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Interaction of hydrocortisone with ATP and adenosine on nerve-mediated contractions of frog skeletal muscle

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ABSTRACT

The inhibitory effects of ATP and adenosine on the nerve-mediated contractile responses of isolated sartorius muscle of the frog, *Rana ridibunda*, evoked by electrical field stimulation (EFS) were studied using pharmacological organ-bath technique. The effects of hydrocortisone applied *in vitro* and *in vivo* on contractility of sartorius muscle were also examined. ATP (100 μM) significantly reduced the amplitude of contraction to EFS of sartorius muscle, while pyridoxalphosphate-6-azonphenyl-2′,4′-disulfonic acid (PPADS; 10 μM), a P2 receptor antagonist, abolished inhibitory effect of ATP. A similar inhibitory effect of adenosine (100 μM) was fully antagonized by 8-(*p*-sulfophenyl)-theophylline (8-SPT, 100 μM), a P1 receptor antagonist. Incubation of the tissue with hydrocortisone (10 μM) caused a slight, but significant, decrease of muscle contractions. After incubation of muscle preparations with both hydrocortisone and ATP, no inhibition of muscle contractility was registered. A single injection of hydrocortisone (100 mg/kg) 12 h prior to experiments to frogs did not significantly change the nerve-mediated contractility of isolated sartorius muscle; however, it abolished the inhibitory action of ATP without changing inhibitory activity of adenosine. After treatment of frogs with hydrocortisone for 14 days (100 mg/kg/day), both ATP and adenosine retained their inhibitory action on EFS-induced contractions of the muscle, and their effects were antagonized by PPADS and 8-SPT, respectively. It is concluded that hydrocortisone has antagonistic actions against the inhibitory effects of ATP at the frog neuromuscular junction, although this effect is lost following long-term treatment with hydrocortisone.

1. Introduction

P2 receptors, which consist of a family of ligand-gated ion channel P2X receptors and a family of G-protein-coupled P2Y receptors, are widely distributed in animal and human tissues (Burnstock and Knight, 2004; Burnstock, 2007). These receptors are activated by ATP (the principal endogenous agonist) and some other natural purine and pyrimidine nucleotides and their analogs. It has been shown that P2 receptors are involved in regulation of many physiological processes in central and peripheral nervous systems, as well as functions of cardiovascular, urogenital, respiratory and gastrointestinal systems (Burnstock, 2006, 2007). In contrast to smooth muscle, in adult skeletal muscle, agonists and antagonists of P2 receptors directly cause neither contraction nor relaxation. However, it was found that ATP can modulate the effectiveness of neuromuscular transmission by prejunctional inhibition (Giniatullin and Sokolova, 1998; Galkin et al., 2001; Sokolova et al., 2003) or facilitation (Salgado et al., 2000) of transmitter release at the neuromuscular junction. ATP can also act as a postjunctional facilitator of transmission (Ribeiro, 1977).

It is widely accepted now that steroid hormones realize their effects not only by binding with specific intracellular receptors, but also by some other non-genomic ways (Haller et al., 2008; Song and Buttgereit, 2006). Non-genomic effects of steroids occur rapidly and can be due to interactions with membrane lipids, proteins, receptors and intracellular second-messenger systems (Watson and Gaetchu, 1999; Zor et al., 1991). At frog neuromuscular junction, the acute (non-genomic) effect of hydrocortisone was facilitation, while long-term treatment with this glucocorticosteroid was inhibition of multiquantal end-plate currents induced by motor nerve stimulation (Giniatullin et al., 2000).

The aim of this study was to evaluate the effects of hydrocortisone on P2 receptor-mediated inhibition of contractions of frog skeletal muscle.

2. Methods

2.1. General procedure

Experiments were carried out on *Rana ridibunda* frogs at room temperature (20–22 °C) during the months of September to March. The sartorius muscles were dissected free and suspended vertically for isometric recording of mechanical activity in 10 ml organ baths filled with the Ringer solution containing (in mM): NaCl 113.0; KCl

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2.5; $CaCl_2$ 1.8 and a sufficient quantity of $NaHCO_3$ to adjust pH to 7.3. An initial load of 1 g was applied to the muscles, which were then allowed to equilibrate to the bath conditions for 30 min.

Electrical field stimulation (EFS) was provided by a Digitimer MultiStim D330 stimulator via two platinum wire rings 2.5 mm in diameter, 15 mm apart, through which the muscles were threaded. Contractile activity of the tissue was elicited by applying rectangular impulses at a frequency of 1 Hz, 0.5 ms length and 10 V amplitude and recorded for 30 s. Responses of the tissue were registered isometrically by a Linton FSG-01 (UK) force displacement transducer, acquired by a Biopack (UK) MP100WSW Data Acquisition System and displayed on a PC screen. The mean of the amplitude of all the individual twitches recorded during 30 s (30 twitches) was calculated and used as a single data (see Fig. 1).

To ensure that the stimulation parameters cause nerve-mediated contractions, preliminary experiments with tubocurarine, a nicotinic cholinoceptor antagonist, were carried out. We found that in the presence of tubocurarine (10 μM) there was no response of the muscle to EFS ($n = 6$, results are not shown).

2.2. Initial control procedure

After equilibration to the bath conditions, the tissue was stimulated several times by EFS with 10 min intervals in-between until reproducible contractile responses were recorded; all the following contractions were compared with this initial response, which was taken as a 100%. To ensure the stability of contractions and survival of the tissue during the experiments, preliminary control series of experiments were carried out, in which isolated tissue was challenged several times with the intervals of 10 to 30 min without induction of any substances into the bath but regularly changing the Ringer solution. Results revealed no significant changing of the tissue response during 3 h (average experimental time, $n = 6$, results are not shown).

After stable, reproducible contractions evoked by EFS were obtained, the initial contractile responses of the isolated frog sartorius muscle to EFS were recorded. The amplitude of this initial response was 83.7 $\pm$3.3% ($n = 23$) relative to the contraction evoked by KCl at a concentration of 240 mM. For any given muscle preparation, its initial response was taken as a 100%, and all the following contractile responses of the tissue were calculated as a percentage of this initial response.

2.3. Effects of ATP and adenosine in the absence and the presence of antagonists

Tissues were incubated for 10 min with a given concentration of ATP or adenosine, and responses to EFS were recorded without changing the bath solution. The 10 min interval was chosen as a result of preliminary experiments, where we found that the prominent effect of ATP (100 μM) on frog muscle contraction is registered after 10 min of incubation ($n = 6$, results are not shown). After washout, the tissue was incubated for 20 min with either pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS, a P2 receptor antagonist, 10 μM) or 8-(*p*-sulfophenyl)theophylline (8-SPT, a P1 receptor antagonist, 100 μM) plus 10 min with a corresponding agonist and the stimulation-evoked responses of the tissue were recorded again. In control experiments, the tissue was incubated only with one of the antagonists for 30 min and the contractile responses of the muscle to the EFS were registered.

2.4. Effects of ATP and adenosine in the presence of hydrocortisone

After the control muscle responses to the EFS and the initial effects of the agonists (ATP or adenosine) were tested, the tissue was incubated with hydrocortisone (10 μM) for 20 min plus with the same agonist for 10 min and responses to the EFS were registered again. Then the tissue was washed and incubated again with hydrocortisone together with PPADS in case of ATP or 8-SPT in case of adenosine for 20 min plus 10 min with a corresponding agonist, and the final stimulation-evoked responses of the tissue were recorded. In control experiments, the tissue was incubated only with hydrocortisone for 30 min.

2.5. Acute and subchronic hydrocortisone treatment

Frogs were weighed and accommodated into individual cupping glasses. The animals were divided into two equivalent groups: one group received hydrocortisone suspension (100 mg/kg/day) by subcutaneous injection into dorsal lymphatic sack while another group received the similar volume of the vehicle (0.5% solution of lidocaine). In subchronic treatment, injections were made daily once a day for 14 days, in the morning, animals being sacrificed 12 h after the last injection. In case of acute treatment, animals were killed after 12 h of the single injection of hydrocortisone (or vehicle).

2.6. HPLC study of ATP concentrations in the organ bath

In order to check possible degradation of ATP in the organ bath solution during incubation time, we carried out a set of experiments to measure changes in ATP concentrations in the bath solution using high-pressure liquid chromatography (HPLC). Skeletal muscle preparations were placed into the organ bath as described above. Then ATP was added to the bath to produce a final concentration of 100 μM. Immediately after addition, a sample of bath solution (100 μl) was taken for analysis (zero point), while the second sample (100 μl) of the same bath solution was taken after 10 min of incubation. Samples were immediately analysed by HPLC.

The HPLC system (Perkin Elmer, Series 200, USA) was coupled with a UV/Vis detector, and chromatograms were registered using a computer. For separation of nucleotides, a 25 cm long Spherisorb column (Hichrom, UK) was used, with an inner diameter of 0.46 mm. The column was washed for 24 h using mobile phase consisting of a 0.2 M solution of

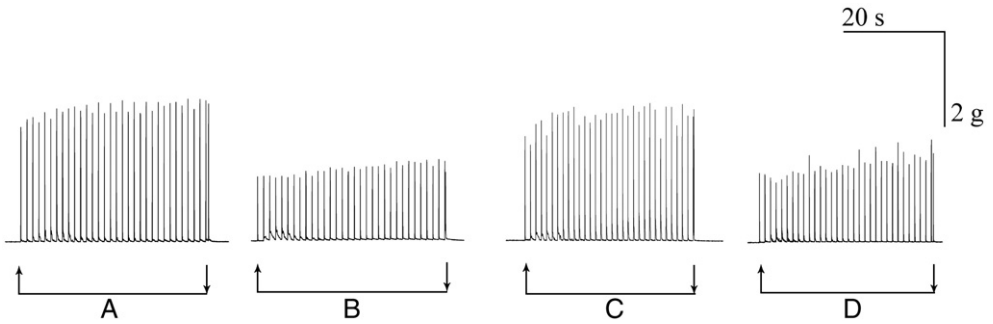

Fig. 1. Sample mechanogram of the contractions of frog sartorius muscle evoked by electrical field stimulation (EFS). A — initial contractions of the muscle evoked by EFS, B — contractions in the presence of ATP (100 μM), C — contractions in the presence of ATP after incubation with PPADS (10 μM), D — contractions in the presence of ATP after incubation with 8-SPT (100 μM). Arrows indicate start and finish of EFS.

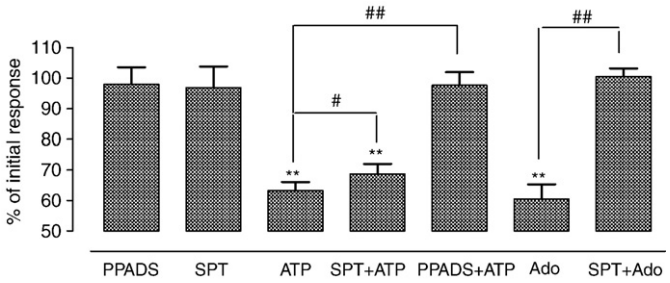

Fig. 2. Effects of PPADS (10 μM), 8-sulfophenyl-theophylline (SPT, 100 μM), ATP (100 μM), adenosine (Ado, 100 μM) and their combinations on contractile responses of isolated frog sartorius muscles evoked by electrical field stimulation (EFS). Data are presented as a percentage of initial tissue responses to EFS. Data shown are means and vertical bars indicate S.E.M., $n = 6–14$ (** — $P<0.01$ with initial response; # — $P<0.05$, ## — $P<0.01$ with corresponding group without antagonist, Student's paired t-test).

KH_2PO_4 with the addition of methanol up to a final concentration of 3% (pH 6.0). ATP detection was carried out at a flow rate of 1.5 ml/min, wave length 260 nm and a sample volume of 20 μl. The ATP concentration was calculated by comparing the area under the ATP peak on the experimental chromatograms with the corresponding area of the control chromatograms for standard solutions of ATP.

2.7. Drugs used

Adenosine 5′-triphosphate sodium salt (ATP), adenosine, hydrocortisone acetate and tubocurarine were obtained from Sigma. PPADS tetrasodium salt was provided by Tocris Cookson. 8-(*p*-sulfophenyl)-theophylline (8-SPT) was supplied by RBI. Hydrocortisone microcrystalline suspension for injections and lidocaine were obtained from a local chemist's shop.

2.8. Analysis of results

Student's *t*-test (paired and unpaired) was used for comparing the effects of agonists and antagonists in *in vitro* experiments. Nonparametric Kruskal–Wallis test was used to analyze results of *in vivo* experiments. A probability of less than 0.05 was considered significant. Data are presented as mean ± standard error of mean (n) where n is the number of muscle preparations.

2.9. Ethics

Before taking for the experiments, frogs were contained at 15 °C in a 100 l aquarium, 1/10 charged with water that was changed daily. Frogs were killed by decapitation and destruction of spinal cord, causing a minimum of physical suffering according to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes.

3. Results

3.1. Effects of ATP on EFS-induced contractions

ATP at a concentration of 10 μM inhibited contractions of frog sartorius muscles evoked by EFS, decreasing the amplitude of the contractions to 92.9 ± 0.9% ($n = 14$) compared to the initial response. The inhibitory effect of ATP was concentration-dependent, being 89.5 ± 1.0% ($n = 14$) and 63.3 ± 4.2% ($n = 12$) at concentrations of 30 μM and 100 μM, respectively. All these data were significantly different from the corresponding initial control values ($P<0.05$, Student's paired *t*-test). Incubation of the tissue with the P2 receptor antagonist, PPADS (10 μM), did not significantly change the amplitude of EFS-evoked muscle contractions, however the antagonist abolished the inhibitory effects of ATP (100 μM) returning the amplitude of contractions close to its initial level (97.7 ± 4.3%; $n = 10$) (Figs. 1 and 2).

Preincubation of tissues with the P1 receptor antagonist, 8-SPT (100 μM), caused a slight, but significant, decrease of the inhibitory action of ATP on EFS-evoked muscle contractions (Figs. 1 and 2).

3.2. Effects of adenosine on EFS-induced contractions

Similar to ATP, incubation with adenosine (100 μM) significantly decreased contractile responses of the muscle preparations evoked by EFS, the amplitude being 60.5 ± 4.8% ($n = 11$) relative to controls ($P<0.05$, Student's paired *t*-test). The P1 receptor antagonist, 8-SPT (100 μM), had no direct effect on muscle contractions, but fully reversed the inhibitory effects of adenosine, such that the amplitude of contractions in the presence of the antagonist were not statistically different from controls (100.5 ± 2.7%; $n = 6$) (Fig. 2). The P2 receptor antagonist, PPADS (10 μM), did not significantly change the inhibitory effect of adenosine on EFS-evoked contractions.

3.3. Effects of hydrocortisone in vitro

Incubation of the tissue with hydrocortisone (10 μM) caused a slight, but significant, decrease of muscle contractions, the amplitude being 83.1 ± 6.4% ($n = 14$) compared to controls ($P<0.05$, Student's paired *t*-test). However, when hydrocortisone was used together with ATP (100 μM), the contractile responses to EFS were not significantly different from the control values. The addition of PPADS together with hydrocortisone and ATP did not significantly change this phenomenon (Fig. 3).

Interestingly, hydrocortisone did not affect the inhibitory action of adenosine: contractile responses to EFS in the presence of adenosine alone or in combination with hydrocortisone were statistically identical. When tissue was incubated with hydrocortisone and 8-SPT together, the inhibitory affects of adenosine were abolished (Fig. 3).

3.4. Effects of acute hydrocortisone treatment

In the vehicle-treated group, ATP (100 μM) markedly reduced the contractile responses of the frog sartorius muscle evoked by EFS, compared with control values (65.1 ± 5.9%; $n = 4$, $P<0.05$, Student's paired *t*-test); the degree of the ATP-induced inhibition in this group was comparable with that in normal, non-treated animals (see Section 3.1.).

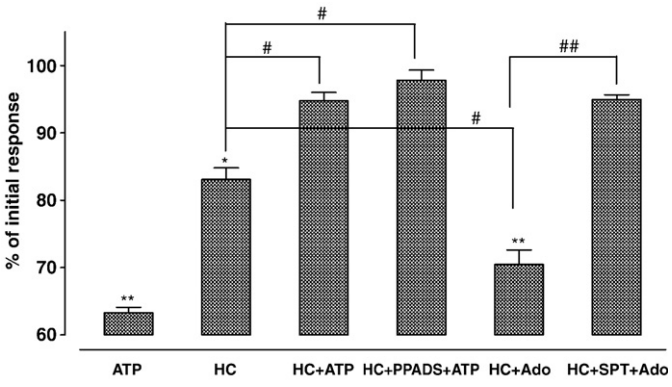

Fig. 3. Contractile responses of isolated frog sartorius muscles evoked by electrical field stimulation (EFS) in the presence of ATP (100 μM), hydrocortisone (HC, 10 μM) and in combinations of hydrocortisone with ATP (100 μM), PPADS (10 μM), adenosine (Ado, 100 μM) and 8-SPT (100 μM). Data are presented as a percentage of initial tissue responses to EFS. Data shown are means and vertical bars indicate S.E.M., $n = 8–12$ (* — $P<0.05$, ** — $P<0.01$ with initial response; # — $P<0.05$, ## — $P<0.01$ between groups, Student's paired *t*-test).

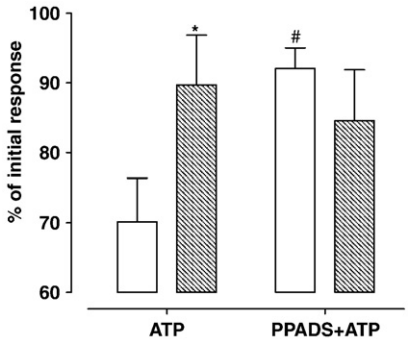

Fig. 4. Effects of ATP (100 μM) in the absence and presence of PPADS (10 μM) on electrical field stimulation (EFS)-evoked contractile responses of sartorius muscles of frogs injected by vehicle (Control, open bars) or a single dose of hydrocortisone (100 mg/kg, dashed bars). Data are presented as a percentage of initial tissue responses to EFS. Data shown are means and vertical bars indicate S.E.M., $n=4$–7. (* — $P<0.05$ with Control, Kruskal–Wallis test; # — $P<0.05$ with corresponding group in the absence of PPADS, Student's paired t-test).

However, in the hydrocortisone-treated animals, ATP had no significant effects on EFS-evoked contractions, the amplitude was $97.2\pm3.3\%$ ($n=4$) of its initial level (Fig. 4). The Kruskal–Wallis test revealed a statistical difference between effects of ATP in vehicle and hydrocortisone injected groups ($P<0.05$). Addition of PPADS (10 μM) abolished the inhibitory effects of ATP in the control group, but did not change that in the experimental group (Fig. 4).

In contrast to ATP, adenosine inhibited the contractile responses of the muscles in both vehicle and hydrocortisone-treated groups to a similar degree, the amplitude of contractions was $69.6\pm2.4\%$ ($n=4$) and $62.4\pm6.8\%$ ($n=4$), respectively; these figures were not significantly different from the effects of adenosine in intact animals (see Section 3.2., $P>0.05$, Kruskal–Wallis test). In both groups, preincubation of the tissue with 8-SPT (100 μM) antagonized the effects of adenosine, so that contractile effects of the muscles were similar to their initial control response (Fig. 5).

3.5. Effects of subchronic hydrocortisone treatment

In both vehicle and hydrocortisone-treated animals, ATP (100 μM) caused a significant and statistically identical inhibition of EFS-evoked contractile responses, with amplitudes of $70.8\pm3.3\%$ ($n=6$) and $69.5\pm5.4\%$ ($n=6$) of their initial responses, respectively. These figures were not statistically different from the effect of the similar concentration of ATP in non-treated animals (see Section 3.1., $P>0.05$, Kruskal–Wallis test). In both groups, PPADS antagonized the effects of ATP, returning the amplitude of contractions close to its initial values (Fig. 6).

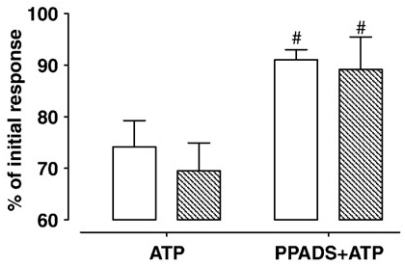

Fig. 6. Effects of ATP at a concentration of 100 μM in the absence and presence of PPADS (10 μM) on electrical field stimulation-evoked contractile responses of sartorius muscles of frogs injected by vehicle (Control, open bars) or hydrocortisone (100 mg/kg/day, dashed bars) for 14-days. Data are presented as a percentage of initial tissue responses to EFS. Data shown are means and vertical bars indicate S.E.M., $n=4$–7. (# — $P<0.05$ with corresponding group in the absence of PPADS, Student's paired t-test).

Similarly, the inhibitory effects of adenosine were not statistically different in placebo and hydrocortisone-treated groups ($68.9\pm4.2\%$; $n=5$ and $64.8\pm5.8\%$; $n=6$, respectively, $P>0.05$, Kruskal–Wallis test), and these again were not significantly different from the effects of adenosine in non-treated animals (see Section 3.2., $P>0.05$, Kruskal–Wallis test). Incubation of the tissue with 8-SPT led to a significant reduction of the inhibitory effects of adenosine in both animal groups (Fig. 7).

3.6. Degradation of ATP in the organ bath

After incubation of tissues for 10 min with ATP at an initial concentration of 100 μM only approximately 5% of the ATP was degraded during the 10 min incubation time; this change, however, was statistically significant (Table 1).

4. Discussion

In the present study, we have demonstrated that hydrocortisone antagonized P2 receptor-mediated inhibition of the nerve-stimulated frog skeletal muscle contractions, both *in vitro* and *in vivo*.

It is well known that ATP is released from motor nerve endings together with acetylcholine (Cunha and Sebastiao, 1993; Silinsky, 1975). However, unlike the autonomic nerve system, where ATP and other agonists of P2 receptors cause direct stimulatory or inhibitory effects on a wide range of smooth muscle tissues (Burnstock and Knight, 2004), in the mature somatic nervous system ATP as a co-mediator has

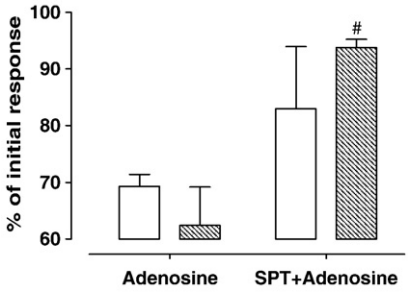

Fig. 5. Effects of adenosine (100 μM) in the absence and presence of 8-sulfophenyl-theophylline (SPT, 100 μM) on electrical field stimulation-evoked contractile responses of sartorius muscles of frogs injected by vehicle (Control, open bars) or a single dose of hydrocortisone (100 mg/kg, dashed bars). Data are presented as a percentage of initial tissue responses to EFS. Data shown are means and vertical bars indicate S.E.M., $n=4$–7. (# — $P<0.05$ with corresponding group in the absence of SPT, Student's paired t-test).

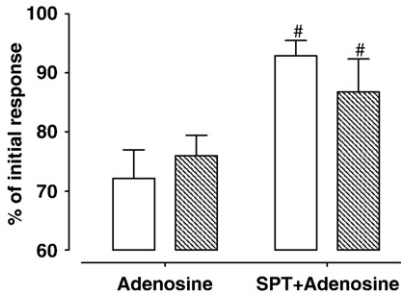

Fig. 7. Effects of adenosine at a concentration of 100 μM in the absence and presence of 8-sulfophenyl-theophylline (SPT, 100 μM) on electrical field stimulation-evoked contractile responses of sartorius muscles of frogs injected by vehicle (Control, open bars) or hydrocortisone (100 mg/kg/day, dashed bars) for 14-days. Data are presented as a percentage of initial tissue responses to EFS. Data shown are means and vertical bars indicate S.E.M., $n=4$–7. (# — $P<0.05$ with corresponding group in the absence of SPT, Student's paired t-test).

Table 1
Changes in the ATP concentration in the organ bath solution after 10 min incubation with frog skeletal muscle at a room temperature ($n=8$)

Initial concentration added	Concentration found at zero point, μM		Concentration found after 10 min of incubation, μM	
	$M \pm m$	%	$M \pm m$	%
100 μM	97.04 ± 1.64	100	92.08 ± 1.16[a]	94.9

[a] $p<0.05$, Student's unpaired t-test.

modulatory effects on acetylcholine release at both presynaptic and postsynaptic levels. It was shown that at rat motor nerve terminals the stable analogue of ATP, β,γ-imido-ATP, facilitated acetylcholine release probably via presynaptic P2 receptors (Salgado et al., 2000). In contrast, at frog neuromuscular junction, ATP inhibits transmitter release through the action on metabotropic P2Y receptors (Giniatullin and Sokolova, 1998; Sokolova et al., 2003) by inhibiting Ca^{2+} entry into the nerve terminal (Grishin et al., 2005).

In the present study, we confirmed an inhibitory action of ATP on neuromuscular transmission by recording the mechanical activity of nerve-stimulated frog sartorius muscle. We found that the effect of ATP was fully antagonized by PPADS, a non-selective antagonist of P2 receptors, which supports the involvement of P2 receptors in the effect of ATP. However, we also found that 8-SPT, a non-selective antagonist of P1 receptors, produced some antagonism against the effect of ATP. This is probably due to partial degradation of ATP to adenosine, which takes place in the bath solution during the 10 min incubation (see Table 1).

Adenosine, like ATP, caused inhibition of EFS-evoked contractions of frog skeletal muscle, its effect was antagonized by 8-SPT and not affected by PPADS, which indicates an involvement of P1 (adenosine) receptors. Our observation is consistent with the earlier findings of Giniatullin and Sokolova (1998), who demonstrated electrophysiologically that ATP and adenosine inhibited transmitter release at the frog neuromuscular junction through distinct presynaptic receptors.

It is generally accepted that the main effects of steroid hormones are due to binding with specific cytoplasmic receptors and the consequent stimulation of nucleic acid production and protein synthesis. However, a substantial body of evidence has been accumulated to support the importance of non-genomic effects of steroids, which can be exerted by direct action on membrane proteins — both ion channels and G protein-coupled receptors (Haller et al., 2008). Rapid, non-genomic effects of steroid hormones have been shown in many tissues, for example, guinea-pig bronchial muscle (Sun et al., 2006), human neutrophils (Liu et al., 2005), human bronchial epithelium (Urbach et al., 2002), endocrine pancreatic cells (Sutter-Dub, 2002) and in the central and peripheral nervous systems (Makara and Haller, 2001; Johnson et al., 2005).

In our study, we tested both genomic long-term and non-genomic rapid effects of hydrocortisone on ATP-induced inhibition of muscle contractions. When frogs were treated for 14 days with hydrocortisone, we did not find any significant changes of either ATP or adenosine-induced inhibition of frog skeletal muscle contractility. However, when frog skeletal muscles were tested after 12 h of a single hydrocortisone injection, we found a significant antagonism with effects of ATP, but not adenosine, on the amplitude of muscle contractions. Although 12 h probably is enough time to produce nuclear action of steroids, we suggest that this antagonism is non-genomic in nature. Our suggestion is supported by similar results obtained in *in vitro* experiments, where hydrocortisone was directly added into the bath solution. In these experiments, genomic effects of hydrocortisone on nerve terminals can be excluded, not only due to the short period of incubation with the tissue (30-60 min), but also by the absence of motoneuron soma under these conditions.

The results of acute effect of hydrocortisone on frog muscle contraction are rather puzzling. Hydrocortisone and ATP, being added separately, both inhibited the contractile responses of the muscle, however, when we used these substances together, we did not observed any significant inhibition of contraction. Several possibilities can be introduced to explain this phenomenon.

It is well known that some of P2X receptor subtypes are highly susceptible to desensitization after long exposition to high concentration of agonists (Roberts et al., 2006). One can suggest that hydrocortisone might significantly increase the sensitivity of these receptors and thus cause the development of rapid desensitization. However, this looks unlikely in our conditions since earlier Sokolova et al. (2003) established that presynaptic P2Y, but not P2X, subtypes are involved in ATP-induced inhibition of transmitter release in frog neuromuscular junction. Another possibility could be a fast (and total) degradation of ATP due to a hydrocortisone-induced significant increase of the activity of ecto-ATPase — an effective extracellular enzyme found in many tissues (Gendron et al., 2002). We think that it is also unlikely since in our biochemical assays we found that only about 5% of ATP is degraded during incubation time, and hardly hydrocortisone can increase the rate of enzyme activity so drammatically.

It is possible that the hydrocortisone-ATP antagonism is due to different (opposite?) modification of intracellular levels of some second messengers. It was established that at the frog neuromuscular junction ATP produces an inhibitory action on transmitter release via presynaptic P2Y receptors coupled to multiple intracellular cascades including phosphatidylinositide-specific phospholipase C, phospholipase A_2, protein-kinase C and cyclooxygenase mechanisms (Sokolova et al., 2003). Similarly, it was shown that rapid, non-genomic effects of glucocorticoids on nerve terminals are results of changes of intracellular concentrations of inositol-triphosphate, diacylglycerol and arachidonic acid (Solito and Parente, 1989, Zor et al., 1991).

Yet another possible mechanism of antagonism between ATP and glucocorticoids is involvement of Ca^{2+} and Ca^{2+} channels. Earlier we have shown that at the frog neuromuscular junction ATP inhibits Ca^{2+} entry into the nerve terminal (Grishin et al., 2005), and a similar effect was found for glucocorticoids at C2C12 skeletal muscle cells (Passaquin et al., 1998). In contrast to that, increase of intracellular calcium level as a result of stimulation of P2Y and P2X receptors was demonstrated in endothelial (Duchene and Takeda, 1997) and glial cells (James and Butt, 2002). It has also been shown that glucocorticoids inhibit ATP-induced Ca^{2+} influx in HT4 neuroblastoma cells (Han et al., 2005) and in endothelial cells (Rogers et al., 2002).

We think that the involvement of second messengers and/or Ca^{2+} in the realization of hydrocortisone-ATP antagonism is more realistic. Meanwhile, one more possibility is the co-existence of two separate, but interrelated mechanisms which somehow (reciprocally?) inhibited the effect of each other. Further experiments are needed to evaluate the role of all these putative mechanisms.

The antagonism of hydrocortisone with ATP which we revealed in our experiments might have some physiological and pharmacological significance during inflammation. It is known that ATP being liberated from destructed cells during inflammation has a proinflammatory activity through a combination of actions: release of histamine from mast cells, provoking production of prostaglandins, and the production and release of cytokines from immune cells (Di Virgilio et al., 1998). It is accepted now that antiinflammatory action of glucocorticoids is not only due to the well-defined genomic way but also due to some less known non-genomic mechanisms (Goulding, 2004). It is believed, for example, that the non-genomic mechanisms are involved in antiinflammatory action of corticosteroids during so called "pulse therapy" — short-course high-dose treatment regimen (Sinha, Bagga, 2008). We suggest that antagonism with ATP could be one of such possible non-genomic mechanisms of acute antiinflammatory action of glucocorticosteroids. The loss of hydrocortisone antagonism against ATP action after subchronic treatment is possibly due to development of genomic effects of the corticosteroid.

In conclusion, in this study we have shown that hydrocortisone has a clear antagonistic effect on ATP-induced inhibition of EFS-evoked

contractions of frog skeletal muscle. This effect is not due to a genomic action of the glucocorticosteroid, although the exact mechanism of the antagonism needs further investigation.

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