DYNAMICS OF CHANGES IN RAT BRAIN AND ERYTHROCYTE ATPase ACTIVITY IN HYPOXIC HYPOXIA

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UDC 612.273.2-08:612.822.1.015.1+612.111.1.015.1

The metabolic action of hypoxia on the enzyme systems of active ion transport depends on the duration of exposure. The activity of Na, K-ATPase is sharply reduced during short periods of hypoxia (15 min) in the microsomes of the brain and in the erythrocytes. With an increase in the exposure to hypoxia an increase in Na, K-ATPase activity is observed (in the erythrocytes after an exposure of 1.5 h in the pressure chamber, in the microsomes after 2 h). The results are evidence of considerable functional changes in the cell membranes during exposure to acute hypoxic hypoxia.

KEY WORDS: hypoxic hypoxia; brain microsomes; erythrocytes; Na, K-ATPase.

In hypoxia, together with marked changes in energy metabolism, destructive changes in cell components are found relatively early: disorganization of membranous structures, swelling of the mitochondria, loss of the regular molecular heterogeneity of the cytoplasm, and so on [1, 3-5, 10, 15]. The evident cause of these changes is a redistribution of electrolytes in the tissues which, in turn, is responsible for the disturbance of active energy-dependent ion transport through the membranous structures of the cell [4, 15]. The writers previously showed a change in the Na, K-ATPase activity in a homogenate of brain tissue and in erythrocytes [4] during hypoxic hypoxia.

In the investigation described below changes in ATPase activity were studied in brain microsomes and in erythrocytes during hypoxia lasting for different periods of time.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred rats weighing 150-200 g. The animals were kept under fairly rigorous conditions of hypoxia ("ascent" in a pressure chamber to 9.5 km) for 5, 15, 90, 120, and 180 min. Immediately after "descent" the animals were decapitated, mixed blood was taken for analysis, the cerebral hemispheres were quickly removed and the tissue homogenized at 0-4°C in 20 volumes of 0.25 M sucrose with 0.005 M tris-HCl, pH 7.6 (in a Teflon homogenizer).

The fraction of heavy microsomes obtained by differential centrifugation (40,000 g, 2 h) was resuspended in 0.2 M tris-HCl, pH 7.6, and frozen and thawed once. The protein content was determined by the microbiuret method [2]. Erythrocytes were twice washed with 4 volumes of 0.14 M NaCl and frozen and thawed once; the protein content was determined by Gornall's method [13] with modifications [12].

Na, K-ATPase activity was determined from the difference between the total ATPase activity and activity after inhibition by strophanthin K ($4 \cdot 10^{-4}$ M). The composition of the incubation medium (1 ml), in millimoles, was as follows: ATP-Na_2 3, $MgCl_2$ 3, NaCl 100, KCl 20, tris-HCl, pH 7.6, 20, 200-400 μg microsomal protein or 10 ± 2 mg erythrocyte protein. After incubation at 37°C (20 min for microsomes, 40 min for erythrocytes) the reaction was stopped with 50% TCA (0.2 : 1). The concentration of inorganic phosphate (P_i) was determined by the method of Fiske and Subbarow [11]. The results were subjected to statistical analysis by Student's method.

Laboratory of Physiology of the Cerebral Circulation, A. L. Polenov Leningrad Neurosurgical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR S. V. Anichkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 11, pp. 38-41, November, 1975. Original article submitted December 31, 1974.

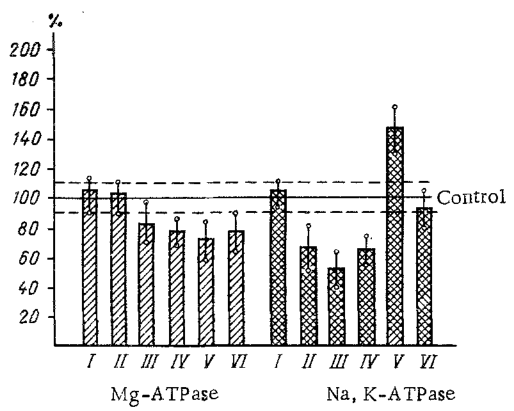

Fig. 1

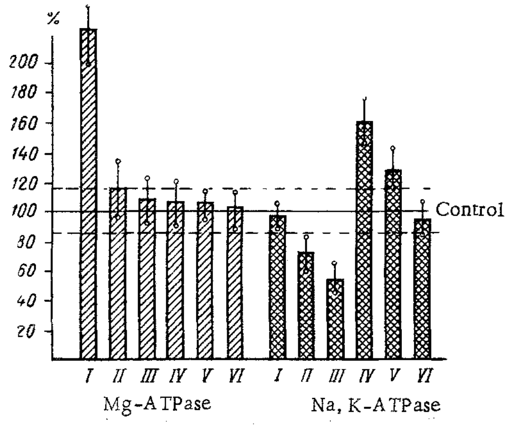

Fig. 2

Fig. 1. Dynamics of changes in ATPase activity of microsomal fractional of brain in hypoxic hypoxia. I) "Ascent—descent;" II) 5 min; III) 15 min; IV) 90 min; V) 120 min; VI) 180 min in pressure chamber. Activity of enzymes in intact animals taken as 100%: for Mg-ATPase 5.77 ± 0.33 μmoles P_i/mg protein/h and for Na, K-ATPase 3.65 ± 0.23 μmoles P_i/mg protein/h.

Fig. 2. Dynamics of changes in ATPase activity of erythrocytes in hypoxic hypoxia. Legend as in Fig. 1. Activity under normal conditions: Mg-ATPase 0.126 ± 0.006 μmoles P_i/mg protein/h; Na, K-ATPase 0.073 ± 0.005 μmoles P_i/mg protein/h.

EXPERIMENTAL RESULTS AND DISCUSSION

Hypoxia of the severity created under the experimental conditions used caused marked changes in the Na, K-ATPase of the brain microsomes (Fig. 1). After exposure for 5 min to a "high altitude" the decrease in the activity of this enzyme system was statistically significant. The greatest decrease in Na, K-ATPase activity was observed after hypoxia for 15 min. During a further period in a rarefied atmosphere an increase in the activity of this enzyme was observed. After a stay of 2 h at the specified altitude, Na, K-ATPase activity was 1.5 times higher than the control. A further increase in the period of hypoxia led to restoration of the normal Na,K-ATPase activity.

Mg-ATPase activity fell gradually (by 25%) with an increase in the period of hypoxia. As the control, the effect of pressure dropped in the pressure chamber during "ascent" and "descent" (760-215-760 mm Hg) without exposure to a "high altitude" was investigated. The results showed that "ascent–descent" had no effect on the ATPase activity of the rat brain microsomes. Consequently, the observed changes in ATPase activity did not depend on drops of pressure, but were caused by oxygen deficiency.

Na, K-ATPase activity of the erythrocyte membranes showed practically the same changes under the influence of hypoxia as in the experiments with the brain microsomes. The initial phase of inhibition of Na, K-ATPase activity of the erythrocytes was replaced after hypoxia for 90-120 min by a phase of activation, which in turn was followed by a return to normal (Fig. 2). Control tests ("ascent–descent") caused no change in Na, K-ATPase activity of the erythrocytes. Pressure drop activated Mg-ATPase considerably. This response of Mg-ATPase activity can be regarded as a manifestation of a nonspecific response of the erythrocyte membranes to pressor action.

The results of these experiments thus indicate a considerable change in the activity of the transport Na, K-ATPase of the brain and erythrocytes. The rapid (5 min after the beginning of hypoxia) decrease in activity of Na, K-ATPase of the brain microsomes is in good agreement with observations of other workers on early changes in metabolic processes in the tissues in hypoxia [6, 8]. The subsequent increase in activity of the enzyme system (after 90 min) was evidently a sign of the activation of adaptive mechanisms, leading later (after 120 min) to hypercompensation. The results showing activation of Na, K-ATPase of the brain during hypoxia for 2 h agree with histological observations [3, 7, 9] indicating considerable changes in the ultrastructural characteristics of nerve tissue in hypoxic hypoxia lasting 2 h. The authors cited observed an increased number of free and membrane-bound ribosomes in the cytoplasm of neurons together with swelling of the mitochondria, while the cristae remained intact. These changes, in these workers' opinion, are not pathological and they regard them as a morphological feature of increased functional activity of these organelles and of a strain on the energy resources of the nerve cells. The reduced activity of brain Na, K-ATPase in the later stages (after hypoxia for 3 h) must probably be regarded as indicating collapse of the compensatory mechanisms. There is alternative evidence in support of this view – after exposure of rats to the degree of hypoxia used in the present experiments for 4 h, 90% of the animals died. Electron-microscopic investigations [3, 7] of the brain tissues of animals exposed to hypoxia for 4 h showed a decrease in the features of physiological neuronal activity, an increase in the number of lysosomes, and vacuolation of the cytoplasm on account of swelling of the cisterns of the endoplasmic reticulum.

Active transport of Na^+ and K^+ ions in erythrocytes is known to be maintained by glycolytic ATP resynthesis [14, 16]. This evidently explains the later inhibition of ATPase of the erythrocyte membranes and the more marked supercompensation of its activity.

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