



Temporal changes of calbindin expression in the nodulus following unilateral labyrinthectomy in rats



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HIGHLIGHTS

- The calbindin expression in the ipsi and contralateral side was found to decrease 6 hr after UL.
- Asymmetric calbindin expression was found between ipsi and contralateral nodulus 24 hr after UL.
- Forty-eight hours after UL, calbindin expression returned to the control level.
- And asymmetric expression in both noduli also subsided.

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ABSTRACT

Following unilateral vestibular deafferentation, many of the oculomotor and postural symptoms, such as spontaneous ocular nystagmus and head tilt, gradually abate over time in a process known as 'vestibular compensation'. Although many experimental studies have indicated a role for the cerebellum during vestibular compensation, the effects of unilateral labyrinthectomy (UL) on cerebellar function and the role of cerebellum in post-lesional plasticity remain unclear. Thus, we investigated the temporal changes of calbindin expression in the ipsilateral and contralateral nodulus to the lesion side during vestibular compensation following UL in rats. Change of calbindin expression in the nodulus was measured by immunohistochemistry at 2, 6, 24 and 48 hr following UL. The staining intensity of calbindin-positive Purkinje cells in the ipsilateral and contralateral nodulus to the lesion side was found to decrease 6 hr after UL compared with the control and asymmetric calbindin expression between ipsilateral and contralateral nodulus 24 hr after UL. Forty-eight hours after UL, calbindin expression returned to the control level, and asymmetric expression in both noduli also subsided. It is suggested that the regulation of calbindin expression may facilitate synaptic plasticity by adjusting the efficacy of biochemical responses of Purkinje cells according to the changes in neuronal activity in the vestibular nuclear complex during the early phase of vestibular compensation. Thus, the results revealed that the nodulus has a role during vestibular compensation through Purkinje cells.

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1. Introduction

Following unilateral vestibular deafferentation, many of the oculomotor and postural symptoms that persist in the absence of head movement, such as spontaneous ocular nystagmus and head tilt, gradually abate over time in a process known as 'vestibular compensation' [9,21]. Recent studies have identified several

mechanisms of plasticity in vestibular nuclei during vestibular compensation such as rebalancing of bilateral excitability of vestibular nuclei through reciprocal commissural inhibitory system [20], GABA neurotransmission system in the commissural system of bilateral vestibular nuclei [4], and neurogenesis and cell proliferation in the vestibular nuclei [10]. Although many experimental studies have indicated a role for the cerebellum during vestibular compensation, the effects of unilateral labyrinthectomy (UL) on cerebellar function and the role of the cerebellum in post-lesional plasticity still remain unclear. A functional role of the cerebellum in vestibular compensation was introduced by previous studies that reported lesions of the flocculonodular, posterior, and anterior lobes retarded vestibular compensation [15], and the ipsilateral flocculus to the lesion side was essential for the increase in the

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intrinsic excitability of medial vestibular nucleus (MVN) neuron after UL [16]. Also, we have reported that the cerebellar flocculus may play a role in vestibular compensation through calbindin-positive Purkinje cells of the flocculus [14].

Calbindin, one of the calcium-binding proteins, is a cytosolic intracellular Ca^{2+} regulator that restricts Ca^{2+} -mediated signals in the cytoplasm [13]. Moreover, calbindin-D 28k was reported to be a more reliable marker of human Purkinje cells than standard Nissl stains [22]. In the present study, temporal changes of calbindin expression in the ipsilateral and contralateral nodulus to the lesion side during vestibular compensation were examined to investigate the post-lesional plasticity of the nodulus following UL in rats.

2. Materials and methods

2.1. Animal preparation

Adult male Sprague-Dawley rats (Saemtaco Biokorea Co., Suwon, Korea), weighing 250–300 g, were used in this study. Experimental animals were divided into control ($n = 5$) and experimental ($n = 20$) groups. The experimental group contained 5 animals at different time points of 2, 6, 24 and 48 hr after UL. The ossicular bones in the middle ear cavity of control animals were removed by a retroauricular approach, but the membranous labyrinth was left intact. Control animals were sacrificed immediately after surgery and then used for immunohistochemical staining. The Institutional Ethical Committee of Hallym University on the Experimental Use of Animals approved all the procedures used.

2.2. Labyrinthectomy

After anesthesia with isoflurane (Ilsung Co., Seoul, Korea), the ossicular bones in the middle ear cavity were removed to open the oval window via a ventral approach. A small opening was made around the oval window under a surgical microscope. The membranous labyrinth was removed surgically through this opening using a small, right-angled hook, and was aspirated with a suction pump. UL was confirmed by the appearance of spontaneous nystagmus and postural asymmetry after recovery from anesthesia. Left labyrinthectomy was performed in the experimental group.

2.3. Immunohistochemistry

After deep anesthesia with an intraperitoneal injection of pentobarbital sodium (100 mg/kg), the animals were perfused transcardially with 0.9% saline and fixed with 4% paraformaldehyde dissolved in a phosphate-buffered saline solution (PBS, pH 7.4) containing 0.05 M Na_2HPO_4 and 0.137 M NaCl. The animals were then decapitated and their brains were removed, post-fixed, rinsed with PBS, and immersed in 30% sucrose solution for 2 days. The sucrose-embedded brain was sectioned at a thickness of 40 μm using a cryostat. The sections were then washed three times with PBS and mounted on gelatin-coated slides. Following washing with PBS, the sections were processed for immunohistochemical detection of calbindin using mouse anti-calbindin antibody (Chemicon, Billerica, USA). Free-floating sections were incubated in PBS (4 °C) containing anti-calbindin antiserum (1:1000 dilution), 0.3% Triton X-100, 0.05% bovine serum albumin, and 1.5% normal horse serum for 48 hr. Using the Vectastain-Elite kit (Vector Laboratories, Burlingame, USA), the sections were incubated for 1 hr with biotinylated horse anti-mouse IgG secondary antibody (1:200), followed by incubation with avidin–biotin–peroxidase complex (1:100). Both incubations were carried out at room temperature. The sections were then treated with 0.02% 3,3'-diaminobenzidine

Table 1

ANOVA result of calbindin expression among control, 2 hr, 6 hr, 24 hr and 48 hr after UL ipsilateral and contralateral side, respectively.

	df	F	p
Ipsilateral side	4	5.218	0.001
Contralateral side	4	3.821	0.006

tetrahydrochloride and 0.01% H_2O_2 for 3 min. Next, the sections were washed and examined by microscopy.

2.4. Data analysis

Four slides of same level in the nodulus were selected using the guidance of the atlas [19]. Staining intensity in the stained Purkinje cells was obtained by measuring the optical density of calbindin-positive neurons in the diverse visual region of the nodulus. The optical density of the stained neurons was quantitatively assessed by micro-densitometry using an image analyzer (Multiscan, Miami, USA). The voltage-related changes in optical density were evaluated before the measurement of densitometry. After this, optical voltage from the linear portion of the S-shaped voltage-related optical density curve was obtained. During the full measurement of optical density, the optical voltage level was unchanged. The results of microscopic densitometry were expressed in arbitrary densitometric units. All data are presented as means $\pm$ SD. Statistical significance of differences was assessed using the paired *t*-test for comparison of calbindin expression between ipsilateral and contralateral side and one-way ANOVA (SPSS 17.0) for comparison of calbindin expression among control, 2 hr, 6 hr, 24 hr and 48 hr after UL in the ipsilateral and contralateral side, respectively. Values of $p < 0.05$ were considered significant.

3. Results

In control animals without UL, calbindin immunoreactivity in the nodulus was observed in the somata and dendrites of many Purkinje cells as well as in the somata of some molecular layer interneurons. Calbindin-positive Purkinje cells were aligned in a single row in the nodulus. The staining intensity of calbindin-positive Purkinje cells was symmetrical between the right and left sides of the nodulus (Fig. 1) and ANOVA results of calbindin expression among control, 2 hr, 6 hr, 24 hr and 48 hr after UL are summarized Table 1.

Two hours after UL, the expression of calbindin showed a similar pattern to that of control animals, but 6 hr after UL, a marked reduction of calbindin expression was observed in both ipsilateral and contralateral nodulus to the lesion side compared to controls ($p < 0.05$).

Twenty-four hours after UL, the staining intensity of calbindin-positive Purkinje cells increased close to the level of control animals, but asymmetric expression between the ipsilateral and contralateral nodulus was observed. That is, the staining intensity of calbindin-positive Purkinje cells was significantly smaller in the ipsilateral nodulus to the lesion side than the contralateral nodulus ($p < 0.05$) (Table 2).

Forty-eight hours after UL, the calbindin expression of Purkinje cells in the ipsilateral and contralateral nodulus to the lesion side reverted to the level of control animals, and the asymmetric expression between the ipsilateral and contralateral nodulus disappeared (Figs. 1 and 2).

4. Discussion

This study demonstrated transient changes in the calbindin expression of Purkinje cells in the cerebellar nodulus. Calbindin

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