Histamine Excites Rat Superior Vestibular Nuclear Neurons via Postsynaptic H₁ and H₂ Receptors in vitro

Qian-Xing Zhuang Yong-Hui Wu Guan-Yi Wu Jing-Ning Zhu Jian-Jun Wang

Department of Biological Science and Technology and State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing, PR China

Key Words

Histamine • Superior vestibular nucleus • Histamine receptors • Vestibular reflexes

Abstract

The superior vestibular nucleus (SVN), which holds a key position in vestibulo-ocular reflexes and nystagmus, receives direct hypothalamic histaminergic innervations. By using rat brainstem slice preparations and extracellular unitary recordings, we investigated the effect of histamine on SVN neurons and the underlying receptor mechanisms. Bath application of histamine evoked an excitatory response of the SVN neurons, which was not blocked by the low-Ca²⁺/high-Mg²⁺ medium, indicating a direct postsynaptic effect of the amine. Selective histamine H₁ receptor agonist 2-pyridyl-ethylamine and H₂ receptor agonist dimaprit, rather than VUF8430, a selective H₄ receptor agonist, mimicked the excitation of histamine on SVN neurons. In addition, selective H₁ receptor antagonist mepyramine and H₂ receptor antagonist ranitidine, but not JNJ777120, a selective H₄ receptor antagonist, partially blocked the excitatory response of SVN neurons to histamine. Moreover, mepyramine together with ranitidine nearly totally blocked the histamine-induced excitation. Immunostainings further showed that histamine H₁

and H₂ instead of H₄ receptors existed in the SVN. These results demonstrate that histamine excites the SVN neurons via postsynaptic histamine H₁ and H₂ receptors, and suggest that the central histaminergic innervation from the hypothalamus may actively bias the SVN neuronal activity and subsequently modulate the SVN-mediated vestibular functions and gaze control.

Copyright © 2012 S. Karger AG, Basel

Introduction

Among the vestibular disorders, pathologic nystagmus is a symptom which often accompanies vertigo and motion sickness. Interestingly, a recent investigation reported an efficacy of the histaminergic drug, betahistine dihydrochloride, in not only antivertigo and anti-motion sickness but also improving oculomotor activity, including increased gain during pursuit movements and faster and more accurate saccades [1]. In fact, antihistaminergic drugs have been used for clinical treatment of vestibular-related diseases, including vertigo, emesis and motion sickness, for almost a century. The therapeutic mechanisms are involved not only in the peripheral vestibular system, such as the labyrinth in the inner ear [2, 3], but

also in the central vestibular nuclear complex, including at least the medial (MVN) and lateral (LVN) vestibular nucleus [4–6].

Immunohistochemical studies have already revealed a moderately dense direct histaminergic projection from the tuberomammillary nucleus of the hypothalamus to the vestibular nuclei in many species, including rat, cat and pigeon [7–9]. Nevertheless, histaminergic fiber distribution in the vestibular nuclei shows spatial variations, with significantly heavier labeling in the superior vestibular nucleus (SVN) and MVN than in the LVN and descending vestibular nucleus [9]. On the other hand, molecular, autoradiographic and pharmacological studies have also demonstrated that the LVN and MVN are both endowed with histamine H_1 , H_2 and/or H_3 receptors [10, 11]. Among the four nuclei in the central vestibular nuclear complex, the SVN, together with the MVN, receives fibers predominantly from the semicircular canals and sends fibers through the medial longitudinal fasciculus rostrally to oculomotor centers and caudally to the spinal cord [12, 13]. Both of these two nuclei actually hold a key position in vestibulo-ocular reflexes and gaze control [12, 14–16], and are closely related to nystagmus [17, 18].

In recent years, the role of histamine/hypothalamic histaminergic innervation in the MVN neuronal activity and the MVN-mediated vestibular compensation has received increased attention [4, 19, 20]; however, the action of histamine on the SVN, another key vestibular nucleus primarily involved in reflexes controlling gaze, still remains enigmatic. Thus, by using extracellular recordings and immunostainings, this work was designed to investigate the effect of histamine on neurons in the SVN and the underlying receptor mechanism. The results demonstrated that histamine excited the SVN neurons via activation of both postsynaptic H_1 and H_2 receptors.

Material and Methods

Slice Preparations and Incubations

Sagittal brain slices (400 μm thickness) containing the SVN were prepared from Sprague-Dawley rats (120–250 g) of either sex. Rats were decapitated under sodium pentobarbital (40 mg/kg) anesthesia. After carefully removing the skull, the brain extending from the obex to the superior colliculus was rapidly removed into ice-cold artificial cerebrospinal fluid (ACSF, composition in mM: 124 NaCl, 5 KCl, 1.2 NaH_2PO_4 , 1.3 MgSO_4 , 26 NaHCO_3 , 2 CaCl_2 and 10 D-glucose) equilibrated with 95% O_2 /5% CO_2 . According to the rat brain atlas of Paxinos and Watson [21], the sagittal slices [22] containing the SVN, cerebellum and part of the midbrain (including colliculi) were cut (fig. 1a) with a vibraslicer (VT 1200S, Leica, Wetzlar, Germany) at 4°C. The slices

were subsequently transferred into a recording chamber, which was continuously perfused with 95% O_2 /5% CO_2 oxygenated ACSF (pH 7.4, $33 \pm 0.2^\circ\text{C}$, flow rate 1.5–2 ml/min). All slices were incubated for at least 40 min before neuronal electrophysiological recordings. All experiments completely conformed to the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications 80-23, revised 1996). All efforts were made to minimize the number of animals used and their suffering.

Electrophysiological Recordings, Data Acquisition and Statistical Analysis

The SVN was visually identified with the aid of a stereomicroscope (SD-3045F; Olympus, Tokyo, Japan) and spontaneous unitary activity of the SVN neurons was recorded extracellularly from the slices by using glass microelectrodes filled with 2 M NaCl (resistance 5–10 $\text{M}\Omega$). Before bath application of histaminergic compounds at known concentrations, the discharge rate of the recorded neuron was observed for at least 40 min to assure stability. In some experiments, low- Ca^{2+} /high- Mg^{2+} medium was used to decrease presynaptic neurotransmitter release. In these cases, the concentration of Ca^{2+} was lowered to 0.3 mM and Mg^{2+} was raised to 9.0 mM [23–27]. Histamine or histamine receptor agonist was added to the perfusing ACSF to stimulate the recorded SVN neuron for a test period of 1 min. After each stimulation, cells were given at least 20 min for recovery and preventing receptors from desensitization. If the SVN neuron responded to the stimulation, the perfusing medium was switched from normal ACSF to the ACSF containing histamine receptor antagonist(s). After the slice was equilibrated with the ACSF containing the antagonist(s) for at least 15 min, histamine or histamine receptor agonist was reapplied and the effect of antagonist(s) on the response of SVN neuron to histamine or histamine receptor agonist was observed.

The neuronal discharges of single units were amplified and displayed conventionally, and fed into a window discriminator simultaneously. The standard rectangle pulses (5 V, 1 ms) triggered from the spikes were sent through an interface (1404 Plus, CED, Cambridge, UK) to a laboratory computer, which was used to analyze the discharge rate online by the software Spike 2 (CED). Peristimulus time histograms (sampling interval: 1 s) and the interspike intervals (sampling interval: 1 ms) distributions of neuronal discharges were generated by the computer to assess the effects of histamine and histamine receptor agonists on the SVN neurons. Drug-induced effects on spontaneous unitary activity of SVN neurons were considered to be substance specific provided they were reversible and reproducible. The response magnitude of a neuron to the stimulation of histamine or histamine receptor agonist was calculated as the percentage change in the cell's peak discharge rate following stimulation with respect to its basal firing rate. All data were expressed as means $\pm$ SEMs. Student's *t* test was employed for statistical analysis of the data and *p* values of <0.05 were considered to be significant.

Immunofluorescence

The experimental procedures for immunostaining followed our previous work [28]. Rats (weighing 150–200 g) were deeply anesthetized with sodium pentobarbital and perfused transcardially with 100 ml normal saline, followed by 450–500 ml 4% paraformaldehyde in 0.1 M phosphate buffer. Subsequently, the brain

Fig. 1. Histamine directly/postsynaptically excited the SVN neurons. **a** Diagram of sagittal brainstem sections shows the area of the SVN investigated in this study. 4V = 4th Ventricle; IN = interpositus nucleus; IVN = inferior vestibular nucleus; SCP = superior cerebellar peduncle. **b** Oscilloscope traces show the action potentials of a recorded SVN neuron and the neuron's response to 10 μM histamine. The mean firing rate of the neuron before, during and after application of histamine was 22.6, 29.8 and 22.9 spikes/s, respectively. **c** Histograms show that the SVN neuron exhibited a concentration-dependent excitatory response to 1, 3 and 10 μM histamine stimulation. **d** Interspike intervals show that histamine shortened the intervals of spikes of the SVN neuron. **e** Histograms show the histamine-induced excitation on the same neuron when the slice was equilibrated with low- Ca^{2+} /high- Mg^{2+} medium. In this and the following figures, the short horizontal bars above the data indicate the 1-min period of application of histamine or histamine receptor agonists, and the long horizontal bars denote the exposure of the slice to the low- Ca^{2+} /high- Mg^{2+} medium or histamine receptor antagonists.

was removed, trimmed and postfixed in the same fixative for 12 h at 4°C, and then cryoprotected with 30% sucrose for 48 h. Frozen coronal sections (25 μm thickness) containing the SVN were obtained by using a freezing microtome (CM 3050S, Leica) and mounted on gelatin-coated slides. The slices were rinsed in

phosphate-buffered saline containing 0.1% Triton X-100 (PBST) and then incubated in 10% normal bovine serum in PBST for 30 min. Sections were incubated overnight at 4°C with primary antibodies to histamine H_1 , H_2 or H_4 receptor, respectively: a rabbit anti- H_1 receptor polyclonal antibody (1:50; Santa Cruz Biotech-

Fig. 2. The averaged concentration-response curves at group data level showing the effects of histamine, selective histamine H_1 , H_2 and H_4 receptor agonists (2-PyEA, dimaprit and VUF8430) on the SVN neurons, and the effects of selective histamine H_1 , H_2 and H_4 receptor antagonists (mepyramine, ranitidine and JNJ777120) on the histamine-induced excitation. Numbers in parentheses denote the number of cells tested in each experiment. Data are presented as mean $\pm$ SEM.

nology, Santa Cruz, Calif., USA), a rabbit anti-histamine H_2 receptor polyclonal antibody (1:50; Santa Cruz Biotechnology), or a rabbit anti-histamine H_4 receptor polyclonal antibody (1:50; Santa Cruz Biotechnology). After a complete wash in PBS, these sections were incubated in the Alexa 488-conjugated secondary antibody solutions (goat anti-rabbit 1:2,000; Invitrogen, Carlsbad, Calif., USA) for 2 h at room temperature in the dark. The slides were washed and mounted in UltraCruz mounting medium (Santa Cruz Biotechnology). All micrographs were taken with an inverted laser scanning confocal microscope (FV1000; Olympus). Incubations replacing the primary antiserum with control immunoglobulins and/or omitting the primary antiserum were used as negative controls.

Histaminergic Reagents

Stocks solutions of histaminergic compounds were made in distilled water, and dilutions were freshly prepared in ACSF and equilibrated with 95% O_2 /5% CO_2 before the slices were superfused. The histaminergic compounds used in this experiment included histamine (Sigma, St. Louis, Mo., USA), highly selective histamine H_1 receptor antagonist mepyramine (Tocris, Bristol, UK), highly selective histamine H_2 receptor antagonist ranitidine (Tocris), highly selective histamine H_1 receptor agonist 2-pyridylethylamine (2-PyEA; Tocris) and highly selective histamine H_2 receptor agonist dimaprit (Tocris). JNJ777120, a highly selective histamine H_4 receptor antagonist, and VUF8430, a highly selective histamine H_4 receptor agonist, were kind gifts from Dr. Rob Leurs (VU University Amsterdam, Amsterdam, The Netherlands). The concentrations of histaminergic reagents were chosen according to previous reports [4, 6, 23–27, 29, 30].

Results

Histamine Directly Excites the SVN Neurons in a Concentration-Dependent Manner

One hundred and twenty SVN neurons with tonic spontaneous firing activity were extracellularly recorded from 55 brainstem slices of rats in the present study. The spontaneous firing rate of the SVN neurons ranged from 5 to 80 spikes/s and the mean firing rate was 25.32 ± 0.86 spikes/s, both of which are similar to the previous study on the SVN neurons in vivo [31] and slightly higher than the MVN neuronal spontaneous firing rate in vitro [4, 32, 33].

All of the 120 recorded SVN neurons (120/120, 100%) sampled from various subregions of the SVN (fig. 1a) were excited by the histamine stimulation (1–30 μ M; fig. 1b). As illustrated in figure 1c (see also fig. 2), the recorded SVN neuron showed a concentration-dependent excitation to 1, 3 and 10 μ M histamine with peak firing values of 24.5, 26.8 and 29.8 spikes/s, i.e. 8.4, 18.6 and 31.9% increments in the peak discharge rate compared with its basal firing rate of 22.6 spikes/s ($p < 0.05$ or 0.01), respectively. Furthermore, interspike intervals revealed that during the period of histamine-induced excitation, the interspike intervals of SVN neurons were significantly shortened ($p < 0.05$ or 0.01; fig. 1d). The results indicate that histamine is capable of exciting SVN neurons.

To exclude the possibility that the excitatory response of SVN neurons was indirectly induced by the effect of histamine on presynaptic elements, we tested the effects of histamine on the SVN cells when the normal ACSF had been replaced with a low- Ca^{2+} /high- Mg^{2+} medium ($n = 5$). The results showed that the low- Ca^{2+} /high- Mg^{2+} medium did not block the histamine-induced excitation ($p > 0.05$; fig. 1e vs. c), although the spontaneous firing rates of some tested neurons were slightly decreased, which might be related to a disturbance of normal Ca^{2+} concentration in local milieu and/or to actions of Mg^{2+} on intracellular Ca^{2+} -dependent processes [23, 32, 34, 35]. This result suggests a direct postsynaptic excitatory effect of histamine on the SVN neurons.

The Histamine-Induced Postsynaptic Excitation on SVN Neurons Is Comediated by Histamine H_1 and H_2 Receptors

Since the histamine H_1 , H_2 and H_4 receptors are all postsynaptic whereas H_3 receptors are presynaptic [36, 37], we further used histamine receptor agonists and antagonists to examine which postsynaptic histamine receptor(s) mediated the histamine-induced excitation on

Fig. 3. Effects of histamine receptor agonists on the SVN neurons and effects of histamine receptor antagonists on the histamine-induced excitation. **a** Concentration-related excitatory responses of a recorded SVN neuron to histamine. **b–d** The effects of selective histamine H_1 receptor agonist 2-PyEA, H_2 receptor agonist dimaprit and H_4 receptor agonist VUF8430 on the same

cell. **e–h** The effects of selective histamine H_1 receptor antagonist mepyramine, H_2 receptor antagonist ranitidine and H_4 receptor antagonist JNJ777120 on the histamine-induced excitations on the same SVN neuron. Note that mepyramine combined with ranitidine blocked the histamine-induced excitation almost totally.

SVN neurons. The results showed that both the selective H_1 receptor agonist 2-PyEA (3–100 μM) and selective H_2 receptor agonist dimaprit (3–100 μM) mimicked the excitatory effect of histamine on SVN neurons ($n = 49$ and 56, respectively; fig. 3b, c, see also fig. 2), whereas the selective H_4 receptor agonist VUF8430 (3–100 μM) did not elicit any response in SVN neurons ($n = 12$; fig. 3d, see also fig. 2). As shown in figure 3, the recorded SVN neurons exhibited concentration-related excitatory responses to

histamine (1, 3, and 10 μM , with 23.6, 35.1 and 48.7% increases in the peak discharge rate compared with its basal firing rate, $p < 0.05$ or 0.01; fig. 3a), 2-PyEA (3, 10 and 30 μM , with 11.1, 21.5 and 33.4% increases in the peak discharge rate compared with its basal firing rate, $p < 0.05$ or 0.01; fig. 3b) and dimaprit (3, 10 and 30 μM , with 3.5, 17.2 and 29.0% increases in the peak discharge rate compared with its basal firing rate, $p < 0.05$ or 0.01; fig. 3c) but not VUF8430 (3, 10 and 30 μM , $p > 0.05$; fig. 3d).

Fig. 4. The blocking effect of histamine H_1 receptor antagonist mepyramine and H_2 receptor antagonist ranitidine on selective histamine H_1 and H_2 receptor agonists 2-PyEA- and dimaprit-mimicked excitations on the SVN neurons. **a** A concentration-dependent excitation induced by 1, 3 and 10 μM histamine on a recorded SVN neuron. **b, c** The excitatory response of the neuron to 2-PyEA and the blocking effect of mepyramine on 2-PyEA-induced excitations. **d, e** The excitatory response of the same neuron to dimaprit and the blocking effect of ranitidine on the dimaprit-induced excitations.

On the other hand, mepyramine (1 μM), a selective histamine H_1 receptor antagonist, and ranitidine (1 μM), a selective histamine H_2 receptor antagonist, effectively blocked the excitatory responses of SVN neurons ($n = 20$ and 12, respectively) to histamine (1–30 μM ; fig. 3e, f), but selective histamine H_4 receptor antagonist JNJ777120 (1 μM) did not influence the excitatory effects of histamine on the neurons ($n = 10$; fig. 3g). As illustrated in figure 3, on the same recorded SVN neuron, mepyra-

mine (1 μM) and ranitidine (1 μM) significantly reduced the 23.6, 35.1 and 48.7% increases in the peak firing rate evoked by 1, 3 and 10 μM histamine to 8.3, 14.9 and 22.7% ($p < 0.05$ or 0.01, fig. 3e vs. a) and 7.1, 13.7 and 18.3% ($p < 0.05$ or 0.01; fig. 3f vs. a), respectively; however, JNJ777120 (1 μM) did not influence the histamine-induced excitatory responses, i.e. the cell's responses to histamine remained at the same levels as with the control experiments (22.2, 34.5 and 47.3% increase in the peak firing rate, $p > 0.05$; fig. 3g). Furthermore, mepyramine (1 μM) combined with ranitidine (1 μM) blocked the histamine-induced excitation almost totally ($n = 8$; fig. 3h).

As summarized in figure 2, the SVN neurons exhibited a concentration-dependent excitatory response to the stimulation of histamine, 2-PyEA and dimaprit rather than VUF8430. In addition, mepyramine, ranitidine and a combination of both rather than JNJ777120 pushed the concentration-response curve of histamine down to the lower level, indicating a comediation mechanism of both H_1 and H_2 receptors.

To confirm the receptor mechanisms underlying the histamine-induced excitation on SVN neurons, the effect of histamine receptor antagonists on agonist-mimicked responses was further examined. The results showed that mepyramine (1 μM) significantly blocked the concentration-related excitation mimicked by 10, 30 and 100 μM 2-PyEA (fig. 4c vs. b), and ranitidine (1 μM) remarkably antagonized the concentration-related excitation mimicked by 10, 30 and 100 μM dimaprit (fig. 4e vs. d). Furthermore, the additive effects of coapplication of 2-PyEA and dimaprit on the SVN neurons are shown in figure 5. The averaged concentration-response curves are plotted in figure 6.

Table 1 summarizes the effects of histamine and histamine receptor agonists on SVN neurons and the effects of histamine receptor antagonists on histamine- or agonist-induced excitations. The data strongly suggest that it is H_1 and H_2 receptors, rather than H_4 receptors, mediate histamine-induced excitatory responses on SVN neurons.

Both Histamine H_1 and H_2 Receptors Are Expressed in the SVN

To map the distribution of histamine receptors in the SVN, we performed immunostaining of the rat brainstem slices containing the SVN with an antibody against histamine H_1 , H_2 and H_4 receptors, respectively. We found that histamine H_1 and H_2 rather than H_4 receptors were localized in the SVN (fig. 7). These observations are consistent with the above-mentioned electrophysiological

cal results and prove that histamine excites the SVN neurons through activation of both histamine H₁ and H₂ receptors.

Discussion

The central histaminergic system solely originates from the tuberomammillary nucleus of the hypothalamus but extensively innervates almost the whole brain including various subcortical motor structures [36–38]. It holds a key position in the regulation of many basic physiological functions, including the sleep-wake cycle, energy and endocrine homeostasis, synaptic plasticity and learning, as well as motor control [36–39]. Thus, the central histaminergic system/histamine has been considered as a general modulator for whole brain activity.

Intriguingly, neuroanatomical and immunohistochemical studies have also revealed a direct histaminergic innervation on the central vestibular nuclear complex [7–9], and the electrophysiological studies further reported an excitatory effect of histamine on neurons in both the MVN and LVN [4, 6]. However, these studies on the role of histamine/the histaminergic system in vestibular neurons and vestibular-related functions neglect the SVN, an important subnucleus in the vestibular nuclear complex and playing a critical role in the vestibulo-ocular reflexes and gaze control. In the present study, we demonstrated that histamine excited the rat SVN neurons by mediation of both histamine H₁ and H₂ receptors.

Although histamine exerts a uniform excitatory effect on neurons in all of the LVN, MVN and SVN, the receptor mechanisms underlying the histamine-induced excitation on neurons in the three vestibular nuclei are different. In the LVN, histamine depolarizes the neurons via only postsynaptic histamine H₂ receptors [6], whereas in the MVN not only postsynaptic histamine H₁ and H₂ receptors, but also presynaptic histamine H₃ receptors are involved in the action of histamine on the neurons [4, 19]. In this study, low-Ca²⁺/high-Mg²⁺ medium did not affect the excitatory response of SVN neurons to histamine (fig. 1e), suggesting that the histamine-induced excitation was due to a direct postsynaptic effect of the amine on the cells, but not an indirect effect induced by presynaptic neural events. Therefore, selective agonists and antagonists for postsynaptic histamine H₁, H₂ and H₄ receptors were applied to determine the receptor mechanisms on the SVN neurons. Mepyramine and ranitidine, selective antagonists for histamine H₁ and H₂ receptors, separately blocked the histamine-induced excitation in part and

Fig. 5. The additive excitatory effect of coapplication of histamine H₁ and H₂ receptor agonists 2-PyEA and dimaprit on a single SVN neuron (a–d).

Fig. 6. The averaged concentration-response curves at group data level showing the effects of histamine, 2-PyEA, dimaprit, and co-application of 2-PyEA and dimaprit on the SVN neurons, and the effects of mepyramine (1 μM) and ranitidine (1 μM) on 2-PyEA- and dimaprit-induced excitation, respectively. Numbers in parentheses denote the number of cells tested in each case. Data are presented as mean ± SEM.

Fig. 7. Confocal photomicrographs showing histamine receptor immunoreactivity in the SVN in rats. Histamine H_1 receptor- (a1–3) and H_2 receptor-immunolabeled neurons (b1–3) were detected in the SVN, but H_4 receptor-immunolabeled neurons (c1–3) were not detected. d1–3 Negative staining controls. The second and third images in each series are higher magnifications of the square in the preceding image. Scale bars: a–d1 = 990 μ m; a–d2 = 270 μ m; a–d3 = 20 μ m. LR4V = Lateral recess of the 4th ventricle.

Table 1. Comparison of the effects of histamine and histamine receptor agonists on SVN neurons, and the effects of histamine receptor antagonists on histamine- or agonist-induced excitation on the SVN neurons

	Cells, n	% of response
Cells excited by histamine	120	100 (120/120)
Cells excited by 2-PyEA	49	100 (49/49)
Cells excited by dimaprit	56	100 (56/56)
Cells which responded to VUF8430	12	0 (0/12)
Cells excited by coapplication of 2-PyEA and dimaprit	10	100 (10/10)
Histamine-induced excitation blocked by mepyramine	20	100 (20/20)
Histamine-induced excitation blocked by ranitidine	12	100 (12/12)
Histamine-induced excitation blocked by JNJ777120	10	0 (0/10)
2-PyEA-induced excitation blocked by mepyramine	10	100 (10/10)
Dimaprit-induced excitation blocked by ranitidine	10	100 (10/10)

together antagonized the excitation nearly totally (fig. 2, 3). Moreover, 2-PyEA and dimaprit, selective agonists for histamine H_1 and H_2 receptors, separately mimicked the histamine-induced excitation in a concentration-dependent manner (fig. 2, 3), and coapplication of 2-PyEA and

dimaprit evoked an additive excitatory effect (fig. 5, 6). Also, the 2-PyEA- and dimaprit-mimicked excitations on SVN neurons were blocked by mepyramine and ranitidine, respectively (fig. 4, 6). On the other hand, VUF8430 and JNJ777120, selective agonist and antagonist for his-

tamine H₄ receptors, had no effect on the SVN neurons and histamine-induced excitation (fig. 2, 3). Furthermore, immunostaining results revealed that only histamine H₁ and H₂ receptors, and not H₄ receptors, were expressed in the SVN (fig. 7). These results clearly demonstrate that histamine H₁ and H₂ receptors rather than H₃ and H₄ receptors mediate the histamine-induced excitatory effect on rat SVN neurons.

The vestibular nuclei in the brainstem are a sensorimotor complex that integrates vestibular, visual and motor signals to make compensatory eye and head movements as well as postural adjustments [13]. Among the four major vestibular nuclei, the SVN receives inputs predominantly from the semicircular canals and sends axons to the oculomotor nucleus, the cerebellar nodulus, uvula and flocculus, and the ventral posterior nuclear complex of the thalamus, which in turn projects to the cortical areas relevant to vestibular sensations [12]. Consequently, slightly different from the other vestibular nuclei which are also essential for posture adjustments of the head and/or body, the SVN primarily participates in vertical vestibulo-ocular reflexes and holds a key position in gaze control [12, 14–16], together with the MVN. Therefore, the present results that histamine directly excites the SVN neurons suggest that the histaminergic innervation from the hypothalamus on vestibular nuclei may actively participate in stabilizing not only head and posture through the MVN and LVN, but also gaze through the MVN as well as the SVN.

Besides the vestibular nuclear complex in the brainstem, various important subcortical motor structures have been found to receive and be modulated by direct hypothalamic histaminergic projections, including the cerebellum [23, 26], the red nucleus [24], the substantia nigra [40, 41], the neostriatum [42] and the globus pallidus [25] in the basal ganglia. Interestingly, without exception, neurons in all these subcortical motor centers are uniformly excited by histamine. Since most histaminergic endings (varicosities) do not typically form synaptic specializations and all of the histamine receptors are metabotropic [36, 37, 43], we suggest that histamine or histaminergic inputs from the hypothalamus to these central motor structures may not transmit fast concrete signals, but act as a biasing force to influence electrophysiological properties of these motor neurons and hold their excitability and responsiveness to an appropriate level. Consequently, the central histaminergic system may ultimately regulate movements. In fact, in the cerebellum, histamine/the histaminergic afferents regulate the cerebellar nuclear neuronal activity and improve motor balance and motor coordi-

ination during ongoing movements [26, 44]. Also, depletion of brain histamine or knockout of histamine receptors alters ambulatory activity and reduces exploratory behavior [45–47]. Thus, we speculate that the hypothalamic histaminergic system/histamine may actively participate in central motor control by extensive modulation of the sensorimotor integration in circuits through various subcortical motor structures including the SVN.

In conclusion, we found a direct excitatory action of histamine on the SVN neurons, which is mediated by both postsynaptic histamine H₁ and H₂ receptors. Through biasing neuronal activity in the SVN, the histaminergic system may be involved in gaze control and contribute to the generation of appropriate compensatory eye movements and stable vision during head rotations. The modulation of the hypothalamic histaminergic system on the SVN may constitute an important and functional component of the vestibular motor control, including not only postural stability of both the head and body, but also compensatory eye movements. On the other hand, the modulation of the histaminergic system on the SVN cannot be ignored in the symptomatic treatment of vestibular-related diseases (including nystagmus), and the postsynaptic histamine receptors in central vestibular nuclei may be potential targets for clinic therapy.

Acknowledgements

We thank Dr. Rob Leurs (VU University Amsterdam, Amsterdam, The Netherlands) for his generous gifts of histamine H₄ receptor agonist and antagonist. This work was supported by grants 31070959, 31071021, 31171050, J1103512 and NSFC/RGC Joint Research Scheme 30931160433 from the National Natural Science Foundation of China; RFDP grant 20100091110016, NCET Program and Fundamental Research Funds for the Central Universities 1094020806 and 1095020821 from the State Educational Ministry of China; grant BK2011014 from the Natural Science Foundation of Jiangsu Province, China. This work was also supported by grant 9112020802 of '985 Project' from Nanjing University.

References

- 1 Matsnev EI, Sigaleva EE: Efficacy of histaminergic drugs in experimental motion sickness. *J Vestib Res* 2007;17:313–321.
- 2 Soto E, Chávez H, Valli P, Benvenuti C, Vega R: Betahistidine produces post-synaptic inhibition of the excitability of the primary afferent neurons in the vestibular endorgans. *Acta Otolaryngol* 2001;154(suppl):19–24.
- 3 Chávez H, Vega R, Soto E: Histamine H₃ receptors modulate the excitatory amino acid receptor response of the vestibular afferents. *Brain Res* 2005;1064:1–9.

- 4 Wang JJ, Dutia MB: Effects of histamine and betahistidine on rat medial vestibular nucleus neurons: possible mechanism of action of anti-histaminergic drugs in vertigo and motion sickness. *Exp Brain Res* 1995;105:18–24.
- 5 Lacour M, van de Heyning PH, Novotny M, Tighilet B: Betahistidine in the treatment of Ménière's disease. *Neuropsychiatr Dis Treat* 2007;3:429–440.
- 6 Zhang J, Han XH, Li HZ, Zhu JN, Wang JJ: Histamine excites rat lateral vestibular nuclear neurons through activation of postsynaptic H₂ receptors. *Neurosci Lett* 2008;448:15–19.
- 7 Schwartz JC, Arrang JM, Garbarg M, Pollard H, Ruat M: Histaminergic transmission in the mammalian brain. *Physiol Rev* 1991;71:1–51.
- 8 Steinbusch HW: Distribution of histaminergic neurons and fibers in rat brain: comparison with noradrenergic and serotonergic innervation of the vestibular system. *Acta Otolaryngol* 1991;479(suppl):12–23.
- 9 Tighilet B, Lacour M: Distribution of histaminergic axonal fibers in the vestibular nuclei of the cat. *NeuroReport* 1996;7:873–878.
- 10 Yabe T, de Waele C, Serafin M, Vibert N, Arrang JM, Mühlethaler M, Vidal PP: Medial vestibular nucleus in the guinea-pig: histaminergic receptors. II. An in vivo study. *Exp Brain Res* 1993;93:249–258.
- 11 Vizuete ML, Traiffort E, Bouthenet ML, Ruat M, Souil E, Tardive-Lacombe J, Schwartz JC: Detailed mapping of the histamine H₂ receptor and its gene transcripts in guinea-pig brain. *Neuroscience* 1997;80:321–343.
- 12 Highstein SM, Holstein GR: The anatomy of the vestibular nuclei. *Prog Brain Res* 2006;151:157–203.
- 13 Straka H, Vibert N, Vidal PP, Moore LE, Dutia MB: Intrinsic membrane properties of vertebrate vestibular neurons: function, development and plasticity. *Prog Neurobiol* 2005;76:349–392.
- 14 Wentzel PR, De Zeeuw CI, Holstege JC, Gerrits NM: Inhibitory synaptic inputs to the oculomotor nucleus from vestibulo-ocular reflex-related nuclei in the rabbit. *Neuroscience* 1995;65:161–174.
- 15 Zhang Y, Partsalis AM, Highstein SM: Properties of superior vestibular nucleus neurons projecting to the cerebellar flocculus in the squirrel monkey. *J Neurophysiol* 1993;69:642–645.
- 16 Zhang Y, Partsalis AM, Highstein SM: Properties of superior vestibular nucleus flocculus target neurons in the squirrel monkey. II. Signal components revealed by reversible flocculus inactivation. *J Neurophysiol* 1995;73:2279–2292.
- 17 Arnold DB, Robinson DA, Leigh RJ: Nystagmus induced by pharmacological inactivation of the brainstem ocular motor integrator in monkey. *Vision Res* 1999;39:4286–4295.
- 18 Pierrot-Deseilligny C, Milea D: Vertical nystagmus: clinical facts and hypotheses. *Brain* 2005;128:1237–1246.
- 19 Bergquist F, Dutia MB: Central histaminergic modulation of vestibular function – a review. *Acta Physiol Sin* 2006;58:293–304.
- 20 Tighilet B, Mourre C, Trottier S, Lacour M: Histaminergic ligands improve vestibular compensation in the cat: behavioural, neurochemical and molecular evidence. *Eur J Pharmacol* 2007;568:149–163.
- 21 Paxinos G, Watson C: *The Rat Brain in Stereotaxic Coordinates*, ed 6. San Diego, Academic Press, 2007.
- 22 Korte GE, Friedrich VL Jr: The fine structure of the feline superior vestibular nucleus: identification and synaptology of the primary vestibular afferents. *Brain Res* 1979;176:3–32.
- 23 Shen B, Li HZ, Wang JJ: Excitatory effects of histamine on cerebellar interpositus nuclear cells of rat through H₂ receptors in vitro. *Brain Res* 2002;948:64–71.
- 24 Chen K, Zhu JN, Li HZ, Wang JJ: Histamine elicits neuronal excitatory response of red nucleus in the rat via H₂ receptor in vitro. *Neurosci Lett* 2003;6:25–28.
- 25 Chen K, Wang JJ, Yung WH, Chan YS, Chow BKC: Excitatory effect of histamine on neuronal activity of rat globus pallidus by activation of H₂ receptors in vitro. *Neurosci Res* 2005;53:288–297.
- 26 He YC, Wu GY, Li D, Tang B, Li B, Ding Y, Zhu JN, Wang JJ: Histamine promotes rat motor performances by activation of H₂ receptors in the cerebellar fastigial nucleus. *Behav Brain Res* 2012;228:44–52.
- 27 Wu GY, Han XH, Zhuang QX, Zhang J, Yung WH, Chan YS, Zhu JN, Wang JJ: Excitatory effect of histamine on rat spinal motoneurons by activation of both H₁ and H₂ receptors in vitro. *J Neurosci Res* 2012;90:132–142.
- 28 Zhang J, Li B, Yu L, He YC, Li HZ, Zhu JN, Wang JJ: A role for orexin in central vestibular motor control. *Neuron* 2011;69:793–804.
- 29 Lim HD, Adami M, Guaita E, Werfel T, Smits RA, de Esch IJ, Bakker RA, Gutzmer R, Coruzzi G, Leurs R: Pharmacological characterization of the new histamine H₄ receptor agonist VUF 8430. *Br J Pharmacol* 2009;157:34–43.
- 30 Breunig E, Michel K, Zeller F, Seidl S, Weyhern CW, Schemann M: Histamine excites neurones in the human subnucleus lexis through activation of H₁, H₂, H₃ and H₄ receptors. *J Physiol* 2007;583:731–742.
- 31 Licata F, Li Volsi G, Maugeri G, Ciranna L, Santangelo F: Serotonin-evoked modifications of the neuronal firing rate in the superior vestibular nucleus: a microiontophoretic study in the rat. *Neuroscience* 1993;52:941–949.
- 32 Dutia MB, Johnston AR, McQueen DS: Tonic activity of rat medial vestibular nucleus neurones in vitro and its inhibition by GABA. *Exp Brain Res* 1992;88, 466–472.
- 33 Saito Y, Takazawa T, Ozawa S: Relationship between afterhyperpolarization profiles and the regularity of spontaneous firings in rat medial vestibular nucleus neurons. *Eur J Neurosci* 2008;28:288–298.
- 34 Stehle J: Effects of histamine on spontaneous electrical activity of neurons in rat suprachiasmatic nucleus. *Neurosci Lett* 1991;130:217–220.
- 35 Yu L, Zhang XY, Zhang J, Zhu JN, Wang JJ: Orexins excite neurons of the rat cerebellar nucleus interpositus via orexin 2 receptors in vitro. *Cerebellum* 2010;9:88–95.
- 36 Haas H, Panula P: The role of histamine and the tuberomammillary nucleus in the nervous system. *Nat Rev Neurosci* 2003;4:121–130.
- 37 Haas HL, Sergeeva OA, Selbach O: Histamine in the nervous system. *Physiol Rev* 2008;88:1183–1241.
- 38 Brown RE, Stevens DR, Haas HL: The physiology of brain histamine. *Prog Neurobiol* 2001;63:637–672.
- 39 Zhu JN, Yung WH, Chow BKC, Chan YS, Wang JJ: The cerebellar-hypothalamic circuits: potential pathways underlying cerebellar involvement in somatic-visceral integration. *Brain Res Rev* 2006;52:93–106.
- 40 Korotkova TM, Haas HL, Brown RE: Histamine excites GABAergic cells in the rat substantia nigra and ventral tegmental area in vitro. *Neurosci Lett* 2002;320:133–136.
- 41 Zhou FW, Xu JJ, Zhao Y, LeDoux MS, Zhou FM: Opposite functions of histamine H₁ and H₂ receptors and H₃ receptor in substantia nigra pars reticulata. *J Neurophysiol* 2006;96:1581–1591.
- 42 Sittig N, Davidowa H: Histamine reduces firing and bursting of anterior and intralaminar thalamic neurons and activates striatal cells in anesthetized rats. *Behav Brain Res* 2001;124:137–143.
- 43 Diewald L, Heimrich B, Busseberg D, Watanabe T, Haas HL: Histaminergic system in co-cultures of hippocampus and posterior hypothalamus: a morphological and electrophysiological study in the rat. *Eur J Neurosci* 1997;9:2406–2413.
- 44 Song YN, Li HZ, Zhu JN, Guo CL, Wang JJ: Histamine improves rat rotarod and balance beam performances through H₂ receptors in the cerebellar interpositus nucleus. *Neuroscience* 2006;140:33–43.
- 45 Onodera K, Yamatodani A, Watanabe T, Wada H: Neuropharmacology of the histaminergic neuron system in the brain and its relationship with behavioral disorders. *Prog Neurobiol* 1994;42:685–702.
- 46 Inoue I, Yanai K, Kitamura D, Taniuchi I, Kobayashi T, Niimura K, Watanabe T: Impaired locomotor activity and exploratory behavior in mice lacking histamine H₁ receptors. *Proc Natl Acad Sci USA* 1996;93:13316–13320.
- 47 Toyota H, Dugovic C, Koehl M, Laposky AD, Weber C, Ngo K, Wu Y, Lee DH, Yanai K, Sakurai E, Watanabe T, Liu C, Chen J, Barbier AJ, Turek FW, Fung-Leung WP, Lovenberg TW: Behavioral characterization of mice lacking H₃ receptors. *Mol Pharmacol* 2002;62:389–397.