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Research report

Immunocytochemical and stereological analysis of $GABA_B$ receptor subunit expression in the rat vestibular nucleus following unilateral vestibular deafferentation

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Abstract

The process of behavioral recovery that occurs following damage to one vestibular labyrinth, vestibular compensation, has been attributed in part to a down-regulation of $GABA_B$ receptors in the vestibular nucleus complex (VNC) ipsilateral to the lesion, which could potentially reduce commissural inhibition from the contralateral VNC. In this study, we tested the possibility that this occurs through a decrease in the expression of either the $GABA_{B1}$ or $GABA_{B2}$ subunits of the $GABA_B$ receptor. We used Western blotting to quantify the expression of these subunits in the VNC at 10 h and 50 h following unilateral vestibular deafferentation (UVD) or sham surgery in rats. We then used immunocytochemistry and stereological counting methods to estimate the number of neurons expressing these subunits in the MVN at 10 h and 2 weeks following UVD or sham surgery. Compared to sham controls, we found no significant changes in either the expression of the two $GABA_B$ receptor subunits in the VNC or in the number of MVN neurons expressing these $GABA_B$ receptor subunits post-UVD. These results suggest that $GABA_B$ receptor expression does not change substantially in the VNC during the process of vestibular compensation.

Theme: Motor systems and sensorimotor integration
Topic: Vestibular system

Keywords: GABA; GABA(B) receptors; Vestibular compensation; Unilateral vestibular deafferentation; Unilateral labyrinthectomy; Vestibular nucleus

1. Introduction

It has been suggested that $GABA_B$ receptors contribute to the vestibular compensation process that follows the loss of function of one vestibular labyrinth (unilateral vestibular deafferentation, UVD) [9,10,17,29]. Since vestibular compensation is associated with a gradual recovery of spontaneous resting activity to the vestibular nucleus complex (VNC) on the ipsilateral side, one possibility is that a down-regulation of $GABA_B$ receptors in the ipsilateral VNC could release neurons on this side from tonic GABAergic inhibition by the contralateral VNC, via the brainstem commissures.

At present, there are few data to support this hypothesis. Using RT-PCR, Horii et al. observed a significant increase in the expression of the $GABA_{B1a}$ receptor gene in the ipsilateral VNC at 6 h post-UVD; however, this increase had disappeared by 50 h post-operative [15]. By contrast, Eleore et al. [8], using in situ hybridization, reported no change in $GABA_{B1}$ receptor gene expression in the medial vestibular nucleus (MVN) during the development of vestibular compensation. Systemic injections of the $GABA_B$ receptor agonist, baclofen, and the selective antagonist, CGP56433A, have been shown to alter the expression of spontaneous nystagmus (SN) and vestibulo-ocular reflex

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function following UVD [18–20]. However, because of the use of systemic injections, it is not clear whether these effects were due to the action of the drugs in the VNC [9].

The main evidence in support of the hypothesis that $GABA_B$ receptors down-regulate in the ipsilateral VNC during vestibular compensation comes from in vitro brainstem slice studies. Dutia and colleagues observed an increase in the EC50 of baclofen to inhibit the spontaneous activity of neurons in the ipsilateral rostral MVN at 4 h following UVD; this effect persisted even 7–10 days later [17,29]. In addition, at 4 h post-UVD, neurons in the contralateral rostral MVN showed a significant decrease in their EC50 for baclofen [17,29]. Dutia and colleagues [10,17,29] suggested, therefore, that pre- and post-synaptic $GABA_B$ receptors may contribute to the recovery of resting activity in the ipsilateral VNC during vestibular compensation by undergoing some form of down-regulation, for example, reduced expression.

The $GABA_B$ receptor is a G-protein coupled receptor which requires the combination of one of the $GABA_{B1}$ subunits (either the $GABA_{B1a}$ or $GABA_{B1b}$ isoform) with the $GABA_{B2}$ subunit to be functional (see Refs. [1,2,9] for reviews). RT-PCR, in situ hybridization and immunohistochemical studies have demonstrated the existence of $GABA_B$ receptor mRNA and protein in the VNC (respectively) [3,6,8,9,13–15,21] and in vitro electrophysiological studies have shown that these receptors are functional (e.g., Refs. [7,9,17,26,27,29]). However, only Eleore et al. [8] have examined $GABA_B$ receptor protein expression in the MVN during the development of vestibular compensation and they found no change in $GABA_B$ expression between 5 h and 8 days post-UVD in rats, using immunohistochemistry with an antibody labeling both the $GABA_{B1}$ and $GABA_{B2}$ subunits. We decided to investigate this issue further using both Western blotting and immunocytochemistry with stereology to quantify $GABA_B$ receptor subunit expression in the VNC at different times following UVD. We used selective antibodies for the $GABA_{B1}$ and $GABA_{B2}$ subunits to quantify their expression independently. Stereological analysis was used because it provides an unbiased estimate of the number of cells expressing a receptor and also because glial cells can be excluded. This technique has not been used previously in the analysis of receptor changes in the VNC during vestibular compensation.

2. Methods

Data were obtained from a total of 40 male Wistar rats (250–320g). Twenty animals were used for the Western blotting study and 20 were used for the immunocytochemical study. The animals were maintained on a 12–12 h light/dark cycle with food and water available ad libitum. All experiments were carried out in accordance with the regulations of the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals. Every effort was made to minimize animal usage and discomfort.

2.1. Western blotting

The aim of these experiments was to perform a quantitative analysis of the expression of the two $GABA_B$ subunits in the whole ipsilateral and contralateral VNCs. Animals were randomly divided into 4 groups (n = 5 in each): sham surgery controls and UVD, at each of the 10 h and 50 h time points post-operative. The post-surgery times were based on previous studies of behavioral compensation in rats: 10 h represents the acute, uncompensated stage, when SN is vigorous and postural asymmetry is severe; by 50 h, static symptoms, such as SN, have substantially compensated, but dynamic reflex deficits still remain (see Ref. [4] for review). It was confirmed that, at 10 h post-UVD, rats displayed vigorous SN and postural asymmetry that had compensated substantially by 50 h.

Animals were anesthetized with 0.3 mg/kg fentanyl citrate and 0.3 mg/kg medetomidine hydrochloride (Domitor, Novartis, UK); 1 ml of xylocaine (with 1:10,000 adrenaline) was injected around the wound margins. Once the pedal reflex was absent, rats were fixed in a custom-made nose bar to hold the head steady. Using an otolaryngological microscope, the right tympanic bulla was exposed using a retro-auricular approach. After removing the tympanic membrane, the malleus and incus, the vestibule was visualized. The stapedial artery was cauterized at two points, and the cochlea removed using a dental drill with a fine burr. Following destruction of the auditory apparatus, the horizontal, anterior and posterior semicircular canal ampullae and the utricle and saccule of the vestibular labyrinth were opened and aspirated [5,30]. At the end of the surgery, antibiotic cream (Mupirocin) was topically applied to the opened labyrinth, the wound sutured and the temporal bone sealed with dental cement. Carprofen was injected s.c. (5 mg/kg) (Rimadyl; Pfizer) for post-operative analgesia. Previous studies have shown that this procedure results in a complete destruction of the labyrinth, which has been verified histologically [5]. Sham control surgery consisted of exposing the temporal bone and carefully drilling below it without damaging the vestibular or auditory apparatus. At 10 h or 50 h post-surgery, all animals were decapitated without anesthesia. The brains were immediately dissected out and placed into ice-cold 0.9% saline for 50 s. The left and right VNCs were rapidly removed, frozen on dry ice and stored at −84 °C.

At the time of the assay, protease-inhibitory buffer containing 50 mM Tris–HCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1.5 mM pepstatin A and 0.2 mM leupeptin (pH 7.4), (1:10, w/v) was added to the samples on ice which were then homogenized using ultrasonification (Sonifier cell disrupter B-30, Branson Sonic Power, USA) and centrifuged at 12,000 × g for 10 min at 4 °C (BHG Hermle Z-229 centrifuge, Germany). Part of the supernatant was used to determine protein concentrations based on the Bradford method using a Bio-Rad protein assay dye reagent concentrate and Spectramax microplate