Role of Protein Kinase C in the Effect of ATP on Contractile Function of the Isolated Strip from Mouse Diaphragm

A. Yu. Teplov, S. N. Grishin*, A. L. Zefirov*, and A. U. Ziganshin**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 4, pp. 389-391, April, 2006
Original article submitted June 24, 2005

We studied the effects of adenosine and ATP on contractile function of the isolated strip from mouse diaphragm. ATP significantly increased the strength of muscle contraction induced by carbachol. Adenosine had no effect on carbachol-induced muscle contraction. P_2 receptor antagonist suramin abolished the effect of ATP. The positive chronotropic effect of ATP was not observed after treatment with specific protein kinase C inhibitor chelerythrine. Our results indicate that the effect of ATP on contractile function of mouse diaphragm is realized via protein kinase C.

Key Words: *mouse diaphragm; ATP; P_2 receptors; protein kinase C*

Purine nucleotides modulate function of muscle tissue. Much attention was given to the effects of ATP and its analogues on smooth muscles [2,7]. However, little is known about the mechanism underlying the action of purines on skeletal muscle (SM). Published data show that exogenous ATP *in vitro* increases the strength of mouse diaphragm contraction [8]. The effect of purines on SM contraction is probably related to modulation of the cholinergic response via changes in non-quantum [4] and quantum secretion of acetylcholine [5]. P_2 receptors play an important role in this process. However, the role of intracellular messengers in this process is poorly understood.

This work was designed to study the role of protein kinase C (PKC) in the effect of ATP on contractile activity of the isolated strip from mouse diaphragm.

MATERIALS AND METHODS

Experiments were performed on male and female mice weighing 25-32 g. The animals were killed by bloodletting under light ether anesthesia. The diaphragm muscle was isolated. The muscle was placed in a thermostatic bath. Isometric tension was achieved by prior stretching of SM at force of 500 mg over 40 min. The muscle was perfused with Krebs solution at 20-21°C. Diaphragm contraction was induced by carbachol (CCh). CCh in a submaximal concentration of 200 μM was added to the bath at 30-min intervals. Perfusion was stopped during CCh treatment. Contraction was recorded using a photoelectric transducer [1] and a direct writer. We recorded the strength of muscle contraction and the rate of contraction (as the ratio of muscle contraction force to the time-to-maximum tension).

Study was performed in 3 series.

In series I we studied the effect of ATP on the strip of mouse diaphragm. Parameters of contraction were compared before and after 5-min incubation of the muscle in an ATP-containing solution (100 μM). In series II we recorded parameters of contraction before and after 15-min perfusion with specific PKC inhibitor chelerythrine (50 μM). In series III we evaluated the influence of combined treatment with chelerythrine (15-min perfusion, 50 μM) and ATP (5-min incubation, 100 μM).

After each series the muscle was washed with a perfusion solution not less than for 90 min to

Department of Pathophysiology, *Department of Normal Physiology, **Department of Pharmacology, Pharmaceutical Faculty, Kazan State Medical University. ***Address for correspondence:*** alikteplov@mail.ru. A. Yu. Teplov

0007-4888/06/1414-0407 © 2006 Springer Science + Business Media, Inc.

recover its contractile activity. During this period the muscle was contracted in response to CCh treatment at 30-min intervals.

RESULTS

Under control conditions ATP increased the strength of CCh-induced contraction of the diaphragm strip to 126.8% ($p<0.001$, Table 1). The rate of muscle contraction increased to 187% ($p<0.01$, Table 1).

Adenosine (100 μM) treatment under conditions similar to those in experiments with ATP had no effect on CCh-induced muscle contraction.

Perfusion with suramin (100 μM) abolished the effect of ATP on CCh-induced contraction of the diaphragm muscle.

Perfusion of the muscle with chelerythrine decreased the strength of contractions to 65.7% ($p<0.05$, Table 2). The rate of contractions decreased to 70.8% ($p<0.001$, Table 2).

PKC blockade with chelerythrine followed by incubation with ATP decreased the strength of diaphragm contraction to 68.9% ($p<0.05$, Table 3). The rate of muscle contraction increased to 354% ($p<0.05$, Table 3).

The observed changes in the test parameters were reversible.

ATP modifies functional properties of SM by producing a direct effect on various stages of myocyte contraction and indirectly (via the sympathetic system, when purines operating as neurotransmitters simulate the sympathetic effect).

ATP affects quantum secretion of acetylcholine [5], inhibits non-quantum secretion of the transmitter [4], and modulates postsynaptic ATP-sensitive cation channels.

Our experiments on the isolated strip of mouse diaphragm showed that incubation with ATP increases the strength and rate of CCh-induced contraction (Table 1). Studying the contractile response of the isolated muscle to agonist administration showed that ATP affects postsynaptic structures of the muscle.

The postsynaptic effects on mouse diaphragm are realized similarly to other SM (*i.e.*, via P_2 receptors). This conclusion is derived from published data and results of our experiments. The effect of ATP was abolished by P_2 receptor antagonist suramin. The action of adenosine is realized via P_1, but not via P_2 receptors [3]. In our experiments substitution of ATP for adenosine had no effect on CCh-induced contraction of the diaphragm.

The role of PKC in ATP-mediated modulation of contractile function of mouse diaphragm was evaluated by recording the strength and rate of muscle contraction under various conditions of treatment using specific PKC inhibitor chelerythrine (Tables 1-3). ATP increased (187%), while chelerythrine decreased the rate of contraction (by 71%). The rate of contraction increased more significantly after combined treatment with ATP and chelerythrine in similar concentrations over the same period (354 *vs*. 187%). Opposite changes in the velocity of contraction indicate that PKC plays a role in contractile activity of mouse diaphragm modulated by ATP.

The involvement of PKC in choline-mediated excitation and contraction modulated by ATP is important for the function of respiratory muscles and mechanisms of fatigue. Studying the effect of purines on SM will elucidate the mechanisms for regulation of the contractile apparatus in SM under normal conditions and in various models of experimental pathology.

TABLE 1. CCh-Induced (200 μM) Contraction of the Isolated Strip of Mouse Diaphragm after 5-min Incubation with ATP (100 μM; *n*=10)

Parameter	Before incubation	After incubation	*p*
FT, sec	10.7±1.1	7.3±0.5	0.05
For, mV	335.2±93.4	426.2±110.0	0.001
Voc, mV/sec	25.6±5.6	47.8±8.0	0.01

Note. Here and in Tables 2 and 3: For, force of muscle contraction; Voc, velocity; FT, ratio of For to the time-to-maximum tension.

TABLE 2. CCh-Induced (200 μM) Contraction of the Isolated Strip of Mouse Diaphragm before and after 15-min Perfusion with Chelerythrine (50 μM; *n*=7)

Parameter	Before perfusion	After perfusion	*p*
FT, sec	8.0±0.5	7.5±0.5	—
For, mV	230±30	151±19	0.05
Voc, mV/sec	28.8±3.8	20.4±2.6	0.001

TABLE 3. CCh-Induced (200 μM) Contraction of the Isolated Strip of Mouse Diaphragm before and after Combined Treatment with Chelerythrine (15-min Perfusion, 50 μM) and ATP (5-min Incubation, 100 μM; *n*=7)

Parameter	Before combined treatment	After combined treatment	*p*
FT, sec	7.8±0.9	3.6±0.8	0.05
For, mV	161.0±28.2	111.0±22.8	0.05
Voc, mV/sec	16.2±3.8	57.4±10.8	0.05

REFERENCES

1. R. Kh. Akhmerzyanov and E. B. Fillipov, *Fiziol. Zh. SSSR*, **72**, No. 3, 387-390 (1986).
2. G. Burnstock, *Curr. Top. Med. Chem*., **4**, No. 8, 793-803 (2004).
3. G. Burnstock and G. E. Knight, *Int. Rev. Cytol*., **240**, 31-304 (2004).
4. A. V. Galkin, R. A. Giniatullin, M. R. Mukhtarov, *et al*., *Eur. J. Neurosci*., **13**, No. 11, 2047-2053 (2001).
5. R. A. Giniatullin and E. M. Sokolova, *Ross. Fiziol. Zh. Im. I. M. Sechenova*, **84**, No. 10, 1132-1138 (1998).
6. S. J. Hong and C. C. Chang, *J. Neurophysiol*., **80**, No. 5, 2550-2558 (1998).
7. C. H. V. Hoyle, *Autonomic Neuroeffector Mechanism*, Eds. G. Burnstock and C. H. V. Hoyle, Harwood (1992), pp. 367-407.
8. A. Yu. Teplov, S. N. Grishin, A. M. Deviataev, and A. L. Zefirov, *J. Muscle Res. Cell. Motility*, **24**, Nos. 4-6, 332 (2003).