Role of P2X Receptors in Positive Inotropic Effect of Rat Myocardium during Ontogeny

A. A. Zverev, T. A. Anikina, and F. G. Sitdicov

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Experiments with selective agonists and antagonists of purinoceptors allowed us to evaluate the subtype of P2X receptors. We showed that the myocardium of 14-100-day-old rats contains functionally active $P2X_1$ receptors. These receptors are involved in the realization of the positive inotropic effect of the atria and ventricles. Selective $P2X_1$ receptor agonist β,γ-methylene-ATP induced a dose-dependent increase in the strength of atrial and ventricular contractions. $P2X_1$ receptor antagonist TNP-ATP abolished the effect of the agonist in rats of all age groups.

Key Words: *P2X receptors; ontogeny; myocardial contractility*

There are 7 subtypes of P2X receptors. $P2X_1$-$P2X_5$ receptors were identified in rat heart. These receptors have different localization in the myocardium. Immunohistochemical study of the heart from adult rats revealed $P2X_2$ and $P2X_5$ receptors on cardiomyocyte sarcolemma [6]. $P2X_1$ and $P2X_3$ receptors were shown to be localized near synaptic contacts between neurons and cardiomyocytes. $P2X_1$, $P2X_2$, $P2X_{2/3}$, and $P2X_4$ receptors are located in rat atrium. The ventricles mainly contain $P2X_4$ receptors. Little is known about localization of these receptors in the heart during ontogeny. For example, $P2X_3$ receptors were revealed in human embryonic heart; a variety of subtypes of P2X purinoceptors were identified [10]. Previous studies revealed variations in the expression of P2Y receptors during ontogeny [7].

Extracellular ATP receptors and muscarinic receptors are the first functionally active membrane receptors identified during embryogeny. However, ontogeny of these receptors is poorly understood. Purinergic regulation of the heart was studied only in adults and during neonatal development [4].

Department of Anatomy, Physiology, and Human Health Protection, Tatar State Humanitarian Pedagogical University, Kazan. ***Address for correspondence:*** alekcei5@rambler.ru. A. A. Zverev

Our previous *in vivo* and *in vitro* experiments showed that cardiac P2X receptors are involved in positive chronotropic and inotropic responses during the early postnatal ontogeny [1,2].

Here we evaluated the subtype of P2X receptors that are involved in the myocardial inotropic response. Age-related differences in functional activity of receptors were studied.

MATERIALS AND METHODS

Myocardial contractility was studied *in vitro* on myocardial strips from albino rats. Contractile function of the myocardium was evaluated after treatment with β,γ-methylene-ATP (β,γ-m-ATP) in 3 increasing concentrations. The study was performed on a PowerLab device (ADInstruments) equipped with a MLT 050/D force transducer (ADInstruments).

The animals were narcotized with urethane. The hearts were removed and placed in a Petri dish with working solution under oxygenation and ESL-2 stimulation. Myocardial strips were prepared. The preparation was immersed in a 10-ml reservoir. The working solution contained 119.8 μmol/liter NaCl, 5.4 μmol/liter KCl, 1.8 μmol/liter $CaCl_2$, 1.05 μmol/liter $MgCl_2$, 0.42 μmol/liter NaH_2PO_4, and 5.05 μmol/liter glucose (95% O_2 and 5% CO_2). pH was main-

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tained at 7.3-7.4 by adding Trizma base buffer and acid buffer (Sigma) to the solution. The strips were stimulated via platinum electrodes (6 and 10 pulses for 14-, 56-, and 100-day-old animals, pulse duration 5 msec).

The results were recorded on a personal computer with Chart 5.0 software. Immersion of the preparations in a reservoir was followed by the "running-in period" of 40-60 min. Muscle strips gained an optimal tension over this period. Basal contractility was studied over 10 min after the "running-in period". Muscle contractility was studied over 20 min after addition of β,γ-m-ATP to the working solution. After β,γ-m-ATP stimulation, the test preparations were washed 3 times with the working solution for 10 min. Basal contractility was estimated for each dose of β,γ-m-ATP. The strength and time of β,γ-m-ATP-induced contractions were expressed in percent of the basal level. Blockade of $P2X_1$ and $P2X_2$ receptors was induced by TNP-ATP. The significance of differences was evaluated by Student's *t* test ($p<0.05$). Experiments were performed with Sigma reagents.

RESULTS

β,γ-m-ATP is a synthetic analogue of ATP. This compound acts as a selective P2X1 receptor agonist [3]. $P2X_1$, $P2X_3$, and $P2X_{2/3}$ receptors are sensitive to β,γ-m-ATP. This unstable compound degrades in many tissues over 1 min with the formation of an adenosine analogue [8].

Myocardial contractility was studied after treatment with selective agonist of $P2X_1$ and $P2X_3$ receptors β,γ-m-ATP in concentrations of 10^{-15}-10^{-6} M. β,γ-m-ATP in concentrations of 10^{-13}-10^{-9} M caused a dose-dependent contraction of atrial and ventricular strips from rat myocardium during ontogeny. This selective agonist in 2 concentrations produced a positive inotropic effect on the ventricles in rats of all age groups. β,γ-m-ATP in concentrations of 10^{-12}-10^{-13} M induced contraction in rat pups aging 14 and 21 days. The strength of ventricular contractions increased most significantly after addition of β,γ-m-ATP in a concentration of 10^{-12} M (31.2±4.7 and 24.5±3.3% in 14- and 21-day-old animals, respectively; $n=17$, $p<0.01$). β,γ-m-ATP in a concentration of 10^{-13} M increased myocardial contractility by 16.1±8.1 ($n=10$) and 14.9±4.1% ($n=9$, $p<0.05$), respectively. Increasing the concentration of β,γ-m-ATP to 10^{-11} M was accompanied by a significant decrease in ventricular contraction in 21-day-old rats (by 10.8± 0.5%; $n=9$, $p<0.05$), but had no effect on preparations from 14-day-old animals ($n=9$).

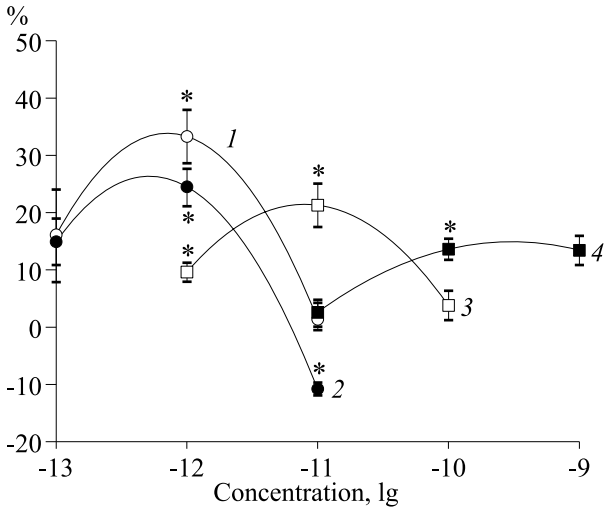

Fig. 1. Effect of β,γ-m-ATP on contractility of isolated strips from heart ventricles of growing rats: 14-day-old animals (*1*), 21-day-old animals (*2*), 56-day-old animals (*3*), and 100-day-old animals (*4*).

β,γ-m-ATP in concentrations of 10^{-12} and 10^{-11} M was effective in 56-day-old animals. Ventricular contractility increased most significantly after treatment with β,γ-m-ATP in a concentration of 10^{-11} M (21.4±3.8%; $n=9$, $p<0.05$). The agonist in a concentration of 10^{-12} M increased ventricular contractility by 9.6±1.5% ($n=9$, $p<0.05$). β,γ-m-ATP in a concentration of 10^{-10} M had the strongest effect on 100-day-old rats. Myocardial contractility in these animals increased by 13.6±1.8% ($n=9$, $p<0.05$, Fig. 1).

The atrial response to β,γ-m-ATP in these concentrations differed from the ventricular response. Addition of β,γ-m-ATP in a concentration of 10^{-12} M increased the strength of atrial contractions in animals aging 14 and 21 days (by 12.1±1.7 and 4.4±0.9%, respectively; $n=20$, $p<0.05$). The con-

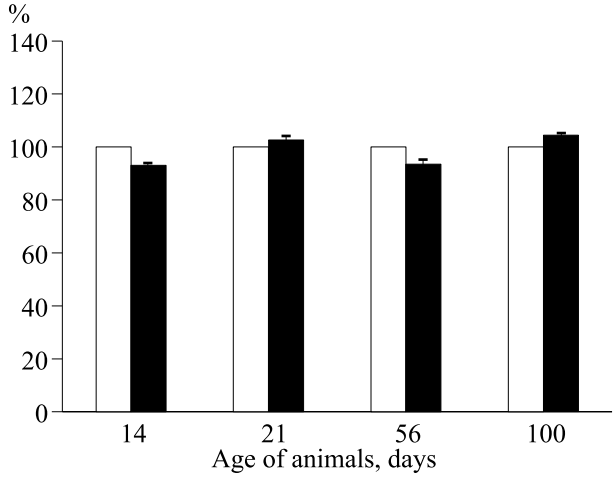

Fig. 2. Effect of β,γ-m-ATP after blockade of $P2X_1$, $P2X_2$, and $P2X_{2/3}$ receptors with TNP-ATP. Light bars, initial values; dark bars, after blockade.

centrations of β,γ-m-ATP inducing the maximum contractile response of atrial myocardium in 56- (10^{-11} M, 7.7±1.5%; n=10, p<0.05) and 100-day-old animals (10^{-10} M, 5.3±0.8%; n=10, p<0.05) were lower by one and two orders of magnitude, respectively. Increasing the concentration of β,γ-m-ATP was followed by reduction of atrial contractility in rats of all age groups. The agonist in a concentration of 10^{-12} M decreased myocardial contractility in 14- and 21-day-old animals by 8.5± 2.7 and 4.6±1.0%, respectively (n=20, p<0.05). Atrial contractility in 56-day-old rats decreased by 7.7±0.9% after treatment with β,γ-m-ATP in a concentration of 10^{-10} M. Addition of β,γ-m-ATP in a concentration of 10^{-9} M was followed by a decrease in atrial contractility of 100-day-old rats by 10.2±1.7% (n=16, p<0.05). β,γ-m-ATP in high concentrations produced a negative effect on atrial contractility due to degradation into adenosine, whose effect is realized via P1 receptors.

Selective antagonist TNP-ATP (2',3'-*o*-(2,4,6-trinitrophenyl)adenosine-5'-triphosphate) was used to evaluate the subtype of P2X receptors. The study involved β,γ-m-ATP in concentrations, which were most effective for animals aging 14 (10^{-12} M), 21 (10^{-12} M), 56 (10^{-11} M), and 100 days (10^{-10} M). TNP-ATP is a potent antagonist of $P2X_1$, $P2X_{2/3}$, and $P2X_3$ purinoceptors in the heart [10]. This compound in low nanomolar concentration is used as a noncompetitive antagonist to characterize native $P2X_1$, $P2X_{2/3}$, and $P2X_3$ purinoceptors [5].

Selective P2X1 receptor antagonist TNP-ATP completely abolished the effect of β,γ-m-ATP on contractility of the atrial and ventricular myocardium in rats of all age groups. Addition of the agonist after TNP-ATP-induced blockade had little effect on contractility of myocardial strips (Fig. 2).

Our results indicate that the positive inotropic effect of β,γ-m-ATP is realized via $P2X_1$ and $P2X_{2/3}$ receptors. The dose-dependent effect of this compound provides support to previous data on high sensitivity of cardiac P2X receptors during early postnatal ontogeny.

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