METABOLISM IN RAT TISSUE DURING PROLONGED ARTIFICIAL HYPOTHERMIA

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UDC 612.013-064].015.3

Prolongation of the duration of artificial hypothermia, produced by a combination of premedication and external cooling in rats, from 24 to 29 h led to no significant changes in the carbohydrate and phosphorus metabolism of the brain, but intensified changes in conformation of the brain proteins. The glycogen content in the liver and muscles was very low during prolonged hypothermia for 24-29 h, but hyperglycemia was maintained in the blood. Some animals showed a sharp decrease in the blood level of nonesterified fatty acids after hypothermia for 29 h.

The mortality rate among nonhibernating homoiothermic animals in a state of reduced activity due to hypothermia increases if the duration of this state exceeds 24 h. There is some evidence that death of the animals is linked with the onset of dyscoordination between metabolic processes in the tissues [14, 22].

Changes in metabolism in the tissues of rats under artificial hypothermia not exceeding 24 h in duration were investigated previously by the writers [3, 5, 9, 11]. The object of the present investigation was to study changes in the matabolism in the brain, liver, and muscle tissues and blood of rats with an increase in the duration of hypothermia from 24 to 29 h.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 150-250 g. Artificial hypothermia was produced by Timofeev's method [8, 12]. After administration of the lytic mixture and tubocurarine, the rats were placed in a ventilated chamber at a temperature of −10°C, when their body temperature fell to 18-20°C. The animals were then transferred to a chamber in which the temperature was 16-18°C, and their body temperature was kept at the level of 18-22°C for 24-29 h.

The rats were decapitated and the electrophoretic mobility of the soluble proteins [7] of the brain tissues and their ultraviolet absorption spectra [13] were determined. Sugar in the blood was estimated by the Hagedorn-Jensen method, and ketone bodies and nonesterified fatty acids were determined as described in [2] and [19] respectively. The remaining tests were carried out on the tissues frozen in situ in liquid oxygen. The total protein amido groups [6], the total content of ATP and ADP as readily hydrolyzed phosphorus, and the contents of creatine phosphate [1], inorganic phosphate [21], glucose [10], glycogen [11], and lactic acid [15] in the brain tissue were determined. The glycogen content in the liver and muscle tissues [4] and the glucose [10] and lipid [16] content in the liver also were determined.

EXPERIMENTAL RESULTS

Prolongation of the time during which the rats were in a state of hypothermia from 24 to 29 h led to no significant changes in the carbohydrate and phosphorus metabolism of the brain (Table 1). The changes observed were evidence of profound changes in the conformation of the brain proteins during prolongation

(Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 71, No. 4, pp. 53-55, April, 1971. Original article submitted June 5, 1970.

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TABLE 1. Effect of Prolongation of Hypothermia on Content of Components of Carbohydrate and Phosphrus Metabolism and State of Proteins in Rats' Brain

Component tested	Statistical index	Normal	State of hypothermia	
			24 h	29 h
Glucose (in mg%)	M±m	68.6±4.2	91.6±4.6	73.1±10.2
	n	7	10	7
Glycogen (in mg%)	M±m	74.4±4.3	72.1±4.2	83.8±4.3
	n	7	10	4
Lactic acid (in mg%)	M±m	25.4±2.1	34.5±3.1	36.9±5.6
	n	8	9	8
ATP + ADP (in mg% readily hydrolyzed phosphorus)	M±m	18.2±0.88	20.6±0.69	20.2±0.63
	n	16	15	10
Creatine phosphate (in mg% phosphorus)	M±m	7.7±0.24	11.3±0.66	10.3±0.31
	n	15	14	6
Inorganic phosphorus (in mg%)	M±m	13.3±0.75	16.3±1.43	12.9±0.75*
	n	16	10	11
Total amino-groups of proteins (in mg%)	M±m	100	95.3±2.1	88.4±2.5*
	n	14	8	7
Electrophoretic mobility of proteins (in %)	M±m	100	92.6±2.7	109.8±2.9*
	n	10	8	5
Intensity of ultraviolet absorption spectra of proteins in region 245-270 nm (in %)	M±m	100	85.6±1.0	83.5±4.7*
	n	10	10	5

Note. Here and in Table 2 the value of P is calculated relative to hypothermia for 24 h. The significant differences are indicated by asterisks ($P \leq 0.05$).

TABLE 2. Effect of Prolongation of Hypothermia on Content of Components of Carbohydrate and Lipid Metabolism in the Liver, Muscles, and Blood of Rats

Component tested	Statistical index	Normal	State of hypothermia	
			24 h	29 h
Liver				
Glucose (in g%)	$M \pm m$	0,78±0,06	0,92±0,10	0,89±0,03
	n	7	13	12
Glycogen (in g%)	$M \pm m$	5,31±0,37	0,35±0,07	0,23±0,05
	n	8	13	14
Lipids (in g%)	$M \pm m$	3,88±0,68	7,80±0,46	6,38±0,30*
	n	5	6	13
Skeletal muscles				
Glycogen (in mg%)	$M \pm m$	447±18	236±12	233±24
	n	7	7	15
Blood				
Sugar (in mg%)	$M \pm m$	103±8	177±20	160±13
	n	8	7	13
Ketone bodies (in mg%)	$M \pm m$	1,66±0,10	11,20±1,42	8,39±0,86
	n	7	7	6
Nonesterified fatty acids (in μeq/liter)	$M \pm m$	899±26	946±96	778±58 240±10*
	n	5	5	7 3

of hypothermia. However, the slight nature of the changes discovered both in carbohydrate and phosphorus metabolism and in the state of the brain proteins suggests that they most probably had no significant effect on the mortality among the animals during hypothermia of this duration.

An increase in the duration of hypothermia from 24 to 29 h caused no significant changes in the glucose or glycogen concentrations in the liver, and although the decrease in the lipid concentration was significant, it was nevertheless very small (Table 2). The glycogen content in the muscles after hypothermia for 29 h still remained at the same low level as after hypothermia for 24 h.

Despite the prolongation of hypothermia, hyperglycemia was maintained in the rats' blood, possibly as a result of gluconeogenesis and also of the very low tissue demand of glucose, since during cooling mainly lipids are utilized as is seen during hypothermia and hibernation [17, 20]. Whatever the case, although other investigators [22] when prolonging hypothermia in rats discovered a state of hypoglycemia, causing death of the animals, this was not observed in the present experiments. After hypothermia for 29 h, the concentration of ketone bodies in the blood still remained increased, in accordance with the suggested predominant utilization of lipids in metabolism during hypothermia.

The concentration of nonesterified fatty acids in the blood plasma after hypothermia for 29 h differed only slightly in 7 rats from that discovered at the end of hypothermia for 24 h, while in 3 rats it was sharply reduced. These were the rats whose condition was the most serious: their respiration was slow and hardly perceptible, and their muscle tone was very weak.

Hence, of the metabolic parameters studied, only the deficiency of nonesterified fatty acids in the blood could be directly connected with death of the rats when hypothermia was prolonged beyond 24 h.

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