EFFECT OF DEEP HYPOTHERMIA ON CARBOHYDRATE AND PHOSPHORUS METABOLISM IN BRAIN TISSUE

G. A. Nechaeva

Laboratory of Biochemistry of the Nervous System (Head – Prof. G. E. Vladimirov), I. P. Pavlov Institute of Physiology (Director – Academician K. M. Bykov), Academy of Sciences, USSR, Leningrad

(Presented by Academician K. M. Bykov)

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In a number of investigations [6, etc.] it has been shown that when the body temperature of warm-blooded animals is artificially lowered, the rate of oxidative processes, both in the whole organism and in the tissues of the brain, is distinctly reduced. Despite this fact, the concentration of such energy-yielding substances as adenosinetriphosphate (ATP) remains at the normal level; the balance of oxidative and glycolytic processes also is not disturbed [1 – 4, etc.]. In lowering the body temperature of animals, the authors mentioned did not, as a rule, study the effect of temperatures below 20°, since the animals quickly died from cardiac or respiratory arrest when body temperature was further reduced.

Our purpose in the present study was to ascertain whether disturbances occur in the balance of oxidative and glycolytic processes and in the maintenance of a high level of energy-yielding substances, when the level of body temperature is reduced still further than in the works mentioned above. Such studies are of interest in view of the fact that a special method of warming has been described which permits animals to recover even after their body temperature has been reduced almost to 0°, with cessation of respiration and cardiac activity [5].

METHOD

Cats were used as experimental animals. Efforts to carry out cooling of animals as described by O. N. Savchenko [4] in experiments on rabbits (injection of 70 mg/kg of amobarbital sodium, and placing in ice water) were unsuccessful, since respiration and cardiac activity stopped when the body temperature was lowered to approximately 20°.

The following method of achieving deep hypothermia in cats was more successful. The cat was placed in a 16-liter glass vessel, surrounded by ice. A piece of cotton moistened with ether was placed on the cat's nose. As soon as the animal fell asleep, the ether-filled cotton was removed and the vessel was covered with a lid. Carbon dioxide exhaled by the cat into the closed vessel exerted a narcotic effect, cutting off the activity of the temperature-regulating centers in the first period of hypothermia. During the cooling process, the animal was taken out from time to time for measurement of temperature, and was also watched for respiration and cardiac activity (as indicated by the pulse). During the first hour, the body temperature fell 4°, on the average. After three or four hours, more rapid cooling was begun, by surrounding the cat with ice-filled bags in an open vessel. It was thus possible to reduce the body temperature to 14° while maintaining respiration and heartbeat.

When cooling was complete (five to six hours after the beginning of the experiment), the cat's head was frozen by immersion in liquid oxygen; the animal was decapitated, and the brain was removed and ground in a mortar. The brain proteins were precipitated with trichloroacetic acid (2 ml of 10% CCl_3COOH and 2 ml of water were added to 1 gm of tissue), and the protein-free filtrate was analyzed for inorganic phosphorus (by the method of Fiske and Subbarow), ATP labile phosphate (as readily hydrolyzable phosphorus in a mercury precipitate), phosphocreatine (Alekseeva's method), and lactic acid (method of Friedemann, Cotonio, and Shaffer), with precipitation of proteins with zinc hydroxide (Vladimirov and Urinson's method) and precipitation of carbohydrates with a copper sulfate-calcium hydroxide mixture.

RESULTS

To have the necessary data for a comparison under the conditions used for freezing the brain, we performed the first group of experiments on animals with normal body temperature (Table 1).

The results obtained are marked by a small scatter around the mean, which enables us to conclude that the methods used for freezing the brain and preparing brain tissue for analysis are satisfactory. The results of experiments on animals with reduced temperature are presented in Table 2.

Comparison of the mean values in Tables 1 and 2 shows that they are very close to each other. Thus, when the body temperature of the experimental animals is reduced more than 20°, the level of all the constituents investigated is the same as it is if the temperature is normal.

TABLE 1. Content of Inorganic Phosphorus, ATP Labile Phosphorus, Phosphocreatine Phosphorus, and Lactic Acid in Brain Tissue of Cats with Normal Temperature

No.	Phosphorus (mg %)			Lactic acid (mg %)
	inorganic	ATP	phosphocreatine	
1	16	16,2	8	30
2	11	12,5	9	32
3	13	11,7	7	29
4	14	12,4	7	33
5	13,7	12,5	7,6	29
Mean	13.5	13	7.7	31

In several instances, in spite of the precautionary measures taken, cardiac arrest occurred. When this fact was discovered, the animal was immediately frozen. The period of time between the moment of cardiac arrest and freezing apparently did not exceed 2 - 4 minutes; nevertheless, marked changes were observed in all the investigated components in such cases, particularly in the concentrations of phosphocreatine and lactic acid (Table 3).

TABLE 2. Content of Inorganic Phosphorus, ATP Labile Phosphorus, Phosphocreatine Phosphorus, and Lactic Acid in Brain Tissue of Cats with Reduced Temperature

No.	Body temperature	Phosphorus (mg %)			Lactic acid (mg %)
		inorganic	ATP	phosphocreatine	
1	24°	25	10,6	4,8	27
2	21°	13	11	6,8	28
3	16°	16	11,5	9	—
4	14°	12,2	12,7	6,6	27,3
5	14°	12	11,4	7	24
6	15°	12,5	11,7	7,5	26
Mean	—	15,1	11,5	7	26,4

In the literature one encounters the assumption that hypothermia is accompanied by brain hypoxia, even when cardiac activity and respiration are maintained. O. N. Savchenko [4] showed that there is no hypoxia of the brain in rabbits when the body temperature is lowered to 22 - 20°. In our experiments on cats at lower temperatures, in every case in which respiration and cardiac activity were maintained, no signs of hypoxia were observed; the level of such energy-yielding substances as ATP and phosphocreatine was not reduced, and breakdown of phosphorus compounds and accumulation of lactic acid did not occur. But as soon as the heartbeat stopped, all the measured indices of carbohydrate and phosphorus metabolism showed a markedly hypoxic condition during the first two to four minutes: The phosphocreatine concentration fell to zero in every case, without exception; the ATP concentration dropped 50%; and the levels of inorganic phosphorus and lactic acid doubled.

In spite of the low body temperature and the slowing of biochemical processes under these conditions, these changes occurred with extreme rapidity - within two to four minutes. This gives us a better understanding of the results of the investigation of Andjus and Smith [5], who were able to maintain life in rats whose body temperature was reduced almost to zero, with cardiac arrest. Recovery was possible only in those cases in which warming was begun in the region of the heart, by means of ultrahigh frequency currents.

The supplying of oxygen and glucose to the brain, begun while the body temperature is still very low, plays a decisive role in restoring the normal balance of oxidative and glycolytic processes and the requisite reserve of energy-yielding substances, and thereby, in restoring brain activity.

TABLE 3. Content of Inorganic Phosphorus, ATP Labile Phosphorus, Phosphocreatine Phosphorus, and Lactic Acid in Brain Tissue of Cats in Hypothermia With Cardiac Arrest

No.	Body temperature	Phosphorus (mg %)			Lactic acid (mg %)
		inorganic	ATP	phosphocreatine	
1	20	32	5,6	0	58
2	15	30	5	0	60
3	13	22,5	7,1	0	55

SUMMARY

When the body temperature of cats is reduced to as low as 14° C, the concentrations of inorganic phosphorus, ATP, phosphocreatine, and lactic acid in the brain tissue remain unchanged. This points to an absence of hypoxia or any other condition that might disturb the balance between glycolytic and oxidative processes. Arrest of the cerebral blood supply, even for a short time, under hypothermia results in breakdown of energy-yielding substances; this should be taken into consideration when the animal is to be brought back to the normal temperature level.

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