Neuroscience Letters 558 (2014) 180–185

Contents lists available at ScienceDirect

Neuroscience Letters

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

Vulnerability of the vestibular organs to transient ischemia: Implications for isolated vascular vertigo

Jin Ok Lee[a,1], Seong-Ho Park[b,1], Hyo Jung Kim[a], Min Sun Kim[c], Byung Rim Park[c], Ji-Soo Kim[b,*]

[a] Biomedical Research Institute, Seoul National University Bundang Hospital, Republic of Korea
[b] Department of Neurology, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam, Republic of Korea
[c] Department of Physiology, Wonkwang University School of Medicine and Brain Research Institute at Wonkwang University, Iksan, Republic of Korea

HIGHLIGHTS

- Rats well tolerate global cerebral ischemia up to 20 min during 4-vessel occlusion.
- Among the vestibular structures, the medial vestibular nucleus (MVN) is most vulnerable to ischemia in rats.
- The frequent occurrence of isolated vascular vertigo in human is supported by the selective ischemic vulnerability of MVN confirmed in this experiment.
- The 4-vessel occlusion adopted in the study may be easily utilized to induce posterior circulation ischemia in rats.

ARTICLE INFO

Article history:
Received 28 July 2013
Received in revised form 1 November 2013
Accepted 12 November 2013

Keywords:
Ischemia
Vertigo
Vestibular nucleus
Rat

ABSTRACT

The aim of this study was to elucidate the mechanism of isolated vascular vertigo by determining selective and relative ischemic vulnerability of the vestibular structures using a global hypoperfusion model in rats. Sprague-Dawley male rats weighing 330–350 g were subjected to transient global ischemia of the brain using a 4-vessel-occlusion (4VO) model. After permanent occlusion of both vertebral arteries (VA) using electrocauterization, both common carotid arteries (CCAs) were occluded for 5–20 min with ligation. One hour after reperfusion of the CCAs, the animals were sacrificed and subjected to c-Fos staining of the entire cerebellum, brainstem, and vestibular ganglion. The rats in the sham group received the same surgical procedures except the vessel ligation. With 4VO for 5–15 min, both the sham and experimental groups showed a weak and scarce c-Fos expression in the medial vestibular nucleus (MVN), neuron Y, and cochlear nucleus. After 4VO for 20 min, only the MVN began to show a significant difference in the number of c-Fos positive neurons between the experimental and sham groups (33.7 ± 17.7 vs.7.1 ± 5.1, Wilcoxon rank test, $p = 0.005$). With 4VO for up to 20 min, c-Fos positive neurons were not found in other areas of the brainstem and cerebellum, including the superior, lateral, and spinal vestibular nuclei, the vestibular ganglion, the cerebellar cortex, and the deep cerebellar nuclei. The vestibular structures appear to be vulnerable to ischemia more than any other structures in the brainstem and cerebellum. Of the vestibular structures, the MVN is most vulnerable to ischemic insults in rats. These findings are consistent with the common findings of vertigo as an initial and isolated symptom of posterior circulation ischemia in human.

1. Introduction

Dizziness and vertigo are common symptoms of vertebrobasilar ischemia (VBI), and may be isolated [1,2,25]. In contrast to the dictum that episodes of vertigo without other neurological symptoms should suggest a disorder other than VBI [8], vertigo was the initial symptom in 48% of patients with VBI and was isolated in 17% of patients with posterior inferior cerebellar artery (PICA) infarction [15].

The isolated vertigo observed in VBI may be ascribed to selective involvement of the terminal branch supplying the vestibular end organs [6] or to relative vulnerability of the central vestibular structures to ischemia [21]. However, there have been only a few studies that investigated selective vulnerability of the vestibular organs to ischemia [10,16], and no study compared the ischemic vulnerability between the peripheral and central vestibular structures.

* Corresponding author at: Department of Neurology, Seoul National University College of Medicine, Seoul National University Bundang Hospital, 300 Gumi-dong, Bundang-gu, Seongnam-si, Gyeonggi-do 463-707, Republic of Korea.
Tel.: +82 31 787 7463; fax: +82 31 719 6828.
E-mail address: jisookim@snu.ac.kr (J.-S. Kim).
[1] These authors equally contributed to this study.

0304-3940/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.11.016

In this study, we aimed to determine the selective ischemic vulnerability of the vestibular structures by adopting a generalized hypoperfusion model in rats.

2. Materials and methods

2.1. Animals

Sprague-Dawley male rats weighing 330–350 g were randomly assigned to either the sham ($n = 5$) or the experimental group ($n = 14$). The study was in accordance with the guidelines regarding the care and use of animals for experimental procedures, and was approved by the Institutional Animal Care and Use Committee of Seoul National University Bundang Hospital. All efforts were made to minimize the number of animals used and their suffering.

2.2. Experimental procedures

We induced posterior circulation ischemia by modifying the 4-vessel-occlusion (4VO) technique described in detail previously [27]. Briefly, all animals were anesthetized with an intraperitoneal injection of Zoletil (30 mg/kg, VIRBAC, France) and Rumpun (10 mg/kg, Bayer, Korea). The first incision was made behind the occipital bone directly overlying the first two cervical vertebrae. The paraspinal muscles were separated from the midline. Then, the right and left alar foramina of the first cervical vertebra were exposed. An electrocautery needle (Bowie electrocautery Jorgensen laboratories, Inc., USA), 0.5 mm in diameter, was inserted through each alar foramen, and both vertebral arteries were electrocauterized and permanently occluded. After then, while the rats were placed in the supine position, a 2.5 cm midline skin incision was made on the neck, and both common carotid arteries (CCAs) were isolated and 3.0 silk surgical sutures were loosely placed around the arteries and were tightened simultaneously in both sides. Subsequently, the incision was closed with a single suture.

After various occlusion time (5, 10, 15, or 20 min), the sutures were removed from the CCAs and restoration of the carotid blood flow was confirmed by direct observation. In the sham group, the rats underwent the same procedures except the vessel occlusion. During the procedures, the animals were monitored for the body temperature, respiration pattern, and responsiveness.

2.3. Histopathologic studies

One hour after the reperfusion of both CCAs, the animals were sacrificed and subjected to immunohistochemical studies. Briefly, the animals were deeply anesthetized with intraperitoneal injection of Zoletil and Rumpun, and sequentially perfused through the ascending aorta with 300 ml of normal saline, and 300 ml of 4% formaldehyde. Then, the animals were decapitated, and the perfused brain and inner ear were removed for overnight fixation in the same fixative. After then, the posterior brain including the cerebellum and brainstem was processed further for conventional paraffin block. And then, serial sections were obtained from −9 to −12.0 mm relative to the bregma with a 20 μm thickness at 200 μm intervals. The inner ear was decalcified in 10% EDTA solution (pH 7.4) at 4 °C on a rotator and processed for conventional paraffin block. The inner ears including the vestibular ganglion were also sectioned with a 7 μm thickness.

2.4. Immunohistochemistry for c-Fos

For antigen retrieval, the sections of the brain and inner ear provided for c-Fos immunohistochemistry were heated for 25 min and 15 min each in 10 mM citrate buffer within a microwave.

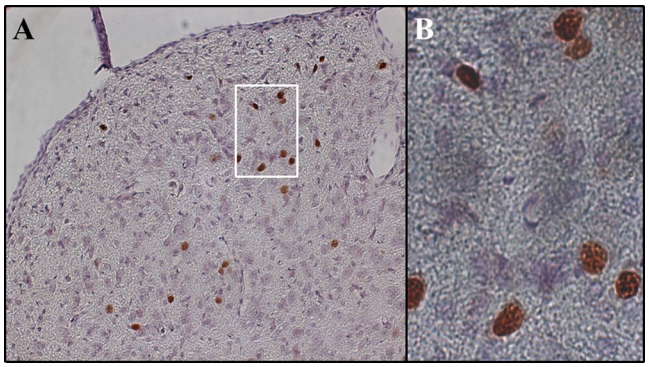

Fig. 1. (A) Expression of c-Fos protein in the medial vestibular nucleus (MVN). To confirm that c-Fos was positive in the neurons and not in other types of cells, the MVN was counterstained with Harris' hematoxylin after the immunohistochemistry. (B) A magnified view of the boxed area in (A). Co-staining of Harris' hematoxylin and c-Fos protein is evident in the nuclear region of the neurons.

Then, the sections were incubated for 15 min with 3% hydrogen peroxide (H_2O_2) and blocked with 3% normal goat serum for one hour. Then the sections were incubated overnight at 4 °C with c-Fos polyclonal antibody (1:1000, Santa Cruz Biotechnology, Inc., USA). At the following day, the sections were incubated with a biotinylated secondary antibody (goat anti-rabbit, 1:500, DAKO, Denmark). These were followed by an avidin–biotin peroxidase method (ABC Elite Kit, Vector Laboratories, USA) and processed with 3,3′-diaminobenzidine (DAB, DAKO, Denmark), which resulted in a brown reaction product. After the DAB reaction, the sections were dehydrated and cover-slipped with Permount (Fisher Chemical, USA). The supraoptic nucleus region inducing c-Fos positive neurons by injection of hypertonic saline served as a positive staining control [12]. Also, to confirm that c-Fos is positive in the neurons and not in other types of cells, the MVN with positive c-Fos was counterstained with Harris' hematoxylin after the immunohistochemistry (Fig. 1).

2.5. Quantitative analysis

The sections were examined and images were acquired with a Zeiss Axioskop 40 microscope (Carl Zeiss Micorscopy, Germany) to localize and count c-Fos positive neurons. Based on the previously developed atlas [9,26], the brown c-Fos positive neurons were captured and digitized using an imaging analysis software adopted in Zeiss Axio observer microscope (Carl Zeiss Micorscopy, Germany) at the levels of the superior vestibular nucleus (SVN, bregma −9.96 mm), lateral vestibular nucleus (LVA, bregma −11.04 mm), spinal vestibular nucleus (SpVN, bregma −11.76 mm), medial vestibular nucleus (MVN, bregma −11.76 mm), and cerebellum. The c-Fos positive cells on either side of the section were counted independently, so that any asymmetry in the sectioning procedure would not affect the accuracy of the cell count.

2.6. Statistical analyses

All the quantitative data are presented as the mean ± SD. The number of labeled cells within MVN, neuron Y and cochlear nucleus were compared between the sham and 4VO for 20 min groups using the Wilcoxon rank test. The $p < 0.05$ were considered statistically significant.

3. Results

3.1. Ischemic tolerability and behavioral responses during the procedures

Rats usually well tolerated the surgical procedures. When we determined ischemic tolerability of rats using 4VO, 4VO for more