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ORIGINAL PAPER

The Study of Na^+, K^+-ATPase Activity of Rat Brain during Crush Syndrome

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Abstract Crush syndrome (CS) results from severe traumatic damage to the organism that is characterized by stress, acute homeostatic failure of the tissues, and myoglobinuria with severe intoxication. This leads to an acute impairment of kidneys and heart. The peripheral and central nervous systems are also the subject of significant changes in CS. Na^+, K^+-ATPase is a critical enzyme in neuron that is essential for the regulation of neuronal membrane potential, cell volume as well as transmembrane fluxes of Ca^{++} and Excitatory Amino Acids. In the present study, Na^+, K^+-ATPase activity of rat brain regions [Olfactory lobes (OL), Cerebral cortex (CC), Cerebellum (CL), and Medulla oblongata (MO)] during CS was investigated. Experimental model of CS in albino rats was induced by 2-h of compression followed by 2, 24, and 48-h of decompression of femoral muscle tissue. In this study, we have observed elevation in Na^+, K^+-ATPase activity above normal/control levels in all parts of brain (OL: 34.4%; CC: 1.0%; CL: 3.3% and MO: 45%) during 2-h compression in comparison to controls.

Keywords Na^+ · K^+-ATPase in crush syndrome · ATPases in brain

Introduction

Crush syndrome (CS) is a composite pathological condition developed after compression failure of skeletal soft tissues that results from severe traumatic damage to the organism characterized by stress, pain, acute hemodynamic shock, myoglobinuria, and total intoxication [1, 2]. Serious damage has been observed in the function and structure of kidneys. In most cases, such disturbances lead to a high level of mortality, which is caused by acute heart failure or acute renal insufficiency [3].

The peripheral and central nervous systems are also the subject of significant changes in CS. Facial nerve crush during different stages of postnatal development causes loss of neurons, showing a direct correlation between the injury and the age of the experimental animals [4]. The most marked changes in the nervous system have been found during the decompression period. Quantitative and ultrastructure alterations, as well as neuron cell death has been discovered 3–6 months after the crushing of the sciatic nerve, depending on the time and force of crushing [5]. The most important damage resulting from the crushing of the spinal cord is axonal damage.

Na^+, K^+-ATPases' isozymes exhibit a complex pattern of expression and structural heterogeneity, which is stringently regulated allowing the enzyme activity to match the cell requirements under the physiological and pathological conditions. Na^+, K^+-ATPase activity is known to decrease in cerebral ischemia [6], in epilepsy [7], and in various neurodegenerative disorders [8]. However, there is hardly any report on changes in Na^+, K^+-ATPase activity during CS. Na^+, K^+-ATPase is a critical enzyme in neurons which is likely to be affected by CS [5] and is also essential for the regulation of neuronal membrane potential, cell volume [9] as well as transmembrane fluxes of Ca^{++} and Excitatory Amino Acids whose transport is linked to that of Na^+ [8]. Therefore, it was felt appropriate to investigate the changes in Na^+, K^+-ATPase activity from four functionally important regions of brain viz. olfactory lobes (OL), cerebral cortex (CC), cerebellum (CL), and medulla oblongata (MO) of rats subjected to CS.

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Experimental procedure

Experiments were carried out on Swiss albino rats (100–150 g). CS was induced by unilateral compression of femoral tissues of anesthetized rats on a special press exerting a force of 100 kg per kg of animal weight, for a period of 2 h. The animals were divided into five groups. Each group consisted of five animals, Group I: an intact group, without exposure to compression; Group II: exposed to 2 h of compression; Group III: exposed to 2 h of compression, followed by decompression for 2 h; Group IV: exposed to 2 h of compression, followed by decompression for 24 h; Group V: exposed to 2 h of compression, followed by decompression for 48 h.

Preparation of synaptic plasma membrane from brain

The whole brain was rapidly removed and placed in ice-cold 0.32 M sucrose, blotted dry and immediately transferred to an ice chamber. Then OL, CC, CL, and MO were dissected to prepare synaptic plasma membranes according to the method of Jones and Matus [10], with some modifications. Each brain tissue was homogenized in ten volumes of a 0.32 M sucrose solution containing 5 mM HEPES and 1 mM EDTA. The homogenate was centrifuged at 1,000 *g* for 20 min and the supernatant removed and centrifuged at 12,000 *g* for a further 20 min. The pellet was then resuspended in hypotonic buffer (5.0 mM Tris–HCl buffer and pH 8.1), incubated at 0°C for 30 min, and applied on a discontinuous sucrose density gradient consisting of successive layers of 0.3, 0.8, and 1.0 M. After centrifugation at 69,000 *g* for 2 h, the fraction at the 0.8–1.0 M sucrose interface was taken as the membrane enzyme preparation.

Mg^{2+}-ATPase and Na^+, K^+-ATPase activity assay

The reaction mixture for the Mg^{2+}-ATPase and the Na^+, K^+-ATPase assays contained 5.0 mM $MgCl_2$, 80.0 mM NaCl, 20.0 mM KCl, and 40.0 mM Tris–HCl buffer, pH 7.4, in a final volume of 200 ml. The reaction was started by the addition of ATP (disodium salt, vanadium free) to a final concentration of 3.0 mM. Mg^{2+}-ATPase was assayed under the same conditions with the addition of 1.0 mM ouabain. Na^+, K^+-ATPase activity was calculated by the difference between the two assays [11]. Released inorganic phosphate (Pi) was measured by the method of Gomorri [12]. Enzyme specific activities were expressed as μ mol Pi released per min per mg of protein.

Protein determination

Protein was measured by the method of Lowry et al. [13] using bovine serum albumin as standard.

Statistical analysis

All values represented in graph are expressed as mean ± SD. Statistical significance was determined by using Student's *t*-test to compare respective controls against experimental groups. $P < 0.05$ was considered statistically significant. One-way and two-way ANOVA was done with the help of Analyze-it Program for General and Clinical statistics Version 1.73.

Results

In general the CS has promoted increase in Na^+, K^+-ATPase activity in all four regions of brain of rats.

Olfactory lobes: Na^+, K^+-ATPase of OL increased in Group II (2-h compression) by 34.5% (*t*-test and $P < 0.001$), 47.1% (*t*-test and $P < 0.001$) in Group III (2-h compression followed by 2-h decompression), 1.3-fold (*t*-test and $P < 0.001$) in Group IV (2-h compression followed by 24-h decompression), and 41.4% (*t*-test and $P < 0.001$) in Group V (2-h compression followed by 48-h decompression) in comparison to Group I rats (Fig. 1).

Mg^{2+}-ATPase activity of OL decreased after exposure to compression and compression followed by decompression periods (*t*-test-Group II: 0.39-fold and $P < 0.001$; Group III: 0.6-fold and $P < 0.001$; Group IV: 0.63-fold and $P < 0.001$; Group V: 0.77-fold and $P < 0.001$).

Changes in Na^+, K^+-ATPase activity due to compression, compression followed by decompression (against plain control) was significant at $P < 0.0001$ (one-way ANOVA: 4, 24; $F = 1534.51$). Variations in Na^+, K^+-ATPase activity due to compression only (against plain control) was significant at $P < 0.0001$ (one-way ANOVA: 1, 9; $F = 1425.08$). Variations in Na^+, K^+-ATPase activity during decompression periods (against Group II) were significant at $P < 0.0001$ (one-way ANOVA: 3, 19; $F = 1534.51$).

Cerebral cortex: all groups showed significant elevations in Na^+, K^+-ATPase activity of CC (*t*-test-Group II: 1% and $P < 0.001$; Group III: 83.2% and $P < 0.001$; Group IV: 1.2-fold and $P < 0.001$; Group V: 26.7% and $P < 0.001$) in comparison to Group I (plain control). However, Mg^{2+}-ATPase activity showed 0.65-fold (*t*-test and $P < 0.001$) decrease in Group II, 0.53-fold (*t*-test and $P < 0.001$) decrease in Group III, 0.74-fold (*t*-test and $P < 0.001$) decrease in Group IV, and 0.82-fold (*t*-test and $P < 0.001$) decrease in Group V (Fig. 2).

Changes in Na^+, K^+-ATPase activity due to compression, compression followed by decompression (against plain control) was significant at $P < 0.0001$ (one-way ANOVA: 4, 24; $F = 2304$). Variations in Na^+, K^+-ATPase activity due to compression only (against plain control)

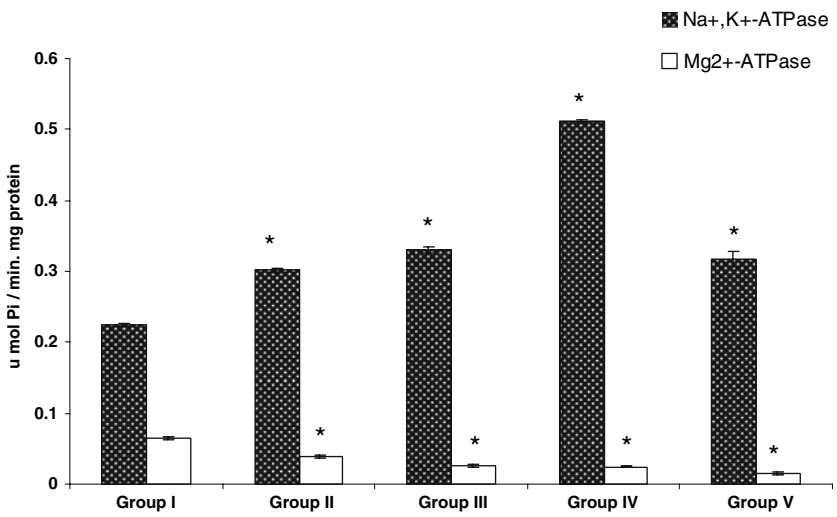

Fig. 1 Changes in Na^+, K^+-ATPase activity, Mg^{+2}-ATPase activity of olfactory lobes of rats subjected to compression and decompression. Data are mean ± SD for five independent experiments performed. *Asterisk* different from control (Group I), $P < 0.001$ (Student's *t*-test)

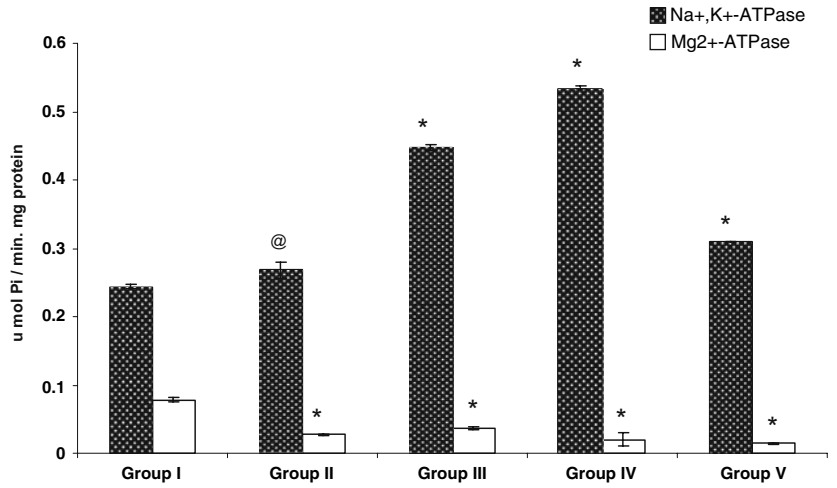

Fig. 2 Changes in Na^+, K^+-ATPase activity, Mg^{+2}-ATPase activity of cerebral cortex of rats subjected to compression and decompression. Data are mean ± SD for five independent experiments performed. *Asterisk* different from control (Group I), $P < 0.001$; @, $P < 0.005$ (Student's *t*-test)

was significant at $P = 0.0018$ (one-way ANOVA: 1, 9; $F = 20.97$). Variations in Na^+, K^+-ATPase activity during decompression periods (against Group II) were significant at $P < 0.0001$ (one-way ANOVA: 3, 19; $F = 1858.36$).

Cerebellum: the cerebellar Na^+, K^+-ATPase activity increased by 3.3% (*t*-test and $P < 0.001$) in rats of Group II, 1.1-fold (*t*-test and $P < 0.001$) in Group III, 79.4% (*t*-test and $P < 0.001$), and 86.4% (*t*-test and $P < 0.001$) in rats of Groups IV and V, respectively (Fig. 3). Whereas, cerebellar Mg^{2+}-ATPase activity reduced in Group II (0.64-fold; *t*-test and $P < 0.001$), Group III (0.41-fold; *t*-test and $P < 0.001$), Group IV (0.63-fold; *t*-test and $P < 0.001$), and Group V (0.66-fold; *t*-test and $P < 0.001$) in comparison to Group I.

Changes in Na^+, K^+-ATPase activity due to compression, compression followed by decompression (against plain control) was significant at $P < 0.0001$ (one-way ANOVA: 4, 24; $F = 3060.51$). Variations in Na^+, K^+-ATPase activity due to compression only (against plain control) was significant at $P = 0.0008$ (one-way ANOVA: 1, 9; $F = 27.047$). Variations in Na^+, K^+-ATPase activity during decompression periods (against Group II) were significant at $P < 0.0001$ (one-way ANOVA: 3, 19; $F = 2112.71$).

Medulla oblongata: Groups II, III, IV, and V rats showed 0.45, 1.38, 2.31, and 2.13-fold increase (*t*-test and $P < 0.001$) in Na^+, K^+-ATPase activity of MO, respectively, in comparison to Group I (Fig. 4). MO of Groups II, III, IV, and V showed 48% (*t*-test and $P < 0.001$), 34% (*t*-test and $P < 0.001$), 55% (*t*-test and $P < 0.001$), and 48% (*t*-test and $P < 0.001$) reduction of Mg^{2+}-ATPase activity in comparison to Group I (Fig. 4).

Changes in Na^+, K^+-ATPase activity due to compression, compression followed by decompression (against plain control) was significant at $P < 0.0001$ (one-way ANOVA: 4, 24; $F = 22446.21$). Variations in Na^+, K^+-ATPase activity due to compression only (against plain control) was significant at $P < 0.0001$ (one-way ANOVA: 1, 9; $F = 2302.56$). Variations in Na^+, K^+-ATPase activity

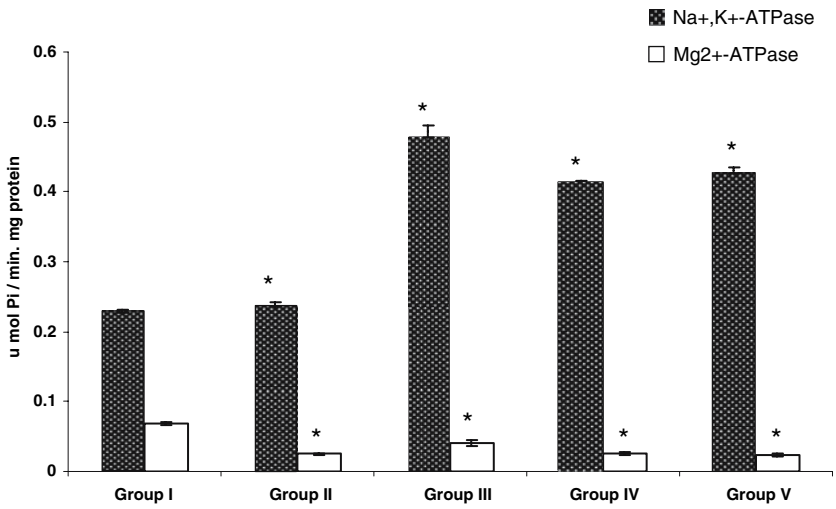

Fig. 3 Changes in Na^+, K^+-ATPase activity, Mg^{+2}-ATPase activity of cerebellum of rats subjected to compression and decompression. Data are mean ± SD for five independent experiments performed. *Asterisk* different from control (Group I), $P < 0.001$ (Student's *t*-test)

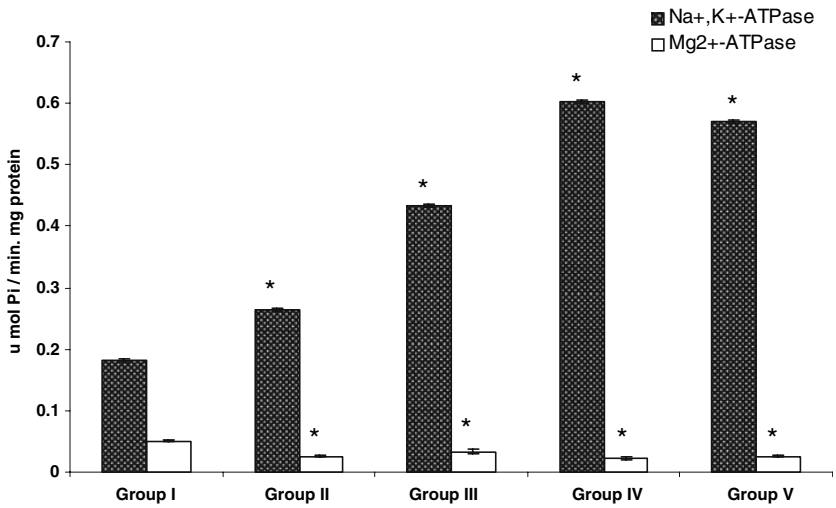

Fig. 4 Changes in Na^+, K^+-ATPase activity, Mg^{+2}-ATPase activity of medulla oblongata of rats subjected to compression and decompression. Data are mean ± SD for five independent experiments performed. *Asterisk* different from control (Group I), $P < 0.001$ (Student's *t*-test)

during decompression periods (against Group II) were significant at $P < 0.0001$ (one-way ANOVA: 3, 19; $F = 13831.45$).

Besides, two-way ANOVA of changes observed in Na^+, K^+-ATPase activity between all the groups (Groups I, II, III, IV, and V) and all four brain regions (OL, CC, CL, and MO) were significant at $P < 0.0001$ (12, 99; $F = 1095.58$).

Two-way ANOVA of reductions observed in Mg^+-ATPase activity between all the groups (Groups I, II, III, IV, and V) and all four brain regions (OL, CC, CL, and MO) were significant at $P < 0.0001$ (12, 99; $F = 31.94$).

Discussion

The pathology of the CS appears after the compression of soft tissues. CS results from severe traumatic damage to the organism, which is characterized by, stress, acute homeostatic failure of the tissues, and myoglobinuria with severe intoxication. This leads to an acute impairment of kidneys and heart. However, the nervous system does not escape damage. The peripheral and CNS are significantly affected [14]. Facial nerve crush injury during postnatal development promotes neuronal injury or loss indicating correlation between the injury and age of experimental animal [4]. Most of the significant changes in CNS are found during decompression period. Even, 3–6 months after the crushing of sciatic nerve, several ultrastructural changes and neuronal death are observed, depending upon the time and force of crushing [5]. The development of CS is associated with stress which leads to many biochemical changes in CNS such as: (1) alterations in protein synthesis [15], (2) Changes in alanine–glutamate ratio of brain [16], (3) increase in adenosine deaminase activity [17]. In the present study, we are reporting the changes occurring in Na^+, K^+-ATPase Activity. Brain witnesses active oxidative metabolism mostly required to maintain cellular Na^+/K^+ gradients crucial for nerve impulse propagation, neuro-

transmitter release, and cation homeostasis [9]. Na^+ exit from neurons requires its movement against a concentration gradient and membrane potential through Na^+ pump. Na^+ pump and Na^+, K^+-ATPase enzyme are mechanical and chemical manifestations of one system, which is required to prevent cell membrane rupture due to osmotic pressure [18]. In the present study, we have observed increase in Na^+, K^+-ATPase activity above normal/control levels in all parts of brain during compression and decompression phases of CS. Due to the compression related stress on soft tissue and sciatic nerve, non-selective stretch/stress, channels of neurons might have activated, partly also due to high-affinity glutamate uptake by brain due to CS [14]. Opening of these channels would flood the cytosol of the neurons with cations like Ca^{++} and Na^+ down their chemical gradients followed by water causing cell swelling, thereby activating Na^+–K^+ pumps to a maximum extent to restore the cell volume. Also, the release of some substances due to stress produced by compression could be contributing to the changes in Na^+, K^+-ATPase, and Mg-ATPase activities. Similar cascade of events have been reported for myocytes in traumatic rhabdomyolysis by Better and Stein [19]. The elevation of Na^+, K^+-ATPase activity also indicates the well being of neuronal plasma membrane and synaptic plasma membranes. Maintenance of Na^+, K^+-ATPase activity is critical for normal brain function and increase in Na^+, K^+-ATPase indicate elevated brain function. Particularly during CS the compressed nerves could be sending rapid signals of stress thereby activating brain to release some endogenous peptides, which could be modulating ATPase activity. The increase of Na^+, K^+-ATPase activity at the end of 2-h decompression by many times to that observed in compression phase of CS as well as to that found in controls indicate a rapid attempt by the neurons to restore the normalcy of cell volume along with the mitigation of the effects of toxic substances distributed in the brain owing to stress related compromises of blood-brain barriers. Subsequently the degree of cell swelling and number of affected neurons as well as number of neurons restored to normalcy would decide the degree of activation vis-à-vis fluctuations of Na^+, K^+-ATPase activities.

Mg^{2+}-ATPase is an important energy linked enzyme and the inhibition of which reduces oxidative phosphorylation. In cells, intracellular concentration of Mg^{2+}-ATPase is always found at higher concentration and the energy molecules are found as Mg-ATP^{2-}. ATP takes a very important role in many enzymatic reactions by releasing its phosphate group and consequently the synthesis of ATP is always equal to that of its break down because cells are known to maintain constant level of energy molecules in a steady state. Results of the present study show inhibition of Mg^{2+}-ATPase activity in all four-brain regions of rats subjected to crush in comparison to control groups. Inhibition in the activity of Mg^{2+}-ATPase suggests induction of energy crisis through disruption in oxidative phosphorylating process. The level at which Mg^{2+}-ATPase inhibited well indicates that compression has disrupted the balance that is maintained between equal rate of synthesis and utilization of energy. Under stressful conditions, animals are known to utilize large quantity of energy in order to withstand the impact of stress. The results indicate that the rate of utilization of energy exceeds the rate of energy synthesis under stress. This augments the energy crisis leading to non-availability of energy or less energy for carrying out normal physiological activities. It appears that the energy crisis induced by compression through inhibition of Mg^{2+}-ATPase further affects ATPase systems and disrupts ionic movements, which normally affects nerve impulse transmission. The results of Mg^{2+}-ATPase showing significant inhibition in its activity in all brain regions explains the level at which energy producing system is made ineffective to produce energy for amine synthesizing neurons and active transport [20, 21]. The inhibition of Mg^{2+}-ATPase during CS could be attributed to some substance/s released during the stress produced by compression, which could be reacting with the Mg component of Mg^{2+}-ATPase. However, it needs further investigation to identify such substances and their mechanism of action on ATPases in general.

Conclusions: (1) Na^+, K^+-ATPase activity elevates during compression in all regions of brain except. (2) 2 and 24 h of decompression promotes many fold increase of Na^+, K^+-ATPase activity in all regions of brain except OL. (3) CS inhibits Mg^{2+}-ATPases.

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