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Different effects of ATP on the contractile activity of mice diaphragmatic and skeletal muscles

Sergey N. Grishin [a,b], Alexander Y. Teplov [c], Alexander V. Galkin [a], Alexander M. Devyataev [c], Andrey L. Zefirov [a], Marat A. Mukhamedyarov [a], Airat U. Ziganshin [d], Geoffrey Burnstock [e], András Palotás [a,f,g,h,*]

[a] *Department of Physiology, Kazan State Medical University, ul. Butlerova 49, R-420012 Kazan, Russia*
[b] *Department of Human and Animal Physiology, Kazan State University, ul. Kremlevskaya 18, R-420008 Kazan, Russia*
[c] *Department of Pathophysiology, Kazan State Medical University, ul. Butlerova 49, R-420012 Kazan, Russia*
[d] *Department of Pharmacology, Kazan State Medical University, ul. Butlerova 49, R-420012 Kazan, Russia*
[e] *Autonomic Neuroscience Institute, Royal Free and University College Medical School, London, UK*
[f] *Department of Psychiatry, Albert Szent-Györgyi Medical and Pharmaceutical Center, Faculty of Medicine, University of Szeged, H-6725 Szeged, Semmelweis u. 6, Hungary*
[g] *Division of Cardiac Surgery, Center for Cardiology, Albert Szent-Györgyi Medical and Pharmaceutical Center, Faculty of Medicine, University of Szeged, H-6720 Szeged, Pécsi u. 4, Hungary*
[h] *Asklepios-Med Bt. (private practice and research center), H-6722 Szeged, Kossuth Lajos sgt. 23, Hungary*

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Abstract

Apart from acetyl-choline (Ach), adenosine-5′-trisphosphate (ATP) is thought to play a role in neuromuscular function, however little information is available on its cellular physiology. As such, effects of ATP and adenosine on contractility of mice diaphragmatic and skeletal muscles (m. extensor digitorum longa—MEDL) have been investigated in *in vitro* experiments. Application of carbacholine (CCh) *in vitro* in different concentrations led to pronounced muscle contractions, varying from 9.15 ± 4.76 to 513.13 ± 15.4 mg and from 44.65 ± 5.01 to 101.46 ± 9.11 mg for diaphragm and MEDL, respectively. Two hundred micromolars of CCh in both muscles caused the contraction with the 65% (diaphragm) to 75% (MEDL) of maximal contraction force—this concentration was thus used in further experiments. It was found that application of ATP (100 μM) increased the force of diaphragmatic contraction caused by CCh (200 μM) from 335.2 ± 51.4 mg ($n = 21$) in controls to 426.5 ± 47.8 mg ($n = 10$; $P < 0.05$), but decreased the contractions of MEDL of CCh from 76.6 ± 6.5 mg ($n = 26$) in control to 40.2 ± 9.0 mg ($n = 8$; $P < 0.05$). Application of adenosine (100 μM) had no effect on CCh-induced contractions of these muscles.

Resting membrane potential (MP) measurements using sharp electrodes were done at 10, 20 and 30 min after the application of ATP and adenosine. Diaphragm showed depolarization from 75 ± 0.6 down to 63.2 ± 1.05, 57.2 ± 0.96 and 53.6 ± 1.1 mV after 10, 20 and 30 min of exposition, respectively (20 fibers from 4 muscles each, $P < 0.05$ in all three cases). Adenosine showed no effect on diaphragmatic MP. Both agents were ineffective in case of MEDL.

The effects of ATP in both tissues were abolished by suramin (100 μM), a P2-receptor antagonist, and chelerythrin (50 μM), a specific protein-kinase C (PKC) inhibitor, but were not affected by 1*H*-[1,2,4]-oxadiazolo-[4,3-α]-quinoxalin-1-one (ODQ, 1 μM), a guanylyl-cyclase inhibitor, or by adenosine-3,5-monophosphothioate (Rp-cAMP, 1 μM), a protein-kinase A (PKA) inhibitor.

Besides the action on contractile activity, ATP (100 μM) led to a significant ($P < 0.001$) depolarization of diaphragm muscle fibers from 74.5 ± 2.3 down to 64 ± 2.1, 58.2 ± 2.2 and 54.3 ± 2.4 mV after 10, 20 and 30 min of incubation, respectively. Incubation of MEDL with the same ATP concentration showed no significant change of MP.

Denervation of the muscles for 28 days led to a decrease of CCh-induced contractions of diaphragm down to 171.1 ± 34.5 mg ($n = 11$, $P < 0.05$), but increased the contractile force of MEDL up to 723.9 ± 82.3 mg ($n = 9$, $P < 0.01$). Application of ATP elevated the contractility of denervated diaphragm caused by CCh up to normal values (311.1 ± 79.7 mg, $n = 6$, $P > 0.05$ versus control), but did not significantly affect of contractility of MEDL, which became 848.1 ± 62.7 mg ($n = 6$).

* Corresponding author. Tel.: +36 30 255 6225.
E-mail address: palotas@nepsy.szote.u-szeged.hu (A. Palotás).

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These results show that the effects of ATP on both diaphragmatic and skeletal muscles are mediated through P2Y receptors coupled to chelerytrin-sensitive protein-kinase C.

Keywords: Adenosine; ATP; Denervation; Diaphragm; Muscle; M. extensor digitorum longa; P2-receptors

1. Introduction

Adenosine-5′-trisphosphate (ATP) is an important co-neurotransmitter (Fredholm, 1995; Harden et al., 1995; Ralevic and Burnstock, 1998). Purine nucleotides and nucleosides acting via different types of purinoceptors are known to regulate the function of various cell types, including smooth and striated muscles (Ralevic and Burnstock, 1998). It has been shown that ATP and its analogues may cause contractions of some smooth muscles (urinary bladder, vas deferens, uterus, most blood vessels), while relaxing others (duodenum, taenia coli, some blood vessels) (Hoyle, 1992). It was established that contractile effects of purine nucleotides on smooth muscles are usually associated with stimulation of the purinergic $P2X_1$- and/or $P2X_2$-receptors, which are ligand-gated ion channel receptors, whereas relaxant effect of nucleotides is mainly due to stimulation of a G-protein-coupled $P2Y_1$- and/or $P2Y_2$-receptors (Abbracchio and Burnstock, 1994; Burnstock, 2002). However, the role of P2-receptors in skeletal muscles is less known. Several subtypes of metabotropic P2Y-receptors are known to be expressed in mammalian skeletal muscles during development (Cheung et al., 2003). Furthermore, purine nucleotides can modulate cholinergic responses in adult skeletal muscle by affecting non-quantal (Galkin et al., 2001) and quantal (Giniatullin and Sokolova, 1998) Ach-release via pre-synaptic P2-receptors, and therefore play crucial roles in neuromuscular transmission.

The aim of this study was to compare the effects of ATP on contractile activity of two different striated muscles from mice, often used for investigation, namely diaphragm and musculus extensor digitorum longa (MEDL).

2. Materials and methods

Experiments were conducted on diaphragm and MEDL preparations from 130 adult mice of both sexes with the body weight of 15–34 g. After anesthesia in a special box using ether, mice were decapitated. Muscle preparations, approximately 5 × 10 mm, were made shortly after decapitation. Isolated muscle preparations (for details of the preparation see Galkin et al., 2001) were placed into 2 mL chambers and constantly perfused with Ringer–Krebs solution bubbled with 95% O_2 and 5% CO_2. Ringer–Krebs solution contained the following substances (in mM): NaCl, 120.0; KCl, 5.0; $CaCl_2$, 2.0; $MgCl_2$, 1.0; $NaHCO_3$, 23.0; NaH_2PO_4, 1.0; glucose, 11.0.

Isometric conditions were attained by pre-stitching the muscle with 500 mg weight for 30–40 min. To evoke the muscle contraction, different concentrations of carbacholine (CCh) – a stable analogue of Ach – were added to the superfusing solution.

To assess the effects of ATP and drugs applied after initial contractions in response to CCh the tissue was washed for 30 min and then incubated for 15 min with a given agonist or antagonists and then CCh was added once more to evaluate the effects.

To measure the depolarization of skeletal muscles sharp glass electrodes (tip resistance 8–12 MΩ, filled with 2.5 M KCl) were used. Each diaphragm was dissected into two parts (left and right): both were tested separately. Membrane potentials were measured in 20–30 fibers of each muscle and results were averaged.

In case of denervation, the phrenic nerve was intersected and the animal was kept in a cage for 28 days. After this period, the usual protocol was applied.

Data were recorded using optomechanical coupling and further analyzed with a help of statistical software (Microcal Origin 6.1, Microsoft Excel). The temporal (time to maximal amplitude, time of amplitude half decrease) as well as kinetic (contraction force) characteristics of muscle contraction were calculated. Parametric Student's *t*-test was performed using Microcal Origin 6.1.

All compounds used in this study were purchased commercially. 1*H*-[1,2,4]-Oxa-diazolo-[4,3-α]-quinoxalin-1-one (ODQ), a selective inhibitor of NO-sensitive quanylyl-cyclase, was obtained from Tocris Cookson (UK). ATP (disodium salt), adenosine, adenosine-3,5-monophosphothioate (Rp-cAMP) – a protein-kinase A (PKA)-inhibitor, chelerythrin chloride – a non-isoform-specific protein-kinase C (PKC)-inhibitor, and the rest of the chemicals were purchased from Sigma (USA).

3. Results

3.1. Muscle contraction in response to different CCh concentrations

Under control conditions application of CCh to both muscles led to prolonged (60–80 s) muscle contraction (Fig. 1). Since the experiments were conducted under the constant perfusion, the applied CCh concentration was rapidly washed out, that allowed us to apply CCh many times and average the obtained values.

The muscle force did not vary more than 10% between each experiment. In some muscles a run-down effect was observed, when the contraction force decreased by more than 10%. These experiments were excluded from the statistical analyses.

Various concentrations of CCh were tested on both muscles to estimate the dose–response curve. Interestingly, even though the muscles probably comprise different types of muscle fibers, the dose–response curve appeared to be similar (Fig. 2). The lowest concentration of CCh used (50 μM) led to measurable contraction of both muscles. Further increase of concentration led to the increase of contraction force with saturation at about 400–500 μM (Fig. 2).

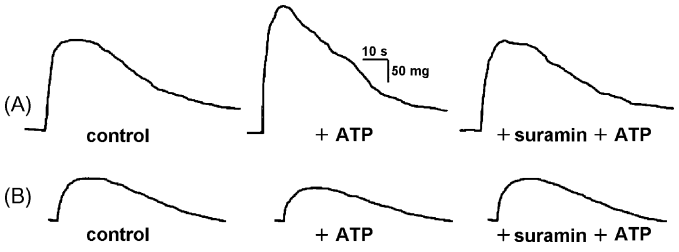

Fig. 1. The effect of ATP (100 μM) and its attenuation by suramin (500 μM) on contractile activity of mouse diaphragm (A) and musculus extensor digitorum longa (B) evoked by the application of 200 μM CCh.

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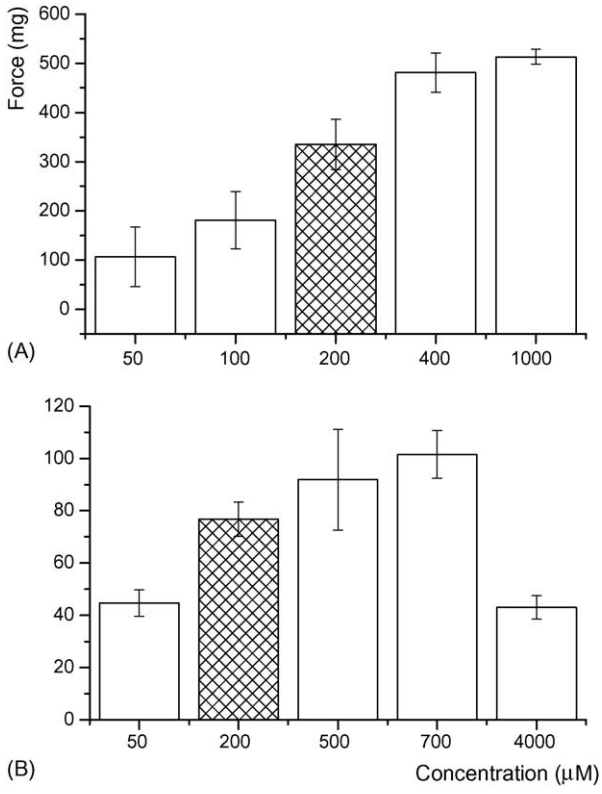

Fig. 2. Effects of different CCh levels on diaphragm ($n = 21$) and MEDL ($n = 26$). The concentrations, used in further experiments, are shown as hatched bars: (A) diaphragm and (B) MEDL.

To avoid the non-specific (non-nicotinic receptor mediated) effects of high levels of CCh, the concentration evoking 60–70% of maximal response (200 μM) were used in both cases. Application of this concentration led to muscle contraction with the force of 335.2 ± 51.4 mg (65% of full response, $n = 21$) and 76.6 ± 6.5 mg (70% of full response, $n = 26$) for the diaphragm and MEDL, respectively (Fig. 2).

3.2. Effects of purines on the contractile activity of intact diaphragm and MEDL

In diaphragm, ATP in a concentration of 100 μM caused a significant increase of muscle contraction force to 426.5 ± 47.8 mg ($P < 0.05$; $n = 10$), i.e. to 127% of control (Figs. 1 and 3). When ATP was applied to MEDL, the contraction force decreased to 40.2 ± 9.0 mg ($P < 0.05$; $n = 8$; Figs. 1 and 3), i.e. 52.2% of original strength.

To exclude the possibility that ATP does not act on its own receptors, but either via its less phosphorilated derivatives or in a non-specific mode, the following tests were performed: (1) the effects of adenosine in the same concentrations (100 μM) were tested, however no effect on the diaphragmatic or MEDL contraction was observed (Figs. 3 and 4) and (2) 500 μM of suramin, a non-selective P2-receptor antagonist was applied (Nakatsuka and Gu, 2001; Giniatullin et al., 2005). The presence of suramin abolished the effects of ATP, confirming its action on its own P2-receptors (Figs. 1, 3 and 4).

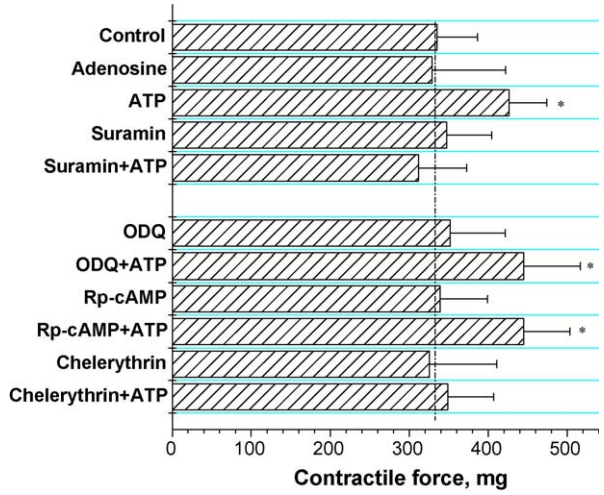

Fig. 3. This figure delineates the effect of different substances on the contractile force of mouse diaphragm evoked by 200 μM of CCh. The agents were used at the following concentrations: ATP, 100 μM; adenosine, 100 μM; suramin, 500 μM; ODQ, 1 μM; Rp-cAMP, 50 μM; chelerythrin, 50 μM. $^{*}P < 0.05$ against control.

3.3. Metabolic pathways

Having proved that the effects of ATP on muscles are mediated via P2-receptors, but not via breaking down to adenosine and affecting P1-receptors, we tried to find a possible cascade of second messengers which conveys the signal to the intracellular structures. One report (Mukhtarov et al., 2000) has implicated the role of NO-cascade in the regulation of muscle metabolism. To assess whether the ATP-induced effects were coupled to NO-cascade, we investigated the effects of ODQ, a specific inhibitor of guanylyl-cyclase (Garthwaite et al., 1995). ODQ in a concentration of 1 μM neither changed the contraction force (Figs. 3 and 4), nor blocked the inhibitory action of ATP when applied concomitantly. This indicates that NO-cascade most likely does not play any role in this scenario.

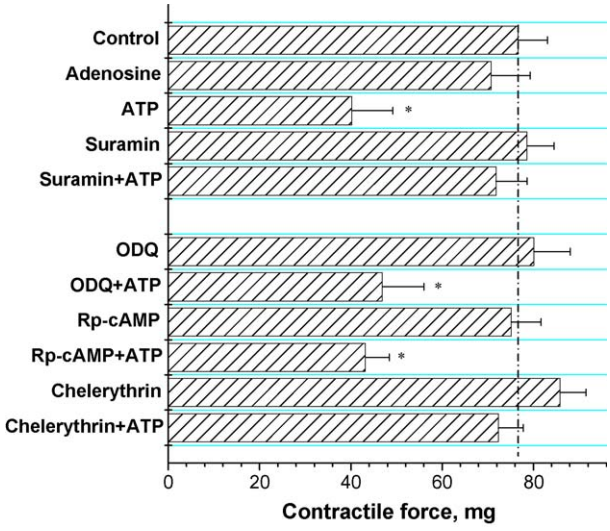

Fig. 4. Effects of different agents on contractile force of mouse musculus extensor digitorum longa evoked by 200 μM of CCh. $^{*}P < 0.05$ against control.

Possible implications of other metabolic pathways were also tested. In the presence of 100 μM Rp-cAMP, a specific inhibitor of PKA (Dostmann et al., 1990), the contraction force remained unchanged. ATP, applied simultaneously, still had an effect on both muscles (Figs. 3 and 4). Since it has been previously shown by our team (Galkin et al., 2001) that ATP can exert its effect via PKC-coupled cascades, we also tested the newly described specific PKC-inhibitor chelerythrin (Bull and Barnett, 2002). Chelerythrin *per se* in the concentration of 50 μM did not affect muscle contractions, but abolished all effects of ATP on both MEDL and diaphragm (Figs. 3 and 4). These data suggest that the effects of ATP on muscle contraction are mediated via second messenger cascades involving chelerytrin-sensitive PKC.

3.4. Effects of denervation

To gain insight into the process of regulation of muscle contraction after Ach-receptor degradation caused by denervation, we carried out experiments on the muscles denervated for 28 days. Denervated diaphragm showed decreased contractile ability on CCh application: the force constituted 171.1 ± 34.5 mg which was approximately 50% of intact muscle force (Fig. 5A). Interestingly, ATP was still able to increase the contraction to approximately the same extend as in intact muscle (by 43%).

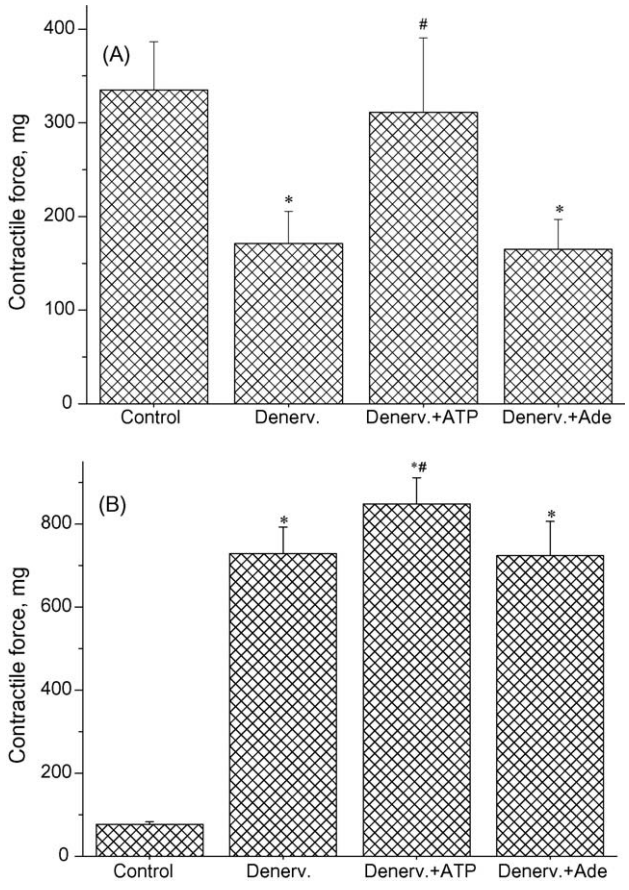

Fig. 5. Effects of ATP (100 μM) and adenosine (100 μM) on the contractility of mouse diaphragm (A) and musculus extensor digitorum longa (B) after 28 days of denervation. $^{*}P < 0.05$ against control, $^{\#}P < 0.05$ against denervated.

MEDL showed drastic increase of contractile ability after denervation. The contraction force increased to almost 1000% (from 76.6 ± 6.5 to 723.9 ± 82.3 mg). Under these conditions, the effects of ATP on MEDL reversed—it acted as a promotor of contractile activity, leading to moderate (but significant) increase of MEDL contraction by 17% (Fig. 5B).

3.5. Effects of purines on the muscle membrane potential

One possible mechanism of ATP action on muscle contraction could be the depolarization of muscle membrane, leading to more ready and pronounced contraction (Hong et al., 1997). Therefore we performed sharp-electrode experiments to measure the resting MPs in the muscles used.

Initial MP was 74.2 ± 0.7 and 72.6 ± 0.8 mV in diaphragm and MEDL, respectively (12 muscles, 20 fibers each). Incubation of muscle in Ringer–Krebs solution for 30 min

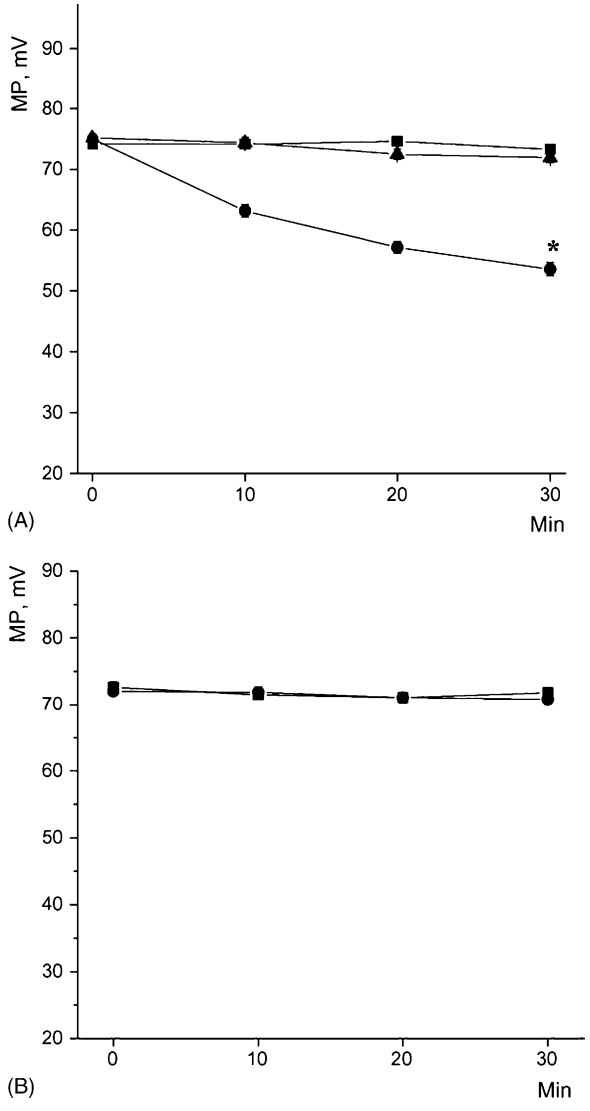

Fig. 6. Muscles after 30 min of incubation with ATP (circles), ATP with suramin (triangles), and control (squares): (A) diaphragm and (B) musculus extensor digitorum longa. $^{*}P < 0.001$ both against control and ATP + suramin (4 muscles for every experiment, 20 fibers each).

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showed a little run-down effect, inasmuch as the resting potential decreased to 73.3 ± 0.8 and 71.8 ± 0.8 mV for diaphragm and MEDL (Fig. 6). In all experiments this effect was not more than 1 mV or 1.5% of initial potential.

ATP application to diaphragmatic muscle for 30 min evoked membrane depolarization to 53.6 ± 1.1 mV (4 muscles, 40 fibers each), which was significantly ($P < 0.001$) different from the control values (27% of decrease, Fig. 6A). ATP application to MEDL did not have an effect on the membrane potential (Fig. 6B).

To check the specificity of this ATP action, the common protocol was used in this case also. Incubation of diaphragm with adenosine did not have any effects on MP, whereas concomitant application of ATP and suramin abolished these actions, proving that these effects are ATP and P2-receptor mediated.

4. Discussion

The skeletal muscle comprises several types of cells and structures: nerve endings, Schwann cells, endothelium, infiltrating immune cells, etc. Almost all of these cells express purinoceptors (Burnstock, 2002). As such, it is difficult to evaluate the effects of ATP on skeletal muscle, especially measuring the contraction which is regulated by many factors.

The common method to investigate the contractile properties of the muscles is to use the electric field potential (EFS) for stimulation (Hong and Chang, 1998). It has been previously shown by our team that ATP is able to inhibit frog sartorius muscle contractions with strong temperature dependence, if the muscle is stimulated using EFS (Ziganshin et al., 2005). This is mostly attributable to the fact that EFS causes muscle contraction by stimulating the nerve endings in the muscle and the ATP effects on muscle contraction in this case are due to well-known pre-synaptic inhibitory effect of ATP on synaptic transmission (Giniatullin and Sokolova, 1998; Ziganshin et al., 2005).

Since the main goal of our investigation was to estimate the direct effect of ATP on skeletal muscles, a nicotinic receptor agonist was used. We selected CCh, the stable analogue of Ach, which acts as nicotinic receptor agonist at least as effective as Ach itself (Akk and Auerbach, 1999) and often used in similar experiments (Volkov et al., 2001).

Since carbachol, a substance similar to CCh, is widely used as muscarinic agonist, we had to consider this in our experiments. Muscarinic receptors are expressed mostly pre-synaptically and modulate synaptic transmission, as well as lead to Ach-release from pre-synapse (for review see Volpicelli and Levey, 2004). This release may constitute the neurogenic part of muscle contraction, however it is reasonable to believe that this contribution is of low significance, because the application of adenosine, which is shown to inhibit synaptic transmission (Giniatullin and Sokolova, 1998; Grishin et al., 2005), did not change the force of muscle contraction. As such, the application of CCh enables us to separate pre- and post-synaptic effects of ATP in that it directly activates the post-synaptic Ach-receptors and leads to muscle contraction. This approach allows us to constrain possible targets of ATP action to the skeletal muscle only and investigate the direct action of ATP to muscle fibers.

Mice diaphragm and MEDL are distinctively differentiated skeletal muscles with well-distinguished properties, such as the dissimilar contractile force of both muscles. MEDL, which is much smaller and thinner, has the force of about 1/4 of that of diaphragm—a strong muscle. Interestingly, the contraction of both MEDL and diaphragm lasts for almost the same time (60–80 s, depending on the preparation) and shows no fatigue.

Since MEDL muscle fibers are smaller in general, it was expected that they have lower amount of Ach-receptors and thus they need lower concentration of CCh to be activated. The experiments presented in this report, however, show the striking similarity in concentration curves in both muscles. The smaller size of MEDL muscles is probably compensated by the higher density of Ach-receptors in this type of muscle. This similarity allowed us to perform further experiments without correcting for CCh concentrations.

One of the possible mechanisms that might account for the pronounced facilitating ATP action in the diaphragmatic contraction might be the altered resting MP which may increase the excitability of the muscle membrane.

ATP elicits inward currents and depolarizes muscle membranes of mature mouse skeletal muscle (Hong et al., 1997, mouse diaphragm preparation) that makes them more excitable and low concentration of CCh may lead to increased Ca^{2+}-influx and thus stronger muscle contraction. The shift of membrane potential to depolarization also activates voltage-sensitive Na^{+}-channels, hence elevates muscle tone, which in turn alters contractile forces. These excitatory effects are antagonized by suramin and are similar to the effects of ATP observed for visceral and vascular smooth muscle (Honoré et al., 1989; Benham and Tsien, 1987), developing cultured skeletal muscle (Kolb and Wakelam, 1983; Thomas and Hume, 1990) and cardiac muscle (Friel and Bean, 1988). In all these cells, exogenously applied ATP increases membrane permeability via opening membrane channels gated by the nucleotide. It is thus likely that mature skeletal muscles, such as MEDL and diaphragm, also express such ion channels operated by ATP, although the concentration of ATP required to activate them may be higher and vary between the different types of muscles.

To prove this suggestion we conducted a series of sharp-electrode measurements of MP in the native and ATP-treated muscles. The resting MP did not significantly differ between the two muscles, but diaphragm incubated with ATP led to 27% decrease of MP, as expected. The MP fell even after 30 min of incubation, and the exponential approximation of the decay yielded the value of 51 mV as the saturation point (29% of decrease). This MP decrease was clearly different from the run-down, which never exceeded 1.5% in either measured muscles. This confirms the hypothesis that ATP acts on the muscular MP.

The depolarization of the membrane can be reached either by closing K^{+}-channels, or by opening Na^{+}-permeable structures, however it is still not clear how the signal from a P2-receptor is transmitted to the channel. Previous studies suggested (Kolb and Wakelam, 1983; Thomas and Hume,

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1990) that these are P2X-coupled channels, where the signal is carried by G-proteins. Other studies (Cheung et al., 2003) proposed the role of the P2Y-receptor. To distinguish between these two pathways we tried to selectively inhibit different P2Y-coupled second messenger cascades to evaluate if this may affect the ATP signal. Inhibiting PKC abolished this effect, suggesting that a candidate for the post-synaptic target is the P2Y-receptor.

Revealing the mechanism of ATP-induced facilitation of diaphragmatic contraction poses the question: what could the mechanism in case of MEDL be? Both effects of ATP seem to share the same underlying mechanism, i.e. both are mediated through P2-receptors (blocked by suramin), and coupled to specific PKC (sensitive to chelerythrin application).

In the literature there are some data about the depressive ATP effects on the contractile activity of mouse diaphragm in high concentrations (1 mM, Hong et al., 1997). The effect can be explained taking into account the fact that many subtypes of P2-receptors tend to desensitize fast (Rettinger and Schmalzing, 2004). Desensitization of these receptors upon high ATP concentration leads to closure of the cation channels (which are open in normal conditions) and repolarization of muscular membrane, thus reversing the effects of ATP. Broadly in the line with our experiments, this would lead to the hyperpolarization of muscle membrane of MEDL. Unfortunately, this hypothesis could not be proven in our experiments, however might be explained by suggesting either that (1) the desensitization of ATP receptors does not lead to the closure of normally opened channels, but rather changes the probability of normally shut channels to open up upon CCh application; or (2) the mechanism of ATP action in this case is completely different.

Another interesting phenomenon we found is the change of ATP effects after denervation. It is known that denervation leads muscles and neuromuscular terminals undergo a specific sequence of events leading to disintegration of synaptic function, scattering of the receptors along the pre- and post-synaptic membranes and thinning out of nicotinic receptors and ligand-gated channels (Salpeter et al., 1986; Shyng and Salpeter, 1990; Szabó et al., 2003). The hypersensitivity of denervated skeletal muscles to CCh is a rare but well-described phenomenon, shown in skeletal muscles of frogs (Miledi, 1990a) and rats (Miledi and Slater, 1970). This effect is thought to be attributable to increased quantity of nicotinic receptors on the post-synaptic side (e.g. muscle cell membrane) (Miledi, 1990b).

These changes in post-synaptic receptor quantities could lead to a scenario when higher ATP concentration is required to cause desensitization, as in the case of native/intact muscles. Under these conditions the depressive effects of ATP on MEDL due to fast desensitization of ATP-receptors are reversed.

The level of depolarization by ATP apparently changed after denervation, since the augmentation of contractile activity by ATP (43%) was higher than in intact muscles (27%). This augmentation might be explained by the presence of more densely expressed nicotinic receptors on the post-synaptic side; accordingly, the same CCh concentration leads to higher depolarization and stronger Ca^{2+}-influx, leading to more effective contractile machinery.

The most interesting finding of our experiments is the description of the post-synaptic ATP-activated system which modulates muscle contraction. As it is known, ATP acts differently at pre- and post-synaptic levels. On pre-synapse, it decreases mediator release in a dose–response manner in a large variety of concentrations starting from 1 μM (Giniatullin and Sokolova, 1998). Acting on post-synapse, ATP leads to facilitation of muscle contraction. The mechanism of the facilitation is complex and involves P2Y-receptors coupled to PKC. The activation of PKC on post-synapse may lead to opening of Na^+ or closing of K^+-channels, which eventually leads to the change in resting MP. Depending upon the concentration of ATP, these changes in the MP could lead to either increase or decrease the contractility of the muscle. The fact that ATP has opposing effects on mice diaphragm at 100 μM must be considered strongly by the scientists who investigate signal transduction in neuromuscular junction in isolated nerve-muscular preparations, enabling electrical nerve stimulation and registration of post-synaptic currents or muscle contractile force, because these two effects may mask one another.

When taken together with our previously published data, we may conclude that there are three global systems which regulate nerve-muscular transduction where ATP plays a crucial role: (1) ATP regulates the quantal mediator release from pre-synapse (Giniatullin and Sokolova, 1998); (2) it also inhibits non-quantal mediator release (Galkin et al., 2001); (3) it controls the contraction of muscle acting on post-synaptic ATP-sensitive cation channels.

5. Conclusions: looking at ATP through the clinician's eyes

Neurotransmission is the release of a cocktail of signaling compounds which act on pre- and post-synaptic membranes. In the nerve terminals of vertebrate skeletal muscles, ATP is co-stored and co-released quantally with Ach in a ratio of ∼1:5 (Silinsky and Redman, 1996). This implies that motor activity is accompanied by the pulsatile release of ATP. P2-purinoceptors form the principal extracellular target for ATP (Ralevic and Burnstock, 1998). P2X receptors are ligand-gated ion-channels, which lead to a flux of cations (Na^+, K^+ and Ca^{2+}) across the plasma membrane when activated (North, 2002), whereas P2Y receptors are G-protein-coupled receptors, which mainly activate phospholipase C, leading to the formation of inositol-1,4,5-triphosphate (IP_3) and mobilization of intracellular calcium ($[Ca^{2+}]_i$) (Ralevic and Burnstock, 1998; North, 2002).

Extracellular ATP participates in important biological processes like neurotransmission in the peripheral and central nervous system, the regulation of cardiac function, platelet aggregation, immune response and pain (Fredholm, 1995; Harden et al., 1995; Ralevic and Burnstock, 1998). By eliciting rises in $[Ca^{2+}]_i$, it may also efficiently activate various cell types, including myocytes. Apart from co-localizing of ATP with neurotransmitters at central and peripheral cholinergic and bioaminergic synapses (Zimmermann, 1994), ATP is released by glial cells to modulate neuronal activity (Newman, 2003).

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It has also been suggested that ATP may also stimulate glial proliferation and activate microglial cells after brain injury (Abbracchio et al., 1994; Neary et al., 1994), suggesting a neuro-modulatory role of ATP. In line with this theory, adenosine-uptake inhibitors have been shown to elicit neuroprotective effects (van Wylen et al., 1986), e.g. propentofylline attenuates the progression of Alzheimer's disease (Rother et al., 1998), and dipyridamole, acting mainly on platelets, is used in the secondary prevention of stroke (Picano and Abbracchio, 1998).

In the neuromuscular junction, ATP has been shown to regulate the gene expressions of Ach-receptors (AchR) and Ach-esterase (AchE) via a mitogen-activated protein-kinase pathway (Choi et al., 2001, 2003). AchE has been shown to be a crucial therapeutic target in some brain diseases such as Alzheimer's dementia (Grisaru et al., 1999). By being a co-transmitter and modulator of the cholinergic system, ATP might also have clinical implications in these neuro-psychiatric disorders with defective cholinergic activity, and also in maladies of the musculo-skeletal system with cholinergic deficiency, including myasthenia gravis. However, in order to understand the significance of neuron-derived ATP in supplementing Ach as a possible alternative neurotransmitter, or its potential to modulate the post-synaptic apparatus, and therefore to consider ATP as a novel therapeutic approach, further studies are required.

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