PHARMACOLOGY AND TOXICOLOGY

ATP as Modulator of Carbacholine Effect on Contractility of Rat Myocardium in Postnatal Ontogeny

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We studied combined effect of 2-m-ATP, P_2 receptor agonist, and carbacholine, muscarinic M_2 cholinoreceptor agonist, on contractility of rat myocardium during the postnatal ontogeny. Activation of P_2 receptors can stimulate or attenuate the effects of carbacholine depending on animal age. 2-m-ATP potentiates the inhibitory effect of carbacholine on myocardial contractility in 14- and 100-day-old rats. In 21-day-old rats, activation of P_2 receptors prevented the negative effect of carbacholine on myocardial contractility. Activation of muscarinic M_2 receptors inhibited the inotropic effect of purine in all age groups.

Key Words: *P_2 receptors; muscarinic cholinoceptors; myocardium; contractility; ontogeny*

ATP is present in vesicles together with acetylcholine or norepinephrine and is involved in transmission of nerve pulses, being released from nerve terminals together with the main transmitters; in other words, it is a co-transmitter. Previous studies confirmed co-secretion of norepinephrine, acetylcholine, and ATP from sympathetic and parasympathetic nerves and the capacity of ATP to modulate nerve transmission in the heart [4,8] by stimulating or attenuating the effects of classical transmitters.

By their functions, purine receptors can be attributed to modulator receptors, determining purine effects on the functions of other receptors. It can be hypothesized that the direct and modulator effects of ATP are determined by maturation of the central, efferent sympathetic and parasympathetic regulatory effects on the heart, its receptor system, in other words, depend on the stages of biological maturation of the organism.

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According to Dr. Burnstock's hypothesis [6], ATP functioned as the transmitter at the very first stages of evolution, long before the appearance of the main transmitters. ATP is assumed to be one of the most phylogenetically ancient neurotransmitters. This hypothesis is supported by the fact that the extracellular effect of ATP is detected in primitive organisms, including bacteria and algae [6]. Purine receptors are among the first in the ontogeny. Along with muscarinic cholinoreceptors, extracellular ATP receptors are the first functionally active membrane receptors detected during the period of embryo formation [13].

The modulating effect of ATP on cardiac work regulation attracts now special attention. Patch-clamp studies on the guinea pig atrial myocytes showed that P_2 receptor agonists reversibly reduced the acetylcholine-dependent current (K^+ current) by inhibiting the acetylcholine-dependent K^+ channels via PTX-sensitive G protein [12]. Studies of the effects of extracellular ATP on muscarinic cholinoreceptors in atrial cells showed that stimulation of P_2 receptors induced biphasic changes (an increase followed by a decrease)

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in acetylcholine-dependent K^+ current. In other words, ATP potentiated and then abolished shortening of the action potential caused by carbacholine (CC), which confirmed ATP modulation of the chronotropic and inotropic effects of parasympathetic nerves [10].

MATERIALS AND METHODS

Experiments were carried out on albino rats aged 14, 21, 56, and 100 days. The amplitude of isometric contractions of myocardial strips was recorded on a PowerLab device with Chart 5.0 software. The preparations were fixed vertically with one end attached to MLT 050/D force pickup, the other to the support. Each preparation was plunged in an individual reservoir (10 ml) filled with Krebs working solution of the following composition (mM): 133 NaCl, 4.7 KCl, 0.6 $MgCl_2$, 1.35 NaH_2PO_4, 2.5 $CaCl_2$, and 7.8 glucose at 28°C and carbogen (95% O_2 and 5% CO_2). In order to maintain pH at the level of 7.35-7.40, the basal and acid Trizma buffers (Sigma) were added. The preparations were stimulated through platinum electrodes.

2-m-ATP (P2X and $P2Y_1$ cardiac receptors agonist) served as the P_2 receptor agonist. We previously detected a dose-dependent positive inotropic effect of 2-m-ATP on myocardial contractility [1]. Carbacholine, a stable analog of acetylcholine, served as muscarinic receptor agonist. The amplitude of contractions of atrial and ventricular myocardial strips was recorded and the force of contraction was evaluated in percent of the initial contraction taken as 100%.

The modulating effect of 2-m-ATP on CC effect was evaluated by recording the effect of CC over 20 min, after which the strips were washed, basal myocardial contractility was recorded over 10 min, after which CC was added and 2-m-ATP was added for 10 min. A series of experiments was carried out with an opposite order of the substance addition (CC was added in the presence of 2-m-ATP).

The significance of differences was evaluated by paired and unpaired Student's *t* tests ($p<0.05$).

RESULTS

The effect of CC (stable agonist of mainly muscarinic M_2 cholinoreceptors) on myocardial contractility was studied for concentrations of 10^{-7}-10^{-4} M. Carbacholine in a concentration of 10^{-5} M caused a negative inotropic effect on the myocardium of rats aged 14, 21, 56, and 100 days. Activation of M_2 receptors coupled with PTX-sensitive G proteins (G_i/G_o) leads to a decrease in cAMP content in the cell, which results in reduction of Ca^{2+} current intensity and attenuation of contraction force. This effect is also due to stimulation of K^+ currents at the expense of direct relationship between the G protein β,γ-subunit with the channel [14]. According to our data, the response of atrial and ventricular myocardial contractility to CC varies during the postnatal ontogeny. Atrial contractility of 14-day-old rats decreased by 15.4±4.3% in comparison with the basal level. At the age of 21 days, atrial contractility decreased by 39.1±5.2% in response to CC addition, while in animals aged 56 and 100 days it decreased by 30.5±5.8 and 25.0±5.7%, respectively (Fig. 1). CC reduced the contractility of ventricular myocardium by 19.4±4.3% in rats aged 14 days, by 37.0±4.2% in those aged 21 days, and by 36% in comparison with the initial level in animals aged 56 and 100 days. In our experiments on isolated atrial and ventricular strips CC exhibited different efficiency, depending on animal age, which suggests changes in functional activity of M_2 receptors in the ontogeny. For example, the reaction of M_2 cholinoreceptors determining atrial and ventricular contractility under the effect of CC decreased from the age of 14 to 100 days. The least reduction was recorded in animals aged 14 days ($p<0.05$) with still incomplete sympathetic innervation of the heart, while the maximum decrease was recorded in rats aged 21 days ($p<0.05$) in comparison with the age of 100 days. Many scientists think that the parasympathetic effects are poorly pronounced in newborn animals. A significant increase of cholinergic effects on the cardiac chronotropic function is observed in rats from week 3 to week 10 of the postnatal ontogeny [3]. It is noteworthy that the sympathetic regulation of the heart is formed during weeks 3-6, and the first and maximum elevation of heart rate was recorded in rats of this age (weeks 3-4) [2]. Experimental study of age-associated changes in M_2 cholinoreceptors in humans and animals showed a reduction of the density of M_2 cholinoreceptors [5,7,11], paral-

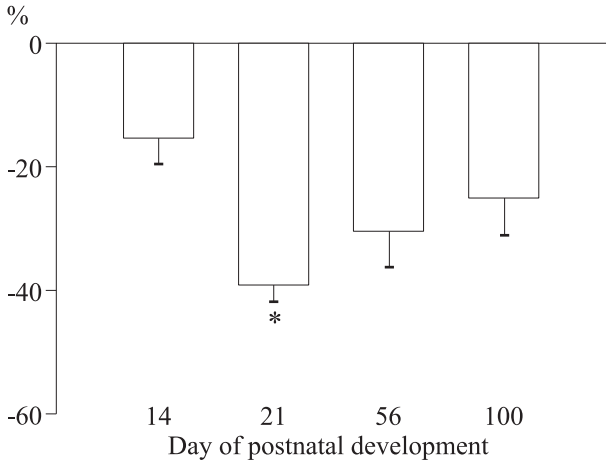

Fig. 1. The effect of CC on rat atrial myocardial contractility in the postnatal ontogeny. Ordinate: force of myocardial contractions in response to CC, %. *$p<0.05$ compared to rats aged 14 and 100 days.

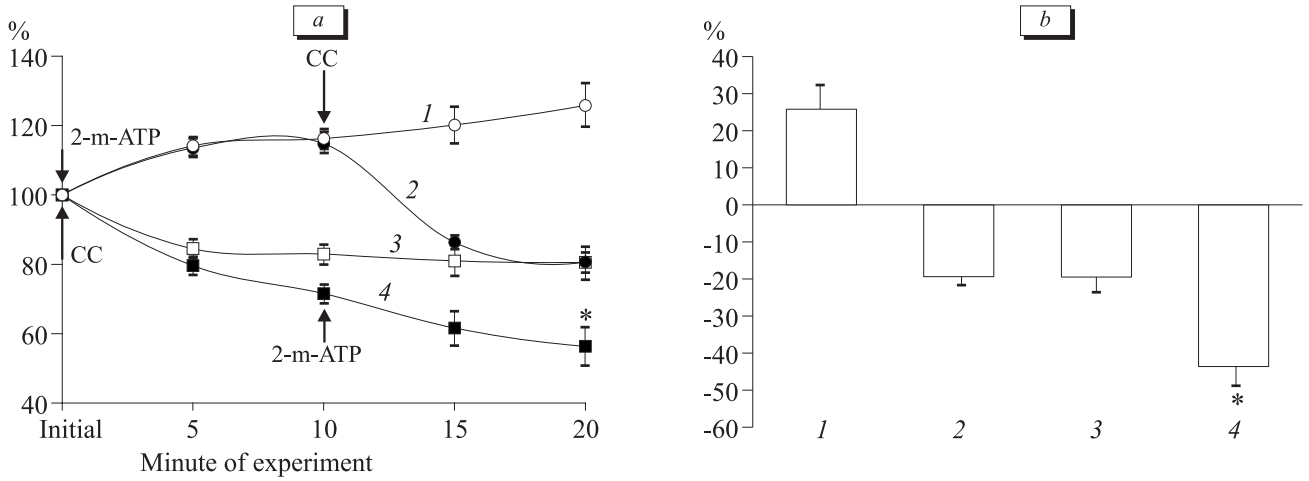

Fig. 2. Combined effects of 2-m-ATP and CC (*a*) and the resultant effect of 2-m-ATP and CC modulation (*b*) of ventricular myocardial contractility of 14-day-old rats. Here and in Fig. 3: *1*) 2-m-ATP (control); *2*) CC in the presence of 2-m-ATP; *3*) CC (control); *4*) 2-m-ATP in the presence of CC. **p*<0.05 compared to *3*.

leled by a reduction of CC capacity to adenylate cyclase inhibition [9]. Our data on changes in myocardial contractility in response to exogenous CC confirm that the number and functional activity of M_2 cholinoreceptors decrease with age.

In order to detect the capacity of cardiomyocyte cholinergic and purinergic receptors to interactions, we studied the effects of 2-m-ATP (stable agonist of P2X and P2Y1 receptors) under conditions of developing inhibitory inotropic effect of CC (M_2 cholinoreceptor agonist). Another series of experiments was carried out with an opposite order of the substances addition: CC in the presence of developing positive inotropic effect of P_2 receptor agonist.

In control 14-day-old rats, CC reduced atrial contractility by 15.4% and ventricular contractility by 19.4%. Addition of 2-m-ATP potentiated the inhibitory effect of CC on atrial and ventricular contractility by 31.5±5.2 and 43.6±5.3%, respectively (Fig. 2, *a*), which was significantly higher than its effect in the control (p<0.05). This effect was significantly superior to the inhibitory effect of CC alone. Addition of a stable ATP analog potentiated the CC effect by about 54%. In the control, 2-m-ATP in a concentration of 10^{-8} M increased the rate of atrial contractions by 15.3±2.0%, of ventricular ones by 25.8±6.2%. It is noteworthy that under conditions of opposite order of substance addition (CC addition in the presence of 2-m-ATP) 2-m-ATP failed to stimulate myocardial contractility in all age groups. The negative inotropic effect of CC developed in the presence of 2-m-ATP: 20.9±1.9% in the atria and 19.3±2.3% in the ventricles of 14-day-old rats, thus reaching the control values for CC (Fig. 2, *b*).

In control animals aged 21 days, CC reduced atrial contractility by 39.1%, ventricular one by

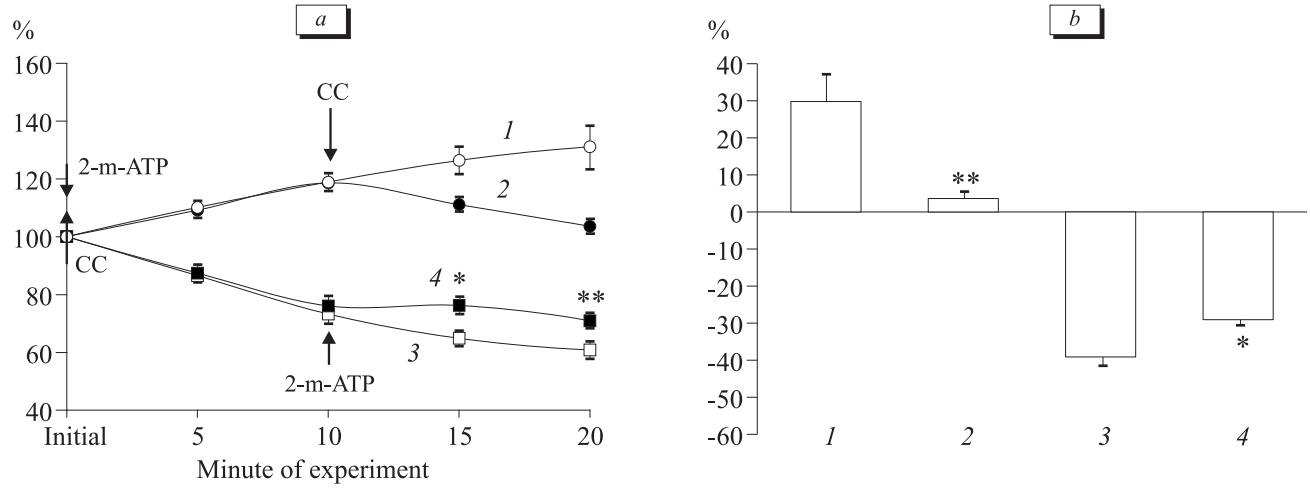

Fig. 3. Combined effects of 2-m-ATP and CC (*a*) and the resultant effect of 2-m-ATP and CC modulation (*b*) of atrial myocardial contractility of 21-day-old rats. **p*<0.05, ***p*<0.01 compared to 3.

37.0%. Addition of 2-m-ATP reduced the CC effect by 29.0±1.2% in the atria and by 28.4±3.4% in the ventricles (Fig. 3, *a*), which was significantly lower than its effect in the control ($p<0.05$). In the control, P_2 receptor agonist in a concentration of 10^{-7} M stimulated atrial and ventricular myocardial contractility by 31.3%. The inhibitory effect of CC on myocardial contractility was reduced in experiments with opposite order of substance application (CC in the presence of P_2 receptor agonist) and was just 3.6±1.7% in the atria and -7.7±1.4% in the ventricles, which was significantly lower than CC effect in the control ($p<0.001$; Fig. 3, *b*).

In control 56-day-old rats, CC decreased the force of atrial contractions by 30.5% and ventricular contractions by 36%. Stimulation of P_2 receptors potentiated the negative inotropic effect of CC by 35.0±2.6% in the atria and by 41.3±3.6% in the ventricles. In the control, 2-m-ATP in a concentration of 10^{-9} M increased atrial and ventricular myocardial contractility by 12.6%. Addition of CC in the presence of P_2 receptor activation led to the development of rapid inhibitory effect of CC, which manifested from the first minute after its addition and within 5 min reached the CC effect in the control.

In control 100-day-old rats, CC reduced atrial and ventricular myocardial contractility by 25 and 36%, respectively. Addition of 2-m-ATP in the presence of CC potentiated the effect of CC by 30.9±1.7% in the atria and reduced it by 25.70±2.25% in the ventricles, without reaching the control level ($p<0.05$). In experiments with opposite order of substance application, CC reduced the contractility of atrial myocardium by 19.4±1.5% and ventricular myocardium by 24.0±2.5%, which was significantly lower than CC effect in the control ($p<0.05$).

Hence, combined effect of 2-m-ATP (P_2 receptor agonist) and of CC (M_2 cholinoceptor agonist) on myocardial contractility can be stimulatory or attenuating, depending on the age of rats. Stimulation of P_2 receptors potentiated the inhibitory effect of CC on atrial and ventricular myocardial contractility in 14-day-old rats and ventricular myocardial contractility in 100-day-old rats. In 21-day-old rats, activation of P_2 receptors prevented the negative effect of CC on myocardial contractility. Activation of M_2 cholinoreceptors inhibited the positive inotropic effect of purine in all age groups.

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