ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Jul; 21 (7): 617 - 622
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NMDA receptor mechanism involved in arterial baroreflex[1]

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KEY WORDS *N*-methyl-*D*-aspartate receptors; baroreflex; solitary nucleus; medulla oblongata; excitatory amino acids

ABSTRACT

AIM: To study the roles of *N*-methyl-*D*-aspartate (NMDA) receptors in arterial baroreflex. **METHODS**: Experiments were performed in 17 urethane-anaesthetized male Sprague-Dawly rats. Twenty-seven rostral ventrolateral medulla (RVLM) neurons were electrophysiologically identified as putative presympathetic neurons. Responses of these neurons to baroreflex activation were used as an index to observe the effects of 3-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid (CPP) microinjected (0.1 μL, 50 mmol·L^{-1}) into the nucleus tractus solitarii (NTS) or the caudal ventrolateral medulla (CVLM) ipsilaterally. **RESULTS**: In the NTS, CPP eliminated or attenuated the inhibition of these neurons induced by aortic nerve stimulation, but the inhibition induced by elevation of arterial pressure was not eliminated and the cardiac cycle-related rhythm of spontaneous discharge still existed. Whereas in the CVLM, CPP eliminated not only the inhibition of these neurons induced by aortic nerve stimulation, but the inhibition induced by blood pressure elevation and the cardiac cycle-related rhythm of spontaneous discharge disappeared. **CONCLUSION**: NMDA mechanism is importantly involved in the mechanism of baroreflex both in the NTS and in the CVLM; the barosensitive neurons in the NTS project to ipsilateral CVLM.

INTRODUCTION

The presympathetic neurons in rostral ventrolateral medulla (RVLM) are related to arterial baroreflex, and they could be inhibited by stimulation of the aortic nerve, silenced by elevation of blood pressure, and have a cardiac cycle-related rhythm of spontaneous discharge. Aortic nerve fibres in mammals like rats and cats are known to terminate in the nucleus tractus solitarii (NTS) at the dorsal medial subnucleus[1]. Ample evidence has shown that excitatory amino acids may be the transmitters of arterial baroreflex[2-7]. By observing parameters of cardiovascular dynamics, many researchers have found that in the NTS or the caudal ventrolateral medulla (CVLM), glutamate receptor antagonists block arterial baroreflex. But as these parameters fluctuate physiologically and are susceptible to numerous factors, the usefulness of these studies is limited.

In our previous study[8], the responses of the presympathetic neurons in the RVLM towards activation of peripheral afferent proved to be a sensitive index to study cardiovascular reflexes and corresponding transmitters. In the present study, 27 RVLM neurons were identified as putative presympathetic neurons. Their responses to stimulation of the aortic nerve were recorded. Using the responses as an index, the effects of 3-(2-Carboxypiperazin-4-yl) propyl-1-phosphonic acid (CPP), a selective *N*-methyl-*D*-aspartate (NMDA) receptor antagonist from Sigma were recorded, after its microinjection into the NTS or the CVLM.

MATERIALS AND METHODS

Animals, anaesthesia, and surgical procedures Experiments were performed in 17 adult male Sprague-Dawly rats (Animal Center, Shanghai Institute of Family Planning, grade Ⅱ, Certificate No 152) weighing 280 - 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 of the arterial pressure or for administration of various agents. Following tracheostomy, each rat was paralyzed with gallamine triethiodide (4 mg/kg, iv) and was mechanically ventilated with oxygen-enriched room

[1] Project supported by the Research Foundation for Medical Sciences of PLA (the 9th Five-year Program, No 96M072)
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Received 1999-04-22 Accepted 2000-01-20

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Jul; 21 (7)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

air (12 mL/kg body weight, 60 – 70 strokes/min). Urethane (1.1 g/kg body weight) was injected intravenously to maintain surgical anaesthesia. 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 and was then covered with warm mineral oil. The rectal temperature was maintained at 37 ℃ with a thermostatic heating lamp.

Extracellular recording of the RVLM presympathetic neuron activities With the dorsal surface of the medulla in horizontal position, spontaneous activities of the RVLM neurons on either side were extracellularly recorded by means of glass microelectrodes (tip diameter about 1 μm, 4 – 10 MΩ impedance). The glass microelectrodes were filled with 2 % pontamine sky blue dissolved in 0.5 mol/L sodium acetate. Recordings were made 2.5 – 2.9 mm rostral to the obex, 1.6 – 1.9 mm lateral to the mid-line, and 2.9 – 3.5 mm below the dorsal surface of the medulla. Spontaneous spikes were preamplified (100 – 3000 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.

Activation of baroreflex Arterial baroreflex was activated by electrical stimulation of the left aortic nerve, which in rats contains only baroreceptor afferent fibers. The central cut end of the left aortic nerve was mounted on a pair of bipolar Ag/AgCl hook electrode 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) 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 which 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 RVLM presympathetic neuron was identified, bolus intravenous injections of phenylephrine (10 μg/kg) were administered to elevate blood pressure so as to activate arterial baroreflex.

Microinjection of agents into the NTS or the CVLM Under the guidance of the sterotoxic apparatus, a glass micropipette (tip diameter 25 – 50 μm) was inserted into the NTS (0.5 mm rostral to the obex, 0.5 mm lateral to the mid-line, and 0.3 – 0.5 mm below the dorsal surface of the medulla) or the CVLM at the level of the obex (1.6 – 1.9 mm lateral to the mid-line, 2.8 – 3.2 mm below the dorsal surface of the medulla). It was filled with artificial cerebrospinal fluid (ACSF) or solution of CPP (50 mmol/L, dissolved in ACSF, pH 7.38) and attached to a 1-μL syringe through a catheter. The agents were given by pressure microinjection in a volume of 0.1 μL. The sites for microinjection were ipsilateral to the putative RVLM presympathetic neuron to be tested. At the end of the experiment, the glass micropipette was removed from the brain tissue, filled with 2 % pontamine sky blue and returned to the same brain area as that of agent microinjection. Dye solution 0.1 μL was injected for subsequent histological identification.

Analysis of neuronal signals *The digitized* neuronal signals corresponding to unit spikes from the window discriminator, together with the arterial pressure pulses and the TTL pulses derived from the stimulator delivering currents to the aortic nerve, were fed into a computer and analyzed by means of integrated activity histogram, peristimulus time histogram, and arterial pressure pulse triggered time histogram. Spontaneously active RVLM neurons that were inhibited by stimulation of the aortic nerve, silenced by elevation of blood pressure and had a cardiac cycle-related rhythm were identified as putative presympathetic neurons and were tested further.

Experimental protocol Once a putative RVLM presympathetic neuron was identified, its control response to aortic nerve stimulation was recorded by means of peristimulus time histogram. Thereafter CPP was microinjected into the NTS or the CVLM. After each application of agents, the neuronal response was tested intermittently until its recovery. After completion of each neuron test, the recording site was marked by microiontophoretic deposit of PSB (15 μA of cathodal current for 15 min) from the recording electrode.

Histological procedure By the end of the experiment, the animal was killed by intravenous injection of excessive anesthetic and perfused transcardially with saline and 10 % formalin, and coronal sections of the medulla (50 μm) were made, and the sites for recording or microinjection were reconstructed from the dyespots by referring to *Paxinos and Watson*' atlases (1986).

Statistic analysis The results were expressed as $\bar{x} \pm s$ and analyzed by paired t-test.

RESULTS

Totally 27 neurons were identified as putative RVLM

presympathetic neurons. Fig 1 shows arterial baroreflex related properties of a typical putative RVLM presympathetic neuron. In the peristimulus time histogram, stimulation of the aortic nerve caused an inhibition of (46 ± 21) ms onset latency and (55 ± 12) ms duration, the maximally inhibited firing probability in percentage of base line being (94.3 ± 2.2) %.

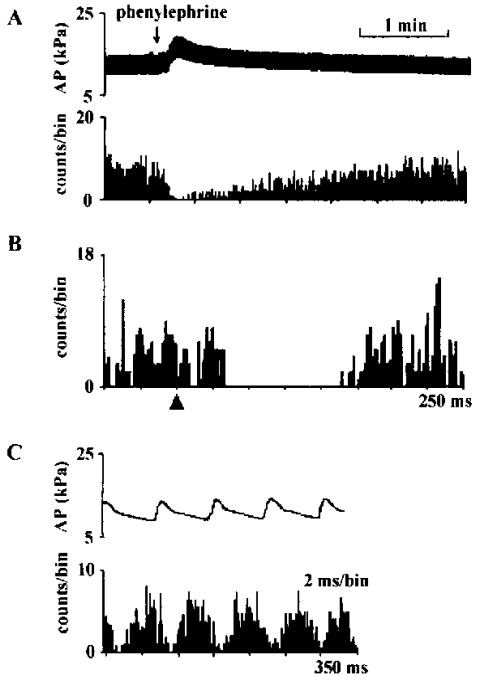

Fig 1. Arterial baroreflex related properties of a typical putative RVLM presympathetic neuron. A, inhibition of spontaneous discharge by blood pressure elevation. B, Peristimulus time histogram during stimulation of the aortic nerve, ▲ represents the time at which stimulation was delivered. C, blood pressure pulse triggered time histogram of spontaneous discharge, showing the cardiac cycle-related rhythm.

Effects of CPP microinjection into the NTS on the neuronal responses of putative RVLM presympathetic neurons to baroreflex activation

Of the 12 putative presympathetic neurons tested in 8 rats, microinjection of CPP into the NTS eliminated or attenuated the inhibition induced by aortic nerve stimulation (Fig 2, A and B). This effect occurred within 10 s after the microinjection, persisted for about 10 – 15 min, and then disappeared gradually.

In addition, this protocol caused an immediate increase in mean arterial blood pressure, from the base line

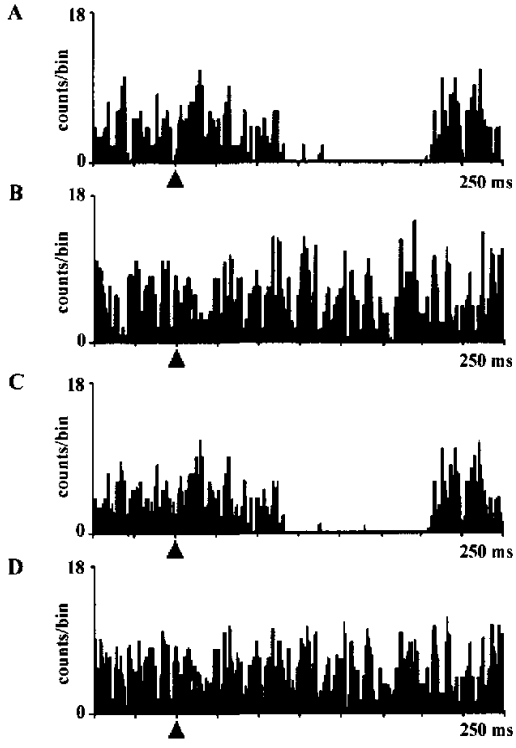

Fig 2. A and B, peristimulus time histogram of a putative RVLM presympathetic neuron before (A) and after (B) CPP microinjection into the NTS. C and D, peristimulus time histogram of this neuron before (C) and after (D) CPP microinjection into the CVLM.

of (104.5 ± 4.3) mmHg (1 mmHg = 0.133 kPa) to the highest of (119.2 ± 4.7) mmHg ($P < 0.01$, $n = 12$). This increase occurred in seconds and usually persisted for about 10 min. The spontaneous discharge rate of these neurons also increased, from the base line of (13.9 ± 2.6) pulses/s to the highest of (17.9 ± 2.4) pulses/s ($P < 0.01$, $n = 12$). This increase occurred almost simultaneously with the microinjection and persisted for about 15 minutes. However, regarding the spontaneous discharge of these neurons, the inhibition induced by elevation of arterial pressure, though attenuated, was not eliminated (Fig 3A), and the cardiac cycle-related rhythm still existed (Fig 3C), even when the neuronal inhibition induced by aortic nerve stimulation was completely blocked in peristimulus time histogram.

Effects of CPP microinjection into the CVLM on the neuronal responses of putative RVLM presympathetic neurons to baroreflex activation

Of the 15 putative presympathetic neurons tested in 9

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Jul; 21 (7)
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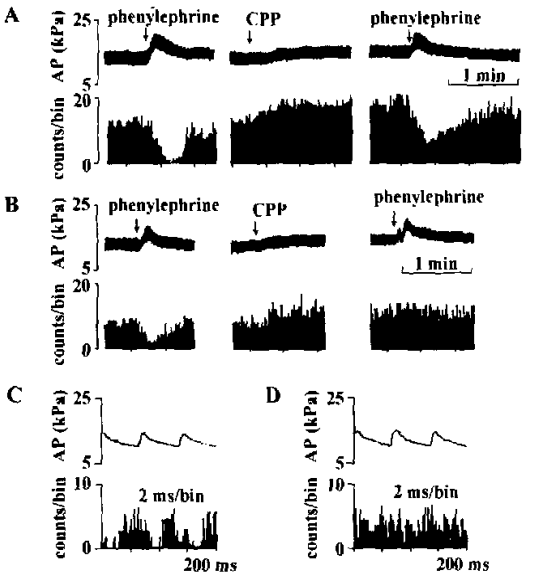

Fig 3. A and B, effect of CPP microinjection into the NTS (A) or the CVLM (B) on the inhibition of spontaneous discharge of putative RVLM presympathetic neurons induced by blood pressure elevation. Left, before CPP microinjection. Middle, effect of CPP microinjection on the spontaneous discharge. Right, after CPP microinjection. C and D, blood pressure pulse triggered time histogram of spontaneous discharge after CPP injection into the NTS (C) or the CVLM (D).

rats, microinjection of CPP into the CVLM eliminated the inhibition induced by aortic nerve stimulation (Fig 2C, 2D). Inaddition, this protocol also caused an immediate increase of mean arterial blood pressure, from the base line of (101.6 ± 5.2) mmHg to the highest of (122.2 ± 4.9) mmHg ($P < 0.01$, $n = 15$). The spontaneous discharge rate of these neurons also increased, from the base line of (13.7 ± 3.6) pulses/s to the highest of (19.5 ± 2.7) pulses/s ($P < 0.01$, $n = 15$). The onset latencies, duration of all these effects were similar to the effects of CPP in the NTS. However, regarding the spontaneous discharge of these neurons, the cardiac cycle-related rhythm, as well as the inhibition induced by elevation of arterial blood pressure, was eliminated (Fig 3B, 3D).

In the control experiments, microinjection of CSF into the NTS or the CVLM had no detectable effects on the baseline discharge or the baroreflex activation responses of putative RVLM presympathetic neurons.

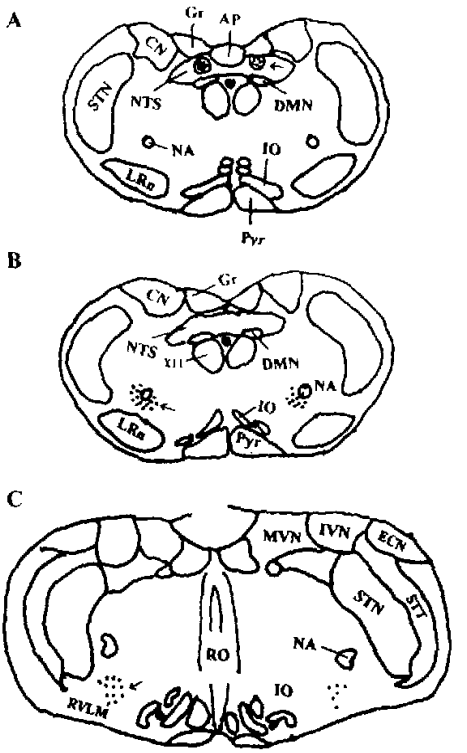

Fig 4. (A) Sites (arrow-pointed black points) of CPP microinjection in the NTS. (B) Sites (arrow-pointed black points) of CPP microinjection in the CVLM. (C) Sites of recorded putative presympathetic neurons. Abbreviations: AP, area postrema; CN, nucleus cuneatus; DMN, dorsal motor nucleus of the vagus; ECN, external cuneate nucleus; Gr, nucleus gracilis; IO, inferior olive; IVN, inferior vestibular nucleus; LRn, lateral reticular nucleus; MVN, medial vestibular nucleus; NA, nucleus ambiguus; NTS, nucleus tractus solitarious; Pyr, pyramidal tract; RO, nucleus raphe obscurus; RVLM, rostral ventrolateral medulla; STN, spinal trigeminal nucleus; STT, spinal trigeminal tract; XII, hypoglossal nucleus.

DISCUSSION

There are controversies over the role of NMDA and non-NMDA receptors in baroreflex[9-11]. In the present study, CPP at a comparative dose, reported to selectively antagonize NMDA[12,13], was applied to the NTS or the CVLM, and responses of putative RVLM presympathetic neurons to baroreflex activation were used as an index to test the role of NMDA receptors.

In the NTS, CPP attenuated, and in some cases

eliminated the inhibition of the putative RVLM presympathetic neurons to aortic nerve stimulation. This was different from previous studies reporting that NMDA antagonists achieved only partial blockage of baroreflex, and non-NMDA receptors played a predominant role[9,10]. The reason is not clear, but the differences may have resulted from the dose, chemical characteristics of the NMDA antagonists applied, sites of injection, and particularly the reliability of the indexes employed. In the CVLM, CPP in all cases eliminated the inhibition of these neurons to aortic nerve stimulation. This was consistent with previous studies reporting that NMDA antagonists completely blocked baroreflex[3]. Moreover, barosensitive neurons in the NTS of single side were reported to inhibit ipsilateral RVLM presympathetic neuron[14]. Our results suggest that this may be due to their termination in ipsilateral CVLM.

CPP in the NTS did not affect the inhibition induced by blood pressure elevation and the cardiac cycle-related rhythm of spontaneous discharge, but did so the CVLM. The following reasons may account for this difference. All the synapses of baroreflex except those of aortic nerve were not blocked by the microinjected CPP in the NTS, such that baroreflex was not completely eliminated. In another test, microinjections of such a dose of CPP into the commissural subnucleus of the NTS, a site at which most fibers of the sinus nerve terminate, only had slight or no effect on the inhibition of RVLM presympathetic neurons by aortic nerve stimulation.

In conclusion the present study has demonstrated that NMDA mechanism is importantly involved in the mechanism of baroreflex both in the NTS and in the CVLM, and that the barosensitive neurons in the NTS project to ipsilateral CVLM.

ACKNOWLEDGEMENTS We are very grateful to Professor ZHANG Shun-Xing for his help in revision of the manuscript. The instructions from Professors FU Wei-Jun and LU Zhen-Dong are indispensable.

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ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Jul; 21 (7)
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NMDA 受体机制在动脉压力反射中的介导作用[1]

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关键词 *N*-甲基-*D*-天门冬氨酸受体；压力反射；孤束核；延髓；兴奋性氨基酸

目的：研究 *N*-甲基-*D*-天门冬氨酸（NMDA）受体机制在动脉压力反射中的作用. **方法**：头端延髓腹外侧区（RVLM）前交感神经元（presympathetic neuron）与动脉压力反射相关，它们可被电刺激主动脉神经或升高动脉血压所抑制，其自发放电具有心性节律. 根据这一特性，本研究用电生理学方法在 17 只雄性 SD 大鼠鉴定了 27 个假想的（putative）RVLM 前交感神经元. 以这些神经元对电刺激主动脉神经的反应为指标，观察在同侧孤束核（NTS）或尾端延髓腹外侧区（CVLM）微注射选择性 NMDA 受体拮抗剂 CPP（0.1 μL，50 mmol/L）的作用. **结果**：在 NTS 微注射 CPP 可完全阻断或减弱电刺激主动脉神经引起的神经元抑制，但血压升高引起的神经元抑制不能完全被消除，神经元放电的心性节律仍然存在；在 CVLM，CPP 不仅完全阻断电刺激主动脉神经引起的神经元抑制，而且阻断血压升高引起的神经元抑制，神经元放电的心性节律消失. **结论**：NMDA 受体机制在动脉压力反射中起着重要的介导作用；单侧孤束核的压力敏感神经元向单侧 RVLM 投射.

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