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THE EFFECT OF CHLORPYRIFOS UPON ATPase ACTIVITY OF SARCOPLASMIC RETICULUM AND BIOMECHANICS OF SKELETAL MUSCLE CONTRACTION

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We investigated the effect of chlorpyrifos, an organophosphate insecticide, on Ca^{2+},Mg^{2+}-ATPase activity of sarcoplasmic reticulum and on contraction dynamics (force and length changes) of Rana temporaria m. tibialis anterior muscle fiber bundles. All of the used concentrations of chlorpyrifos (10^{-6} to 10^{-5} M) caused decrease of Ca^{2+},Mg^{2+}-ATPase activity. The inhibition of Ca^{2+},Mg^{2+}-ATPase activity by chlorpyriphos in concentrations of 10^{-6} M to $7.5 \cdot 10^{-6}$ M is due to permeation of sarcoplasmic reticulum rather than due to direct enzyme inhibition by organophosphate insecticides. The inhibitory properties of the compound were higher at increased concentration and exposure timeframes. Chlorpyrifos in concentration range of 10^{-6} to $7.5 \cdot 10^{-6}$ M causes changes in muscle fiber response force that were more pronounced than changes in contractile length. We demonstrated inhibition of Ca^{2+},Mg^{2+}-ATPase activity caused by noncholinergic effects of chlorpyriphos. It is possible to conclude that influence of organophosphate insecticides happens not only in the neuromuscular transmission but also on the level of subcellular structures.

K e y w o r d s: chlorpyrifos, ATPase activity of sarcoplasmic reticulum, muscle contraction.

Chlorpyriphos (O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) is an organophosphate insecticide (OPI) widely used in household, agricultural and industrial applications in over 100 countries in the last 40 years. OPI may enter organism via dermal, oral or respiratory pathways and cause poisoning affecting neuromuscular signal exchange resulting in skeletal muscle pathologies [1-4].

There is as yet no universally accepted theory of mechanism of pathological changes that cause damage to muscle functioning under effects of OPI and other anthropogenic factors [5-8]. The toxicity of organophosphate insecticides is explained by irreversible inhibition of acetylcholine esterase leading to acetylcholine accumulation and excessive activation of cholinergic receptors [9, 10].

Generally, the research of OPI's effects on muscle contraction is centered on endpoint change in muscle strength, and the dynamic developments in these processes remain unstudied. The equilibrium stable state of muscle contraction under effect of external substances may vary within wide margins due to differences in concentrations and timeframes [7, 11, 12], which makes it difficult to give adequate interpretations to the results. Thus, we paid special attention to periods it takes muscle to reach equilibrium stable state of contraction under effect of the investigated chlorpyriphos compound.

The kinetics and dynamics of OPI poisoning is known to depend not only on different dosage, but also on elimination half-life of OPI [8, 9, 11, 12, 16]. The calculations of effects' duration upon organism or a particular organ are complicated due to variations in elimination time.

Studies of properties of particular muscles in living organism are difficult to perform, as measurements of mechanical kinetic muscle properties are tied to their changes due to efferent and humoral regulatory effects and fatigue [15]. The contractile processes in poikilotherms are known to be slower than in homoiotherms, which make a number of research objectives required for the correct registration of dynamic parameters of muscle contraction easier to achieve. Therefore, we chose muscle fiber bundles from m. tibialis anterior of Rana temporaria as experimental subjects.

The aim of this study was to establish the concentrational variations of chlorpyriphos' effects on ATPase activity of sarcoplasmic reticulum (SR) and biomechanical contractile properties of skeletal muscle (SM), and to analyze the progression of these changes over time depending on concentrations of the compound.

Materials and Methods

The contractile force of SM fiber bundles of m. tibialis anterior muscle fibers from a hind leg of Rana temporaria frog was determined with a tensometer device [11, 12].

Ca^{2+},Mg^{2+}-ATPase activity of SR was determined by Fiske and Subbarow method [13]. Protein concentration was determined by Bradford assay [14].

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 $P \leq 0.05$.

Results and Discussion

The experimental data obtained demonstrates inhibition of Ca^{2+},Mg^{2+}-ATPase activity of SR under effect of chlorpyriphos in concentrations 10^{-6} to 10^{-5} M (Table 1). Decreased Ca^{2+},Mg^{2+}-ATPase activity may result from permeation of SR membrane due to intensive lipid peroxidation [17]. It may be explained by the capacity of these substances to enter cell through the plasma membrane and disrupt intracellular processes. According to our results, the possible molecular mechanisms underlying chlorpyriphos toxicity include inhibition of SR Ca^{2+},Mg^{2+}-ATPase activity and unbalancing of intracellular Ca^{2+} homeostasis.

Changes in activity of ATPases under influence of OPI may be an important factor in cellular dysfunctions due to disruptions in transmembrane cation exchange [17, 19]. The authors [19] demonstrated inhibition of Ca^{2+},Mg^{2+}-ATPase and Na,K-ATPase activity of sarcolemma and of protein kinase A (cAMP-dependent protein kinase) under effect of OPI as potential biological mechanism inhibiting neuromuscular impulse exchange. It has been also demonstrated that decreased Ca^{2+},Mg^{2+}-ATPase activity may result from damaged SR membrane and not from direct OPI effect on the enzyme [20].

It is therefore sensible to assume that one of the factors of complex OPI effect on skeletal muscle function is the inhibition of SR Ca^{2+},Mg^{2+}-ATPase activity, which results in decreased contractility of the muscle, as described in [21]. One of the important changes happening in muscle fibers in fatigue is increased Ca^{2+} concentration caused due to decreased uptake of this ion by SR [22].

Our results demonstrate that chlorpyriphos causes significant changes to contractile parameters of muscle fibers in concentrations of 10^{-6} to 10^{-5} M (Fig. 1). The compound had no significant effect on contractile properties of muscle fibers in concentrations below 10^{-6} M. Chlorpyriphos introduced into incubation medium in concentrations above 10^{-5} M caused total arrest of muscle fiber contractions within 12 min of the experiment; consequently, we considered investigation of its effect in such concentrations impractical.

Chlorpyriphos introduction into incubation medium in concentration of 10^{-6} M caused uniform decrease in contractile parameters of SM for the entire duration of stimulus. The maximum inhibition of muscle productivity was observed starting from 21st min of the experiment and was 82.8 ± 1.9% of control (Fig. 1, *A*). Changes in muscle length were

Table 1. The effect of chlorpyrifos solutions on Ca^{2+},Mg^{2+}-ATPase activity of SR from m. tibialis anterior muscle fibers of Rana temporaria (M ± m, n = 10)

Enzyme	Chlorpyriphos concentration, M					
	Control	10^{-6}	$2.5 \cdot 10^{-6}$	$7.5 \cdot 10^{-6}$	$5 \cdot 10^{-6}$	10^{-5}
Ca^{2+},Mg^{2+}-ATPase activity, nM of P_i × ×min^{-1}×mg of protein	246.8 ± 1.5	234.6 ± 1.2*	211.3 ± 2.5*	203.3 ± 1.9*	193.5 ± 2.1*	186.5 ± 2.1*

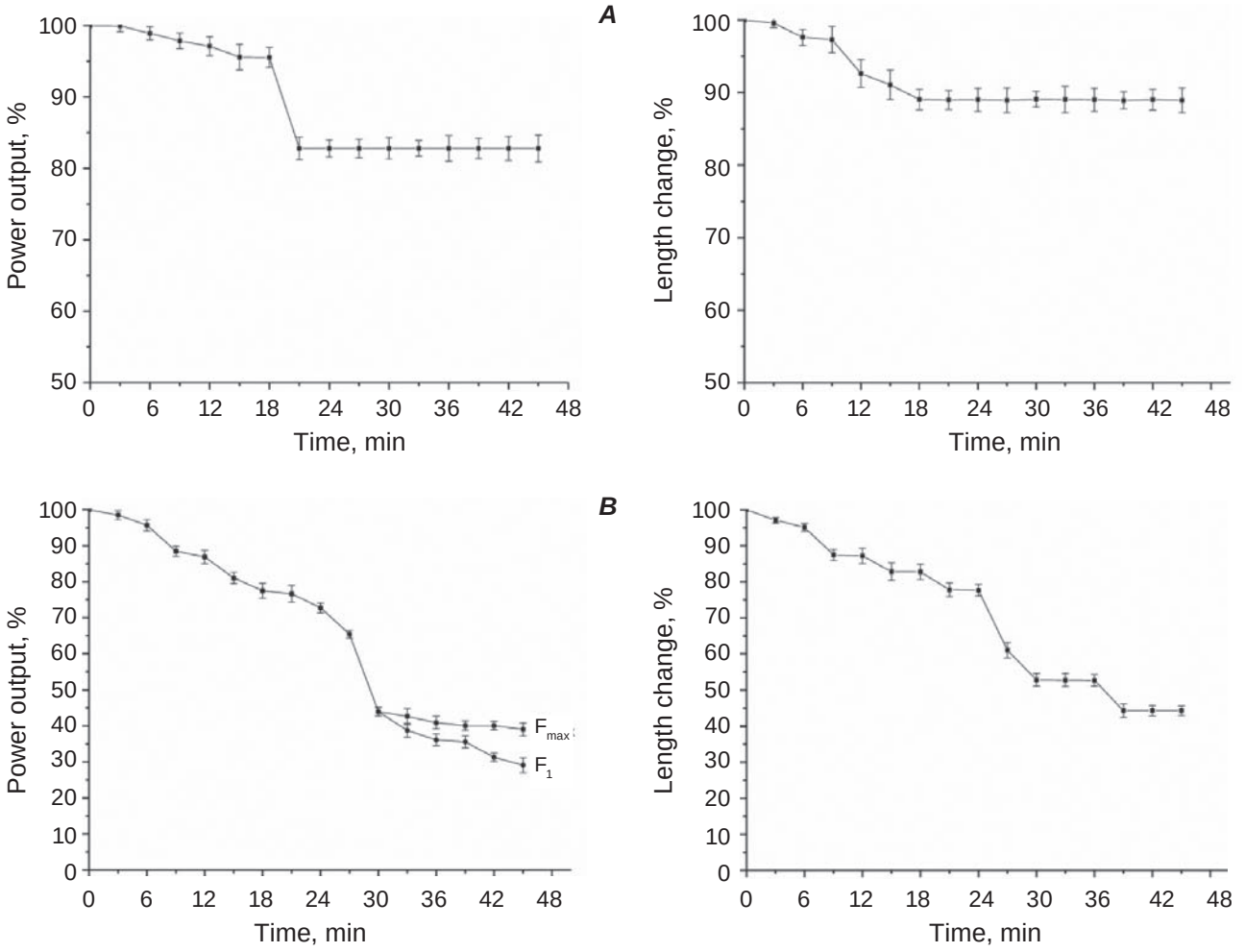

Fig. 1. The effect of chlorpyriphos in concentrations of 10^{-6} M (A) and $2.5 \cdot 10^{-6}$ M (B) upon dynamic contraction properties of m. tibialis anterior as function of exposure time (M ± m, n = 10). F_{max} denotes maximum power output; F_1 denotes changes in SM fiber strength at level corresponding to equilibrium stable state

less pronounced and constituted 89.0 ± 1.7% of control.

Chlorpyriphos in concentration of $2.5 \cdot 10^{-6}$ resulted in significantly more pronounced inhibition of SM contraction activity (Fig. 1, *B*). The maximum strength decrease is denoted as F_{max} (Fig. 1, *B*) and constituted 39.0 ± 1.8% on the increase stage after 30 min incubation with chlorpyriphos, and denoted as F_1 in equilibrium stable state, in which the decrease was 29.1 ± 2.1% from control values.

Length changes under effect of chlorpyriphos ($2.5 \cdot 10^{-6}$ M) were less pronounced than changes in power output. The maximum inhibition was 44.3 ± 1.3% of control values beginning on 39th min of the experiment. It must be noted that the length of muscle changed uniformly for the entire duration of the stimulus.

There were no marked changes in dynamic parameters for chlorpyriphos concentration of $5 \cdot 10^{-6}$ M in comparison to the effects of the compound in concentration of $2.5 \cdot 10^{-6}$ M. Nevertheless, we observed noticeable fluctuations in strength of response to stimulus. The muscle fibers were unable to maintain a uniform strength of response after 30th min of the experiment. The maximum inhibition of power output was 38.9 ± 1.8% and was detected on the 45th min of the experiment (Fig. 2, *A*). The maximum decrease in contraction length was found on 54th min of incubation, and constituted 40.3 ± 2.1% from that of control.

The effect of chlorpyriphos in concentration of $7.5 \cdot 10^{-6}$ caused maximum inhibition of SM fibers power output after 30th min of exposure (Fig 2, *B*). The contraction strength was at 20.0 ± 2.3% of that

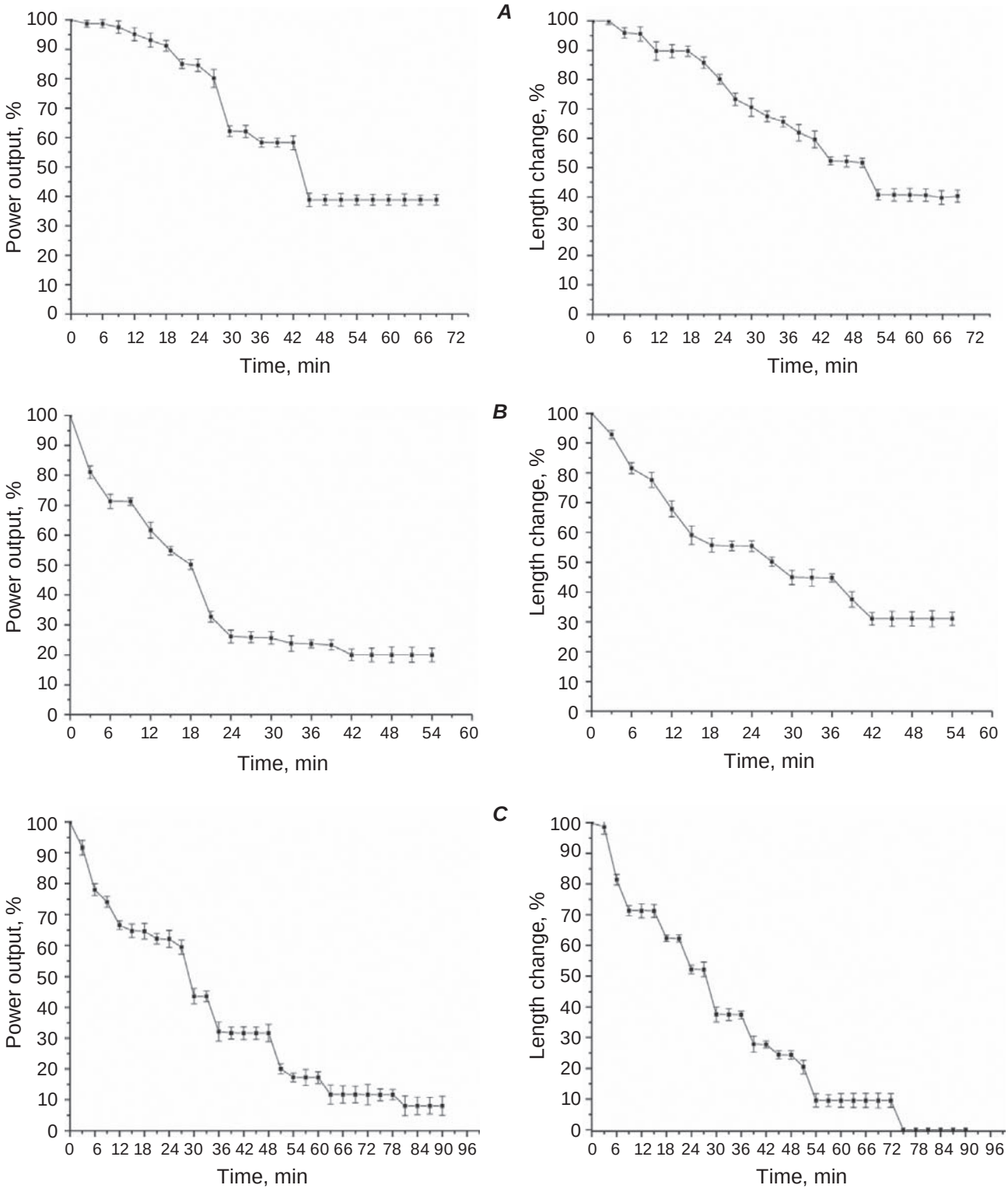

Fig. 2. The effect of chlorpyriphos in concentrations of $5 \cdot 10^{-6}$ M (A), $7.5 \cdot 10^{-6}$ (B), and 10^{-5} M (C) upon dynamic contraction properties of m. tibialis anterior as function of exposure time (M ± m, n = 10)

of control. The inhibitive effect of chlorpyriphos on muscle power output was observed immediately after addition of the compound to incubation buffer. The power output level decreased by 20% in comparison to control within three min of the beginning of exposure, and the corresponding decrease in con-

traction length was detected beginning on 6th min the maximum decrease in contraction length was 31.0 ± 2.2% from the control values. The decrease in contraction length reached stable level on 42nd min of the exposure to chlorpyriphos. Therefore, this dynamic parameter is less sensitive to inhibitive effects of chlorpyriphos in concentration of $7.5 \cdot 10^{-6}$ M than contraction strength.

Chlorpyriphos in concentrations of 10^{-5} M caused nearly total inhibition of muscle contractile activity. The maximum decrease in contraction strength was observed after 63rd min of chlorpyriphos exposure and was 8.1 ± 3.1% from that of control (Fig. 2, *C*).

There was no detectable change in muscle length during contractions under chlorpyriphos exposure after 75th min of the experiment.

Therefore, these results demonstrate concentration-dependent effect of chlorpyriphos upon muscle contraction dynamics. It should be noted that muscle power output changed more noticeably under exposure to chlorpyriphos in concentrations of 10^{-6} to $7.5 \cdot 10^{-6}$ than muscle contraction length (Fig. 3).

The contractile length of muscle under chlorpyriphos exposure in concentration of 10^{-5} M was undetectable, and the muscle power output was no higher than 10% of that of control. On the other hand, the differences between contraction strength inhibition and changes in contraction length were not significant only in under effects of chlorpyriphos in concentration of $5 \cdot 10^{-6}$ M (Fig. 3).

We interpret the experimental data as demonstrating the marked inhibition in dynamic parameters of SM fibers contraction due to noncholinergic effects of chlorpyriphos. Muscle's inability to maintain stable contraction strength under tetanic contraction indicates not only the variability in effects of different chlorpyriphos concentrations upon contractile activity, but also differences in molecular mechanisms of generation of response strength and in propagation of dynamic muscle movements [6, 22-25]. The observed decrease in dynamic parameters of contraction results probably from direct influence of chlorpyriphos on myofilaments unmediated by acetylcholine esterase. These results allow us to propose a mechanism of decrease in muscle contractile function under OPI exposure that is independent of their cholinergic effect. This is in accordance with results [26], which show that under low doses of OPI changes in SM are observable despite nearly no detectable decrease in acetylcholine

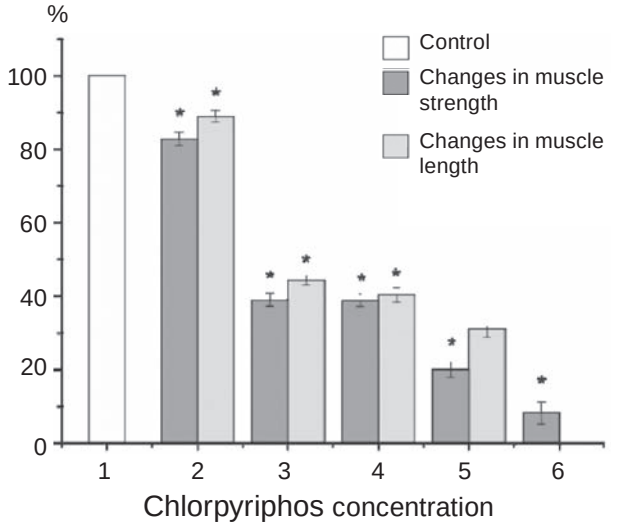

*Fig. 3. Maximum decrease in dynamic parameters of contraction of m. tibialis anterior under stimulus (30 Hz 3000 ms pulse) and chlorpyriphos exposure. 1 – control, 2 to 6 – chlorpyriphos concentrations 10^{-6}; $2.5 \cdot 10^{-6}$; $5 \cdot 10^{-6}$; $7.5 \cdot 10^{-6}$; 10^{-5} M, correspondingly (M ± m, n = 10); * denotes changes significant with P < 0.05 in comparison to control*

esterase activity. The demonstrated inhibition of SR Ca^{2+},Mg^{2+}-ATPase activity and dynamic contraction properties of m. tibialis anterior from *Rana temporaria* due to noncholinergic effects of chlorpyriphos indicates that disruptions in SM function under OPI exposure happens not just in the neurotransmission, but on cellular and subcellular levels as well.

ВПЛИВ ХЛОРПІРИФОСУ НА АКТИВНІСТЬ АТРази САРКОПЛАЗМАТИЧНОГО РЕТИКУЛУМА ТА БІОМЕХАНІКУ СКОРОЧЕННЯ СКЕЛЕТНИХ М'ЯЗІВ

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Вивчали вплив фосфорорганічного інсектициду – хлорпірифосу – на активність Ca^{2+},Mg^{2+}-АТРази саркоплазматичного ретикулума (СР) і на біомеханіку скорочення скелетних м'язів (m. tibialis anterior) жаби *Rana temporaria*. Показано, що активність Ca^{2+},Mg^{2+}-АТРази СР

пригнічувалась хлорпірифосом у діапазоні концентрацій від 10^{-6} до 10^{-5} М. Активність Ca^{2+},Mg^{2+}-АТРази знижувалась у діапазоні від 10^{-6} до $7{,}5{\cdot}10^{-6}$ М найімовірніше внаслідок порушення цілісності мембрани СР. Показано, що інгібіторні властивості хлорпірифосу посилювалися як за підвищення концентрацій, так і у разі збільшення тривалості його дії. У концентраціях від 10^{-6} до $7{,}5{\cdot}10^{-6}$ М хлорпірифос змінював силову відповідь м'язових волокон вираженіше порівняно зі зміною довжини скорочення. Встановлено пригнічення Ca^{2+},Mg^{2+}-АТРази СР внаслідок нехолінергічних ефектів дії хлорпірифосу.

Ключові слова: хлорпірифос, АТРазна активність саркоплазматичного ретикулума, м'язове скорочення.

ВЛИЯНИЕ ХЛОРПИРИФОСА НА АКТИВНОСТЬ АТРазы САРКОПЛАЗМАТИЧЕСКОГО РЕТИКУЛУМА И БИОМЕХАНИКУ СОКРАЩЕНИЯ СКЕЛЕТНЫХ МЫШЦ

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Исследовали влияние фосфорорганического инсектицида – хлорпирифоса – на активность Ca^{2+},Mg^{2+}-АТРазы саркоплазматического ретикулума (СР) и на биомеханику сокращения скелетных мышц (m. tibialis anterior) жабы *Rana temporaria*. Показано, что активность Ca^{2+},Mg^{2+}-АТРазы СР угнеталась хлорпирифосом в диапазоне концентраций от 10^{-6} до 10^{-5} М. Активность Ca^{2+},Mg^{2+}-АТРазы снижалась в диапазоне концентраций от 10^{-6} до $7{,}5{\cdot}10^{-6}$ М вероятнее всего в результате нарушения целостности мембраны СР. Показано, что ингибиторные свойства хлорпирифоса усиливались как при повышении концентраций, так и при увеличении продолжительности его действия. В концентрациях от 10^{-6} до $7{,}5{\cdot}10^{-6}$ М хлорпирифос изменяет силовой ответ мышечных волокон более выражено по сравнению с изменением длины сокращения. Установлено угнетение Ca^{2+},Mg^{2+}-АТРазы саркоплазматического ретикулума в результате нехолинергических эффектов воздействия хлорпирифоса.

Ключевые слова: хлорпирифос, АТРазная активность саркоплазматического ретикулума, мышечное сокращение.

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