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Uridine triphosphate prolongs action potential duration of guinea pig papillary muscles via $P2Y_2$ purinoceptors[1]

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KEY WORDS *uridine triphosphate; action potentials;* papillary muscles; guinea pigs

ABSTRACT

AIM: To study the electrophysiologic effects of uridine triphosphate (UTP) on the guinea pig papillary muscles *in vitro* and purinoceptors related with the action of UTP. **METHODS**: Intracellular microelectrode method was used to record action potentials (AP) in guinea pig papillary muscles. **RESULTS**: UTP, adenosine triphosphate (ATP), and adenosine diphosphate (ADP) prolonged the action potential duration (APD) concentration-dependently in guinea pig papillary muscles. The potency order was UTP = ATP > ADP. There was cross-desensitization between the response to ATP and that to UTP, and neither Ado nor α,β-MeATP caused great change in AP of the papillary muscles. The prolongation of APD by UTP was not affected by sustained perfusion with aminophylline. As an osmotic pressure control equivalent to UTP 3 mmol/L, ceftriaxonum 3 mmol/L or NaCl 9 mmol/L induced a marked but slight prolongation of APD. **CONCLUSION**: UTP produced APD prolongation through specific and nonspecific actions, and the specific response to UTP was mediated by $P2Y_2$ purinoceptors.

INTRODUCTION

Adenosine triphosphate (ATP) and uridine triphosphate (UTP) function as extracellular signalling molecules to regulate a myriad of physiologic responses by cell surface P2 purinoceptors. In cardiovascular system, ATP and UTP play important physiologic and pathophysiologic roles. P2X and P2Y receptors are purinoceptor subtypes generally accepted as responsible for the effects of ATP in cardiovascular system. Recently it has been found that UTP decreases vascular tone both in an endothelium-dependent and independent manner[1]. UTP-induced release of vasodilator compounds such as NO from the endothelium of cardiac blood vessels has been demonstrated, and UTP also affects vascular smooth muscles directly to cause vasodilatation. A recent article suggested that UTP induced vasoconstriction via $P2Y_2$-purinoceptors in the isolated pulmonary vascular bed of the rat, and liberated intracellular calcium via $P2Y_4$-purinoceptors in cells cultured from guinea pig cardiac endothelium[2]. So far there is no available data on electrophysiologic actions of UTP. The present study was designed to investigate the effect of UTP on action potentials of guinea pig papillary muscles, and to characterize the purinoceptor contribution to the electrophysiologic action of UTP.

MATERIALS AND METHODS

Drugs Uridine triphosphate (UTP), adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine (Ado), α,β-methylene ATP (α,β-MeATP) and aminophylline were all provided by Sigma Co. Ceftriaxonum natrium was provided by Guangzhou Baiyunshan Pharmaceutical General Factory (China). All drugs were dissolved in normal saline (NS).

Tissue preparation Guinea pigs of either sex weighing (300 ± 20) g, provided by Experimental Animal Center of Hebei Medical University (Grade Ⅱ, Certificate № 04064), were stunned. The hearts were excised and immersed into aerated Krebs-Henseleit solution (K-H solution). Papillary muscles of the right ventricle were isolated and pinned in a 1.5 mL tissue bath. The muscles were perfused with K-H solution (35 ℃ ± 0.5 ℃, pH 7.2 ~ 7.4) gassed with 95 % O_2 + 5 % CO_2 at a rate of 4 mL/min. The K-H solution contained

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(mmol/L): NaCl 118, KCl 4.7, $CaCl_2$ 2.52, $MgSO_4$ 1.64, KH_2PO_4 1.18, $NaHCO_3$ 24.88, glucose 10. The preparation was stimulated by square waves at 1 ms duration, 1.5 times threshold voltage, and 1 Hz frequency from an electric stimulator (SEN-3201, Nihon Kohden).

Recording and analysis of AP After a stabilization in K-H solution for 1 h, the glass microelectrode filled with KCl 3 mol/L was inserted into the papillary muscle to record the electrical signal intracellularly. The parameters including resting potential (RP), amplitude of AP (APA), overshoot (OS), maximal rate of depolarization (V_{max}) of AP and AP duration at 50 % and 90 % repolarization (APD_{50} and APD_{90}) were on-line analyzed by a microcomputer system MAP2, a program designed by the Department of Physiology, Hebei Medical University.

Method of administration Agonists were given with a microsyringe (50 μL) to the tissue bath directly because P2 purinoceptor agonists were quite easy to cause desensitization of the receptors, and antagonists were given with constant perfusion. AP were recorded respectively before administration and at 10 s, 20 s, 30 s, 1 min, 2 min, 4 min, 8 min, and 30 min after administration. One preparation was stimulated with just one agonist. The interval of agonist administration at each concentration was 30 min. The data obtained were observed in the same cell in each preparation.

Statistical analysis Results were expressed as $\bar{x} \pm s$, and the responses to agonists were calculated as a percentage of the control obtained immediately before addition of the respective agonist concentration. Paired t test was used to evaluate the significant difference between the data before and after agonist, and two way ANOVA was used in the experiments with antagonists and the comparison between UTP and ATP or ADP. $P < 0.05$ were considered statistically significant.

RESULTS

The microelectrode was kept steadily in the same cell for 120 min, and the parameters of AP recorded at 30, 60, 90, 120 min did not show marked difference from the beginning ($P > 0.05$, Tab 1). NS as a solvent control had no marked effect on any parameters of the AP ($P > 0.05$, $n = 5$, data not shown).

Effects of UTP, ATP, ADP, Ado, and α,β-MeATP on AP in papillary muscles UTP, ATP, and ADP were injected respectively into the tissue bath at the concentration of 0.1, 0.3, 1, and 3 mmol/L. All the three drugs concentration-dependently prolonged APD_{50} and APD_{90} ($P < 0.05$ and 0.01), and had no marked effects on other parameters of AP ($P > 0.05$, Tab 2). The lowest concentrations of UTP, ATP, and ADP to produce about 10 % prolongation of APD_{50} were 1.0, 1.0, and 3.0 mmol/L respectively. The effect of UTP was not different from that of ATP at each comparable concentration ($P > 0.05$), but the effects of UTP or ATP were more potent than those of ADP ($P < 0.05$, Tab 2). Responses to agonists reached the peak values at 10 ~ 20 s after administration, and disappeared within 10 min. Neither Ado (0.1, 0.3 mmol/L, $n = 5$) nor α,β-MeATP (0.03 mmol/L, $n = 5$) caused changes in the parameters of AP in the papillary muscles ($P > 0.05$, data not shown).

Influence of aminophylline on the effects of UTP in papillary muscles Preparations were superfused with K-H solution containing P1 purinoceptor antagonist aminophylline 0.1 mmol/L for 20 min, and the parameters of AP of papillary muscles were not changed markedly ($P > 0.05$, Tab 2). UTP was injected to the tissue bath at 0.1, 0.3, 1, and 3 mmol/L, and APD_{50} and APD_{90} were also prolonged concentration-dependently. Comparison of the data between UTP alone and UTP in the preparation treated with aminophylline did not show

Tab 1. Parameters of action potentials in guinea pig papillary muscles in control experiment. $n = 5$ animals. $\bar{x} \pm s$.

Time/min	RP/mV	APA/mV	V_{max}/V·s^{-1}	APD_{50}/ms	APD_{90}/ms
0	−80.9 ± 2.0	142 ± 4	155 ± 7	141.0 ± 2.9	177 ± 3
30	−80 ± 4	142 ± 7	156 ± 10	140.0 ± 2.6	176 ± 7
60	−79 ± 4	143 ± 5	155 ± 9	140 ± 4	176 ± 7
90	−80 ± 6	142 ± 6	155 ± 9	141.0 ± 2.6	177 ± 4
120	−80.6 ± 2.9	142 ± 5	155 ± 9	142.2 ± 2.4	175 ± 4

Tab 2. Effects of UTP, ATP, and ADP on action potentials in papillary muscles and influence of aminophylline (0.1 mmol/L) on the effects of UTP. $n = 5$ animals. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* before agonist. $^eP < 0.05$ *vs* UTP group. $^hP < 0.05$ *vs* ATP group.

Drug/mmol·L^{-1}		Percentage changes/%				
		RP	APA	V_{max}	APD_{50}	APD_{90}
UTP	0.1	+1.1±4.7	−0.3±1.0	+0.2±0.9	+2.6±3.2	+2.2±3.0
	0.3	+0.6±1.4	+1.0±2.0	+2.1±3.8	+5.0±2.7^b	+3.9±2.2^b
	1.0	−0.4±2.3	−1.6±1.8	−0.8±3.3	+9.4±4.5^c	+8.4±3.4^c
	3.0	+0.4±2.4	−3.9±3.3	−2.8±7.8	+18.1±6.9^c	+17.1±6.0^c
ATP	0.1	−0.1±3.0	+0.0±0.9	−1.3±2.6	+2.8±2.0^b	+2.5±1.6^b
	0.3	+0.2±2.1	−0.7±0.7	−0.2±1.8	+7.2±3.5^c	+5.6±2.5^c
	1.0	−0.5±2.0	−1.2±1.3	+0.2±2.2	+10.2±4.8^c	+8.4±3.4^c
	3.0	+0.1±3.8	−5.8±3.3	−5.0±4.9	+24.3±8.3^c	+21.0±7.0^c
ADP	0.1	−2.3±1.5	+0.0±1.2	−0.2±0.8	+1.2±1.1	+1.2±1.2
	0.3	−0.5±1.4	−0.1±0.4	−0.4±0.6	+1.1±0.7^{beh}	+1.2±0.7^{beh}
	1.0	−1.0±1.3	−0.3±1.4	−1.9±2.5	+5.4±1.1^c	+5.2±1.1^c
	3.0	+1.8±2.9	−1.3±2.0	−0.9±1.2	+9.9±1.3^{ceh}	+9.3±1.9^{ceh}
Aminophylline treatment						
		+2.4±4.0	+0.4±2.2	−2.4±6.8	−0.2±2.4	−0.7±3.4
Aminophylline treatment						
UTP	0.1	−1.2±1.2	−0.1±2.0	+0.6±4.1	+1.5±3.3	+1.2±3.2
	0.3	−1.1±1.5	−0.4±0.7	−0.4±1.2	+5.4±4.8	+4.4±4.3
	1.0	−0.0±2.4	−0.6±1.3	+1.1±3.3	+9.8±7.5^b	+8.1±5.7^b
	3.0	−1.5±3.8	+0.1±1.0	−2.5±5.0	+16.8±7.7^c	+15.7±6.9^c

difference in prolongation of APD_{50} and APD_{90} (Tab 2).

Influence of sustained perfusion with ATP on the effects of UTP in papillary muscles After preparations were perfused with K-H solution containing ATP 1 mmol/L for 20 min − APD_{50} and APD_{90} were prolonged by 3.2 % and 2.6 % respectively ($P < 0.05$, Tab 3). UTP 1 mmol/L did not affect the parameters of AP in papillary muscles perfused with ATP ($P > 0.05$). Increase of UTP concentration to 3 mmol/L prolonged APD_{90} of the papillary muscles only by 11.9 % ($P < 0.01$, Tab 3).

Effect of osmotic pressure on the AP in papillary muscles Ceftriaxonum, molecular weight of which is near to UTP, was used as the osmotic pressure control. One molecule of UTP salt contained 3 natrium, therefore NaCl equivalent to the natrium concentration of UTP salt was used as a Na ionic control. Ceftriaxonum at 3 mmol/L prolonged APD_{50} and APD_{90} in papillary muscles by 11.7 % and 8.8 % respectively ($P < 0.01$), but had no effect on AP at other concentrations ($P > 0.05$, Tab 4). NaCl at 9 mmol/L prolonged APD_{50} and APD_{90} by 6.6 % and 5.1 % respectively ($P < 0.01$), but had no effect on AP at 3 mmol/L ($P > 0.05$, Tab 4).

DISCUSSION

UTP, ATP, and ADP concentration-dependently prolonged APD of AP in the guinea pig papillary muscles, and the potency order was UTP = ATP > ADP. Ado and α, β-MeATP at concentrations used had no marked effect. P1 purinoceptor antagonist aminophylline did not alter the effect of UTP, and there was a cross-desensitization between the responses to ATP and to UTP. These results indicated that the prolongation of APD in guinea pig papillary muscles by UTP was mediated by $P2Y_2$ purinoceptors.

Pelleg[3] reported that ATP elicited a dose-dependent shortening of APD_{50} in atrium of the anesthetized dog, but this effect of ATP was mediated to a large extent by a vagal reflex and to a lesser extent by Ado, the product of enzymatic degradation from ATP. In the mammalian (human, cat, and guinea pig) atrium, Ado increases outward potassium current mediated by P1 purinoceptors,

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Tab 3. Effect of UTP on action potentials in papillary muscles perfused with ATP 1 mmol/L. $n = 5$ animals. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* before agonist.

Drugs/mmol·L^{-1}	Percentage changes/%				
	RP	APA	V_{max}	APD_{50}	APD_{90}
ATP treatment	−6.0±7.9	−1.8±2.3	+2.8±6.0	+3.2±1.7^b	+2.6±1.7^b
ATP treatment					
UTP 1.0	+0.1±3.6	−0.5±2.7	−3.1±5.6	+2.6±2.8	+2.8±2.6
3.0	+0.8±1.6	+0.2±1.1	−2.0±4.4	+11.9±3.3^c	+11.9±3.3^c

Tab 4. Effect of ceftriaxonum and NaCl on action potentials in papillary muscles. $n = 5$ animals. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* before agonist.

Drugs/mmol·L^{-1}	Percentage changes/%				
	RP	APA	V_{max}	APD_{50}	APD_{90}
Ceftriaxonum 0.1	+0.6±1.2	+0.2±0.9	+1.8±2.1	+0.4±0.8	+0.6±0.8
0.3	−0.6±0.8	+0.6±0.8	−1.0±1.6	+1.3±1.0	+0.9±0.9
1.0	−0.2±2.2	−0.8±0.7	+0.0±2.1	+0.9±1.4	+0.6±1.2
3.0	+0.3±2.0	−1.8±1.8	+0.1±4.0	+11.7±2.7^c	+8.8±2.5^c
NaCl 3.0	−1.2±0.7	−0.2±0.6	−0.3±1.4	+0.6±1.5	+0.3±1.4
9.0	−0.1±2.6	+0.4±1.2	+3.1±4.7	+6.6±3.1^b	+5.1±2.5^b

and results in shortening of APD and hyperpolarization of RP. In mammalian ventricular myocardium, Ado does not cause this effect[4-6]. Song and Belardinelli[7] did not observe significant electrophysiologic changes in guinea pig isolated ventricular myocytes superfused with ATP 0.01 ~ 0.1 mmol/L. Results of the present study, however, showed that both UTP and ATP at concentrations above 0.1 mmol/L produced concentration-dependent prolongation of APD_{50} and APD_{90} in guinea pig papillary muscles. Bolus injection of the drugs to the tissue bath and higher concentration of the drugs might be accounted for different results of the present study from those of the others[7].

It is well known that P2 purinoceptors have the characteristic of tachyphylaxis. In this study, ATP 1 mmol/L injected to the tissue bath prolonged APD_{50} by 10.2 %, but the prolongation of APD_{50} was only by 3.2 % in the preparation superfused with ATP 1 mmol/L for 20 min, which demonstrated that constant superfusion with higher concentration of ATP was not suitable for observation of the responses to ATP or to UTP as agonists. Therefore, we adopted the way of bolus injection of the agonists to the tissue bath in the present experiments. Parameters of AP in time or solvent control experiments were in accordance with the observations reported[8-10].

The concentration of UTP administered in this study was much higher. Consequently, influence of osmotic pressure on the cellular electrophysiology was analyzed. Ceftriaxonum, whose molecular weight (661.59) was similar to that of UTP (589.1) or ATP (551.1), was used as osmotic pressure control. Ceftriaxonum only at 3 mmol/L prolonged APD_{50} and APD_{90} respectively by 11.7 % and 8.8 %, suggesting that effects of UTP and ATP on APD at 3 mmol/L were partially related to an influence of osmotic pressure. One molecule of UTP contained 3 positive ions of natrium, and NaCl at 3 mmol/L equivalent to UTP 1 mmol/L in natrium ion concentration had no influence on the AP. On the other hand, in the cross-desensitization experiments between UTP and ATP in preparations superfused continuously with ATP 1 mmol/L, the prolongation of APD by UTP 1 mmol/L was abolished, and by UTP 3 mmol/L, APD_{50} was decreased from 18.1 % (in normal preparations, Tab 2) to 11.9 % (in desensitized preparations, Tab 3), the value of the latter was equivalent to ceftriaxonum at 3 mmol/L (11.7 %, Tab 4). Thus UTP at a concentration less than 3 mmol/L produced a pharmacologic action on APD of papillary muscles, and UTP at 3 mmol/L prolonged APD_{50} and APD_{90} through both specific and unspecific mechanisms.

A selective P1 purinoceptor agonist Ado at 0.1 − 0.3 mmol/L did not cause great changes in APD in papillary

muscles, and aminophylline which selectively antagonizes P1 purinoceptors[6,11] at 0.1 mmol/L, did not affect the responses to UTP, suggesting that P1 purinoceptors did not contribute to the prolongation of APD by UTP. Previously, we reported that in dog and rabbit splenic arteries, a dose ratio of α,β-MeATP to ATP to induce equivalent vasoconstrictive responses mediated by $P2X_1$ purinoceptors was 1∶100 ~ 1∶300[12,13]. In the present study, ATP produced a marked effect at 0.3 mmol/L, but α,β-MeATP did not induce any effect at 0.03 mmol/L, suggesting that the prolongation of APD by UTP was not related to $P2X_1$ purinoceptors. On the other hand, potency order for prolongation of APD was UTP = ATP > ADP, and α,β-MeATP was much less effective, which was consistent with the potency order of $P2Y_2$ receptors[14]. In conclusion, UTP produces a prolongation of APD by specific and nonspecific actions, the specific response to UTP is mediated by $P2Y_2$ purinoceptors, and the nonspecific response is due to the influence of osmotic pressure of UTP at the highest concentration.

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尿苷三磷酸通过 $P2Y_2$ 尿嘌呤受体延长豚鼠乳头状肌动作电位时程[1]

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关键词 尿苷三磷酸; 动作电位; 乳头状肌; 豚鼠

目的: 研究尿苷三磷酸(UTP)对豚鼠乳头状肌的电生理作用, 及 UTP 作用的相关受体. **方法**: 利用细胞内微电极技术记录豚鼠乳头状肌跨膜电位. **结果**: UTP、ATP 和 ADP 均可浓度依赖性延长豚鼠乳头状肌动作电位时程(APD). 激动剂的效应强度序列为 UTP = ATP > ADP, 且 UTP 和 ATP 的作用存在交叉脱敏现象. Adenosine (Ado) 和 α,β-methylene ATP (α,β-MeATP) 对豚鼠乳头状肌动作电位各参数均无影响, 氨茶碱持续灌流亦不影响 UTP 的作用. 与 UTP 3 mmol/L 等渗的 ceftriaxonum (3 mmol/L)或 NaCl (9 mmol/L)可显著但轻微地延长 APD. **结论**: UTP 延长豚鼠乳头状肌 APD 的作用由特异性和非特异性两种作用组成, 前者与 $P2Y_2$ 受体有关.

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