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Agmatine inhibits carotid sinus baroreflex in anesthetized rats

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KEY WORDS agmatine; carotid sinus; baroreflex; blood pressure; imidazoline receptors; alpha-2 adrenergic receptors; idazoxan

ABSTRACT

AIM: To study the effect of agmatine (Agm) on carotid sinus baroreflex. **METHODS**: The functional parameters of baroreflex were measured by perfusing the carotid sinus in anesthetized rats. **RESULTS**: (1) Agm 1, 5, and 10 mmol/L shifted the functional curve of carotid sinus baroreflex to the right and upwards in a concentration-dependent manner with a reduction in peak slope and a reflex decrease in mean arterial pressure, indicating that Agm exerted an inhibitory effect on the carotid baroreflex. (2) The inhibitory effect of Agm (5 mmol/L) on baroreflex was eliminated by pretreatment with idazoxan (Ida, 0.1 mmol/L), an α_2-adrenoceptor (α_2-AR) and imidazoline receptor (IR) antagonist, and partially blocked by yohimbine (Yoh, 15 μmol/L), a selective α_2-AR antagonist. (3) N^G-nitro-*L*-arginine methyl ester (*L*-NAME, 500 μmol/L), an NOS inhibitor, did not affect the inhibitory effect of Agm. **CONCLUSION**: Agm inhibits carotid baroreflex via IR and α_2-AR.

INTRODUCTION

Agmatine has been identified as an endogenous clonidine-displacing substance (CDS) in bovine brain and other tissues. Li *et al* have formulated that Agm is an endogenous agonist at imidazoline receptor (IR) and a noncatecholamine ligand at α_2-adrenoceptor (α_2-AR), and may act as a neurotransmitter[1]. Agm is widely distributed in mammalian tissues including heart, blood vessels, and brain[2], suggesting the involvement of Agm in the regulation of cardiovascular and neural functions[3]. A previous study from our laboratory demonstrated that intravenous administration of Agm resulted in decrease of heart rate, blood pressure, cardiac output, and myocardial contractility in the anesthetized rat[4]. Agm inhibits the electrical activities of guinea pig papillary muscle, pacemaker cells in rabbit SA node and AV node, and human atrial fibers[5-8]. It also inhibits the afterdepolarizations induced by isoproterenol in guinea pig papillary muscles and human atrial fibers[9,10]. However, whether or not Agm affects the arterial baroreceptor function remains unknown. The present study was undertaken to examine the effects of Agm on carotid baroreflex by perfusing the isolated carotid sinus and the mechanism involved.

MATERIALS AND METHODS

Sprague-Dawley rats (♂ weighing 350 ± 20 g, n = 24, Grade Ⅱ, Certificate № 04036) obtained from the Experimental Animal Center of Hebei Province. Rats were anesthetized with ip urethane 625 mg/kg. The trachea was cannulated for ventilation. The femoral artery was cannulated for recording blood pressure (BP) with a transducer (MPU-0.5A, Nihon Kohden).

Perfusion of isolated carotid sinus The perfusion of isolated carotid sinus area was carried out with a method modified by our laboratory[11]. The intrasinus pressure (ISP) was monitored by a pressure transducer (MPU-0.5A) and controlled by a peristaltic pump.

After perfusion of the left carotid sinus, ISP was kept at 13.3 kPa for 20 min and then was lowered to 0 rapidly, from which ISP was elevated to 33.3 kPa in the form of pulsatile ramp by regulating the speed of peristaltic pump, which was automatically controlled by a program designed by our laboratory[12]. It took 0.5 min for ISP to be increased from 0 to 33.3 kPa. ISP and BP were simultaneously recorded on a polygraph (RM-6200, Nihon Kohden). This process was repeated at an interval of 5 min to check the stability of the baroreflex. Reproducibilty of the experimental preparation was documented by the recurrent drop of BP in response to the increase in ISP.

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Experimental protocols By perfusing left carotid sinus with Krebs-Henseleit (K-H) solution and elevating ISP, the functional curve for the ISP-BP relation was constructed, and the functional parameters of baroreflex such as threshold pressure (TP), saturation pressure (SP), equilibrium pressure (EP), peak slope (PS), reflex decrease of BP (RD), and operating range (OR) were determined. TP was the ISP at which BP began to decrease in response to the increase of ISP. SP was the ISP at which BP just showed no further reflex decrease with an increase in ISP. EP was the ISP which equalled to systemic BP. OR was calculated as the difference of SP minus TP.

Before administration of the drugs, the K-H solution was used as a control. The experiments consisted of 4 groups. (1) The effect of Agm on carotid baroreflex (n = 18): ISP was fixed at 13.3 kPa for 20 min with K-H solution as control, and the parameters of baroreflex were measured. Then K-H solution containing Agm (1, 5, or 10 mmol/L) was used to perfuse the isolated carotid sinus for 40 min, followed by measurements of the parameters again. Finally, the carotid sinus was perfused with K-H solution to washout Agm; (2) Effect of idazoxan (Ida 0.1 mmol/L) on the response of carotid baroreflex to Agm (n = 6): Parameters were examined following the application of Agm before and after pretreatment with Ida; (3) Effect of yohimbine (Yoh, 15 μmol/L) on the results produced by Agm (n = 6): The procedure followed was the same as in group (2); (4) Effect of N^G-nitro-*L*-arginine methyl ester (*L*-NAME, 500 μmol/L) on the action of Agm (n = 6): The procedures followed was the same as in group (2).

Drugs Agmatine, idazoxan, *L*-NAME (Sigma) and yohimbine (Tianjin Chemical Co, China) were all dissolved in distilled water.

Statistics All data were expressed as $\bar{x} \pm s$ and evaluated by paired and unpaired t-test. $P < 0.05$ was considered significant.

RESULTS

Effects of Agm on carotid sinus baroreflex By perfusing the left carotid sinus with K-H solution and elevating ISP from 0 to 33.3 kPa, BP was reflexly decreased. Agm induced obvious changes in baroreflex parameters, which appeared within 15 min after perfusing isolated carotid sinus with K-H solution containing agmatine, and reached the peak at 20 min, and disappeared 30 min after washout. Agm induced the decrease of RD and PS concentration-dependently (Tab 1). Moreover, Agm 5, 10 mmol/L increased TP ($P < 0.01$) and also decreased OR ($P < 0.05$) (Tab 1).

Agmatine shifted the functional curve of the baroreflex to the right and upward in a concentration-dependent manner (Fig 1), and such a result indicates the inhibitory effect of Agm on carotid baroreflex (Fig 2).

Effect of Ida on Agm responses Ida 100 μmol/L *per se* did not induce any change in functional parameters of baroreflex, but completely blocked the effects of Agm 5 mmol/L (Tab 2).

Effect of Yoh on Agm responses Yoh 15 μmol/L *per se* had no effects on baroreflex, but partially blocked the actions of Agm 5 mmol/L (Tab 2).

Effects of *L*-NAME on Agm responses *L*-NAME 500 μmol/L *per se* did not induce any change in functional parameters of baroreflex and the effects of Agm 5 mmol/L on the baroreflex were not affected by *L*-NAME (Tab 2).

Tab 1. Effect of Agm on carotid baroreflex in rats. $\bar{x} \pm s$. $n = 6$. **Agm: agmatine.** $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* **control.** $^dP > 0.05$, $^eP < 0.05$ *vs* **Agm 1 mmol/L.** $^fP > 0.05$, $^gP < 0.05$ *vs* **5 mmol/L.**

Drugs	TP/kPa	EP/kPa	SP/kPa	OR/kPa	PS	RD/kPa
Control	7.8±0.4	14.7±0.5	25.0±0.6	17.2±0.7	0.44±0.07	6.4±0.5
Agm (1 mmol/L)	8.2±0.6^a	14.0±0.8^a	25.3±0.7^a	16.7±0.8^a	0.36±0.06^a	5.2±0.8^a
Control	7.8±0.7	15.6±0.9	24.9±0.5	17.1±0.6	0.43±0.05	6.2±0.6
Agm (5 mmol/L)	9.4±1.1^{ce}	14.8±0.6^{ad}	24.7±0.8^{ad}	15.3±0.4^{bd}	0.23±0.08^{ce}	3.3±0.8^{ce}
Control	7.4±0.5	14.3±0.6	24.2±0.4	16.8±0.5	0.41±0.09	6.7±0.9
Agm (10 mmol/L)	10.0±0.6^{cg}	15.2±0.4^{ag}	25.6±0.6^{ag}	15.2±0.6^{bg}	0.19±0.06^{ch}	2.7±1.2^{ch}

EP: equilibrium pressure. OR: operating range. PS: peak slope. RD: reflex decrease of blood pressure. SP: saturation pressure. TP: threshold pressure.

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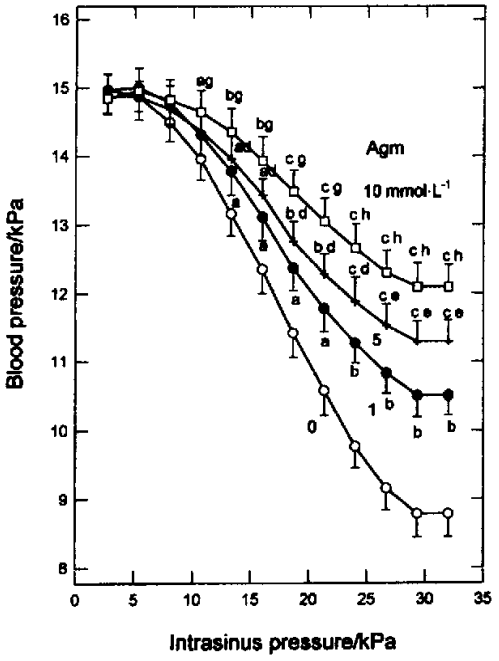

Fig 1. Effects of different concentrations of Agm on the functional curves of carotid baroreflex in anesthetized rats. $n = 6$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^dP > 0.05$, $^eP < 0.05$ *vs* Agm 1 mmol/L. $^fP > 0.05$, $^hP < 0.05$ *vs* Agm 5 mmol/L.

DISCUSSION

Agmatine, a decarboxylated arginine metabolite, is reported to be a locally synthesized clonidine-displacing substance (CDS) in bovine brain. That Agm might be relevant to vascular function was derived from the following observations: (1) Agm is synthesized and stored in vascular endothelium and smooth muscle cells, and (2) vascular smooth muscle and endothelium express its receptors (IR and α_2-AR)[13]. Intravenous injection of agmatine decreased systemic arterial pressure and systemic vascular resistance[4]. However, it is uncertain whether the systemic vasodilator responses to agmatine are centrally or peripherally mediated. Intracisternal agmatine concentration-dependently increased sympathetic nerve activity and arterial pressure and blocked baroreflex[14]. The present study is the first to provide the evidence that agmatine is able to affect carotid sinus baroreflex. In our experiment, the technique of perfusing carotid sinus was used and the activity of baroreceptors was altered by changing ISP with agmatine restricted to local sinus area to observe its direct action on carotid sinus baroreceptors. Agmatine shifted the functional curve of carotid sinus baroreflex to the right and upwards, with reduction in PS, RD and increase in TP, thereby indicating that agmatine inhibited carotid baroreflex.

The type of receptor mediating inhibitory effects of agmatine on carotid baroreflex remains to be defined. The present study showed that as the carotid sinus was pretreated with idazoxan, an antagonist at IR and α_2-AR, the inhibitory effect of agmatine on carotid baroreflex was completely abolished, thereby implying the involvement of IR and/or α_2-AR. All IR agonists, including agmatine, also possess comparable affinity for α_2-AR, and in many cases, stimulation of either IR or α_2-AR produces identical physiological responses[15]. Yohimbine, a selective α_2-AR antagonist, could partially block the effects of agmatine. Therefore, it is suggested that the inhibitory action of agmatine is mediated by IR and α_2-AR.

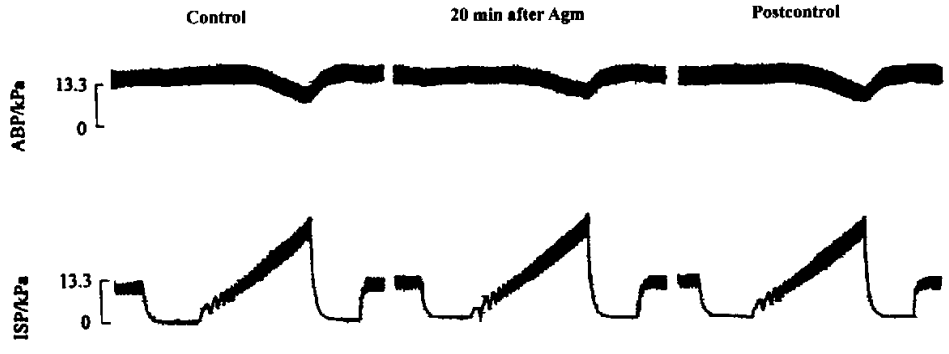

Fig 2. Inhibitory effect of Agm on the carotid baroreflex in anesthetized rats ABP: arterial blood pressure. ISP: intrasinus pressure.

Tab 2. Effects of pretreatment with Ida (0.1 mmol/L), *L*-NAME (500 μmol/L) and Yoh (15 μmol/L) for 20 min on the responses of carotid baroreflex to Agm (5 mmol/L). $\bar{x} \pm s$. $n = 6$. Agm: agmatine. Ida: idazoxan *L*-NAME: N^G-nitro-*L*-arginine methyl ester. Yoh: yohimbine. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^dP > 0.05$, $^eP < 0.05$ *vs* Agm (5 mmol/L).

Groups	TP/kPa	EP/kPa	SP/kPa	OR/kPa	PS	RD/kPa
Control	7.6±0.7	13.5±0.6	23.7±0.7	16.1±0.5	0.43±0.05	6.5±0.8
Agm	9.7±0.4^c	13.3±0.4^a	24.1±0.5^a	14.4±0.8^b	0.22±0.07^c	3.3±0.7^c
Ida	7.7±0.4^a	13.6±0.9^a	24.0±0.8^a	16.3±0.4^a	0.44±0.08^a	6.6±1.1^a
Ida + Agm	7.4±0.6^{af}	13.6±0.8^{ad}	23.2±1.0^{ad}	15.8±0.7^{ae}	0.42±0.04^{af}	6.6±0.9^{af}
Control	7.4±0.7	14.2±0.6	23.2±0.8	15.8±0.5	0.41±0.06	6.6±0.7
Agm	10.0±0.6^c	14.6±0.8^a	24.9±0.7^a	14.9±0.4^a	0.23±0.08^c	3.2±0.5^c
Yoh	7.6±0.4^a	14.0±1.0^a	23.8±0.9^a	15.7±0.9^a	0.43±0.05^a	6.4±0.8^a
Yoh + Agm	8.8±0.7^{be}	14.7±0.5^{ad}	24.8±0.5^{bd}	16.0±0.7^{ad}	0.33±0.09^{be}	4.9±0.8^{be}
Control	7.8±0.6	13.9±0.7	23.7±0.7	15.9±0.8	0.44±0.07	6.3±0.5
Agm	9.4±0.5^c	14.3±0.6^a	24.0±0.9^a	15.6±0.5^a	0.25±0.08^c	3.1±0.6^c
L-NAME	7.7±1.2^a	14.1±0.8^a	23.5±0.4^a	15.8±0.5^a	0.45±0.09^a	6.4±0.6^a
L-NAME + Agm	9.2±0.8^{cd}	14.5±0.6^{ad}	24.1±0.6^{ad}	14.9±0.7^{ad}	0.24±0.05^{cd}	3.3±0.7^{cd}

Agmatine might be a precursor for NO generation as its effects can be completely abolished by *L*-NAME, a NO synthase inhibitor[16]. On the basis of this viewpoint, agmatine could induce an increased production of NO[17]. On the contrary, Galea *et al*[18] showed that agmatine was a competitive NO synthase inhibitor, but not a precursor for NO. In the present experiment, *L*-NAME did not affect the inhibitory effect of agmatine, suggesting that NO might not be involved in the action of agmatine on carotid baroreflex.

In conclusion, agmatine exhibited inhibitory effects on carotid baroreflex, which was mediated by IR and α_2-AR.

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胍丁胺抑制麻醉大鼠颈动脉窦压力反射

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关键词 胍丁胺；颈动脉窦；压力反射；血压；咪唑啉受体；肾上腺素 α_2 受体；咪唑克生

目的：观察胍丁胺对颈动脉窦压力感受器反射的影响．**方法**：利用灌流左颈动脉窦方法，观察胍丁胺对麻醉大鼠压力反射机能参数的影响．**结果**：(1) 胍丁胺 1，5，10 mmol/L 均使颈动脉窦压力反射机能曲线向右上方移位，曲线最大斜率和反射性平均动脉压下降幅度均减小，提示胍丁胺对压力感受器反射有抑制作用；(2) 预先应用咪唑啉受体(IR)和肾上腺素能 α_2 受体(α_2-AR)拮抗剂咪唑克生(idazoxan，0.1 mmol/L)，则可完全阻断胍丁胺 5 mmol/L 的效应．预先应用 α_2 受体拮抗剂育亨宾(yohimbine，15 μmol/L)，则可部分阻断其效应；(3) 预先应用 NOS 抑制剂 *L*-NAME (500 μmol/L)，对胍丁胺的抑制作用无影响．**结论**：胍丁胺对颈动脉窦压力反射有抑制作用，并由咪唑啉受体和 α_2 受体介导．

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