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Short Communication

Foot-shock Stimulation Decreases the Inhibitory Action of ATP on Contractility and End-plate Current of Frog Sartorius Muscle

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Abstract

Background and Objective: Inhibitory action of ATP on contractions of frog isolated sartorius muscle was shown earlier, while hydrocortisol prevented that effect of ATP. The aim of this study was to investigate whether ATP also inhibits the contractility of sartorius muscle on stress-induced animals. **Materials and Methods:** The influence of ATP on contractions and end-plate current (EPC) of sartorius muscles isolated from frogs exposed to electric foot-shock stimulation (EFSS) was analyzed. **Results:** In the muscles of frogs exposed to EFSS, the inhibitory effect of ATP on contractions was significantly decreased and on EPC was abolished. The level of cortisol was much higher in the blood obtained from EFSS animals comparing with control ones. **Conclusion:** The EFSS has inhibitory action on the effect of ATP on the frog sartorius muscle and that could be due to increase of cortisol blood level.

Key words: Frog sartorius muscle, foot-shock, cortisol, ATP, contractions, end-plate current

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There is much evidence now that ATP is a co-transmitter and a neuromodulator in the peripheral and central nervous systems influencing many important body functions. In skeletal muscles, ATP released with acetylcholine (ACh) can modify exocytosis of ACh by negative feedback mechanism¹ as well as increase the sensitivity of postsynaptic nicotinic receptors².

It is well known that steroid hormones do not influence the contractile responses of striated muscles, although they can regulate the release of the neurotransmitters leading to inhibition or facilitation of end-plate current (EPC)³. For example, steroid hormones can lead to calcium influx reduction in C2C12 striated cell muscles⁴, while estrogen hormones cause ATP-induced Ca²⁺ flux declining⁵. The ATP and adenosine significantly inhibited the contractions and reduced the amplitude of end-plate currents in frog isolated sartorius muscle⁶. Results of the earlier experiments showed that the inhibitory actions of ATP but not adenosine can be prevented by hydrocortisol *in vivo* and *in vitro*⁷. Those experiments lead to hypothesis that corticosteroids might antagonize the effects of ATP by some non-genomic influence.

In order to study whether that glucocorticoid-ATP antagonism can be seen also in *ex vivo* experiments, this paper investigated the influence of high level of glucocorticoid concentration in blood (induced by the acute stress condition-foot-shock stimulation) on actions of ATP and adenosine on contractions and EPC of frog sartorius muscle.

MATERIALS AND METHODS

General procedures: Experiments were carried out on 48 *Rana ridibunda* frogs at the temperature of 20-24°C from September until March. Ringer solution composition was (in mM): NaCl 113.0, KCl 2.5, CaCl₂ 1.8 and NaHCO₃ to bring pH up to 7.3-7.4.

Electrical foot-shock stimulation (EFSS): One hour before the experiment, each animal was placed in a separate home cage to wait the exposure to a foot-shock stimulation. That was done in a stimulation cage with 30×30 cm metal floor connected to electric stimulator with 50 cm height transparent plastic walls. A series of three foot-shock stimulation (0.7 mA for 3 sec with a 15 min inter-shock interval) were administered. After the last series, the frog was left in the shock device for 30 min and then returned to a

home cage. Frogs were killed 2 h after the last stimulation and used to isolate sartorius muscles. Control animals were also placed into the stimulation cage but no electric foot-shock was provided.

Contractile responses measurement: The isolated muscles were hanged vertically in organ baths filled with the 10 mL of the Ringer solution. An initial tension of 1.0 g was applied to the muscles and 30 min was given to the muscles to equilibrate to the experimental conditions.

Stimulation of the muscles was provided by a pair of platinum rings 2.5 mm in diameter and 15 mm apart through which the muscles were placed. Electrical stimulation applied using a Digitimer Multi Stim D330 module (UK) by rectangular electrical impulses (1 Hz, 0.5 ms, 10 V) and stimulation time was 30 sec. Muscle responses were registered in isometric condition by a force displacement transducer (Linton FSG-01, UK), acquired by a digital data acquisition system (Biopack MP100WSW, USA) and recorded by a personal computer.

Initially electrical stimulation to the muscles was applied every 10 min till responses become stable. These initial responses were taken as initial controls (100%) relative to which all the experimental contractions measured.

The stability of contraction was tested in preliminary experiments where muscles were electrically stimulated 5 times with 10-30 min gaps in-between, during which no drugs or agents were used. Experiments showed that there were no significant changes in muscle contractility during this period.

EPC measurement: A muscle was divided and cut across to eliminate any mechanical responses. Muscle band was fixed with a point to a silicone holder on the lower of a Lucite camera with a 2.5 mL of Ringer solution. The EPC were induced with a frequency of 1/30 Hz with one strong stimulation of the nerve. Synaptic currents were written through the voltage clamp technique by two electrodes with a holding potential of -40 mV. Intracellular electrodes were filled with 2.5 M KCl and were given the resistance of 3-5 MΩ. All drugs were addressed through solution providing tubes with a speed of a 2 mL min⁻¹. Values were normalized according to initial EPC that were assumed to be a 100%.

Cortisone level measurement: Blood was taken by a syringe directly from the frog's heart. The concentration of cortisol in the serum was measured using LIAISON Kit (Biomedica Gruppe, Austria).

Substances used: Adenosine 5'-triphosphate (ATP) and adenosine were supplied by Sigma.

Analysis of results: Student's one sample two tailed test to compare medians to a control 100% value was used. Student's unpaired test (two tailed) to compare cortisone concentrations was used. Values were calculated as Mean \pm Standard mean error (n) where n was a muscle sample for mechanical contraction or synapses for EPC.

Ethics: Frogs lived in an aquarium (100 L), partly filled with a water (15°C) that was replaced daily. Animals were killed by beheading, after that the spinal brain was broken down.

The Ethical committee of Kazan State Medical University approved this study (September, 2012 protocol).

RESULTS

Contractile responses: In control experiments ATP and adenosine (both at a concentration of 100 μ M) significantly inhibited the contractions of frog sartorius muscles evoked by electrical field stimulation which were $77.6 \pm 2.3\%$ (n = 24) and $81.3 \pm 2.4\%$ (n = 23) of their initial values, correspondingly (Fig. 1).

In frogs exposed to EFSS inhibitory action of ATP attenuated and was $86.7 \pm 6.0\%$ (n = 7), that figure was not significantly different from the initial value. In contrast, after EFSS inhibitory action of adenosine was not significantly affected being $67.5 \pm 9.2\%$ (n = 4) still being significantly different from initial value (Fig. 1).

End-plate current (EPC): Motor nerve excitation (0.03 Hz) induced EPC with the amplitude of 199 ± 12 nA (n = 11) on muscles with a holding potential of -40 mV.

In sartorius muscles of control frogs ATP (100 μ M) significantly reduced amplitude of EPC in a reversible way to $66.1 \pm 1.1\%$ (n = 28) of its initial value. The similar degree of depression ($70.4 \pm 1.3\%$ (n = 27) was observed after adenosine (100 μ M) application (Fig. 2).

In frogs after EFSS, inhibitory action of ATP almost totally abolished amplitude of EPC being $94.1 \pm 6\%$ (n = 11) which was statistically identical to initial value. In similar conditions, adenosine (100 μ M) decreased the amplitude of EPC to $72.3 \pm 5\%$ (n = 6) (Fig. 2).

Cortisol level: The concentration of cortisol in blood serum obtained from foot-shocked frogs was 1.33 ± 0.05 mkg dL⁻¹

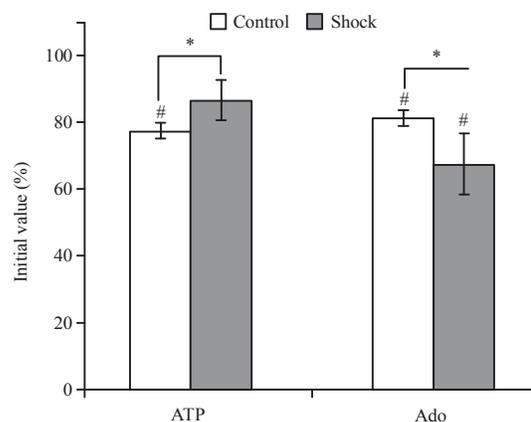


Fig. 1: Amplitude of contractions of frog sartorius muscles elicited by electrical field stimulation in the presence of ATP (100 μ M) or adenosine (Ado, 100 μ M) in control conditions and in frogs exposed to electrical foot-shock stimulation (Shock). Figures are calculated as a percentage to initial values before applications of ATP or adenosine (100%). Values were mean, vertical bars were S.E.M., n = 7-24 (*p<0.05 compared with initial response, #p<0.05 compared with control)

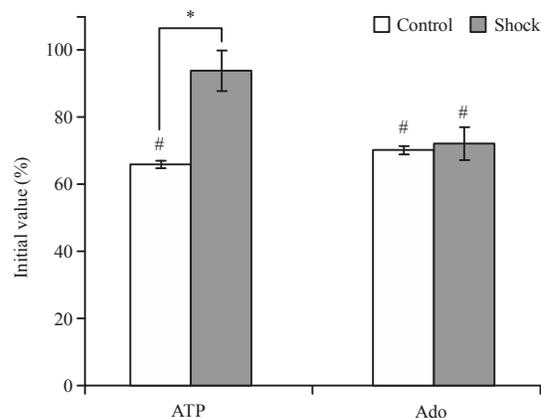


Fig. 2: End plate currents of frog sartorius muscles elicited by electrical field stimulation in the presence of ATP (100 μ M) or adenosine (Ado, 100 μ M) in control conditions and in frogs exposed to electrical foot-shock stimulation (Shock). Figures are calculated initial values (%) before applications of ATP or adenosine (100%). Values are mean, vertical bars are S.E.M., n = 7-24 (*p<0.05 compared with initial response, #p<0.05 compared with control)

(n = 20), while for blood obtained from control frogs, that were not exposed to foot-shock stimulation, it was

0.64±0.06 mkg dL⁻¹ (n = 28) and Student's unpaired test showed a significant difference between these values (p<0.001).

DISCUSSION

This study demonstrated that inhibitory effect of ATP on contractions and EPC of frog sartorius muscles was reduced if frogs were preliminary put on acute stress condition like electric foot-shock. Interestingly, inhibitory actions of adenosine were not affected at similar experimental conditions. Since the increase of cortisol level in the blood of foot-shocked frogs was also revealed, it is suggested that these results are once more support the view about glucocorticoids-ATP antagonism, which has been shown earlier⁷.

The possible mechanisms of antagonism of glucocorticoids with ATP are not well understood currently. One possibility is involvement in intracellular second messenger production since at the frog neuromuscular junction ATP inhibited the transmitter release via presynaptic P2Y receptors coupled to multiple intracellular cascades⁸.

Another possible mechanism of ATP-glucocorticoids interactions can be involvement of Ca²⁺ movements. It is known that at the frog neuromuscular junction ATP inhibits Ca²⁺ entry into the nerve terminal⁹ and a similar effect was found for glucocorticoids at C2C12 skeletal muscle cells⁴. It has also been shown that glucocorticoids inhibit ATP-induced Ca²⁺ influx in HT4 neuroblastoma cells¹⁰ and in endothelial cells¹¹. The involvement of second messengers and changes of cell calcium level is considered currently as the more realistic mechanisms of the cortisol-ATP antagonism.

The interaction of glucocorticoids with ATP might have some physiological and pharmacological significance during inflammation. It is known that ATP released from damaged cells during stress and inflammation has a pro-inflammatory 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 cells¹². It is accepted now that anti-inflammatory action of glucocorticoids is not only due to the well-defined genomic way but also due to some less known non-genomic mechanisms¹³. It is suggested that antagonism with ATP could be one of such possible non-genomic mechanisms of acute anti-inflammatory action of glucocorticoids.

CONCLUSION

This study verified that foot-shock stimulated frogs have higher blood cortisol levels and ATP, in contrast to adenosine did not considerably modify contractions and EPC of sartorius muscles isolated from these animals.

SIGNIFICANCE STATEMENT

This study showed that ATP, unlike in control conditions, did not considerably inhibit contractions and EPC of sartorius muscles isolated from foot-shock stimulated frogs. This effect is at least partially might be due to higher blood cortisol levels in foot-shock stimulated frogs than in controls.

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