Inhibition by agmatine on spontaneous activity of rabbit atrioventricular node cells

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

ABSTRACT

AIM: To study the effects of agmatine on spontaneous activity of atrioventricular (AV) node and its action mechanisms. METHODS: Action potentials in AV node cells were recorded using intracellular microelectrode technique. RESULTS: Agmatine not only reduced the amplitude of action potential (APA), maximal rate of depolarization (Vmax), velocity of diastolic (phase 4) depolarization (VDD), and rate of spontaneous firing (RSF), but also prolonged 90% duration of action potential (APD90) in a concentration-dependent manner. The effects of agmatine (10 mmol/L) could be blocked completely by pretreatment with idazoxan (0.1 mmol/L), an imidazoline receptor (IR) and alpha-2 adrenergic receptor (alpha-2-AR) antagonist. Pretreatment with Nω-nitro-L-arginine methyl ester (L-NAME, 0.5 mmol/L), a nitric oxide (NO) synthase inhibitor, did not affect the effects of agmatine on AV node cells. Elevation of Ca2+ concentration (5 mmol/L) in perfusate antagonized the effects of agmatine (10 mmol/L). L-mevalonate (30 μmol/L), an ATP-sensitive potassium channel opener, inhibited the prolonging effects of agmatine on repolarization. CONCLUSION: The inhibitory effects of agmatine on spontaneous activity of AV node cells in rabbits were likely mediated by IR and/or alpha-2-AR, and were related to the reduction in calcium influx and potassium efflux.

INTRODUCTION

Agmatine (4-aminobutyl guanidine), an amine resulting from decarboxylation of L-arginine by arginine decarboxylase, has been identified as an endogenous clonidine-displacing substance (CDS) in bovine brain and other tissues. It has been well established that agmatine is an endogenous agonist at imidazoline receptors (IR) and a ligand for alpha-2-adrenergic receptors (alpha-2-AR), and may act as a putative neurotransmitter[1]. The fact that agmatine is presented in heart[2] raises the possibility that it may influence the cardiac function. Intravenous administration of agmatine to anesthetized rats reduced heart rate, blood pressure, cardiac output and myocardial contractility[3,4]. Moreover, our previous study showed that agmatine could exert a negative chronotrophic effect on sinoatrial node of rabbits in vitro[5]. The atrioventricular (AV) node is vital to normal cardiac function. The AV node constitutes the only region where electrical excitation can pass from atria to ventricles, and the relatively slow electrical conduction through this region ensures complete ventricular filling before ventricular contraction. Furthermore, if the sinoatrial node fails, then the AV node can act as pacemaker for the ventricles by virtue of its own spontaneous rhythm. Till now, little is known about the effects of agmatine on AV node. The present study was undertaken to investigate the effects of agmatine on spontaneous activity of AV node and its action mechanisms.

MATERIALS AND METHODS

AV node preparations The experiments were performed in vitro in 30 preparations obtained from the hearts of rabbits provided by Experimental Animal Center of Hebei Province. Rabbits of either sex, weighing 2.3 kg ± 0.2 kg, Class I, Certificate No. 04037 were killed with a single blow on the head and the hearts were removed and placed in cold (0 - 4℃) oxygenated (100% O2) Tyrode solution. The right ventricle was slit open near the apex, and the incision was carefully extended toward the tricuspid valve following the chordae tendineae of the papillary muscles. The tricuspid valve
was cut through and the right atrium slit open on its lateral wall to expose the right side of the interatrial septum. The area of the AV node could then be identified using anatomical landmarks. The ventricles and left atrial tissues were discarded. The final preparation contained right atrial tissues consisting of the interatrial septum, the right atrial appendages with the muscularis pectinatis, the septal leaflets of the tricuspid valve, the central fibrous body, and the small portion of the ventricular septum just below the bundle of His. The preparations were pinned down on a thin silicon disc with the endocardial surface oriented upwards and kept in a thermostatically controlled glass superfusion system.

Superfusing solution The Tyrode solution was prepared with deionized, distilled water and had the following composition (mmol/L): NaCl 130.0, NaHCO₃ 25.0, KCl 4.0, MgCl₂ 1.0, CaCl₂ 1.8, Na₂HPO₄ 1.8, and glucose 11.1. It was oxygenated with 100 % O₂ and maintained at 35.5 °C with pH of 7.38 ± 0.04.

Electrical recording The transmembrane potentials were recorded by means of 3 mol/L KCl-filled micropipettes (tip diameter less than 0.5 μm), coupled to a high input impedance amplifier (MEZ 8201, Nihon Kohden). The amplified signals were fed to the A/D converter and processed by a microcomputer. Maximal diastolic potential (MDP), amplitude of action potential (APA), 90% of duration of action potential (APD₉₀), maximal rate of depolarization (Vmax), rate of spontaneous firing (RSF), and velocity of diastolic (phase 4) depolarization (VDD) were analyzed by the microcomputer.

Experimental protocols The experiments started after the preparations were equilibrated for 60 min in the Tyrode solution at a perfusion rate of 4 mL/min. The preparations were beating spontaneously. The action potentials were taken before and at 1, 5, 10, 15, and 20 min after drug administration.

The experiments consisted of 5 groups: (1) The effects of agmatine on spontaneous activity of AV node cells. The animals were randomly divided into 4 subgroups; control, agmatine 5, 10, 15 mmol/L; (2) Effect of idazoxan (0.1 mmol/L) on the response of AV node cells to agmatine (10 mmol/L); (3) Effect of N⁵-nitro-L-arginine methyl ester (L-NAME, 0.5 mmol/L) on the response of AV node cells to agmatine (10 mmol/L); (4) The effect of high Ca²⁺ (5 mmol/L) on the action of agmatine; (5) Effect of ATP-sensitive potassium channel opener lemakalim (30 μmol/L) on the agmatine-induced changes in repolarization of AV node cells. In subgroup 2–5, the effects of agmatine were observed at 10 min following pretreatment with idazoxan, L-NAME, high Ca²⁺, and Lemakalim.

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

Statistics Experimental data were expressed as x ± s. Statistical comparisons were performed using t test.

RESULTS

Action potential configurations of AV node cells Using microelectrode, action potentials from spontaneously active node cells were recorded. AV node cells had a mean rate of spontaneous activity of 75 ± 16 beat/min. The mean MDP was (−61 ± 7) mV, and most cells exhibited relatively slow action potential upstroke [Vmax = (5.8 ± 1.4) V/s]. The mean APA was (75 ± 7) mV, and APD₉₀ was (203 ± 38) ms (Fig 1, Tab 1).

![Fig 1. Effects of idazoxan, L-NAME, high Ca²⁺, and lemakalim (Lem) on the responses of AV node cells in rabbits to agmatine (Agm). A) Control. B) Agm 10. C) Idazoxan 0.1 + Agm 10. D) L-NAME 0.5 + Agm 10. E) High Ca²⁺ 5 + Agm 10. F) Lem 0.03 + Agm 10 mmol/L.](image-url)

Effects of agmatine on spontaneous activity
of AV node cells VDD was slowed down by agmatine in a concentration-dependent manner (Table 1). RSF began to decrease after 5 min of perfusion with the Tyrode solution containing agmatine (10 mmol/L). The changes in RSF induced by agmatine paralleled those of VDD. Agmatine 5 mmol/L only induced a slight increase in APD₃₀, while at 10, 15 mmol/L, induced a marked increase in APD₃₀ and decreased Vₙₐₓ and APA, in a concentration-dependent manner (Table 1).

Idazoxan 0.1 mmol/L could completely block the above-mentioned effects induced by agmatine 10 mmol/L, whereas L-NAME (0.5 mmol/L) could not. Elevation of Ca²⁺ concentration (up to 5 mmol/L) in perfusate abolished the inhibitory effects of agmatine 10 mmol/L on spontaneous activity of AV node cells (Fig 1, Tab 2).

Effects of lemakalin on prolonged repolarization of AV node cells induced by agmatine Lekamalin 30 μmol/L could antagonize the prolonged repolarization of action potential of AV node cells induced by agmatine 10 mmol/L. Agmatine 10 mmol/L induced an increase in APD₃₀ from (208 ± 32) to (233 ± 28) ms (n = 5, P < 0.01), with a net change of (24.8 ± 5.4) ms. After pretreatment with lemakalin 30 μmol/L, agmatine increased the APD₃₀ of AV node cells from (208 ± 32) to (216 ± 33) ms with a net change of (7.8 ± 2.4) ms, which was significantly different from that induced by agmatine alone (P < 0.01). The final concentration of ethyl alcohol solvent for lemakalin in perfusate was 0.13%, which had no effect on APD₃₀ of the action potentials of AV node cells in control [(203 ± 34) vs (207 ± 30) ms, n = 4, P > 0.05].

DISCUSSION

AV node cells have been functionally subdivided into three regions called AN (atrioventricular), N (nodal), and NH (nodal-His) cells. The electrophysiologic features of N and NH cells are low resting potential and low maximum upstroke velocity. Action potential configurations recorded from rabbit AV node cells in this experiment exhibited the characteristics of N or NH cells.

| Table 1. Inhibitory effects of agmatine (Agm) on spontaneous activity of atrioventricular node cells in rabbits. n = 5. x ± s. *P < 0.05, **P < 0.01 vs control. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|     | MDP (mV) | APA (mV) | Vₙₐₓ (V/s) | VDD (mV/s) | RSF (beat/min) | APD₃₀ (ms) |
| Control | -61 ± 7 | 75 ± 7 | 5.8 ± 1.4 | 27 ± 7 | 75 ± 16 | 208 ± 38 |
| Agm (mmol/L) |  |  |  |  |  |  |
| 5 | -60 ± 5* | 74 ± 5* | 5.6 ± 1.1* | 26 ± 7* | 73 ± 17* | 210 ± 40* |
| 10 | -59 ± 6* | 71 ± 7* | 5.1 ± 1.3* | 20 ± 4* | 61 ± 11* | 230 ± 34* |
| 15 | -58 ± 7* | 69 ± 9* | 4.5 ± 1.2* | 17 ± 4* | 55 ± 9* | 243 ± 35* |

| Table 2. Influences of idazoxan (Ida, 0.1 mmol/L), L-NAME (0.5 mmol/L), and high Ca²⁺ (5 mmol/L) on the agmatine (Agm, 10 mmol/L)-induced inhibitory effects on spontaneous activity of rabbit atrioventricular node cells. n = 16. x ± s. *P < 0.05, **P < 0.01 vs control. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|     | MDP (mV) | APA (mV) | Vₙₐₓ (V/s) | VDD (mV/s) | RSF (beat/min) | APD₃₀ (ms) |
| Control | -57 ± 7 | 72 ± 9 | 5.5 ± 1.2 | 25 ± 7 | 71 ± 17 | 210 ± 34 |
| Agm | -57 ± 6* | 69 ± 8* | 4.9 ± 0.9* | 18 ± 5* | 53 ± 15* | 230 ± 33* |
| Ida + Agm | -56 ± 8* | 71 ± 9* | 5.2 ± 0.9* | 24 ± 6* | 68 ± 13* | 213 ± 31* |
| Control | -62 ± 8 | 77 ± 8 | 5.2 ± 1.0 | 26 ± 7 | 77 ± 18 | 212 ± 32 |
| Agm | -60 ± 8* | 72 ± 8* | 4.5 ± 1.0* | 18 ± 5* | 60 ± 15* | 235 ± 27* |
| L-NAME + Agm | -59 ± 8* | 71 ± 10* | 4.4 ± 1.1* | 19 ± 5* | 61 ± 16* | 234 ± 28* |
| Control | -59 ± 6 | 75 ± 8 | 5.3 ± 1.4 | 29 ± 7 | 81 ± 19 | 209 ± 36 |
| Agm | -57 ± 7* | 69 ± 10* | 4.5 ± 1.0* | 20 ± 5* | 65 ± 14* | 235 ± 37* |
| High Ca²⁺ + Agm | -58 ± 6* | 71 ± 8* | 5.2 ± 1.2* | 26 ± 6* | 79 ± 17* | 212 ± 35* |
Studies of rabbit AV node preparations demonstrate that cells in the N and NH regions are capable of generating spontaneous action potentials.

The major finding of this study is that agmatine could concentration-dependently decrease $V_{\text{max}}$, APA, VDD, and RSP of action potential of rabbit AV node cells. Based on the fact that $I_{\text{Ca}}$ contributed significantly to the action potential upstroke of AV node cells in rabbits\(^9\), the decrease in APA might be attributed to the reduction of $I_{\text{Ca}}$. Elevation of calcium concentration in perfusate antagonized the above-mentioned inhibitory effects of agmatine in our experiment. Therefore, the inhibitory effects of agmatine on $V_{\text{max}}$, APA, VDD, and RSP of AV node cells might be due to the blockade of calcium influx. Recently, imidazoline binding sites (IBS), receptors, and their endogenous ligands have been identified in cardiovascular tissues of various species, and I$_2$-IBS have been shown to exist on cardiac myocytes\(^10\). As an antagonist at I$_1$, I$_2$, and o$_2$-AR\(^11\), idazoxan markedly inhibited the effects of agmatine on AV node cells in our experiment, implying that IR and/or o$_2$-AR were involved in the inhibitory effects of agmatine on AV node cells.

Morrissey et al\(^{12}\) indicated that agmatine markedly stimulated nitric oxide (NO) production (determined as nitrite formation) in cultured bovine pulmonary artery endothelial cells and induced vasodepressor response. Contrary to the findings with agmatine, Syenets et al\(^{13}\) showed that CDS failed not only to induce NO release from rat aorta endothelial cells, but also to relax the isolated rat aorta; in contrast, it even produced a contraction. In our study, L-NNAME did not affect the inhibitory effects of agmatine on spontaneous activity of AV node cells, suggesting that NO might not be involved in the action mechanism of agmatine.

In this study, action potential duration of AV node cells was prolonged as the concentration of agmatine was increased. This effect might be related to a reduction in potassium currents. Lemakalin (an opener of ATP-sensitive potassium channel) attenuated the agmatine-induced prolongation of repolarization of AV node cells, suggesting that agmatine possessed the ability to inhibit ATP-sensitive potassium channel in AV node cells, and hence supports the conception of Chan et al\(^{14}\). However, the involvement of other potassium currents can not be excluded. These results merit further investigation by patch-clamp experiments.

In summary, agmatine exhibited inhibitory effects on spontaneous activity of AV node cells in rabbits, which may be attributed to reduction in calcium influx and potassium efflux and may be mediated by IR and/or o$_2$-AR.

REFERENCES

肌钙蛋白抑制兔房室结细胞的自发活动

李晓艳，段会茹，何瑞荣（河北医科大学基础医学研究所生理室，石家庄 050017，中国）

关键词：肌钙蛋白；房室结；咪唑啉；肾上腺素 α2-受体；钙通道；钾通道

目的：研究肌钙蛋白（Agn）对兔房室结细胞自发活动的影响及其作用机制。方法：应用玻璃微电极方法

法。结果：Agn 不仅剂量依赖地抑制房室结细胞自发活动的 Vm，APA 和 VDD，RSF；而且延长 APDα；idazoxan 能明显抑制 Agn 的作用；而 L-NAME 不能影响 Agn 的作用；提高灌流液中的 Ca2+ 浓度可对抗 Agn 的作用；ATP-敏感性钾通道开放剂（lemakalim）可拮抗 Agn 延长 APDα 的作用。结论：Agn 对房室结细胞自发活动的抑制作用由咪唑啉受体和/或肾上腺素 α2-受体介导，并与 Ca2+ 内流和 K+ 外流减少有关。

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