ბინოიდების პოტენციური ეფექტურობა ტკივილისა და ნევროლოგიური დარღვევების შემსუბუქებაში. თუმცა, ენდოკანაბინოიდური სისტემის მონაწილეობა ასევე ასოცირდება არასასურველ გვერდით მოვლენებთან, მათ შორის აღსანიშნავია შემეცნებითი და ემოციური ფუნქციების დათრგუნვა, ტოლერანტობისა და დამოკიდებულების განვითარება და პრეპარატის მოხსნის სინდრომი.

კვლევის მიზანი იყო იმის დადგენა, განუვითარდებოდათ თუ არა მამრ თაგვებს ტოლერანტობა დელტა-9-ტეტრაჰიდროკანაბინოლითა (THC)- და კანაბინოლის მჟავით (CBNA)-ინდუცირებული ანტინოციცეფციის მიმართ.

მექანიკური და ტემპერატურის ქცევითი ტესტების გამოყენებით გამოვლინდა, რომ THC და CBNA სისტემურ (ინტრაპერიტონეალური) შეყვანამ გამოიწვია ძლიერი ანტინოციცეფცია ექსპერიმენტის პირველ დღეს. თუმცა, მომდევნო ოთხი დღის განმავლობაში ქცევითი მაჩვენებლები, რომლებიც დაკავშირებულია მექანიკურ და თერმულ სტიმულებთან, თანდათან დაქვეითდა, რაც მიუთითებს ტოლერანტობის განვითარებაზე ამ პრეპარატების მიმართ.

ამრიგად, კანაფის ორი ძირითადი კომპონენტი, THC და CBNA, ხასიათდება თაგვებში ტოლერანტობის განვითარებით მათი განმეორებითი ინტრაპერიტონეალური ინიექციებისას.

Na, K-ATPase AND Cl-ATPase REGULATION BY DOPAMINE

Chkadua G., Tsakadze L., Shioshvili L., Nozadze E.

Iv. Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia

The neurotransmitter (NT)-dependent regulatory system of Na, K-ATPase, and Cl-ATPase have been discovered in the synaptic membranes of the rat brain. This regulation is implemented with noradrenaline (NA), dopamine (DA), serotonin (5HT), and acetylcholine (ACh). The action of NA, DA, and 5HT, in turn, is regulated by the factor found in the synaptosomal cytosol fraction (SFa). The addition of SFa abolishes Na, K-ATPase inhibition induced by the action of the above-mentioned NTs, leading to the enzyme activation [9,6].

Regulation of the Cl-ATPase and Na/K-ATPase with an NT and SFa has functional importance because it is specific for the synaptic membranes, while SFa is localized exclusively in the synaptic cytosol. The effect of NT and SFa action on the Na, K-ATPase is characterized by tissue specificity [9,3]. The effect is different in various regions of the brain and different types of synapses [9]. The ratios of activatory/inhibitory mechanisms and their depths at different stages of ontogenesis and in the animals of different habitats vary as well [10]. However, the molecular mechanisms of the NT and SFa action on Cl-ATPase and Na, K-ATPase are yet unknown. Clarification of these mechanisms will provide more information on the functional role of this regulation.

The material presented in this work is an endeavor to elucidate the above problem; we have investigated the action of dopamine on Cl-ATPase and Na, K-ATPase transport stoichiometry.

Material and methods. The synaptic fraction from rat brains served as the Cl-ATPase and Na, K-ATPase preparation, which was collected between the 1.2-0.9 *M* sucrose layers [2]. The protein concentration was evaluated by the Lowry method [14], the inorganic phosphorus by the modified Fiske-Subbarow [4], and the Kazanova-Maslova method [7]. The Na, K-ATPase activity (*V*) was assessed as the ouabain-sensitive part of the total ATPase activity, in μmolP_i h^{-1} *(mg protein)*$^{-1}$.

The standard reaction medium for Na/K-ATPase assay contained: 2 mM ATP, 2 mM $MgCl_2$, 140 mM NaCl, 5 mM KCl and 50 mM Tris-HCl, pH 7,7. Assessment of the Mg-ATPase was conducted in the incubation medium containing 0,2 mM ouabain, 2mM ATP, 2 mM $MgCl_2$, 145 mM KCl, and 50 mM Tris-HCl, pH 7,7. With respect to Mg-ATP, the dissociation constant adopted was 0.085mM [1,10]. For Cl-ATPase reagent medium always contained 30 mM Tris–Malate (pH 7.65), 0.4 mM EGTA, and 0.3 mM ethacrynic acid (the specific inhibitor of Cl-ATPase [5,15]. Cl-ATPase was measured as the difference between Cl^--containing incubation and ethacrynic acid-containing media.

The Cl-ATPase and Na, K-ATPase enzyme systems reaction is a function of many physiological ligand sands. Each one may exert an activating or inhibiting action on the enzyme. To analyze the enzymatic reaction's initial velocity, it is required to get V = f (MgATP, Mg_f^{2+}, ATP_f, Cl^-) function for the Cl-ATPase and V = f (MgATP, Mg_f^{2+}, ATP_f, Na^+, K^+) for the Na, K-ATPase to the one variable function, where the values of other ligands are constant.

In case the conditions are unchanged during the reaction, with the enzyme functional unit structure being stable, the initial velocity would be a one-variable function and would be reflected by the following analytical formula:

$$V = e_0 \frac{x^n \sum_{i=0}^{p} \alpha_i x^i}{\sum_{i=0}^{S} \beta_i x^i} \quad S = n + m + p$$

where α_1 and β_1 are the sum of products of individual velocity coefficients and constant ligands' concentrations. X is a variable ligand concentration; e_0- is the enzyme overall concentration. n, m, and p represent power parameters and are positive integers. n is the number of sites for essential activators, m is the number of

sites assigned for full effect inhibitors, while p is the number of sites of partial effect modifiers [11]. To define n, m, and p parameters and correspondingly, decipher the molecular mechanism of the multi-sited enzyme systems a special method was used [11].

The principle of defining n parameter's numerical value is based on the following property of the function

$$U(r,t)=\left[\frac{t^{n}\sum_{i=0}^{S}\beta_i t^{-i}}{\sum_{i=0}^{P}\alpha_i t^{-i}}\right]^{\frac{1}{r}}$$ (U=1/V; t=1/x) (x- is concentration of variable ligand)

For U (r, t) function there is an open interval (t_0; +∞) (t_0 is for U (r,t) function the final turning point), where it has a concave shape at r<n, it has a convex shape when r>n, while when r=n U(r,t) function has an asymptote [8,1,11]. Mean approximation coefficient (MV) and correction coefficient (CC) allow for the estimation of the existence of the asymptote and let us define the n parameter. The theory and method for defining m parameter are identical to those of n parameter [11], only argument of U(r,t) function should be replaced by x (x is the concentration of variable ligand). To define the numerical quantity of the p parameter a table is presented [11].

Ethics approval: Rats experienced no suffering before death, as their death was caused by decapitation. All experiments were approved by the animal care and use committee at the Iv. Beritashvili Center of Experimental Biomedicine (N05/04.04.2021).

Results and discussion. In the case of relatively small Mg_f^{2+}, ATP_f, and high substrate (S) concentrations, the Na, K-ATPase system works in the so-called OPS regime, while in the case of a low substrate and high free Mg^{2+} concentrations, the so-called OPM regime operates [1,13].

In the OPS regime, transport of Na^+ and K^+ occurs simultaneously at a constant stoichiometry (Na^+/K^+=3/2), while in the OPM regime transport is sequential and characterized by variable stoichiometry [1,13]. In the OPM regime, in the case of high, ([K^+]>100 mM) concentration, the number of sites required for the essential activators for Na^+ alters and becomes equal to four, while transport of three Na^+ ions may be followed by transport of one K^+ ion.

For obtaining a more detailed picture of the action of any modifiers on the Na, K-ATPase, it is essential to study its working in every regime of operation. With this goal in mind, the action of the Na, K-ATPase regulating factor, NT, should be studied in both the MgATP dependent -OPS and Mg^{2+} dependent - OPM regimes. Specifically, it was necessary to study the mechanism of Na^+ and K^+-induced Na, K-ATPase activation without and after the addition of DA. It was also intended to determine the number of sites for essential activators (n).

Na/K-ATPase activity dependence upon K^+ concentration in *u(r, t)/t* coordinate system in the OPS regime under influence of DA is presented on Fig.1. The incubation medium was [MgATP]=1,628 mM, [ATP_{free}]=0, 372 Mm, [Mg_{free}]=0,372mM, [NaCl]=147mM. In all case, linearization was attained at r=1, showing that the number of essential activators for K^+ ions equals one.

In the OPS regime under the influence of DA, the transport stoichiometry of K^+ changes and corresponds to the OPM regime stoichiometry (n=1) (Fig.1). Therefore, we can infer that under the influence of the DA, the enzyme system is converted from the OPS to the OPM regime, and the electrogenicity coefficient changes.

As shown in Fig. 1, with increased concentration of DA, the intercept on the ordinate increases, the slope increases, and the straight lines cross the abscissa at the same point, which indicates that, in this case, DA is a nonspecific inhibitor [11].

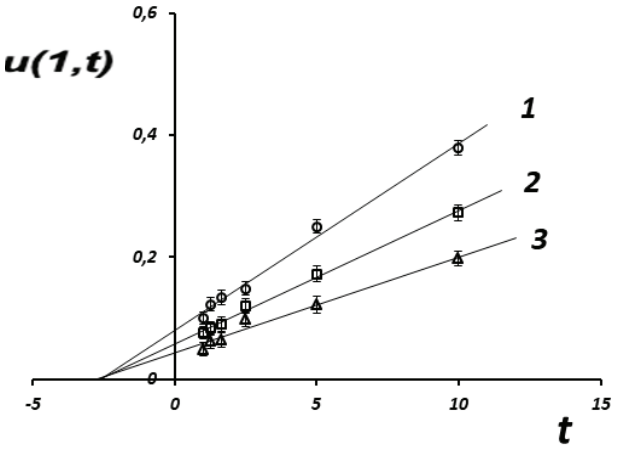

Fig. 1. Influence of different concentrations of DA on Na/K-ATPase in the OPS regime during activation with K^+; incubation medium was: [DA]=0.01mM (3), 0.05 mM (2), 0.1mM (1); [NaCl]=147 mM, [MgATP]=1.628 mM, [Mg]=[ATP]=0.372 mM. u (r, t)= f (t) (r=1; u=1/V; t=1/[KCl]).

Curve 1: [DA]=0.100 mM; r=1

Curve 2: [DA]=0.050 mM; r=1

Curve 3: [DA]=0.01 mM; r=1

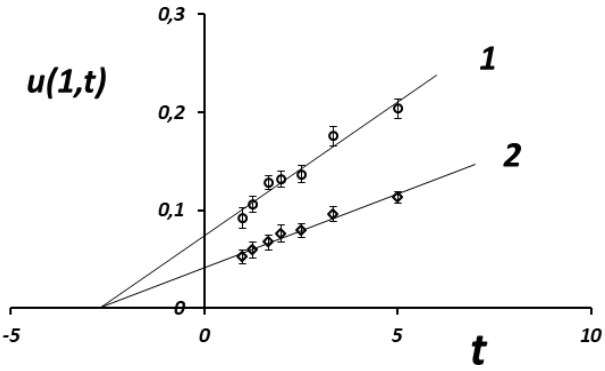

Fig. 2. Dependence of Na/K-ATPase activity upon K^+ concentration in the OPM regime, in the absence of [DA]=0 (2) and after addition of [DA]=0.05mM (1). Incubation medium was: [NaCl]=147 mM; [MgATP]=1.8mM, [Mg]=3mM, [ATP]=0.05mM. u (r, t)=f(t) (r=1; u=1/V; t=1/[KCl]).

Curve 1: [DA]=0.05 mM; r=1

Curve 2: [DA]=0. r=1

Na/K-ATPase activity dependence upon K^+ concentration in *u(r, t)/t* coordinate system in the OPM regime is shown on Fig.2. The reaction medium was: [MgATP]=1,8 mM, [ATP_{free}]=0, 05 Mm, [Mg^{2+}_{free}]= 3 mM, [NaCl]=147mM. According to the data obtained in both cases, when [DA]=0 (curve 2) and [DA]=0,05 mM (curve 1), linearization occurs at the r=1 value, which shows that in the OPM regime the number of the essential activators for K^+ is unchanged.

Investigation of the K^+ activation mechanism of Na, K-ATPase system in OPM regime (Fig. 2) demonstrates that under the action of DA linearity occurs when r=1, so the number of the essential activators for K^+ equals one. In this regime, the slope increases, and the straight lines intercept the abscissa at a common point, which shows that in this regime DA again acts as a nonspecific inhibitor [11]. These data, as well as the results shown in Fig 1, support the earlier suggestion that DA transforms the Na, K-ATPase from the OPS into the OPM regime.

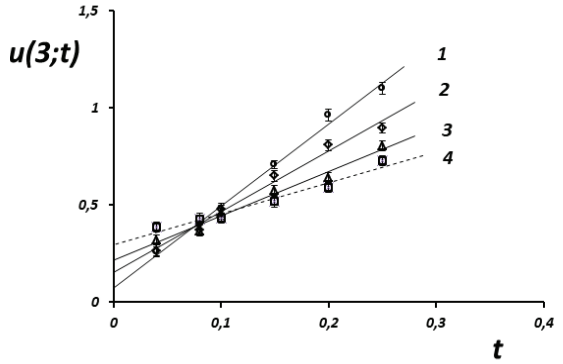

Fig. 3. Dependence of Na/K-ATPase activity upon Na^+ concentration in the OPS regime, in the absence of DA (1) and after DA (2;3;4) addition. Incubation medium: [MgATP]=1.628 mM;, [Mg]=[ATP]=0.372, [KCl]=125 mM. u (r, t)= $\sqrt[3]{u}$ =f (t) (r=3; u=1/V; t=1/[NaCl]).

Curve 1: [DA]=0, r=3

Curve 2: [DA]=0.01mM, r=3

Curve 3: [DA]=0.05mM; r=3

Curve 4: [DA]=0.1mM, r=3

Fig 3 shows the dependence of the Na, K-ATPase activity upon Na^{2+} concentration in *u(r, t)/t* coordinate system, in the OPS regime, in the absence of DA and following the addition of DA to the incubation medium. The OPS regime was determined by the following constant of the incubation medium: [Mg]/[ATP]=2/2 mM, [MgATP]=1,628 mM, $[Mg^{2+}]$=0.372 mM, $[ATP_f]$=0.372 mM, [KCl]=147 mM. Fig. 3 shows that the function has an asymptote only when r=3, both in the absence of DA (curve 1) and in the presence of different concentrations of DA: 0.01mM (curve 2), 0.05mM (curve 3), and 0.1mm (curve 4), which confirms that the number of the Na^+ essential activators does not change in this regime after the addition of DA, and equals three. As follows from Fig. 3, during Na^+-induced activation of the Na, K-ATPase, in the OPS regime and at either concentration of DA in the incubation medium, crossing of the straight lines always occurs in the first quadrant.

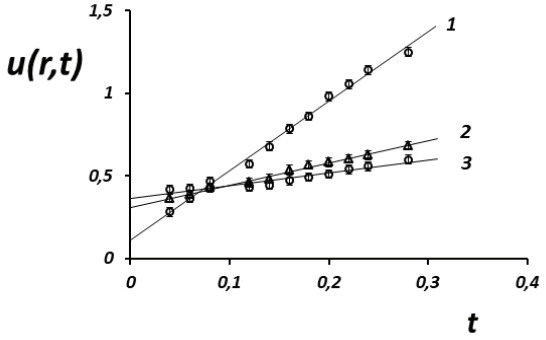

Fig. 4. Dependence of the Na/K-ATPase activity upon Na^+ concentration in the OPS regime, [DA]=0, r=3 (curve 1). In the OPM regime, when [DA]=0, r=4 (curve 3), and when [DA]=0.05 mM, r=3 (curve2). (In the OPS regime, reaction medium was: [MgATP]=1.628mM, [Mg]=[ATP]=0.372, [KCl]=125mM. u (r, t)=$\sqrt[3]{u}$=f (t) (r=3; u=1/V; t=1/[NaCl]). in the OPM regime Incubation medium was: [KCl]=147 mM; [S]=1.8mM, [Mg]=3mM, [ATP]=0.05mM, u (r, t)= $\sqrt[3]{u}$ =f (t) (r=3 or 4; u=1/V; t=1/[NaCl]).

Curve 1: OPS, [DA]=0, r=3

Curve 2: OPM: [DA]=0,05mM, r=3

Curve 3: OPM: [DA]=0, r=4

From this study of Na^+ activation mechanism in the OPS regime (Fig3), it is apparent that all four straight lines, where DA=0 (curve 1) and where DA=0.01mM, 0.05mm, and 0.1 mM (curves 2, 3, and 4), cross in the first quadrant of the coordinate system, which implies that DA- induced activation occurs at low concentration of Na^+, while at high Na^+ concentration inhibition occurs [11]. As follows from Fig. 3, during Na^+-induced activation of the Na, K-ATPase, in the OPS regime and at either concentration of DA in the incubation medium, crossing of the straight lines always occurs in the first quadrant and the number of sites for essential activators for Na^+ always equals three. When working in the same regime, in the case of K^+ activation, the number of the essential activators for K^+ equals two, whereas upon adding DA, it changes and becomes one (Fig. 1). This confirms that the DA is a modifier, the action of which may transfer the enzyme system from the OPS into the OPM regime because n=1 (in the case of K^+) is characteristic of the OPM regime. When the DA is added in the OPM regime, the number of the Na^+ essential activators is n=3 (Fig.4 curve2). Thus, it could be considered that the straight lines 2, 3, and 4 plotted in Fig 3 may correspond to the OPM regime. This probability is supported by the fact that in the OPM regime under influence of DA the number of sites for essential activators for Na^+ equals not four but three (Fig.4 curve2).

From the experimental results, it is evident that DA acts as a modifier bringing the Na, K-ATPase from the OPS into the OPM regime. The addition of DA changes the Na: K stoichiometry from 3:2 to 3:1, and thus the electrogenicity coefficient changes.

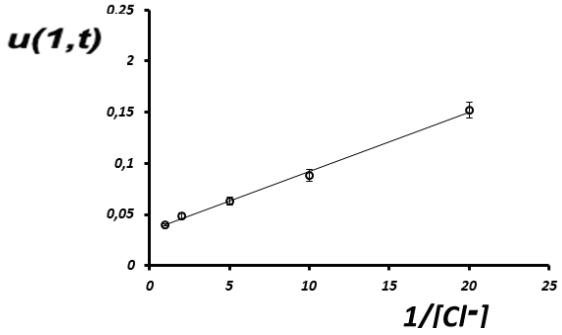

Fig. 5. Dependence of Cl-ATPase activity upon Cl^- concentration, Incubation medium was: [MgATP]=1.8mM, $[ATP_f]=[Mg_f]$=0.2mM

Fig. 5 shows Cl-induced activation of Cl-ATPase. The incubation medium was: MgATP=1.8mM, ATP_f=Mg_f=0.2mM. Linearity of the experimental curve occurs when r=1

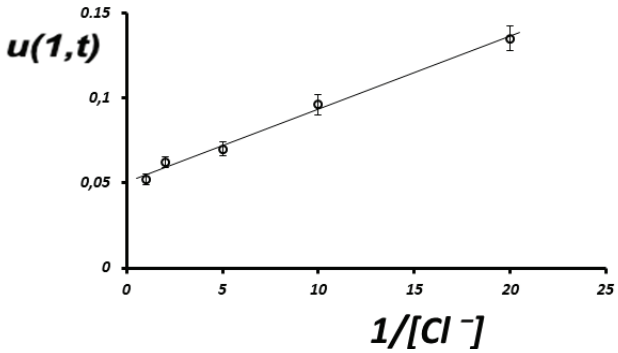

Fig. 6. Influence of DA on Cl-ATPase activity during activation with Cl^-. Incubation medium was: [MgATP]=1.8mM, $[ATP_f]=[Mg_f]$=0.2mM, [DA]=0.05mM

Fig. 6 shows the effect of DA on Cl^- activation of Cl-ATPase. The incubation medium was: MgATP=1.8mM, ATP_f=Mg_f=0.2mM, DA=0.05mM. Linearity of the experimental curve occurs when r=1, which confirms that the number of the Cl^- essential activators does not change after the addition of DA and equals one (Fig. 5, 6).

In conclusion, DA does not change the essential activator number of Cl^- and it remains 1, however brings the Na, K-ATPase from the OPS to the OPM regime. It was determined that the OPM regime is characteristic of the brain synaptic membranes Na, K-ATPase only and is not found in kidney tissue, where the Na, K-ATPase works in just one (OPS) regime, with Na^+: K^+ stoichiometry equal to 3:2 [12].

It is suggested that this transition from one regime to the other is induced by different isomeric states of the Na, K-ATPase [13], which would be subject to further study.

From the previous study, it was known, that neurotransmitters are modulators of Na, K-ATPase, and Cl-ATPase systems, as they change their activity. This study reveals a new mechanism of the DA effect. From the experimental results, it is evident that DA acts as a modifier bringing the Na, K-ATPase from the OPS into the OPM regime. DA changes the Na: K stoichiometry from 3:2 to 3:1, and thus the electrogenicity coefficient changes. DA does not change stoichiometry of Cl^- transport.

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SUMMARY

Na, K-ATPase AND Cl-ATPase REGULATION BY DOPAMINE

Chkadua G., Tsakadze L., Shioshvili L., Nozadze E.

Iv. Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia

The influence of dopamine (DA) on the Cl-ATPase and Na, K-ATPase in the synaptic membrane fraction of the rat brain has been investigated. The fraction was obtained by differential centrifugation. To analyze the function of Na, K-ATPase, and Cl-ATPase, we have applied the method of kinetic analysis of multi-sited enzyme systems, which has been described as the only method used for kinetic investigation of multi-sited enzyme systems.

Dopamine does not alter the stoichiometry of Cl^- transport. The number of the Cl^- sites intended for the essential activator (n) remains 1. During Na, K-ATPase activation with K^+, with the application of DA, it was found that the number of the K^+ sites intended for the essential activators (i.e. the stoichiometry of K^+ transport) changes, and instead of two becomes one. The impact of DA on the mechanism of Na^+-activation of Na, K-ATPase results in the unchanged number of essential activators for Na^+ (n=3). The ratio of Na: K stoichiometry changes from 3:2 to 3:1 and so the electrogenicity coefficient is changed.

Keywords: Cl-ATPase, Na, K-ATPase, neurotransmitter, dopamine, regulation, stoichiometry.

РЕЗЮМЕ

РЕГУЛЯЦИЯ Na, K-АТРазы И Cl-АТРазы ДОФАМИНОМ

Чкадуа Г.Н., Цакадзе Л.Г., Шиошвили Л.Ш., Нозадзе Э.Г.

Центр экспериментальной биомедицины им. И. Бериташвили, Тбилиси, Грузия

Изучено влияние дофамина (DA) на Cl-АТРазу и Na/K-АТРазу, локализованных в синоптических мембранах головного мозга белых крыс, полученных методом дифференциального центрифугирования.

Для изучения указанных АТРаз использовался метод кинетического анализа для многоучастковых фермертативных систем.

В ходе исследования установлено, что дофамин не меняет стехеометрию транспорта Cl^- и число участков, предназначенных для обязательных активаторов (n), остается равным 1. Что касается Na/K-АТРазы, активированной ионами K^+, установлено, что стехеометрия транспорта K^+ и, соответственно, число участков, предназначенных для обязательных активаторов, меняется, т.е. вместо двух это число равно одному.

При активации ионами Na^+ число участков, предназначенных для обязательных активаторов, не меняется и равно

трем. Следовательно получено соотношение 3:1. Соответственно этому меняется коэффициент электрогенности.

რეზიუმე

Na/K-ATPაზას და Cl-ATPაზას რეგულაცია დოფამინით

გ. ჭკადუა, ლ. წაქაძე, ლ. შიოშვილი, ე. ნოზაძე

ივ. ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თბილისი, საქართველო

შესწავლილია დოფამინის (DA) გავლენა თეთრი ვირთაგვას თავის ტვინის სინაფსურ მემბრანებში ლოკალიზებულ Cl-ATPაზასა და Na/K-ATPაზაზე. სინაფ-სური მემბრანების ფრაქცია მიღებულია დიფერენციალური ცენტრიფუგირების მეთოდით. Cl-ATPაზასა და Na/K-ATPაზას შესწავლისას გამოყენებული იყო მრავალუბნიანი ფერმენტული სისტემების კინეტიკური ანალიზის მეთოდები, რაც წარმოადგენს მრავალუბნიანი ფერმენტული სისტემების მოქმედების მოლეკულური მექანიზმის შესწავლის ერთადერთ მეთოდს.

დადგენილია,რომ დოფამინი არ ცვლის Cl^--ის ტრანსპორტის სტექიომეტრიას და Cl^--ისათვის განკუთვნილი აუცილებელი აქტივატორების რიცხვი (n) რჩება 1-ის ტოლი. დოფამინის თანაობისას Na/K-ATP-აზას K^+-ით აქტივაციის შესწავლისას დადგენილია, რომ K^+-ის, როგორც აუცილებელი აქტივატორებისათვის განკუთვნილი უბნების რიცხვი და, შესაბამისად, K^+-ის ტრანსპორტის სტექიომეტრია იცვლება და 2-ის ნაცვლად ხდება 1-ის ტოლი. Na,/K-ATPაზას Na^+-ით აქტივაციაზე დოფამინის გავლენა არ იწვევს Na^+-ის აუცილებელი აქტივატორებისათვის განკუთვნილი უბნების რიცხვის ცვლილებას (n=3). ამრიგად, DA-ის გავლენით Na/K-ATPაზას Na:K სტექიომეტრია იცვლება და ნაცვლად 3:2, ხდება 3:1. შესაბამისად, იცვლება ელექტროგენულობის კოეფიციენტიც.

DYNAMICS OF ULTRASTRUCTURAL REARRANGEMENTS OF SKELETAL MUSCLE FIBROBLASTS AFTER SIMULATED GUNSHOT SHRAPNEL WOUNDS

[1]Mikhaylusov R., [2]Negoduyko V., [1]Pavlov S., [3]Oklei D., [1]Svyrydenko L.

[1]Kharkiv Medical Academy of Postgraduate Education; [2]Military Medical Clinical Center of the Northern Region of the Ministry of Defense of Ukraine; [3]V.N. Karazin Kharkiv National University, Ukraine

The issues of diagnostics, treatment, and rehabilitation of surgical patients of gunshot wounds, in spite of the centuries-long history of fire weapon using, continues to be a topical issue of modern medicine [6,16]. Studies show that even increasing the reservation of a person does not fully guarantee the preservation of life and health after exposure to fragmentation ammunition [18].

When performing peace-support missions, counter-terrorism operations, in local military conflicts from 40 to 72% of injuries are shrapnel, of which more than 50% are blind, most commonly affects the lower limbs and the most massively damaged muscle tissue [3,5,8,12].

Muscle tissue regeneration after extensive injuries affects for regenerative process in terms of gunshot wounds and rehabilitation of the injured and is an important medical and social issue. After significant gunshot damage to the soft tissues, has been observed a longstanding and defective recovery of skeletal muscles with replacement dead fibers by conjunctive tissue [11,13]. Connective tissue cells – fibroblasts – perform an important function in wound healing, are capable of division, growth, and movement, synthesize extracellular matrix, secrete precursors of collagen, elastin, and mucopolysaccharides [9,17].

The question of the duration of reparative processes, full-fledged tissue restoration, and the formation of mature scar tissue after a gunshot shrapnel wound remains unclear. One of the methods allowing determine in-depth the recovery of the functioning and structure of tissues at the subcellular level is the submicroscopic [1,10].

To study the peculiarities of rearrangements of skeletal muscle fibroblasts surrounding the wound canal at various times after an experimentally modeled gunshot shrapnel wounds.

Material and methods. Experimental modeling of gunshot shrapnel wounds of soft tissues has been carried out on 20 laboratory animals – breeding rabbits of the breed «Chinchilla». The mass of animals was 2200-3000 g. The average weight of animals was 2620±120 g. To laboratory animal was caused a gunshot wound in the sartorius area with the gun «Fort-12» caliber 9 mm, with a reinforced cartridge loaded with cut-off (without a cap) metal screws SMK 3.5×9.5 («self-tapping screw») mass 0.9-1.1 g, from a distance of 3.0 m. The initial velocity of the fragment was 305 m/s. Gunshot shrapnel wounds were simulated in a certified shooting range while meeting the safety requirements.

Laboratory animals were kept in a vivarium of the Kharkiv Medical Academy of Postgraduate Education in standardized conditions using natural light and a standard diet, with free access to water and food, in accordance with the international rules of the “Guide for the Care and Use of Laboratory Animals” [7]. Experimental work was carried out in accordance with European animal handling requirements [2,4].

Research protocols using laboratory animals were approved by the local ethics committee of the Military Medical Clinical