Opposite Effect of ATP on Contraction Force of Tonic and Phasic Skeletal Muscles in Frogs

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Experiments *in vitro* showed that ATP and adenosine equally suppressed contractions of frog *m. sartorius*, which belongs to the phasic type muscles. Adenosine receptors antagonist 8-SPT abolished the effect of adenosine, but did not change the effect of ATP. This fact proves the independence of signaling pathways of these purines. ATP produced an opposite effect on the tonic muscle *m. cruralis* and increased the force of its contraction. Adenosine produced an inhibitory effect on the force of *m. cruralis* contration. In this case, 8-SPT also eliminated the effect of adenosine, but did not change the effect of ATP. The potentiating effect of ATP was blocked by suramin, a nonselective antagonist of P2 receptors, which attests to their involvement into the effects of this purine. The opposite effects of purinergic regulation reflect fundamental differences in functional organization of phasic and tonic muscular systems. It was hypothesized that the increase in contraction force under the effect of ATP is a mechanism providing maitenance of the contracted state of tonic muscle without appreciable metabolic costs.

Key Words: *tonic and phasic muscles; contraction parameters; ATP; adenosine*

Tonic muscle fibers are cross-striated muscle fibers, components of skeletal muscles, characterized by the capacity to develop and maintain long-term stable (non-oscillatory) contracted state (contracture). This capacity is provided by certain structural and functional peculiarities of cell systems and innervation.

Tonic neuromuscular system is highly developed in muscles of amphibians in terms of both quantity and manifestation of specific tonic properties. In higher vertebrates, the total number of tonic neuromotor units in skeletal muscles and their role for the tonic function decreased. In mammals, they completely disappeared from the locomotor muscles, but participate in the work of special muscle structures associated with functioning of sensory organs [8].

In tonic fibers, intracellular calcium stores are sufficient to provide contraction and extracellular calcium entry is not needed for activation and maintenance of contraction. Thus, significant differences in the electromechanical coupling in phasic and tonic fibers are mainly determined by quantitative difference in the ratio of calcium binding and release rates in calcium stores. The effect of ATP, an important endogenous modulators of myoneural transmission, is known to be calcium-dependent, in contrast to the effects of its final product adenosine [4]. It therefore can be expected that ATP will differently modulate the functional state of phasic and tonic muscles.

The aim of this work was to study the mechanism of exogenous purine influence on contraction force of tonic (*m. cruralis*) and phasic (*m. sartorius*) muscles of lake frogs.

MATERIALS AND METHODS

Experiments were carried out on the preparations of tonic muscles *m. cruralis* (MC) of lake frogs *Rana radibunda* (n=73) at room temperature (20-24ºC) during September–March. Control experiments were

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performed on *m. sartorius* (MS) which contains only phasic muscle fibers.

Frogs were killed by decapitation and spinal cord destruction. MC and MS were isolated and mounted vertically in a 10-ml tank filled with Ringer's solution (in mM) 113.0 NaCl, 2.5 KCl, 1.8 $CaCl_2$, and $NaHCO_3$ in amount sufficient to bring pH of the solution to 7.3. The muscles were stretched with 1 g preliminary load and left for 30 min for adaptation.

The muscles were put through two platinum rings (2.5 mm in diameter). The distance between the rings was 15 mm. Electrical stimulation through the rings was performed using MultiStim D330 or Grass S9 simulator. Muscle contractions were induced by stimulation with electric field (SEF; 1 Hz rectangular pulses, 0.5 msec duration and 10 V amplitude) for 30 seconds. The force of contractions was registered with a Linton FSG-01 isometric sensor of mechanical activity. The analog signal was digitized and processed by MP100WSW data collection system (Biopack Systems). Mean value for all contractions over 30 sec (30 responses) was treated as a single outcome. The contractile responses were normalized according to the maximal contraction caused by the solution of KCl (240 mM) at the end of the experiment.

After 30-minute rest, the tissue was stimulated several times with 10-min interval with an electric field to induce stable contractions. All subsequent contractions were assessed with respect to this initial response, taken as 100%. In a preliminary set of experiments, the tissue was stimulated several times with intervals from 10 to 30 minutes with regular replacement of bathing solution in order to ensure stability of contractions. No significant changes in tissue response within 3 h were revealed (n=6).

The neuromuscular preparation was incubated with a certain concentration of ATP or adenosine for 10 min, and the responses to SEF were recorded. According to electrophysiological experiments on frog MS, the inhibitory effects of ATP and adenosine on the MC are recorded starting from the 10th minute of incubation with the agonist. Therefore incubation with the agonists in pharmacological experiments lasted for 10 min.

In experiments with ATP, washing with fresh solution was followed by tissue incubation with suramin (a nonselective antagonist of P2 receptors, 100 μM). In experiments with adenosine, the tissue was incubated with 8-SPT and then with the appropriate agonist (10 min), with subsequent registration of contractile responses. In the control, the tissue was incubated with an antagonist for 30 min and muscle responses to SEF were recorded.

After recording of control responses to SEF and assessment of the effects of agonists (ATP or adenosine), the tissue was incubated with suramin (ATP) and 8-SPT (adenosine) for 20 min and with the corresponding agonist for 10 min. ATP, adenosine, 8-SPT, and suramin were used in a concentration of 100 μM and tubocurarine was used in a concentration of 10 (all substances were purchased from Sigma).

RESULTS

The pattern of the contractile responses of frog MS greatly varied at different frequencies of SEF. The muscle has no time to relax before the next contraction when the frequencies surpassed 4 Hz, which led to saw-tooth tetanus. Potentiation of the force of the contractions was observed at frequencies below 0.2 Hz. Therefore 1 Hz frequency was chosen, at which stable single contractions were observed throughout the whole experiment.

To exclude direct stimulation of muscle fibers, 10 V voltage was used. After 5-min preincubation of the tissue with tubocurarine, SEF caused no contractile responses. Subsequent increase in voltage to 100 V led to direct stimulation of the muscle with stronger responses in comparison with control contractions.

Adenosine suppressed contractions to 81.1±2.4% (n=22, p<0.05; Student's t test for related variables). ATP reversibly suppressed contractions of MS caused by SEF (to 77.8±2.4%, n=12) in comparison with control taken as 100% (p<0.05; Student's t test for related variables).

Adenosine receptor antagonist 8-SPT [2,9] suppressed the inhibitory effect of adenosine on contractile responses of the muscle (100.5±2.7% of control; n=6, p>0.05). At the same time, 8-SPT had no effect on the inhibitory effect of ATP, which excluded the effect of adenosine deriving from ATP cleavage. In our previous studies [11] we showed that ATP reduces the force of contraction of frog MS through P2 receptors.

The pattern of MC contractile responses also greatly varied upon changes in SEF frequency. The muscle has no time to relax before the next contraction, when frequencies above 3 Hz were used. The saw-tooth tetanus was obtained as a result of incomplete summation of contractions. When the frequency of stimulation surpassed 30 Hz, continuous persistent contraction appeared which was superimposed by discrete waves. Upon cessation of stimulation, this contraction remains for a long time in isotonic mode of recording. Potentiation of the force of the contractions was obtained when frequencies in tenths of Hz were used. Therefore 1 Hz frequency was chosen at which stable single contractions were observed throughout the entire experiment. This frequency was used in the experiments with MS as it was mentioned earlier.

First, using tubocurarine application we assured that stimulation with selected parameters acts on MC indirectly.

In experiments with MC, the force of contraction decreased to 82.1±6.3% (n=5, p<0.05) from the control after application of adenosine in bathing Ringer's solution, which in general reproduced the effect of this purine on the contraction force of frog phasic muscles. No significant changes in temporal parameters of contraction were observed.

Adenosine receptor antagonist 8-SPT eliminated the effect of adenosine on the contractile responses of the muscle. Thus, the force of MC contraction after application of adenosine in the presence of 8-SPT was 102.0±4.1% (n=5, p>0.05).

Adenosine effect on the force of contraction was reversible; after washout with Ringer's solution, the amplitude of muscle responses returned to the baseline values (n=5, p>0.05). This confirmed previously established involvement of adenosine receptors into the regulation of neuromuscular transmission in skeletal muscles [10].

After application of ATP, the force of MC contraction increased to 123.7±7.7% (n=6, p <0.05) in comparison with control, *i.e.* the effect was opposite to the action of this purine in phasic muscles.

In the next set of experiments we identified receptors involved in this unexpected effect. Preincubation with 100 μM of nonselective P2 receptor antagonist suramin [2,9] completely eliminated the effect of ATP. Thus, the force of MC contraction to ATP against the background of suramin application was 104.1±4.7% (n=6, p>0.05) in comparison with the control.

In tonic fibers, the intracellular calcium stores completely provide contraction (no need in extracellular calcium entry for activation and maintenance of contractions), therefore blockade of transmembrane calcium ion channels with ATP may not inhibit contraction force. However, it not the only explanation of the observed unexpected effect of ATP on contraction force of lake frog tonic muscle.

One of the most important differences between the two types of the studied skeletal muscles is their innervation. The tonic muscle fibers are innervated by thin nerve fibers with a diameter of 2-4 μ and conduction speed <6 m/sec; they form synapses at several points (from 4 to 12) along the fiber [6]. Innervation can be supplied from 1 or 2 to 3 motoneurons. The nerve terminal has a configuration very different from that in phasic fibers and known as "bunch of grapes" [7]. Each synaptic zone of the tonic fiber is much smaller than in phasic one, but the total area of all the synaptic zones in the tonic fiber is approximately equal to the area of synaptic zone of phasic fiber of the corresponding size. Synapses of tonic fibers have lower cholinesterase activity [3].

Tonic fibers are characterized by specific expression of the summation of synaptic potentials during rhythmic stimulation, which is largely determined by protraction of individual potentials [1]. Taking into account rapid decrease in the amplitude of discrete synaptic potentials, further maintenance of high continuous depolarization is probably determined by accumulation of the neurotransmitter in the synaptic zones in the course of repetitive stimulation. Small cholinesterase activity of the postsynaptic membrane of tonic fibers contributes to this phenomenon. The amplitude of synaptic potentials during frequent stimulation significantly decreased. Hence the balance between the release of neurotransmitter at the postsynaptic membrane and its replenishment at the presynaptic membrane during rhythmic stimulation of the nerve is set here at a lower level than in phasic fibers. The data on less intensive replenishment of mediator in the nerve terminals of thin nerves of tonic system agree with presented experimental data. Indeed, we did not observe inhibitory myoneural purinergic negative feedback regulation typical of phasic muscles [4,10], but we did observe positive regulation, where ATP contributes to accumulation of muscle strength. Using the corresponding mode of stimulation of survived nerve terminals in muscle preparation we proved that the place of application of this effect was the neuromuscular synapse. Earlier, the positive feedback realized by the action of extracellular ATP on presynaptic P2 receptors was observed only in experiments on diaphragmatic muscle [5] also characterized by polyneuronal innervation.

We should note that adenosine, a product of ATP cleavage in the synaptic cleft, producing its own independent action, retained its inhibitory effect on the tonic muscle. Thus, the action of two effective purines are opposite in the synapse of tonic muscles and the final effect is difficult to predict in case of rhythmic stimulation and requires further experimental studies.

Opposite effects of purinergic regulation reflect fundamental differences in functional organization of the phasic and tonic muscular systems. We make the assumption that the increase in contraction force under the influence of ATP is a mechanism promoting maitenance of the contracted state of tonic muscle without significant energy expenditures.

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REFERENCES

1. G. I. Blokhina and A. L. Zefirov, *Fiziol. Zh. USSR*, **70**, No. 2, 157-165 (1984)
2. A. U. Ziganshin and L. E. Ziganshina, *P2 receptors: a Promising Target for Future Drugs* [in Russian], Moscow (2009).

3. B. Csillik, I. Schneider, and C. Kalman, *Acta Neuroveg.* (Wien), **22**, 212-224 (1961).
4. S. Grishin, A. Shakirzyanova, A. Giniatullin, *et al.*, *Eur. J. Neurosci.*, **21**, No. 5, 1271-1279 (2005).
5. S. N. Grishin, A. Y. Teplov, A. V. Galkin, *et al.*, *Neurochem. Int.*, **49**, No. 8, 756-763 (2006).
6. S. W. Kuffler and R. W. Gerard, *J. Neurophysiol.*, **10**, No. 6, 383-393 (1947).
7. S. G. Page, *J. Cell. Biol.*, **26**, No. 2, 477-497 (1965).
8. L. D. Peachey and A. F. Huxley, *J. Cell. Biol.*, **13**, 177-180 (1962).
9. V. Ralevic and G. Burnstock, *Pharmacol. Rev.*, **50**, No. 3, 413-492 (1998).
10. E. M. Sokolova, S. Grishin, A. V. Shakirzyanova, *et al.*, *Eur. J. Neurosci.*, **18**, No. 5, 1254-1264 (2003).
11. A. U. Ziganshin, R. R. Kamaliev, S. N. Grishin, *et al.*, *Eur. J. Pharmacol.*, **509**, Nos. 2-3, 187-193 (2005).