

Electrophysiologic effects of agmatine on pacemaker cells in sinoatrial node of rabbits

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KEY WORDS agmatine; sinoatrial node; electrophysiology; imidazoles; alpha 2- adrenergic receptors; calcium channels; potassium channels

ABSTRACT

AIM: To study the electrophysiologic effects of agmatine (Agm) on pacemaker cells in sinoatrial (SA) node. **METHODS:** Parameters of action potential (AP) in SA node were recorded using intracellular microelectrode technique. **RESULTS:** Agm not only slowed down the amplitude of action potential (APA), maximal rate of depolarization (V_{max}), velocity of diastolic (phase 4) depolarization (VDD), and rate of pacemaker firing (RPF), but also prolonged 90 % duration of action potential (APD_{90}) in a concentration-dependent manner. The effects of Agm ($10 \text{ mmol} \cdot \text{L}^{-1}$) could be blocked completely by pretreatment with idazoxan ($0.15 \text{ mmol} \cdot \text{L}^{-1}$), an α_2 -adrenergic receptor (α_2 -AR) and imidazoline receptor (IR) antagonist. Pretreatment with *N*^G-nitro-*L*-arginine methyl ester (*L*-NAME, $1 \text{ mmol} \cdot \text{L}^{-1}$), an NOS inhibitor, did not affect the electrophysiologic effects of Agm on pacemaker cells in SA node. Elevation of Ca^{2+} concentration ($5 \text{ mmol} \cdot \text{L}^{-1}$) in perfusate antagonized the effects of Agm ($10 \text{ mmol} \cdot \text{L}^{-1}$). Lemakalim (Lem, $30 \mu\text{mol} \cdot \text{L}^{-1}$), an opener of ATP-sensitive potassium channels, partially inhibited the prolonging effect of Agm on repolarization. **CONCLUSION:** The electrophysiologic effects of Agm on pacemaker cells in SA node were likely attributed to the reduction in calcium influx and potassium efflux and mediated by α_2 -AR and IR.

INTRODUCTION

Agmatine (Agm) has been identified as an endogenous clonidine-displacing substance (CDS) in mammalian brain. Agmatine, an endogenous agonist at imidazoline receptors (IR) and a noncatecholamine ligand at α_2 -adrenergic receptor (α_2 -AR), may act as a neurotransmitter^[1]. It has been known that Agm was widely distributed in mammalian tissues including heart, blood vessels, and brain^[2], suggesting that Agm could act on cardiovascular system and nervous center^[3]. Recently, we have found that Agm (iv) decreased heart rate, blood pressure, cardiac output, and myocardial contractility in the anesthetized rats^[4], which are consistent with the results from Gao *et al*^[5]. Agmatine could also reduce calcium influx of AP in guinea pig papillary muscles *in vitro*^[6]. However, the effects of Agm on SA node has not yet been elucidated. The purpose of the present study was to investigate the electrophysiologic effects of Agm on SA node and its action mechanism(s).

MATERIALS AND METHODS

Preparation Rabbits ($\uparrow \uparrow$, $n = 25$, weighing $2.4 \pm s 0.3 \text{ kg}$, grade II, Certificate No 04037, provided by Experimental Animal Center of Hebei Province) were stunned by heavy blow on the head 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 atrio-ventricular node. The upper part of the crista terminalis was cut to open the superior vena cava to expose the SA node. The preparation were removed and immediately perfused with K-H solution of the following composition: NaCl 118.0, NaHCO_3 25.0, KCl 4.8, MgSO_4 1.6, CaCl_2 1.8, and

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glucose $11.1 \text{ mmol} \cdot \text{L}^{-1}$. This solution was buffered to $\text{pH } 7.39 \pm 0.03$ by saturation with 100 % O_2 gas, and temperature was maintained at $35.0 \pm 0.5 \text{ }^\circ\text{C}$.

Electrophysiological measurements The transmembrane AP was recorded from pacemaker cells in SA with a glass microelectrode filled with $\text{KCl } 3 \text{ mol} \cdot \text{L}^{-1}$ (a tip resistance of $10 - 30 \text{ M}\Omega$), coupled to a high input impedance amplifier (MEZ 8201, Nihon Kohden). The amplified signals were fed to the A/D convertor and processed by a microcomputer. Maximal diastolic potential (MDP), amplitude of action potential (APA), 90 % of duration of action potential (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 AP were stored into a diskette.

Experimental protocols The preparation was equilibrated for 60 min in the K-H solution at $4 \text{ mL} \cdot \text{min}^{-1}$ before intracellular recordings. The AP was recorded before and at 1, 5, 10, 20, and 30 min after application of drugs. The changes of AP were recorded 10 min after application of Agm. The preparation was washed with the K-H solution to observe the recovery of AP.

The experiments consisted of 5 groups: (1) The electrophysiologic effects of Agm on SA node pacemaker cells. The animals were divided into 5 subgroups: control, Agm 1, 5, 10, 15 $\text{mmol} \cdot \text{L}^{-1}$; (2) Effect of idazoxan ($0.15 \text{ mmol} \cdot \text{L}^{-1}$) on the electrophysiologic response of pacemaker cells to Agm ($10 \text{ mmol} \cdot \text{L}^{-1}$); (3) Effect of *L*-NAME ($1 \text{ mmol} \cdot \text{L}^{-1}$) on the electrophysiologic response of pacemaker cells to Agm ($10 \text{ mmol} \cdot \text{L}^{-1}$); (4) The effect of high Ca^{2+} ($5 \text{ mmol} \cdot \text{L}^{-1}$) on the actions of Agm; (5) Effect of ATP-sensitive potassium channel opener lemakalim (Lem, $30 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) on the Agm-induced changes of repolarization of pacemaker cells in SA node. In subgroup 2 - 5, the effects of Agm were observed at 10 min following pretreatment with idazoxan, *L*-NAME, high Ca^{2+} , and Lem, respectively.

Drugs Agm, idazoxan, and *L*-NAME (Sigma Co, USA). Lemakalim (Leo Pharmaceutical Products Ltd, Denmark). Agmatine, idazoxan, and *L*-NAME were dissolved in distilled water. Lemakalim was dissolved in 99 % ethyl alcohol and diluted in the

distilled water.

Statistical analysis Data were presented as $\bar{x} \pm s$ and analyzed by ANOVA or by *t*-test.

RESULTS

Effects of Agm on automaticity of pacemaker cells in SA node VDD was slowed down by Agm in a concentration-dependent manner (Tab 1). RPF began to decrease after 5 min of perfusion with K-H solution containing Agm $10 \text{ mmol} \cdot \text{L}^{-1}$. The changes in RPF induced by Agm paralleled to those of VDD. The effects of Agm $10 \text{ mmol} \cdot \text{L}^{-1}$ on VDD and RPF could be blocked completely by pretreatment with idazoxan $0.15 \text{ mmol} \cdot \text{L}^{-1}$, but not by *L*-NAME $1 \text{ mmol} \cdot \text{L}^{-1}$ (Tab 2, Fig 1).

Elevation of Ca^{2+} concentration ($5 \text{ mmol} \cdot \text{L}^{-1}$) in perfusate decreased the inhibitory actions of Agm ($10 \text{ mmol} \cdot \text{L}^{-1}$) on the automaticity of pacemaker cells in SA node (Tab 2).

Effects of Agm on transmembrane potentials of pacemaker cells in SA node Agm $5 \text{ mmol} \cdot \text{L}^{-1}$ only induced a slight increase in APD_{90} , while at 10, 15 $\text{mmol} \cdot \text{L}^{-1}$ induced a marked increase in APD_{90} and decreased V_{max} and APA in a concentration-dependent manner (Tab 1). Idazoxan ($0.15 \text{ mmol} \cdot \text{L}^{-1}$) could completely block the above-mentioned effects induced by Agm ($10 \text{ mmol} \cdot \text{L}^{-1}$), but *L*-NAME ($1 \text{ mmol} \cdot \text{L}^{-1}$) could not (Tab 2). Elevation of Ca^{2+} concentration in perfusate could also antagonized the actions of Agm on pacemaker cells (Tab 2).

Effects of lemakalim on response of repolarization of pacemaker cells to Agm Lemakalim ($30 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$), an ATP-sensitive potassium channel opener, could partially antagonize the prolonged repolarization of AP of the pacemaker cells induced by Agm $10 \text{ mmol} \cdot \text{L}^{-1}$. Agmatine at $10 \text{ mmol} \cdot \text{L}^{-1}$ induced an increase in APD_{90} from 177 ± 19 to $203 \pm 18 \text{ ms}$ ($n = 5$, $P < 0.01$), with a net change of $25.8 \pm 3.8 \text{ ms}$. While pretreatment with Lem $30 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$, Agm increased APD_{90} from 177 ± 19 to $187 \pm 17 \text{ ms}$ with a net change of $9.6 \pm 2.6 \text{ ms}$, which was significantly different from that induced by Agm alone ($P < 0.01$).

Tab 1. Electrophysiologic effects of agmatine (Agm) on pacemaker cells in rabbit sinoatrial node.
 $n = 5$, $\bar{x} \pm s$. ^a $P > 0.05$, ^b $P < 0.05$, ^c $P < 0.01$ vs control.

	MDP/mV	APA/mV	$V_{max}/V \cdot s^{-1}$	VDD/mV·s ⁻¹	RPF/beat·min ⁻¹	APD ₅₀ /ms
Control	-59 ± 7	64 ± 8	4.5 ± 1.2	53 ± 8	147 ± 10	169 ± 17
Agm/mmol·L ⁻¹						
1	-58 ± 6 ^a	63 ± 7 ^a	4.5 ± 1.2 ^a	54 ± 9 ^a	147 ± 16 ^a	170 ± 17 ^a
5	-58 ± 6 ^a	62 ± 6 ^a	4.4 ± 1.2 ^a	52 ± 10 ^a	143 ± 12 ^a	175 ± 16 ^b
10	-56 ± 6 ^a	59 ± 6 ^b	3.3 ± 0.9 ^c	46 ± 8 ^c	120 ± 9 ^c	195 ± 18 ^c
15	-54 ± 7 ^a	58 ± 7 ^b	2.9 ± 1.0 ^c	41 ± 7 ^c	110 ± 8 ^c	220 ± 20 ^c

Tab 2. Effects of idazoxan (Ida, 0.15 mmol·L⁻¹), L-NAME (1 mmol·L⁻¹), and high Ca²⁺ (5 mmol·L⁻¹) on the agmatine (Agm, 10 mmol·L⁻¹)-induced electrophysiologic changes of pacemaker cells in rabbit sinoatrial node.
 $n = 15$. $\bar{x} \pm s$. ^a $P > 0.05$, ^b $P < 0.05$, ^c $P < 0.01$ vs control.

	MDP/mV	APA/mV	$V_{max}/V \cdot s^{-1}$	VDD/mV·s ⁻¹	RPF/beat·min ⁻¹	APD ₅₀ /ms
Control	-59 ± 7	63 ± 6	4.3 ± 1.1	55 ± 11	146 ± 13	172 ± 20
Agm	-56 ± 8 ^a	59 ± 6 ^b	3.5 ± 0.8 ^c	42 ± 7 ^b	121 ± 13 ^c	199 ± 15 ^c
Ida	-58 ± 6 ^a	63 ± 7 ^a	4.2 ± 0.8 ^a	54 ± 10 ^a	141 ± 12 ^a	178 ± 18 ^a
Ida + Agm	-57 ± 6 ^a	62 ± 6 ^a	4.1 ± 1.0 ^a	52 ± 12 ^a	139 ± 12 ^a	178 ± 18 ^a
Control	-61 ± 9	65 ± 10	4.8 ± 1.4	57 ± 9	149 ± 15	168 ± 17
Agm	-58 ± 7 ^a	61 ± 8 ^b	3.5 ± 1.0 ^c	47 ± 7 ^c	126 ± 13 ^c	196 ± 19 ^c
L-NAME	-58 ± 7 ^a	63 ± 7 ^a	4.8 ± 1.1 ^a	57 ± 7 ^a	150 ± 16 ^a	167 ± 16 ^a
L-NAME + Agm	-55 ± 5 ^a	59 ± 5 ^b	3.7 ± 0.8 ^b	45 ± 8 ^c	126 ± 14 ^c	198 ± 16 ^c
Control	-64 ± 9	69 ± 8	4.9 ± 1.3	48 ± 9	141 ± 12	176 ± 19
Agm	-60 ± 7 ^a	65 ± 8 ^b	3.7 ± 1.1 ^c	39 ± 9 ^c	122 ± 9 ^c	202 ± 17 ^c
High Ca ²⁺	-65 ± 7 ^a	70 ± 8 ^a	5.3 ± 1.3 ^b	52 ± 10 ^b	148 ± 14 ^b	173 ± 19 ^a
High Ca ²⁺ + Agm	-62 ± 8 ^a	67 ± 9 ^a	4.6 ± 1.0 ^a	45 ± 10 ^a	137 ± 12 ^a	181 ± 16 ^a

DISCUSSION

The present study demonstrated that Agm could concentration-dependently decrease V_{max} , APA, VDD, and RPF of AP in pacemaker cells in SA node of rabbits. Based on the fact that the action potential upstroke of pacemaker cells in SA node is generated by I_{Ca} , the decrease in APA may be attributed to the reduction of I_{Ca} . Likewise, the decrease of VDD, and RPF may result from the reduction in I_{Ca} ^[7,8]. Elevation of calcium concentration in perfusate antagonized the above-mentioned inhibitory effects of Agm. Therefore, it indicated that the inhibitory effects of Agm on V_{max} , APA, VDD, and RPF of pacemaker cells might be due to the blockade of calcium influx, which was consistent with the results of our recent report^[6].

Likungu *et al* showed that there were α_2 -AR and IR in the heart and noradrenaline released from the

human heart was inhibited not only *via* presynaptic α_2 -AR but also *via* presynaptic IR^[9]. As an α_2 -AR and IR antagonist^[10], idazoxan markedly inhibited the electrophysiologic effects of Agm on pacemaker cells in SA node, suggesting that α_2 -AR and IR were involved in the effects of Agm on pacemaker cells.

Agmatine might be a precursor for NO generation and its effects could be completely abolished by L-NAME, an NO synthase inhibitor, or endothelium denudation^[11]. From this point of view, Agm could induce an increase in the production of NO, thereby leading to an increase of intracellular cGMP with a subsequent reduction in intracellular calcium^[12]. On the contrary, Galea *et al*^[13] showed that Agm was a competitive NO synthase inhibitor, but not a precursor for NO. In the present study, L-NAME did not affect the electrophysiologic effects of Agm on pacemaker cells in SA node, suggesting that NO might not be involved, which remains to be further established.

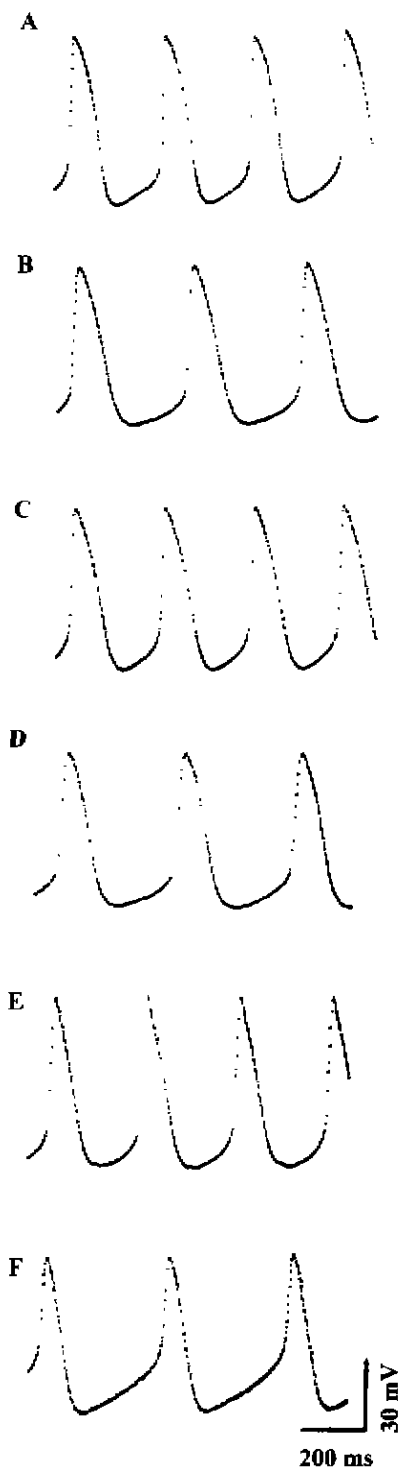


Fig 1. Effects of idazoxan, L-NAME, high Ca²⁺, and lemakalim(Lem) on electrophysiologic responses of pacemaker cells in SA node of rabbits to Agm. A) Control. B) Agm 10. C) idazoxan 0.15 + Agm 10. D) L-NAME 1 + Agm 10. E) high Ca²⁺ + Agm 10. F) Lem 0.03 + Agm 10 mmol·L⁻¹.

In this study, action potential duration of pacemaker cells was prolonged as the concentration of Agm was increased. This action might be related to a reduction in potassium currents. In the present study, lemakalim (an opener of ATP-sensitive potassium channels) partially attenuated the prolonging effect of Agm on repolarization of pacemaker cells. It is likely that Agm was a blocker of ATP-sensitive potassium channel, as suggested by Chan *et al*^[14]. However, the involvement of other potassium currents cannot be excluded. This issue merits further investigation by patch-clamp experiments.

In summary, Agm exerts a negative chronotropic action and induces a delayed repolarization of pacemaker cells in SA node, which may be due to reduction in calcium influx and potassium efflux and mediated by α_2 -AR and IR.

REFERENCES

- 1 Li G, Regunathan S, Barrow CJ, Eshraghi J, Cooper R, Reis DJ. Agmatine; an endogenous clonidine-displacing substance in the brain. *Science* 1994; 263: 966-9.
- 2 Raasch W, Regunathan S, Li G, Reis DJ. Agmatine, the bacterial amine, is widely distributed in mammalian tissues. *Life Sci* 1995; 56: 2319-30.
- 3 Li XT, He RR. Research progress on agmatine. *Prog Physiol Sci* 1998; 29: 335-8.
- 4 Li XT, He RR. Hemodynamic effects of agmatine and its cellular mechanism in anesthetized rats. *Acta Physiol Sin* 1999; 51; in press.
- 5 Gao Y, Gumusel B, Koves G, Prasad A, Hao Q, Hyman A, *et al*. Agmatine, a novel endogenous vasodilator substance. *Life Sci* 1995; 57: PL83-6.
- 6 Li XT, He RR. Electrophysiological effects of agmatine on guinea pig papillary muscles *in vitro*. *Acta Physiol Sin* 1999; 51; in press.
- 7 Watanabe EI, Honjo H, Boyett MR, Kodama I, Toyama J. Inactivation of the calcium current is involved in overdrive suppression of rabbit sinoatrial node cells. *Am J Physiol* 1996; 271 (5 Pt 2): H2097-107.
- 8 An RH, He RR. Electrophysiological effects of m-nisoldipine and nisoldipine on pacemaker cells in sinoatrial node of rabbits. *Acta Pharmacol Sin* 1991; 12: 36-9.
- 9 Likungu J, Molderings GJ, Gothert M. Presynaptic imidazoline receptors and α_2 -adrenoceptors in the human heart: discrimination by clonidine and moxonidine. *Naunyn-Schmiedeberg's Arch Pharmacol* 1996; 354: 689-92.

- 10 Hieble JP, Ruffolo RR Jr. Possible structural and functional relationships between imidazoline receptors and alpha 2-adrenoceptors. *Ann NY Acad Sci* 1995; 763: 8-21.
- 11 Ishikawa T, Misonou T, Ikeno M, Sato K, Sakamaki T. *N^ω*-hydroxyagmatine; a novel substance causing endothelium-dependent vasorelaxation. *Biochem Biophys Res Commun* 1995; 214: 145-51.
- 12 Brady AJ, Warren JB, Poole-Wilson PA, Williams TJ, Harding SE. Nitric oxide attenuates cardiac myocyte contraction. *Am J Physiol* 1993; 265 (1 Pt 2): H176-82.
- 13 Galea E, Regunathan S, Eliopoulos V, Feinstein DJ, Reis DJ. Inhibition of mammalian nitric oxide synthases by agmatine, an endogenous polyamine formed by decarboxylation of arginine. *Biochem J* 1996; 316 (Pt 1): 247-9.
- 14 Chan SL, Cable HC, Morgan NG. Agmatine, a putative endogenous ligand at imidazoline binding sites, is a weak potentiator of insulin secretion. *Pharmacol Commun* 1995; 7: 29-41.

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关键词 胍丁胺; 窦房结; 电生理学; 咪唑啉; 肾上腺素 α_2 -受体; 钙通道; 钾通道

药理

目的: 研究胍丁胺(Agm)对兔窦房结起搏细胞的电生理效应及其作用机制。 **方法:** 应用玻璃微电极方法。 **结果:** Agm 不仅能剂量依赖地抑制兔窦房结起搏细胞的 V_{max} , APA 和 VDD, RPF; 而且能延长 APD₉₀; idazoxan 能明显抑制 Agm 的电生理效应; 而 L-NAME 不能影响 Agm 的电生理效应; 提高灌流液中的 Ca^{2+} 浓度可对抗 Agm 的作用; ATP-敏感性钾通道开放剂(Iemakalim)可部分拮抗 Agm 延长 APD₉₀ 的作用。 **结论:** Agm 对窦房结的电生理效应由肾上腺素能 α_2 -受体和咪唑啉受体介导, 并与 Ca^{2+} 内流和 K^+ 外流减少有关。

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胍丁胺对兔窦房结起搏细胞的电生理效应

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