Neuroscience Letters 563 (2014) 70–74

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Comparative analysis of vestibular receptor and baroreceptor inputs to the nucleus tractus solitarius following acute hypotension in conscious rats

Xian Jiang[a,b], Li-Wei Li[a], Yan Lan[a], Yan-Zhao Yang[a], Guang-Shi Jin[c], Min Sun Kim[d], Byung Rim Park[d,*], Yuan-Zhe Jin[a,1]

[a] Department of Physiology and Pathophysiology, Yanbian University College of Medicine, Yanji 133002, China
[b] Department of Otolaryngology, Yanbian University College of Clinical Medicine, Yanji 133000, China
[c] Department of Cerebral Surgery, Yanbian University College of Clinical Medicine, Yanji 133000, China
[d] Department of Physiology, Wonkwang University School of Medicine and Brain Science Institute at Wonkwang University, Iksan 570-749, Korea

HIGHLIGHTS

- c-Fos protein following acute hypotension was measured in NTS of conscious rats.
- Activation in NTS following acute hypotension is largely due to baroreceptor inputs.
- And moderately due to the signals originating from vestibular receptors.
- And at least partly dependent on the other inputs except vestibular and baroreceptors.
- c-Fos protein expression was localized to caudal portion of NTS in BL and SAD groups.

ARTICLE INFO

Article history:
Received 7 December 2013
Received in revised form 14 January 2014
Accepted 20 January 2014

Keywords:
Vestibular receptor
Baroreceptor
c-Fos protein
Nucleus tractus solitarius
Acute hypotension
Conscious rat

ABSTRACT

Blood pressure is maintained by the interaction between the arterial baroreflexes and the vestibulocardiovascular reflexes during postural changes. In this study, the influence of the vestibular receptors on the maintenance of blood pressure following acute hypotension was quantitatively compared with the role of baroreceptors in terms of c-Fos protein expression in the nucleus tractus solitarius (NTS). Expression of c-Fos protein in the NTS was measured in conscious rats that had undergone bilateral labyrinthectomy (BL) and/or sinoaortic denervation (SAD). Expression of c-Fos protein increased significantly in the NTS in the sham group after sodium nitroprusside (SNP) administration. However, the BL, SAD, and SAD + BL groups showed significant decreases in c-Fos protein expression compared to that of the sham group. The SAD group showed relatively more reduction in c-Fos protein expression than the BL group, and the SAD + BL group showed the least expression among the three experimental groups. The c-Fos protein expression in the NTS following acute hypotension was localized to the caudal portions of the nuclei in the BL and SAD groups. These results suggest that the role of vestibular receptors in maintaining blood pressure following acute hypotension is less potent than that of the baroreceptors but more potent than other afferent inputs in conscious rats. In addition, afferent signals for maintaining blood pressure originating from the vestibular receptors and the baroreceptors may converge in the caudal portion of the NTS.

1. Introduction

Peripheral vestibular receptors in the inner ear control autonomic outflow as well as postural equilibrium in response to movements and gravitational forces [28]. Excitation of the vestibular system induces functional changes to the cardiovascular system, including modulation of blood pressure, pulse rate, baroreceptor reflex, and blood flow to the extremities [21]. Electrical or selective natural stimulation of the peripheral vestibular receptors induces an increase in sympathetic nerve activity [12,26]. Hypotension induced by head-up tilting is augmented by vestibular lesions in cats, which indicates that vestibular inputs play an important role in maintaining arterial blood pressure during postural changes [6,10]. Moreover, neurons from the medial and inferior vestibular nuclei project to the nucleus tractus solitarius(NTS), which controls

* Corresponding author. Tel.: +82 63 850 6773; fax: +82 63 852 6108.
E-mail addresses: byungp@wku.ac.kr (B.R. Park), y-z-jin@ybu.edu.cn (Y.-Z. Jin).
[1] Tel.: +86 433 2660586; fax: +86 433 2659795.

0304-3940/$ – see front matter © 2014 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2014.01.040

autonomic responses including those related to cardiovascular function during head movement [2,27].

The baroreflex is a feedback control system for maintaining arterial blood pressure during hemorrhage, postural changes, and exercise. Signals from the baroreceptors are conveyed by branches of the glossopharyngeal and vagus nerves to the NTS, which is an essential component of the circuitry mediating the baroreceptor reflex [24]. The NTS analyzes the context of input signals and initiates baroreflex responses by virtue of its influence on sympathetic premotor neurons in the rostral ventrolateral medulla (RVLM) [5]. This causes sympathetic preganglionic neuronal activity to decrease, thereby lowering blood pressure [8].

The baroreflex and vestibulosympathetic reflex may coordinate to maintain blood pressure during movement [7]. An additive effect of the baroreflex and vestibulosympathetic reflex on sympathetic nerve activity in muscles during head-down vestibular stimulation and lower body negative pressure was revealed in humans. We also reported that both of the vestibular receptors and baroreceptors cooperate with each other during acute hypotension, and the afferent signals from the vestibular receptors and the baroreceptors may integrate in the RVLM to facilitate the maintenance of blood pressure [16]. Although both vestibular receptors and baroreceptors cooperate to maintain blood pressure, the role of each receptor in the maintenance of blood pressure has not been quantitatively clarified.

Studies on acute hypotension-induced responses of the brainstem are critical to understand the mechanism underlying orthostatic hypotension. Previous studies in our laboratory have found that acute hypotension increases neuronal activity, expression of c-Fos protein, and glutamate release in the vestibular nuclei [15,17,22]. These effects were eliminated by the removal of peripheral vestibular receptors, which indicates that acute hypotension influences the vestibular neuronal activity by augmenting the afferent signals from the peripheral vestibular receptors. These findings suggest that afferent signals from the peripheral vestibular receptors in acute hypotension are transduced to the RVLM through the NTS to maintain blood pressure.

A population of neurons expressing c-Fos protein responds to acute hypotension in the NTS, a primary integrator of baroreceptor afferent input and vestibular afferent input. Therefore, evaluation of c-Fos protein expression would reflect the quantitative role of each receptor in maintaining blood pressure, since c-Fos protein is sensitive, inducible, and is a high-resolution marker of individual cell groups and extended neural systems that are activated by various stimuli [19]. However, the expression of c-Fos protein in the central nervous system could also be greatly affected by physiological stimuli induced by anesthetics. Therefore, to clarify the role of vestibular receptors and baroreceptors and to quantify their relative influence on the regulation of blood pressure during acute hypotension, the expression of c-Fos protein in the NTS was analyzed in conscious rats with bilateral labyrinthectomy and/or baroreceptor unloading.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (Changchun, China) weighing 220–250 g were used. All animal protocols and procedures described were approved by the institutional ethical committee on experimental use of animals. The animals had free access to food and water. Efforts were made to minimize the number of animals used and suffering. The rats were divided into four groups for immunohistochemical analysis ($n = 8$ per group): a sham group, in which both the vestibular-end organs and sinoaortic baroreceptors were intact; the bilateral labyrinthectomy (BL) group, in which the sinoaortic baroreceptors were intact, but a bilateral labyrinthectomy was performed; the sinoaortic denervation (SAD) group, in which the sinoaortic baroreceptors were denervated, but the vestibular end organs remained intact; and the BL + SAD group, in which both the bilateral vestibular end organs and sinoaortic baroreceptors were removed.

2.2. Labyrinthectomy

A chemical labyrinthectomy was performed as described previously [9,14]. Briefly, 100 μL of sodium arsanilate (100 mg/mL) was intratympanically injected into the bilateral middle ear of the rats under isoflurane anesthesia (Ilsung Co.; Seoul, Korea), which chemically damaged the membranous labyrinth. The damage to the epithelial cells in peripheral vestibular receptors was confirmed by immunohistochemical staining. As a control, saline, instead of sodium arsanilate solution, was injected intratympanically in the sham and SAD rats. The labyrinthectomies were performed 48 h prior to experimentation.

2.3. Sinoaortic denervation

Under isoflurane anesthesia, the carotid sinus nerve was sectioned bilaterally following a midventral incision in the neck, and the internal, external, and common carotid arteries were stripped of connective tissue at the level of bifurcation and painted with 10% phenolethanol to denervate the carotid sinus. For aortic arch denervation, the aortic arch nerve was severed bilaterally proximal to its junction with the vagus nerve [25]. In the sham and BL groups, the rats received similar cervical incisions while leaving nerves, vessels, and baroreceptors intact. After the surgery, the animals breathed spontaneously without significant changes in respiratory rhythm. SAD was performed 24 h prior to experimentation.

2.4. Acute hypotension

Two heparinized polyethylene tubes were inserted into the femoral artery for recording the blood pressure, and into the femoral vein for sodium nitroprusside (SNP) infusion, under isoflurane anesthesia. The tubes were guided toward the skull percutaneously, fixed into the skull, and connected to the tubes of a cybernation metabolism cage to allow free movement in a conscious state during the experiment. The blood pressure was recorded from the unilateral femoral artery using a pressure transducer and physiography (Grass model 7400; USA). SNP was infused in 3 min at a dose of 15 μg/kg/min, and blood pressure decreased by 30–40 mmHg during this period.

2.5. Immunohistochemistry

An immunohistochemical analysis of c-Fos protein expression was performed as described previously [13,17]. Deep anesthesia was induced with an overdose of sodium pentobarbital, and the animals were perfused transcardially with 500 mL of 0.9% NaCl at 4 °C and then perfused with 500 mL of 4% paraformaldehyde dissolved in 0.1 M phosphate buffer (pH 7.4) at 4 °C. The brains were dissected and post-fixed with paraformaldehyde fixative solution for 4 h at room temperature. The fixed brains were immersed in 30% sucrose in phosphate-buffered saline (PBS) for 2 days at 4 °C for cryoprotection. Tissue sections of 20-μm thickness were obtained using a freezing microtome (Leica; Nubloch, Germany), incubated for 30 min with 0.3% H_2O_2, rinsed three times for 5 min with 0.01 M PBS, and then incubated with 1% Triton X-100 for 30 min After a brief wash, the tissues were incubated for 30 min with PBS containing 5% bovine serum albumin (PBS + BSA), and then incubated