Role of Purinoceptors in Cardiac Function in Rats during Ontogeny

T. A. Anikina, F. G. Sitdikov, E. Yu. Khamzina, and G. A. Bilalova

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Intravenous injection of exogenous ATP (10^{-4} M) to rats aging 21, 56, and 100 days increased the heart rate by the 15th sec postinjection. Stable ATP analogue α,β-methylene-ATP in an equimolar concentration increased the heart rate in rats aging 56 and 100 days (by the 15th second after treatment), but had no effect on 21-day-old animals. α,β-Methylene-ATP in a concentration of 10^{-7} M increased the heart rate in 21-day-old rat pups, which attests to high sensitivity of P2 purinoceptors. Administration of ATP and α,β-methylene-ATP after treatment with nonselective purinoceptor antagonist suramin did not increase the heart rate in rats of different age groups. Infusion of ATP and its stable analogue after administration of selective P2Y receptor antagonist reactive blue 2 increased the heart rate in rats of different age groups. These changes reflect activation of P2X receptors in the heart.

Key Words: *heart; heart rate; ATP; purinoceptors; rat*

ATP is an intracellular energy substrate. In addition to other nucleotides, ATP can regulate various intracellular processes by modulating activity of specific P2 purinoceptors. There is strong evidence that ATP produces chronotropic, inotropic, and arrhythmogenic effects on the heart. The action of ATP is realized via the direct effect on cardiomyocytes and modulation of the regulatory mechanisms in the heart [3,6]. An immunohistochemical study of rat heart showed that cardiomyocyte sarcolemma contains a considerable number of inotropic P2X2 and P2X5 receptors. P2X1 and P2X3 receptors are located near the synaptic contact between neurons and cardiomyocytes [3]. Purines modulate activity of the cardiovascular system. ATP in low doses causes short-term tachycardia. However, ATP in high doses suppresses cardiac function and causes atrioventricular blockade [8]. Previous experiments showed that ATP has a direct effect on the heart (independently on its conversion into adenosine). *In vivo* studies of the effect of ATP on cardiac function produced contradictory results. The purinergic regulation of cardiac function was studied only during the neonatal development. Previous experiments showed that intravenous infusion of ATP produces a positive chronotropic effect on the heart, but does not change stroke volume (SV). This effect is associated with activation of P2 purinoceptors, but not with the influence of products formed during ATP hydrolysis, because P1 receptor agonist adenosine was ineffective [1].

Here we studied age-related differences in the sensitivity of purinoceptors in rat heart to exogenous ATP and its stable analogue α,β-methylene-ATP.

MATERIALS AND METHODS

Experiments were performed on outbred albino rats aging 21, 56, and 100 days (late milk-feeding, pubertal, and maturity periods, respectively). The animals were divided into 4 groups: group 1, ATP;

Department of Human Anatomy, Physiology, and Health Protection, State Training University, Kazan. ***Address for correspondence:*** fgsitdikov@mail.ru. F. G. Sitdikov

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group 2, α,β-methylene-ATP; group 3, ATP and α,β-methylene-ATP after pretreatment with suramin; and group 4, ATP and α,β-methylene-ATP after pretreatment with reactive blue 2. The animals were narcotized with urethane (1.2 mg/kg) and fixed to a surgical table. The right femoral artery was catheterized under a MBS-2 binocular microscope. Differential rheogram and ECG were recorded. The data were recorded on a complex electrophysiology device and analyzed by means of Digger software. This procedure allowed us to obtain the results of ECG recording and variation pulsometry. ATP and α,β-methylene-ATP (10^{-4} M, 0.02 mg/kg, Sigma), suramin (20 mg/kg, Sigma; 15-30 sec treatment), and reactive blue 2 (5 mg/kg) were infused into the femoral vein [5]. The test parameters were recorded over 10-15 min after infusion.

The results were analyzed by pairwise Wilcoxon test (SigmaStat software).

RESULTS

The heart rate (HR) in rats aging 21 (n=8, p<0.01), 56 (n=9, p<0.01), and 100 days (n=9, p<0.01) increased 15 sec after intravenous infusion of exogenous ATP, but decreased by the 30th second after treatment (Fig. 1). HR in rats aging 21, 56, and 100 days returned to normal by the 10th, 5th, and 1st minute, respectively, and remained unchanged over 10 min. SV did not change in rats of different age groups. The mode amplitude (AMo) and variation range (ΔX) reflect activity of the sympathetic and parasympathetic regulatory mechanisms for cardiac function, respectively. The test parameters in rats aging 21, 56, and 100 days remained unchanged over 10 min.

Repeated treatment with ATP increased HR in rats of different age groups, which illustrates reproducibility of the observed effect.

The unstable compound ATP is rapidly hydrolyzed into adenosine with ecto-ATPase. The effect of adenosine is realized via P1 receptors. We used stable ATP analogue α,β-methylene-ATP, which serves as a P2X receptor agonist. P2X and P2Y receptors differ in the sensitivity to several analogues of ATP. Affinity of P2X receptors for agonists increases in the following order: ATP<β,γ-methylene-ATP<α,β-methylene-ATP [2]. HR in rats aging 56 (n=7, p<0.01) and 100 days (n=6, p<0.01) increased 15 sec after administration of α,β-methylene-ATP in an equimolar concentration (Fig. 1). HR in 56- and 100-day-old animals returned to normal by the 15th and 2nd minute, respectively, and remained practically unchanged over 15 min. The time to recovery of HR after treatment with exogenous ATP and α,β-methylene-ATP decreased with an increase in the age of animals, which reflects maturity of the parasympathetic regulatory mechanism.

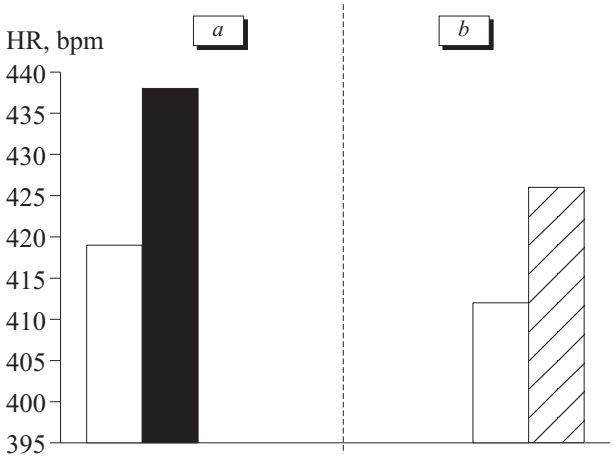

Fig. 1. Heart rate in 56-day-old rats after administration of ATP (*a*) and α,β-methylene-ATP (*b*). Light bars, baseline level; dark bars, ATP administration; shaded bars, α,β-methylene-ATP administration.

These changes were not observed in 21-day-old rat pups. Administration of exogenous α,β-methylene-ATP in concentrations of 10^{-5} and 10^{-6} M had no effect on HR. HR increased 15 sec after infusion of α,β-methylene-ATP in a concentration of 10^{-7} M (0.00002 mg/kg, n=8, p<0.05), but decreased by the 1st minute after treatment. α,β-Methylene-ATP in a concentration of 10^{-7} M had little effect on SV, ΔX, and AMo. Similarly to ATP, exogenous α,β-methylene-ATP produced a short-term effect on rats of different age groups. These changes were probably associated with rapid desensitization of P2X receptors. The time to recovery of HR after infusion of α,β-methylene-ATP exceeded that observed in experiments with ATP.

Experiments with nonselective P2 purinoceptor antagonist suramin were performed to establish the effect of ATP on P2 purinoceptors [4,6,7]. Cardiac function remained unchanged over 60 min after administration of suramin. Infusion of ATP and α,β-methylene-ATP after pretreatment with suramin had no effect on HR, SV, AMo, and ΔX in rats of different age groups.

Selective P2Y receptor antagonist reactive blue 2 was used to evaluate the subtype of purinoceptors mediating the positive chronotropic effect of exogenous ATP [5]. Administration of ATP and α,β-methylene-ATP after pretreatment with reactive blue 2 increased HR in rats aging 21, 56, and 100 days (by the 15th second, n=6, p<0.001). The changes in HR produced by infusion of α,β-methylene-ATP were most significant in 56-day-old animals.

Our results show that ATP in a concentration of 10^{-4} M increases HR in rats of different age groups. α,β-Methylene-ATP in an equimolar concentration increases HR in 56- and 100-day-old animals. Studying the dose-effect relationship showed that exogenous α,β-methylene-ATP in a concentration of 10^{-7} M increases HR in 21-day-old rat pups, which attests to high sensitivity of P2X receptors during early postnatal ontogeny. The increase in HR is not accompanied by changes in SV. Infusion of ATP and its stable analogue α,β-methylene-ATP after pretreatment with selective P2Y receptor antagonist reactive blue 2 increases HR in rats of different age groups, which reflects activation of P2X receptors in the heart.

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