Enzymatic Characteristics of Creatine Kinase System under Conditions of Cerebral Circulatory Disorders

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Changes in the catalytic properties of cytoplasmic and mitochondrial creatine kinase were studied at various periods of cerebral circulatory disorders. Cerebral ischemia was accompanied by activation of free radical processes, changes in the dimer/octamer ratio for mitochondrial creatine kinase, and appearance of new catalytic properties of isoenzymes.

Key Words: *creatine kinase; brain; membrane; ischemia; free radical oxidation*

The regulatory capacity of enzymes and functional activity of cell structures play the major role in the regulation of energy metabolism in the brain during ischemia. Creatine kinase (CK) plays a key role in energy processes in the cell. This enzyme catalyzes the formation of a macroergic compound creatine phosphate. There are two CK isoforms in the brain, cytoplasmic (cCK) and mitochondrial CK (mCK). Activity of mCK is mainly determined by the interaction with the mitochondrial membrane [12]. mCK exists in two oligomeric forms (octamer and dimer) that are capable of undergoing mutual transitions [10]. cCK is present in the cell only as a dimer. Energy deficiency during ischemia is mainly related to regulation of oxidative enzymes. Our previous studies revealed significant changees in the catalytic properties of phosphokinases during acute cerebral ischemia [2,3]. However, little is known about functional activity of enzymes during chronic cerebral ischemia.

Here we studied the catalytic properties of CK isoenzymes and intensity of free radical oxidation (FRO) in various periods of cerebral circulatory disorders.

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MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 150-180 g. Cerebral ischemia was produced by bilateral ligation of the common carotid arteries. The animals were anesthetized with nembutal (30 mg/kg intraperitoneally). The brain tissue was examined 30 min, 18 h, and 3 days after surgical impairment of cerebral hemodynamics.

The mitochondrial fraction of the brain was isolated by differential centrifugation [1].

mCK was dissociated by incubation of the total mitochondrial fraction and mitochondrial membrane pellet with substrates for the transition-state analogue complex ($MgCl_2$, ADP, KNO_3, and creatine) at 4°C for 2 h.

CK activity was measured spectrophotometrically in the coupled system of pyruvate kinase and lactate dehydrogenase [9].

The intensity of FRO was estimated by the method of H_2O_2, Fe^{2+}-induced chemiluminescence on a BChL-07 biochemiluminometer. The characteristics of chemiluminescence were recorded for 30 sec. The total luminescence yield (S), maximum flash amplitude (Imax), and tg2 index characterizing antioxidant potential were used as integral parameters of chemiluminescence.

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Protein concentration was measured by the method of Bradford [8].

The results were analyzed by means of BIOSTAT software. The significance of differences between the samples was evaluated by Student's *t* test. The data are presented as $M \pm m$.

RESULTS

Cerebral ischemia was followed by changes in activity of CK isoenzymes (Table 1). Activity of mCK in all fractions from treated animals was much lower than in intact specimens. mCK activity in the total mitochondrial fraction and mitochondrial membrane pellet was reduced by 21% after 30-min ischemia. Therefore, acute ischemia is accompanied by a decrease in mitochondrial enzyme activity. However, activity of cCK slightly increased under these conditions.

Increasing the duration of cerebral ischemia to 18 h was accompanied by changes in activity distribution for mCK and cCK. Activity of cCK progressively increased. Activity of mCK under these conditions was higher than in acute ischemia. However, mCK activity in animals of the treatment group remained lower than in intact specimens.

Increasing the duration of cerebral ischemia to 3 days was accompanied by further increase in mCK activity. CK activity in the total mitochondrial fraction was 1.4-fold higher than during acute 30-min ischemia. Moreover, activity of CK in treated rats was slightly higher than in intact animals. The membrane-bound enzyme activity in these rats increased by 22% (as compared to acute ischemia) and did not differ from that in intact animals.

Ischemia had a modulatory effect on interaction of mCK with mitochondrial membrane. The products of abnormal metabolism during cerebral ischemia and hypoxia are characterized by membrane toxicity and can stimulate FRO [6,7]. Neuronal membranes contain a considerable amount of unsaturated lipids. Low activity of antioxidant enzymes and formation of free radicals in neurochemical reactions provide conditions for lipid oxidation and induce protein modification (*e.g.*, enzyme modification). To evaluate the state

TABLE 1. Activity Distribution of CK Isoenzymes during Cerebral Ischemia (nmol creatine/mg/min)

Experimental conditions		Total mitochondrial fraction	Mitochondrial membranes	Cytoplasmic fraction
Intact animals		562.00±45.56	579.50±37.57	528.60±25.61
		(*n*=30)	(*n*=30)	(*n*=30)
Cerebral ischemia	30 min	442.60±33.81*	467.10±42.50*	600.90±27.85
		(*n*=16)	(*n*=16)	(*n*=16)
	18 h	495.50±32.67	521.10±58.52	618.50+30.96*
		(*n*=16)	(*n*=16)	(*n*=16)
	3 days	597.50±55.47^{+}	573.43±36.17^{+}	653.70±68.15*
		(*n*=12)	(*n*=12)	(*n*=12)

Note. Here and in Tables 2 and 3: *$p<0.05$ compared to intact animals; $^{+}p<0.05$ compared to 30-min ischemia.

TABLE 2. Characteristics of Chemiluminescence in the Total Mitochondrial Fraction of the Brain (pulses/30 sec)

Experimental conditions		Maximum flash amplitude (Imax)	Total yield of slow flash (S)	tg2 index
Intact animals		58.00±1.85	566.22±28.58	15.00±0.75
		(*n*=30)	(*n*=30)	(*n*=30)
Cerebral ischemia	30 мин	70.33±4.15*	702.70±54.02*	17.50±1.20
		(*n*=16)	(*n*=16)	(*n*=16)
	18 h	95.45±4.28*$^{+}$	981.50±32.72*$^{+}$	20.73±1.21*$^{+}$
		(*n*=16)	(*n*=16)	(*n*=16)
	3 days	96.17±9.47*$^{+}$	960.80±85.21*$^{+}$	20.29±2.54*
		(*n*=12)	(*n*=12)	(*n*=12)

TABLE 3. Distribution of Oligomeric mCK during Cerebral Ischemia

Experimental conditions		Total mitochondrial fraction, percentage of dimers	Mitochondrial membranes, percentage of dimers
Intact animals		68.04±5.07	67.58±4.01
		(*n*=30)	(*n*=30)
Cerebral ischemia	30 min	77.83±9.14	82.49±6.52*
		(*n*=16)	(*n*=16)
	18 h	55.93±3.73	60.10±4.73
		(*n*=16)	(*n*=16)
	3 days	44.49±2.61*+	48.31±3.23*+
		(*n*=12)	(*n*=12)

of membranes, the intensity of FRO and antioxidant properties of the brain tissue were estimated in various periods of ischemia (Table 2). Various characteristics of chemiluminescence (maximum flash amplitude and total yield of slow flash) in the mitochondrial fraction were elevated during various periods of cerebral ischemia. These changes reflect activation of free radical processes in the brain. The intensity of FRO increased by 40% after 18-h ischemia (relative to intact animals) and was 30% higher than under conditions of 30-min ischemia. Parameters of FRO (Imax and S) remained practically unchanged by the 3rd day. They did not differ from the corresponding parameters after 18-h ischemia.

Acute exposures (*e.g.*, ischemia) were not only followed by damage to cell membrane structures and activation of FRO, but also induce defense systems of the organism (for example, the antioxidant system) [5]. The tg2 index serves as a criterion for the antioxidant potential of the cell. The tg2 index in the early stage of ischemia did not differ from that in intact animals. However, the antioxidant activity of the brain tissue was elevated after ischemia for 18 h and 3 days. Our conclusion was derived from the increase in this index. These data indicate that the prooxidant/antioxidant ratio returns to normal with increasing in the duration of cerebral circulatory disorder. The observed changes are probably related to activation of defense protein synthesis, which increases the resistance of membrane structures to the adverse effect of ischemia.

The major problem in the involvement of cell structures in the regulation of enzyme activity is the dependence of enzyme properties on the association of this enzyme with the membrane under conditions of functional changes in the organism. mCK is associated with mitochondrial membranes due to the forces of electrostatic and hydrophobic interaction. Under *in vivo* conditions, the mitochondrial enzyme is bound to membranes or exists in a free form in the intermembrane space. In the solution, mCK is presented by two oligomeric forms (dimer and octamer). They are characterized by dynamic equilibrium [4]. The transition-state analogue complex of mCK was induced to evaluate the ratio between oligomeric forms of this enzyme in the total mitochondrial fraction and mitochondrial membrane pellet under conditions of cerebral circulatory disorders (Table 3).

In mitochondria from intact animals, mCK exists as a mixture of two oligomeric forms (dimer and octamer; 68 and 32%, respectively). Cerebral ischemia changes the dimer/octamer ratio. This ratio is shifted toward the formation of dimers after 30-min ischemia. Phospholipids serve as the structural elements of membranes that are bound to mCK. Membrane-binding properties of mCK depend strongly on the protein dimer/octamer ratio and degree of lipid oxidation. Activation of FRO during acute ischemia is probably followed by partial dissociation of octamers to dimers.

Increasing the duration of ischemia to 18 h was followed by an increase in the octamer ratio in both fractions. By the 3rd day, this ratio was 50% in the total mitochondrial fraction and mitochondrial membrane. Published data show that octameric mCK contributes to the appearance and strengthening of contact sites, which increases the efficiency of energy formation in brain mitochondria, consolidates the membrane structure, and determines the resistance of membranes to the adverse effect of hypoxia [11]. The existence of two oligomeric forms of this enzyme probably maintains the near-equilibrium state of reaction in a wide range of physiological conditions.

Our results indicate that cerebral ischemia is accompanied by opposite changes in activities of mCK and cCK. Catalytic properties of mCK depend on the functional interaction with mitochondrial membranes. Acute ischemia impairs enzyme interaction with the mitochondrial membrane. Increasing the duration of

ischemia is not only followed by injury and dysfunction, but also activates the defense systems in the nervous tissue. The resistance of membrane structures increases in the later period. These changes manifested in activation of mCK and change in the dimer/octamer ratio toward the formation of octamer. Therefore, mCK gains new properties under conditions of oxygen deficiency in nerve cells. The absence of significant changes in functional properties of the cytoplasmic isoenzyme, increase in activity of this isoenzyme, and strong variations in activity of mCK reflect the progression of adaptive processes during prolonged impairment of cerebral circulation.

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