Role of P2X and P2Y Receptors in Rat Myocardial Contractility during Ontogeny

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Stable agonist of P2 receptors 2-methylthio-ATP and selective antagonists of P2X and P2Y receptors PPADS and reactive blue-2 were used for evaluation of the role of P2 receptors in positive contractile reaction of atrial and ventricular myocardium in rats. PPADS significantly moderated the effects of 2-methylthio-ATP in 14-, 21-, and 56-day-old rat pups, but potentiated them in 100-day-old rats. Under conditions of reactive blue-2 treatment, the positive effect of the agonist was preserved in the atria and ventricles in all age groups and was age-dependent.

Key Words: *P2X and P2Y receptors; myocardial strips; contractility; ontogenesis*

The atria of mature rats contain primarily $P2X_1$, $P2X_2$, and $P2X_4$ receptors, while in ventricles $P2X_4$ receptors predominate. $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors were also found in rat hearts [9]. P2X receptors are cation-selective ionic channels regulating the entry of Na^+, K^+, and Ca^{2+} ions into cells and responsible for rapid response to ATP.

Our previous *in vivo* experiments on rats demonstrated the involvement of cardiac P2X receptors into positive chronotropic reaction and their high sensitivity during the early postnatal ontogeny [1].

P2Y receptors are slow metabotropic receptors coupled with G-proteins. In the whole neonatal heart, $P2Y_{1,2,4,6}$ receptors are expressed, $P2Y_1$ receptors predominate. Expression of P2Y receptors in cardiomyocytes varies during ontogeny. For example, expression of $P2Y_1$, $P2Y_2$, and $P2Y_6$ receptors increases with age [5,8], while $P2Y_4$ receptors are absent in cardiomyocytes of adult rats.

We studied age-related peculiarities of functional activity of cardiac P2X and P2Y receptors involved in myocardial contraction of rats aging from 14 to 100 days.

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MATERIALS AND METHODS

Contractile activity of the myocardium in response to 2-methylthio-ATP (2MeSATP) applied in maximum effective concentration was examined *in vitro* on the myocardium strips isolated from atria and ventricles of albino rats (n=120) under conditions of selective blockade of P2 receptors. The experiments were carried out on a PowerLab setup (ADInstruments) equipped with an MLT 050/D tension transducer (ADInstruments).

The rats were narcotized with urethane, the thorax was opened, and the heart was rapidly isolated, placed into a Petri dish with oxygenated physiological solution (with ESL-2 stimulator switched on). The myocardial strips were cut and fixed vertically to the wall and a transducer. Each strip was placed into individual 10-ml chamber with working solution containing (in mM): 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. For maintaining pH within 7.3-7.4, basic and acid Trizma buffers (Sigma) were added. The strips were stimulated via platinum electrodes with 5-msec pulses presented at the rate of 6 or 10 Hz for 14-, 21-, 56- or 100-day-old ratsy. Three series of experiments were carried out (n=10 in each age group).

The myograms were recorded on a PC using Chart 5.0 software. After immersion of the strips into the chambers, they were gradually stretched to an optimal tension over 40-60 min (stabilization period). The optimal tension was determined as the point were further stretching was accompanied by a decrease in the contractile force. The initial contraction parameters (control) were recorded for 10 min and then the action of selective P2 receptor antagonists was studied for 20 min.

Agonist of P2 receptors 2MeSATP (Sigma) was applied in a working concentration and changes in the contraction of muscle strips were assessed after washout. Changes in the contraction force in response to 2MeSATP application were calculated in percents of initial value (100%). PPADS (pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid, Sigma), a selective and stable antagonist of P2X receptors, was used in a concentration of 10^{-5} M [7]; P2Y receptors were blocked with anthraquinone derivative reactive blue-2 (RB-2, 1.5 μM) [3].

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

RESULTS

ATP, an agonist of all subtypes of P2 receptors, produces a short-lasting effect due to its rapid breakdown by extracellular nucleotidases [2]. By contrast, 2MeSATP is a stable agonist of cardiac P2X and P2Y receptors [7]. In most *in vitro* experiments, ATP is not the strongest agonist; it is weaker than 2MeSATP: 2MeSATP>ATP>α,β-MeSATP. 2MeSATP is the most effective agonist of $P2X_1$, $P2X_2$, $P2X_5$, and $P2Y_1$ receptors in the heart.

We demonstrated a positive dose-dependent inotropic action of 2MeSATP on myocardial contractility in 14-, 21-, 56-, and 100-day-old rats. In 14-day-old rats, the maximum contractile effect was observed after application of 10^{-8} M 2MeSATP: the agonist increased atrial contraction by 15.40±2.06% ($p<0.05$) and enhanced ventricular contraction by 25.8±6.2% ($p<0.01$). 2MeSATP shortened the duration of atrial and ventricular contractions by 14.7±3.1% ($p<0.05$). At this age, the total duration of contraction decreased due to a decrease of the relaxation period of atrial and ventricular strips. In 21-day-old rat pups, 2MeSATP (10^{-7} M) increased the contraction force of atrial and ventricular strips by 31.3±7.4% ($p<0.01$) and lengthened the duration of contraction by 15.0±5.1% ($p<0.01$). The prolongation of total contraction-relaxation period resulted from an increase in the duration of the contractile phase. In 56-day-old rats, 2MeSATP (10^{-9} M) increased the contraction force in atria and ventricles by 11.6±0.6% ($p<0.05$) and 13.30±0.89% ($p<0.05$), respectively. In 100-day-old rats, 2MeSATP (10^{-6} M) produced no significant changes in the contraction force of atria and ventricles (the positive inotropic effect was 6.50±0.23%).

For identification of P2 receptors involved in purinergic positive contractile effect, we used selective blockers of P2X and P2Y receptors. In the presence of these antagonists, the action of 2MeSATP in most effective concentrations was tested: 10^{-8} M for 14-day-old rats, 10^{-7} M for 21-day-old rats, 10^{-9} M for 56-day-old rats, and 10^{-6} M for 100-day-old rats.

PPADS, a widely used P2-antagonist, demonstrates pronounced blocking action on cardiac $P2X_1$, $P2X_2$, $P2X_4$, and $P2X_5$ receptors, but does not mo-

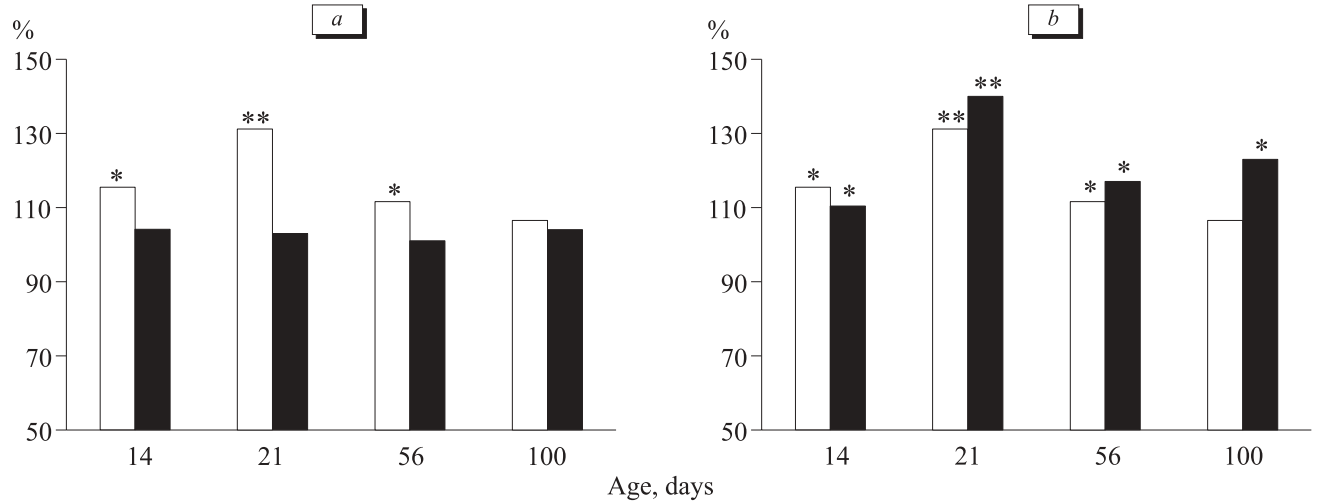

Fig. 1. Force of atrial contraction after receptor blockade with PPADS (*a*) and RB-2 (*b*). Here and in Fig. 2: application of 2MeSATP (open bars), application of P2 receptor antagonists. **p*<0.05, ***p*<0.01 compared to the control (100%).

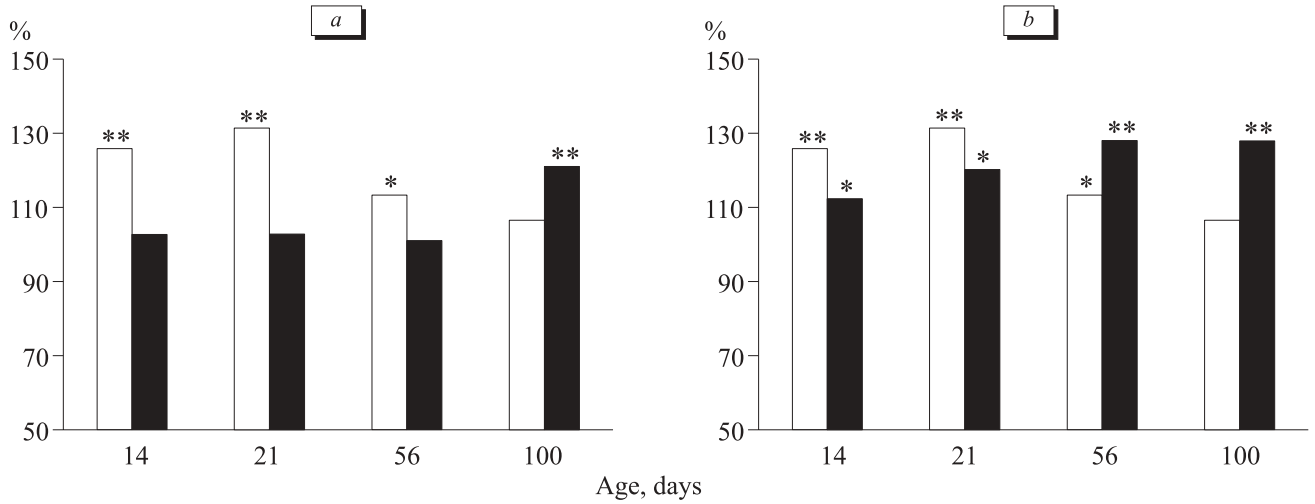

Fig. 2. Force of ventricular contraction after receptor blockade with PPADS (*a*) and RB-2 (*b*).

dulate the effects mediated via P2Y receptors [3]. In our experiments, this selective P2X-antagonist virtually completely prevented the effect of 2MeSATP on myocardial contractility in 14-, 21-, and 56-day-old rats. In atria and ventricles of 14-day-old rat pups, the effects of the agonist in a concentration of 10^{-8} M were 2.3±0.2% and 4.1±0.3%, respectively. In PPADS-treated 21-day-old rat pups, 2MeSATP (10^{-7} M) increased the contraction force of the atria and ventricles by 3.0±0.1%. In 56-day-old rats, the reaction of the atria and ventricles to 2MeSATP in a concentration of 10^{-6} M against the background of PPADS virtually disappeared. In PPADS-treated 100-day-old rats, the reaction of the atria was only 4.0±0.2%, while contractility of ventricular myocardium increased by 21.0±0.9% ($p<0.05$). Hence, in 100-day-old rats, P2Y receptors are involved in positive contractile reaction of ventriclular myocardium. In our experiments, PPADS significantly decreased the effects of 2MeSATP in 14-, 21-, and 56-day-old rat pups (Figs. 1, 2); the role of P2Y receptors in atrial and ventricular contractility at the early stages of ontogeny is minor.

For evaluation of functional activity of P2X receptors, we blocked P2Y receptors with RB-2. This agent produces an antagonizing effect within the very narrow concentration range and limited incubation time (<60 min). Beyond these limits, the non-specific effects of RB-2 become significant [1,4].

Under conditions of P2Y receptor blockade, 2MeSATP significantly increased the contraction force in atria and ventricles in all age groups: in 14-day-old rat pups by 10.4±0.4 and 12.30±0.32%, respectively ($p<0.05$). The duration of contraction decreased by 11.50±0.52% ($p<0.05$) due to shortening of the relaxation phase. In 21-day-old rat pups, the maximum effects of 2MeSATP was recorded in atria (40.0±2.3%, $p<0.01$), while its effect in the ventricles was less pronounced (20.2±1.8%, $p<0.01$). In 56- and 100-day-old rats, the contractility of muscular strips from ventricles increased by 28% and 27%, respectively ($p<0.01$) and from atria by 17% and 23% ($p<0.05$). The duration of contraction did not change significantly.

Under conditions of P2Y receptor blockade, the positive effect was preserved in atria and ventricles in all age groups, which indicates the involvement of P2X-reveptors in the realization of the positive chronotropic effect of 2MeSATP in the heart. However, functional activity of P2X receptors varies at different stages of ontogeny. In our experiments on isolated muscle strips from the atria and ventricles, 2MeSATP under conditions of P2Y receptor blockade produced different effects depending on animal age. In 21-day-old rat pups, the force of atrial contraction and total duration of their contraction under the action of agonist and during blockade of P2Y receptors was significantly greater ($p<0.05$) than in 100-day-old rats. In 56- and 100-day-old animals, the force of ventricular contraction measured under conditions of functionally active P2X receptors was significantly higher ($p<0.05$) than in 14-day-old pups. Hence, the role of P2X receptors in positive contractile reaction decreases in the atria, but increases in the ventricles during the period from 14 to 100 days. The age-related peculiarities of functional activity of P2X and P2Y receptors involved in myocardial contractility attest to heterochronous maturation of cardiac receptor system and to changes in the density and sensitivity of various receptor subtypes during ontogeny.

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