EFFECT OF HYPOTHERMIA ON ATP-ASE ACTIVITY OF RAT BRAIN TISSUE

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Artificial lowering of the body temperature is currently being used on an increasing scale in different branches of medicine. Hypothermia has a marked beneficial effect in the course of certain surgical operations and leads to improvement in the course of the postoperative period [1].

On the other hand, hypothermia is known to act as a damaging factor on biological membranes, leading to changes in activity of enzymes bound with them [3].

Thus hypothermia has both positive and negative effects. Its further study is therefore urgent and necessary.

The aim of this investigation was to study the effect of hypothermia on activity of the plasma membrane enzyme Na,K-ATPase during short- and long-term deep hypothermia in fractions and homogenate of the rat cerebral cortex.

EXPERIMENTAL METHOD

The experiments were carried out on male laboratory albino rats weighing 180-200 g. Hypothermia was induced by keeping the animals in cold Plexiglas chambers, in the jacket of which water at a temperature of 6-7°C circulated. The body temperature was lowered in the course of 60 min to a rectal temperature of 19-20°C. In the 1st series of experiments the rats were decapitated when it reached 20°C. In the 2nd and 3rd series of experiments the state of hypothermia was prolonged for 1 and 3 h respectively. Control animals were immobilized for 1 h in chambers which were not cooled. The cerebral cortex was taken for investigation, and synaptosomal and mitochondrial fractions were isolated from it by differential centrifugation in a sucrose density gradient [4], and a 10% homogenate also was prepared in 0.32 M sucrose, 0.015 M Tris-HCl (pH 7.4), and $0.8 \cdot 10^{-3}$ M EDTA.

ATPase activity was judged by accumulation of P_i [5] in the incubation medium. Na,K-ATPase activity was calculated as the difference between total and Mg-ATPase activity, determined in the presence of $2 \cdot 10^{-4}$ M ouabain. Specific enzyme activity was expressed in μmoles P_i/mg protein/h.

Under the conditions used a linear dependence of Na,K-ATPase activity on protein concentration was observed until 600 mg, and also on incubation time until 300 min.

Protein was determined by Lowry's method [6], using human serum albumin as the standard.

EXPERIMENTAL RESULTS

The results of measurement of Na,K-ATPase and Mg-ATPase activity of the homogenate and synaptosomal and mitochondrial fractions of the cerebral cortex of intact rats are given in Table 1. Na,K-ATPase activity was 1.8 times and 4.9 times higher in the synaptosomal fraction than in homogenate and mitochondrial fraction respectively. Meanwhile Mg-ATPase activity in the mitochondrial fraction was 1.9 and 2.6 times higher respectively than in the synaptosomal fraction and homogenate. These results are in agreement with those obtained by other workers [2].

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TABLE 1. ATPase Activity (μmoles P_i/mg protein/h) of Different Fractions of Rat Cerebral Cortex (M ± m)

Fractions	Total ATPase	Mg-ATPase	Na, K-ATPase
Homogenate (19)	16,76±0,64	9,91±0,45	6,51±0,28
Synaptosomal fraction (6)	25,42±0,86	13,48±0,26	11,61±0,80
Mitochondrial fraction (6)	27,74±2,09	25,38±2,18	2,36±0,65

Legend. Number of animals in experiment shown in parentheses.

TABLE 2. Effect of Hypothermia of Varied Duration on ATPase Activity (μmoles P_i/mg protein/h) of Homogenate and Subcellular Fractions of Rat Cerebral Cortex (M ± m)

Fractions	Activity of		
	total ATPase	Mg-ATPase	Na, K-ATPase
Hypothermia at 20°C			
Synaptosomal fraction (6)	30,81±2,03*	14,09±1,11	16,72±0,96*
Mitochondrial fraction (5)	24,37±2,72	20,08±2,54*	4,29±0,36
Hypothermia prolonged for 1 h			
Homogenate (7)	17,66±1,17	12,48±1,00	5,10±1,19
Synaptosomal fraction (12)	31,76±1,46*	16,21±0,66*	16,39±1,16*
Mitochondrial fraction (11)	32,67±2,05*	22,27±2,06*	7,09±0,51*
Hypothermia prolonged for 3 h			
Homogenate (10)	19,22±1,04	11,04±0,50	8,18±0,72
Synaptosomal fraction (5)	26,91±1,78*	13,99±1,33	12,92±0,48*
Mitochondrial fraction (5)	21,36±1,24	17,24±0,68*	4,11±1,19

Legend. Significant differences ($p < 0.05$) indicated by asterisk.

The effect of hypothermia on Na,K-ATPase and Mg-ATPase activity is shown in Table 2. The results show that a short-term form of body temperature leads to an increase in activity of synaptosomal and mitochondrial Na,K-ATPase activity by 44.0 and 81.8% respectively. Mg-ATPase activity did not change significantly. With an increase in the duration of hypothermia to 1 h, Na,K-ATPase activity in the synaptosomal fraction fell a little, but in the mitochondrial fraction it increased even more, so that the increase was threefold compared with the control. Mg-ATPase activity increased by 25.9% in the homogenate and by 18.1% in the synaptosomal fraction. In the mitochondrial fraction, however, Mg-ATPase activity did not change significantly. A further increase in the duration of hypothermia to 3 h was not accompanied by any significant change in Na,K-ATPase activity in the synaptosomal and mitochondrial fractions compared with the control, but a tendency for it to increase persisted. However, in the homogenate of the cerebral cortex there was a significant increase of 25% in Na,K-ATPase activity. Meanwhile, Mg-ATPase activity was unchanged in the synaptosomal fraction and homogenate and significantly increased in the mitochondrial fraction.

The increase in Na,K-ATPase activity in subfractions of the cerebral cortex of rats during hypothermia at 20°C can be explained primarily by compensation of changes in membrane properties during cooling of the animal, leading to destabilization of ionic gradients between the internal and external medium. Meanwhile, the increase in Na,K-ATPase activity is evidence also that hypothermia itself, under the conditions specified above, does not injure this very important membrane enzyme system. The fact that changes in Mg-ATPase activity were not significant likewise is evidence that the chief process undergoing compensation during hypothermia is that related to Na^+, K^+ gradients.

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EFFECTS OF HYPOXIA ON PHOSPHOINOSITIDE METABOLISM AND THE ADENYLATE CYCLASE SYSTEM IN ENDOTHELIAL CELL CULTURES

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The fact is not in dispute that vascular tone is regulated through the direct participation of endothelial cells (EC). The endothelium plays a particularly important role in maintenance of the necessary level of the circulation in vitally important organs when the body is in a state of oxygen insufficiency [6, 8]. The possible existence of pO_2-chemoreceptors in the EC of arteries has been discussed [3]. However, the problem of the mechanism of changes taking place in EC during hypoxia remains unclear. This is because of difficulties in differentiation of reactions arising in EC under the influence of a low pO_2 from the effects of the large quantity of mediators circulating in the blood in hypoxia. There is thus a need for the investigations of regulatory processes during hypoxia in endothelial cell cultures, which preserve their basic properties during passage [8]. The key factor in the regulation of cell metabolism is the system of secondary messengers, responsible for transmitting a broad spectrum of external signals through the cell membrane [1, 2]. An important place among them is occupied by the adenylate cyclase complex and phosphoinositide (PI) metabolism. The active metabolites of these complex regulatory systems are cyclic 3′,5′-adenosine monophosphate (cAMP), inositol-1,4,5-triphosphate (IP_3), and diacylglycerol (DAG). IP_3 is known to intensify the release of Ca^{2+} from the endoplasmic reticulum, whereas DAG activates protein kinase C (PKC), which phosphorylates a large number of structural and functional proteins, modifying cellular activity [2, 4].

The effect of hypoxia and of Ca-mobilizing hormones on the system of secondary messengers was investigated in cultured EC from human blood vessels.

EXPERIMENTAL METHOD

EC were isolated from the human umbilical vein by perfusion of the vessel with 0.1% collagenase, and were cultured in medium 199 with Earle's salts ("Flow Lab," Great Britain), with 20% human serum, endothelial growth factor from human brain (200 μ/ml), and with heparin (100 μ/ml) [9]. Membrane fractions of EC were isolated from the confluent cell layer after the 2nd-5th passage. The cells were resuspended in buffer containing 10 mM HEPES, 150 mM NaCl, 1 mM EDTA, pH 7.5, at 4°C. The cells were then frozen in liquid nitrogen, thawed, homogenized with a ground glass homogeniz-

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