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Effects of agmatine on afterdepolarizations induced by isoproterenol in guinea pig papillary muscles

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KEY WORDS agmatine; papillary muscles; electrophysiology; isoproterenol; nitroprusside; idazoxan; imidazoles; alpha-2 adrenergic receptors; calcium

ABSTRACT

AIM: To study the effects of agmatine (Agm) on early afterdepolarizations (EAD) and delayed afterdepolarizations (DAD) induced by isoproterenol (Iso) in guinea pig papillary muscles. **METHODS**: EAD and DAD were recorded using intracellular glass microelectrode technique. **RESULTS**: (1) EAD and DAD induced by Iso 20 nmol·L^{-1} were markedly inhibited by pretreatment with Agm 1.0 - 2.0 mmol·L^{-1} in a concentration-dependent manner. (2) N^G-nitro-*L*-arginine methyl ester (*L*-NAME, 0.5 mmol·L^{-1}), a NOS inhibitor, did not affect the inhibitory effects of Agm (1.0 mmol·L^{-1}) on EAD and DAD induced by Iso. (3) The inhibitory effects of Agm (1.0 mmol·L^{-1}) on EAD and DAD induced by Iso (20 nmol·L^{-1}) were eliminated by pretreatment with idazoxan (Ida, 0.1 mmol·L^{-1}), an alpha-2 adrenergic receptor (α_2-AR) and imidazoline receptor (IR) antagonist. **CONCLUTION**: The inhibitory effects of Agm on EAD and DAD induced by Iso in papillary muscles is related to the reduction in calcium influx and mediated by α_2-AR and/or IR.

INTRODUCTION

Afterdepolarizations cause cardiac arrhythmias. Early afterdepolarizations (EAD) are a type of triggered activity that can develop in heart muscle before action potential (AP) repolarization completed[1]. Delayed afterdepolarizations (DAD) are oscillations in membrane potential that occur after repolarization of an AP and that are caused by that AP[2]. Stimulation of β-adrenergic receptors by isoproterenol (Iso) can induce either EAD or DAD. Ca^{2+} release from the sarcoplasmic reticulum is a factor in the development of EAD and DAD, thus pointing to activation of a nonspecific Ca^{2+}-activated channel as a common mechanism for afterdepolarizations[3]. Agmatine (Agm) has been identified as an endogenous clonidine-displacing substance (CDS) in mammalian brain. Li *et al*[4] formulated that Agm is an endogenous agonist at imidazoline receptor (IR) and a noncatecholamine ligand at α_2-adrenergic receptor (α_2-AR), and may act as a neurotransmitter. It has been known that Agm is widely distributed in mammalian tissues including heart, blood vessels and brain[5], suggesting that Agm could act on cardiovascular system and nervous center[6]. Recently, we have found that Agm could not only reduce calcium influx of AP in guinea pig papillary muscles *in vitro*[7], but also reduce calcium influx of pacemaker cells in sinoatrial node[8]. However, the effects of Agm on EAD and DAD induced by Iso in guinea pig papillary muscles have not yet been elucidated. The purpose of this study was to observe the effects of Agm on EAD and DAD induced by Iso.

MATERIALS AND METHODS

Materials Guinea pigs (♂ ♀, n = 48, weighing 405 g ± s 26 g, grade Ⅱ, Certificate № 04040, provided by Experimental Animal Center of Hebei Province) were stunned and the hearts were superfused with oxygenated modified Tyrode's solution (0 - 4 ℃). Isolated papillary muscles of right ventricle were perfused with modified Tyrode's solution:

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NaCl 130.0, KCl 4.8, NaH_2PO_4 1.8, $MgCl_2$ 1.05, $CaCl_2$ 1.8, $NaHCO_3$ 25.0, and glucose 5.5 mmol·L^{-1}. This solution was buffered to pH 7.4 by gassing 100 % O_2 at 36 ℃.

The preparation was stimulated through a bipolar electrode at a control basic cycle length (BCL) of 2000 ms (1 ms rectangular pulse and two times threshold intensity) from the stimulator (SEN-3201, Nihon Kohden). The transmembrane AP was recorded by a glass microelectrode filled with KCl 3 mol·L^{-1} (a tip resistance of 10 – 30 MΩ), 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. Iso was used to induce EAD and DAD.

Experimental protocols The preparation was equilibrated for 60 min in the modified Tyrode's solution at a rate of 4 mL·min^{-1} before intracellular recordings. The experiments consisted of 4 groups: (1) Induction of EAD and DAD by Iso: under the BCL of 2000 ms, amplitudes of EAD and DAD, durations of DAD were measured when the preparation was perfused with modified Tyrode's solution containing Iso (20 nmol·L^{-1}). (2) Effects of Agm on the EAD and DAD induced by Iso: the preparation was perfused with modified Tyrode's solution containing Agm (0.5, 1.0, or 2.0 mmol·L^{-1}) for 10 min followed by perfusion with modified Tyrode's solution containing Iso (20 nmol·L^{-1}). This group of experiment was undertaken to evaluate the inhibitory effect of Agm on EAD and DAD induced by Iso. (3) Effects of N^G-nitro-*L*-arginine methyl ester (*L*-NAME) on the actions of Agm: after *L*-NAME (0.5 mmol·L^{-1}) was perfused with modified Tyrode's solution for 10 min, the protocol of the second group was undertaken again. (4) Effects of idazoxan (Ida) on the actions of Agm: after Ida (0.1 mmol·L^{-1}) was perfused with modified Tyrode's solution for 10 min, the protocol of the second group was undertaken again.

Using a program designed by our department, the following parameters of EAD and DAD were defined automatically by an on-line microcomputer analyzing system: (1) Amplitude of EAD: the amplitude from the onset of EAD to the peak of EAD[9]; (2) Amplitude of DAD: the amplitude from the onset of DAD to the peak of DAD; (3) Duration of DAD: the time from the onset of DAD to the end of DAD; (4) TD (total duration): the time from the onset of upstroke of AP to the end of EAD. (5) TOP (take-off potential): the lowest point in the repolarization phase when the first EAD starts[10].

Drugs Agmatine, idazoxan, *L*-NAME (Sigma Co, USA), and Iso were dissolved in modified Tyrode's solution.

Statistical analysis Data were analyzed by ANOVA or by Fisher exact test. All data were presented as $x \pm s$.

RESULTS

EAD and DAD induced by Iso In the presence of Iso (20 nmol·L^{-1}), 92 % EAD and DAD were elicited under BCL of 2000 ms. The amplitude of EAD and DAD were (12.2 ± 2.9) mV and (9.2 ± 2.0) mV, respectively (Fig 1, Tab 1).

Effects of Agm on EAD and DAD induced by Iso At Agm 0.5 mmol·L^{-1}, EAD, DAD, TD, and TOP were not influenced. When the concentration of Agm was increased to 1.0 and 2.0 mmol·L^{-1}, the above parameters were decreased (Fig 1, Tab 1).

Effects of *L*-NAME on actions of Agm The effects of Agm (1.0 mmol·L^{-1}) on Iso (20 nmol·L^{-1})-induced EAD and DAD were not affected by *L*-NAME (0.5 mmol·L^{-1}) (Tab 1).

Effects of Ida on actions of Agm The effects of Agm (1.0 mmol·L^{-1}) on Iso (20 nmol·L^{-1})-induced EAD and DAD were blocked completely by Ida (0.1 mmol·L^{-1}) (Tab 1).

DISCUSSION

The present study demonstrated that Agm could concentration-dependently inhibit EAD and DAD induced by Iso in guinea pig papillary muscles. Song *et al*[11] proved that stimulation by Iso of I_{Ca} was transiently enhanced in guinea pig ventricular myocytes. The increase in Iso-stimulated I_{Ca} was associated with a prolongation of AP duration and induction of arrhythmogenic effect. As Agm could reduce calcium influx of AP in guinea pig papillary muscles[7], therefore, the inhibitory effects of Agm on EAD and DAD induced by Iso might be due to the blockade of calcium influx.

Agm might be precursor for NO generation and its

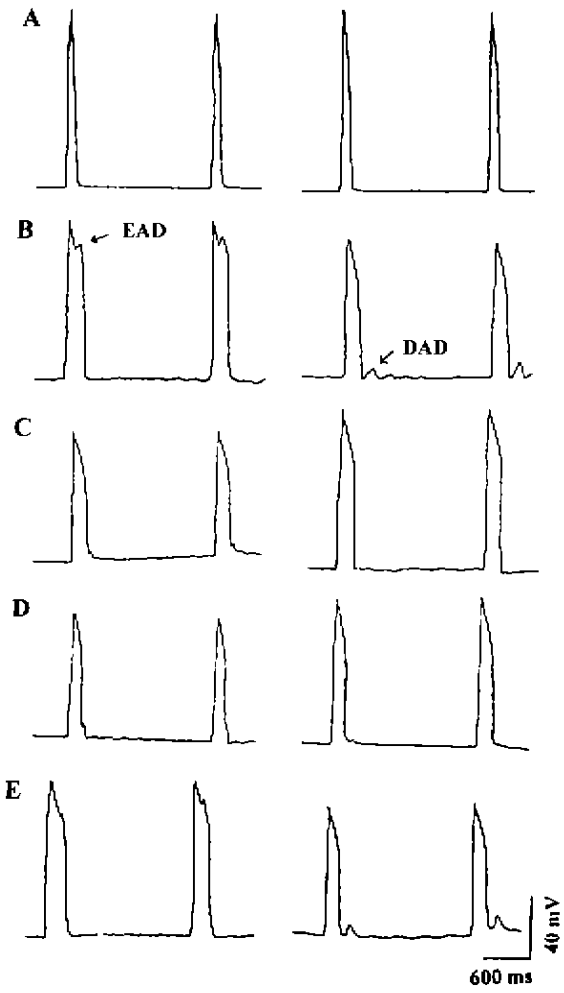

Fig 1. Effects of agmatine (Agm) on EAD or DAD induced by Iso and influence of *L*-NAME and Ida on the actions of Agm (basic cycle length 2000 ms). A: Control; B: Iso 20 nmol·L^{-1}; C: Agm 1.0 mmol·L^{-1} + Iso 20 nmol·L^{-1}; D: *L*-NAME 0.5 mmol·L^{-1} + Agm 1.0 mmol·L^{-1} + Iso 20 nmol·L^{-1}; E: Ida 0.1 mmol·L^{-1} + Agm 1.0 mmol·L^{-1} + Iso 20 nmol·L^{-1}.

effects could completely be abolished by *L*-NAME, a NO synthase inhibitor, or endothelium denudation[12]. From this point of view, Agm would induce an increase in the production of NO, thereby lead to increase in intracellular levels of cGMP with a subsequent reduction in intracellular calcium[13]. On the contrary, Galea *et al*[14] 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 inhibitory effects of Agm on EAD and DAD induced by Iso, suggesting that NO might not be involved.

Likungu and his colleagues[15] demonstrated that norepinephrine released from the human heart was inhibited not only by presynaptic α_2-AR but also by presynaptic IR. Most recently, imidazoline binding sites and receptors and their endogenous ligands have been identified to exist on cardiac myocytes[16]. As an α_2-AR and IR antagonist[17], Ida markedly inhibited the effects of Agm on EAD and DAD induced by Iso in guinea pig papillary muscles, suggesting that α_2-AR and/or IR were involved in the inhibitory effects of Agm on EAD and DAD induced by Iso.

In summary, Agm exerts an inhibitory effects on EAD and DAD induced by Iso in guinea pig papillary muscles, which is related to the reduction in calcium influx and mediated by α_2-AR and/or IR.

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Tab 1. Effects of agmatine (Agm), *L*-NAME, and idazoxan (Ida) on early afterdepolarizations (EAD) and delayed afterdepolarizations (DAD) induced by isoproterenol (Iso) 20 nmol·L^{-1} in guinea pig papillary muscles (basic cycle length 2000 ms). TD: the time from the onset of upstroke of AP to the end of EAD. TOP: the lowest point in the repolarization phase when the first EAD starts. $n = 64$ papillary muscles from 48 guinea pigs. $\bar{x} \pm s$.
$^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* Iso (20 nmol·L^{-1}).
$^{d}P > 0.05$, $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* Iso (20 nmol·L^{-1}) + Agm (1.0 mmol·L^{-1}).

Drugs	n	Incidence of EAD/%	Amplitude of EAD/mV	TD /ms	TOP/mV	Amplitude of DAD/mV	Duration of DAD/ms
Iso	12	92	12.2±2.9	384±39	−12.4±2.9	9.2±2.0	169±15
Agm (mmol·L^{-1})							
0.5 + Iso	10	90^{a}	11.5±2.4^{a}	379±34^{a}	−14.7±2.8^{a}	8.8±1.7^{a}	164±12^{a}
1.0 + Iso	12	33^{c}	4.6±1.0^{c}	276±30^{c}	−60.5±9.5^{c}	2.6±0.8^{c}	68±12^{c}
2.0 + Iso	12	17^{c}	1.4±0.6^{c}	214±23^{c}	−81.5±6.4^{c}	1.2±0.5^{c}	28±9^{c}
Iso + Agm (1.0 mmol·L^{-1}) + *L*-NAME (0.5 mmol·L^{-1})	9	44^{bd}	4.4±1.3^{cd}	272±32^{cd}	−58.8±10.2^{cd}	2.2±0.8^{cd}	67±11^{cd}
Iso + Agm (1.0 mmol·L^{-1}) + Ida (0.1 mmol·L^{-1})	9	89^{ae}	12.0±2.4^{af}	371±32^{af}	−13.4±3.1^{af}	9.4±1.9^{af}	172±19^{af}

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胍丁胺对异丙肾上腺素诱发豚鼠乳头状肌后除极的影响

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关键词 胍丁胺；乳头状肌；电生理学；异丙肾上腺素；硝普盐；咪唑克生；咪唑类；肾上腺素能 α_2 受体；钙

目的：研究胍丁胺(Agm)对异丙肾上腺素(Iso)诱发豚鼠乳头状肌早发和晚发后除极(EAD 和 DAD)的影响及其作用机制. **方法**：细胞内玻璃微电极技术. **结果**：(1) Agm 明显抑制 Iso 诱发的 EAD 和 DAD；(2) 预先应用 NOS 抑制剂 *L*-NAME 0.5 mmol·L^{-1}，不能影响 Agm 1.0 mmol·L^{-1}对 Iso 20 nmol·L^{-1}诱发 EAD 和 DAD 的抑制作用；(3)预先应用咪唑啉受体(IR)和肾上腺素能 α_2 受体(α_2-AR)拮抗剂 idazoxan 0.1 mmol·L^{-1}则可阻断这种作用. **结论**：Agm 对 Iso 诱发 EAD 和 DAD 的抑制作用由 α_2-AR 和/或 IR 介导，并与钙内流减少有关.

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