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Original Article

Role of Histamine H1 Receptors in Vestibular Nucleus in Motion Sickness

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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-

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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 anesthesia 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

aversion

As showed in Table 1, the mean SS intake volume of the MS(+)/Drug(−) group (8.8 ml) was significantly less than that of the MS(−)/Drug(−) group (15.1 ml) ($P < 0.01$), indicating successful induction of conditioned taste aversion apparently in response to motion stimulation. The mean SS intake volume of the MS(−)/Drug(+) group (14.8 ml) was similar to that of the MS(−)/Drug (−) group, showing that 0.25 mg promethazine had little effect on SS intake in the rat. The mean SS intake volume of MS(+)/Drug(+) group (9.6 ml) was greater than that of the MS(+)/Drug(−) group ($P < 0.01$), but less than that of the MS(−)/Drug(−) or MS (−) /Drug (+) group ($P < 0.01$), indicating that the conditioned taste aversion (MS) induced by motion stimulation was inhibited partially by anti−MS drug promethazine.

Motion stimulation increased H1R expression in the vestibular nucleus

Immunofluorescence staining showed positive expression of H1R in the vestibular nucleus (Figure 1a), which was enhanced by motion stimulation (Figure 1b) but not significantly affected by promethazine, with (Figure 1d) or withou exposure to motion stimulation (Figure 1c).

Table 1 Sodium saccharin solution intake by rats (ml, *Mean ± S.D*)

Group	n	Sodium saccharin solution intake
MS(−)/Drug(−)	6	15.1 ± 0.4
MS(+)/Drug(−)	6	8.8 ± 0.3*
MS(−)/Drug(+)	6	14.8 ± 0.6△
MS(+)/Drug(+)	6	9.6 ± 0.3*△#
F value		346.82
P value		<0.01

*Compared with the MS(−)/Drug(−) control group, $P < 0.01$; △Compared with the MS(+)/Drug(−) group, $P < 0.01$; # Compared with the MS(−)/Drug (+) group, $P < 0.01$. MS: motion stimulation; Drug: promethazine

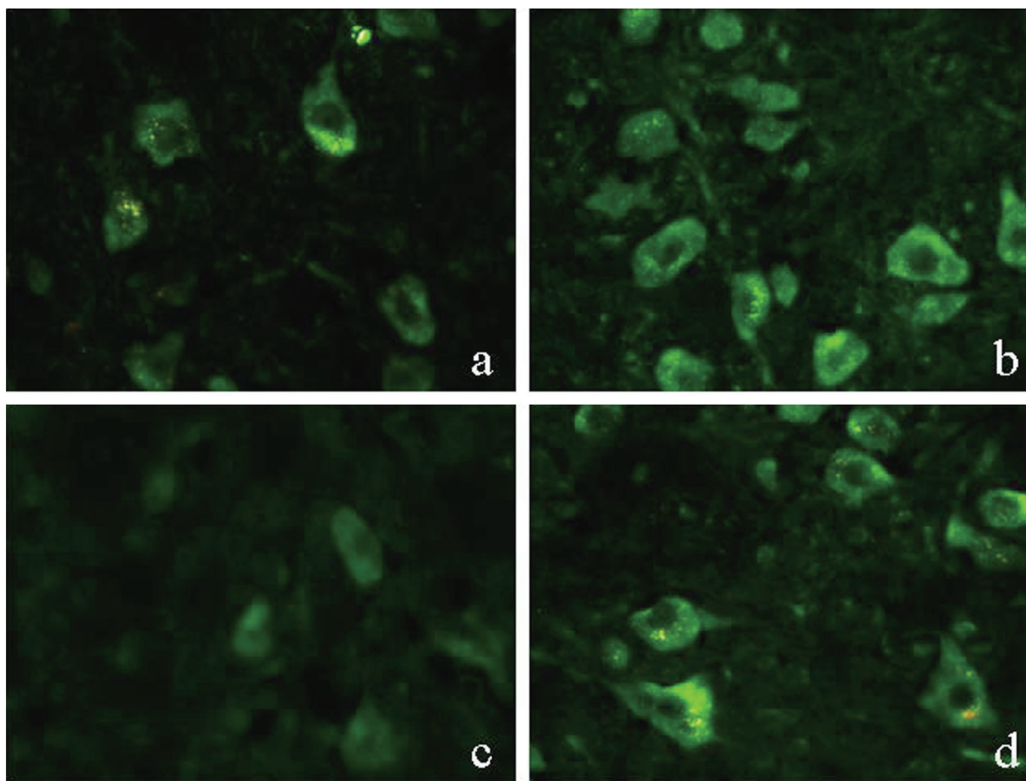

Figure 1 Immunofluorescent staining of H1R in the vestibular nucleus. (a) Positive staining of H1R in an animal without motion stimulation or anti−MS drug (promethazine). (b) Staining in an animal who has received motion stimulation but no promethazine showing significantly enhanced staining of H1R compared with (a). (c) Staining in an animal with promethazine treatment but no motion stimulation showing immunofluorescent staining similar to (a), indicating little effects by promethazine on H1R expression. (d) Staining in an animal who received promethazine (0.25 mg) before motion stimulation showing immunofluorescent staining similar to (b), indicating that increased H1R expression by motion stimulation is not inhibited significantly by promethazine. × 400.

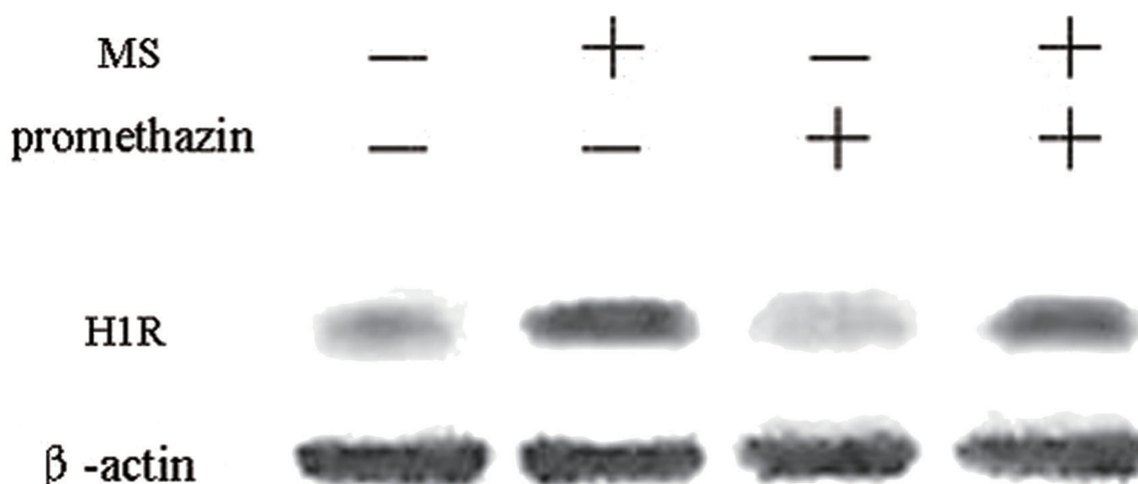

Figure 2 Western blot analysis of H1R expression in brainstem tissues at vestibular nucleus level. Control rats without motion stimulation (MS) or anti-MS drug treatment showed positive expression of H1R in brainstem tissue at vestibular nucleus level (Line 1). Motion stimulation enhanced the expression (Line 2). Promethazine had no significant influence on H1R expression regardless of motion stimulation (Line 4 and Line 3). β-actin was used as loading control. The membrane is representative of two repeated experiments.

Western blot analysis also showed expresseion of H1R protein at the vestibular nucleus level (Figure 2, Line 1), which was not significantly affected by promethazine treatment (Figure 2, Line 3). Motion stimulation increased expression of H1R protein (Figure 2, Line 2), which was not significantly inhibited by promethazine (Figure 2, Line 4).

Discussion

MS is considered a response of nervous system to motion stimulation, involving various nervous structures including the vestibular system, vomiting center, brain stem network, cerebellum, hypothalamus as well as the autonomic nervous system.[4, 9] Motion stimulation is detected by vestibular hair cells that are located in the vestibular end organs of the inner ears. Under motion stimulation, vestibular hair cells in the semicircular canal and otolith receptors are activated. The hair cells in the otolith receptors are sensitive to linear acceleration. Angular accelerations stimulate the hair cells in the semicircular canals. Vestibular afferent signals from the hair cells in the semicircular canal and otolith receptors are transmitted to the neurons in vestibular nuclei in the brainstem, in turn to other structures in brain. Vestibular information also projects to autonomic nuclei, raising vestibulo-autonomic responses. In addition to direct inputs from vestibular afferents, the vestibular nuclei receive substantial projections from cortical, cerebellar, and other brain stem structures. These structures provide the vestibular nuclei with numerous extra-vestibular cues. The vestibular nucleus plays a key role in vestibular information signaling.

The neurochemical and neuropharmacological mechanisms of MS have been studied extensively, but the underlying mechanisms and signaling pathways are not completely understood. Some experimental and clinical studies indicate that neurotransmitters, such as acetylcholine, noradrenaline and histamine, play important roles in the development of MS. [10, 6] It is believed that histamine and its receptors in central nerve system are involved in the development of the symptoms of MS, including emesis. [6]

Histamine is distributed extensively, but unevenly, throughout the brain. [11] Histamine is a multifunctional messenger that acts mainly as a neurotransmitter or neuromodulator in the brain. The role of histamine as a neurotransmitter was finally established in 1980s.[11,12]. Immunohistochemistry studies have shown that there are histaminergic neurons in the brains of rats, which are concentrated in the hypothalamus-autonomic nervous system. [13] The vestibular nuclei have, like other brain stem structures, histamine fiber innervation. [14] However, the exact roles of histamine and histamine-containing neurons in these areas in MS are not clear. Some experimental and

clinical studies indicate that vestibular functions are regulated directly or indirectly by the histaminergic nerve system. Histamine has been shown to modulate the hair-cell-afferent synaptic transmission, acting both at the pre- and the post synaptic terminals. [15] There is study evidence indicating that histamine acts as a hair cell transmitter in frog semicircular canal. [16] A report showed that histamine levels in the hypothalamus and pons-medulla oblongate increased significantly following MS in rats and the increase was suppressed after the rats were given a-fluoromethylhistadine, an irreversible inhibitor of histamine-forming enzyme (a histadine decarboxylase).[17] These findings indicate an important role of histamine in MS.

The effects of histamine as a neurotransmitter or neuromodulator result from its binding to specific histamine receptors. Up to now at least four subtypes of histamine receptors, including H1, H2, H3 and H4 receptors, have been found. [18] Recently the expressions and locations of histamine receptors in inner ear have been identified. Studies have showed expressions of H1, H2, and H3 receptor proteins and mRNAs in rat cochleae. [19] Immunohistochemical studies have showed that H1, H2, and H3 receptors exist in rabbit endolymphatic sac. [20] H1 receptors have also been found in the semicircular canal sensory epithelia of frog and mouse. [21] These findings suggest that histamine receptors have a central role in functions of the inner ear including peripheral vestibular organs. Of the four types of histamine receptors, H1R is related more closely to MS. H1R can be found in the hypothalamus, nucleus tractus solitarius, dorsal motor nucleus of vagus nucleus, nucleus ambiguous, lateral vestibular nucleus, and emetic center. [22, 23] It is believed that H1Rs are involved in the development of MS symptoms including emesis. [6, 17]

In the present study, both immunofluorescence staining and western blot analysis showed expression of H1R that was enhanced by motion stimulation. Our findings again indicate that H1Rs in the vestibular nucleus are associated with MS.

Anti-histamine drugs (H1R blockers) are commonly used in the treatment of vertigo and MS, althogu their action sites and underpinning mechanisms remain poorly defined. [21] The sites of action for these drugs are thought to include the vestibular receptors, vestibular nuclei, cerebellum, reticular area and vomiting center. [24]

Promethazine, an H1R blocker, can effectively prevent MS.[24] In the present study, conditioned taste aversion (representing presence of MS) induced by motion stimulation was partially inhibited by promethazine at a dose of 0.25 mg, although promethazine did not significantly affect MS-stimulated expressions of H1R in the vestibular nuclei. This is probably due to that promethazine plays its anti-MS role by competing the binding sites on H1R with histamine rather than by inhibiting the expression of H1R in the vestibular nuclei.

Mechanisms of MS are yet to be completely understood and effective interventions for prevention of MS are yet to be developed. [3] We hope that the present study helps to understand the neuropharmacological mechanisms and signalling pathways of MS, and facilitate development of treatments to prevent MS in the future.

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