EFFECT OF HYPOTHERMIA ON BRAIN GLUTAMATE DEHYDROGENASE ACTIVITY

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KEY WORDS: brain; hypothermia; enzyme; activity; glutamate dehydrogenase.

Hypothermia is widely used in surgical practice [2] and also in experimental biology [3, 5]. The main mechanism of the protective action of general cooling of the body is lowering the velocity of metabolic reactions in the organs and tissues to correspond to the degree of hypothermia. However, under these circumstances coordination between the velocities of enzymic reactions is lost [3, 5] and the relations between nitrogen and carbohydrate metabolism in the brain are disturbed [3, 5, 6, 8, 9]. A central place in the regulation of the nitrogen and carbohydrate metabolism of the brain is occupied by glutamic acid [12]. One of the most important pathways of this regulation is activity of glutamate dehydrogenase (GDH), which synthesizes glutamic acid and takes part in ammonia formation [13]. As previous investigations in the writers' laboratory [5] showed, in rats with hypothermia sharp changes take place in glutamic acid metabolism and ammonia accumulation in brain tissues.

It was accordingly decided to study the effect of hypothermia of different depths and durations on GDH activity in the brain.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 200-250 g. Hypothermia was induced in special chambers, through the jacket of which cold water (4°C) circulated. The rectal temperature was lowered uniformly so that after 15-20 min it reached 30°C and after 50-60 min it reached 19-20°C. In order to study prolonged hypothermia, the body temperature of the rats was kept at 30 and 20°C for 1 h. Intact animals served as the control. The control and experimental rats were decapitated and the brain (cerebral hemispheres) was quickly removed and transferred into the necessary volume of phosphate buffer, pH 7.8. A Potter–Elvehjem homogenizer with Teflon pestle was used to prepare the homogenate. GDH activity in extract of 20% homogenate was determined spectrophotometrically in the reductive amination reaction. The composition of the reaction mixture was as follows: 0.2 ml of 0.2 mM NADH solution, 0.2 ml of 0.2 mM α-ketoglutarate solution, 0.2 ml extract of homogenate, and 0.2 ml of 0.33 M NH_4Cl solution. The final volume of the mixture was made up to 3 ml with a solution of 0.067 M phosphate buffer, pH 7.8, containing 5 mM EDTA. The reaction was started by adding NADH and it was measured in the course of 1-2 min. Activity was expressed in nanomoles NADH/mg protein/min. The protein concentration in the samples was determined by Lowry's method [10]. Enzymic activity was measured during incubation at a temperature of 37°C, and also at a temperature corresponding to the body temperature of the cooled animal (30 and 20°C).

EXPERIMENTAL RESULTS

As Table 1 shows, a fairly high GDH activity was found in the tissues of the cerebral hemispheres of intact rats at all incubation temperatures studied. Lowering the incubation temperature of the brain tissue homogenates from normothermic animals from 37 to 20°C was accompanied by considerable inhibition of enzyme activity: The decrease at 30°C was 27.6% and at 20°C it was 45.1% compared with the initial level of activity measured at 37°C.

During moderate hypothermia (30°C), when the rats were in an excited state, GDH activity in the cerebral hemispheres was very slightly increased at all incubation temperatures studied (37, 30, and 20°C). The GDH activity in homogenates incubated at 30 and 20°C in these experiments was 22.5 and 50.0% lower respectively than in homogenates incubated at 37°C.

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TABLE 1. Glutamate Dehydrogenase Activity of Cerebral Hemispheres of Rats (in nmoles NADH/mg protein/min) during Hypothermia (M ± m, data of 6-7 experiments)

Experimental conditions	Incubation temperature of homogenates, °C		
	37	30	20
Control (normothermia)	69,38±2,69	50,21±2,40	38,10±1,70
Hypothermia 30°C immediately	73,20±3,53	56,66±4,50	36,66±2,48
P	>0,05	>0,05	>0,05
after 1 h	78,57±1,84	60,66±1,82	44,64±1,52
P_1	>0,05	<0,05	<0,05
Hypothermia 20°C immediately	78,90±3,90	64,28±2,43	52,90±2,66
P_2	>0,05	>0,05	<0,05
after 1 h	106,00±3,60	82,70±3,95	56,00±1,10
P_3	<0,05	<0,05	<0,05

Legend. P) Significance of difference compared with control, P_1, P_2, and P_3) compared with previous stage of hypothermia.

Prolonging the moderate hypothermia for 1 h led to a more marked increase in brain GDH activity. For instance, activity of the enzyme in samples incubated at 37, 30, and 20°C was 13.2, 20.8, and 17.0% respectively higher than in control normothermic rats. However, the degree of fall in GDH activity when the incubation temperature was lowered at this stage of hypothermia remained almost within the limits of its decrease in the control animals and those subjected to short-term hypothermia (22.7 and 42.2%).

In deep hypothermia (20°C), when the rats were in a state of cold "anesthesia," GDH activity in the brain differed only in samples incubated at a temperature corresponding to the body temperature of the cooled animal (20°C); it was 18.6% higher than that in rats exposed to moderately prolonged hypothermia (30°C).

Prolonging deep hypothermia for 1 h led to a considerable increase in brain GDH activity. For instance, when tissues from the cerebral hemispheres were incubated at 37, 30, and 20°C this increase was 25.9, 26.7, and 21.0% respectively compared with activity in rats subjected to prolonged hypothermia at 30°C.

The degree of the fall in brain GDH activity depending on lowering of the incubation temperature of the tissue samples likewise was almost unchanged during prolongation of the state of deep hypothermia (this fall at an incubation temperature of 30°C amounted to 22.0%, and at 47.1% compared with activity of the enzyme incubated at 37°C).

It can thus be concluded that lowering the body temperature of an animal leads to an increase in GDH activity in its brain tissues, which can be detected on incubation at temperatures of 37, 30, and 20°C. It is an interesting fact that, as our observations showed, under certain conditions of hypothermia glutamate dehydrogenase activity, if measured at a lower incubation temperature, is either higher than or the same as that measured at a higher temperature.

In previous investigations in the writers' laboratory [4, 5] high activity of mitochondrial enzymes of nitrogen metabolism was found in the brain of hypothermic animals when incubated at lower temperatures. It is known [11] that the succinate dehydrogenase activity of brain mitochondria of hypothermic animals, determined at lower incubation temperatures, is greater than that determined at high temperatures. However, the mechanism of this process has not been explained. It may be that the increase in brain GDH activity in the present experiments was partly the result of changes in permeability of the mitochondria for glutamic acid in hypothermia. Although hypothermia does not affect capillary permeability for amino acids, it nevertheless does have a considerable action on their cellular permeability in the brain [8]. Correlation has been shown between the activity of enzymes of glutamic acid metabolism and the concentration of this substance in the brain in various hypothermic states [5]. Increased GDH activity in brain tissues at low body temperatures

evidently ensures synaptic contact with a sufficient quantity of the mediator (glutamic acid), and this can be regarded as an adaptive reaction.

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EFFECT OF TRIFLUOPERAZINE, A CALMODULIN INHIBITOR, ON CALCIUM ACTIVATION OF PHOSPHORYLASE IN RABBIT SKELETAL MUSCLE GLYCOSOMES

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Glycogen–protein particles structurally bound with the membranes of the sarcoplasmic reticulum (glycosomes), containing Ca^{++}-sensitive phosphorylase kinase and phosphorylase phosphatase, have been isolated from rabbit skeletal muscle by fractionation in acetone and by acid precipitation and centrifugation [6]. In the presence of ATP, Ca^{++} ions have been shown to induce flash activation of phosphorylase in the same way as activation of Ca,Mg-ATPase [2]. These two systems are closely interconnected in the glycosome, and for that reason calcium activation of phosphorylase is quickly replaced by inhibition because of active Ca^{++} transport inside the sarcoplasmic reticulum (SR) [1]. Various Ca-dependent enzymes, including phosphorylase kinase and Ca,Mg-ATPase, receive Ca^{++} through the Ca-binding low-molecular-weight protein calmodulin [1, 8], which is inhibited by trifluoperazine (TFP) [5].

The object of this investigation was to study the character of calcium activation of phosphorylase in glycosomes isolated from skeletal muscle when calmodulin was inhibited by TFP.

EXPERIMENTAL METHOD

Glycosomes were isolated by the method in [4]. Rabbit muscle proteins (150 g) were homogenized in 2.5 volumes of 4 mM EDTA, pH 7.3, at 0°C. The homogenate was centrifuged at 4000g for 40 min at 0°C. The pH of the supernatant was adjusted to 6.1, and 10 min later it was centrifuged at 4000g for 30 min at 0°C. The residue was resuspended in an equal volume of buffer (100 mM sodium β-glycerophosphate + 4 mM EDTA, pH 8.2) and made up to a final volume of 25 ml with buffer containing 50 mM sodium glycerophosphate + 4 mM EDTA,

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