Possible Mechanisms for the Effect of Protein Sensitization on Functional Properties of the Isolated *m. soleus* and *m. EDL* from Mice

A. Yu. Teplov, A. M. Farkhutdinov, O. V. Teplov, S. N. Grishin*, and M. M. Minnebaev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 9, pp. 262-265, September, 2010
Original article submitted June 1, 2009

We studied possible involvement of ATP in the influence of protein sensitization on contractile function and non-quantum secretion in the end-plate of isolated skeletal muscles from mouse leg. The dynamic vector of muscle contraction force was shown to correlate with changes in non-quantum secretion of acetylcholine under various conditions of experimental pathology. However, the degree of these changes was lower in sensitized animals. It can be hypothesized that the ATP-induced variability in functional properties of slow muscles during protein sensitization reflects the development of resistance to external loads. The adaptive changes in fast muscles during protein sensitization are not associated with the ATP-mediated mechanisms of excitation.

Key Words: *skeletal muscle; contractile functions; non-quantum secretion of acetylcholine; protein sensitization; ATP*

The variability of functional properties of skeletal muscles during sensitization of the body was reported for the motor [4,6] and respiratory muscles in allergic diseases [10,11,14]. The mechanisms of these changes primarily involve the excitatory processes in the muscle fiber membrane [1]. *In vitro* experiments on slow and fast skeletal muscles from mouse leg confirmed this suggestion. Muscle contraction in response to cholinergic receptor agonist carbachol (CC) and non-quantum secretion of acetylcholine (ACh) in the end-plate zone (H-effect) change significantly during protein sensitization (PS).

Previous studies showed that exogenous ATP can reversibly change the contractile function and H-effect of skeletal muscles in nonsensitized animals [7,9]. This effect is probably related to the influence of ATP on cholinergic excitation in muscle fibers. ATP is an endogenous functional modulator of the striated muscles [5]. It is involved in the induction of the immune response [12]. Taking into account these data, we studied the effect of exogenous ATP on functional properties of the isolated slow and fast leg muscles from ovalbumin-sensitized mice (PS).

Here we studied the effect of exogenous ATP on contractile function and non-quantum secretion of ACh in the end-plate of the fast (*m. extensor digitorum longus*, *m. EDL*) and slow (*m. soleus*) skeletal muscles of the leg from intact or sensitized mice.

MATERIALS AND METHODS

Contractile function of a striated muscle from an experimental animal was studied *in vitro* by measuring contractile activity under isometric conditions on a photoelectric transducer [2].

The muscle preparation was put in a temperature-controlled bath. One end of the muscle was attached to a sensor of a photoelectric transducer.

Department of Pathophysiology, Kazan State Medical University; *Department of Television and Multimedia Systems, National Research University, Kazan, Russia. ***Address for correspondence:*** AlikTeplov@.mail.ru. A. Yu. Teplov

0007-4888/11/1503-0295

The other end was fixed in the bath. The muscle was stretched at 20-21°C for 20-30 min under conditions of constant perfusion with Krebs solution. The force of muscle tension was sufficient to reach the isometric conditions. Muscle contraction was induced by CC. CC in a final dose of 5×10^{-4} (*m. soleus*) or 7×10^{-4} M (*m. EDL*) was added to the bath using a microdispenser after termination of perfusion. Contractile function was estimated from the force of muscle contraction (mg).

Non-quantum secretion of ACh was measured with glass microelectrodes (resistance 8-12 MΩ) filled with 2.5 M KCl [7]. Acetylcholinesterase was inhibited by armin ("Tatkhimfarmpreparaty"). N-Cholinoceptor antagonist d-tubocurarine (TBC) in a final concentration of 10^{-5} M was applied to the muscle for 8-12 min. The difference between the membrane potentials (MP) before and after application of TBC corresponds to non-quantum secretion of ACh (H-effect).

The methods of mouse sensitization with a mixture of ovalbumin and aluminum hydroxide gel (Sigma) and control of sensitization were described in details [3].

The effect of ATP (Boehringer Mannheim Gmbh) was studied by comparing contraction indexes before and after 5-min perfusion with a solution containing this substance in a specified molar concentration (10^{-4} M). The time of ATP action depended on the duration of perfusion.

The results were analyzed statistically (BIOSTATISTICA). The data on intact (control) and sensitized (treated) animals were analyzed by parametric and nonparametric tests. The differences were significant at $p<0.05$. The results were expressed as $X\pm Sx(n)$ (X, arithmetic mean; Sx, average error; and n, number of observations).

RESULTS

The force of CC-induced muscle contraction (*m. soleus*) in intact mice was 180.5±6.8 mg (n=8). This parameter increased to 224.3±12.9 mg after incubation with ATP (n=8, $p<0.01$). Studying the dynamics of non-quantum secretion of AC showed that resting MP (-70.9±1.7 mV under basal conditions, n=150) increased to -75.9±1.3 mV (n=150) in the presence of TBC. Therefore, the H-effect under control conditions was 5.0±0.7 mV. Resting MP increased from -70.5±0.4 mV (n=150) to -71.5±0.3 mV (n=150) after incubation with ATP in the presence of TBC. The H-effect decreased to 1.0±0.5 mV under these conditions ($p<0.05$).

The force of CC-induced muscle contraction (*m. EDL*) in intact mice was 72.2±19.5 mg. This parameter decreased to 52.4±11.0 mg after incubation with ATP (n=8, $p<0.05$). Resting MP increased from -72.3±0.6 mV (basal level, n=150) to -77.4±1.6 mV (n=150) in the presence of TBC. Therefore, H-effect under control conditions was 5.1±0.4 mV. After incubation with ATP in the presence of TBC, resting MP increased from -72.5±0.7 mV (n=150) to -77.3±1.1 mV (n=150). The H-effect remained practically unchanged under these experimental conditions (4.8±0.5 mV).

The force of CC-induced muscle contraction (*m. soleus*) in sensitized mice was 235.67±19.55 mg (n=6). Incubation with ATP was accompanied by an increase in this parameter to 264.33±21.09 mg (n=6, $p<0.01$). The resting MP increased from -69.4±0.9 mV (basal level, n=150) to -72.5±1.0 mV in the presence of TBC (n=150). The H-effect under control conditions was 3.1±0.6 mV. After incubation with ATP in the presence of TBC, the resting MP increased from -69.0±0.5 mV (n=150) to -71.1±0.5 mV (n=150). The H-effect decreased to 2.1±0.5 mV ($p<0.05$).

The force of CC-induced muscle contraction (*m. EDL*) in sensitized mice was 59.5±3.3 mg. Incubation with ATP was followed by a decrease in this parameter to 44.5±3.3 mg (n=6, $p<0.01$). Studying the non-quantum secretion of ACh showed that the resting MP in the presence of TBC increases from 73.9±0.5 mV (basal level, n=150) to -79.7±1.7 mV (n=150). Therefore, the H-effect under control conditions was 5.8±0.5 mV. After incubation with ATP in the presence of TBC, resting MP increased from -74.0±0.8 mV (n=150) to -79.3±1.4 mV (n=150). Therefore, the H-effect remained practically unchanged under these experimental conditions (5.3±0.5 mV).

Preliminary perfusion with suramin (100 μM) abolished the influence of ATP on CC-induced contraction and non-quantum secretion of ACh in muscles from intact and sensitized mice. Adenosine in a concentration similar to that of ATP had no effect on non-quantum secretion of ACh and contractile activity of muscles from intact and sensitized mice.

PS of mice is a common experimental model to study the pathogenesis of allergic diseases [10,11,14], which allows us to evaluate functional changes in the striated muscles during allergy. Previous studies showed [4] that functional changes in slow and fast muscles of the mouse leg during PS primarily concern the choline-mediated excitatory processes in the muscle fiber. These changes are opposite for *m. soleus* and *m. EDL*. Functional variability of the test muscles during PS is probably associated with the mechanisms of the release of synaptic transmission cofactors. It was shown that ATP is involved in induction of the immune response [15]. Taking into account these data, we proposed that purines play a role in functional changes of striated muscles during PS. Hence,

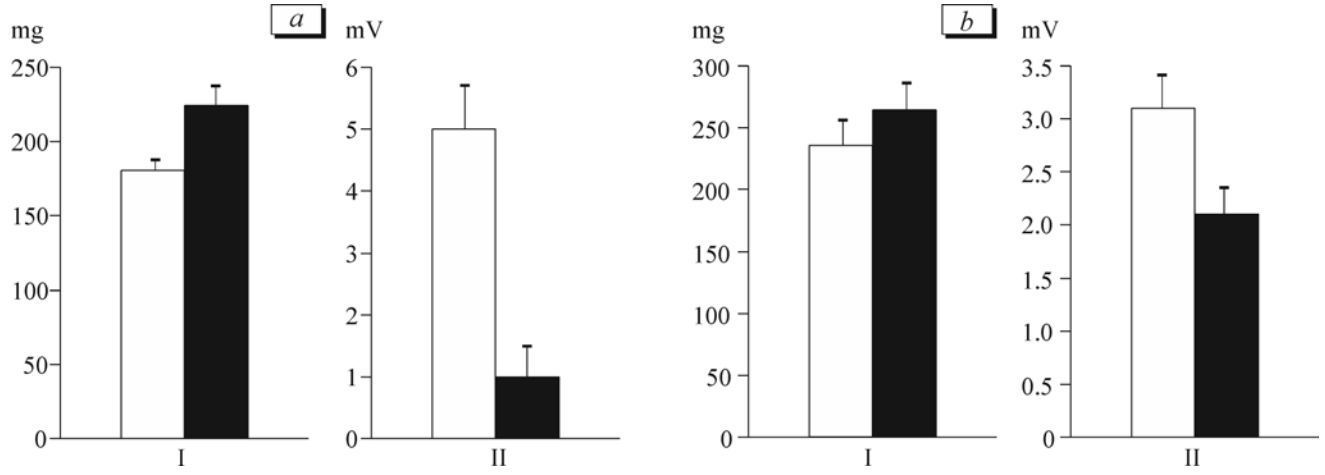

Fig. 1. Functional properties of isolated *m. soleus* from intact (*a*) and sensitized (*b*) mice before (light bars) and after treatment with ATP (dark bars). Here and in Fig. 2: I, force of CC-induced contraction; II, H-effect.

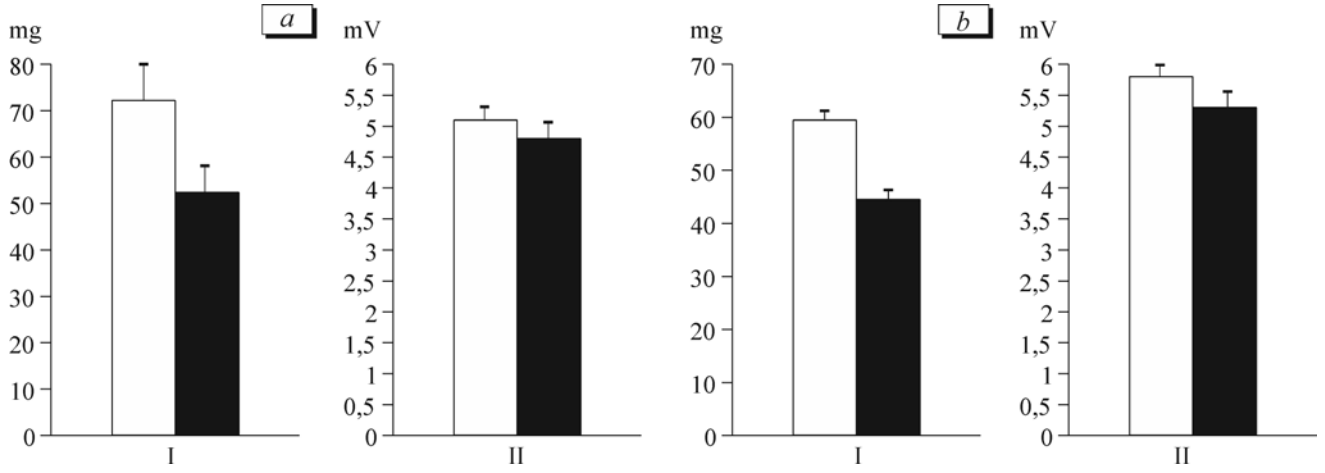

Fig. 2. Functional properties of solated *m. EDL* from intact (*a*) and sensitized (*b*) mice before (light bars) and after treatment with ATP (dark bars).

we studied the dynamics of functional changes in *m. soleus* and *m. EDL* from intact and sensitized mice before and after incubation with ATP.

It should be emphasized that studying the influence of exogenous ATP on various skeletal muscles is an urgent problem. Little is known about the regulatory role of purines for these muscles (as distinct from the smooth and cardiac muscles).

Our results indicate that ATP increases the force of CC-induced contraction in *m. soleus*, but decreases this parameter in *m. EDL*. Non-quantum secretion of ACh decreases in *m. soleus*, but remains unchanged in *m. EDL*. The force dynamic vector and H-effect in *m. soleus* after incubation with ATP are similar to those in muscles of the PS group. We conclude that an increase in the contraction force results from the rise in the sensitivity of the postsynaptic membrane to the cholinergic agonist (Fig. 1, *a*). ATP has a similar effect on the dynamics of these properties in *m. soleus* during sensitization. These data show that the effects of purines on slow muscles in intact and sensitized mice are mediated by similar mechanisms (Fig. 1, *b*).

However, the contraction force of *m. soleus* from intact and sensitized mice increases to 124.3 and 112.2%, respectively. The H-effect for this muscle from intact and sensitized mice decreases to 20 and 67.7%, respectively, after treatment with ATP. The ATP-induced change in functional properties of *m. soleus* was less pronounced in sensitized mice (as compared to control animals). It can be suggested that ATP plays a role in functional changes in slow muscles during PS.

The contraction force of *m. EDL* decreased similarly in intact animals (to 72.6%) and sensitized mice (to 74.8%). No significant changes were observed in the H-effect of ATP-treated *m. EDL* in intact and sensitized mice. This parameter decreased by 94.1 and 91.4%, respectively. No changes were revealed in the contraction force and H-effect in both groups of animals after ATP treatment. Therefore, purines are not involved in

the mechanisms of PS-induced changes in contractile function of mouse fast muscles (Fig. 2, *a*, *b*).

The effect of ATP on contractile activity of both muscles in mice is similar to the influence of this agent on the majority of skeletal muscles. This effect is realized via P_2-receptors. This conclusion is confirmed by published data and results of our experiments. A P_2-receptor antagonist suramin abolishes the effect of ATP under various experimental conditions. The effect of adenosine is realized via P_1-receptors for adenosine [7]. The force of CC-induced muscle contraction and H-effect did not change after the substitution of ATP for adenosine.

There is a variety of probable mechanisms for the influence of ATP under these experimental conditions. They include a direct effect of purines on contractile structures, secretion of the transmitter, intracellular messengers [7,8], and function of ATP-dependent potassium channels [15]. It can be suggested that ATP is involved in some stages of the immune response. For example, ATP increases the specific immune response by stimulating the production of IL-1. Extracellular ATP stimulates the production of active caspase-1 during the immune response, which provides secretion of biologically active IL-1. Hyperexpression of the P_2X_7 receptor results in secretion of mature IL-1β [12].

PS of mice is an experimental model of allergy, which allows us to study the mechanisms of pathological and compensatory changes in the skeletal muscles. We showed that sensitization of animals is followed by an increase in the force of CC-induced contraction of *m. soleus*. The ATP-induced change in functional properties of *m. soleus* was less pronounced in sensitized mice (as compared to control animals). These data reflect the development of resistance to external loads in the slow muscle from the mouse leg. The reasons for adaptive changes in the fast muscle during PS are not associated with the ATP-mediated mechanisms of excitation.

REFERENCES

1. A. D. Ado, N. V. Stomakhina, L. M. Tuluevskaya, and V. N. Fedoseeva, *Byull. Eksp. Biol. Med.,* **98**, No. 7, 84-86 (1984).
2. R. Kh. Akhmetzyanov and E. B. Fillipov, *Phisiol. Zh. SSSR*, No. 3, 387-390 (1986).
3. I. S. Gushchin, A. I. Zebreva, and N. L. Bogush, *Pathol. Phisiol. Eksper. Ther.*, No. 4, 18-23 (1986).
4. A. Yu. Teplov, S. N. Grishin, and A. L. Zefirov, *Byull. Eksp. Biol.,* **147**, No. 5, 487-492 (2009).
5. G. Burnstock, *Trends Pharmacol. Sci*., **27**, No. 3, 166-176 (2006).
6. J. A. Florendo, J. F. Reger, and P. K. Law, *Exp. Neurol*., **82**, No. 2, 404-412 (1983).
7. A. V. Galkin, R. A. Giniatullin, M. R. Mukhtarov, and I. Svandova, *Eur. J. Neurosci*., **13**, No. 11, 2047-2053 (2001).
8. R. A. Giniatullin, R. N. Khazipov, T. I. Oranska, *et al., J. Physiol*., **466**, 105-114 (1993).
9. S. Grishin, A. Teplov, A. Galkin, *et al., Neurochem. Int*., **49**, No. 8, 756-763 (2006).
10. W. Ji, X. Chen, C. Zhengrong, *et al., Int. Immunopharmacol.,* **8**, No. 9, 1190-1195 (2008).
11. S. H. Kim and Y. C. Lee, *J. Pharm. Pharmacol*., **61**, No. 3, 353-359 (2009).
12. S. Mariathasan and M. Monack, *Nat. Rev. Immunol*., **7**, No. 1, 31-40 (2007).
13. M. Solle, J. Labasi, D. Perregaux, *et al., J. Biol. Chem.,* **276**, No. 1, 125-132 (2001).
14. A. Teplov, S. Grishin, M. Mukhamedyarov, *et al., Exp. Physiol.,* **94**, No. 2, 264-268 (2009).
15. T. L. Tsai, S. Y. Chang, C. Y. Ho, *et al., J. Appl. Physiol.,* **106**, No. 5, 1584-1592 (2009).