Electrophysiologic effects of adenosine triphosphate on rabbit sinoatrial node pacemaker cells via P1 receptors[1]

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ABSTRACT

AIM: To study the electrophysiologic effects of adenosine triphosphate (ATP) on rabbit sinoatrial node pacemaker cells and the receptors related with the action of ATP. **METHODS:** Intracellular microelectrode method was used to record the parameters of action potential (AP) in the rabbit sinoatrial nodes. **RESULTS:** ATP (0.1-3 mmol/L) decreased the rate of pacemaker firing (RPF) by 16 %-43 % and velocity of diastolic depolarization (VDD) by 33 %-67 %, increased the amplitude of AP (APA) by 6 %-9 % and maximal rate of depolarization (V_{max}) by 30 %-76 %, shortened APD_{50} by 7 %-12 % and APD_{90} by 6.3 %-9 %, concentration-dependently. The effects of ATP, adenosine (Ado), and adenosine diphosphate at the same concentration on AP were not different from each other significantly. Neither uridine triphosphate nor , α,β-methylene ATP had significant electrophysiologic effects on the sinoatrial node of rabbits. Both the electrophysiologic effects of ATP and Ado on pacemaker cells were inhibited by P1 receptor antagonist aminophylline 0.1 mmol/L ($P<0.05$) in a closely similar manner, and the effects of ATP were not affected by P2 receptor antagonist reactive blue 2 at 0.05 mmol/L ($P>0.05$). **CONCLUSION:** There are no functional $P2X_1$ and $P2Y_2$ receptors on pacemaker cells of the rabbit sinoatrial nodes, and the electrophysiologic effects of ATP in the rabbit sinoatrial node pacemaker cells are mediated via P1 receptors by Ado degraded from ATP.

INTRODUCTION

Extracellular adenosine triphosphate (ATP) has widespread effects on physiologic activities, and plays important physiologic and pathophysiologic roles in cardiovascular system. ATP exerts transient physiologic and pharmacologic effects in the organism because it can be rapidly metabolized to adenosine (Ado) by ectotriphosphatase, ectodiphosphatase, and 5'-nucleotidase[1]. Pelleg *et al* reported that the negative chronotropic and dromotropic responses to ATP in the anesthetized canine were partly due to its degradation to Ado[2]. On the other hand, ATP also produced cardiac effects via P2 receptors on the cell surface[3]. In the isolated guinea pig atria, ATP produced not only negative inotropic effects mediated by P1 receptors, but also positive inotropic effects mediated by P2 receptors[3]. Recently, we demonstrated that ATP and uridine triphosphate (UTP) produced concentration-dependent prolongation of 50 % repolarization (APD_{50}) and 90 % repolarization (APD_{90}) in the guinea pig papillary muscles with the same potency, and there was cross-desensiti-

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zation between the response to ATP and that to UTP, indicating that there were functional $P2Y_2$ receptors in the guinea pig papillary muscles[4]. Although ATP has been applied in clinics as an antiarrhythmic drug[5], there has been no report about the effects of ATP on pacemaker cells in the sinoatrial node of rabbits *in vitro*. The present study was designed to investigate the electrophysiologic action of ATP and UTP on the sinoatrial node pacemaker cells of rabbits *in vitro* by intracellular microelectrode method.

METERIALS AND METHODS

Reagents Uridine triphosphate (UTP), adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine (Ado), α, β-methylene ATP (α, β-meATP), aminophylline, and reactive blue 2 were all provided by Sigma Chemical Co (St Louis, USA). All drugs were dissolved in distilled water.

Tissue preparation Male New-Zealand white rabbits (2.3 kg±0.3 kg), provided by Experimental Animal Center of Hebei Medical University (Certificate No 0059), were stunned by a blow, then exsanguinated. The hearts were excised and immersed into aerated Krebs-Henseleit (K-H) solution (mmol/L: NaCl 118, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 10, pH 7.3-7.4). Sinoatrial node preparation was isolated and pinned in a 1.5-mL tissue bath. The preparation was perfused with K-H solution (35.0-35.5 ºC) saturated with 95 % O_2+5 % CO_2 at a rate of 4 mL/min.

Recording and analysis of action potential (AP) After the preparation was equilibrated for 60 min in the K-H solution, the glass microeletrode filled with KCl 3 mol/L was inserted into pacemaker cell to record the electrical signal intracellularly. The parameters including amplitude of AP (APA), maximal rate of depolarization (V_{max}), APD_{50}, APD_{90}, rate of pacemaker firing (RPF), maximal diastolic potential (MDP) and velocity of diastolic depolarization (VDD) were on-line analyzed by a microcomputer system MAP2, a program designed by the Department of Physiology, Hebei Medical University[4].

Method of administration Agonists were given with a microsyringe (50 μL) to the tissue bath directly in order to avoid the desensitization of P receptors, and antagonists were given with constant perfusion[4]. AP were recorded respectively before and at 10 s, 20 s, 30 s, 1 min, 2 min, 4 min, 8 min, and 30 min after administration of agonists. One preparation was stimulated with just one agonist, and the interval of agonist administration at different concentrations was 30 min. The data were obtained from the same cell in each preparation.

Statistical analysis Data were expressed as mean±SD, and the response to agonist was calculated as a percentage of the control value obtained immediately before administration of each agonist concentration. Paired *t* test was used to evaluate the significant difference between the data before and after agonist. Unpaired *t* test was used in the experiments with antagonists and the comparison between ATP and Ado or ADP. $P<0.05$ was considered statistically significant.

RESULTS

Responses to UTP, ATP, ADP, Ado, and α,β-meATP in the sinoatrial node pacemaker cells. The microelectrode was kept steadily in the same cell for 120 min, and the parameters of AP recorded at 30, 60, 90, and 120 min did not change markedly ($P>0.05$, Tab 1). ATP (0.1, 0.3, 1, and 3 mmol/L) decreased RPF by 16 %-43 %, and VDD by 33 %-67 %, increased APA by 6 %-9 % and V_{max} by 30 %-76 %, and shortened APD_{50} by 7 %-12 % and APD_{90} by 6.3 %-9 % ($P<0.05$, $P<0.01$, Tab 2). ADP or Ado at the same concentration as ATP produced similar effects on the pacemaker cells, and there was no statistically difference between the effects of ATP and ADP or Ado at the same concentration (0.1 or 0.3 mmol/L) ($P>0.05$, Tab 2).

Tab 1. Parameters of action potential of the sinoatrial node pacemaker cells in control experiment. *n*=5. Mean±SD.

Time/ min	MDP/ mV	APA/ mV	V_{max}/ $V\cdot s^{-1}$	VDD/ $mV\cdot s^{-1}$	APD_{50}/ ms	APD_{90}/ ms	RPF/ $beat\cdot min^{-1}$
0	-61±6	66±7	6.4±1.3	47±6	94±7	153±12	154±10
30	-60±7	67±8	6.9±1.0	49±6	97±8	154±11	159±9
60	-60±6	66±6	7.0±1.2	50±6	97±8	154±11	159±9
90	-60±5	66±6	6.9±1.2	50±6	96±7	155±12	159±8
120	-60±4	66±5	6.6±1.1	51±6	95±7	154±11	157±8

Responses to each agonist reached the peak values at 20-30 s after administration, and disappeared within 10 min. Neither UTP (0.1-3.0 mmol/L) nor α, β-meATP (0.03 mmol/L) caused significant changes of

Tab 2. Effects of ATP, UTP, ADP, Ado, and α, β-meATP on action potential of pacemaker cells in the rabbit sinoatrial node. *n*=5. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* before agonist. [d]*P*>0.05 *vs* ATP group.

Drug/ mmol·L^{-1}		Percentage changes/%					
		APA	V_{max}	VDD	APD_{50}	APD_{90}	RPF
ATP	0.1	+6±3[b]	+30±20[b]	-33±10[c]	-7±4[b]	-6.3±2.5[b]	-16±7[c]
	0.3	+7±4[b]	+32±20[b]	-43±13[c]	-7.1±2.7[b]	-7±3[b]	-22±8[c]
	1.0	+8±4[c]	+48±29[b]	-50±8[c]	-11±7[b]	-9±4[b]	-31±14[c]
	3.0	+9±4[c]	+76±32[c]	-67±16[c]	-12±5[b]	-9±4[b]	-43±13[c]
ADP	0.1	+5.1±1.3[bd]	+25±5[bd]	-36±10[cd]	-6±4[bd]	-6±4[bd]	-15±8[cd]
	0.3	+6.5±2.5[bd]	+29±18[bd]	-47±10[cd]	-7±4[bd]	-7±5[bd]	-20±8[cd]
	1.0	+7.6±1.7[bd]	+42±17[bd]	-53±18[cd]	-10±6[bd]	-8±3[cd]	-29±12[cd]
	3.0	+7.9±2.6[bd]	+69±22[cd]	-64±10[cd]	-10±5[cd]	-8.5±2.2[cd]	-38±8[cd]
Ado	0.01	+2.6±1.0[b]	+13±8[b]	-14±8[b]	-1.6±2.3[a]	-1.2±1.6[a]	-7±4[b]
	0.03	+2.9±0.6[b]	+17±10[b]	-19±8[b]	-2.8±2.4[a]	-2.8±1.9[a]	-11±8[b]
	0.1	+3.8±1.7[bd]	+27±16[bd]	-27±9[cd]	-5±3[bd]	-5.8±1.3[bd]	-16±10[bd]
	0.3	+7±4[bd]	+38±23[bd]	-39±13[cd]	-6.7±1.2[bd]	-7.0±2.6[bd]	-24±12[bd]
UTP	0.1	+0.7±1.2[a]	+0.4±2.8[a]	-1±3[a]	+1±3[a]	-1.4±2.0[a]	+0.2±1.4[a]
	0.3	- 0.7±2.9[a]	+1.8±2.3[a]	-1.5±2.0[a]	+0.5±2.4[a]	-1.2±2.0[a]	-1.5±1.3[a]
	1.0	+0.4±2.3[a]	-0.8±1.2[a]	-2.8±2.4[a]	+1.3±2.2[a]	-1.2±2.3[a]	-1.4±1.2[a]
	3.0	+0.5±1.9[a]	-0.8±1.4[a]	-2.4±2.6[a]	-1.1±2.4[a]	-1.2±2.6[a]	-1.3±1.5[a]
α, β-meATP	0.03	+0.5±0.9[a]	+1±4[a]	-3±9[a]	-1±3[a]	-1.4±2.9[a]	-4±8[a]

AP in the sinoatrial node pacemaker cells (*P*>0.05, Tab 2). Even though the concentration of α,β-meATP was increased to 100 mol/L, it did not affect the electrophysiological activity of the rabbit sinoatrial node pacemaker cells (*n*=4, data not shown).

Influence of aminophylline on the electrophysiologic responses to ATP and Ado in the sinoatrial node pacemaker cells Preparations were superfused with K-H solution containing aminophylline 0.1 mmol/L for 20 min, and RPF and VDD of the preparations were increased by 23 %-24 % and 22 %-24 %, respectively (*P*<0.05, *P*<0.01, Tab 3). In the preparation treated with aminophylline 0.1 mmol/L, the effects of ATP were inhibited significantly in comparison with those in untreated preparations (*P*<0.05, *P*<0.01, Tab 3). There was no statistically difference between the responses to ATP and those to Ado in the preparations treated with aminophylline (*P*>0.05, Tab 3).

Influence of reactive blue 2 on the electrophysiologic response to ATP in the sinoatrial node pacemaker cells Preparations were superfused with K-H solution containing reactive blue 2 (0.05 mmol/L) for 20 min, and the parameters of AP did not change markedly (*P*>0.05, Tab 3). The electrophysiologic responses to ATP (0.1 and 0.3 mmol/L) in the preparations treated with reactive blue 2 were not significantly different from those in the untreated preparations (*P*>0.05, Tab 3).

DISCUSSION

The present study demonstrated that ATP, ADP and Ado decreased RPF and VDD, increased APA and V_{max}, and shortened APD_{50} and APD_{90} concentration-dependently. Effects of the same concentrations (0.1, 0.3 mmol/L) of ATP, ADP and Ado were equivalent. UTP (0.1-3 mmol/L) and α,β-meATP (0.03 or 0.1 mmol/L) did not markedly affect AP of the sinoatrial node pacemaker cells. P1 receptor antagonist aminophylline at 0.1mmol/L significantly decreased both the effects of ATP and Ado on parameters of AP of the pacemaker cells, and P2 receptor antagonist reactive blue 2 at 0.05 mmol/L did not change the effects of ATP significantly.

P receptors are subdivided into P1 receptors and P2 receptors. Ado is a physiologic agonist of P1 receptors, and has no effects on P2 receptors[6,7]. ATP and UTP activate $P2Y_2$ receptors with a same potency, and ADP activates the same receptors with lower potency than ATP[8,9]. ATP and α,β-meATP are the agonists of P2X receptors, but α,β-meATP has no effect on P2Y receptors[8,9].

Pelleg *et al* reported that the negative chronotropic and dromotropic responses to ATP in the anesthe-

Tab 3. Effects of ATP and Ado on action potentials of pacemaker cells in the rabbit sinoatrial node perfused with aminophylline or reactive blue 2. *n*= 5. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* before agonist. [d]*P*>0.05, [e]*P*<0.05 *vs* ATP alone. [g]*P*>0.05 vs ATP group pretreated with aminophylline.

Drug/mmol·L^{-1}		Percentage changes/%					
		APA	V_{max}	VDD	APD_{50}	APD_{90}	RPF
ATP	0.1	+6±3[b]	+30±20[b]	-33±10[c]	-7.0±4[b]	-6.3±2.5[b]	-16±7[c]
	0.3	+7±4[b]	+32±20[b]	-43±13[c]	-7.1±2.7[b]	-7±3[b]	-22±8[c]
Aminophylline treatment (0.1 mmol·L^{-1})							
		-2.2±1.5[a]	-2.3±0.9[a]	+22±6[c]	+2.4±1.4[a]	+1.9±1.4[a]	+24±8[c]
ATP	0.1	+1.3±1.6[ae]	+10±7[bd]	-18±5[ce]	-1±3[ae]	-2.6±2.0[ae]	-7±5[be]
	0.3	+2.1±1.7[ae]	+22±8[bd]	-27±8[ce]	-3±4[ad]	-2.9±2.3[ae]	-9±6[be]
Aminophylline treatment (0.1 mmol·L^{-1})							
		-2.2±1.5[a]	-2.3±1.4[a]	+24±3[b]	+2.3±1.5[a]	+2.5±2.3[a]	+23±6[b]
Ado	0.1	+2.0±1.6[ag]	+13±4[bg]	-16±10[bg]	-3±3[ag]	-2.7±2.0[ag]	-6±4[bg]
	0.3	+2.6±1.8[ag]	+24±14[bg]	-23±6[bg]	-3±3[ag]	-3.5±2.5[ag]	-8±7[bg]
Reactive blue 2 treatment (0.05 mmol·L^{-1})							
		-1.2±1.7[a]	-1.3±0.9[a]	-2.4±2.3[a]	+1.8±1.3[a]	-1.2±1.6[a]	-1.3±1.4[a]
ATP	0.1	+6±4[bd]	+28±20[bd]	-35±12[cd]	-7±3[bd]	-6.8±2.3[bd]	-18±12[cd]
	0.3	+6.5±1.4[bd]	+29±20[bd]	-47±14[cd]	-8±4[bd]	-7.2±2.6[bd]	-23±11[cd]

tized canine were mainly caused by vagal involvement, and partly resulted from its degradation to Ado[2]. Since the nucleotidases in cardiac cells degraded ATP, ADP, and AMP to Ado rapidly[10], the negative chronotropic responses to Ado were equivalent to or more potent than those to ATP administered into the sinus nodal artery of the canine[11]. In the present study, the same concentration of ATP and Ado produced equivalent effects on the parameters of AP in the sinoatrial node pacemaker cells, and both the responses to ATP and Ado were inhibited by P1 receptor antagonist aminophylline in a closely similar manner. It has been reported that Ado and its derivatives induce bradycardia in the canine, rat and rabbit[12-14]; and decrease RPF and VDD, increase APA and V_{max}, and shorten APD_{50} and APD_{90} in the isolated guinea pig sinoatrial nodes[15]; which are consistent with the presently observed responses to Ado in the rabbit sinoatrial nodes. From the results in this study and those reports by others, it is suggested that the electrophysiologic responses to ATP in the rabbit sinoatrial node pacemaker cells are mediated via P1 receptors by Ado degraded from ATP.

Recently we reported that both ATP and UTP prolonged APD via $P2Y_2$ receptors in the guinea pig papillary muscle cells with the same potency, and there was a cross-desensitization between the response to ATP and that to UTP[4]. Mantelli *et al* showed that the positive inotropic response to ATP was mediated via P2 receptors in the isolated guinea pig atria, which was inhibited by P2 receptor antagonist reactive blue 2, and that a selective agonist of both $P2X_1$ and $P2X_3$ receptors α, β-meATP also produced the similar response[3]. Previously, we reported that in the canine and rabbit splenic arteries, a dose ratio of ATP to α, β-meATP to induce equivalent vasoconstrictive responses mediated by $P2X_1$ receptors was 100:1-300:1[16-18]. In the present study, however, reactive blue 2 did not change the effects of ATP on the parameters of AP, and both α, β-meATP and UTP did not produce any effects on the parameters of AP in the rabbit sinoatrial node pacemaker cells, indicating that the effects of ATP on the parameters of AP were impossibly mediated via $P2X_1$ or $P2Y_2$ receptors. Furthermore, the fact that effects evoked by the same concentration (0.1and 0.3 mmol/L) of ATP, ADP and Ado were equivalent was not consistent with the potency order of agonists for any cloned P2 receptor subtypes. In conclusion, there are no functional $P2X_1$ and $P2Y_2$ receptors on pacemaker cells of the rabbit sinoatrial nodes, and the electrophysiologic responses to ATP in the pacemaker cells are mediated via P1 re-

ceptors by Ado degraded from ATP.

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