Involvement of P2Y Receptors in Myocardial Contractile Activity of Rats during Postnatal Ontogeny

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We studied the effect of uridine 5'-triphosphate in concentrations of 10^{-10}-10^{-6} M on myocardial contractile activity in 7-100-day-old rats. Analysis of isometric contraction of myocardial strips showed that uridine 5'-triphosphate reduced the strength of myocardial contraction in rats of all age groups. In 21- and 100-day-old rat pups, exogenous uridine 5'-triphosphate produced a stronger inhibitory effect than in 7-day-old animals. The negative inotropic effect of UTP was abolished under conditions of $P2Y_4$ purinoceptor blockade with reagent blue-2. These data indicate that the effect of UTP on the myocardium is realized via $P2Y_4$ purinoceptors.

Key Words: *P2 purinoceptors; heart; uridine 5'-triphosphate; ontogeny*

Similarly to ATP, uridine 5'-triphosphate (UTP) plays a role of the signal molecules and is co-localized in the vesicles with the major transmitter. UTP is involved in nerve signal transduction and acts as a co-transmitter. The protective effect of UTP under conditions of cardiac ischemia was evaluated [7,14]. UTP is significantly released from cardiomyocytes under pathophysiological conditions, including coronary occlusion, hypoxia, myocardial infarction, and arrhythmia. These disturbances are accompanied by cell death and release of ATP and UTP into the extracellular matrix, which results in activation of P2 purine receptors (PR).

Metabolism of pyrimidine nucleotides (*e.g.*, UTP) in the heart is poorly understood. The concentration of these nucleotides in the myocardium is very low [8]. The content of uracil nucleotides in the myocardium is lower than that of adenine nucleotides (*i.e.*, ATP). The ATP/UTP ratio in the heart is 1:10 or 1:16 [10].

A large body of evidence exists to support the presence of extracellular UTP and uracil nucleotides activate several subtypes of P2Y receptors [10]. P2Y receptors are metabotropic receptors. There are 8 subtypes of P2Y receptors in the heart.

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$P2Y_{1,2,4,6,11,12,13}$ receptors were shown to exist in the heart, cardiac myofibroblasts [9,10,16], individual cardiomyocytes [9,10,13], and endothelial and smooth muscle cells of the vessels [12]. $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors are activated by UTP.

Our previous studies showed that P2X receptor agonists ATP, α,β-methylene ATP, and β,γ-methylene ATP produce a positive inotropic effect, which is realized via $P2X_1$ receptors [1].

Here we studied the effect of UTP in various concentrations on myocardial contractile activity (MCA) in rats during early postnatal ontogeny and identified the subtype of P2Y receptors involved in MCA.

MATERIALS AND METHODS

MCA was evaluated *in vitro* on myocardial strips from albino rats aging 7, 21, and 100 days. The study was performed on a PowerLab device (ADInstruments) with a MLT 050/D force sensor (ADInstruments).

The experiment was conducted in accordance with the bioethical principles. The animals were anesthetized. The heart was rapidly removed and placed in a Petri dish with oxygenated working solution. An ESL-2 stimulator was switched on. Myocardial strips were prepared. The samples were fixed in a vertical position. One end of the sample was connected to a force

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sensor, while the other was connected to a supporting point. Each sample was embedded into an individual reservoir with working solution containing (in μmol/liter) 119.8 NaCl, 5.4 KCl, 1.8 $CaCO_3$, 1.05 $MgCl_2$, 0.42 $NaHPO_4$, and 5.05 glucose (95% O_2 and 5% CO_2 was delivered through the solution). The basic and acid buffers Trizma (Sigma) were added to maintain a pH value of 7.3-7.4. The strips from animals aging 7, 21, and 100 days were stimulated via platinum electrodes with a frequency of 6 and 10 pulses and duration 5 msec, respectively.

The curve was recorded on a personal computer with Chart 5.0 software. The samples were plunged into a reservoir and after 40-60-min "running-in period" the parameters of contraction were recorded under basal conditions (10 min) and after addition of UTP (Sigma) in one of the concentrations to a working solution (30 min). After stimulation with UTP, the samples were washed 3 times with working solution for 10 min. Baseline parameters of contraction were recorded for each dose of UTP. 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

By their sensitivity to agonists, P2Y receptors are pharmacologically divided into 3 groups. Group 1 receptors ($P2Y_1$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$) are activated by adenine nucleotides (*i.e.*, ATP and ADP). Group 2 receptors ($P2Y_6$) are stimulated by uranyl nucleotides UTP and UDP. Group 3 receptors ($P2Y_2$ and $P2Y_4$) respond both to uracil and adenine nucleotides [9,10].

A highly sensitive method to measure the concentration of extracellular UTP (subnanomolar concentrations) was developed recently [6]. In our experiments, the agonist was used in concentrations of 10^{-6}-10^{-10} M to evaluate the dose-dependent effect. Exogenous UTP in these concentrations produced a negative inotropic effect on the atria and ventricles from animals aging 7, 21, and 100 days.

UTP in concentrations of 10^{-10}, 10^{-8}, 10^{-7}, and 10^{-6} M produced the same effect, which was manifested in a 6-8% decrease of MCA in the atria and ventricles ($p<0.05$; Fig. 1). UTP also decreased MCA in 21-day-old rat pups. UTP in concentrations of 10^{-10} and 10^{-7} M was most potent in inhibiting the atrial MCA (19-22%, $n=8$; $p<0.05$). UTP in concentrations of 10^{-10} and 10^{-8} M most significantly reduced ventricular MCA (18-22%, $n=8$; $p<0.05$). The effect of UTP in concentrations of 10^{-10} and 10^{-6} M was most significant in adult rats. The strength of atrial and ventricular contractions decreased by 18-21% ($n=6$; $p<0.05$).

Extracellular UTP in near-physiological concentrations had a stronger inhibitory effect in animals aging 21 and 100 days than in 7-day-old rats ($p<0.05$).

In the next series we evaluated the subtype of P2Y receptors that mediated these effects of UTP. To estimate a possible role of $P2Y_4$ receptors in MCA, we studied the effect of UTP after blockade with reagent blue-2 (1.5 μM) [4]. Suramin and PPADS are not potent antagonists of $P2Y_4$ receptors [10,11].

Reagent blue-2 increased MCA of the atria and ventricles. UTP was added after stabilization of the antagonist effects. The parameters were recorded for 30 min. The negative inotropic effect of UTP in animals aging 7, 21, and 100 days was not observed after incubation of the myocardium with reagent blue-2. Addition of UTP was not followed by the decrease in MCA after $P2Y_4$ receptor blockade (Fig. 2).

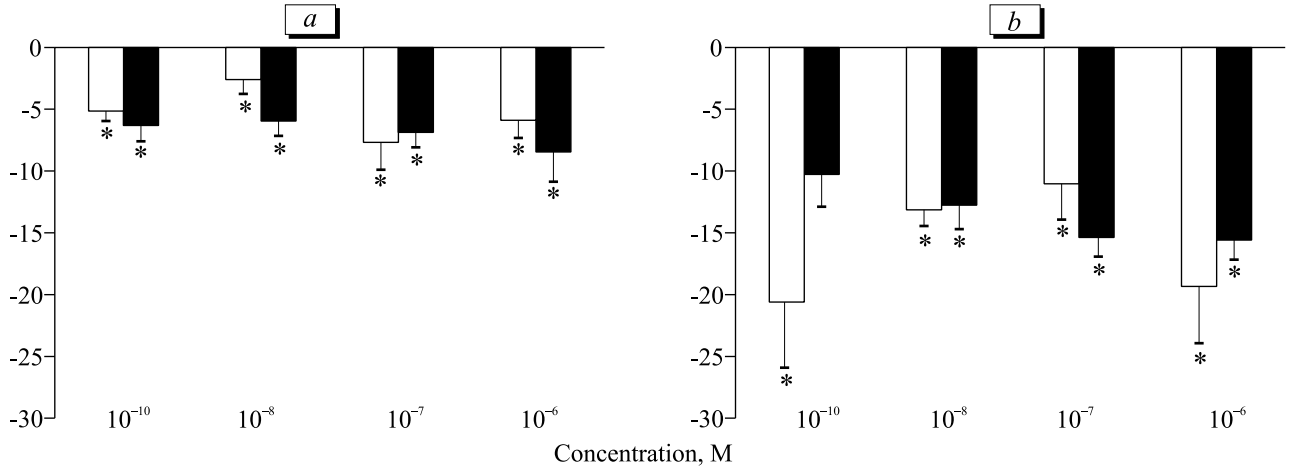

Fig. 1. Effect of UTP in various concentrations on the strength of myocardial contraction in the atria and ventricles from rats aging 7 (*a*) and 100 days (*b*). Here and in Fig. 2: light bars, atria; dark bars, ventricles. *$p<0.05$ compared to the baseline value. Ordinate: strength of myocardial contraction in percentage of the baseline value (100%).

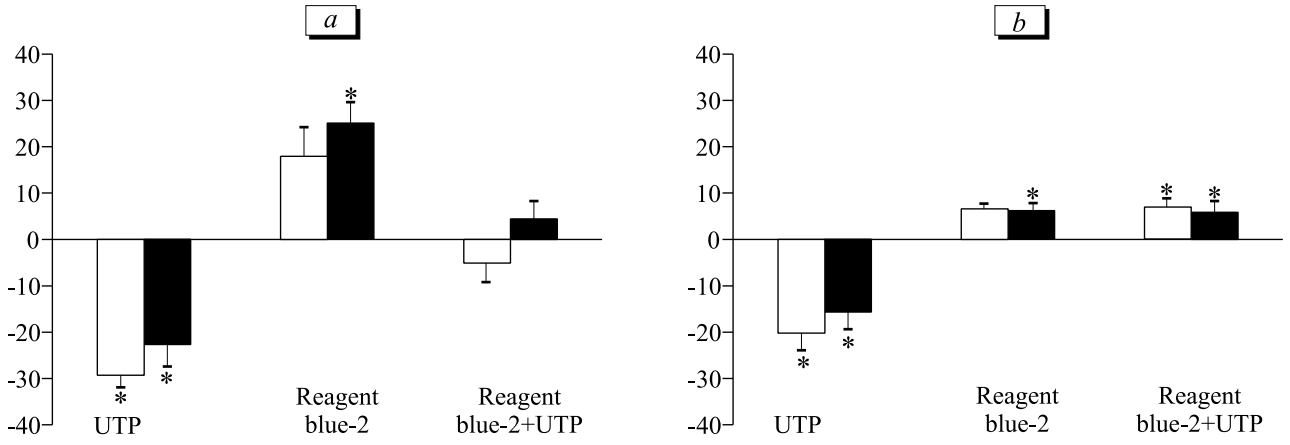

Fig. 2. Effect of UTP on the strength of myocardial contraction in the atria and ventricles from rats aging 21 (*a*) and 100 days (*b*) under control conditions and after blockade with reagent blue-2.

Our results indicate that the inhibitory effect of UTP in rats aging 7, 21, and 100 days (early ontogeny) is realized via $P2Y_4$ receptors. Previous studies showed that UTP produced the opposite effects on cardiac activity. For example, UTP in concentrations of 10^{-6}-10^{-3} M reduced the strength of myocardial contraction in the right atrial auricle by 7-10% [2]. Other authors reported that UTP increased MCA in rats [5,9,10]. The dual effect of UTP on MCA of rat atria and ventricles was described elsewhere [5]. The majority of P2Y receptors ($P2Y_{1,2,4,6,11}$) are coupled to $G_{q/11}$ protein, which activates phospholipase C with the formation of inositol triphosphate, elevation of intracellular Ca^{2+} concentration, and increase in MCA [9]. A negative inotropic effect can be also observed during activation of $P2Y_2$ and $P2Y_4$ receptors that are activated by ATP and UTP and coupled to $G_{i/o}$ protein. UTP inhibits adenylate cyclase due to activation of $G_{i/o}$ protein, which results in the reduction of cAMP synthesis and decrease in the Ca^{2+} influx into the cell. These changes are followed by the decrease in MCA [9].

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