碱治疗，测量小鼠肝 GAG 的含量，显微镜观察感染后第6，10，13 wk 小鼠肝脏组织切片。**结果**：感染6 wk 以后，小鼠肝 GAG 含量显著高于未感染的对照组。GAG 含量在第10 wk 达到最高峰，约为正常水平的6倍；感染组为56±9 μg/g，无感染组为10±1 μg/g。10 wk 后，无感染组 GAG 水平开始下降。秋水仙碱治疗使感染10 wk 小鼠的 GAG 含量比感染对照组降低了约50 %，即22±3 μg/g，说明在第10 wk 秋水仙碱不能使 GAG 含量下降到正常水平以下。 **结论**：秋水仙碱可能通过抑制肉芽组织的形成从而降低被日本血吸虫感染的小鼠肝 GAG 的含量。

关键词 日本血吸虫；秋水仙碱；葡糖胺基聚糖类；肝

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Jan; 16 (1): 42 - 46

Endogenous adenosine and ATP-sensitive potassium channel modulate anoxia-induced electrophysiological changes of 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*)

AIM: To investigate the electrophysiological effects of adenosine deaminase (ADase, an enzyme converting adenosine to inosine and ammonia), 8-phenyltheophylline (8-PT, a non-selective antagonist of adenosine receptors) and glibenclamide (Gli, a potent blocker of ATP-sensitive K^+ channels) on anoxic pacemaker cells of SA node. **METHODS**: Anoxia of pacemaker cells in SA node of guinea-pig was induced by perfused for 20 min with a modified K-H solution gassed with 100 % N_2 deprived of glucose. Parameters of action potentials including 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 recorded using intracellular microelectrodes. **RESULTS**: Anoxia increased MDP, APA, and V_{max} and decreased VDD, RPF in a time-dependent manner. ADase 10 $U \cdot L^{-1}$, 8-PT 0.1 $\mu mol \cdot L^{-1}$ and Gli 10 $\mu mol \cdot L^{-1}$ significantly attenuated the electrophysiological changes of pacemaker cells in sincatrial node induced by anoxia. **CONCLUSION**: Endogenous adenosine and ATP-sensitive K^+ channels may play an important role in the generation of anoxic bradycardia in guinea pigs.

KEY WORDS anoxia; adenosine; adenosine deaminase; glyburide; potassium channels; sinoatrial node; electrophysiology

Endogenous adenosine is primarily formed from dephosphorylation of AMP that may occur intracellularly or extracellularly[1] and may be derived from hydrolysis of s-adenosylhomocysteine (SAH)[2]. In hypoxic myocardium, adenosine release from SAH hy-

Received 1993-07-12 Accepted 1994-06-06

[1] Project supported by the Natural Science Foundation of Hebei Province, № 393125.

drolysis did not increase[4], but that from AMP dephosphorylation increased[4]. During anoxia and ischemia, adenosine release increased about 50 times more than that during normoxia[5]. Our previous study suggested that the selective A_1 adenosine receptor agonists, N^6-cyclopentyladenosine (CPA) and [R]-N^6-[1-methyl-2-phenylethyl] adenosine (R-PIA) exerted a negative chronotropic action on the pacemaker cells in sinoatrial (SA) node of guinea pigs and their effects were adenosine-receptor dependent and mainly modulated by activation of ATP-sensitive K^+ channels coupled to adenosine receptors[6]. Hypoxia and ischemia also exhibited a negative chronotropic action on SA node[7,8]. We hypothesized that endogenous adenosine and ATP-sensitive K^+ channels were involved in the hypoxia-induced bradycardia. To examine this hypothesis, the electrophysiological effects of adenosine deaminase (ADase, an enzyme converting adenosine to inosine and ammonia), 8-phenyltheophylline (8-PT, a non-selective antagonist of adenosine receptors) and glibenclamide (Gli, a potent blocker of ATP-sensitive K^+ channels) on anoxic pacemaker cells in SA node of guinea pig were investigated.

MATERIALS AND METHODS

Guinea pigs of either sex weighing 0.38±*s* 0.06 kg were decapitated and the hearts were superfused with Krebs-Henseleit (K-H) solution. The right atrium was dissected carefully for the preparation of SA node. Preparations included the intercaval region and a small part of the interatrial septum but not the atrioventricular node. The upper part of the crista terminalis was cut to open the superior vena cava to expose the SA node. The preparation was mounted on a perforated silicon rubber block in a tissue bath.

The glass microelectrode was inserted into pacemaker cells in SA node. The transmembrane potential of pacemaker cells 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 microcomputer. Parameters of action potentials (AP) were stored into a diskette.

In the first part, electrophysiological effects of anoxia on pacemaker cells in SA node were examined. After recording 3 control AP, the preparation was perfused with a 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}) gassed with 100 % N_2 and deprived of glucose. The AP was recorded 1, 5, 10, 20 min after the anoxic situation. After anoxia for 20 min, some preparations were washed with the modified K-H solution with 100 % O_2 to observe the recovery of AP and others were continuously perfused with anoxic K-H solution until arrest of activity in pacemaker cells.

The second part was undertaken to assess the effects of ADase, 8-PT and Gli on pacemaker cells in SA node. The preparations were divided into 3 groups at random. (1) ADase treatment. The preparations were perfused with oxygenated K-H solution containing ADase (1, 5, and 10 U·L^{-1}) for 10 min and then superfused with anoxic K-H solution with ADase for 20 min. (2) 8-PT treatment (0.01, 0.05, and 0.1 μmol·L^{-1}). (3) Gli treatment (1, 5, and 10 μmol·L^{-1}). The protocols of 8-PT and Gli treated groups were similar to that of ADase treated group. The solvent and resources of 8-PT and Gli were as previously described[9] and ADase (Sigma) was diluted with the modified K-H solution.

The AP expressed as $\bar{x}\pm s$ were analyzed using *F* and *t* test.

RESULTS

Automaticity of anoxic pacemaker cells

In pacemaker cells of SA node superfused with the oxygenated K-H solution, RPF and VDD were 200±11 bpm and 89±8 mV·s^{-1}, respectively. After SA node was superfused

with the anoxic K-H solution. RPF was remarkably decreased in a time-dependent manner. Arrest of activity in pacemaker cells was induced by anoxia for 12 ± 8 min ($n = 8$). VDD did not show any significant changes after 1 min of anoxia but gradually reduced at 5, 10, and 20 min after anoxia. The effect of anoxia in producing a slowing of RPF was parallel to a decrease in VDD. RPF was the most sensitive to anoxia in all parameters examined (Tab 1).

As the preparations were first exposed to oxygenated K-H solution with ADase for 10 min and then superfused with anoxic K-H solution with ADase, the decreases in RPF and VDD induced by anoxia (20 min) were inhibited in a concentration-dependent manner. However, even if the highest concentration (10 $U \cdot L^{-1}$) of ADase was applied, the decreases in RPF and VDD were not completely abolished by ADase (Fig 1).

Similar to ADase, Gli attenuated but did not completely abolish the decreases in RPF and VDD induced by anoxia (20 min) in the 3 concentrations used (Tab 1). 8-PT showed a potent antagonistic effects on the reduction in RPF and VDD induced by anoxia (20 min). The highest concentration (0.1 $\mu mol \cdot L^{-1}$) of 8-PT fully antagonized the effects of anoxia on automaticity of pacemaker cells in SA node.

The relationship between RPF and anoxic time has been well established. ADase (10 $U \cdot L^{-1}$), 8-PT (0.1 $\mu mol \cdot L^{-1}$) and Gli (10 $\mu mmol \cdot L^{-1}$) could shift the relationship curve upwards, thus decreasing the slope of the curve (Fig 2).

Transmembrane potentials of pacemaker cells No significant changes of transmembrane potential variables were seen in anoxia at 1 min, but MDP, APA, and V_{max} were

Tab 1. Electrophysiological effects of adenosine deaminase (ADase), 8-phenyltheophylline (8-PT), and glibenclamide (Gli) on anoxic 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* **anoxia (20 min)**.

	MDP/mV	APA/mV	V_{max}/V·s^{-1}	VDD/mV·s^{-1}	RPF/bpm	APD_{50}/ms
Control	−45±5	48±4	2.4±0.9	89±8	200±11	206±15
Anoxia						
1 min	−47±4^a	52±4^a	3.6±1.2^a	81±6^a	180±12^b	193±10^b
5 min	−50±4^a	55±4^b	8.9±1.3^c	59±6^b	141±15^b	190±17^a
10 min	−59±3^c	64±4^c	11.2±3.0^c	34±10^c	110±19^c	192±21^a
20 min	−58±5^c	64±5^c	11.7±2.7^c	24±7^c	92±11	179±14^c
ADase ($U \cdot ml^{-1}$) + Anoxia (20 min)						
1	−56±7^{cd}	60±5^{cd}	10.9±2.6^{cd}	45±9^{ce}	123±10^{ce}	192±17^a
5	−54±5^{cd}	57±5^{be}	9.3±1.5^{cd}	52±4^{cd}	140±14^{be}	190±19^a
10	−52±4^{be}	56±3^{be}	6.7±0.8^{ce}	70±8^{bd}	166±13^{bf}	194±10^a
8-PT ($\mu mol \cdot L^{-1}$) + Anoxia (20 min)						
0.01	−55±6^{bd}	59±7^{bd}	10.3±1.1^{cd}	44±7^{ce}	126±10^{ce}	188±20^{bc}
0.05	−47±3^{ae}	52±4^{ae}	4.7±1.5^{be}	69±6^{bf}	168±13^{bf}	196±13^{ad}
0.1	−46±4^{af}	50±5^{af}	2.8±0.8^{af}	80±11^{af}	186±11^{af}	194±14^{ae}
Gli ($\mu mol \cdot L^{-1}$) + Anoxia (20 min)						
1	−54±5^{bd}	57±5^{bc}	11.0±2.4^{ce}	39±7^{ce}	121±14^{ce}	192±14^{ad}
5	−49±6^{ae}	52±4^{ae}	5.7±1.7^{ce}	64±6^{bd}	163±12^{bf}	194±11^{ad}
10	−47±4^{af}	50±3^{af}	3.8±1.1^{af}	72±9^{bf}	176±17^{bf}	197±18^{ad}

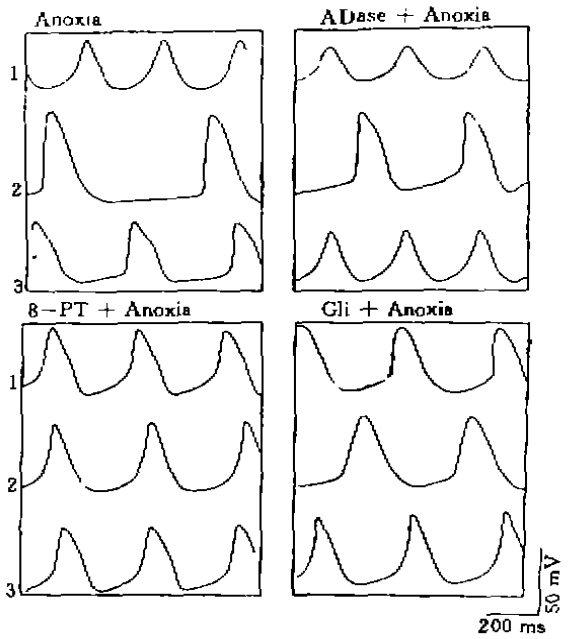

Fig 1. Inhibitory effects of ADase (10 U·L^{-1}), 8-PT (0.1 μmol·L^{-1}) and Gli (Gli 10 μmol·L^{-1}) on the electrophysiological responses of pacemaker cells in SA node of guinea pig to anoxia 20 min. 1) control, 2) treatment, 3) washout for 30 min.

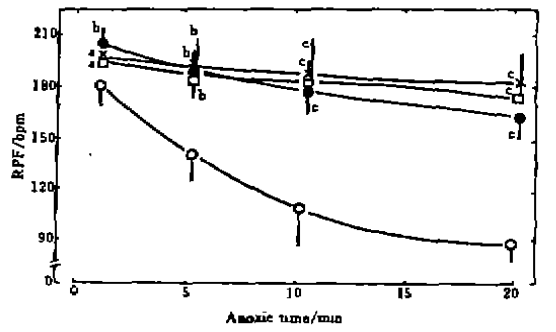

Fig 2. Inhibitory effects of ADase, 8-PT and Gli on anoxia-induced slowing of RPF. $n = 8$, $\bar{x} \pm s$. Anoxia (○); ADase (10 U·L^{-1}) + anoxia (●); 8-PT (0.1 μmol·L^{-1}) + anoxia (△); Gli (10 μmol·L^{-1}) + anoxia (□). $^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* anoxia.

gradually increased by anoxia at 5, 10, and 20 min. APD_{90} was decreased by anoxia only at 20 min (Tab 1).

ADase 10 U·L^{-1} inhibited the increases in APA and V_{max}, and the decrease in MDP of the pacemaker cells induced by anoxia (20 min). ADase 1 U·L^{-1} did not show any observable effect. The decrease in APD_{90} induced by anoxia was not obviously affected by ADase at all 3 concentrations ($P > 0.05$).

High concentrations of 8-PT (0.1 μmol·L^{-1}) and Gli (10 μmol·L^{-1}) also inhibited the changes of transmembrane potentials induced by anoxia (except for APD_{90}), more potent than those of ADase ($P < 0.05$).

DISCUSSION

In our previous studies, adenosine analogue—CPA exerted a negative chronotropic action on the pacemaker cells in SA node of guinea pigs[6]. The present study indicated that the anoxia-induced endogenous adenosine release might also play an important role in the development of anoxia-induced bradycardia in guinea pigs. This concept was supported by the following facts: 1) The changes of electrophysiological variables of pacemaker cells in SA node of guinea pigs induced by anoxia were similar to those of CPA (selective adenosine A_1-receptor agonists)[2] in the same species. 2) ADase, an enzyme converting adenosine to inosine and ammonia showed the significant antagonistic effect on the electrophysiological changes of pacemaker cells in SA node induced by anoxia. 3) 8-PT, an adenosine receptor blocker completely abolished the anoxia-induced electrophysiological changes of pacemaker cells in SA node.

Gli, a drug known to inhibit ATP-sensitive K^+ channels[10,11], attenuated the bradycardia induced by exogenous adenosine and its analogues *in vivo* and *in vitro*[8,9]. Depletion or reduction of intracellular ATP, as might appear during anoxic or ischemic stress, could open ATP-sensitive K^+ channels in cardiac muscles[12]. Our present results that Gli inhibited the electrophysiological changes of

pacemaker cells in SA node induced by anoxia suggested that ATP-sensitive K^+ channels might also modulate the above-mentioned changes. ATP-sensitive K^+ channels may be coupled to adenosine receptor via GTP-binding proteins[13]. Thus, it was assumed that anoxia induced the increase of endogenous adenosine and the reduction of ATP, thereby activating the ATP-sensitive K^+ channels which might be involved in the electrophysiological changes of pacemaker cells induced by anoxia.

REFERENCES

1 Worku Y, Newby AC. The mechanism of adenosine production in rat polymorphonuclear leukocytes. Biochem J 1983; **214**: 325—30.

2 Achterberg PW, de Tombe PP, Harmsen E, de Jong JW. Myocardial s-adenosylhomocysteine hydrolase is important for adenosine production during normoxia. Biochim Biophys Acta 1985; **840**: 393—400.

3 Deussen A, Möser G, Schrader J. Contribution of coronary endothelial cells to cardiac adenosine production. Pflugers Arch 1986; **406**: 608—14.

4 Bardenheuer H, Whelton B, Sparks HV Jr. Adenosine release by the isolated guinea pig heart in response to isoproterenol, acetylcholine, and acidosis; the minimal role of the vascular endothelium. Circ Res 1987; **61**: 594—600.

5 Kitakaze M, Hori M, Kamada T. Role of adenosine and its interaction with α-adrenoceptor activity in ischaemic and reperfusion injury of the myocardium. Cardiovasc Res 1993; **27**: 18—27.

6 Li YL, He RR. Electrophysiological effects of N^6-cyclopentyl-adenosine and [−]-N^6-[phenylisopropyl]-adenosine on pacemaker cells in sinoatrial node of guinea pigs. Acta Pharmacol Sin 1993; **14**: 414—17.

7 Baba N, Leighton RF, Weissler AM. *Experimental cardiac ischemia.* Observation of the sinoatrial and atrioventricular nodes. Lab Invest 1970; **23**: 168—78.

8 Senges J, Mizutani T, Pelzer D, Brachmann J, Sonnhof U, Kubler W. Effect of hypoxia on the sinoatrial node, atrium, and *atrioventricular node in the rabbit heart.* Circ Res 1979; **44**: 856—63.

9 Li YL, He RR. Effects of 8-phenyltheophylline and glibenclamide on cardiovascular responses to adenosine and [−]-N^6(phenylisopropyl)adenosine in anesthetized rats. Chin J Physiol Sci 1994; **10** (2): 97—104.

10 Ashcroft FM. Adenosine 5′-triphosphate-sensitive potassium channels. Annu Rev Neurosci 1988; **11**: 97—118.

11 Sturgess NC, Ashford MLJ, Cook DL, Hales CN. The sulphonylurea receptor may be an ATP-sensitive potassium channel. Lancet 1985; **2**: 474—5.

12 Noma A, Shibasaki T. Membrane current through adenosine-triphosphate-regulated potassium channels in guinea-pig ventricular cells. J Physiol (Lond) 1985; **363**: 463—80.

13 Kirsch GE, Codina J, Birnbaumer L, Brown AM. Coupling of ATP-sensitive K^+ channels to A_1 receptors by G proteins in rat ventricular myocytes. Am J Physiol 1990; **259** (3 Pt 2): H820—26.

内源性腺苷和 K_{ATP} 通道介导缺氧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)

目的：观察腺苷脱氨酶(ADase)，8-苯茶碱(8-PT)和格列苯脲(Gli)在豚鼠缺氧窦房结起搏细胞的电生理效应。 **方法**：以充有100 %氮和无糖的K-H液灌流豚鼠窦房结20 min引起其缺氧。用玻璃微电极技术记录起搏细胞的MDP，APA，APD_{90}，V_{max}，RPF和VDD等动作电位参数。 **结果**：缺氧增加起搏细胞APA，MDP和V_{max}，但减小VDD和RPF。Adase 10 U·L^{-1}，8-PT 0.1 μmol·L^{-1}和Gli 10 μmol·L^{-1}明显缓解缺氧引起的电生理效应。**结论**：内源性腺苷和K_{ATP}通道在缺氧所致窦房结起搏细胞电生理效应中起重要作用。

关键词 缺氧症；腺苷；腺苷脱氨酶；格列苯脲；钾通道；窦房结；电生理学