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Original Research

Effect of ketamine on presympathetic neurons in rostral ventrolateral medulla of rats[1]

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KEY WORDS ketamine; medulla oblongata; neurons; electrophysiology

ABSTRACT

AIM: To study the effects of ketamine on central sympathetic and cardiovascular regulation. **METHODS**: The experiment was performed on 25 urethane-anaesthetized, artificially ventilated adult rats. A total of 32 presympathetic neurons in the rostral ventrolateral medulla (RVLM) were electrophysiologically identified, which had properties of both barosensitivity and projection to the spinal cord. Responses of these neurons to intravenously administrated ketamine (a non-competitive *N*-methyl-*D*-aspartate receptor antagonist) were observed. **RESULTS**: Intravenous injection of ketamine (3, 6, 12 mg/kg body weight) increased the firing rate and blocked the barosensitivity of presympathetic neurons in the RVLM in a dose-dependant manner. **CONCLUSION**: Ketamine could affect the sympathetic outflow by blocking tonic baroreceptor inhibition of the presympathetic neurons in the RVLM.

INTRODUCTION

Ketamine, a commonly used dissociative anesthetic, has been long known to have sympathoexcitatory actions and is able to block the arterial baroreceptor reflex when it is applied intravenously[1,2]. However, the mechanism by which ketamine exerts these actions is still not validated.

Recent pharmacological and electrophysiological studies have shown that the rostral ventrolateral medulla (RVLM) plays a key role in cardiovascular control[3,4]. A group of neurons, namely sympathoexcitatory or presympathetic neurons in the RVLM project, provide tonic excitatory inputs to the sympathetic preganglionic neurons (SPNs) located in the intermediolateral nucleus (IMIN) of the thoracolumbar spinal cord[5,6]. In addition, these neurons are thought to be the final common bulbospinal pathways of the baroreceptor reflex arc, which control the sympathetic vasomotor outflow[7,8]. The present study designed to observe the effect of intravenously applied ketamine on this group of neurons in an attempt to confirm our view that ketamine tonic baroreceptor inhibited the presympathetic neurons in the RVLM. For this purpose, the firing of the presympathetic neurons in the RVLM was recorded extracellularly, and effects of ketamine on both spontaneous activity and barosensitivity of these neurons were analyzed.

MATERIALS AND METHODS

Animals, anesthesia, and surgical procedures Experiments were performed on 25 adult male Sprague-Dawly rats (Animal Center, Shanghai Institute of Family Planning, Grade II, Certificate № 152) weighing between 280 g and 360 g. After induction of anesthesia with nembutal (80 mg/kg, ip), catheters were inserted into the left femoral artery and the left femoral vein for recording the arterial pressure (AP) or for administration of various agents. Following tracheostomy, each rat was paralyzed with gallamine triethiodide (10 mg/kg initially and 4 mg/kg every 30 min, iv) and was mechanically ventilated with oxygen-enriched room air (12 mL/kg body weight, 60 - 70 strokes/min), maintaining end-

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tidal CO_2 at 4 % – 5 %. Urethane was injected intravenously to maintain surgical anesthesia (1.1 g/kg applied in three injections with an interval of 10 min). Anaesthetics were supplemented when necessary. The animal was then fixed in a stereotaxic apparatus (Narishige, Japan). The left aortic nerve was exposed and isolated near its junction with the superior laryngeal nerve. The dorsal surface of the medulla was exposed by occipital craniotomy and partial cerebelectomy. Laminectomy was performed between C1 and T2. A glass-coated tungsten electrode was inserted into the dorsolateral funiculus of T1 segment for antidromic stimulation of spinally projecting neurons in the RVLM. All exposed tissues were covered with warm mineral oil. The rectal temperature was maintained at 37 ℃ with a heating pad and an infrared lamp.

Extracellular recording of the activities of the presympathetic neuron in the RVLM with the dorsal surface of the medulla in horizontal position, spontaneous activities of the RVLM neurons on either side were extracellularly recorded by glass microelectrodes (tip diameter about 1 μm, 4 – 10 MΩ impedance). The glass microelectrodes were filled with 2 % pontamine sky blue (PSB) dissolved in sodium acetate 0.5 mol/L. Recordings were made 2.5 – 2.9 mm rostral to the obex, 1.6 – 1.9 mm lateral to the midline, and 2.9 – 3.5 mm below the dorsal surface of the medulla. Signals were preamplified (100 – 3000 Hz bandpass) and subsequently fed into a window discriminator and simultaneously monitored on an oscilloscope. Corresponding to each spike, the window discriminator generated a digitized pulse for further computerized analysis.

Baroreceptor reflex activation Arterial baroreceptor reflex was activated by electrical stimulation of the left aortic nerve (AN), which contains only baroreceptor afferent fibers in rats[7]. The central cut end of the left AN was mounted on a pair of bipolar Ag/AgCl hook electrodes and was then immersed in a pool of warm mineral oil. Rectangular pulses were delivered to the aortic nerve through a stimulus isolator. Two patterns of stimulation were used: 1) The 0.2 ms duration square wave pulses at 100 Hz and 50 – 500 μA to test the function of the nerve. The current just enough to evoke a slight but visible decrease in arterial pressure was considered to be the threshold current; 2) Tripled (5 ms pulse interval) 0.2 ms square wave at 1 Hz and 5 times threshold current (0.2 – 0.6 mA) to determine barosensitivity of the RVLM neurons. Once a probable presympathetic neuron in the RVLM was identified, bolus intravenous injections of phenylephrine (10 μg/kg) were administered to elevate blood pressure so as to activate arterial baroreflex.

Analysis of neuronal signals The analyzing techniques of neuronal signals employed were integrated activity histogram, peristimulus time histogram, and arterial pressure pulse triggered time histogram. Barosensitivity of the neurons in the RVLM was evaluated based on the integrated activity histogram and peristimulus time histogram. From the integrated activity histogram, the magnitude of the decrease in unit's firing rate in response to elevation of blood pressure was considered as an index of the unit's barosensitivity. From the peristimulus time histogram, which is the addition of 100 – 300 sweeps of 250 ms (50 ms prestimulus time and 200 ms poststimulus time) period's signals, the onset latency of inhibition, duration of inhibition, and inhibition rate due to AN stimulation were taken as other index of the unit's barosensitivity. Results were expressed as $\bar{x} \pm s$. Differences were considered significant at $P < 0.05$ using paired t-test. The presympathetic neurons in the RVLM were identified by the following criteria[9,10]:

1. The spontaneous discharges of the neurons were inhibited by short train stimulation of the AN (3 pulse, 0.2 ms pulse width, 5 ms pulse interval, 50 – 500 μA, 1 Hz) which was displayed as a trough on the poststimulus time histogram (Fig 1A);

2. The spontaneous activities of the neurons decreased when blood pressure was elevated by bolus injection of phenylephrine (10 μg/kg body weight, Fig 1B). Typically, this dose of phenylephrine increased the mean arterial blood pressure by about 35 mmHg, making the unit temporarily silenced;

3. The neuronal fired in a rhythmic pattern that was related to the cardiac cycle as shown by the arterial pulse triggered time histogram (Fig 1C);

4. Antidromic identification of the neurons. The constant onset latency of action potentials evoked by spinal cord stimulation (0.2 ms pulse width, 0.1 – 1 mA, 1 Hz, Fig 1D).

Histological procedure In each animal, one or two recording sites were marked by iontophoretic deposit of PSB (– 15 μA 10 min) from the recording electrode. At the end of each experiment, the animal was perfused transcardially with saline and then 10 % formalin. Coronal sections of the medulla (50 μm) were made and the dye spots were plotted on standard atlas of Paxinos and Watson[11].

RESULTS

Totally 32 neurons were identified as putative

presympathetic neurons in the RVLM. These neurons fired irregularly with firing frequency ranging from 5 to 23 spikes/s (mean 12 Hz ± 5 Hz). Fig 1 shows arterial baroreflex related properties of the presympathetic neurons in the RVLM. These neurons were evidently barosensitive and were therefore considered as the presympathetic neurons, which directly project to the spinal cord and drive the SPNs.

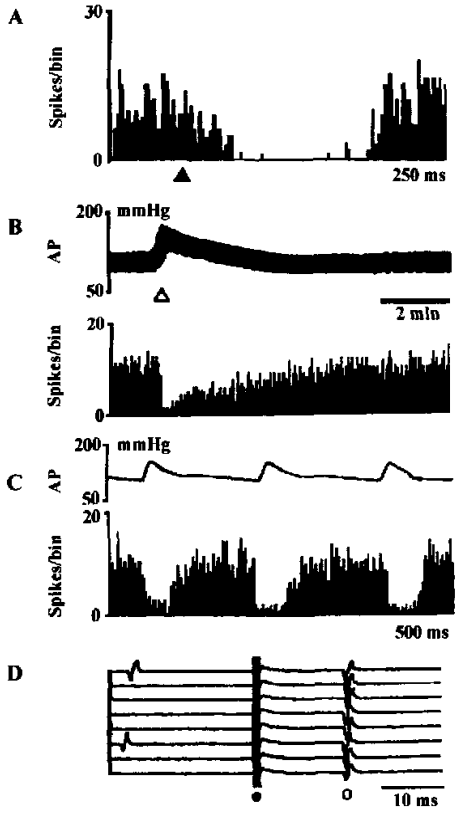

Fig 1. Electrophysiological identification of the presympathetic neuron in the RVLM. A) Inhibition of ongoing activity by short train pulse stimulation of AN (indicated by a filled triangle). The recording is a summation of 300 sweeps. Bin width = 1 ms. B) Inhibition of ongoing activity by intravenous injection of phenylephrine (10 μg/kg, marked by an open triangle) which elevated blood pressure. Bin width = 1 s. C) R-wave (of ECG) triggered average of blood pressure pulse and neuronal activity. The recording is a summation of 300 sweeps. Bin width = 1 ms. D) The constant onset latency of action potentials (marked by an open circle) evoked by spinal cord stimulation (marked by a filled circle).

Effect of ketamine on spontaneous activities of the presympathetic neurons in the RVLM Intravenous infusion of ketamine (10 g/L dissolved in saline, 3,6, or 12 mg/kg body weight) induced dose-dependant decrease in arterial blood pressure and simultaneous increase in the firing rate of the identified presympathetic neurons in the RVLM (Fig 2A, Tab 1). Typically, the blood pressure decreased immediately and dropped to the lowest level within 15 s after injection of ketamine, when it gradually recovered towards the baseline level. Full recovery usually occurred within 5 min after ketamine was administered, although with lower doses, the blood pressure recovered somewhat earlier. The neuronal units were excited after slightly longer latency (about 30 s) and were maximally excited at about 2 min after ketamine injection. Excitation of the neurons lasted for 5 min to 30 min depending on the dosage, which was much longer than the duration of the change in arterial blood pressure.

Effects of ketamine on barosensitivity of the presympathetic neurons in the RVLM Peristimulus time histograms of the presympathetic neurons were repeated before and 5, 10, 20, and 30 min after ketamine or saline (for control) was injected. Usually, stimulation of AN with a burst of three pulses (0.2 ms duration, 5 ms pulse interval, 50 – 500 μA at 1 Hz) resulted in a transient inhibition of neuronal firing (mean onset latency 42 ms ± 4 ms, mean duration of inhibition 51 ms ± 6 ms, inhibitory rate 88 % ± 4 %, $n = 32$) and a small drop in blood pressure as well. This reflex was reproducible throughout the recording period.

After low dose injection of ketamine (3 mg/kg body weight), AN stimulation induced inhibition of unit activity was partially blocked, as indicated by the longer onset latency of inhibition (67 ms ± 6 ms at 5 min after treatment *vs* 45 ms ± 5 ms before treatment, $n = 11$, $P < 0.01$), inhibitory rate in short duration (46 % ± 5 % at 5 min after treatment *vs* 88 % ± 8 % before treatment, $n = 11$, $P < 0.01$). Barosensitivity of these units fully recovered at 15 min after ketamine administration. Higher doses (6 and 12 mg/kg body weight) of ketamine almost completely blocked the AN stimulation induced inhibition of the presympathetic neurons in the RVLM (Fig 2B). These doses also abolished the inhibition of these units by activation of baroreceptors through bolus intravenous injection of phenylephrine (10 μg/kg, Fig 2C). Moreover, the cardiac rhythm of the unit firing also disappeared after these doses of ketamine were administrated (Fig 2D). These effects appeared at less than 5 min after administration of ketamine and persisted till 15 min to

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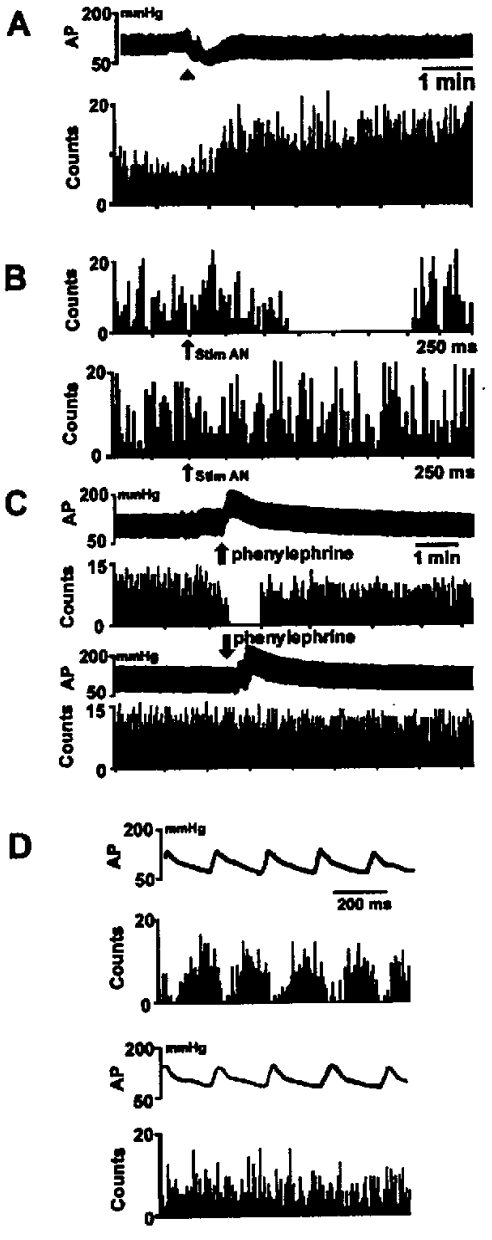

Fig 2. Effects of intravenously administered of ketamine 6 mg/kg on the presympathetic neurons in the RVLM. A) The responses of blood pressure and firing rate of the presympathetic neurons in the RVLM when ketamine was administered (marked by a filled triangle). B, C, and D) The responses of barosensitivity of the presympathetic neurons in the RVLM before (the upper panel) and after (the lower panel) ketamine applied.

25 min after treatment. In each case, partial or full recovery of unit's barosensitivity was observed at 30 min after treatment with ketamine.

Localization of the presympathetic neurons in the RLVM studied Recording sites were reconstructed from the dye spots. Although the region from 2.6 mm to 3.0 mm rostral to the obex was explored, most of the units included in this study were histologically just caudal to the border of the facial nucleus and ventral to the nucleus ambiguus. The recording sites were shown diagrammatically in Fig 3.

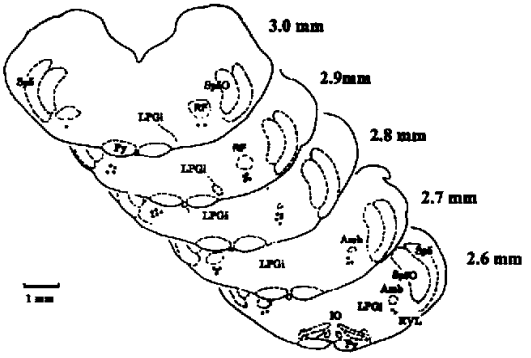

Fig 3. Location of the recording neurons in the RVLM. Recording sites (marked by the black circles) of the presympathetic neurons plotted on each cross section through the rat medulla 2.6 – 3.0 mm rostral to the obex. Amb, ambiguous; IO, inferior olivary nucleus; LPGi, lateral paragigantocelluar nucleus; Py, pyramidal tract; RF, retrofacial nucleus; RVL, nucleus reticularis rostroventrolateralis; Sp5O, spinal trigeminal tract nucleus, oral.

DISCUSSION

The purpose of the study was to investigate the effects of intravenously applied ketamine on the presympathetic neurons in the RVLM which have been reported to be the origin of the vasomotor sympathetic outflow and critical sites for expression of arterial baroreceptor-sympathetic reflex. Criteria for electrophysiological identification of the presympathetic neurons in the RVLM have been dealt with previously and are well established[9,10,12]. The criteria are mainly based on: 1) recording coordinates appropriate for the retrofacial portion of the nucleus paragigantocellularis lateralis; 2) the presence of barosensitivity, in other words, presympathetic neurons should be inhibited by baroreceptor activa-

Tab 1. Mean arterial pressure and the firing rate of the presympathetic neurons in the RVLM after intravenous injection of ketamine. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ **compared with the pretreatment value.**

Group	n	MAP/mmHg		Firing rate/spikes·s^{-1}	
		Before	After	Before	After
3 mg/kg	11	114 ± 7	107 ± 5^b	11 ± 5	12 ± 5^c
6 mg/kg	10	116 ± 7	102 ± 6^c	12 ± 6	14 ± 5^c
9 mg/kg	11	119 ± 6	91 ± 6^c	12 ± 5	16 ± 5^c

tion; 3) the presence of cardiac rhythm; 4) antidromic response following stimulation within thoracic segment of the spinal cord (confirmed by the collision test).

The RVLM neurons investigated in the present study were considered to be putative sympathoexcitatory presympathetic neurons. First, these units were recorded in coordinates as previously reported by others and were confirmed histologically to be located in the region corresponding to the subretrofacial portion of the region paragigantocellularis lateralis. Secondly, these neurons were inhibited both by AN stimulation and by elevation of blood pressure and were therefore obviously barosensitive. Thirdly, these neurons discharged in a rhythmic pattern locked to the cardiac cycle. Finally, these units showed the constant onset latency of action potentials evoked by spinal cord stimulation. Antidromic identification of axonal projections to the thoracolumbar spinal cord is important, as some neurons in the RVLM may be barosensitive but not have spinal axons, and therefore excluded from the presympathetic category. In short, these neurons which are both barosensitive and having spinal axons were likely to be presympathetic neurons contributing to sympathetic outflow.

Bolus intravenous injection of ketamine was found to be able to excite the presympathetic neurons in the RVLM in a dose-dependent manner. Therefore, we believe that ketamine can increase sympathetic outflow by somehow exciting the presympathetic neurons in the RVLM which in turn drive the SPNs in the IML (intermediolateral cell column) of the thoracolumbar spinal cord, although the exact mechanisms remain unclear.

However, excitation of neurons in the RVLM firing by intravenous ketamine might be due, at least in part, to blockade of the tonic baroreflex inhibition since the barosensitivity of these neurons was inhibited by low dose and completely blocked by higher doses of ketamine, which indicates that central transmission of baroreceptor signals was blocked. But which synapses in the baroreflex pathway ketamine affected are beyond this study. It is now clear that baroreceptor afferent fibers terminate on the barosensitive neurons in nucleus tractus solitarius (NTS), which project, probably monosynaptically, to the interneurons in the caudal ventrolateral medulla (CVLM) to form excitatory synapses. The barosensitive interneurons in the CVLM in turn project to the bulbospinal presympathetic neurons in the RVLM to form inhibitory GABAergic synapses[13,14]. Since ketamine is known to be a non-competitive *N*-methyl-*D*-aspartate (NMDA) receptor antagonist[15,16], it is very likely that ketamine blocked the transmission of baroreceptor signals at synapses using glutamic acid as transmitters. Ample evidence has demonstrated that glutamic acid might be the most important neurotransmitter of the first (at the NTS) and second (at the CVLM) order synapses of the arterial baroreceptor pathway[17,18]. Some evidences showed that ketamine and AP-5 (a specific NMDA receptor antagonist) microinjecting into the NTS were able to block the bradycardiac and hypotensive response to baroreceptor activation by traction of the left carotid artery[19]. Another study by Guyenet and his coworkers[20] did show that microinjection of kynurenic acid (a wide spectrum glutamate receptor antagonist) into the CVLM blocked the inhibition of renal sympathetic efferent discharge induced by elevation of blood pressure. In support of these previous reports, we found that CPP (another specific NMDA receptor antagonist) microinjected into the NTS or the CVLM blocked the inhibition of the barosensitive neurons in the RVLM induced by AN stimulation[21,22].

In summary, the present study suggests that the well-known sympathoexcitatory effect of ketamine might be attributed to its ability to excite the presympathetic neurons in the RVLM by blocking tonic baroreceptor inhibition of these neurons.

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氯胺酮对大鼠头端延髓腹外侧区前交感神经元的作用[1]

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关键词 氯胺酮；延髓；神经元；电生理学

目的：研究氯胺酮在中枢交感心血管活动中的调节作用. **方法**：在25只氨基甲酸乙酯麻醉、三碘季铵酚制动并人工通气的雄性SD大鼠中，共细胞外记录到32个头端延髓腹外侧区前交感神经元的自发放电，这些神经元具有压力敏感性和向脊髓投射的特点. 观察选择性NMDA受体拮抗剂氯胺酮对前交感神经元放电的影响. **结果**：静脉注射不同剂量的氯胺酮(3，6，12 mg/kg)能增加RVLM前交感神经元的放电频率，同时能阻断这些神经元的压力敏感性，且具有剂量依赖性的特点. **结论**：氯胺酮通过阻断RVLM前交感神经元压力感受反射的紧张性抑制，从而调节交感心血管活动.

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