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Role of Creatine Kinase – Hexokinase Complex in the Migration of Adenine Nucleotides in Mitochondrial Dysfunction

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1. Introduction

Creatine phosphokinase (CK) (ATP: creatine phosphotransferase, EC 2.7.3.2.) is found in a variety of cells with high and fluctuating energy requirements. It catalyses the reversible transfer of the high-energy-N-phosphoryl group from phosphocreatine to ADP. Creatine kinase connects sites of energy production with sites of energy consumption (Dolder et. al., 2001; Focant et al., 1970; Grossmann et al., 1985; Lipskaya et al., 1989; Walzel et al., 2002; Wyss, 2000).

There are know to be three cytosolic and two mitochondrial isoforms of CK. The more basic mitochondrial creatine kinase $MiCK_b$ is accumulated in mitochondria of cardiac muscle and skeletal muscle. The more acidic mitochondrial creatine kinase $MiCK_a$ was found in the brain (Eppenberger-Eberhardt et al., 1991; Fridman, Roberts, 1994).

Creatine kinase can exist in two interconvertible forms: dimer and octamer (Eriksson et al., 1998; Shen et al., 2002). Creatine kinase binds to the outer leaflet of the entire inner mitochondrial membrane and is specifically enriched in the so-called contact sites where inner and outer membranes are in close proximity (Boero et al., 2003; Chen et al, 1994; Lin et al., al., 1996; Wang et al., 2005).

A change in the octamer/dimer ratio may influence on the association behavior of mitochondrial creatine kinase in general and thus modulate mitochondrial energy flux (Brdiczka, 2003; Dolder et al., 2001; Schnyder et al., 1995).

Mitochondrial creatine kinase forms the functional microcompartment together with the mitochondrial porin (voltage-dependent anion channel) in the outer membrane and as well as the transmembrane protein adenine nucleotide translocase in the inner membrane (Fritz-Wolf et al., 1996; Kaldis, Wallimann, 1994; Schnyder et al., 1988).

Hexokinase (HK) (ATP:D-hexokinase-6-phosphotransferase, EC 2.7.11) is the enzyme with variable cellular localization (Mulichak et al., 1998; Xie & Wilson, 1990).

The type I isoenzyme of mammalian hexokinase is ubiquitously expressed in mammalian tissues but is found particularly at high levels in the brain where it plays an important role in regulating the rate of cerebral glucose metabolism (Schwab & Wilson, 1989; Wilson, 1985).

The major portion of the hexokinase activity in the brain is associated with mitochondria. About 85% of hexokinase is bound to the outer mitochondrial membrane, forming the specific complex with porin (Magnani et al., 1982; Redker et al., 1972; Wilson, 1995). This

physical proximity provides the basis for functional interaction between glucose phosphorylation by hexokinase and mitochondrial ATP production by oxidative phosphorylation with resulting coordination of the glycolytic and oxidative phases of glucose metabolism (Aleshin, 1998; Rosano et al., 1999). The outer mitochondrial membrane protein – porin, which forms the transmembrane channel, is responsible for specific interaction with hexokinase (Aflalo & Azoulay, 1998; Linden et al., 1982; Schlattner et al., 2001; Vyssokikh & Brdiczka, 2003).

The preferential mitochondrial localization of hexokinase in rat brain provides a predominant access to ATP, generated in mitochondria. The ADP produced by hexokinase activity is known to control both membrane potential and reactive oxygen species generation (Rose & Warms, 1967; Smith and Wilson, 1991; Viitanen et al., 1984; Wilson, 1980).

Thus both enzymes – creatine kinase and hexokinase – play an important role in dynamic compartmentation of adenine nucleotides.

Mitochondrial creatine kinase is a key enzyme of oxidative cellular energy metabolism in the brain (Bessman, 1981; Guo et al., 2003; Hemmer et al., 1994; Levin et al., 1990; Takagi et al., 2001; Wallimann et. al., 1992; Wallimnann et al., 1998). Hexokinase is an enzyme involved in the first step of glycolysis. Mitochondrial creatine kinase – hexokinase complex takes part in transport of adenine nucleotides from mitochondria to cytoplasm. Functioning of this complex depends on interaction of enzymes with the mitochondrial membrane and the oligomeric state of mitochondrial creatine kinase.

Mitochondrial dysfunction is one of the main reasons of the pathological changes in cerebral ischemia (Clostre, 2001; Delivoria-Papadopoulos et al., 2007; Fiscum, 2000; Kuznetsov, Margreiter, 2009; Mattson, Liu, 2002; Sas et al., 2007; Siesjo, 1999).

Stroke is a leading cause of disability and death in many countries. Understanding the molecular mechanisms of ischemic injury helps to find the novel therapeutic strategies for stroke. 80% of human strokes are ischemic in origin (Levine et al., 1992; Sappey-Marinier et al., 2002; Ueda et al., 2000).

Thus experimental models of cerebral ischemia have been developed in an attempt to closely mimic the changes that occur during and after human ischemic stroke. Changes in the amount and activity of enzyme proteins are critical factors in regulating intracellular metabolism under ischemic conditions (Cherubini et al., 2000; Dos Santos et al., 2004; Maulik et al., 1999; Rauchova et al., 2002).

According to modern data, membrane-associated enzyme in contrast to soluble enzyme has other catalytic properties (Beutner et al., 1998; da-Silva et al., 2004; Dolder et al., 2001; Kellershohn & Ricard, 1994; Linden et al., 1982; Lyubarev, 1997; Ovadi & Srere, 2000). The reverse adsorption on the mitochondrial membrane is controlled by ions and metabolites thus broadening the regulatory possibility of the cells under hypoxic conditions.

2. Materials and methods

Animals

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 minutes (acute ischemia), 1.5, 4, 18 hours after surgical impairment of cerebral hemodynamics.

The animals were divided into two groups due to their physiological state after acute ischemia (severe and moderate). The severity of ischemia was estimated according to the behavior of rats, the respiratory rate and survival one. In acute severe ischemia the rats after ligation of the common carotid arteries were in severe state: they were passive, in lateral recumbent position, with agonal breathing (20-30 times per minute with respiratory arrest). In acute moderate ischemia the state of animals was satisfactory: they were active, moved in a cage, the respiratory rate was 50-70 times per minute. Due to the increase in the severity of general physiological state of rats in case of long-term ischemia we could not divide the animals into two groups; they were included into one group.

Preparation of brain tissue

The mitochondrial fraction of the brain was isolated by differential centrifugation (Fonyo, Somogy, 1960; Dizhe et al., 2003). The brain tissue was homogenized at 4 ^{0}C in a medium containing 0.32 M sucrose, 10 мM tris-HCl , 1 мM EDTA, pH 7,4. The total tissue homogenate was centrifuge at 2000 g for 10 minutes. The resulting supernatant was collected and centrifuged further at 12 500g for 15 minutes. The pellet containing mitochondria was resuspended in 0.32 M sucrose and centrifuged at 16 500g for 15 minutes. The fraction enriched mitochondria was collected and washed by 0.32 M sucrose.

The mitochondria were then swollen by incubation in distilled water (at a protein concentration of 1 mg) for 30 minutes, followed by centrifugation at 20 000g for 30 minutes. The resulting supernatant was collected for further analysis. The pellet containing mitochondrial membranes was resuspended in 0.32 M sucrose with 0.25 M dithiothreitol, pH 7.4.

Enzyme assay

Creatine kinase activity was measured by the pH-stat method using ADP and creatine phosphate as substrates (Kuby, Noltman, 1962). The velocity of the creatine kinase reaction is estimated by the change in pH. The reaction mixture (3 ml) contained (final concentration): 0.25 M sucrose, 2.5 mM tris-HC1, 12 mM $MgCl_2$, 10 mM KC1, 0.25 mM dithiothreitol, 5 mM creatine phosphate, 2 mM ADP. The reaction was started by addition of 100 µg protein. Then the mixture was titrated by addition of 10 µl 0.1 N HCl.

Creatine kinase activity is expressed as 1 unit corresponds to 1 µg-equ H^+/ min per 1 mg of protein.

Hexokinase activity was measured spectrophotometrically (Felgner, Wilson 1976). The reaction mixture (3 ml) contained (final concentration): 50 mM tris-HC1, pH 8.0, 2 mM glucose, 2 mM ATP, 5 mM $MgCl_2$, 0,25 mM NADP, 0.4 IU/min glucose-6-phosphate dehydrogenase. The reaction was started by addition of 100 µg protein.

Hexokinase activity is expressed as follows: 1 unit corresponds to 1 nmol of NADP transformed/min per 1 mg of protein.

Solubilization of creatine kinase

Mitochondria were resuspended in the proper (0.1M KCl; K-Na phosphate buffer 0.1-1.75 M, 0.5% (v/v) Triton X-100; 0.1% deoxycholate Na) solubilizing solution and incubated for 30 minutes. The samples were centrifuged at 4^{0}C and 20 000g, 60 minutes. Percentage of solubilization was determined as the difference of the activity before and after solubilization of the enzyme.

Solubilization of hexokinase

Mitochondria were resuspended at a protein concentration of 0.5-1 mg/ml in 0.1M tris-HCl pH 6.6, 0.1 M KCl or 0.5% (v/v) Triton X-100. After incubation for 30 minutes on ice, with

occasional mixing, the samples were centrifuged at 4^0C and 20 000g for 30 minutes. Percentage of solubilization was determined as the difference of the activity before and after solubilization of the enzyme.

According to Wilson (2003) there are 2 types of binding sites for hexokinase on brain mitochondria. Hexokinase is readily solubilized from Type A sites by glucose-6-phosphate while hexokinase bound to Type B sites remains bound even in the presence of glucose-6-phosphate.

Mitochondria were resuspended in 2 mM glucose-6-phosphate; tris-HCl buffer, pH 8 and incubated for 30 minutes at the room temperature, and centrifuged 100 000g for 15 minutes. Aliquots of supernatant contain hexokinase Type A.

The sediment of mitochondria which contained hexokinase type B was resuspended again in 0,32 M sucrose, 0.5% (v/v) Triton X-100, 0.1M tris-HCl, pH 8. After incubation for 5 minutes on ice the samples were centrifuged at 20 000g for 10 minutes. The sediment was resuspended again in 0.32M sucrose, 0.1M KCl, 1% (v/v) Triton X-100, 0.1M tris-HCl, pH 8. After incubation for 20 minutes on ice the samples were centrifuged at 20 000g for 10 minutes. The aliquots of supernatant contained hexokinase Type B.

Dissociation of creatine kinase

Mitochondrial creatine kinase 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 ^{0}C for 2 hours (Lipskaya et al., 1989).

The free radical oxidation intensity assay

The intensity of the free radical oxidation (FRO) was estimated by the method of H_2O_2, Fe^{2+}–-induced chemiluminescence on a BChL-07 biochemiluminometer. This method is based on the catalytic decomposition of hydrogen peroxide by ions of metal with variable valency (bivalent iron) (the Phenton reaction). The reaction mixture contained: 0,05 mM Fe_2SO_4, a phosphate buffer and a mitochondrial fraction. The reaction was started by addition of 2 % solution of hydrogen peroxide. Proceeding process of free radical oxidation was registered within 30 seconds. It is the time of the greatest information about its intensity. The ideal curve of the process is presented in figure 1.

The following parameters are the most informative for the estimation of the chemiluminescence intensity: the total luminescence yield (S, enables to estimate a balance between lipid peroxidation and antioxidants), maximum flash amplitude (Imax, shows a potential ability of the biological sample to free radical oxidation), and K index characterizing antioxidant potential were used as integral parameters of chemiluminescence (Kuzmina et al., 2009).

The protein concentration assay

Protein concentration was measured by the method of Bredford (Bredford & Spector, 1978).

Statistical analysis

The data are expressed as mean and standard error of the mean (SEM). The results were analyzed by means of Primer of Biostatistics 4.03 (Glantz, 2005). The significance of differences between the samples was evaluated by Student's test. The level of significance was set at $p<0.05$.

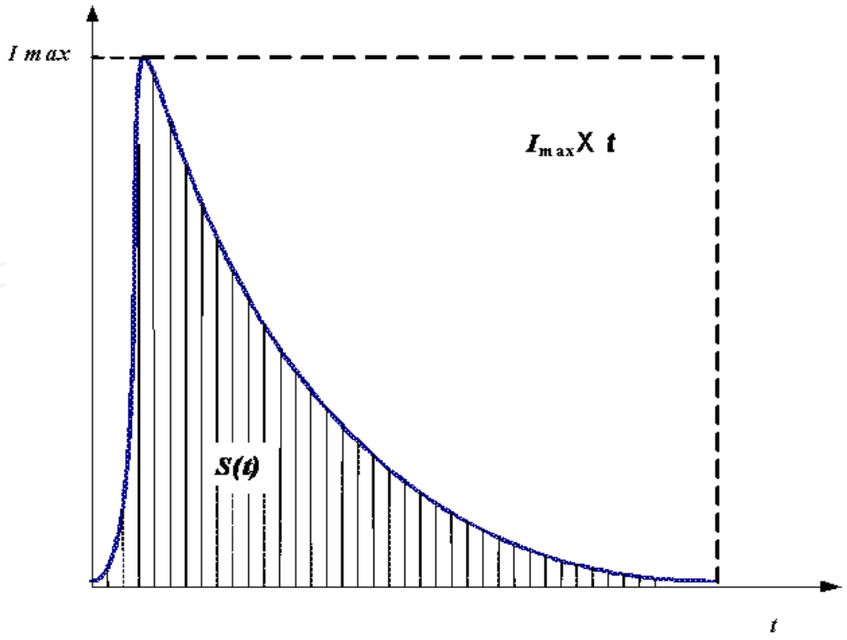

Fig. 1. The kinetic curve of the development of an induced chemiluminescence signal.

3. Results

Two forms of creatine kinase and hexokinase – membrane and soluble – were found in the brain.

The activity of membrane-associated creatine kinase equals to half of the enzyme activity in mitochondria. In contrast hexokinase activity is concentrated on the outer surface of the mitochondrial membrane (Table 1).

Form of the enzyme	Creatine kinase, U/ mg*min.	Hexokinase, U/ mg*min.
Membrane-associated	1.40±0.07 n=23	11.56±0.19 n=12
Soluble	1.78±0.10 n=19	1.53±0.04 n=12

Table 1. The distribution of creatine kinase and hexokinase activity between membrane-associated and soluble forms of the enzymes

Catalytic and kinetic properties of mitochondrial creatine kinase and hexokinase were shown to depend on the interaction with the membrane.

Different solubilizing agents (electrolyte, detergent and the endogenous metabolite glucose-6-phosphate) were used to analyze the character of interaction of hexokinase with the mitochondrial membrane.

All these agents solubilized only a third of the hexokinase activity, and only the sequence of action of electrolyte, detergent and glucose-6-phosphate removed the enzyme from the mitochondrial membrane (Fig. 2). Thus it shows the lability of protein-protein interaction and the possibility of its regulation under the certain pathological conditions.

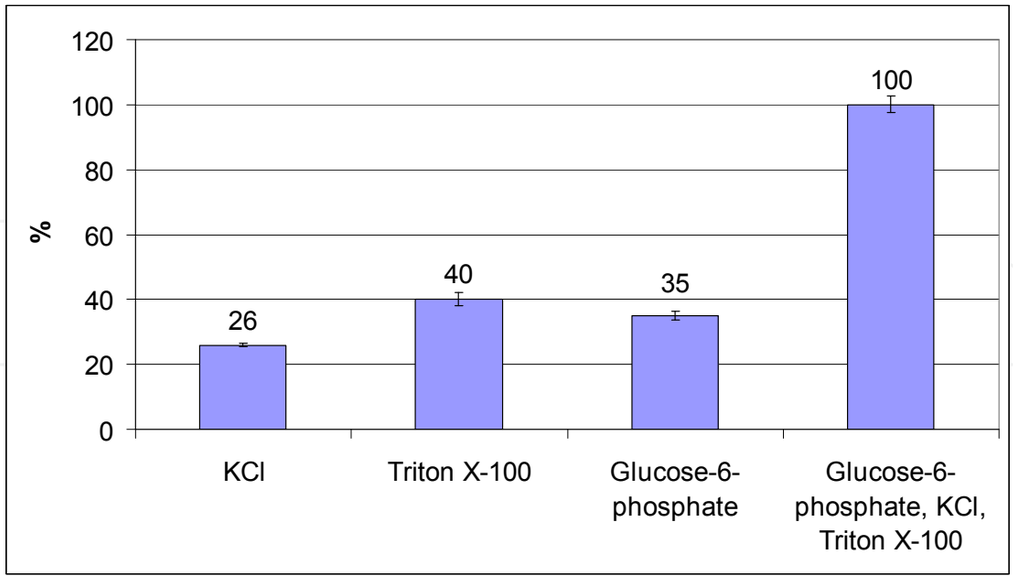

Fig. 2. Solubilization (%) of hexokinase from mitochondrial membranes of intact rats.

Creatine kinase can not dissociate from the mitochondrial membrane even in the presence of the simultaneous action of electrolyte and detergent (Fig. 3).

So the brain creatine kinase exists in different molecular forms: the first – soluble, which is located in the intermembrane space, the second is associated, which is loosely bound to the inner mitochondrial membrane and under the certain solubilizing agents can remove into the intermembrane space, the third form (about 18%) is tightly bound with the membrane.

In mitochondria from intact animals, mitochondrial creatine kinase presents as a mixture of two oligomeric forms (dimer and octamer; 65 and 35%, respectively). We consider that the tightly bound creatine kinase to exist mainly in the contact sites in the octamer form.

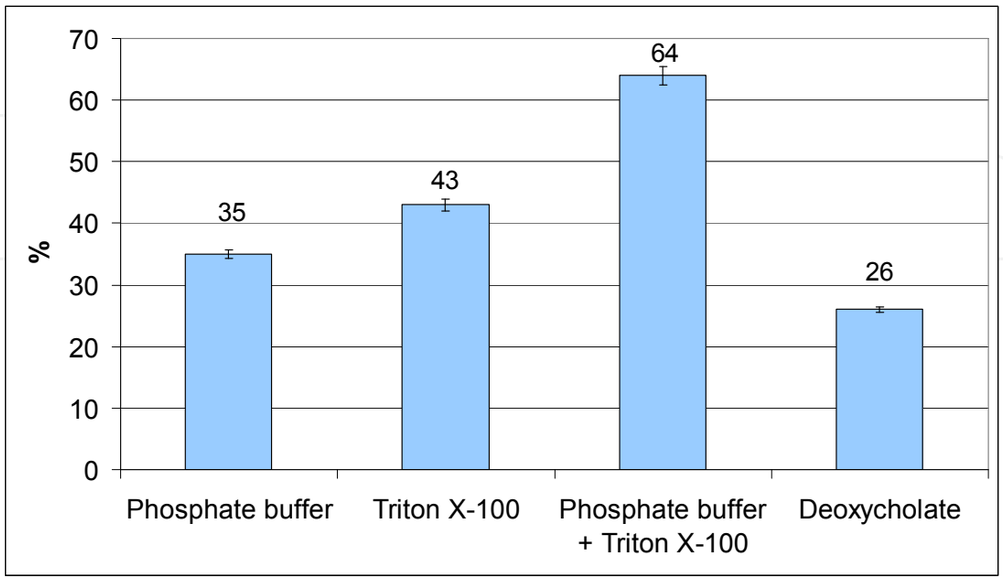

Fig. 3. Solubilization (%) of creatine kinase from mitochondrial membranes of intact rats.

Rebinding of both phosphokinases with the membrane changes their catalytic properties. Binding of hexokinase with the membrane increases the velocity of the reaction in 3 fold, but the kinetic behavior is not changed (Fig. 4).

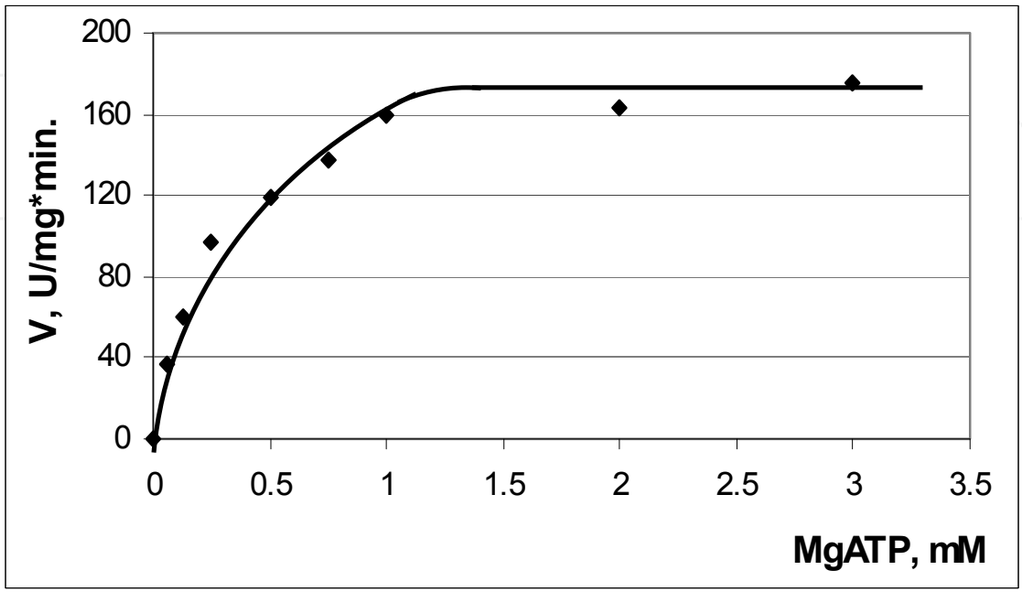

Fig. 4. Dependence of V_0 on concentration of MgATP of membrane-associated hexokinase of intact rats.

Creatine kinase has different types of kinetic behavior (Fig. 5-7). We consider that the membrane associated form of the enzyme binds by ionic interaction with the membrane and the character of the curve reveals the classical kinetic behavior (Fig. 6). The tightly bound

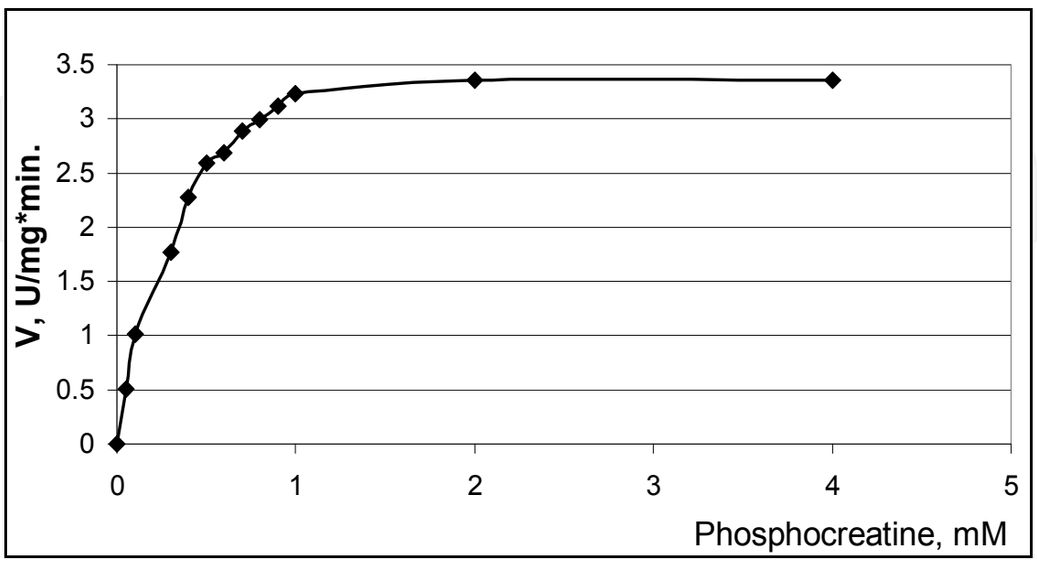

Fig. 5. Kinetic of creatine kinase reaction in total mitochondrial fraction of intact rats.

form of membrane enzyme has the abnormal kinetic behavior due to ionic and hydrophobic interaction (Fig. 7). These data discribe the role of specific microenvironment in the modification of the enzyme properties.

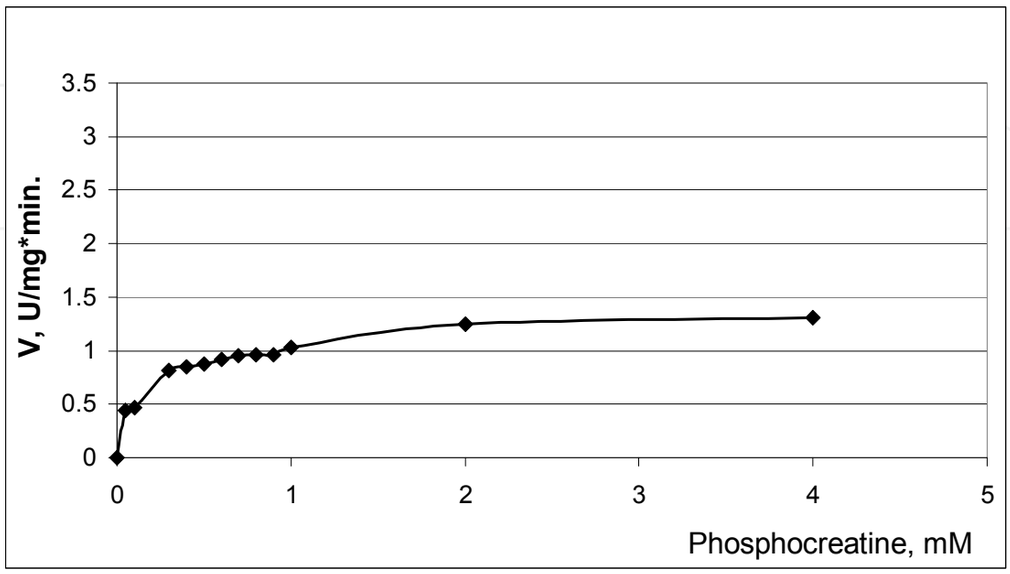

Fig. 6. Kinetic of creatine kinase reaction on mitochondrial membrane of intact rats.

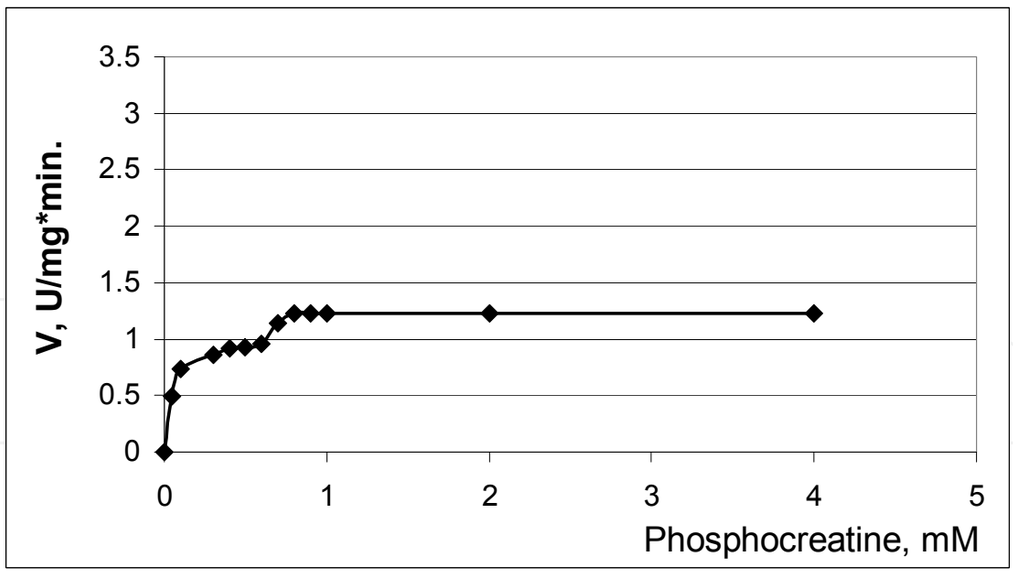

Fig. 7. Kinetic of creatine kinase reaction on mitochondrial membrane after solubilization by phosphate buffer of intact rats.

Thus the catalytic properties depend on the binding with the membrane and this process is controlled by the endogenous metabolites and the functional state of mitochondria.
All forms of hypoxia and ischemia are accompanied by activation of free radical oxidation (Ayer, Zhang, 2008; da-Silva et al., 2004; Kuznetsov, Margreiter, 2009; Meyer et al., 2006;

Wang et al., 2005). As a result of this activation the properties of the mitochondrial membrane-associated enzymes are changed in acute ischemia.
Severe ischemia reduced the binding of the investigated enzymes with the membrane (Fig. 8, 9). The activity of the enzymes decreased 2 fold for creatine kinase and 3 fold for hexokinase. Glucose-6-phosphate and the products of membrane degradation inhibited hexokinase activity in cerebral ischemia (Ishibashi, 1999; Wilson et al., 2000).

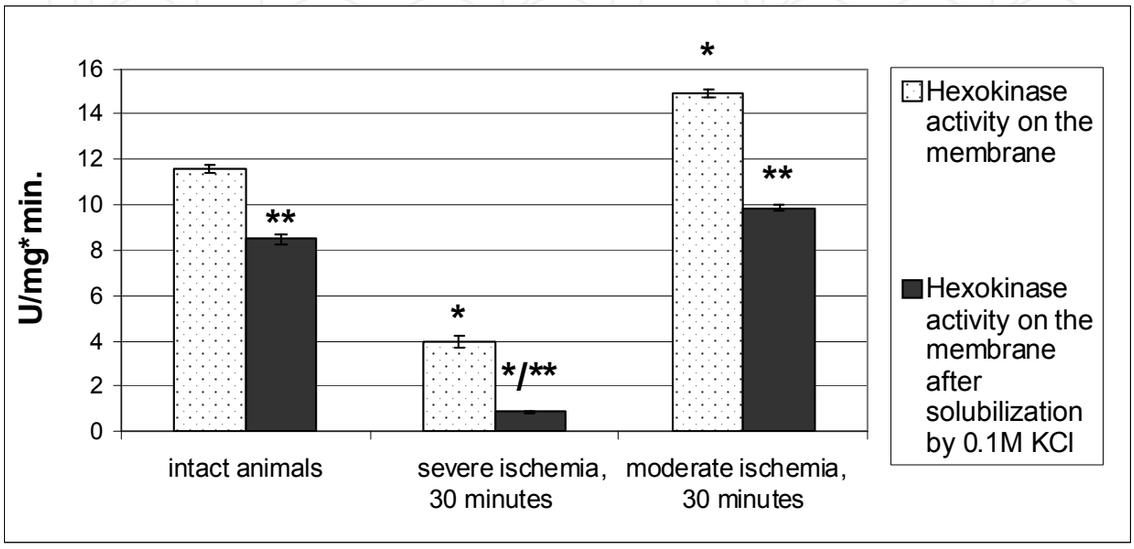

Fig. 8. Membrane associated hexokinase activity on the membrane before and after solubilization by 0.1M KCl of intact rats and in 30 minutes ischemia.

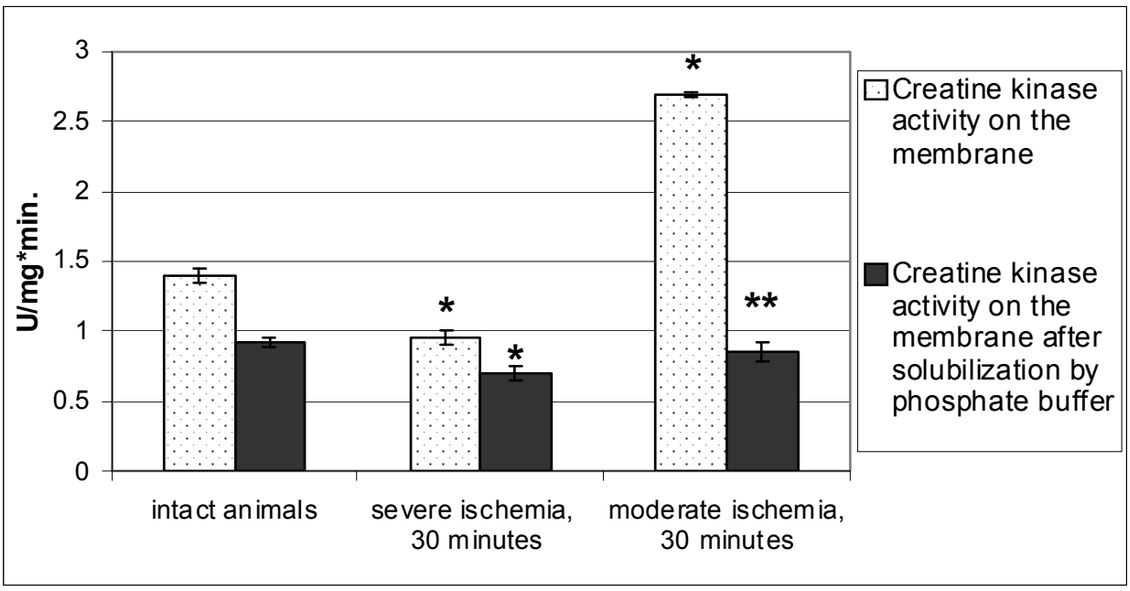

Fig. 9. Creatine kinase activity on the membrane of intact rats and in 30 minutes ischemia before and after solubilization by phosphate buffer.

The bar diagrams display average activities with error bars representing the standard deviation. *p<0.05 versus intact animals. ** p<0.05 versus initial hexokinase activity on the membrane within the same group.
The bar diagrams display average activities with error bars representing the standard deviation. p<0.05 versus intact animals. ** p<0.05 versus initial creatine kinase activity on the membrane within the same group.
In the second group (moderate ischemia) the activity of hexokinase was increased by 29% and by 92% for creatine kinase in comparison with intact animals. After the solubilization of the hexokinase by 0.1 M KCl the enzyme lost 38% of the initial activity. The effect of solubilization for membrane- bound creatine kinase was 69% instead of 35% for the intact rats.
The study of behavior of creatine kinase revealed the modification of its properties in acute ischemia. They differed significantly from those of the intact rats. It is connected with the realization of interconvertible transformation of oligomeric subunits of creatine kinase. This changing in kinetic behavior provides the higher sensitivity of the enzyme to the changes in substrate concentration (Fig. 10-13).

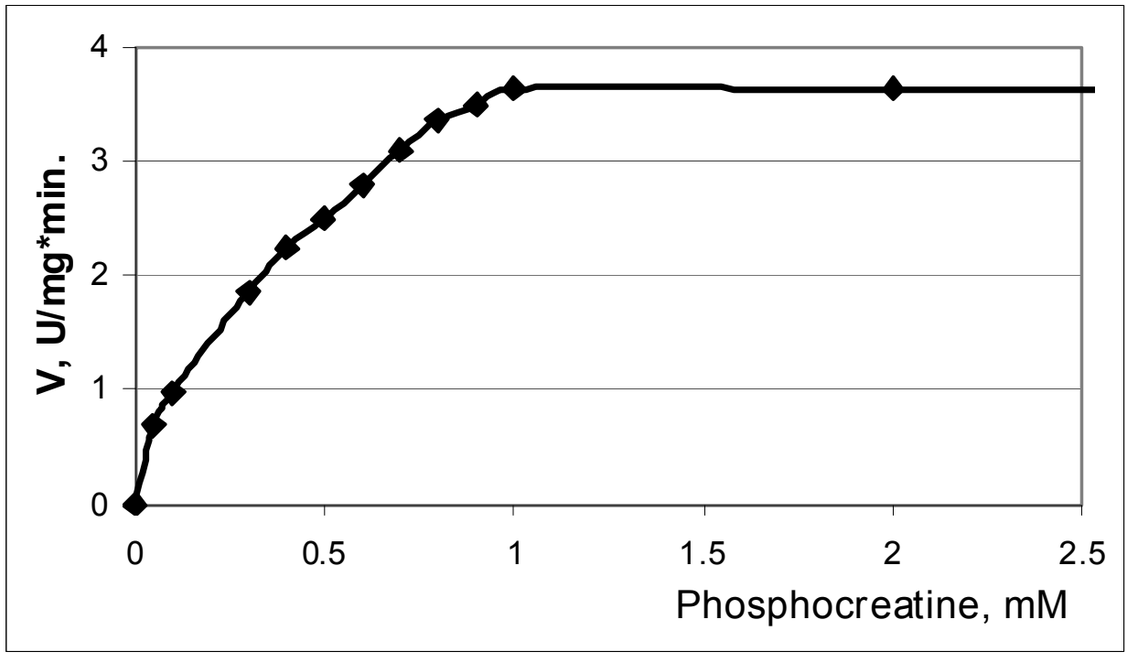

Fig. 10. Dependence of V_0 on concentration of phosphocreatine in total mitochondrial fraction in moderate ischemia

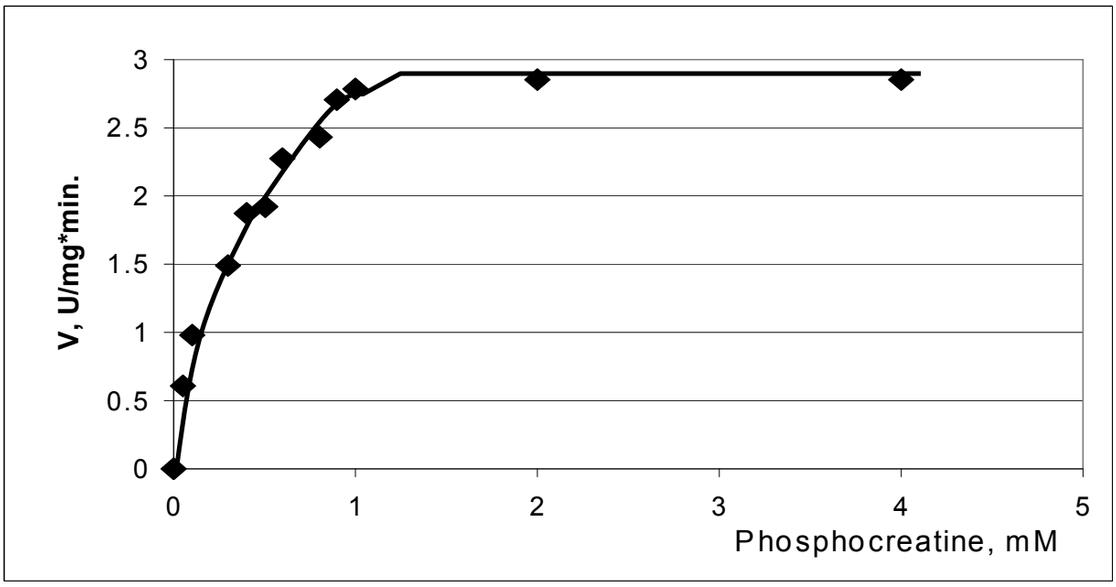

Fig. 11. Dependence of V_0 on concentration of phosphocreatine in fraction of mitochondrial membranes in moderate ischemia

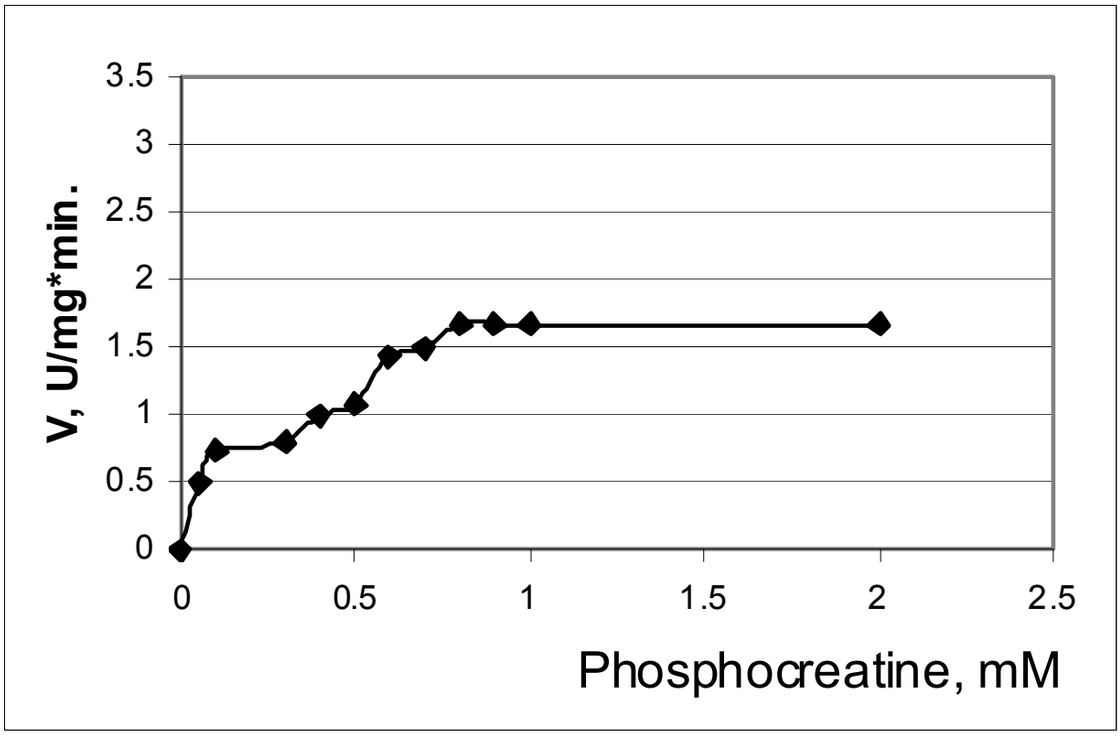

Fig. 12. Dependence of V_0 on concentration of phosphocreatine in total mitochondrial fraction in severe ischemia.

The study of the creatine kinase reaction in the group of animals after severe ischemia showed the abnormal kinetic behavior of the enzyme, the appearance of the intermediate plateau at the low concentration (0.3-0.4 mM) of creatine phosphate, the V_0 decreased 1.4-2 fold in comparison with intact rats.

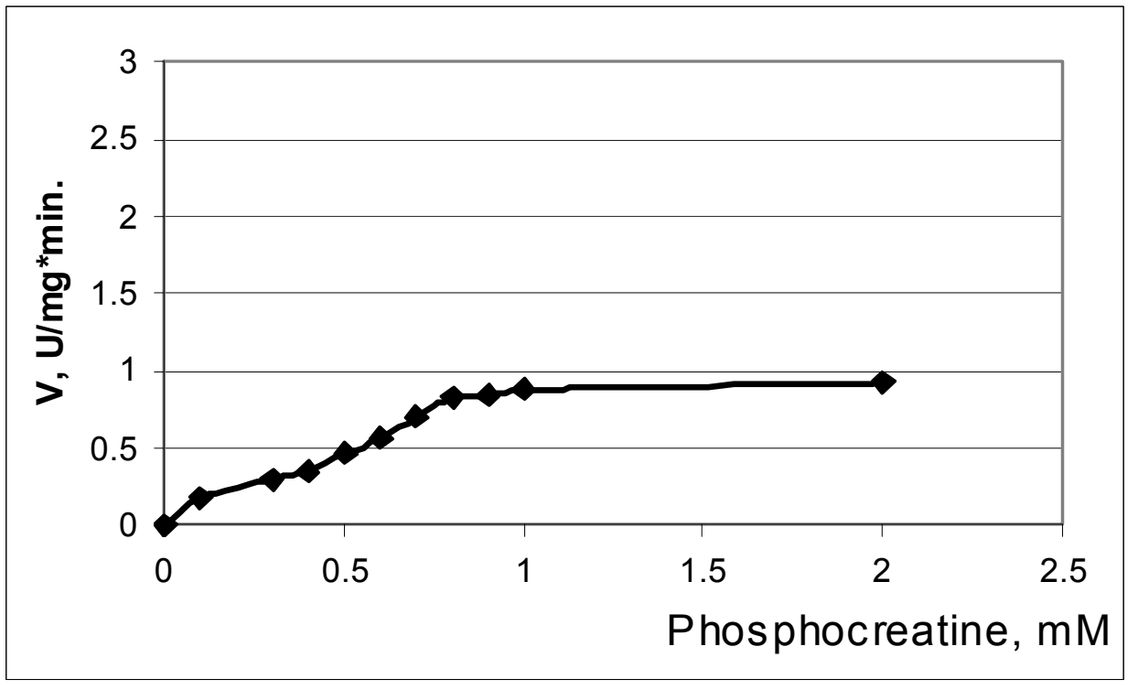

Fig. 13. Dependence of V_0 on concentration of phosphocreatine in fraction of mitochondrial membranes in severe ischemia.

The reversibility of these alterations has been shown during the increasing of duration of ischemia.

Increasing of the duration of cerebral ischemia to 4 and 18 hours was accompanied by changes in activity distribution for hexokinase. The activity of hexokinase progressively increased. The level of activity of the enzyme under these conditions was higher than in acute ischemia. However, the level of hexokinase activity in animals during long-term ischemia remained lower than in intact specimens (Fig. 14).

The bar diagrams display average activities with error bars representing the standard deviation. *p<0.05 versus intact animals. ** p<0.05 versus severe ischemia (30 minutes).

An increase in duration of cerebral ischemia influenced on the adsorption properties of hexokinase. Solubilization of hexokinase by 0.1 KCl was accompanied by decrease in the activity of the enzyme by 78% in acute cerebral ischemia. The percentage of solubilizing enzyme was 37% in 1.5 hours ischemia. It was by 11% higher than in intact specimens. The percentage of solubilizing enzyme was 30% and 27% in 4 hours and 18 hours ischemia, respectively. It did not differ from that in intact animals.

Therefore increase in the duration of cerebral ischemia was followed by an increase in the resistance of membrane structures. These changes were manifested in reduction of hexokinase desorption from the mitochondrial membrane.

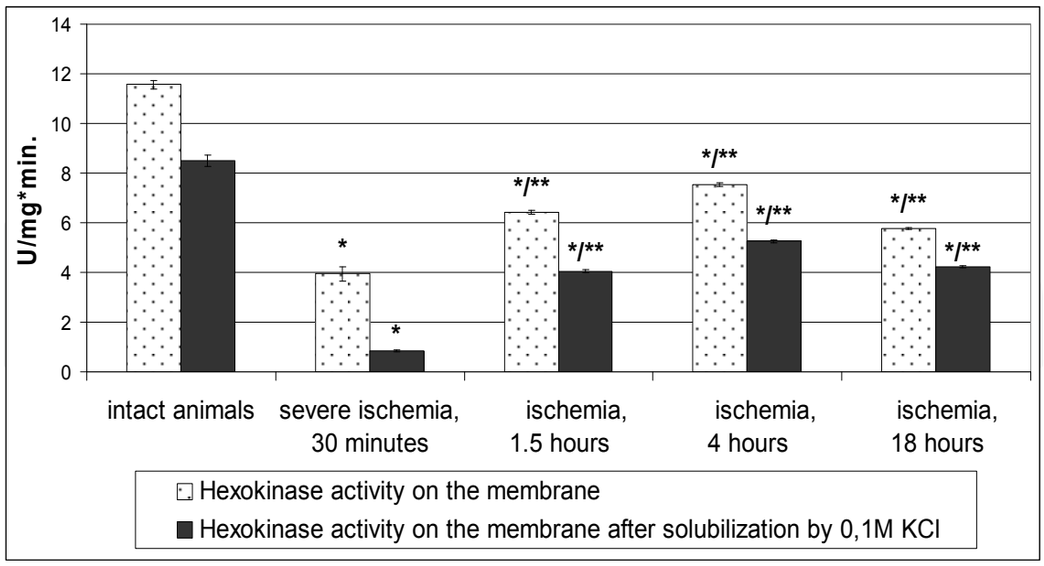

Fig. 14. The activity of membrane-bound hexokinase before and after solubilization by 0.1M KCl in cerebral ischemia

The cerebral ischemia causes the imbalance between reactive oxygen species production and the level of antioxidant defense, which leads to oxidative stress. Neuronal membranes contain a considerable amount of unsaturated lipids. The low level of activity of antioxidant enzymes and formation of free radicals in neurochemical reactions provide conditions for lipid oxidation and induce enzyme modification. To evaluate the state of membranes, the intensity of free radical oxidation and antioxidant properties of the brain tissue were estimated in various periods of ischemia (Table 2).

Experimental groups	Imax, mV	S, imp.*30 sec.	K=1/S
Intact animals	1.02±0.03 n=8	10.64±0.34 n=8	0.094
Ischemia, 30 minutes	1.89±0.07* n=7	17.22±0.98* n=7	0.058
Ischemia, 1.5 hours	1.52±0.04* n=7	15.18±0.91* n=7	0.066
Ischemia, 4 hours	1.47±0.05* n=8	14.55±0.03* n=8	0.068
Ischemia, 18 hours	1.08±0.11 n=8	12.63±0.79* n=8	0.079

*p<0.05 versus intact animals

Table 2. The intensity of free radical oxidation and the activity of antioxidant system in the brain.

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. Total yield of slow flash was 1.6-fold and 1.4-fold higher than in the intact animals in 30 min and 1.5 hours ischemia.

Parameters of free radical oxidation (Imax and S) remained practically unchanged by 4 hours ischemia. The increase in the duration of cerebral ischemia to 18 hours was accompanied by a decrease in the intensity of free radical oxidation. Parameter Imax did not differ from the corresponding parameter in intact animals.

Acute ischemia was not only followed by damage of the cell membrane structures and activation of free radical oxidation, but also induced the antioxidant system (Dziennis et al., 2008; Lai et al., 2003; Perez-Pinzon et al., 2005; Suzuki et al., 1997).

The K index serves as a criterion for the antioxidant potential of the cell. The level of the antioxidant activity of the brain tissue was elevated after ischemia for 1.5 and 4 hours. The conclusion was derived from the decrease in this index. By 18 hours ischemia, the K index did not differ from that in intact animals.

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 kinetic curve for hexokinase was shown to have hyperbola form in various periods of ischemia except for 1.5 hours ischemia (Fig. 15). MgATP did not inhibit hexokinase when increasing the duration of ischemia. By 18 hours, the Km (0.13 mM) is 2-fold lower than in intact animals (0.26 mM) and is 5-fold lower than in 30 minutes ischemia (0.7 mM).

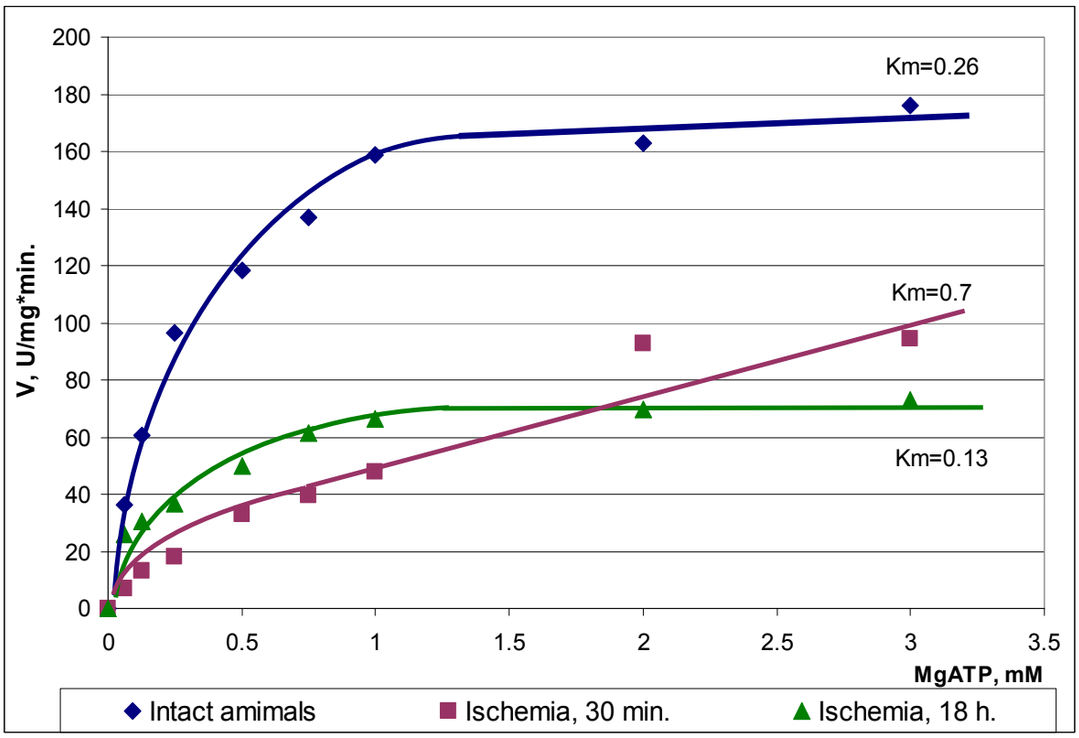

Fig. 15. Kinetic of the hexokinase reaction.

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.

Mitochondrial creatine kinase is associated with mitochondrial membranes due to the forces of electrostatic and hydrophobic interaction. Cerebral ischemia was followed by changes in the activity of associated and tightly-bound mitochondrial creatine kinase (Fig. 16).
The activity of associated mitochondrial creatine kinase increased in comparison with intact animals in 1.5 hours ischemia. However the activity of tightly-bound creatine kinase did not change. The activity of tightly-bound mitochondrial creatine kinase increased and the ratio between two forms of mitochondrial creatine kinase restored in 4 hours ischemia. By 18 hours, the percentage of tightly-bound form of the enzyme reached 90%.

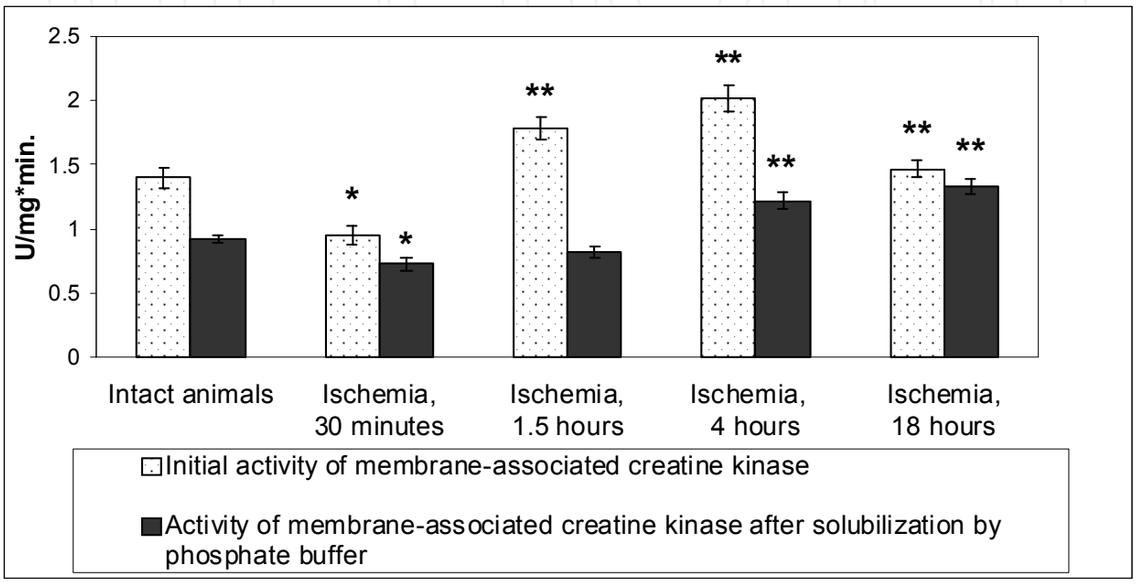

Fig. 16. The activity of membrane-associated creatine kinase before and after solubilization by phosphate buffer in cerebral ischemia.

The bar diagrams display average activities with error bars representing the standard deviation. *p<0.05 versus intact animals. ** p<0.05 versus severe ischemia (30 minutes).
Cerebral ischemia was shown to change the kinetic properties of mitochondrial creatine kinase (Fig. 17). Mitochondrial creatine kinase showed abnormal kinetic with the appearance of intermediate plateau. By 18 hours, the kinetic curve acquired a hyperbola form.
In mitochondria, mitochondrial creatine kinase is presented by two oligomeric forms (dimer and octamer). They are characterized by dynamic equilibrium (Lipskaya et al., 1989). The transition-state analogue complex of mitochondrial creatine kinase was induced to evaluate the ratio between oligomeric forms of this enzyme under conditions of cerebral circulatory disorders.
Cerebral ischemia changes the dimer/octamer ratio. This ratio is shifted toward the formation of dimers after 30-min ischemia (79%). Phospholipids serve as the structural elements of membranes that are bound to mitochondrial creatine kinase. Membrane binding properties of mitochondrial creatine kinase depend strongly on the protein dimer/octamer ratio and degree of lipid oxidation. Activation of free radical oxidation during acute ischemia is probably followed by partial dissociation of octamers to dimers. Increasing of the duration of ischemia to 18 hours was followed by an increase in the octamer ratio (53%).

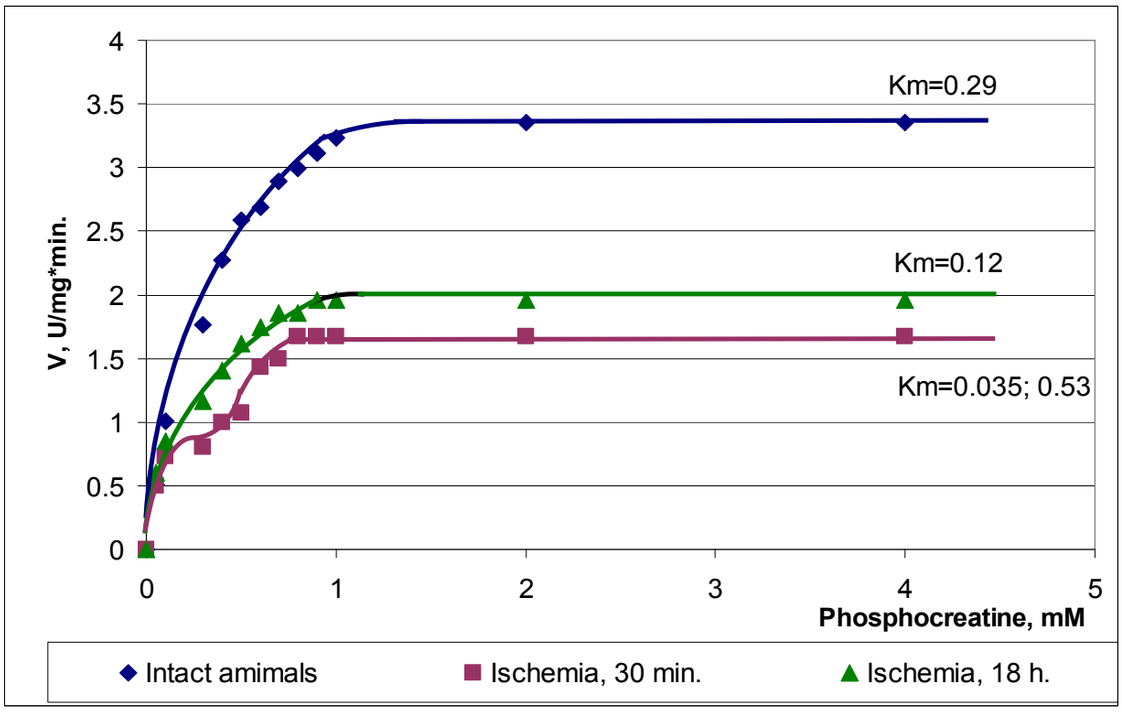

Fig. 17. Kinetic of the membrane-bound creatine kinase reaction

Published data suggest that octameric mitochondrial creatine kinase 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 (Gross, Wallimann, 1995; Koufen et al., 1999; Lenz et al., 2007; Meyer et al., 2006). The existence of two oligomeric forms of this enzyme probably maintains the near-equilibrium state of reaction in a wide range of physiological conditions.

4. Conclusion

The results indicate that catalytic properties of mitochondrial creatine kinase and hexokinase depend on the functional interaction with mitochondrial membranes.

Acute ischemia impairs enzyme interaction with the mitochondrial membrane. Increasing in the duration of ischemia is not only followed by injury and dysfunction, but also activates the defense systems in the nervous tissue. It is manifested in the decrease in the intensity of free radical oxidation, increase in the percentage of tightly-bound mitochondrial creatine kinase, changes in kinetic properties of the enzyme and change in the dimer/octamer ratio toward the formation of octamer for the mitochondrial creatine kinase. These changes stabilize the mitochondrial creatine kinase complex. In contrast, increase in the duration of ischemia is accompanied by the decrease in the hexokinase activity on the membrane in spite the fact that it becomes higher than in acute 30 min ischemia, but the percentage of solubilizing enzyme does not differ from that in intact animals.

Therefore, the long-term ischemia leads to stabilization of the functional interaction between hexokinase and creatine kinase complex with the mitochondrial membranes at a new level,

providing the adequate energy supply of the nervous cells due to the new adaptive conditions.

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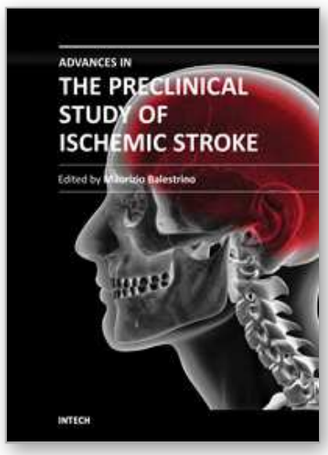

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This book reports innovations in the preclinical study of stroke, including - novel tools and findings in animal models of stroke, - novel biochemical mechanisms through which ischemic damage may be both generated and limited, - novel pathways to neuroprotection. Although hypothermia has been so far the sole "neuroprotection" treatment that has survived the translation from preclinical to clinical studies, progress in both preclinical studies and in the design of clinical trials will hopefully provide more and better treatments for ischemic stroke. This book aims at providing the preclinical scientist with innovative knowledge and tools to investigate novel mechanisms of, and treatments for, ischemic brain damage.

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