Original Article

Role of Histamine H1 Receptors in Vestibular Nucleus in Motion Sickness

HUANG Li-gui[1], WANG En-tong[2], CHEN Wei[2], GONG Wei-xi[2]

1 *Department of Otolaryngology-Head and Neck Surgery, Lushan Hospital of PLA, Jiujiang 332000, China*

2 *Department of Otolaryngology-Head and Neck Surgery, General Hospital of Air Force, Beijing 100142, China*

Abstract **Objectives** To investigate the expression of histamine H1 receptors (H1R) in the vestibular nucleus of brainstem in rats and the role of H1R in motion sickness (MS). **Methods** A total of 24 healthy Sprague-Dawley rats were divided randomly into four groups (n=6 each) which determined if the animals would receive induction of MS or drug (promethazine) treatment: MS(-)/Drug(-); MS(+)/Drug(-); MS(-)/Drug(+ at 0.25 mg); and MS(+)/Drug(+). MS was induced by complex motion stimulation and the conditioned taste aversion was used as a behavioral indicator of MS. The volume of 0.15% sodium saccharin solution (SS) intake within 45 minutes after motion stimulation was measured. H1R in the vestibular nucleus was examined by immunofluorescence staining. The expression of H1R protein in brainstem tissue at vestibular nucleus level was detected by western blot. **Results** The mean SS intake volume in the MS(+)/Drug(-) group (8.8 ml) was significantly less than that of the MS(-)/Drug(-) group (15.1 ml) ($P < 0.01$). The mean SS intake volume of the MS(-)/Drug(+) group (14.8 ml) was similar to that of the MS(-)/Drug(-) group. The mean SS intake volume (9.6 ml) of the MS(+)/Drug(+) group was more than that of the MS(+)/Drug(-)group ($P<0.01$), but less than that of the MS(-)/Drug(-) group or MS(-)/Drug(+) group ($P < 0.01$). Immunofluorescence staining showed positive expression of H1R in the vestibular nucleus of brainstem and the expression was enhanced by motion stimulation. Western blot analysis showed that H1R protein expressed in the brainstem tissue at vestibular nucleus level and the expression also increased significantly after motion stimulation. The MS-induced increase of H1R was not affected significantly by promethazine. **Conclusions** H1Rs exist in the vestibular nucleus in rats and H1R expression is up-regulated by motion stimulation, but not affected by promethazine. The findings indicate that the histaminergic system is involved in MS. Promethazine, as an H1R blocker, may play its anti-MS role by competing the binding site on H1Rs with histamine rather than inhibiting H1R expression.

Key words motion sickness; histamine; H1 receptor; vestibular nucleus; promethazine; rat

Introduction

Motion sickness (MS) induced by various motion stimulation is known by many names, for example, car sickness, sea sickness, air sickness, and space sickness. The incidence of MS varies, depending on the magnitude of the stimulation and the individual susceptibility to the stimulation. MS is experienced by 60%-80% of space travelers. [1] MS is a physiological phenomenon as well as a pathophysiological disorder, which can result in serious of symptoms, including nausea and vomiting. MS not only has major influence on travel activities, but also is a common medical cause of permanent grounding of pilots and student pilots as it interferes with their performance in the air and flight safety.[2] Unfortunately, there are few effective interventions in treating MS. [3]

MS is the responses of central nervous system to unfamiliar motion stimulation transmitted to the vestibular nuclei, archicerebellum, other brainstem structures, autonomic centers and hypothalmus.[4] However, the underlying neurochemical and neuropharmacological mechanisms and signalling pathways of MS are not completely understood. [3] Some studies indicate that neurotransmit-

Correspondence author: WANG En-tong, M.D, Ph.D., Department of Otolarygology-Head & Neck Surgery, General Hospital of Air Force, Beijing 100142, P. R. China;
Email: wang_entong@hotmail.com

ters, such as acetylcholine, noradrenaline and histamine, play important roles in the development of MS. [5, 6] It is believed that histaminergic neuron system in central nerve system is involved in the development of the symptoms and signs of MS. Antihistamine drugs block histaminergic inputs to nervous system via H1 receptors (H1R) to prevent MS. [6] In the present study, we studied the expression of H1R in the vestibular nucleus and the influences of motion stimulation and anti-MS drug promethazine (an antihistamine drug) on the H1R expression in rats. We also investigated the role of H1R in vestibular nucleus in the development of MS and its underlying signal transduction.

Materials & methods

Animals and treatments

Healthy Sprague-Dawley rats (12 males and 12 females, 200 g – 250 g) were used in this study. The rats were randomly divided into four groups (6 rats in each) depending on the treatment: (1) MS(–)/Drug(–)—no motion stimulation or anti-MS drug treatment (the control group); (2) MS(+)/Drug(–)—motion stimulation with no anti-MS drug treatment; (3) MS(–)/Drug(+)—anti-MS drug (promethazine, 0.25 mg, i.p) only with no motion stimulation; and (4) MS(+)/Drug(+)—promethazine treatment at 30 minutes before motion stimulation.

Motion stimulation

MS was induced by complex rotation motion stimulation. [7] Before exposure to motion stimulation, rats were allowed to move freely in the cage in the experimental device. To induce MS, the device was rotated clockwise at an acceleration of 20°/s^2 up to a maximum speed of 120°/s, followed by similar rotation in the reversed direction, and the rotation continued for 60 minutes. Two rats were tested at same time. Conditioned taste aversion was used as a behavioral indicator of MS, i.e. measurement of the intake volume of 0.15% sodium saccharin solution (SS) within 45 minutes after motion stimulation. [8] Paired student's t test was used (CHISS software) in data analysis, and $P < 0.05$ was considered of statistical significance.

Immunofluorescence staining of H1 receptors

Under anesthesis with 10% chloralhydrate, the rat was perfused through the left ventricle with phosphate buffered saline (PBS), followed by a ice-cold fixative containing 4% paraformaldehyde in 0.1 M PBS (pH 7.4). The rat was then decapitated and the brianstems removed and immersed in 4% paraformaldehyde in 0.1 M PBS for 2 hours, followed by dehydration with 15% and 30% sucrose solutions. Cryostat sections (thickness, 10 μm) were prepared at the level of vestibular nucleus. The sections were treated with 5% normal sheep serum diluted in PBS (pH 7.4) for 30 minutes to decrease nonspecific background staining. After rinsing with PBS, the sections were incubated with rabbit polyclonal IgG against H1R (Santa Cruz) at dilution of 1∶50 for 24 hours, followed by triple washes with PBS containg 0.2% Triton X-100, 10 minutes each. This was followed by incubation with goat anti-rabbit IgG with FITC (Invintrogen) at dilution of 1∶200 for 60 minutes at room temperature. After another round of triple washes, the sections were mounted for immunofluorescence microscopic examination for H1R in the vestibular nucleus. Control sections were treated similarly except that the anti-H1R antibodies were replaced by rabbit serum.

Western blot analysis

Harvest of brainstem was similar as described above. The brainstem tissue at the vestibular nucleus level was collected and kept at –80℃ before western blot tests. Cryostat brianstem specimens were lysed with a buffer containing 0.1% SDS, 1% igepal CA-630, 0.5% sodium deoxycholate, 0.01% phenylmethylsulfonyl fluoride, 3% aprotinin and 1 mM sodium orthovanadate for 30 minutes. Identical amounts of protein lysates were dissolved by 12% SDS-PAGE, followed by electroblot analysis onto a nitrocellulose membrane (Invitrogen). After triple rinsing with Tris buffle (20 minutes each), the membrane was blocked with 5% milk PBS for 30 minutes and then probed with rabbit anti-H1R primary antibody (1∶1000) (Santa Cruz) for 24 hours. The membrane was then triple washed and incubated with goat anti-rabbit secondary antibody (IgG) with horseradish peroxidase (HRP) at 1∶3000 dilution (Beijing Zhongshan Goldenbridge Biotechnology Co.) for 1 hour. Immunoblots were detected by an enhanced chemiluminescence (ECL) detection system (Amersham Pharmacia Biotech). β-actin expression was used as control.

Results

Motion stimulation induced the conditioned taste