Effect of Exogenous ATP on Cardiac Activity in Rats

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In vivo effects of exogenous ATP on cardiac activity were studied on adult rats. Intravenous administration of ATP produced a positive chronotropic effect, but did not affect the stroke volume. This was due to activation of type II purine receptors, rather than due to the influence of ATP hydrolysis products, since P1 receptor agonist adenosine was ineffective. The blockade of β-adrenoceptors and muscarinic receptors did not modify the positive chronotropic effect of ATP, which indicated that this action was not realized via sympathetic or parasympathetic nerves. ATP applied against the background of atropine blocade produced a 4-fold increase in the variability of heart rate typical of activation of the parasympathetic myocardial regulation.

Key Words: *heart; heart rate; blood stroke volume; ATP*

Recent studies indicate that ATP plays a role of an excitatory neurotransmitter and neuromodulator in the central and peripheral nervous systems [2,6,12]. Purine compounds control physiological functions of many organs and systems via specific P1 and P2 receptors [12]. Extracellular ATP appears in myocardial ischemia and shock or can be induced by the Ca^{2+}-dependent ATP release (as a cotransmitter) from sympathetic and parasympathetic nerve endings [12].

It was suggested that ATP modulates cholinergic transmission in the heart [5]. Experiments on isolated rabbit heart showed that ATP accelerates heart rate (HR) [15]. However, the physiological role of ATP in the body can differ from that in isolated organ, since the heart is under the complex control of sympathetic and parasympathetic nerves [1,4] and ATP receptors were found not only on cardiomyocytes [8], but also on autonomic nerves [11]. Here we studied *in vivo* effects of ATP and its derivative adenosine on chronotropic and inotropic functions of the heart.

MATERIALS AND METHODS

Experiments were performed on 70 outbred albino rats aging 10-12 weeks. The animals were anesthetized with 25% urethane (1.2 g/kg intraperitoneally). ATP sodium salt (standard final volume 0.075 ml), atropine (0.6 mg/kg), and obsidan (0.8 mg/kg) were injected into the femoral vein. Cardiac activity was recorded on a Conan-2.0c electrophysiological device coupled with a computer. Mathematical analysis of variational pulsogram was performed by the method described elsewhere [1]. Stroke volume (SV) was calculated by the formula [10] with modification [3]. The results were analyzed by Student's t test.

RESULTS

In series I, the effects of intravenous injection of ATP on HR were studied. ATP in a concentration of 10^{-6} M was ineffective, while in a concentration of 10^{-5} M it caused small, but statistically significant increase in HR from 377 to 388 bpm, which lasted for 1 min (n=6, p<0.05). By the end of the 1st minute, HR returned to the control level and remained unchanged over 10 min of observations (Fig. 1). Increasing of ATP concentration to 10^{-4} M enhanced its chronotropic effect (n=6, p< 0.001, Fig. 1). Further increase in the concentration of this purine nucleotide to 10^{-3} M abolished its positive chronotropic effect (n=6, p>0.05). Repeated administration of ATP in concentrations of 10^{-5}-10^{-4} M produced the same positive chronotropic effect. Equivalent volume of physiological saline had no effects on cardiac activity.

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These changes in HR can be related not only to direct effects of ATP on the heart, but also to the influence of adenosine formed during ATP hydrolysis and acting via specific P1 receptors. However, the injection of adenosine in a concentration of 10^{-4} M did not change the major indexes of cardiac function (n=11, p>0.05, Fig. 2, *a*). Thus, the increase in HR was due to direct effects of exogenous ATP, rather than due to the action of products of its hydrolysis.

In series II, the effects of ATP on the function of cardiac muscle were studied in detail. SV and various parameters of variational pulsogram (variational range and mode amplitude) were recorded after administration of 10^{-4} M ATP. SV remained unchanged over 10 min of observations (n=12, p>0.05, Fig. 2, *b*). In the control, variational range and mode amplitude reflecting activity of the parasympathetic and sympathetic

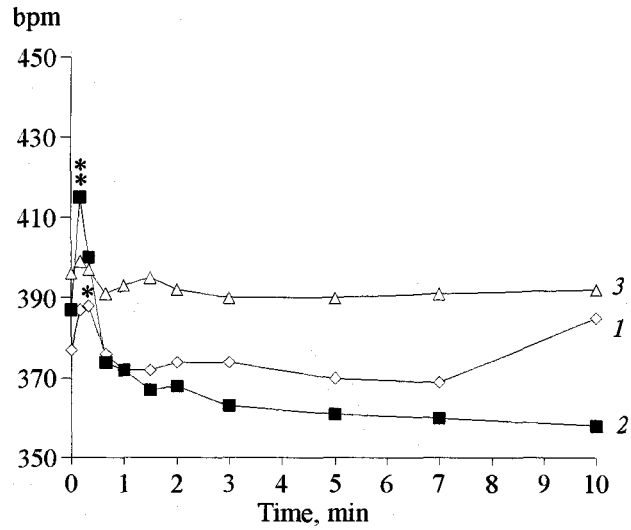

Fig. 1. HR variability in rats intravenously injected with atropine in concentrations of 10^{-5} (*1*), 10^{-4} (*2*), and 10^{-3} M (*3*).

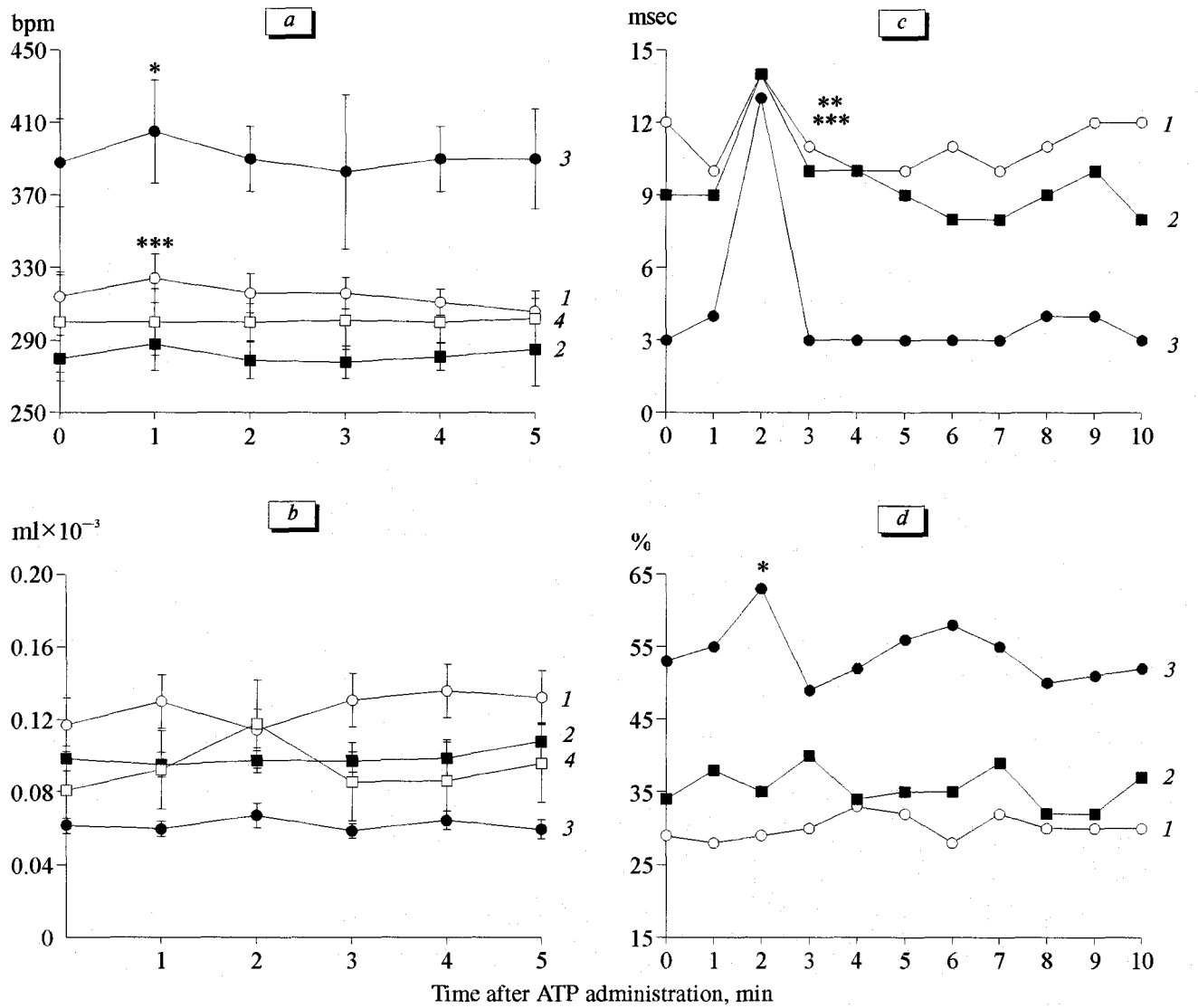

Fig. 2. Dynamics of HR (*a*), stroke volume (*b*), variational range (*c*), and mode amplitude (*d*) after intravenous injection of 10^{-4} M ATP. ATP (*1*), ATP+obsidan (*2*), ATP+atropine (*3*), 10^{-4} M adenosine (*4*). *p<0.0001, **p<0.001, and ***p<0.01 compared to the control.

regulatory mechanisms [1], respectively, practically did not change (Figs. 2, *c*, *d*).

The positive chronotropic action of ATP can be due to its direct effects on the heart or due to activation of sympathetic nerves or suppression of parasympathetic mechanisms regulating cardiac activity. ATP injected in a concentration of 10^{-4} M against the background of cardiac β-adrenoceptor blockade with obsidan elevated HR (as it did in the control series, n=11, p<0.001, 20 sec postinjection). Under the effect of 10^{-4} M ATP applied against the background of muscarinic receptor blockade with atropine, HR also increased by 17.00±0.58 bpm (n=13, p<0.0001) compared to the control (Fig. 2, *a*).

The analysis of variational pulsogram recorded after receptor blockade with obsidan and atropine produced surprising results. In the control, ATP had no effect on variational range (12% by the 20th second, p<0.05), but after the blockade of β-adrenoceptors ATP increased this parameter by 58% 20 sec postinjection (n=11, p<0.01) and against the background of muscarinic receptor blockade by 4 times (n=13, p<0.0001, Fig. 2, *c*).

Positive chronotropic action of exogenous ATP revealed in our studies is consistent with the results of previous experiments on isolated heart [15] and can result from the effects of ATP or products of its hydrolysis (e.g., P1 receptor agonist adenosine) on the heart. However, in our experiments adenosine injected *in vivo* in equimolar concentrations produced no effect typical of ATP. Furthermore, experiments on isolated heart showed that adenosine inhibits, but not stimulates cardiac activity [13].

Positive chronotropic action of ATP can be not only due to its direct effect on the myocardium, but also due to the modulation of activity of sympathetic and parasympathetic nerves. There are data that P2 receptors sensitive to ATP are localized on the membrane of vagus nerve fibers [11] and sympathetic nerve endings [8]. The retainment of positive chronotropic action of ATP after the blockade of β-adrenoceptors and muscarinic cholinergic receptors indicates its direct effect on the myocardium. There are 2 types of ATP P2 receptors: P2X (ionotropic) and P2Y (metabotropic) receptors [12].

P2X receptors are involved in the realization of activating effects of ATP. Recent immunohistochemical assay showed that $P2X_2$ and $P2X_5$ subtypes of ionotropic ATP receptors are present on cardiomyocyte sarcolemma of rat heart, while $P2X_1$ and $P2X_3$ receptors are localized near synaptic contacts between neurons and cardiomyocytes [8]. These subtypes of P2X receptors activate the nonselective ionic channel permeable for Na^+ and Ca^{2+} [7,9,12], thus enhancing cardiomyocyte excitability. Experiments on isolated heart showed that ATP can stimulate L-type Ca^{2+} channels in the myocardium [14]. However, in our experiments the increase in HR was not accompanied by changes in SV. Hence, ATP acts on pacemaker cells, rather than on cardiomyocytes.

Unusual dose-effect dependence and ineffectiveness of high ATP concentrations *in vivo*, indicates rapid desensitization of cardiac P2 receptors [7].

Paradoxical effects of ATP on HR variability confirm direct regulatory action of this nucleotide on the myocardium. Thus, purine compounds act as sympathetic and parasympathetic regulators of cardiac activity.

Extracellular ATP appeared in the cardiac tissue can produce the above mentioned effects mediated by the interaction between ATP and P2 receptors on cardiomyocyte membranes.

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