CHANGES IN CREATINE KINASE ACTIVITY IN THE BRAIN, HEART, LIVER, AND BLOOD PLASMA OF HYPOXIA RATS

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The effect of hypoxic and cytotoxic hypoxia on creatine kinase (CK) activity of the blood plasma, the brain, heart, and liver tissues, and the nuclear and mitochondrial fractions of the brain was studied. Hypoxia causes marked changes in CK activity of all the tissues and organelles studied. The magnitude and direction of the changes in CK activity depend on the type and duration of hypoxia, the organ-specificity of the CK, and disturbance of the permeability of cell membranes.

KEY WORDS: creatine kinase; hypoxia.

Creatine kinase (CK) (ATP: creatine phosphotransferase, EC 2.7.3.2) plays an important role in the metabolism of high-energy phosphorus compounds concerned with ATP resynthesis, and it participates in the control of mitochondrial respiration [10, 13]. In cytotoxic hypoxia CK activity in the heart muscle is reduced but in skeletal muscle it is increased; similar results have been found also in asphyxia [5]. In experimental myocardial infarction an increase in CK activity has been found in heart muscle [1, 6].

The effect of cytotoxic and hypoxic hypoxia of varied duration on CK activity was studied in the brain, heart, liver, and blood plasma.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 150-250 g were used. Cytotoxic hypoxia was induced by subcutaneous injection of sodium nitrite (25 mg/100 g body weight), and the animals were decapitated 25 min later. Hypoxic hypoxia was induced in a pressure chamber at an "altitude" of 9.5 km for 5, 15, 120, and 180 min (the "ascent" and "descent" each took 3 min). Immediately after "descent" the animals were decapitated, the mixed blood was collected, and the cerebral hemispheres, livers, and hearts were removed. The tissues of the brain, heart, and liver were homogenized in 10 volumes of 0.25 M sucrose with 0.005 M tris HCl (at 0°C in a Teflon homogenizer). By differential centrifugation the nuclear and mitochondrial fractions were isolated from the brain homogenate. Tissue extracts were obtained by centrifugation of the homogenates at 40,000g (after removal of the mitochondria and microsomes) [11]. Protein was determined in the samples by the microbiuret method [2] and the CK activity investigated [11].

EXPERIMENTAL RESULTS AND DISCUSSION

In hypoxic hypoxia CK activity was altered in all the tissues studied (Fig. 1). The extent and direction of the change depended on the duration of hypoxia and the specificity of the enzyme. The earliest changes occurred in the activity of brain cytoplasmic CK and blood plasma CK. After a single "ascent–descent," and with a stay at a "high altitude" of not more than 1.5 min, the enzyme activity was lowered ($P < 0.05$). This was probably the result of a decrease in the creatine phosphate concentration in the brain tissue in hypoxia [3, 9]. The increase in enzyme activity after 15 min of hypoxia was evidently the result of hyper-

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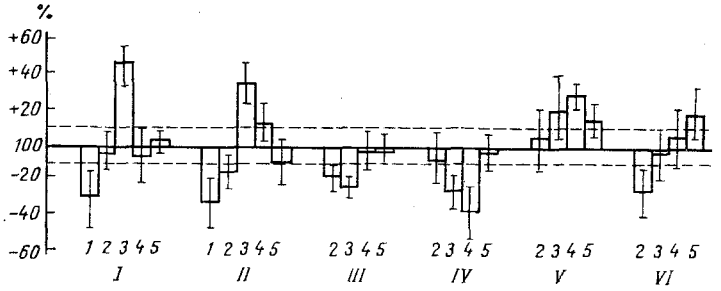

Fig. 1. Effect of hypoxic hypoxia on CK activity in brain, heart, liver, and blood plasma of rats: I) blood plasma; II) brain; III) heart muscle; IV) liver; V, VI) nuclear and mitochondrial fractions of brain; respectively. Duration of exposure in pressure chamber: 1) "ascent–descent"; 2) 5 min; 3) 15 min; 4) 120 min; 5) 180 min. Ordinate, change in CK activity, in %.

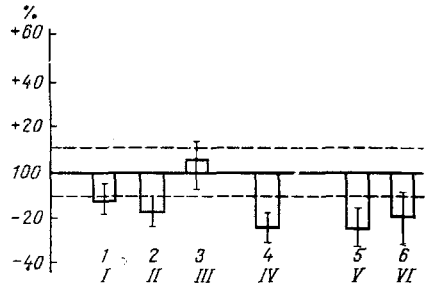

Fig. 2. Action of cytotoxic hypoxia on CK activity of rat brain, heart, liver, and blood plasma: I) brain; II) heart; III) liver; IV) blood plasma; V, VI) nuclear and mitochondrial fractions of brain, respectively. Remainder of legend as in Fig. 1.

adaptation. A study of the action of hypoxia on Na^+, K^+-ATPase also revealed biphasic changes in the enzyme activity in the brain tissue [4]: a sharp decrease in the first 15 min followed by an increase during exposure for 2 h.

Increased CK activity in the blood plasma in pathological states is regarded by most workers as the result of a disturbance of membrane permeability [14, 15]. The decrease in Na^+, K^+-ATPase activity in the microsomal fraction of brain tissue and in erythrocytes during hypoxia for 15 min [4] also points to considerable changes in the membranes. Presumably the decrease in CK activity in the heart muscle and liver during hypoxia for 5 and 15 min was the result of liberation of the enzyme into the blood. After hypoxia for 2-3 h, CK activity in these organs returned to its normal level. No increase in cytoplasmic CK activity above the normal level was observed in the heart and liver tissues.

The differences between changes in CK activity in different tissues during hypoxic hypoxia can probably be explained by the organ specificity of the enzyme and differences in the isoenzyme spectrum in different tissues. CK can perhaps be classed as a constitutive enzyme [7], one which determines the specific enzyme profile of each tissue and which reacts precisely to changes in the conditions of existence of the body cells.

A tendency toward an increase in CK activity at all periods of hypoxia was observed in the nuclear fraction of brain tissue (after exposures of 15 and 120 min this tendency became statistically significant; $P < 0.05$). A decrease in CK activity in the mitochondrial fraction was discovered after 5 min of hypoxia. After 3 h of hypoxia the CK activity was increased.

Experiments have shown that in the mitochondria CK preferentially catalyzes the reaction of formation of creatine phosphate and ADP, but in the cytoplasm the formation of creatine and ATP [12, 16, 17]. The intensity of the process as a whole is determined by the local ATP/ADP ratio [8]. The increased CK activity in the mitochondria during 3 h of hypoxia may perhaps reflect the need for activation of creatine phosphate synthesis; normalization of the creatine phosphate level in the cytoplasm leads to normalization of cytoplasmic CK activity.

In severe cytotoxic hypoxia a sharp decrease in CK activity was found in the brain and heart tissues and also in the nuclear and mitochondrial fractions of the brain and in the blood plasma. CK activity in the liver was unchanged (Fig. 2). The direction and magnitude of the changes in CK activity corresponded on the whole to the primary changes observed in hypoxic hypoxia. The exception was CK activity in the brain nuclei (which had a tendency to increase in hypoxic hypoxia but was lowered in cytotoxic hypoxia) and the CK activity in the liver (which was lowered in hypoxic but remained unchanged in cytotoxic hypoxia). These differences could be associated with the direct effect of sodium nitrite.

Hypoxia thus causes considerable changes in CK activity of the tissues and cell organelles.

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