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Possible Mechanisms for the Effect of Protein Sensitization on Contractile Function of Fast and Slow Muscles in Mice

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We studied the mechanisms underlying the effect of immunobiological reorganization of the organism on contractile function of isolated skeletal muscles from mouse leg (fast muscle, *m. extensor digitorum longus*; and slow muscle, *m. soleus*). Protein sensitization was accompanied by changes in contractile properties of fast and slow skeletal muscles. These changes were differently directed in muscles with various phenotypes. The force of carbachol-induced contraction (cholinergic agonist) increased in the slow muscle, but decreased in the fast muscle. The direction of changes in the force of carbachol-induced contractions under conditions of protein sensitization in skeletal muscles correlates with changes in non-quantal secretion of acetylcholine in the endplate (H-effect). Opposite changes in functional properties of fast and slow muscles from mouse leg during protein sensitization are related to choline-mediated excitation of the muscle fiber membrane. Our results suggest that changes in contractile function of skeletal muscles during protein sensitization are associated with variations in choline-mediated excitation of the muscle fiber membrane and modification of electromechanical coupling.

Key Words: *skeletal muscle; contractile properties; non-quantal secretion; protein sensitization*

Immunobiological reconstruction of the organism is accompanied by changes in morphofunctional characteristics of skeletal muscles (SM) in warm-blooded animals [1]. Contractile function of isolated SM was evaluated in allergic guinea pigs. Studying the mechanisms for contractility variations caused by protein sensitization (PS) in various SM showed that slow and mixed SM can change their contractile properties. It was hypothesized that these changes are related to modification of electrogenic properties of the surface membrane and histochemical profile of SM [3]. Our previous experiments revealed that PS is accompanied by changes in contractile activity of isolated mouse SM [6]. Changes in contractile function during PS were shown to be different in fast and slow muscles of mouse leg. Changes in the force of muscle contraction is partly related to choline-mediated excitation of the SM fiber membrane [6].

Here we studied the mechanism for the effect of PS on contractile function of the isolated fast (extensor digitorum longus muscle, *m. extensor digitorum longus*, *m. EDL*) and slow soleus muscle, *m. soleus* SM from mouse leg. A complex study was conducted on 2 experimental models for choline-mediated excitation of isolated mouse

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SM. We evaluated the influence of PS on the contractile response of SM induced by a humoral cholinergic agonist carbachol. Non-quantal secretion of acetylcholine in the endplate was recorded.

MATERIALS AND METHODS

Experiments were performed on male and female mice weighing 17-22 g. The animals were sensitized with ovalbumin and aluminum hydroxide gel (2 μg dry gel and 150 μg ovalbumin in 0.5 ml physiological saline; intraperitoneally, twice) [4]. The second injection was conducted 14 days after the first treatment. The animals were examined at the peak of sensitization (days 7-14 after the second sensitizing injection). Mechanomyography was performed on the isolated muscle under isometric conditions. SM were stretched with 0.5 g for 20 min under continuous perfusion with Krebs solution. The contraction was recorded with a photoelectric transducer [2]. Cholinergic agonist carbachol was used in concentrations varying from 2×10^{-5} to 3×10^{-3} M. The contractile function was evaluated by carbachol-induced muscle contraction. The force (Poc) and speed of muscle contraction (Voc) were estimated after agonist treatment in maximum and submaximum concentrations. The force of isolated muscle contraction was divided by the weight of this muscle (Poc*, volume of the muscle preparation).

For evaluation of the state of postsynaptic membrane of the muscle fiber in the endplate area [7], non-quantal secretion of acetylcholine was studied using glass microelectrodes (resistance 8-12 MΩ) filled with 2.5 M KCl. Acetylcholinesterase was inhibited with armin and then nicotinic receptor antagonist d-tubocurarine (TB, 10^{-5} M) was applied to the muscle for 8-12 min. The difference between the membrane potentials before and after TB application corresponds to non-quantal secretion of acetylcholine (H-effect).

The experiments were performed at 20-21°C.

We compared the data for muscles from intact (control) and sensitized animals (treatment). The results were analyzed by Student's *t* test.

RESULTS

Table 1 shows biometric parameters for SM and Tables 2 and 3 present the parameters of contraction of isolated mouse *m. EDL* and *m. soleus* after treatment with carbachol in the submaximum and maximum concentrations under control conditions and after PS.

In non-sensitized mouse, treatment with carbachol in the submaximum concentration (7×10^{-4} M) induced *m. EDL* contraction with force and speed of 76.6±6.1 mg (Poc* 9.94±0.39 mg/mm^3) and 14.3±1.6 mg/sec, respectively. After PS, the force of *m. EDL* contraction decreased ($p<0.01$), while the speed of contraction remained practically unchanged (Table 2).

The force of *m. EDL* contraction in response to treatment with carbachol in the maximum concentration (4×10^{-3} M) significantly decreased during sensitization (Table 2).

The study of non-quantal secretion of acetylcholine in muscle fibers of *m. EDL* showed that the resting membrane potential increased from -72.3±0.6 mV (initial, $n=150$) to -77.4±1.6 mV ($n=150$) in the presence of TB. Therefore, the H-effect under control conditions was 5.1±0.4 mV ($n=150$). Under conditions of PS, the initial resting membrane potential was -73.9±0.5 mV ($n=150$). After application of TB under these conditions it increased to -79.7±1.7 mV ($n=150$). Hence, the H-effect increased to 5.8±0.5 mV ($n=150$, $p<0.05$).

In *m. soleus* of non-sensitized mouse, carbachol in the submaximum concentration (5×10^{-4} M) induced contraction with the force and speed of 237.8±20.6 mg (Poc* 35.61±1.67 mg/mm^3) and 13.1±1.0 mg/sec, respectively (Table 3). PS increased the force and speed of *m. soleus* contraction (Table 3).

The force of *m. soleus* contraction in response to treatment with carbachol in the maximum concentration (2×10^{-3} M) significantly increased after sensitization (Table 3).

In the presence of TB, the resting membrane potential increased from -70.9±1.7 mV (initial, $n=160$) to -75.9±1.3 mV ($n=160$). Therefore, the H-effect under control conditions was 5.0±0.7 mV

TABLE 1. Biometric Parameters for Fast and Slow Muscle under Control Conditions (Non-Sensitized Mice) and PS

Group	m. EDL		*m. soleus*	
	length, mm	weight, mg	length, mm	weight, mg
Control	8.21±0.14	7.57±0.21	7.20±0.10	6.53±0.18
Sensitization	9.00±0.25	10.08±0.40	7.85±0.15	6.31±0.24

TABLE 2. Contraction of Isolated Mouse Fast Muscle in Response to Treatment with Carbachol at the Submaximum and Maximum Concentration under Control Conditions and PS

Carbachol concentration	Experimental conditions	Poc, mg	Poc*, mg/mm^3	Voc, mg/sec
Submaximum, 7×10^{-4} M	Control (n=26)	76.59±6.51	9.94±0.39	14.26±1.55
	Sensitization (n=5)	61.92±12.42	5.65±0.82***	13.62±4.09
Maximum, 4×10^{-3} M	Control (n=10)	103.83±15.70	9.82±1.11	11.15±1.97
	Sensitization (n=5)	52.13±14.66***	5.09±0.77**	4.62±1.68*

Note. Here and in Table 3: *p<0.05, **p<0.01, and ***p<0.001 compared to the control.

TABLE 3. Contraction of Isolated Mouse Slow Muscle in Response to Treatment with Carbachol at the Submaximum and Maximum Concentration under Control Conditions and PS

Carbachol concentration	Experimental conditions	Poc, mg	Poc*, mg/mm^3	Voc, mg/sec
Submaximum, 5×10^{-4} M	Control (n=28)	237.77±20.61	35.61±1.67	13.10±0.99
	Sensitization (n=11)	353.25±23.11	54.18±4.99**	16.62±1.50
Maximum, 2×10^{-3} M	Control (n=17)	322.32±30.18	51.50±3.50	24.64±3.65
	Sensitization (n=12)	475.14±52.66*	72.16±7.18*	13.44±2.43*

(n=160). After PS, the initial resting membrane potential was -69.4±0.9 mV (n=150) and after application of TB it increased to -72.5±1.0 mV (n=150). Hence, the H-effect decreased to 3.1±0.6 mV (n=150, p<0.05).

Our results indicate that PS modifies contractile activity of isolated mouse SM (Tables 2 and 3). These changes were shown to be different in fast and slow muscles. It was related to differences in the initial morphofunctional state of these muscles and mechanisms of immunobiological reconstruction of an organism.

Differences in the force of carbachol-induced contractions of SM from non-sensitized animals are probably associated with the composition of fibers. Previous studies showed that mouse *m. soleus* and *m. EDL* contain 50-60% slow fibers and 97-100% fast fibers, respectively [9]. The endplate in slow muscle fibers of *m. soleus* is 3-fold greater than in muscle fibers of *m. EDL* [8]. Taking into account similar biometric parameters of these SM (length and weight, Table 1), the greater force of carbachol-induced contraction of *M. soleus* compared to that of *m. EDL,* probably results from its higher sensitivity to cholinergic agonist due to greater number of cholinoceptors in the synapse.

Sensitization-induced changes in muscle fibers probably concern the outer membrane [1], electron-mechanical coupling [3], or contractile protein system [5]. Variations in contractile properties of mouse leg muscles during PS reflect the complexity of changes in SM under conditions of immunobiological reconstruction of the organism. However, the observed changes were differently directed in two muscles of mouse leg. The observed changes in the force and speed of muscle contractions confirm the existence of differences in functional properties of fast and slow SM. The force and speed of contractions were shown to decrease in the fast muscle (Table 2). The speed of contractions decreased, while the force of contractions increased in the slow muscle (Table 3). Therefore, various changes in both muscles during PS mainly concern the process of choline-mediated excitation in muscle fibers and differ in fast and slow muscles.

Different effects of PS on excitation of the postsynaptic membrane in different muscles were demonstrated in experiments, where non-quantal secretion of acetylcholine was measured in the endplate (H-effect) and the results were compared with changes in the force of carbachol-induced muscle contraction. Under conditions of sensitization, changes in the force of SM contraction correlate with changes in the H-effect. We conclude that the decrease in the force of carbachol-induced contraction of fast muscle is related to reduced sensitivity of postsynaptic structures to the cholinergic agonist, which manifests in potentiation of the H-effect (Fig. 1). The increase in non-quantal secretion of acetylcholine in the synapse is followed by strong desensitization of cholinoceptors on the postsynaptic membrane. Opposite processes take place in the

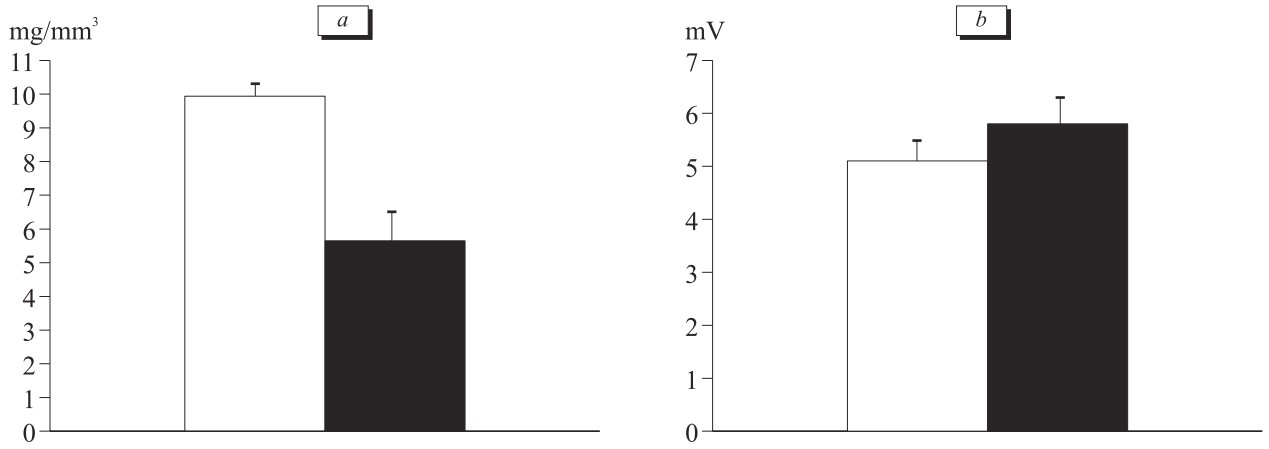

Fig. 1. Effect of PS on functional characteristics of the isolated mouse *m. EDL*. (*a*) Force of carbachol-induced contraction (7×10^{-4} M, *n*=10); (*b*) H-effect (*n*=72). Here and in Fig. 2: light bars, control; dark bars, treatment.

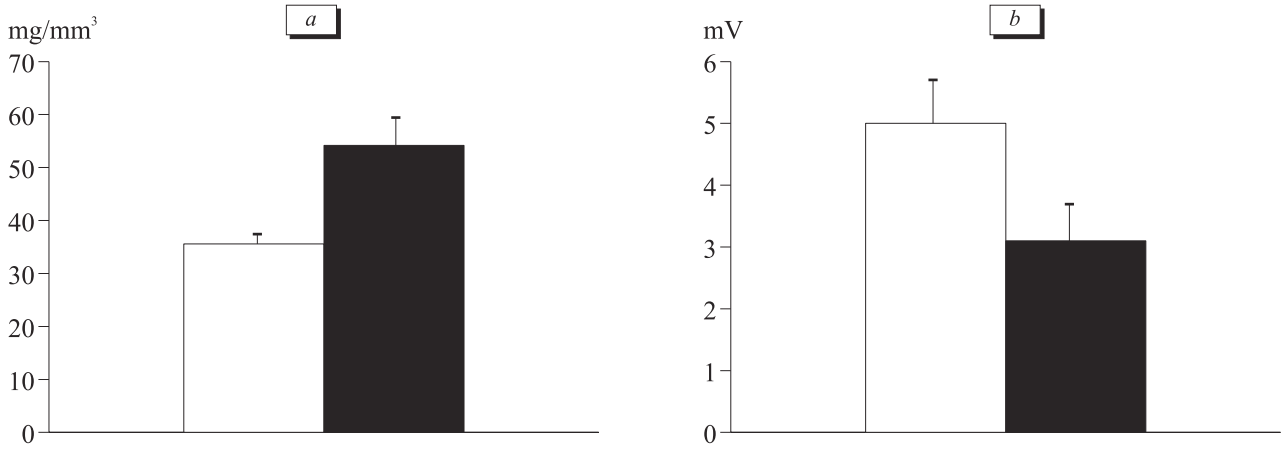

Fig. 2. Effect of PS on functional characteristics of the isolated mouse *m. soleus*. (*a*) Force of carbachol-induced contraction (5×10^{-4} M, *n*=8); (*b*) H-effect (*n*=160).

slow muscle. The increase in the force of carbachol-induced muscle contraction is associated with elevated postsynaptic sensitivity to the cholinergic agonist and attenuation of the H-effect (Fig. 2).

Thus, immunobiological reconstruction of the organism induces changes in contractile function of isolated SM from mouse leg. The force of carbachol-induced contraction decreases in the fast muscle, but increases in the slow muscle. The decrease in the force of fast muscle contraction (*m. EDL*) is associated with reduced sensitivity of the postsynaptic membrane to the cholinergic agonist due to increased non-quantal secretion of acetylcholine in the endplate. The slow muscle (*m. soleus*) is characterized by opposite changes in the force of contraction and non-quantal secretion of acetylcholine. The increase in the force of carbachol-induced slow muscle contraction during PS is related to enhanced sensitivity of the postsynaptic membrane to the cholinergic agonist due to decreased non-quantal secretion of acetylcholine in the endplate. Different changes in contractile function of SM during PS are mainly associated with the dynamics of choline-mediated excitation in the muscle fiber membrane.

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