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Mechanisms of change contraction of function of the muscles *in vitro* at allergic

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ABSTRACT

In this work, mechanisms of influence of protein sensibility of an organism on contractile function of the isolated skeletal muscles of the mouse—"fast"—*musculus extensor digitorum longus*, "mixed"—*musculus diaphragma* and "slow"—*musculus soleus* are investigated. It is shown that at a protein sensitization all "fast", "mixed" and "slow" skeletal muscles change the contractile properties. The vector of these changes for muscles with a various phenotypes carries opposite character. Force of the reduction caused carbacholine at a "slow" and "mixed" muscles increase, at "fast"—decreases. A vector of change of force of reduction on carbacholine at protein sensitization at these skeletal muscles correlates with changes of non-quantum secretion acetylcholine in a zone of a trailer plate. Opposite changes of functional properties of "fast" and "mixed" muscles and "slow" muscles of a shin of the mouse at protein sensitization are caused by dynamics cholinoceptive processes of excitation of membrane muscular fibers. It comes out with the assumption, that change of the contraction functions of skeletal muscles at protein sensitization is caused by changes of cholinoceptive processes of excitation of a membrane of muscular fibers, and other changes in system of electro-mechanical interface.

KEYWORDS

Skeletal Muscle; Contraction Characteristics; Extensor Digitorum Longus; Soleus; Diaphragm; Non-Quantum Secretion; Protein Sensitization

1. INTRODUCTION

It is well-known that airway muscles functional state and, primary diaphragm are essentially changed in bronchial asthma, the disease of allergic origin. We have previously studied the influence of protein sensitization on contraction function of guinea pig isolated diaphragm strip. To be more concrete, its ability to change its contractile functions by increase of force and shortening velocity on carbacholine (CCh) in terms of protein sensitization was shown [1]. To explain this fact, changes of electric features as well as histochemistry profile of muscle fibers were proposed [2]. Furthermore, as the contraction of isolated muscle was initiated by agonist we can suggest the participation of surface membrane cholinergic receptors activation mechanisms in the change of muscle fibers functional features at protein sensitization. Diaphragm is a mixed muscle; it consists of "fast" and "slow" muscular fibers (MF). Recently we showed that protein sensitization changes contraction functions of isolated—"fast" and "slow"—shank mouse skeletal muscles [3]. Dynamics of contraction function of studied skeletal muscles shows remarkable differences. For clarity, cholinoceptive processes of excitement of postsynaptic membrane play an important role in the mechanisms of contraction force change [4]. The ability of mouse diaphragm to change its functions in terms of allergic restructure and its possible mechanisms remain to be determined.

2. EASE OF USE

The purpose of the present study is to reveal cholinoceptive mechanisms in pathogenesis influence of protein sensitization on contraction functions of mouse isolated muscles: "mixed"—*musculus diaphragma* strip (Diaphragm); "fast"—*musculus extensor digitorum longus*

(EDL) and "slow"—*musculus soleus* (Soleus).

The complex researches were performed to investigate this problem. Two experimental models which are characteristic for cholinoceptive processes of excitement of isolated mouse striated muscles were used to study the effects of protein sensitization on: 1) the rates of contraction muscles response, caused by agonist CCh and 2) the level of non-quanta secretion of acetylcholine (Ach) in a zone of a trailer plate (H-effect).

3. MATERIALS AND METHODS

Experiments were conducted on both sexes mice weighing 17 - 22 g. Animals were twice albumin sensitize (OS) with gel hydrate aluminum (2 mkg of dry gel substance + 150 mkg ovalbuminum in 0.5 ml of a physiological solution) parenteral. The second injection was made in 14 days after the first one. Animals were got into experiment on a pique of sensitization—7 - 10 days after the second sensitization injections [5]. Mechanomiographic researches were conducted on a preparation of an isolated muscle in terms of an isometry. Skeletal muscle was stretched during 20 minutes with force of 500 mg at a constant perfusion by a solution Krebs type to maintain isometry and temperature at 20˚C - 21˚C. Contraction was recorded by the photoelectric converter [6]. Agonist CCh was investigated at submaximal concentration: for EDL—7×10^{-4} M, a Diaphragm—2×10^{-4} M, Soleus—5×10^{-4} M. Contraction function was analyzed according muscles contraction parameters on CCh. Muscle contraction force (Poc) and speed (Voc) were estimated. Contraction force developed by isolated muscle was related to its mass (m) (Poc^*—numerically equal volume of a muscular preparation) to get objective information at the force characteristics analysis.

To study a condition of muscle fiber postsynaptic membrane in the zone of a trailer plate non quantum secretion of Ach was studied. It was measured by glass microelectrodes (with the resistance of 8 - 12 MΩ, filled with 2.5 M KCl) [7]. To determine its size armin action acetylcholinesteraze, then on a muscle was eliminated during 8 - 12 minutes application m-cholinergic receptors blockade d-tubocurarine (dTC) (10^{-5} M). The rates difference of membrane potential before and after application dTC corresponds to the rate of non-quantum secretion of ACh (H-effect).

Statistical Analysis

The software package Microcal Origin 5.0 (OriginLab Corp., Northampton, MA, USA) was used for statistical analyses. All data are presented as means ± S.E.M., with significance assessed by Student's *t* test. A p value of less than 0.05 was considered as statistically significant.

4. RESULTS

Contraction parameters of isolated EDL, a Diaphragm strips and mouse Soleus on CCh at submaximal concentration in the control and at protein sensitization are represented in **Tables 1-3**.

For "fast" muscle it is shown that CCh at submaximal concentration caused contraction of with force of 9.94 ± 0.39 mg/mm^3 and speed of 14.26 ± 1.55 mg/s. EDL contraction force decreased, speed practically did not change at protein sensitization (**Table 1**).

Study of non-quantum secretion of ACh in "fast" mouse muscle fiber has shown the following data. Initial rate of membrane potential in the terms of rest was 72.3 ± 0.6 mV (n = 150). But at presence of dTC it increased up to 77.4 ± 1.6 mV (n = 150). Thus, the H-effect in the terms of control makes 5.1 ± 0.4 mV (n = 150). In the terms of protein sensitization initial rate of membrane potential of the rest was 73.9 ± 0.5 mV (n = 150). At presence of dTC it increased up to 79.7 ± 1.7 mB (n = 150). It means that the H-effect value has increased, making in the described terms of experiment 5.8 ± 0.5 mV (n = 150, $p < 0.05$).

In the "mixed" on intact muscle mouse CCh at submaximal concentration caused contraction with force of 49.20 ± 1.75 mg/mm^3 and speed 31.0 ± 1.7 mg/s. Protein sensitization resulted in force increase, speed practically did not change of Diaphragm contraction (**Table 2**).

Study of non-quantum secretion of ACh of "mixed" muscle has shown: the initial rate of membrane potential of the rest was 70.7 ± 1.9 mV (n = 150). At presence of dTC it increased up to 75.9 ± 0.7 mV (n = 150). Thus, H-effect in the control makes 5.2 ± 0.4 mV (n = 150). Initial rate of membrane potential of the rest was 70.0 ± 1.5 mV (n = 150). At protein sensitization at the presence of dTC it increased up to 74.4 ± 0.6 mV (n = 150). Tit means that the H-effect value decreased, making in the described terms of experiment 4.4 ± 0.5 mV (n = 150, $p < 0.05$).

At "slow" intact mouse muscle CCh at submaximal concentration caused contraction with the force of 35.61 ± 1.67 mg/mm^3 and speed of 13.1 ± 1.0 mg/s. Protein sensitization resulted in force and speed increase (**Table 3**).

Study of non-quantum secretion of ACh has shown: the initial rate of membrane potential of rest was 70.9 ±

Table 1. Parameters of isolated *musculus extensor digitorum longus* contraction (X ± Sx) on CCh (7×10^{-4} M) in control and experiment.

Experiment terms	Contraction parameters	
	Poc^* mg/mm^3	Voc, mg/s
Control n = 26	9.94 ± 0.39	14.26 ± 1.55
Experiment n = 5	5.65 ± 0.82***	13.62 ± 4.09

Note: ***p < 0.001.

Table 2. Parameters of isolated *musculus diaphragma* contraction (X ± Sx) on CCh (2×10^{-4} M) in control and experiment.

Experiment terms	Contraction parameters	
	Poc* mg/mm^3	Voc, mg/s
Control n = 10	49.20 ± 1.75	31.0 ± 1.7
Experiment n = 7	58.66 ± 3.97**	32.08 ± 0.89

Note: **p < 0.01.

Table 3. Parameters of isolated *musculus soleus* contraction (X ± Sx) on CCh (5×10^{-4} M) in control and experiment.

Experiment terms	Contraction parameters	
	Poc* mg/mm^3	Voc, mg/s
Control n = 28	35.61 ± 1.67	13.10 ± 0.99
Experiment n = 11	54.18 ± 4.99 ***	16.62 ± 1.50

Note: ***p < 0.001.

1.7 mV (n = 160). But at presence of dTC it increased up to 75.9 ± 1.3 mV (n = 160). Thus, H-effect in the control makes 5.0 ± 0.7 mV (n = 160). Initial rate of membrane potential of rest making 69.4 ± 0.9 mV (n = 150) at protein sensitization at presence of dTC increased up to 72.5 ± 1.0 mV (n = 150). It means that the H-effect value decreased making in the described conditions of experiment 3.1 ± 0.6 mV (n = 150, $p < 0.05$).

5. DISCUSSION

Results of studies indicate that protein sensitization changes contraction function of diaphragm strip as well as isolated shank mouse skeletal muscles (**Tables 1-3**). To clarify, nature of these changes is essentially different for "fast" and "slow" muscles. Basic differences of morphfunctional status of studied objects and its change mechanisms in the process of allergic restructure of an organism can explain this fact.

Presumably, the fiber structure defines the differences in contractile force of observed muscles of nonsensibility animals on CCh. Soleus mouse muscle contains 50% - 60% of "slow" filaments, EDL—97% - 100% of "fast" filaments [8]. Mouse diaphragm, that takes media position, contains 88.6% of fast myosin. Presumably, force differences result from different level of muscle fibers sensibility to CCh that suggests direct dependence on the area of synapse. It is known that the size of a trailer plate of "slow" muscle fiber of soleus mouse muscle is 3 lengths than that of "fast" muscle fiber (EDL) [9]. Considering similarity of biometric parameters (length and mass, **Table 1**) of observed muscles, more sensibility to cholinomediate, caused by greater number of cholinergic receptors in the area of synapse, must result in more contractile force of m soleus and diaphragm on CCh.

Changes of diaphragmatic muscle functional features in terms of protein sensitization suggest that changes in muscle fiber during allergic restructure of an organism are complex. Changes occurring in muscle fiber during sensitization may affect surface membrane [10], electromechanical connected mechanisms [2] or contractile protein system [11]. The absence of corresponding changes of shortening velocity on submaximal concentration of agonist of all the muscles doesn't suggest electromechanical connected system changes. However, a various of force change vector of "fast" muscle from the one side and "mixed" and "slow" muscles from the other side indicate principle difference of change mechanisms of muscles functional features at protein sensitization.

While contractile force (Poc*) of "fast" muscle decreases (**Table 1**), contractile force of "slow" and "mixed" muscles increases (**Tables 2** and **3**). This dynamics confirms the fact that differences of "fast" muscle from the one side and "mixed" and "slow" muscles form the other side at protein sensitization affect, primarily, cholinomediate processes of excitement of muscle fibers manifold effect.

Evidence that protein sensitization is able to affect the mechanisms of excitement of postsynaptic membrane of different muscles in different way comes from comparing muscle contractile force dynamics on CCh with level change of non-quantum secretion in the zone of a trailer plate. Force change vector of observed muscles at protein sensitization correlates with level change of non-quantum secretion of ACh in the zone of a trailer plate (H-effect) (**Figures 1A** and **B**).

We may conclude that the decrease of contractile force of "fast" muscle on CCh results from the decrease of postsynapse sensibility to cholinomediate. The evidence of this fact comes from the increasing H-effect (**Figures 1A** and **B**). Increase of non-quantum secretion of ACh in the zone of synapse contributes intensification of desensitization mechanisms of cholinergic receptors of postsynaptic membrane. Correspondingly, we observe reverse picture about "mixed" and "slow" muscles. Increase of contractile force on CCh results from increase of postsynapse sensibility to CCh. Decreasing H-effect reflects this process (**Figures 2A**, **B** and **3A**, **B**).

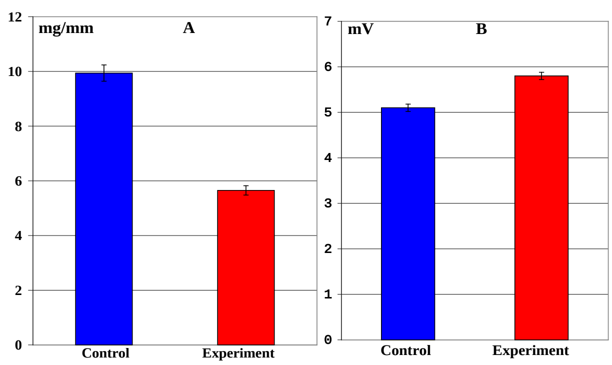

Figure 1. Protein sensitization effect on: A. Contraction force of isolated mouse EDL caused by CCh; B. H-effect value.

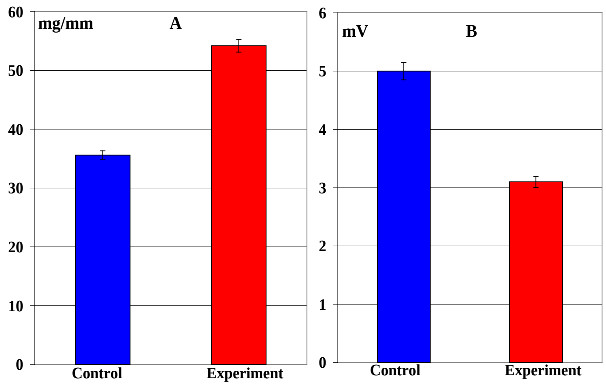

Figure 2. Protein sensitization effect on: A. Contraction force of isolated mouse Soleus caused by CCh; B. H-effect value.

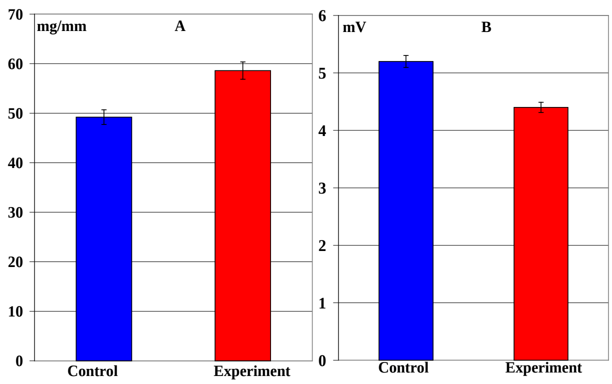

Figure 3. Protein sensitization effect on: A. Contraction force of isolated mouse Diaphragm caused by CCh; B. H-effect value.

Thus, allergic restructure of an organism causes changes of contraction function of isolated mouse skeletal muscles. Contractile force (Poc*) on CCh of "fast" muscle decreases and, correspondingly, of "mixed" and "slow" muscles increases. This rate change occurs from the following. Decrease of contractile force of "fast" muscle (EDL) results from decrease of postsynaptic membrane sensibility to CCh that is caused by increasing of non-quantal secretion of ACh in the zone of a trailer plate. "Mixed" and "slow" muscles show reverse dynamics of contractile force as well as of non-quantum secretion of ACh. Increase of contractile force of these muscles on CCh at protein sensitization results from increasing post-synaptic membrane sensibility to cholinomediate that is caused by decrease of non-quantum secretion of ACh in the zone of a trailer plate. Different changes of contraction function of skeletal muscles at protein sensitization are caused, primarily, by dynamics of cholinomediated processes of membrane excitement of muscle fibers.

6. CONCLUSIONS

Mechanisms plasticity in skeletal muscle for protein sensitization under defined condition cholinomediate post-synaptic membrane. The dynamics of contractile force on CCh all muscles studied pathology correlated with changes in sensitivity to post-synapses ACh, and different types of muscle are the cause of the multi-directional nature of the changes.

In the experimental allergy in the "slow" and "mixed" phase muscles at the base of the development of resistance to long-term external loads are the mechanisms of regulation of their sensitivity to acetylcholine. The processes described above provide increased performance during prolonged physical activity, as well as reduced fatigue of the respiratory muscles during hypoxia that occurs in chronic obstructive pulmonary disease, bronchospastic syndrome and asthma.

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