

# Involvement of P2Y<sub>2,4</sub> 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<sub>2</sub> receptors. R2Y<sub>4</sub> 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<sub>1,2,4,6,11,13</sub> 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<sub>6</sub>>R2Y<sub>1</sub>>R2Y<sub>2</sub>=R2Y<sub>4</sub> [7]. It is known that the expression of R2Y receptors in the myocardium varies and the expression of R2Y<sub>1,2,6</sub> receptors intensifies during ontogeny [11]. However, there is no published data confirming the participa-

tion 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<sub>2</sub> receptors stimulates the synthesis and release of arachidonic acid, prostaglandins, and NO [5]. UTP decreases hypoxia-induced cardiomyocyte death via the activation of R2Y<sub>2</sub> 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<sub>1</sub> receptors and probably of R2Y<sub>6</sub> receptors [8,12]. Literature data indicate that PPADS does not affect human R2Y<sub>2</sub> receptors and rat R2Y<sub>4</sub> receptors, and has moderate blocking effects on P2Y<sub>2,6,11,13</sub> receptors [8].

Taking into account the data that UTP is an agonist of P2Y<sub>2,4,6</sub> receptors, the usage of its antagonist PPADS allows evaluation of functional activity of R2Y<sub>2</sub> receptors. It is possible because PPADS does not block R2Y<sub>4</sub> receptors, and R2Y<sub>6</sub> receptors are conjugated with Gq/11 protein and do not contribute

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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 MgCl<sub>2</sub>, 1.35 mM NaHPO<sub>4</sub>, 2.5 mM CaCl<sub>2</sub>, and 7.8 mM glucose at 28°C, and carbogen (95% O<sub>2</sub> and 5% CO<sub>2</sub>). 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.

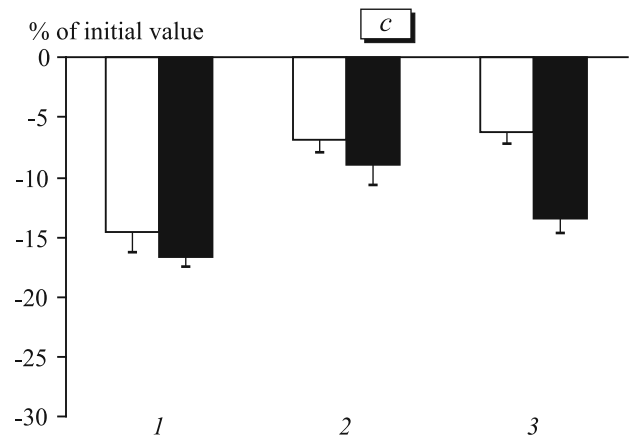
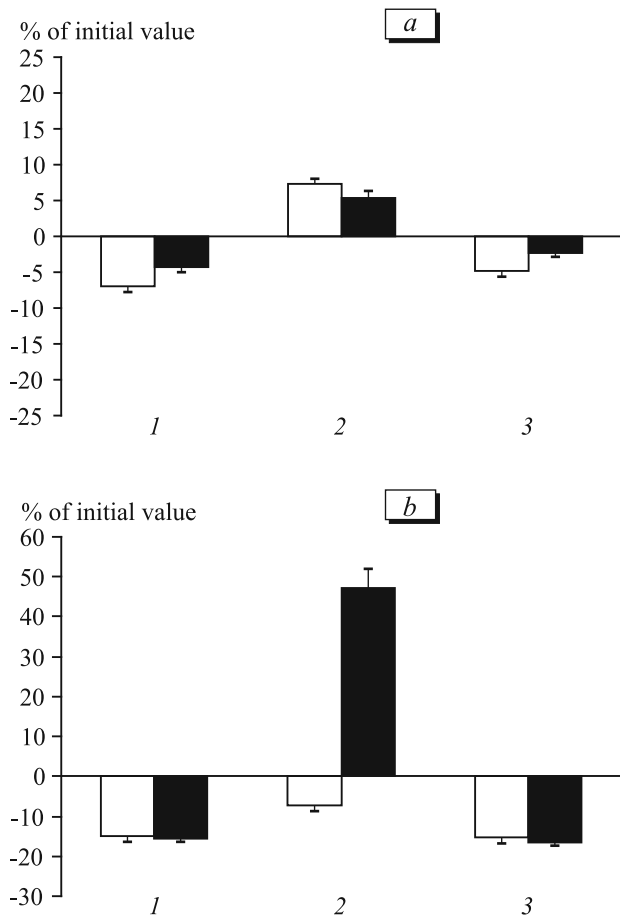
In 100-day-old rats, the blocker had negative inotropic effect. Contraction force decreased by 8.9±1.7% in the atria, and by 6.8±1.1% in the ventricles (as compared to initial level;  $p < 0.05$ ; Table 1).

In accordance to our data, the blocker of R2Y<sub>2</sub> 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 myo-

**TABLE 1.** Effects of PPADS on Myocardial Contractility of the Atria and Ventricles in 7-, 21-, and 100-day-old Rats

Age	PPADS concentration	Contraction force, % of initial value	
		atria	ventricles
Day 7 (n=8)	3×10 <sup>-5</sup> M	107.3±0.7**	105.3±1.0**
Day 21 (n=8)	3×10 <sup>-5</sup> M	92.6±1.4**	147.2±4.8**
Day 100 (n=8)	3×10 <sup>-5</sup> M	91.0±1.8**	93.2±1.1*

**Note.** \* $p < 0.05$ , \*\* $p < 0.01$  in comparison with the initial value.



**Fig. 1.** Effects of UTP on contraction force of atrial (light bars) and ventricular (dark bars) myocardium in 7- (a), 21- (b), and 100-day-old (c) rats under control conditions and after PPADS blockage. 1) UTP effect (control); 2) PPADS effect (control); 3) UTP effect after PPADS addition.

cardial contractility in rats of various ages. In 21-day-old rats, PPADS produced positive inotropic and relaxation effects on ventricular myocardium.

Our results showed that the effects of UTP on myocardial contractility depend on animal age. The most effective concentration of UTP for 7- and 10-day-old rats was  $10^{-8}$  M, and for 21-day-old specimens was  $10^{-7}$  M [1].

The agonist had negative inotropic effect in newborn rats under normal conditions ( $7.0 \pm 0.8\%$  in the atria, and  $4.3 \pm 0.7\%$  in the ventricles). After the blockage of  $R2Y_2$  receptors, the negative inotropic effect of UTP remained unchanged:  $4.9 \pm 0.8\%$  in the atria, and  $2.3 \pm 0.5\%$  in the ventricles (insignificant differences from the control and initial values).

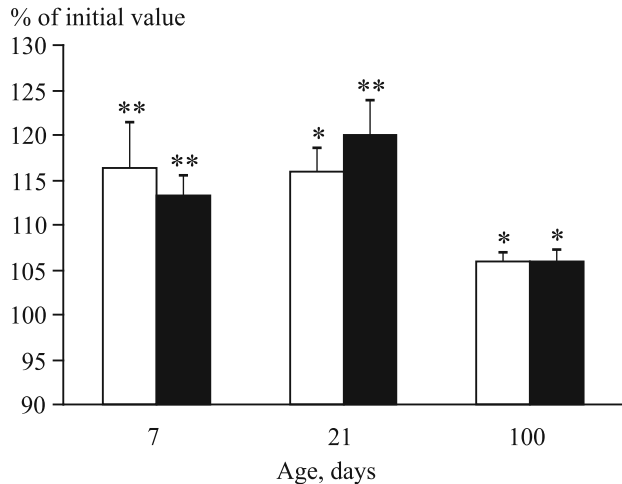
In 21-day-old rats, UTP reduced contraction force by  $15.0 \pm 1.4\%$  in atrial myocardium and by  $15.7 \pm 0.8\%$  in ventricular myocardium in the control, but did not affect contraction force after infusion of the antagonist ( $15.4 \pm 1.4\%$  in the atria, and  $16.5 \pm 0.9\%$  in the ventricles).

In 100-day-old rats, UTP induced a decrease in contraction force by  $14.5 \pm 1.7\%$  in the ventricles and by  $16.6 \pm 0.8\%$  in the atria in the control. After blockage UTP decreased contraction amplitude in ventricular

myocardium from 16 to 6% ( $p < 0.05$ ). The inhibiting effect of UTP decreased by 2.6 times in comparison with the control (Fig. 1).

We previously observed that  $R2Y$  purine receptor agonist UTP has negative inotropic effect, which is mediated by  $R2Y_4$  receptors [1]. The investigation of effects of  $R2Y_4$  receptor blocker reagent blue-2 on myocardial contractility showed that blocker-induced contraction force of myocardial stripes of the atria and ventricles from 7- and 21-day-old rats is significantly higher than from 100-day-old animals ( $p < 0.01$ ). The most significant increase in myocardial inotropy after reagent blue-2 administration was observed in 21-day-old rats and the less significant was found in 100-day-old animals (Fig. 2).

Negative inotropic effect of UTP was not observed after the preliminary blockage of  $R2Y_4$  receptors induced by the incubation of reagent blue-2 with the myocardium from 7-, 21-, and 100-day-old rats. UTP administration after reagent blue-2-induced blockage was followed by a slight decrease in contraction force of atrium and ventricular myocardium in 7-, and 21-day-old rats. In 100-day-old rats UTP administration after the infusion with antagonist reagent blue-2 did not induce a reduction in myocardial contractility



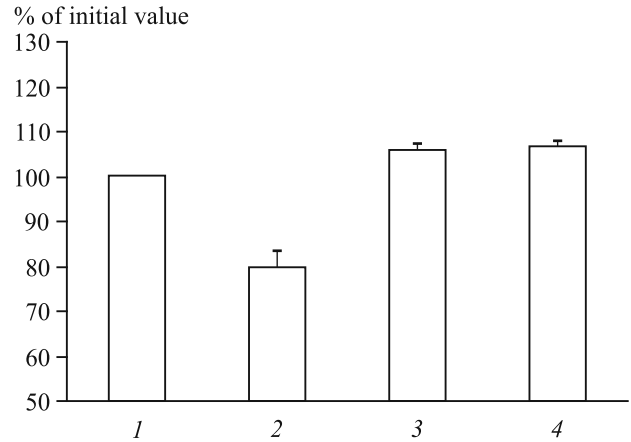
**Fig. 2.** Reagent blue-2 on contraction force of atrial (light bars) and ventricular (dark bars) myocardium in 7-, 21-, and 100-day-old rats. \* $p < 0.05$ , \*\* $p < 0.01$  in comparison with the initial level.

of the atria and ventricles, positive inotropic effect of the blocker remained unchanged (Fig. 3).

Therefore, R2Y<sub>4</sub> receptors contribute to the realization of negative inotropic effect of UTP on myocardial contractility of the atria and ventricles in rats of all age groups. Modulation effect of UTP on myocardial contractility is more pronounced in 100-day-old rats. Inotropic response of the myocardium from 100-day-old rats on UTP is also mediated by R2Y<sub>2</sub> receptors.

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**Fig. 3.** Effects of UTP on contraction force of ventricular myocardium in 100-day-old rats under control conditions and after reagent blue-2-induced blockage. 1) initial level; 2) UTP; 3) reagent blue-2; 4) UTP+reagent blue-2.