Involvement of P2Y\textsubscript{2,4} Receptors in the Regulation of Myocardial Contractility in Growing Rats

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Experiments with R2Y receptor blockers allowed identification of R2Y subtypes mediating the inhibitory effects of uridine triphosphate on myocardial contractility. In 100-day-old animals, the myocardial inotropic response to the administration of uridine triphosphate was mediated by R2Y\textsubscript{2} receptors. R2Y\textsubscript{4} receptors took part in the realization of negative inotropic response to uridine triphosphate in all age groups, but the most pronounced effects of this substance on myocardial contractility were found in 100-day-old rats. It was found that R2Y receptor blockers PPADS and reagent blue-2 affect amplitude-time parameters of myocardial contractility in rats of various ages.

Key Words: purine receptors; heart; ontogeny, myocardial contractility

Intracellular purine and pyrimidine nucleotides (ATP, uridine triphosphate – UTP) serve as a source of energy, take part in the biosynthesis of ribonucleic acids, and contribute to cell vital activity.

The effects of ATP and UTP are mediated by inotropic and metabotropic R2X and R2Y receptors, which serve as the most diverse among known receptor subtypes for classical neurotransmitters [2,3,6]. UTP is an agonist of metabotropic P2Y\textsubscript{1,2,4,6,11,13} receptors in heart cardiomyocytes [5,9,10]. The diversity of purine receptors allows to suggest that one substance can induce various specific signaling depending on the receptor.

Immunohistological analysis reveals age-dependent features of R2Y receptor localization in rat heart. The abundance of these receptors in mature animals decreases in the following order: R2Y\textsubscript{6} > R2Y\textsubscript{2} > R2Y\textsubscript{4} = R2Y\textsubscript{4} [7]. It is known that the expression of R2Y receptors in the myocardium varies and the expression of R2Y\textsubscript{1,2,6} receptors intensifies during ontogeny [11]. However, there is no published data confirming the participation of R2Y receptors in the regulation of myocardial contractility at early stages of ontogeny with immature regulatory pathways in the heart and various stages of development.

Previous studies of R2Y receptors have shown that activation of R2Y\textsubscript{2} receptors stimulates the synthesis and release of arachidonic acid, prostaglandins, and NO [5]. UTP decreases hypoxia-induced cardiomyocyte death via the activation of R2Y\textsubscript{2} receptors [14].

Controversial data on the selectivity of R2Y receptor blockers are shown. R2Y receptor blockers have species and tissue specificity. PPADS, suramin, and reagent blue-2 are the classical blockers for R2Y purine receptors [13]. It is shown that PPADS is an antagonist of R2Y\textsubscript{1} receptors and probably of R2Y\textsubscript{6} receptors [8,12]. Literature data indicate that PPADS does not affect human R2Y\textsubscript{2} receptors and rat R2Y\textsubscript{4} receptors, and has moderate blocking effects on P2Y\textsubscript{2,6,11,13} receptors [8].

Taking into account the data that UTP is an agonist of P2Y\textsubscript{2,4,6} receptors, the usage of its antagonist PPADS allows evaluation of functional activity of R2Y\textsubscript{2} receptors. It is possible because PPADS does not block R2Y\textsubscript{4} receptors, and R2Y\textsubscript{6} receptors are conjugated with Gq/11 protein and do not contribute
to a decrease in myocardial contractility after UTP administration.

Here we determined the subtypes of R2Y receptors, which take part in the regulation of rat myocardial contractility in the ontogeny.

MATERIALS AND METHODS

Experiments were performed on 7-, 21-, and 100-day-old white laboratory rats in accordance with the Rules for Experimental Work with Laboratory Animals. Animals were narcotized with 25% urethane solution (1.2 g/kg of body weight). The amplitude of isometric contraction of myocardial stripes was recorded on PowerLab equipment with Chart 5.0 software. The samples were fixed vertically with one end connected to a MLT 050/D force sensor, while the other connected to a support. Each sample was embedded into an individual reservoir (10 ml) with working Krebs solution: 33 mM NaCl, 4.7 mM KCl, 0.6 mM MgCl₂, 1.35 mM NaHPO₄, 2.5 mM CaCl₂, and 7.8 mM glucose at 28°C, and carbogen (95% O₂ and 5% CO₂). pH was maintained at 7.35-7.40 using basic and acid Trizma buffers (Sigma). The strips from animals aging 7, 21, and 100 days were stimulated via platinum electrodes at a frequency of 6 and 10 pulses and duration 5 msec, respectively. Rat age of 7, 21, and 100 days corresponded to the periods of neonatality, milk feeding, and maturity, and had various maturity degree and various intensity of heart regulations.

After immersion into the reservoir and a 40-60-min “running-in” period, parameters of contraction were recorded under basal conditions for 10 min and after the addition of UTP (Sigma) in one of the concentrations to a working solution for 30 min. After the stimulation with UTP, the samples were washed 3 times with working solution for 10 min and basal parameters were recorded. The agonist was added 20 min after the blocker administration. The strength of UTP-induced contraction was calculated as a percent of baseline value (taken as 100%). The significance of differences was evaluated by parametric paired and unpaired Student’s t test. The differences were significant at p<0.05.

RESULTS

Experiments with various receptor agonists and antagonists allow identification of the subtype of R2Y receptor.

Little is known about the effects of PPADS on myocardial contractility during ontogeny. Thus, we study its effects on atrial and ventricle contractility in 7-, 21-, and 100-day-old rats. The blocker in a dose of 30 μM was used [4].

The effects of the agent on amplitude-time parameters of myocardial contractility were studied for 60 min. The stabilization of contraction parameters was observed at min 10 after blocker addition and this parameter did not change during the following 50 min.

In newborn rats PPADS addition was followed by a positive inotropic effect: 7.3±0.7% in the atria, and 5.3±1.0% in the ventricles (p<0.01).

In 21-day-old rats the study blocker induced a decrease in contraction amplitude of the atria by 7.4±1.4% (p<0.01). The incubation of ventricular myocardium with PPADS was followed by an increase in contraction amplitude by 47.2±4.7% (as compared to the initial; p<0.01). Among the study time parameters of myocardial inotropy, the increase in the rate of contraction and relaxation of ventricular myocardium was most pronounced (by 44.2±3.5 and 40.8±8.9%, respectively, p<0.05). Such parameters as the duration and time of contraction and relaxation underwent minor changes.

In 100-day-old rats, the blocker had negative inotropic effect. Contraction force decreased by 8.9±1.7% in the atria, and by 6.8±1.1% in the ventricles (as compared to initial level; p<0.05; Table 1).

In accordance to our data, the blocker of R2Y receptors PPADS has multidirectional effects on the study parameters of myocardial contractility in 7-, 21-, and 100-day-old rats. R2Y receptor blocker PPADS directly affects the amplitude-time parameters of myocardial contractility.

<table>
<thead>
<tr>
<th>Age</th>
<th>PPADS concentration</th>
<th>Contraction force, % of initial value</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>atria</td>
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<tr>
<td>Day 7</td>
<td>3×10⁻⁵ M</td>
<td>107.3±0.7**</td>
</tr>
<tr>
<td>Day 21</td>
<td>3×10⁻⁵ M</td>
<td>92.6±1.4**</td>
</tr>
<tr>
<td>Day 100</td>
<td>3×10⁻⁵ M</td>
<td>91.0±1.8**</td>
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Note. *p<0.05, **p<0.01 in comparison with the initial value.