ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jul; 23 (7): 627 - 630
http://www.chinaphar.com

Effects of diacetyl guan-fu base A on pacemaker cells in sinoatrial node of rabbits[1]

SUN Hua, ZHANG Qing-Zhu[2], WANG Ru-Bin[3], JI Jian-Bo
(*Department of Pharmacology*; [3]*Department of Organic Chemistry*, *College of Pharmacy*, *Shandong University*, *Jinan 250012*, *China*)

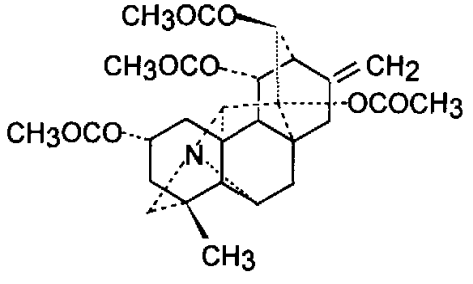
KEY WORDS diacetyl guan-fu base A; sinoatrial node; electrophysiology; action potentials

ABSTRACT

AIM: To study the electrophysiological effects of diacetyl guan-fu base A (DGFA) on pacemaker cells in sinoatrial (SA) node. **METHODS**: Intracellular microelectrode method was used to record parameters of action potential (AP) in SA node of rabbits. **RESULTS**: DGFA could not only slow down spontaneous firing frequency (SFF), mean rate of repolarization (MRR), and rate of diastolic depolarization (RDD), but also prolong diastolic interval (DI) and duration of action potential (APD) in a concentration-dependent manner in SA node. Furthermore, DGFA markedly decreased the maximum rate of depolarization (MRD) with a slight reduce of the amplitude of action potential (APA) and there was no significant effect on the maximal diastolic potential (MDP). The decrease in SFF caused by DGFA was not affected by atropine (0.05 mg/L). **CONCLUSION**: The effects might be due to the reduction of calcium influx and potassium efflux, and the muscarinic receptors were not involved.

INTRODUCTION

Diacetyl guan-fu base A (DGFA), a diacetyl derivative of guan fu base A (GFA) isolated from the root of *Aconitum coreanum*[1], has been proved to be effective in several experimental arrhythmic models[2]. DGFA can significantly antagonize aconitine-induced arrhythmia, $CaCl_2$-induced incidence of ventricular fibrillation, and the arrhythmia induced by coronary ligation in rats, and markedly raise the ventricular fibrillation threshold to electrical stimulation in rabbits[3]. DGFA can also decrease the action potential amplitude and the maximal rate of depolarization of guinea pig papillary muscles[4]. Recently, it has been found that DGFA could block the fast Na^+ channels in isolated ventricular myocytes. However, the effects of DGFA on sinoatrial (SA) node have not been elucidated. The purpose of the present study was to investigate the electrophysiological effects of DGFA on pacemaker cells in SA node and its mechanism.



Diacetyl guan-fu base A

METERIALS AND METHODS

Drugs and agents DGFA was provided by Department of Organic Pharmaceutics, College of Pharmacy, Shandong University. Verapamil was presented by Prof Qian Jia-Qing (Department of Pharmacology, School of Tongji, Huazhong Science and Technology University, Wuhan, China). Atropine was purchased from Sigma. All drugs were dissolved in distilled water.

Preparation Rabbits (♂♀, weighing 2.4 kg ± 0.3 kg, Certificate № 19-025, provided by Experimental Animal Center of Huazhong Science and Technology

[1] Project supported by the Science and Technology Committee of Shandong Province, № 4138842.
[2] Correspondence to Prof ZHANG Qing-Zhu.
Phn 86-531-294-2542. Fax 85-531-2942-019.
E-mail zhangqzh@sd.edu.cn
Received 2001-11-12 Accepted 2002-04-22

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jul; 23 (7)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

University) were stunned by heawy blow on the head and the hearts were superfused with Tyrode's solution. The right atrium was carefully dissected 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[5,6]. The preparations were removed and immediately perfused with Tyrode's solution of the following composition: NaCl 136.9, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42 and glucose 5.55 mmol/L. This solution was maintained at 35 ℃ ± 0.5 ℃ and continuously equili-brated with 95 % O_2 + 5 % CO_2.

Electrophysiological measurements The transmembrane action potential (AP) was recorded from pacemaker cells in SA node with a glass microelectrode filled with KCl 3 mol/L (a tip resistance of 20 – 50 MΩ), coupled to a high input impedance amplifier (MEZ-8021, Nihon Kohden, Japan). The amplified signals were displayed and fed to computer (IBM/PC 386) *via* A/D, D/A converter. Maximum rate of depolarization (MRD), amplitude of action potential (APA), maximal diastolic potential (MDP), mean rate of repolarization (MRR), rate of diastolic depolarization (RDD), and duration of action potential (APD) were analyzed by the microcomputer system (designed by Huazhong Institute of Science and Technology). Parameters of AP were stored into diskettes.

Experimental protocols The preparation was equilibrated for 60 min in Tyrode's solution at 10 mL/min before intracellular recordings. AP was recorded respectively before administration of drugs as control. The preparation was washed with Tyrode's solution to observe the recovery of AP.

The experiments consisted of three groups: (1) The electrophysiological effects of DGFA (4 μmol/L) and verapamil (0.5 μmol/L) on pacemaker cells in SA node. (2) The concentration-response relation of DGFA on SA node pacemaker cells. DGFA 0.3, 1, 3, and 10 μmol/L were cumulatively added to the bath at 15 min interval. AP was recorded at 15 min after administration. (3) Effect of atropine (Atr) on negative chronotropic action of DGFA. The preparation was perfused with Tyrode's solution containing atropine for 30 min, then with Tyrode's solution containing DGFA (0.3, 1, 3, 10 μmol/L). DGFA (0.3, 1, 3, 10 μmol/L) were cumulatively added to the bath at 15 min interval and AP was recorded at 15 min after administration of drugs.

Statistics All data were presented as $\bar{x} \pm s$ and compared with t test. $P < 0.05$ was considered statistically significant.

RESULTS

Effects of DGFA and verapamil on AP of pacemaker cells in SA node At 20 min of perfusion with Tyrode's solution containing DGFA 4 μmol/L, MRD, MRR, RDD, and SFF were slowed down by 34.8 % ($P < 0.01$), 24.3 % ($P < 0.05$), 19.5 % ($P < 0.05$), and 12.5 % ($P < 0.01$), respectively. Furthermore, DI, APD_{50}, APD_{90}, and APD_{100} were prolonged by 20.0 % ($P < 0.01$), 15.1 % ($P < 0.05$), 10.9 % ($P < 0.05$), and 17.5 % ($P < 0.01$), respectively, followed by a slight decrease of APA (Tab 1, Fig 1).

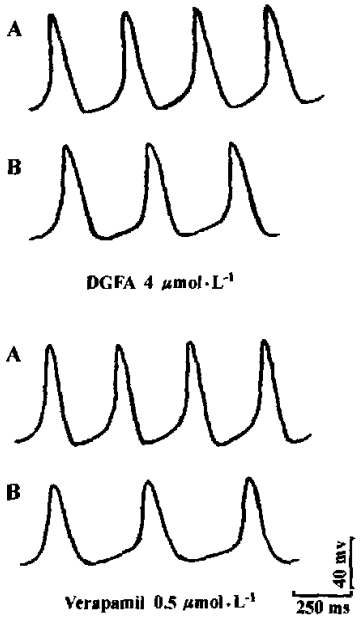


Fig 1. Effects of diacetyl guan fu base A and verapamil on the action potential of dominant pacemaker cells in rabbit sinoatrial node. A: control; B: after treatment of drugs.

At 15 min of perfusion with verapamil 0.5 μmol/L, MRD, MRR, RDD, and SFF were decreased by 47.1 % ($P < 0.05$), 28.6 % ($P < 0.01$), 21.5 % ($P < 0.05$), and 24.5 % ($P < 0.05$), respectively, and DI, DDT, APD_{50}, APD_{90}, and APD_{100} were increased.

Tab 1. Effects of diacetyl guan fu base A 4 μmol/L and verapamil 0.5 μmol/L on the action potential of the dominant pacemaker cells in rabbit sinoatrial node. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

	20 min after		15 min after	
	Control ($n = 8$)	DGFA ($n = 8$)	Control ($n = 7$)	Verapamil ($n = 7$)
APA/mv	63 ± 8	55 ± 9[a]	61 ± 14	42 ± 15[b]
MDP/mv	−58 ± 10	−57 ± 9[a]	−59 ± 12	−57 ± 14
SFF/beat·min^{-1}	168 ± 14	147 ± 13[c]	178 ± 27	143 ± 10[c]
MRD/v·s^{-1}	4.6 ± 1.1	3.0 ± 0.7[c]	3.4 ± 1.2	1.8 ± 1.1[b]
MRR/v·s^{-1}	0.70 ± 0.16	0.53 ± 0.12[b]	0.91 ± 0.10	0.65 ± 0.18[c]
RDD/mv·s^{-1}	70 ± 14	56 ± 10[b]	74 ± 16	58 ± 10[b]
DI/ms	381 ± 41	458 ± 42[c]	341 ± 38	414 ± 39[c]
DDT/ms	276 ± 15	292 ± 19[a]	240 ± 27	280 ± 40[b]
APD_{50}/ms	60 ± 9	69 ± 7[b]	56 ± 8	65 ± 7[b]
APD_{90}/ms	90 ± 10	100 ± 8[b]	82 ± 9	92 ± 4[b]
APD_{100}/m	127 ± 13	150 ± 12[c]	121 ± 13	142 ± 11[c]

Verapamil significantly slowed down APA by 31.1 % ($P < 0.05$), which was different from DGFA (Tab 1, Fig 1).

The concentration-response relationship of DGFA on SA node pacemaker cells Added cumulatively to the bath at 15 min intervals, DGFA 0.3 μmol/L caused gentle changes of the parameters of AP, while DGFA 1, 3, 10 μmol/L slowed down MRD, RDD, SFF, and prolonged APD_{50}, APD_{90} in a concentration-dependent manner. At DGFA 10 μmol/L, APD_{50}, APD_{90} had a shortened trend compared with DGFA 1, 3 μmol/L (Tab 2).

Effect of atropine on negative chronotropic action of DGFA After perfusion with Tyrode's solution containing atropine 0.05 mg/L for 30 min, the effect of DGFA (0.3, 1, 3, 10 μmol/L) on SFF in SA node pacemaker cells was not markedly changed (Fig 2).

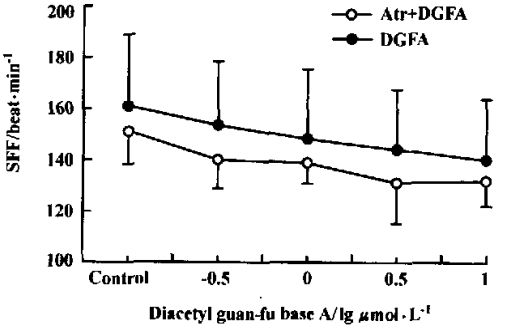


Fig 2. Effects of atropine (Atr) on negative chronotropic action of DGFA in rabbit sinoatrial node. $n = 7$. $\bar{x} \pm s$.

DISCUSSION

Many investigations had demonstrated that DGFA remarkably antagonized several experimental arrhythmia, increased the threshold of ventricular fibrillation and decreased the maximal rate of depolarization of action potential in ventricular myocytes. Ding *et al* found that DGFA might produce its effects by depression on influx of Na^+ and Ca^{2+}[2,3]. By the whole cell patch-clamp

Tab 2. The concentration-response relationship of DGFA on the action potential of the dominant pacemaker cells in rabbit sinoatrial node. $n = 7$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Group/μmol·L^{-1}		APA/mv	MRD/v·s^{-1}	RDD/mv·s^{-1}	SFF/beat·min^{-1}	APD_{50}/ms	APD_{90}/ms
Control		63 ± 6	5.3 ± 1.0	64 ± 10	174 ± 20	63 ± 5	95 ± 11
DGFA	0.3	61 ± 8[a]	4.8 ± 2.1[a]	58 ± 18[a]	166 ± 20[a]	67 ± 7[a]	100 ± 16[a]
	1	61 ± 5[a]	4.2 ± 0.8[b]	51 ± 8[b]	151 ± 19[b]	70 ± 8[a]	106 ± 12[a]
	3	57 ± 5[a]	4.0 ± 1.1[b]	50 ± 14[b]	148 ± 18[b]	74 ± 6[c]	109 ± 10[b]
	10	54 ± 8[b]	3.8 ± 1.0[b]	49 ± 9[b]	144 ± 16[c]	72 ± 9[b]	107 ± 12[b]

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jul; 23 (7)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

technique, our laboratory had demonstrated that DGFA significantly blocked Na^+ influx. It is well known that Ca^{2+} current plays an important role in the phase 0 depolarization and the phase 4 spontaneous diastolic depolarization in pacemaker cells of SA node[7,8]. The present study showed that MRD and RDD were markedly decreased by DGFA, indicating that inhibitory effects of DGFA on depolarization process of pacemaker cells in SA node might be due to the blockade of Ca^{2+} influx. As the process of repolarization of pacemaker cells mainly depended on K^+ efflux[9], DGFA caused a significant prolong of APD_{50}, APD_{90} in a concentration-dependent manner, suggesting that the effect might be related to a reduction in K^+ efflux. In addition, Sakmann and his colleagues demonstrated that muscarinic receptor was important in regulating normal cardiac rhythm and SA node function[10,11]. The results that atropine could not block the effects of DGFA suggested the action of DGFA on SA node was unrelated to muscarinic receptor. We concluded that DGFA exerted a negative chronotropic action and induced a delayed repolarization of pacemaker cells in SA node, which might be due to the blockade of Ca^{2+} influx and K^+ efflux.

REFERENCES

1 Wang YP, Chen WZ. Effects of guan-fu base A on spontaneous rate of isolated sinoatrial node of guinea pig. Acta Pharmacol Sin 1990; 11: 502-5.
2 Ding JX, Zhang SL, Wang RB. Effects of diacetyl guan fu base A on experimental arrhythmia. Chin J Pharm 1996; 27: 165-9.
3 Ding JX, Zhang SL, Wang RB. Effect of diacetyl guan fu base A on isolated rat atrial muscle. Chin Pharmacol Bull 1997; 13: 541-4.
4 Fan Y, Zhang SL, Wang RB. Effects of diacetyl guan fu base A on isolated papillary muscles of guinea pig. Qianwei Pharm Sin 1999; 13: 45-8.
5 Brown HF. Electrophysiology of the sinoatrial node. Physiol Rev 1982; 62: 505-30.
6 Bleeker WK, Mackaay AJC, Masson-Pevet M, Bouman LN, Becker AE. Functional and morphological organization of the rabbit sinus node. Circ Res 1980; 46: 11-21.
7 Lin XG, Chen HZ. Antiarrhythamic drugs. In: Chen X, Chen WZ, Zeng GY, editors. Cardiovascular pharmacology. 2nd ed. Beijing: People's Medical Publishing House; 1997. p 422-7.
8 Vassalle M. The pacemaker current (If) does not play an important role in regulating SA node pacemaker activity. Cardiovasc Res 1995; 30: 309-10.
9 Verheijck EE, van Cinneken AC, Bourier J. Effects of delayed rectifier current blockade by E-4031 on impulse generation in single sinoatrial node myocytes of the rabbit. Circ Res 1995; 76: 607-15.
10 Sakmann B, Noma A, Trautwein W. Acetylcholine activation of single muscarinic K^+ channels in isolated pacemaker cells of the mammalian heart. Nature 1983; 303: 250-3.
11 Zhong N, Qian JQ. Modulation by muscarinic receptor antagonists on negative chronotropic effects of tetrandrine. Acta Pharmacol Sin 1999; 20: 1068-72.

关附乙酯对兔窦房结起搏细胞的作用[1]

R96 A

孙 华，张庆柱[2]，王如斌[3]，纪建波
(山东大学药学院药理学教研室；[3]有机化学教研室，济南 250012，中国)

关键词 关附乙酯；窦房结；电生理学；动作电位

目的：研究关附乙酯(DGFA)对窦房结起搏细胞电生理学的影响. **方法**：采用细胞内微电极技术记录家兔窦房结动作电位(AP)参数. **结果**：DGFA 不仅能够减慢自主性激发频率(SFF)，平均复极化速率(MRR)，舒张期除极化速率(RDD)，而且以剂量依赖性方式延长窦房结舒张期间隔(DI)和动作电位时程(APD). 此外，DGFA 显著降低除极化最大速率(MRD)，并伴有动作电位幅度(APA)的轻微下降，对最大舒张电位(MDP)无显著作用. DGFA 降低自主性激发频率的作用不受阿托品(0.05 mg/L)的影响. **结论**：DGFA 这些作用可能是通过减少钙离子内流及钾离子外流产生的，而非阻断 M 受体.

(责任编辑 韩向晖)