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Electrophysiological effects of N^6-cyclopentyl-adenosine and [—]-N^6-[phenylisopropyl]-adenosine on pacemaker cells in sinoatrial node of guinea pigs[1]

LI Yu-Long, HE Rui-Rong (*Department of Physiology, Institute of Basic Medicine, Hebei Medical College, Shijiazhuang 050017, China*)

ABSTRACT The electrophysiological effects of N^6-cyclopentyladenosine (CPA) and [—]-N^6-[phenylisopropyl]adenosine (*R*-PIA) (both are selective adenosine A_1 receptor agonists) on pacemaker cells in sinoatrial (SA) node of guinea pigs were investigated using intracellular microelectrodes. CPA and *R*-PIA increased the amplitude of action potential, amplitude of the maximal diastolic potential and maximal rate of depolarization (phase 0), but decreased the velocity of diastolic (phase 4) depolarization, the rate of pacemaker firing, and the duration of 90% repolarization in pacemaker cells of guinea pigs. 8-Phenyltheophylline (a nonselective antagonist of adenosine receptors) and glibenclamide (a potent blocker of ATP-sensitive K^+ channels) inhibited the electrophysiological responses of pacemaker cells to CPA. These results suggest that the eletrophysiological changes induced by CPA are adenosine receptor-dependent and mainly mediated by activation of ATP-sensitive K^+ channels coupled to adenosine receptors.

KEY WORDS adenosine; theophylline; glyburide; purinergic receptors; potassium channels; sinoatrial node; electrophysiology

Adenosine exerted an inhibitory action on heart rate in dogs[1], rats[2], and rabbits[3]. Although the adenosine-induced decrease in the rate of diastolic depolarization of mammalian SA node was well documented[4,5], systematic and quantitative studies were not seen in the literature, and its ionic mechanism was not clear.

CPA (N^6-cyclopentyladenosine) and *R*-PIA ([—]-N^6-[phenylisopropyl]-adenosine) are adenosine analogues with the effects similar to those of adenosine in animal hearts. ATP-sensitive K^+ channels may be coupled to adenosine receptors via GTP-binding proteins in rat ventricular myocytes[6]. Glibenclamide (Gli, a potent blocker of ATP-sensitive K^+ channels) may inhibit the adenosine-induced bradycardia[7,8]. Thus, the purpose of our study was to investigate the electrophysiological effects of CPA and *R*-PIA, and to clarify its mechanism and the relationship between ATP-sensitive K^+ channels and adenosine in SA node.

MATERIALS AND METHODS

Guinea pigs of either sex weighing $0.35 \pm s$ 0.04 kg were decapitated and the hearts were superfused with cold balanced salt solution. The preparations included the intercaval region and a small part of the inter-atrial septum but not the atrioventricular node. The upper part of the crista terminalis was cut to open the superior vena cava in order to expose the sinoatrial node. It was mounted on a perforated silicon rubber block in a tissue bath.

The glass microelectrode was inserted into SA node pacemaker cells to record the electric activity. The transmembrane potential from the recording microelectrode was amplified (MEZ-8201), monitored with a memory oscilloscope (VC-11), and fed to the A/D convertor. A microcomputer (Apple-II) was used to process the amplified signals. Maximal diastolic potential (MDP), amplitude of action potential (APA), duration of 90% repolarization (APD_{90}), maximal rate of depolarization (V_{max}), rate of pacemaker firing (RPF), and velocity of diastolic (phase 4) depolarization (VDD) were analyzed by the computer. Parameters of AP were stored into a diskette.

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After recording 3 control action potentials (AP), the preparation was perfused with modified K-H solution: NaCl 125; KCl 5.1; $MgSO_4$ 1.2; $CaCl_2$ 2.1; $NaHCO_3$ 23; glucose 11 mmol·L^{-1}. The AP was recorded at 1, 5, 10, 20, and 30 min after application of drugs. The preparation was washed with the K-H solution to observe the recovery of AP.

The experiment consisted of 3 groups: (1) The electrophysiological effects of CPA and *R*-PIA on SA node pacemaker cells. The animals were divided into 5 subgroups: control, CPA 1, 5, 20 nmol·L^{-1} and *R*-PIA 20 nmol·L^{-1}; (2) Effects of 8-PT (10, 50, and 100 nmol·L^{-1}) on the electrophysiological response of pacemaker cells to CPA (20 nmol·L^{-1}); (3) Effects of Gli (1, 5, and 10 μmol·L^{-1}) on the electrophysiological response of pacemaker cells to CPA (20 nmol·L^{-1}). The solvent and resources of CPA, *R*-PIA, 8-PT, and Gli have previously been described[8].

The changes in parameters of AP expressed as $\bar{x}\pm s$ were analyzed using *t* test. Differences among groups were compared using *F* test.

RESULTS

Effects of CPA and *R*-PIA on automaticity of pacemaker cells in SA node RPF was decreased by CPA in a concentration-dependent manner. VDD began to decrease after 2 min of perfusion with the K-H solution containing CPA, and showed maximal decrease at the 5th min. The maximal decreases in VDD induced by CPA at concentrations of 1, 5, and 20 nmol·L^{-1} were 62, 50, and 40 mV·s^{-1}, respectively. The changes in VDD induced by CPA were parallel to those of RPF. *R*-PIA (20 nmol·L^{-1}) also induced decreases in RPF and VDD (Tab 1).

Effects of CPA and *R*-PIA on transmembrane potentials of pacemaker cells in SA node At 20 nmol·L^{-1}, CPA and *R*-PIA induced decreases in APD_{90} and MDP, and increases in APA and rate (V_{max}) of depolarization on the pacemaker cells. CPA showed no effect on MDP, APA, V_{max}, and APD_{90} at 1 nmol·L^{-1}. The changes in MDP induced by CPA were parallel to those of RPF (Fig 1).

Inhibitory effects of 8-PT and Gli on electrophysiological response of pacemaker cells to CPA 8-PT 10 nmol·L^{-1} did not affect the

Tab 1. Electrophysiological effects of N^6-cyclopentyladenosine (CPA), [−]-N^6-[phenylisopropyl]adenosine (*R*-PIA), 8-Phenyltheophylline (8-PT) and glibenclamide (Gli) on pacemaker cells in SA node of guinea pig. $n=8$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control; $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* CPA (20 nmol·L^{-1}).

Drug/ nmol·L^{-1}		MDP/ mV	APA/ mV	V_{max}/ v·s^{-1}	VDD/ mV·s^{-1}	RPF/ bpm	APD_{90}/ ms
Control		−44±3	47±5	4.6±1.1	70±7	175±10	203±16
CPA							
	1	−48±4^a	52±3^a	5.3±1.8^a	62±8^a	157±12^b	198±14^a
	5	−53±5^b	58±5^b	6.1±2.3^b	50±6^b	134±14^b	189±18^a
	20	−59±5^c	63±4^c	6.9±1.4^c	40±5^c	109±8^c	172±21^b
R-PIA	20	−57±4^{cd}	61±3^{cd}	7.1±2.0^{cd}	42±3^{cd}	113±15^{cd}	177±12^{bd}
CPA 20 + 8-PT							
	10	−54±7^{bd}	59±6^{bd}	6.3±1.3^{bd}	45±6^{bd}	116±13^{cd}	186±18^{ad}
	50	−46±4^{ae}	49±7^{ae}	4.7±1.9^{ae}	67±4^{af}	148±10^{bf}	199±23^{ae}
	100	−40±6^{af}	44±4^{af}	3.8±2.1^{af}	83±10^{ae}	169±14^{af}	223±17^{af}
CPA 20 + Gli (μmol·L^{-1})							
	1	−56±6^{bd}	59±4^{bd}	6.4±1.9^{bd}	44±5^{bd}	116±15^{cd}	179±13^{cd}
	5	−50±4^{ae}	54±5^{ae}	5.7±1.5^{ae}	54±8^{ae}	131±8^{be}	190±12^{ae}
	10	−49±3^{ae}	52±4^{ae}	5.4±0.9^{ae}	58±7^{ae}	142±14^{be}	190±22^{ae}

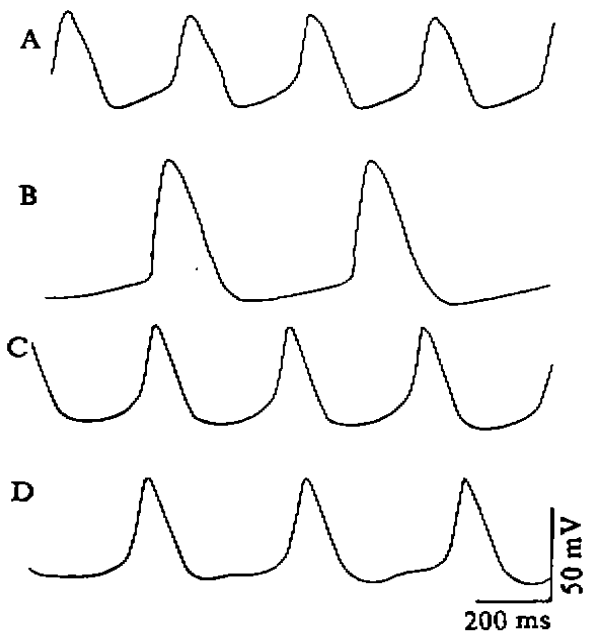

Fig 1. Inhibitory effects of 8-PT and Gli on electrophysiological responses of pacemaker cells in SA node of guinea pig to CPA. A. Control, B. CPA 0.02, C. 8-PT 0.1 + CPA 0.02, D. Gli 10 + CPA 0.02 μmol·L^{-1}.

CPA-induced electrophysiological changes of pacemaker cells in SA node (Tab 1), whereas 50 and 100 nmol·L^{-1} antagonized the electrophysiological responses to CPA (20 nmol·L^{-1}).

Gli 5 and 10 μmol·L^{-1} inhibited the electrophysiological responses to CPA (20 nmol·L^{-1}).

DISCUSSION

The present results confirmed that CPA and *R*-PIA (the selective adenosine A_1-receptor agonists) could concentration-dependently decrease the RPF and increase the APA and V_{max} of pacemaker cells in SA node of guinea pigs. The mechanism underlying the effects of CPA and *R*-PIA on APA and V_{max} remains to be clarified. APD_{90} in SA node pacemaker cells was decreased by CPA and *R*-PIA, suggesting that the repolarization process of SA node cells was sensitive to CPA and *R*-PIA. Since the repolarization process was mainly determined by the potassium current in SA node cells, it was suggested that CPA and *R*-PIA might act on the potassium current.

As a nonselective adenosine receptor antagonist, 8-PT markedly inhibited the eletrophysiological effects of CPA on pacemaker cells in concentration-dependent manner, suggesting that the effects were receptor-dependent.

In the present study, Gli (known to inhibit ATP-sensitive K^+ channels[9,10]) attenuated the negative chronotropic action of CPA in SA node of guinea pigs, an effect consistent with the results obtained in dogs *in vivo*[7]. ATP-sensitive K^+ channels may be coupled to adenosine receptors via GTP-binding proteins in rat ventricular myocytes[6]. Thus, it is likely that activation of ATP-sensitive K^+ channels by CPA via adenosine receptors may be involved in the electrophysiological effects induced by CPA in SA node cells of guinea pigs. As the electrophysiological changes induced by CPA were not fully abolished by Gli, other mechanisms might also be involved.

In summary, CPA exerted a negative chronotropic action on the pacemaker cells in SA node of guinea pigs. The effect was adenosine-receptor dependent and mainly mediated by activation of ATP-sensitive K^+ channels coupled to adenosine receptors.

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环戊腺苷和苯基异丙基腺苷对豚鼠窦房结起搏细胞的电生理效应

李玉龙，何瑞荣 （河北医学院基础医学研究所生理室，石家庄050017，中国）

摘要 利用细胞内微电极技术，观察环戊腺苷(CPA)和苯基异丙基腺苷(*R*-PIA)对豚鼠窦房结起搏细胞电活动的影响. CPA 和 *R*-PIA 不仅能增加豚鼠窦房结起搏细胞 APA、MDP 和 V_{max}，且能减小 VDD、RPF 和 APD_{90}. 8-苯茶碱和格列苯脲能明显地抑制环戊腺苷的电生理效应. 本文结果提示环戊腺苷的电生理效应是受体依赖性的和主要由腺苷受体耦联的 ATP 敏感性钾通道的激活所中介.

关键词 腺苷；茶碱；格列苯脲；嘌呤受体；钾通道；窦房结；电生理学

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吗啡对蟾蜍脊神经节细胞 α-肾上腺素受体敏感性的影响

陈 永[1]，王阿兹[2]，马逸龙[3] （同济医科大学，实验医学研究中心；[3]环境医学研究所，武汉430030，中国）

Effects of morphine on sensitivities of α-adrenoceptors in toad spinal ganglion neurons

CHEN Yong[1], WANG A-Jing[2], MA Yi-Long[3]
(*Research Center of Experimental Medicine; [3]Institute of Environmental Medicine, Tongji Medical University, Wuhan 430030, China*)

ABSTRACT Intracellular recordings were performed on 35 neurons from 35 isolated toad spinal ganglia (SG) and the extracellular free calcium ion activities were measured in another 26 isolated toad SG by Ca^{2+}-selective microelectrodes. The effects of morphine on the sensitivities of α-adrenoceptors were observed. It was found that depolarization of membrane potential induced by norepinephrine (NE 10—100 μmol·L^{-1}) or α_1-adrenoceptor agonist phenylephrine (100 μmol·L^{-1}) was depressed by morphine (27 μmol·L^{-1}). Superfusing SG with opioid receptor antagonist naloxone (100 μmol·L^{-1}) blocked the depressing effect of morphine on NE-induced depolarization. The depressing effect of morphine on NE-induced depolarization was not affected by superfusing SG with α_2-adrenoceptor antagonist yohimbine (5 μmol·L^{-1}). NE (100 μmol·L^{-1}) reduced the extracellular free calcium ion activity while morphine (27 μmol·L^{-1}) increased the extracellular free calcium ion

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[1] Now in *Institute of Liver Diseases, Affiliated Hospital, Hubei College of Traditional Chinese Medicine, Wuhan 430061, China.*

[2] To whom correspondence should be addressed.

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