ЕКСПЕРИМЕНТАЛЬНІ РОБОТИ

ALUMINUM CHLORIDE EFFECT ON Ca²⁺,Mg²⁺-ATPase ACTIVITY AND DYNAMIC PARAMETERS OF SKELETAL MUSCLE CONTRACTION

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We studied enzymatic activity and measured strain-gauge contraction properties of the frog Rana temporaria m. tibialis anterior muscle fascicles during the action of aluminum chloride solution. It was shown that AlCl₃ solutions did not affect the dynamic properties of skeletal muscle preparation in concentrations less than 10^{-4} M. Increasing the concentration of AlCl₃ to 10^{-2} M induce complete inhibition of muscle contraction. A linear correlation between decrease in Ca²⁺,Mg²⁺-ATPase activity of sarcoplasmic reticulum and the investigated concentrations range of aluminum chloride was observed. The reduction in the dynamic contraction performance and the decrease Ca²⁺,Mg²⁺-ATPase activity of the sarcoplasmic reticulum under the effect of the investigated AlCl₃ solution were minimal in pre-tetanus period of contraction.

K e y   w o r d s: aluminum chloride, muscle contraction, Ca²⁺,Mg²⁺-ATPase activity, muscle contractile force, length of muscle fibers.

Aluminum enters the organism with drinking water and has much higher bioavailability than that from other sources; it is also consumed with vegetable food [1]. There is a plethora of data on toxicity of aluminum and other elements for a living organism [2-4]. Pathologies associated with increased aluminum levels in human organism include heart rhythm disorders resulted from its accumulation in the heart muscle. It has been established that aluminum causes specific physiological and biochemical changes in organism of humans and animals, namely disorders of the central nervous system, changes in functional state and development of bone tissue, membrane permeabilization and channel conductance [5].

Aluminum toxicity is attributed to its ability to change concentrations and balance of other ions, e.g. by supplanting other metals, mostly bivalent, from certain enzymes and metalloproteins. It has been established that aluminum may replace magnesium in active sites of enzymes such as phosphodiesterases, acid and alkaline phosphatases. Aluminum was demonstrated to enter myocytes and inhibit Ca²⁺ release from sarcoplasmic reticulum (SR) [1, 3].

The impact of aluminum on muscular system remains poorly understood. There is limited available data, which is primarily of descriptive nature. It has been established that aluminum may inhibit contractile function. The published data gives ground to the assumption that aluminum may affect both neuro-muscular transmission and contractile apparatus itself [6]. Consequently, understanding the effect of aluminum on muscular contraction mechanics may allow better understanding of the mechanisms of action of this metal and the possibilities of its clinical application.

The effects of biologically active substances on terminal changes in power output of the muscle are currently under active investigations. Nevertheless, the dynamics of this process remains largely unstudied. The onset of the equilibrium stable state of contraction under the effect of biologically active substances may vary within wide margins depending not only on the concentrations of the reagents used [7-9], but also on duration of the experiment [10, 11]. This fact complicates the interpretation of the obtained experimental data and may result in severe errors in research planning. Accordingly, an im-

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portant emphasis in our work was made on temporal changes in achievement of equilibrium stable state of contraction under the effect of the investigated compounds. The aim of the study was to investigate the effect of aluminum chloride solutions on Ca^{2+}, Mg^{2+}-ATPase activity of sarcoplasmic reticulum and muscular contraction dynamics of isolated fascicles induced by electrostimulation.

Materials and Methods

The experiments were conducted on *m. tibialis anterior* fascicles of *Rana temporaria* frog. We determined contractile force, change in length and stimulating signal parameters. The experiments were conducted in closed circuit Ringer solution with relaxation period of 3 min. A strain-gauge device was used to determine contractile forces of skeletal muscle fiber bundles [12].

Protein concentration was measured after Bradford [13]. Ca^{2+}, Mg^{2+}-ATPase activity of sarcoplasmic reticulum was studied after Fiske and Subbarow [14].

Incubation medium (1.9 ml) was prepared with the following concentrations in final volume: imidazole – 50 mM, KCl – 100 mM, $MgCl_2$ – 3.5 mM, NaN_3 – 5 mM, EDTA – 3 mM, sodium oxalate – 2 mM, ATP – 3 mM. To this end, we took 0.1 ml imidazole (1 M), 0.2 ml KCl (1 M), 0.1 ml $MgCl_2$ (30 mM), 0.1 ml NaN_3 (1 M), 0.2 ml EDTA (30 mM), 0.2 ml sodium oxalate (20 mM), 0.2 ml ATP (30 mM), and added water to 1.9 ml (test sample) or to 2 ml (control sample).

Test tubes were bathed to 37 °C, and the reaction was started by addition of 0.1 ml of protein (1 mg/ml). The tubes were incubated for 20 min.; the reaction was then stopped by addition of 1.5 ml of cold 10% trichloroacetic acid.

We used the following reagents: reagent 1 – 10% ascorbic acid, freshly prepared; reagent 2 – 0.42% ammonium molybdate in 1 N solution of H_2SO_4; 3 – 1 ml of reagent 1 and 6 ml of reagent 2; 40 µg/ml solution of KH_2PO4. To measure inorganic phosphate produced as a result of enzyme activity, we placed 0.9 ml of supernatant (as a source of P_i) in a test tube and added 2.1 ml of reagent 3. The mix was incubated for 30 min at 37 °C, and optical density determined at $\lambda = 820$ nm.

To facilitate the description and adequate analysis of the results, we attributed various stages of the dynamic response of the muscle to different temporal regions, which correspond to various stages of contractile process. The force response and changes in length were attributed to stages (Fig. 1): F_1 – initiation of the force response of the muscle; F_2 – the muscle force productivity enters a steady level of contraction without a noticeable trend towards either end; F_3 – terminal muscle activity; L_1 – initiation of changes in muscle length; L_2 – the length of the muscle enters a steady level of contraction; L_3 – terminal changes in muscle length, was not analyzed due to noticeable fluctuations even after stimulation ceased. This may be attributed to transitions in rigid composition of muscle fibers caused by abrupt changes in fiber elasticity, which in turn depends on momentary discontinuance in stimulating signal. Registration and adequate analysis of these processes were very complicated. Thus we used the first two, L_1 and L_2, to analyze length change curves in these test series.

In order to establish the margins of concentrations within which the experimental substances display physiological effects influencing dynamic properties of muscle contractions, we investigated concentrations from 10^{-8} to 10^{-4} M. As a result, we demonstrated, that $AlCl_3$ solutions in concentrations of less than 10^{-4} M did not affect performance of skeletal-muscle preparations. As concentrations increased to 10^{-2} M the muscle contractile processes were totally suppressed. Consequently, we used $AlCl_3$ solutions with concentrations of 10^{-4} to 10^{-2} M.

The experiments were done in accordance with guidelines for keeping and work with laboratory animals laid down in the 'European convention for the protection of vertebrate animals used for experimental and other scientific purposes' (Strasbourg, 1986).

The statistical analysis of data was done with variation statistics methods in Origin 7.0 software, using Student's *t*-test. The differences between test and control samples were considered significant at $P \le 0.05$.

Results and Discussion

The experiments using 10^{-4} M $AlCl_3$ demonstrated that muscle contractile force reached steady levels at 4th min of observations during F_1 and was at 99% of control values. It was in F_2 and F_3 on the 12th min at 96.6 and 97.7%, accordingly (Fig. 2, *a*).

The inhibition of changes in length of muscle fibers entered steady level on the 10th min during L_1 in these experimental conditions reaching 93.7% of control, and at the 8th min during L_2 reaching 95% of control. A decrease of dynamic characteristics of muscle contraction under the effect of 10^{-4} M aluminum chloride solution was of linear nature.
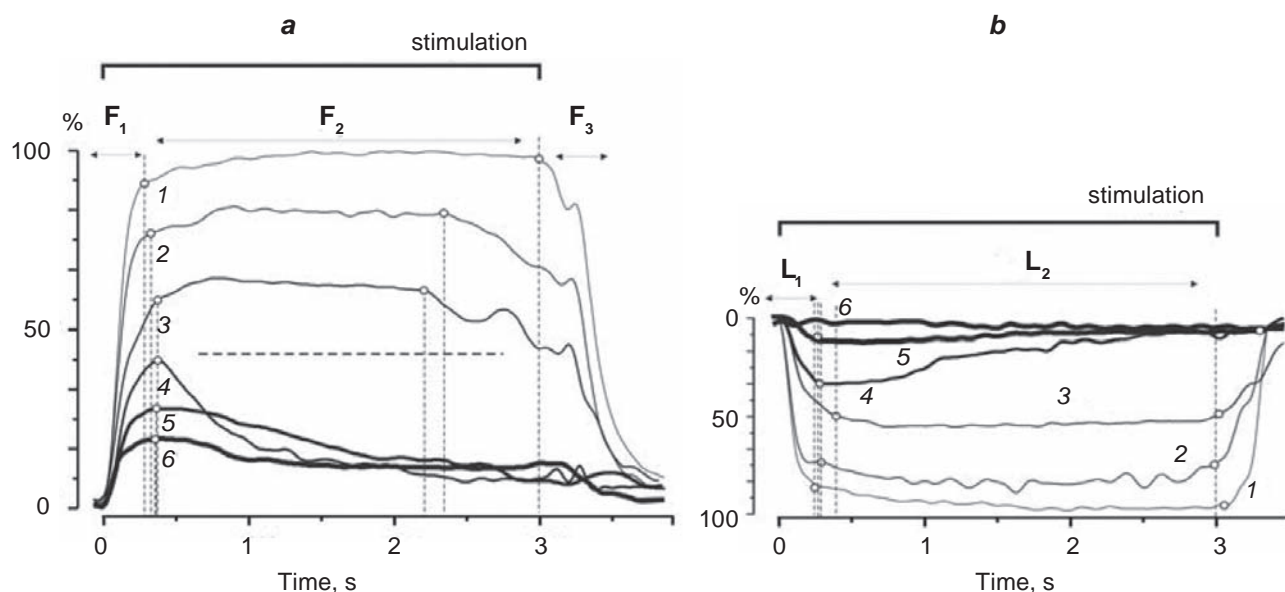
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Fig. 1. Graphical representation of attribution of active muscle's dynamic response to corresponding temporal stages of force response. a – F_1, F_2, F_3, and changes in length; b – L_1, L_2 in contractions of m. tibialis anterior skeletal muscle fibers electrostimulated at 30 Hz for 3 s under effect of $AlCl_3$ in concentrations of 10^{-4}–10^{-3} M. Abscissa – time; ordinate – muscle fiber responses expressed as percent values from that of control (M ± m, n = 10). Relaxation time was 3 min. 1–6 – the curves characterizing changes in force (a) and length (b) of skeletal muscle contraction under effect of $AlCl_3$ in concentrations of $1.4 \cdot 10^{-4}$, $2 \cdot 10^{-4}$, $3.3 \cdot 10^{-4}$, $5 \cdot 10^{-4}$, $6.6 \cdot 10^{-4}$, and 10^{-3} M, accordingly

The results of exposing muscle fiber bundles to 10^{-3} M aluminum chloride solution (Fig. 2, b) demonstrate a statistically significant reduction in muscle contraction parameters during F_2, F_3, L_1 stages.

The maximum decrease in muscle's contractile force was observed after the 10th min during F_1 and was at 92.6% of control values. The maximum decrease in muscle's contractile force during F_2 was at the 12th min and constituted 71.2% from that of control values. The steady level of contraction during F_3 was at the 14th min and was at 71.2% from that of the corresponding control values.

The maximum reduction in contraction of muscle fibers was at the 12th min of the experiment during L_1 and L_2, and constituted 69.1 and 73% of the corresponding control values. The value of changes in muscle fiber length during L_1 was in all instances smaller than that during L_2.

We observed drastic decrease in dynamic properties of contractions in the experiments where 10^{-2} M solutions of $AlCl_3$ were used (Fig. 2, c).

The maximum reduction in muscle contractile force was after the 6th min of stimulation during F_1, F_2, and F_3, and was at 53, 35.6 and 33.9% of correspoding control values.

The maximum inhibition muscle fiber contraction was found after the 6th min of the experiment during L_1 and L_2, and reached zero value. The results of these experiment show the significant linear decrease in Ca^{2+},Mg^{2+}-ATPase activity of sarcoplasmic reticulum (SR) as a result of the effects of all the mentioned concentrations of $AlCl_3$ (Table 1).

Taking into account the profound differences in the effects of aluminum chloride solutions in these concentration margins, we studied the effect of intermediate concentrations of $AlCl_3$ solutions within 10^{-4} to 10^{-3} M. We chose $1.4 \cdot 10^{-4}$, $2 \cdot 10^{-4}$, $3.3 \cdot 10^{-4}$, $5 \cdot 10^{-4}$ and $6.6 \cdot 10^{-4}$ M.

The $AlCl_3$ solution with concentration of $1.4 \cdot 10^{-4}$ M caused maximum decrease in muscle contraction force at the 8th min of the experiment during F_1 and F_2 and constituted 98.4 ± 0.8% and 94.2 ± 1.7%, accordingly. The steady level during F_3 was observed at the 12th min and was at 93% of control value. The curves dependence of muscular contraction force on duration of exposition to the aluminum chloride was of linear nature, both pa-
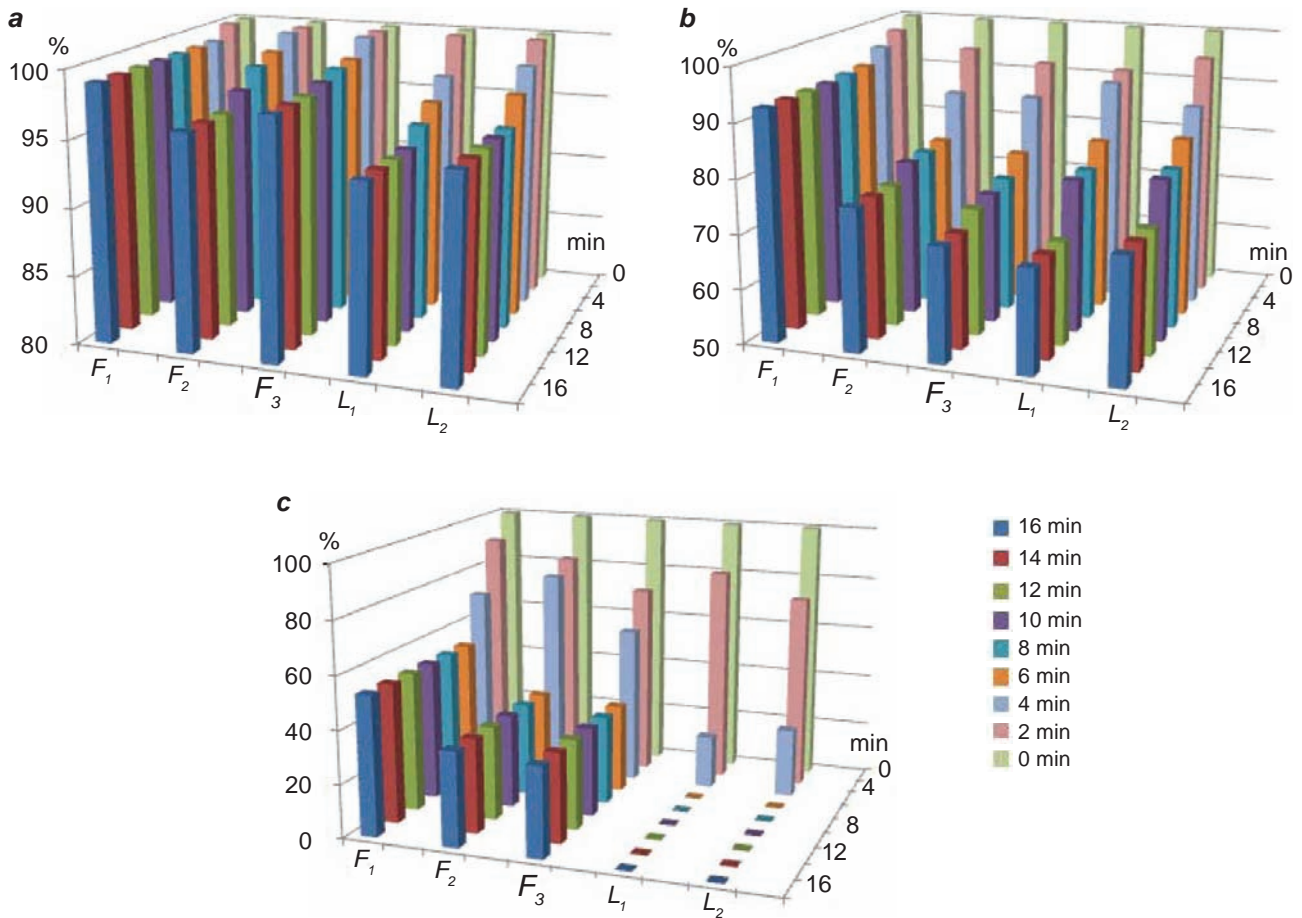
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Fig. 2. The effect of AlCl$_3$ solutions in concentrations of 10^{-4} M (a), 10^{-3} M (b), 10^{-2} M (c) on dynamic properties of contractions caused by electrostimulation at 30 Hz for 3 s, depending on duration of exposition to the reagent (M ± m, n = 10, P ≤ 0.05)

T a b l e 1. Effects of AlCl$_3$ solutions on SR Ca^{2+},Mg^{2+}-ATPase activity of skeletal muscles (M ± m, n = 10)

Enzyme	Control	AlCl$_3$		
		10^{-2} M	10^{-3} M	10^{-4} M
SR Ca^{2+},Mg^{2+}-ATPase activity of skeletal muscles, nmol of P$_i$·mg^{-1} of protein·min^{-1}	245.6 ± 1.4	87.9 ± 4.3*	146.8 ± 3.5*	240.2 ± 2.1*

* P ≤ 0.05

rameters of muscular contractions changed synchronously for the duration of observations.

The most profound changes in length of muscle contractions were at the 10th min of the experiment in L$_1$ and L$_2$ and constituted 94.2 and 94.7% of control values, accordingly.

Experiments with 2·10^{-4} M solution of aluminum chloride (Fig. 3, b) demonstrated that the maximum reduction in muscle contractile force was at the 10th min during F$_1$ and constituted 97.1% of control value. The most profound decrease in muscle

contractile force during F$_2$ and F$_3$ was correspondingly at the 8th and 6th min, and reached 94.1 and 92.3% of control, yet these changes were not statistically significant. The changes in dynamic properties of muscle contraction during these periods were of irregular nature.

We found no significant changes in muscle fibers length under the effect of 2·10^{-4} M solution of aluminum chloride in comparison to the effect of 1.4·10^{-4} M AlCl$_3$ solution (Fig. 3, a and b).

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We detected statistically insignificant decrease in muscle contraction properties in all investigated stages in experiments with $3.3 \cdot 10^{-4}$ M $AlCl_3$ solutions (Fig. 3, c). The maximal reduction of muscle contractile force was found at the 8th min during F_1 and was of 96.2% of control. The most notable decrease in muscle contractile force during F_2 and F_3 was on 12th and 10th min, accordingly, and constituted 92.5 and 85.3% of that of control. The most profound decrease in muscle contraction was during the 10th min in L_1 and L_2, and was correspondingly of 86.1 and 87.1% of control values.

We found decreased force and changes in muscle fiber length in all studied cases in experiments with $5 \cdot 10^{-4}$ M aluminum chloride solution (Fig. 3, d). The maximum in reduction of contractile force was observed at the 10th min during F_1 and was of 96.1% of that of control. The most noticeable decrease in muscle contractile force during F_2 and F_3 was at the 14th and 12th min, accordingly, and was of 90.9% and 85.8% of that of control. The maximum decrease in the length of muscle contraction was at the 14th min in L_1 and L_2 and constituted correspondingly 88.6% and 89% from that of control values.

Aluminum chloride in concentration of $6.6 \cdot 10^{-4}$ M caused a decrease in dynamic properties of contraction (Fig. 3, e). The maximum decrease in muscle contractile force was at the 14th min of the experiment during F_1 and constituted 94.2% of that of control, and at the 12th min during F_2 and F_3, and was accordingly 87 and 82.6%. The dependence of muscle contraction force on the duration of exposition to the effector during F_1, F_2 and F_3 was of linear nature. The maximal statistically significant reduction in muscle fibers contraction was detected at the 14th min during stages L_1 and L_2 and constituted 80.2 and 83.7% of the corresponding control values.

There was a linear decrease in Ca^{2+},Mg^{2+}-ATPase activity of SR as a result of the effects of $AlCl_3$ (Table 2).

Washing of muscle samples with Ringer solution caused restoration of the dynamic properties of contraction to their starting levels in all experimental concentrations of $AlCl_3$ solutions. The duration of the restorative process up to control values depended linearly on the duration of the effector exposition. The time period of washing increased linearly with the concentration of aluminum chloride.

The experimental data show inhibition of Ca^{2+},Mg^{2+}-ATPase activity of SR, with linear dependence on the concentration of $AlCl_3$. The demonstrated inhibition of Ca^{2+},Mg^{2+}-ATPase activity of SR corroborates imbalance in intracellular Ca^{2+} concentrations under the effect of aluminum that has been found by others [3]. The reduction of Ca^{2+},Mg^{2+}-ATPase activity probably results from compromised SR membrane integrity due to activation of lipid peroxidation [1, 3, 18].

We found, in accordance with the obtained results, the irregularities in the effect of aluminum chloride in the investigated concentrations (10^{-4} to 10^{-2} M) on changes in force response and muscle fiber length and inhibiting properties of this compound in concentrations of more than 10^{-4} M. These processes may be attributed to the ability of aluminum ions to permeate sarcolemma [3]. It may be supposed that aluminum ions may affect muscle performance at a level of actin-myosin interaction. The ions of this metal can supposedly supplant magnesium ions in ATP [3, 19]. It is possible that aluminum ions modulate actin-myosin interaction and change the functional properties of actin-myosin complexes of the muscle. There is data of dose-dependent reduction in myosin ATPase activity under the effect of aluminum ions [14]. It has been demonstrated that aluminum ions in concentration of 5 mM inhibited myosin ATPase from heart muscle to half of its maximum level. The inhibition was observed also at concentrations over 5 mM for myosin ATPase from smooth muscle cells [15]. Aluminum ions were

T a b l e 2. Effects of $AlCl_3$ solutions on SR Ca^{2+},Mg^{2+}-ATPase activity of skeletal muscles (M ± m, n = 10)

Enzyme	Control	$AlCl_3$				
		$1.4 \cdot 10^{-4}$ M	$2.0 \cdot 10^{-4}$ M	$3.3 \cdot 10^{-4}$ M	$5.0 \cdot 10^{-4}$ M	$6.6 \cdot 10^{-4}$ M
SR Ca^{2+},Mg^{2+}-ATPase activity of skeletal muscles, nmol of $P_i \cdot mg^{-1}$ of protein $\cdot min^{-1}$	245.6 ± 1.4	228.5 ± 2.1	226.3 ± 2.2	208.6 ± 2.8*	203.3 ± 2.8*	196.5 ± 2.9*

* $P \leq 0.05$

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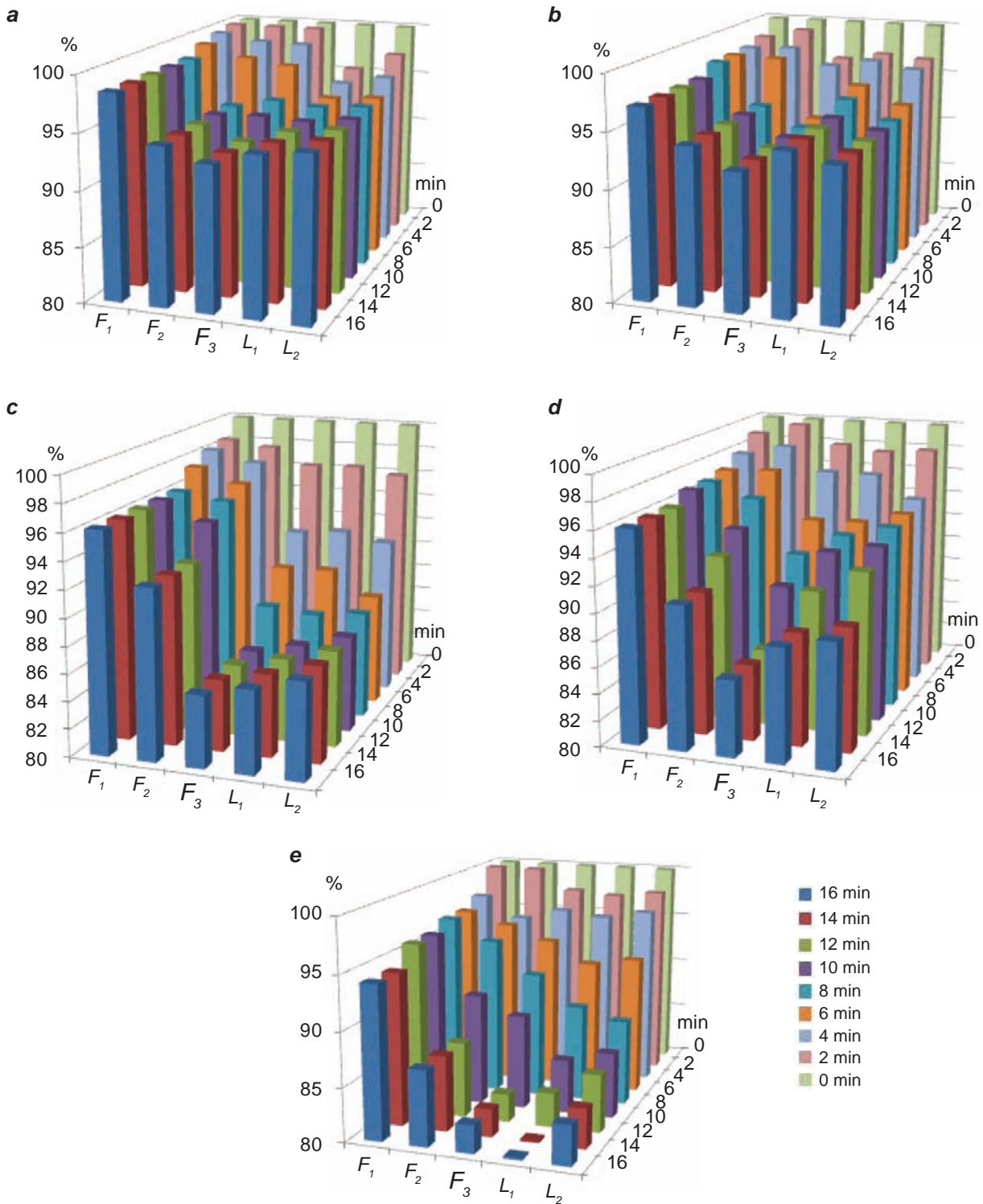*Fig. 3. The effect of AlCl$_3$ solutions in concentrations of 1.4·10^{-4} M (a), 2·10^{-4} M (b), 3.3·10^{-4} M (c), 5·10^{-4} M (d), 6.6·10^{-4} M (e) on dynamic properties of contraction depending on duration of exposition to the reagent (M ± m, n = 10, P ≤ 0.05)*

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shown to exert a notable influence on structural changes in actin-myosin complex during ATP hydrolysis. A decrease in rate of superprecipitation of actin-myosin complex has been found in the presence of aluminum in concentrations of 10^{-4} to 10^{-3} M [16]. This process was totally suppressed in case of aluminum concentration of 10^{-2} M.

Therefore, our results demonstrate that aluminum chloride affects performance of skeletal muscle samples in concentrations 10^{-4} M and higher. Force and length of muscle fiber contractions decreased gradually depending on concentrations of aluminum chloride solution, yet some irregularities in linear dependence characteristics were observed for aluminum chloride concentrations of 10^{-4}, 10^{-3} and 10^{-2} M. There were differences in dependences of reaching the stable state of contraction properties upon experiment duration within margins of the investigated concentrations. Reductions in dynamic properties of contraction under effect of the solutions in investigated concentrations were minimal during F_1.

ВПЛИВ ХЛОРИДУ АЛЮМІНІЮ НА Ca²⁺, Mg²⁺-АТРазну АКТИВНІСТЬ ТА ДИНАМІЧНІ ПАРАМЕТРИ СКОРОЧЕННЯ СКЕЛЕТНИХ М'ЯЗІВ

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Проведено ензиматичні та тензометричні дослідження функціонування волокон скелетного м'яза *tibialis anterior* жаби *Rana temporaria* за дії хлориду алюмінію. Було показано, що розчини AlCl₃ в концентраціях нижчих 10^{-4} моль/л не впливали на динамічні параметри скелетно-м'язових препаратів, за збільшення концентрації до 10^{-2} моль/л відбувалось повне пригнічення процесів скорочення м'яза. Встановлено лінійне зниження Ca²⁺,Mg²⁺-АТРазної активності саркоплазматичного ретикулума за дії всіх досліджуваних концентрацій AlCl₃. Показано, що зменшення динамічних параметрів скорочення і зниження Ca²⁺,Mg²⁺-АТРазної активності саркоплазматичного ретикулума було мінімальним впродовж дотанічного періоду скорочення. Продемонстровано нерівномірний вплив розчинів AlCl₃ різної концентрації на силову відповідь та характер вкорочення м'язових волокон.

К л ю ч о в і с л о в а: хлорид алюмінію, м'язове скорочення, Ca²⁺,Mg²⁺-АТРазна активність, сила скорочення, довжина м'язового волокна.

ВЛИЯНИЕ ХЛОРИДА АЛЮМИНИЯ НА Ca²⁺,Mg²⁺-АТРазную АКТИВНОСТЬ И ДИНАМИЧЕСКИЕ ПАРАМЕТРЫ СОКРАЩЕНИЯ СКЕЛЕТНЫХ МЫШЦ

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Проведены энзиматические и тензометрические исследования сокращения пучков волокон мышцы *tibialis anterior* лягушки *Rana temporaria*. Показано, что растворы AlCl₃ в концентрациях менее 10^{-4} моль/л не влияли на динамические параметры сокращения мышечных препаратов, при увеличении концентрации до 10^{-2} моль/л происходило полное угнетение процессов сокращения мышц. Установлено линейное снижение Ca²⁺,Mg²⁺-АТРазной активности саркоплазматического ретикулума под действием исследуемых концентраций AlCl₃. Показано, что уменьшение динамических параметров сокращения и снижения Ca²⁺,Mg²⁺-АТРазной активности саркоплазматического ретикулума было минимальным на протяжении дотанического периода сокращения. Продемонстрировано неравномерное влияние растворов AlCl₃ различных концентраций на силовой ответ и характер укорачивания мышечных волокон.

К л ю ч е в ы е с л о в а: хлорид алюминия, мышечное сокращение, Ca²⁺,Mg²⁺-АТРазная активность, сила сокращения, длина мышечного волокна.

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