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BRAIN RESEARCH

Research Report

Excitatory pathways from the vestibular nuclei to the NTS and the PBN and indirect vestibulo-cardiovascular pathway from the vestibular nuclei to the RVLM relayed by the NTS

Yi-Ling Cai, Wen-Ling Ma, Jun-Qin Wang, Yi-Qian Li, Min Li**

Department of Military Hygiene, Faculty of Naval Medicine, Second Military Medical University, Shanghai 200433, PR China

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ABSTRACT

Previous studies have confirmed the existence of vestibulo-sympathetic pathways in the central nervous system. However, the exact pathways and neurotransmitters underlying this reflex are unclear. The present study was undertaken to investigate whether the vestibulo-cardiovascular responses are a result of activated glutamate receptors in the caudal vestibular nucleus. We also attempt to verify the indirect excitatory pathways from the vestibular nucleus (VN) to the rostral ventrolateral medulla (RVLM) using a tracing method combined with a vesicular glutamate transporter (VGluTs) immunofluorescence. In anesthetized rats, unilateral injection of L-glutamate (5 nmol) into the medial vestibular nucleus (MVe) and spinal vestibular nucleus (SpVe) slightly increased the mean arterial pressure (MVe: 93.29 ± 11.58 to 96.30 ± 11.66, SpVe: 91.72 ± 15.20 to 95.48 ± 17.16). Local pretreatment with the *N*-methyl-D-aspartate (NMDA)-receptor antagonist MK-801 (2 nmol) significantly attenuated the pressor effect of L-glutamate injected into the MVe compared to the contralateral self-control. After injection of biotinylated dextran amine (BDA) into the MVe and SpVe, and fluorogold (FG) into the RVLM, some BDA-labeled fibres and terminals in the nucleus of solitary tract (NTS) and the parabrachial nucleus (PBN) were immunoreactive for VGluT1 and VGluT2. Several BDA-labeled fibres were closely apposed to FG-labeled neurons in the NTS. These results suggested that activation of caudal vestibular nucleus neurons could induce pressor response and NMDA receptors might contribute to this response in the MVe. The glutamatergic VN-NTS and VN-PBN pathways might exist, and the projections from the VN to the RVLM relayed by the NTS comprise an indirect vestibulo-cardiovascular pathway in the brain stem.

* *Corresponding authors*. Fax: +86 29 83283229.
E-mail addresses: yilingcai1@sohu.com (Y.-L. Cai), Ma_wlmail@sohu.com (W.-L. Ma), linlimin115@hotmail.com (M. Li).

Abbreviations: VN, vestibular nucleus; NTS, nucleus of the solitary tract; PBN, parabrachial nucleus; RVLM, rostral ventrolateral medullar; NMDA, *N*-methyl-D-aspartate; VGluT, vesicular glutamate transporter; IR, immunoreactivity; 4V, fourth ventricle; MVe, medial vestibular nucleus; SpVe, spinal vestibular nucleus; Pr, prepositus nucleus; Sp5, spinal trigeminal tract; IRt, intermediate reticular nucleus; dl, dorsolateral subnucleus; im, intermediate subnucleus; is, interstitial subnucleus; lt, lateral subnucleus; me, medial subnucleus; sup, subpostremal subnucleus; tr, tractus solitarius; vt, ventral subnucleus; elPBN, external lateral parabrachial nucleus; BP, blood pressure; MAP, mean arterial blood pressure; aCSF, artificial cerebrospinal fluid; BDA, biotinylated dextran amine; FG, Fluoro-gold

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1. Introduction

Recent studies have demonstrated that the vestibular system contributes to autonomic regulation during alterations in body position in space (Zhu et al., 2007). Electrical or adequate vestibular stimulation elicits an increase in sympathetic nerve activity in both animal and human subjects (Xu et al., 2002; Kaufmann et al., 2002). A large amount of evidence suggested the existence of vestibulo-sympathetic reflex pathways independent of baroreflex ones in various species (Woodring et al., 1997; Radtke et al., 2000; Ray, 2001; Gotoh et al., 2004). However, the exact pathways and neurotransmitters underlying this reflex in the central nervous system (CNS) are unknown.

Lesions of the central vestibular system can produce a prolonged impairment in posture-related cardiovascular responses while bilateral labyrinthectomy only elicits a transient effect (Jian et al., 1999; Mori et al., 2005). These experiments suggested that the vestibular nucleus (VN) plays a key role in compensatory vestibulo-sympathetic response during movement and posture changes. Studies have indicated that the medial and spinal vestibular nuclei (MVe, SpVe) located caudal to the lateral vestibular nucleus might be the "autonomic region" of the vestibular nucleus complex (Uchino et al., 1970; Shiba et al., 1996; Xu et al., 2002). In anesthetized, tracheotomized and spontaneously breathing rats, electrical stimulation of MVe evoked pressor response (Xu et al., 2002). However, whether this pressor response was a result of activated local neurons or fibres of passage remained unclear. Balaban and Porter reported that neurons in the caudal MVe and SpVe projected directly to nucleus of the solitary tract (NTS) and other autonomic regions, such as the caudal part of parabrachial nucleus (PBN), nucleus ambiguous and dorsal motor nucleus of the vague (Balaban and Porter, 1998). Lesion studies showed that the SpVe and adjacent MVe and the rostral ventrolateral medullary reticular formation were essential for vestibulo-sympathetic reflex during otolith stimulation in cats (Yates and Miller, 1994; Yates et al., 1995). It is well known that the rostral ventrolateral medullar (RVLM) plays a critical role in the sympathetic tone regulation and blood pressure (BP) control, and this area contains tonically active presympathetic neurons innervating sympathetic preganglionic neurons of the intermediolateral cell column monosynaptically (Guyenet, 2006). Although no studies have shown that the vestibular nuclei provide direct projection to the RVLM, physiological studies have confirmed that vestibular related neurons in the RVLM receive indirect vestibular inputs (Dampney et al., 1987; Yates et al., 1991).

In our previous study, we found that phosphate-activated glutaminase (PAG)-immunoreactive VN neurons constituted excitatory glutamatergic VN-NTS and VN-PBN transmission pathways and these pathways were involved in vestibulo-autonomic reflexes during vestibular stimulation (Cai et al., 2007). In addition, there are anatomical and functional evidences for direct excitatory projections from the NTS and PBN to the RVLM in rats (Aicher et al., 1996; Mauad and Machado, 1998; Len and Chan, 1999). Therefore, it is possible that the indirect pathways from the VN to the RVLM may be relayed by the NTS and PBN. Furthermore, if the VN-NTS-RVLM and (or) VN-PBN-RVLM pathways exist, it remains unclear whether glutamate acts as an excitatory neurotransmitter in these projections or not. The present study is undertaken to investigate whether focal administration of excitatory amino acid into the caudal VN can elicit blood pressure response. We also attempt to investigate the indirect excitatory VN-RVLM pathways using retrograde and anterograde tracing method combined with vesicular glutamate transporters (VGluTs) immunofluorescence and confocal laser scanning microscopy.

2. Results

2.1. BP responses to L-glutamate injection into the MVe and SpVe

In group 1, unilateral injection of L-glutamate (5 nmol, $n=11$) into the MVe and SpVe slightly increased mean arterial pressure (MAP) (MVe: from 93.29 ± 11.58 to 96.30 ± 11.66, SpVe: from 91.72 ± 15.20 to 95.48 ± 17.16, $P<0.01$). In a self-control test, injection of the same volume of aCSF into the same region did not alter basal BP. The changes in MAP evoked by aCSF and L-glutamate injection into MVe and SpVe were shown in Table 1. In group 2, pretreatment with aCSF had no effect on the pressor responses caused by microinjection of L-glutamate in the same region of the MVe and SpVe (aCSF pretreatment self-control) (Figs. 1A, B). Local injection with NMDA receptor antagonist MK801 into the controlateral MVe and SpVe had no effect on blood pressure baseline (94.65 ± 17.68 mm Hg vs. 94.05 ± 17.85 mm Hg, 92.35 ± 17.68 mm Hg vs. 92.15 ± 16.75 mm Hg), but significantly attenuated the pressor effect of L-glutamate injected into the same region of MVe afterward compared to the contralateral aCSF pretreatment self-control (Fig. 1C). The changes in MAP evoked by L-glutamate injection into MVe and SpVe of animals pretreated with aCSF on one side (aCSF-L-glutamate) and pretreated with MK801 on another side (MK801-L-glutamate) were shown in Table 1.

Table 1 – The effects of aCSF and L-glutamate unilaterally injected into the MVe and SpVe on changes in mean arterial pressure (ΔMAP) in group 1, and pretreatment with MK801 on changes in mean arterial pressure evoked by L-glutamate in group 2

	ΔMAP (mmHg)	
	MVe	SpVe
Group 1 (n=11)		
aCSF	0.79±0.97	0.88±1.35
L-glutamate	3.01±2.49*	3.76±3.80*
Group 2 (n=10)		
aCSF–l-glutamate	3.09±3.13	3.22±1.68
MK801–L-glutamate	1.10±0.43#	2.72±2.35

Values are means±SD; *$P<0.05$ vs. aCSF self-controls, #$P<0.05$ vs. aCSF pretreatment self-controls (paired t-test).