Effect of ATP and Its Analogs on Contractility of Rat Myocardium during Ontogeny

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The effects of P2-receptor agonists on myocardial contractility were examined in rats aging 14-100 days. ATP and its stable analog 2-methylthio-ATP potentiated the contraction force of isolated myocardial strips in a dose-dependent manner. The agonist concentrations producing the positive inotropic effect increased from days 14 to 100 of life. The efficiency of extracellular purines depends on animal age: in 14- and 56-day rats the positive inotropic effects of ATP and 2-methylthio-ATP were similar, while in 100-day rats ATP produced more pronounced effect than 2-methylthio-ATP.

Key Words: *P2-receptors; myocardium; ATP; contractility; ontogeny*

Immunohistochemical analysis of rat heart revealed abundant presence of P2X2- and P2X5-subtypes of P2-receptors on cardiomyocyte sarcolemma. P2X-receptors are cation-selective ionic channels characterized by virtually equal permeability for Na^+ and K^+ ions and pronounced permeability for Ca^{2+} ions. These channels open in response to micromolar concentration of extracellular ATP and are responsible for rapid cell response to ATP. Activation of P2X-receptors stimulates entry of Ca^{2+} ions into cardiomyocytes. The resultant depolarization of the cell membrane triggers additional Ca^{2+} entry via potential-dependent Ca^{2+}-channels, thus producing a contractile response. P2Y-receptors are also present in the heart and are typical G-protein-coupled receptors.

ATP and 2-methylthio-ATP (2MeSATP) are agonists of all subtypes of P2-receptors. However, the agonistic action of ATP is very short-lasting due to its rapid destruction by extracellular nucleotidases [8].

The age-related peculiarities of functional activity of P2-receptors in the heart are poorly studied. Our previous *in vivo* experiments demonstrated the involvement of exogenous ATP and cardiac P2X-receptors into the positive chronotropic reaction and high sensitivity of these receptors during the early postnatal ontogeny [1].

Here we examined the effect of ATP and its derivatives on contractility of rat myocardium during the postnatal ontogeny.

MATERIALS AND METHODS

Contractile activity of the myocardium was studied *in vitro* on myocardial strips isolated from albino rats. The effect of ATP and 2MeSATP (Sigma) in 3 increasing concentrations on this activity was assessed on a PowerLab setup (ADInstruments) equipped with a MLT 050/D tension transducer (ADInstruments).

The animals were narcotized with urethane, the rat thorax was opened, the heart was rapidly isolated, placed into a Petri dish containing oxygenated physiological solution and connected to ESL-2 stimulator. The myocardial strips were cut and suspended in the vertical position, the lower end being tied to the transducer. Then, each strip was immersed into individual 10 ml chamber perfused with physiological solution containing (in mM):

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119.8 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.05 $MgCl_2$, 0.42 NaH_2PO_4, and 5.05 glucose oxygenated with 95% O_2 and 5% CO_2. To stabilize pH within 7.3-7.4, basic and acid Trizma buffers (Sigma) were added to the solution. The strips were stimulated via platinum electrodes with a 5-msec pulses presented at a rate of 6 or 10 Hz for 14-, 56-, or 100-day-old rats, respectively.

The myograms were recorded on a PC equipped with a Chart 5.0 software. After immersion into the chambers, the strips were gradually stretched to an optimal tension during the stabilization period lasting for 40-60 min. The optimal tension was determined as the point were further increment in stretch was accompanied by a decrease in contraction force. During the following 10 min, the initial parameters of contraction were recorded. Then, ATP or 2MeSATP were applied in one of the tested concentrations and the contraction parameters were recorded for 20 min. P2-receptors agonists ATP and 2MeSATP were applied in different concentrations and changes in contraction parameters were evaluated. Thereafter, the strips were washed with working solutions three times for 10 min. Before application of the next concentration of the agonist, initial contraction parameters were re-determined. The effects of ATP and 2MeSATP on contraction force and duration were calculated in percentage of the control (100%). To block P1-receptors, we used 8-phenyltheophylline in a concentration of 10^{-3} M [6].

The results were analyzed statistically using Student's *t* test.

RESULTS

ATP (10^{-7}-10^{-4} M) induced a dose-dependent contraction of ventricular myocardial strips (Fig. 1, *a*). In 56- and 100-day rats, the maximum effect was observed under the action of ATP in a concentration of 10^{-6} M: contraction force increased by 32.8±6.6 and 25.6±7.8%, respectively (p<0.01, n=7). In 14-day rats, the maximum positive inotropic effect (14.90±2.27%, p<0.01, n=10) was observed at a one-order lower concentration (10^{-7} M). The duration of contraction decreased by 7-10% in 14- and 56-day rats and did not change in 100-day animals.

Increasing ATP concentrations inhibited contractility of the myocardium strips (Fig. 1, *a*). In 100-day rats, 10^{-5} M ATP produced a two-phase effect: the contraction force of ventricular strips first increased by 29.6%±5.6%, but on minute 12 it decreased by 16.0±3.2% (n=10). ATP in a concentration of 10^{-4} M decreased the contraction force by 15.2±3.8% (p<0.01, n=7). In 56-day rats, ATP applied in the concentrations of 10^{-5} M and 10^{-4} M decreased the contraction force by 34.6±4.4 and 19.5±3.4%, respectively, while the duration of contraction decreased by 10.0±1.9% (p<0.01, n=10). In 14-day rats, ATP in a concentration of 10^{-6} M insignificantly decreased the contraction force by 2.3±0.1% (n=10), but in a concentration of 10^{-8} M insignificantly increased it by 5.7±0.3% (n=10).

It was interesting to elucidate the mechanism underlying the negative inotropic effect of high ATP concentrations. The positive effect of ATP in

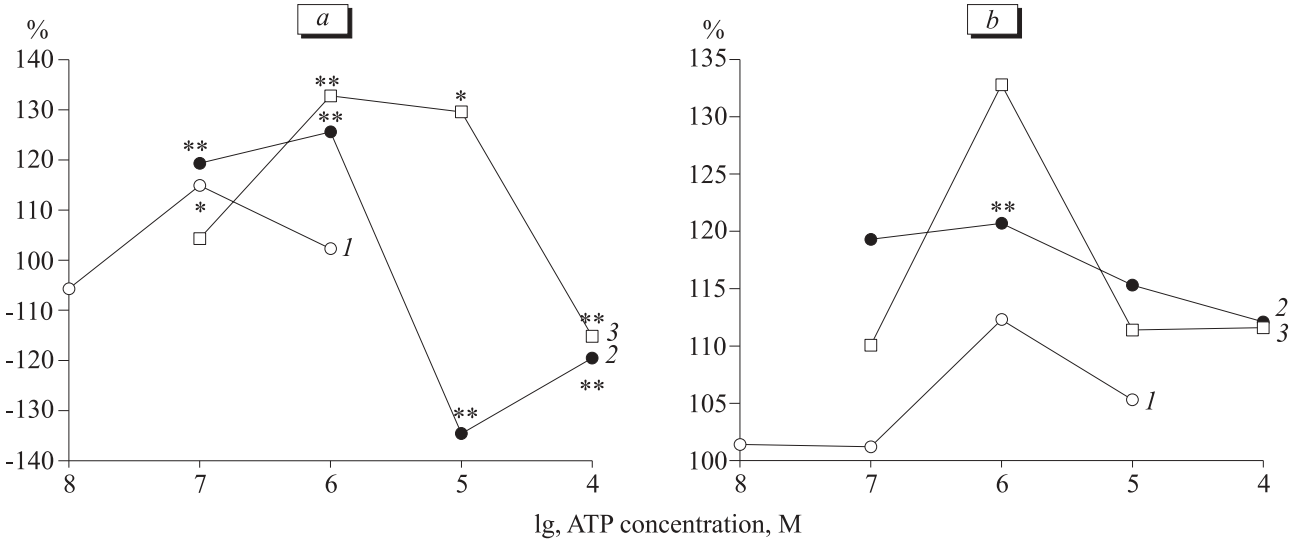

Fig. 1. Effect of ATP on contraction force of ventricular myocardial strips. The agonist was applied in various concentrations either alone (*a*) or under the action of P1-receptor blocker (*b*). Here and in Figs. 2 and 3, the age of rats was 14 (*1*), 56 (*2*) and 100 (*3*) days. *p<0.05, **p<0.01 in comparison with initial level.

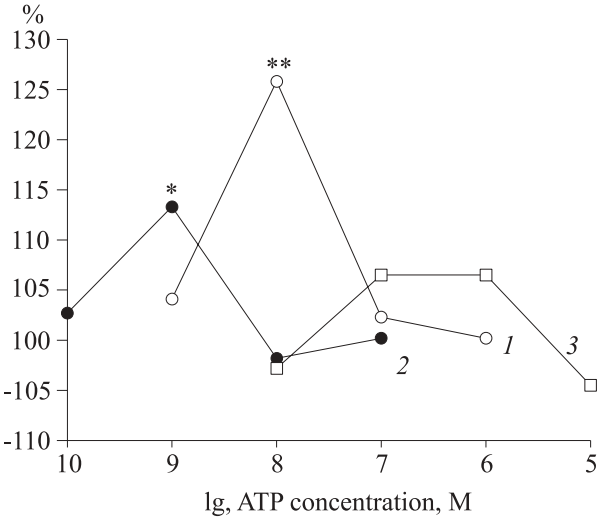

Fig. 2. Effect of 2MeSATP on contraction of myocardial ventricular strips.

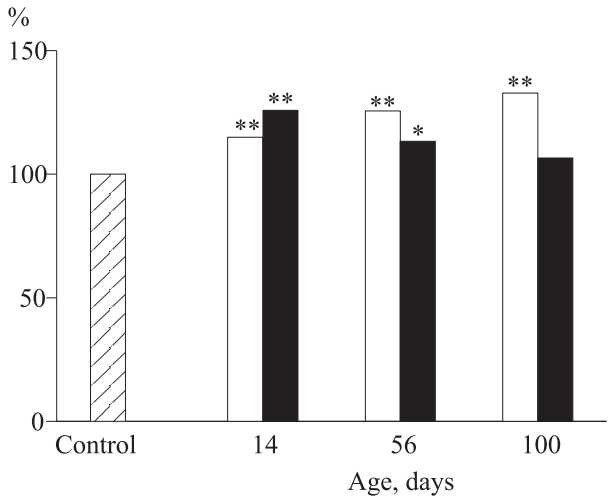

Fig. 3. Effect of ATP (open bars) and 2MeSATP (filled bars) on contractility of myocardial ventricular strips.

cardiovascular diseases is assumed to be related to its degradation to adenosine, which exerts negative chrono- and inotropic effects [2]. However, it is not clear, which of these substances, ATP or adenosine, affects purinergic receptors of the heart. ATP is an unstable substance rapidly hydrolyzed by ectoATPase to adenosine, which exerts its effect via P1-purinoreceptors. However, direct action of ATP on the heart via its own P2-receptors irrespective on its degradation to adenosine was proven. Under conditions of blockade of P1-receptors, exogenous ATP produces a positive inotropic effect in guinea pig left artium probably via activation of P2Y-receptors [6]. ATP increases contractility of guinea pig cardiomyocytes by 26% [7]. The P2-purinoreceptors are the targets for pharmacological preparations. Hence, new drugs producing principally new effects on these receptors can be created [3].

Against the background of P1-receptors blockade with 8-phenyltheophylline, ATP (10^{-6} M) increased the contraction force of ventricular strips in 100- and 56-day rats by 32.8±1.4% ($p<0.01$, $n=5$) and 20.7±3.7% ($p<0.01$, $n=5$), respectively, *i.e.* the effect of ATP remained unchanged (Fig. 1, *b*). In rats of the same age, blockade of P1-receptors reversed the negative inotropic effect of ATP (10^{-5} and 10^{-4} M) into positive one (11.40±2.55%, $n=6$ and 11.60±2.16%, respectively). In ventricular myocardial strips from 14-day rats with blocked P1-receptors, ATP (10^{-7} M) increased the contraction force by 12.30±1.87% ($p<0.05$, $n=10$). Thus, ATP produced a positive inotropic effect via activation of P2-purinoreceptors. Blockade of P1-receptors abolished the negative inotropic effect of ATP applied in concentrations of 10^{-5} and 10^{-4} M. Functional activity of P1-receptors was more pronounced in 100-day rats. Maturation of myocardial P1- and P2-receptors during the ontogeny was heterochronous due to reorganization of structural elements.

In some experiments on isolated organs and tissues, ATP was inferior to 2MeSATP, a stable analog of ATP affecting both P2X- [6] and P2Y-receptors of the myocardium [5].

2MeSATP in concentrations of 10^{-6}-10^{-10} M induced a dose-dependent contraction of atrial and ventricular myocardial strips (Fig. 2). In 100-day rats, these contractions were significantly ($p<0.05$) weaker than those induced by ATP (Fig. 3): the contraction force in response to 2MeSATP (10^{-6} and 10^{-5} M) increased by only 6.50±0.23% ($n=10$) and the duration of contraction did not change significantly. In 56-day rats, 2MeSATP increased the contraction force of atrial and ventricular strips by 11.6±0.6% ($p<0.05$, $n=10$) and 13.30±0.89% ($p<0.05$, $n=10$). The maximum effect was observed after application of 10^{-9} M 2MeSATP ($n=10$). In 14-day rats, 2MeSATP (10^{-8} M) produced a more pronounced effect than ATP and increased the contraction force of atrial and ventricular strips by 15.40±2.06% and 25.8±6.1%, respectively ($p<0.01$, $n=10$, Fig. 3).

In 100-day rats, 2MeSATP in concentrations of 10^{-5} and 10^{-8} M insignificantly decreased the contraction force of ventricular strips by 4.5±0.3 ($n=10$) and 2.8±0.2% ($n=10$) and had little effect on atrial strips. When applied in high or low concentrations to the strips of 14-, 56-, and 100-day rats, 2MeSATP also produced insignificant changes in the force and duration of contraction of atrial and ventricular strips (Fig. 2).

Thus, P2-receptors agonists ATP and 2MeSATP dose-dependently increase contractility of atrial and ventricular myocardium in rats aging 14-100 days. ATP (10^{-6} M) increased myocardial contractility in 100-day rats, whereas the examined concentrations of 2MeSATP produced no significant effects. In 56-day rats, ATP increased myocardial contractility in a concentration of 10^{-6} M, while 2MeSATP produced similar effect in a concentration of only 10^{-9} M. In 14-day rats, the maximum effects were produced by 10^{-7} M ATP and 10^{-8} M 2MeSATP. Therefore, the doses of agonists producing the positive inotropic effect increase with age, which attests to high sensitivity of myocardial P2-receptors during the early postnatal ontogeny. This effect of ATP and 2MeSATP on myocardial contractility depends on animal age.

These phenomena could be related to heterochronous maturation of the cardiac receptor apparatus and consequently, to decreased sensitivity and changes in functional activity and number of various receptors involved in myocardial contraction in animals at various stages of ontogeny.

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