesser extent than respiration should be considered, apparently, as the mobilization of pathways that have lost their essential role for some brain structures, but the potential capabilities that turn out to be sufficient to maintain homeostasis and compensate for the disturbed energy exchange. In this regard, of known interest are the statements of those authors who believe that with deep hypothermia, a different level of metabolism and functional activity of the nervous tissue is established, characterized as an embryonic type.

Conclusions: Each stage of cooling is distinguished by certain quantitative and qualitative relationships between the main pathways for the transformation of the energy substrates of the brain. In the brain of rats during hypothermia, the phase dynamics of the dynamics of the energy substrates of the brain - glucose and glycogen - emerges. At the beginning of cooling, in the stage of excitation, their level decreases, and with deep cooling - an increase.

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