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Histamine immunoreactivity changes in vestibular-lesioned and histaminergic-treated cats

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Abstract

Histamine is likely involved in vestibular function recovery since histaminergic medications are effective in vestibular-related syndromes. We investigated the histamine immunoreactivity changes after unilateral vestibular neurectomy and the effects of betahistine (a partial histamine H_1 receptor agonist and an histamine H_3 receptor antagonist) and thioperamide (a pure histamine H_3 receptor antagonist) treatment in cats. Histamine staining was analyzed in the tuberomammillary and vestibular nuclei through immunohistochemical methods and quantification techniques in light microscopy. Unilateral vestibular neurectomy induced a strong bilateral decrease in histamine immunoreactivity in the vestibular nuclei and a smaller reduction in the tuberomammillary nuclei in both acute (1 week) and compensated (3 weeks, 1 year) cats. One-week thioperamide or betahistine treatment led to a near-total lack of staining in these structures in both lesioned and control cats. One-month betahistine treatment had weaker effects in the compensated cats. We conclude that vestibular lesions reduce histamine staining because of an increase in histamine release in the vestibular and tuberomammillary nuclei, promoting vestibular functions recovery, and betahistine could contribute to this process by acting on both the presynaptic histamine H_3 and postsynaptic histamine H_1 receptors. © 1997 Elsevier Science B.V.

Keywords: Histamine immunoreactivity; Vestibular nucleus; Tuberomammillary nucleus; Unilateral vestibular neurectomy; Thioperamide; Betahistine; (Cat)

1. Introduction

Unilateral lesion of the vestibular system results in a typical postural, locomotor, and oculomotor syndrome in various species. The deficits occurring just after the lesion progressively vanishes in a process referred to as vestibular compensation, which has been extensively investigated both behaviorally and electrophysiologically (see Lacour et al., 1989; Smith and Curthoys, 1989, for reviews). The neurochemical changes related to vestibular compensation are less documented (for review, see Smith and Darlington, 1991; De Waele et al., 1995). Histamine, a neurotransmitter and/or neuromodulator of the mammalian central nervous system (Schwartz et al., 1980; Prell and Green, 1986) is involved in diverse brain functions like sleep and wakefulness (Lin et al., 1990). Recent data reported in the following also suggest that histamine could play a significant role in the processing of sensory information in the vestibular nuclei, the control of vestibular functions and in the recovery process following vestibular lesion.

Histamine- and histidine decarboxylase-immunoreactive neurons are almost exclusively located in the posterior hypothalamus and aggregated in the tuberomammillary nucleus (Pollard and Schwartz, 1987). In contrast, histaminergic projections are widely bilaterally distributed in various regions of the brain (Schwartz et al., 1991). Three types of histamine receptors (for review, see Hill, 1990) have been identified with autoradiographic (Bouthenet et al., 1988; Pollard et al., 1993) and electrophysiological (Kirsten and Sharma, 1976; Phelan et al., 1990) methods. The postsynaptic histamine H_1 and H_2 receptors regulate histamine neurotransmission and neuromodulation whereas the presynaptic histamine H_3 receptors mediate the autoinhibition of histamine release. Selective histamine H_3 receptor ligands strongly modify the histamine turnover in rat brain slices (Arrang et al., 1983, 1987; Garbarg et al., 1989). Histamine H_3 receptor agonists like α-methylhista-

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mine inhibit histamine synthesis in and release from cerebral neurons whereas histamine H_3 receptor antagonists like thioperamide promote this synthesis and release.

Interestingly, histamine-immunoreactive fibers likely representing direct nerve terminals of the histaminergic tuberomammillary cells were found in the vestibular nuclei of mammals. A moderate density of histaminergic fibers was found in the medial nucleus of rats (Takeda et al., 1987; Panula et al., 1989; Steinbusch and Mulder, 1986) and guinea pigs (Airaksinen and Panula, 1988). We recently showed that histaminergic axonal fibers were sparsely distributed in the whole vestibular nuclei complex of the cat, with a significantly higher density in the superior and medial nuclei than in the inferior and lateral nuclei (Tighilet and Lacour, 1996). All the types of histamine receptors were also found in the vestibular nuclei complex through ligand-binding methods (Bouthenet et al., 1988), Northern blot analyses with hybridization probes (Ruat et al., 1991), and selective perfusion of the vestibular nuclei with histamine H_3 receptor agonists and antagonists (De Waele et al., 1992; Yabe et al., 1993). In addition, electrophysiological investigations in vivo pointed to both inhibitory and excitatory responses of cat vestibular nuclei neurones to histamine (Kirsten and Sharma, 1976; Satayavidad and Kirsten, 1977) whereas experiments in vitro in rats (Phelan et al., 1990) and guinea pigs (Serafin et al., 1993) showed only histamine-induced depolarization of the medial vestibular nucleus cells. The excitatory effects of histamine would be mediated by histamine H_1 (Inverarity et al., 1993) and/or H_2 receptors (Serafin et al., 1993; Wang and Dutia, 1995). Finally, histamine receptor ligands modulated static as well as dynamic vestibular functions in vivo (Yabe et al., 1993). These authors reported that after local perfusion on one side of the vestibular nuclei with histamine H_2 receptor antagonists or H_3 receptor agonists, guinea pigs showed stereotyped postural and oculomotor syndromes that mimicked the deficits after unilateral lesion of the peripheral vestibular system. Moreover, they found that histamine H_3 receptor antagonists like thioperamide decreased the guinea pigs' gain in vestibulo-ocular reflex without modifying their degree of alertness.

Vertigo caused by peripheral vestibular lesions in humans is currently treated by medications interfering with the histaminergic system (Rascol et al., 1995). Antihistamines constitute a first class of drugs, which is essentially composed of histamine H_1 receptor blockers. A second class is the histaminergic substances represented by betahistine, a close structural analogue of histamine. Betahistine acts both as an histamine H_3 receptor antagonist and a partial histamine H_1 receptor agonist (Arrang et al., 1985; Timmerman, 1991); its antivertiginous properties have been attributed to blood flow increase that would improve the microcirculation of the internal auditory and vestibular systems (Meyer et al., 1974; Halmagyi, 1992). Betahistine was also found effective in vestibular syndromes unrelated to vascular insufficiency, and several lines of evidence suggested that the histaminergic system influences the vestibular compensation process. Indeed, we demonstrated that compensation of posture and locomotor balance deficits was consistently accelerated in unilateral vestibular neurectomized cats submitted to postoperative betahistine treatment (Tighilet and Lacour, 1995). Betahistine-induced improvement of vestibular compensation was also reported for Ménière's patients (Frew and Menon, 1976; Canty et al., 1981; Bertrand, 1982; Oosterveld et al., 1989; Aantaa, 1991).

The aim of this study was first to analyze the histamine immunoreactivity changes within the vestibular nuclei complex and the tuberomammillary nucleus after unilateral vestibular neurectomy in the cat. We compared the histamine staining in control and lesioned cats through immunohistochemical methods and quantification techniques with an image analyzing computerized system described in our previous study on the normal cat (Tighilet and Lacour, 1996). Immunolabeling of nerve terminals was investigated in the ipsilateral and contralateral vestibular and tuberomammillary nuclei at three survival periods (1 week, 3 weeks, and 1 year). In a second step we investigated the influence of histaminergic substances on histamine immunoreactivity in order to specify the nature of the vestibular lesion-induced changes of histamine staining. The effects of thioperamide, a pure histamine H_3 receptor antagonist that increases histamine turnover, and of betahistine, a weak histamine H_1 receptor agonist and histamine H_3 receptor antagonist used as antivertigo drug, were tested in separate subgroups of control and vestibular lesioned cats.

2. Materials and methods

Experiments were performed on 28 normal pigmented domestic cats (3–4 kg) obtained from the Centre d'Elevage du Contigné, one of the French approved sources. Principles of laboratory animal care and procedures followed the Ministère de l'Agriculture guidelines. Cats were housed under a constant 12 h light-dark cycle.

2.1. Experimental protocol

Histamine immunoreactivity was investigated in the vestibular and tuberomammillary nuclei of 8 intact (control group) and 20 unilateral vestibular neurectomized cats (experimental group). Vestibular lesion-induced changes of histamine immunoreactivity were analyzed at 3 survival time periods: 1 week ($n = 8$), 3 weeks ($n = 6$) and 1 year ($n = 6$) corresponding to the acute (1 week) and compensatory stages (3 weeks, 1 year) of vestibular compensation. The effect of betahistine treatment on histamine immunoreactivity was studied by comparing untreated and treated cats. The same number of animals was used at each of the survival periods (3 treated and 3 untreated cats). The

treated cats received oral administration of betahistine dihydrochloride (50 mg/kg per day) during the first postoperative month. Betahistine treatment was similar to that used in commercial preparation (Serc) and furnished by Solvay Pharma (France); it was composed of methylamino-2-ethyl-pyridine in oral solution (50 mg/ml). Among the 8 control cats, 4 did not receive any pharmacological treatment and 2 were treated with betahistine (50 mg/kg per day) during 1 week. The 2 remaining control cats received intraperitoneal injection (3.5 mg/kg per day) of a pure histamine H_3 receptor antagonist (thioperamide) during 1 week. Thioperamide was also tested on 2 vestibular lesioned cats that received the same daily dose from the time of their lesion to the end of the first postoperative week, when they were killed. We used betahistine and thioperamide in both control and lesioned cats to determine if the unilateral vestibular neurectomy-induced changes of histamine immunoreactivity could be attributed

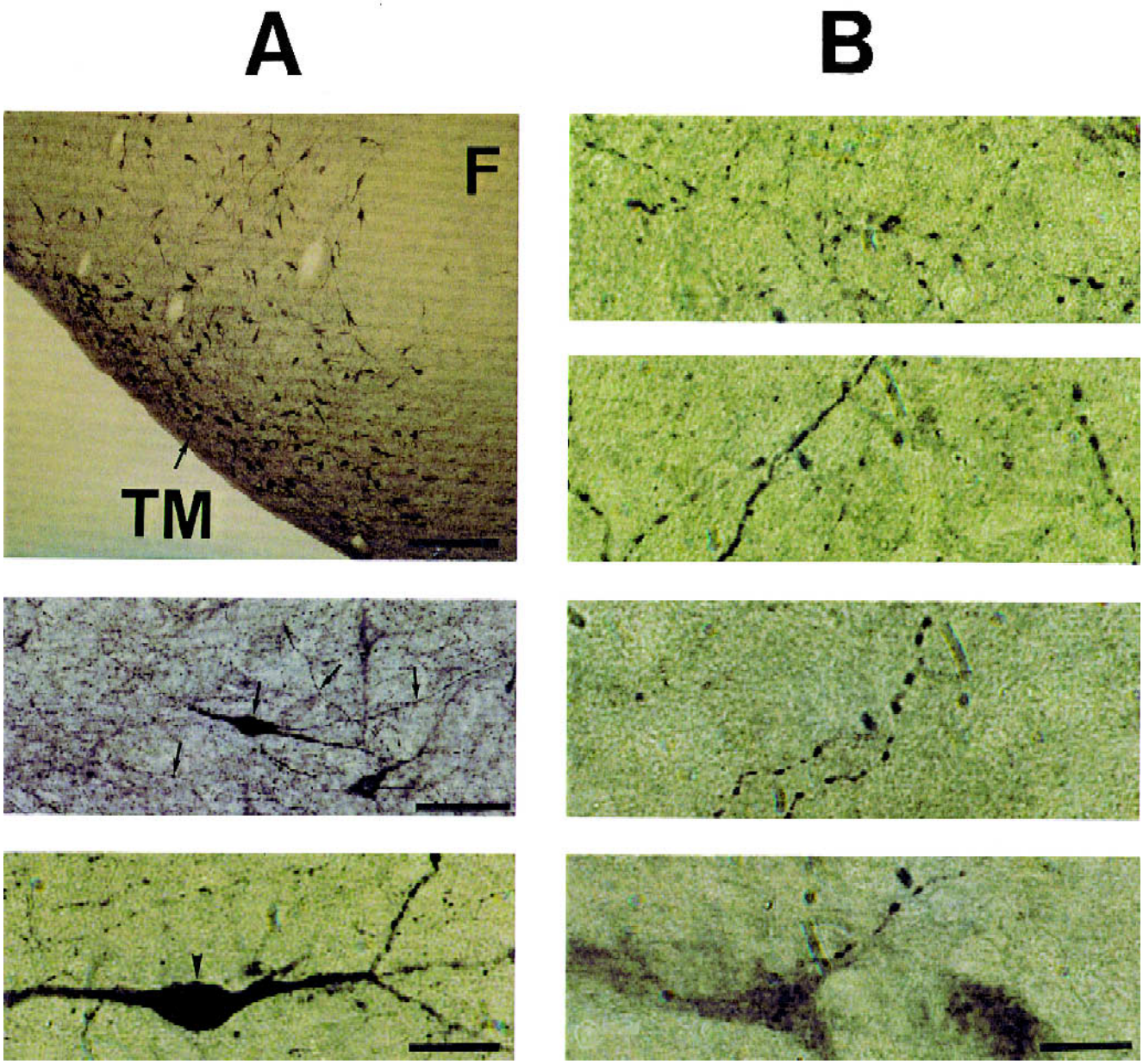


Fig. 1. Histamine immunoreactivity in the control cat. Photomicrograph montages of representative 20 μm frontal brain sections in the posterior hypothalamus region (A) and the vestibular nuclei (B) of one control (untreated and unlesioned) cat. (A) Photomicrographs illustrating the location of labeled histamine immunoreactive neurons and varicosities in the tuberomammillary nucleus (TM). Upper panel: low power magnification (bar = 300 μm) through the rostral part of the TM showing darkly stained neurons (F, fornix); middle panel (bar = 75 μm) and lower panel (bar = 45 μm): higher power magnifications showing the presence of numerous varicosities and the typical morphology of a histamine-containing neuron, respectively. (B) From top to bottom: photomicrographs at high power magnification of sections immunostained for histamine in the superior, medial, inferior and lateral vestibular nuclei, showing histamine-containing axonal fibers in the whole vestibular nuclei complex (bar = 30 μm). Modified from Tighilet and Lacour, 1996.

to an increase or decrease in histamine release and to specify the mode of action of betahistine on the histaminergic system and on the recovery process after unilateral vestibular neurectomy.

2.2. Vestibular neurectomy

Unilateral vestibular neurectomy was carried out under visual control through a dissecting microscope by sectioning the vestibular branches of the 8th cranial nerve after mastoidectomy, partial destruction of the bony labyrinth, and surgical exposure of the internal auditory canal (see Xerri and Lacour, 1981, for more details). Vestibular neurectomy was performed on the left side by sectioning the vestibular nerve medially with respect to Scarpa's ganglion, that is, at a postganglion level, with the cats under fluothane anaesthesia (2%) and in aseptic conditions. Animals were maintained postoperatively under antibiotics (7 days) and pain relievers (1–2 days). The classical posturo-locomotor and oculomotor syndrome seen just after surgery was used to assess the effectiveness of the lesion. Histological controls were done in some animals only because this surgery has been routinely done in the laboratory for many years.

2.3. Tissue preparation and immunohistochemical staining

Tissue preparation and immunohistochemical techniques for histamine staining of the vestibular and tuberomammillary nuclei were carried out according to previously described methods (Panula et al., 1988; Lin et al., 1993; Tighilet and Lacour, 1996). Cats were deeply anaesthetized and perfused through the ascending aorta with 1 l of normal saline solution (0.9%) containing 0.1% heparin, and then with 2 l of ice-cold 0.1 M phosphate buffer (PB, pH 7.4) containing 4% 1-ethyl-3 (3-diaminomethylpropyl) carbodiimide (E-7750; Sigma, St. Louis, MO, USA), 4% paraformaldehyde, and 0.2% picric acid. The brain was cut into several blocks post-fixed overnight at 4°C in the same solution, and then rinsed and cryoprotected in 0.1 M PB containing 10–30% sucrose for 72 h at 4°C. Blocks were rapidly frozen in CO_2 gas and frontally sectioned (20 μm) on a cryostat (Leitz).

Free-floating sections were first incubated for 5 days at 4°C with a rabbit polyclonal antibody against histamine (Delichon Biotechnology, Finland, dilutions 1:5000–10 000) in PB saline containing 0.3% Triton X-100 (PBS-T) and 0.1% sodium azide. Incubations (in PBS-T, at 4°C) in biotinylated goat anti-rabbit IgG (Jackson Immunoresearch Labs, 1:2000) and thereafter in streptavidin-conjugated horseradish peroxidase (Jackson Immunoresearch Labs, 1:40 000) were done after rinsing (2 × 15 min in PBS-T). Brain sections were then immersed in 0.02% 3,3′-diaminobenzidine 4-HCl (DAB) solution containing 0.01% H_2O_2 and 0.6% nickel ammonium sulphate in 0.05 M Tris-HCl buffer (pH 7.6) for 10–12 min at room temperature. This DAB-nickel procedure produced a blue-black staining of the somata, nerve terminals and varicosities containing histamine. Brain sections were prepared for examination by light microscopy. Production, characterization and specificity of the histamine antiserum used in this study (HA 21 C) have been described in our previous work on the distribution of histaminergic axonal fibers in the vestibular nuclei of the intact cat (Tighilet and Lacour, 1996).

2.4. Data quantification

The vestibular and tuberomammillary nuclei were identified through Berman's stereotaxic atlas (Berman, 1968; Berman and Jones, 1982). The histamine immunoreactivity in these structures was quantitatively analyzed by computer-assisted image analysis with a Leitz Aristoplan light microscope equipped with a Lhesa high resolution digital camera (756 × 581 pixels) interfaced to a Power Macintosh 7100 through Chromax card Image software (Biolab, v. 1.55; P. Rage, CNRS, and D. Hagège, Visiosoft) for capture and process of the images. Labeled fibers and varicosities were easily identified through a pixel value thresholding operation which was adjusted for each section, depending on the noise level. As a rule, the histamine antiserum provided a specific dark labeling of the somata, nerve terminals and varicosities with a similar low noise level in all the sections, which provided reproducible measurements. Reproducibility was also assessed by comparing two sets of data collected independently and by looking for interindividual differences in the staining of

Table 1
Statistical analysis of the effects of histaminergic treatments and unilateral vestibular lesion on histamine immunoreactivity in the cat vestibular and tuberomammillary nuclei

Source of variation	df	*F*	*P*
Group (controls/UVN cats)	1	31.5	0.0001 [a]
Treatment (T/NT)	1	502.3	0.0001 [a]
Group × Treatment	1	259.3	0.0001 [a]
Side	1	3.7	0.052 NS
Group × Side	1	3.8	0.051 NS
Treatment × Side	1	1.1	0.28 NS
Group × Treatment × Side	1	1.08	0.29 NS

Repeated-measure analysis of variance on histaminergic surface values (percentage of the total tissue surface after excluding blood vessels). Group (controls: $n = 8$ versus unilateral vestibular neurectomized cats: $n = 20$) and treatment (treated cats: $n = 15$ versus untreated cats: $n = 13$) are the main fixed effects providing the sources of variation among cats, as also illustrated by the significant interaction between these 2 variables. Side of the lesion (ipsilateral versus controlateral) and its interaction with group and treatment are not significant sources of variation. Treatments involving betahistine and thioperamide induce significant differences neither in the control group of cats ($n = 4:2$ betahistine- and 2 thioperamide-treated cats) nor in the unilateral vestibular neurectomized cats tested 1 week after surgery ($n = 5$: 3 betahistine- and 2 thioperamide-treated cats).

[a] Significant differences between variables or their interaction.

control or experimental cats. In addition, quantification was always serially performed on groups of 4 cats including 2 control (1 treated and 1 untreated) and 2 vestibular lesioned (1 treated and 1 untreated) cats in order to reduce possible staining differences due to the tissue preparation and immunohistochemical procedures that had to be repeated in the different subgroups of cats. The surface of the histamine immunoreactive structures was evaluated by subdividing each nucleus into 4–6 adjacent tissue areas (subsampling). The analogue pictures and the corresponding digitized images were displayed simultaneously on two separate Apple monitors for direct comparison. To compensate for uneven illumination of the field, we captured and subtracted digitally from each processed image the image of a blank slide on the microscope. The surface was automatically computed and expressed as a percentage of the total surface of the tissue being analyzed, after excluding stained neurones (tuberomammillary nucleus) and blood vessels (tuberomammillary and vestibular nuclei).

2.5. Statistical analysis

The surface of histamine immunoreactive varicosities was measured in each of the four main vestibular nuclei (medial, superior, inferior and lateral) and in the tuberomammillary nucleus on both sides (left/right for the controls and ipsilateral/contralateral for the lesioned cats) from 40 serial sections per cat. Statistical analysis was

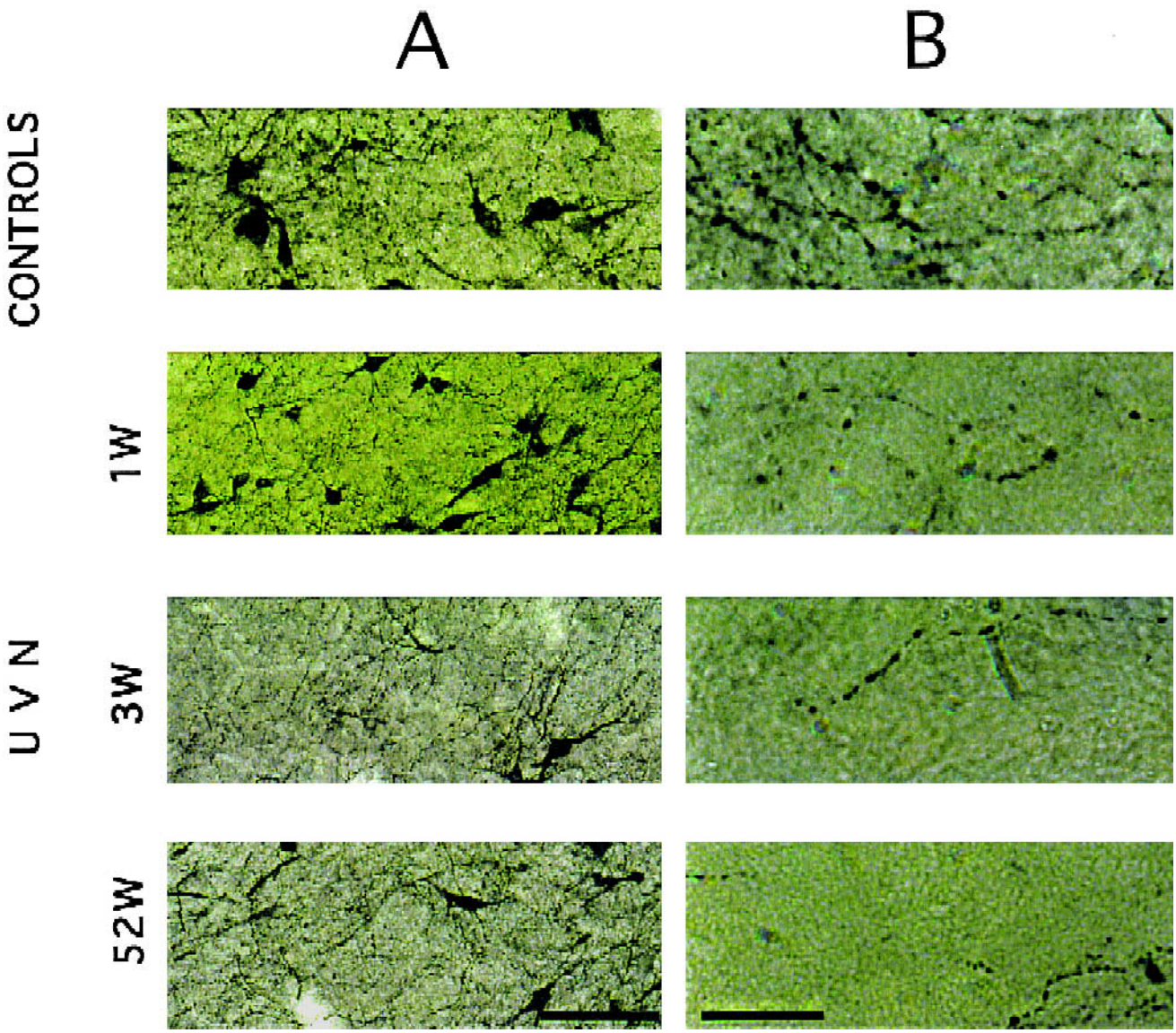


Fig. 2. Histamine immunoreactivity in the cat hypothalamus and vestibular nuclei after unilateral vestibular neurectomy. Photomicrograph montages of representative 20 μm brain sections in the ipsilateral tuberomammillary area (A) and medial vestibular nucleus (B) after unilateral vestibular neurectomy in cats. Each group of panels in A (bar = 75 μm) and B (bar = 30 μm) shows, from top to bottom, the typical staining in these structures in 1 control cat before lesion (controls) and the changes of histamine immunoreactivity in 3 lesioned cats (UVN) killed either 1 week (1 W), 3 weeks (3 W) or 1 year (52 W) after unilateral vestibular neurectomy.

therefore performed on a similar total number of samples for each cat (160–240 values per side) and for each main subgroup of cats (640–960 values per side for the treated and untreated controls and for the vestibular lesioned animals). Statistical evaluation of the results was done by analysis of variance (super-ANOVA) to test interindividual differences as well as side (left vs. right, ipsilateral vs. contralateral), structure (vestibular vs. tuberomammillary nuclei), vestibular lesion (controls vs. lesioned cats), post-operative time (1 week, 3 weeks vs. 1 year) and treatment (treated vs. untreated) effects on histamine staining, and to determine the interactions between these variables. Super-ANOVA was followed by post-hoc analysis with the Scheffe test and the multicomparison Fisher's test (Statview II software). Photomicrographs were made with a Wild Leitz MPS 52 camera and Kodak film.

3. Results

As already described in our previous paper (Tighilet and Lacour, 1996), light microscopy observations in the control cats showed a high histamine staining density in the tuberomammillary nucleus, with darkly labeled neurons and fibers, and a lower histamine staining in the vestibular nuclei, depending on the nuclei themselves and on their rostro-caudal location in the brain stem. As a rule, we observed weak staining in the inferior and lateral nuclei and moderate histamine staining in the medial and superior nuclei. No labeled neurons or non-neuronal cells (mast cells) were recorded in the vestibular nuclei. Histamine immunoreactivity was homogeneously distributed in these structures on both sides, and no significant interindividual differences were found. Photomicrographs of representative sections from the same control cat show the histamine staining for the tuberomammillary nucleus (Fig. 1A) and for each of the four main vestibular nuclei (Fig. 1B).

Strong histamine immunoreactivity changes were recorded after unilateral vestibular lesion as well as after treatment with betahistine or thioperamide. Repeated-measure analysis of variance of the histamine immunoreactive surface measurements demonstrated that group (controls vs. vestibular lesioned cats) and treatment (treated vs. untreated cats) constituted the main fixed effects providing the sources of variation among animals (Table 1). This was corroborated by the significant interaction between these two variables ($P < 0.001$). In contrast, significant differences were found neither for the side (ipsilateral vs. contralateral) nor for its interaction with group and treatment in the lesioned cats.

3.1. Unilateral vestibular lesion-induced changes of histamine immunoreactivity

Histamine immunoreactivity changes characterized by a bilateral reduction of the histamine staining were found in both the vestibular and tuberomammillary nuclei on both sides. This reduction was observed as early as 1 week after surgery and lasted up to the 1-year survival period. Photomicrographs of representative sections from 3 lesioned cats killed either 1 week, 3 weeks or 1 year after the vestibular lesion illustrate this decrease of histamine immunoreactivity in the tuberomammillary nucleus (Fig. 2A) and in the medial vestibular nucleus (Fig. 2B) on the

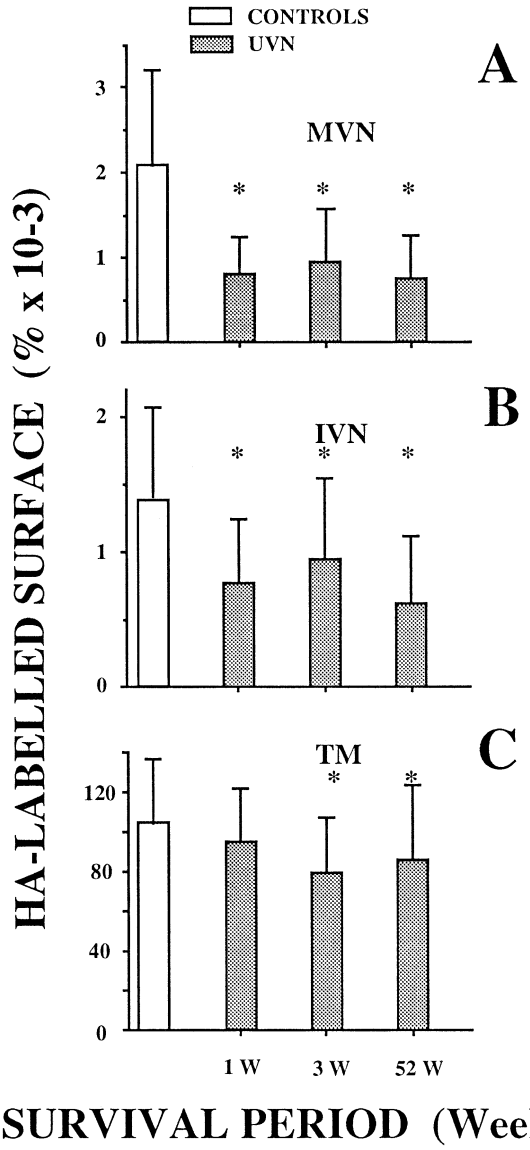


Fig. 3. Quantitative evaluation of the effects of unilateral vestibular neurectomy on histamine staining in the cat hypothalamus and vestibular nuclei. Histograms showing the mean results (±S.D.: vertical bars) in the lesioned cats (UVN: dotted histograms; $n = 9$) at the 3 survival periods (abscissae, in weeks; 3 lesioned cats per postoperative period). Quantification of histamine immunoreactivity is plotted on the ordinates as the ratio in percent of the stained surface to the total tissue surface (blood vessels and labeled neurons excluded). Average data from both the ipsilateral and contralateral sides were pooled for the medial vestibular nuclei (A: MVN), the inferior vestibular nuclei (B: IVN) and the tuberomammillary nuclei (C: TM). * Significant differences ($P < 0.001$) with respect to the controls (open histograms; $n = 4$).

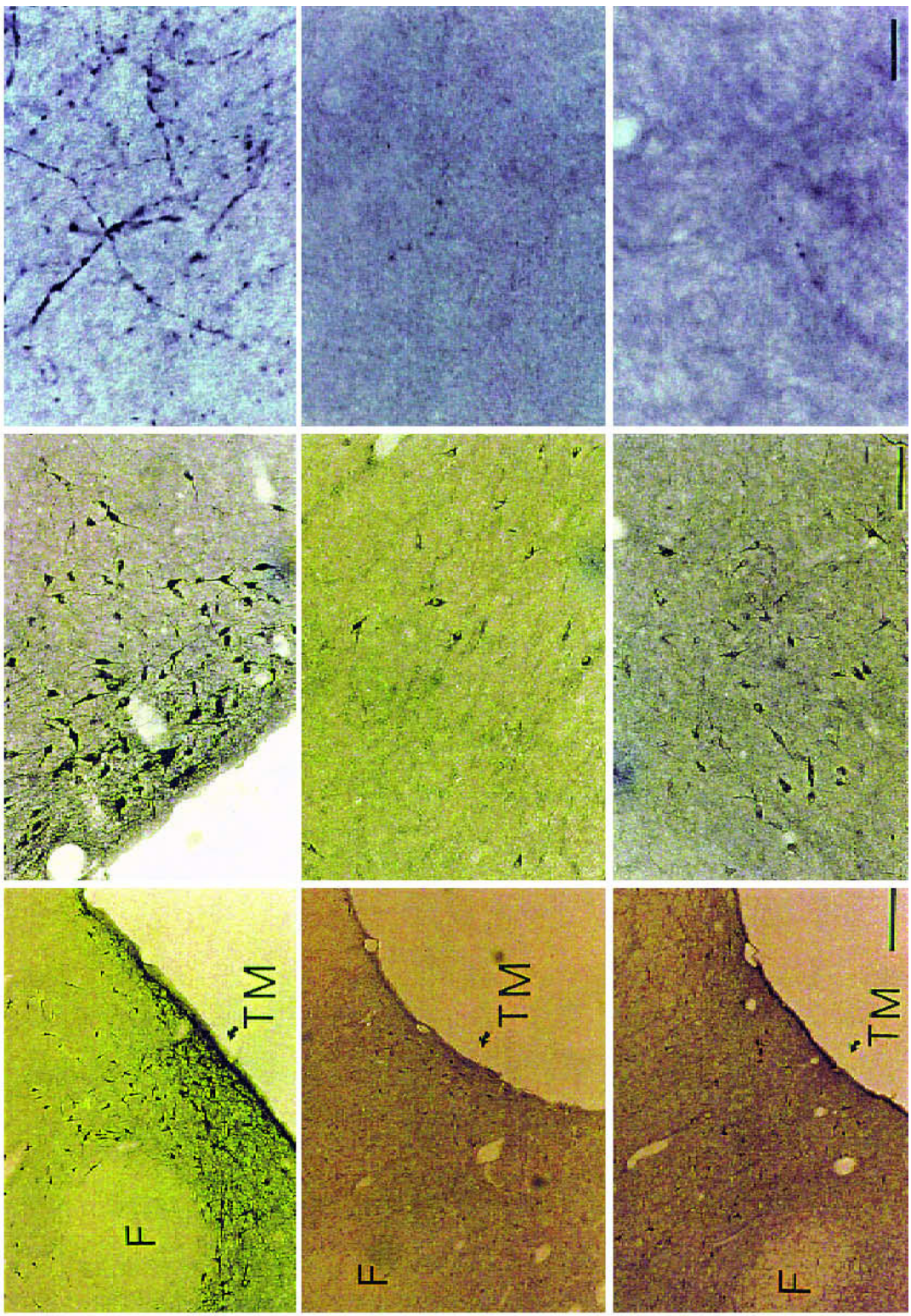


Fig. 4. Histamine immunoreactivity in the hypothalamus and vestibular nuclei of control cats after treatment with histaminergic substances. Photomicrograph montages of representative 20 μm frontal brain sections in the posterior hypothalamus region at low (A: bar = 300 μm) and higher (B: bar = 150 μm) power magnification and in the medial vestibular nucleus (C: bar = 30 μm). A–C, upper panels: data from 1 control, untreated cat showing histamine-containing neurons and varicosities (TM, tuberomammillary nucleus; F, fornix); middle panels: changes of histamine immunoreactivity after 1-week treatment with thioperamide (3.5 mg/kg per day) in a control cat; lower panels: changes of histamine immunoreactivity after 1-week treatment with betahistine (50 mg/kg per day) in another control cat. Note the lack of labeled varicosities and the smaller staining of the TM neurons after treatment with the 2 pharmacological substances.

deafferented side; typical staining recorded in these structures in a control cat is reported for direct comparison. Light microscopy observations showed a reduction in the number of varicosities and/or in the staining density of the individual particles. The total number of swellings on the labeled histaminergic fibers was particularly reduced in the vestibular nuclei. Quantitative evaluation of histamine immunoreactivity and the corresponding statistical analysis confirmed these qualitative findings. The histamine immunoreactive surface was significantly decreased in all the vestibular nuclei as early as 1 week after surgery ($P < 0.001$) and did not change significantly with survival time over the whole postoperative period. On average, the staining was reduced by 50% relative to the controls, with differences in the decrease in histamine staining depending on the vestibular nuclei themselves. There tended to be greater histamine immunoreactivity reduction on the intact rather than on the lesioned side in the vestibular nuclei; it however remained non-significant. Pooled data from both sides showed a mean maximal decrease of 61.7% for the medial vestibular nucleus (Fig. 3A) and a mean minimal reduction of 44.3% for the inferior vestibular nucleus (Fig. 3B) 1 week after surgery relative to the controls. Three weeks and 1 year after vestibular neurectomy, the mean histamine immunoreactive surface was still significantly reduced by 55.1% and 64% for the medial, and by 31.2% and 55.1% for the inferior vestibular nuclei, respectively. The histamine staining was less decreased in the tuberomammillary area (Fig. 3C), but significant differences compared to the controls were observed 3 weeks (24.2% reduction) and 1 year (18.4% reduction) after vestibular lesion.

3.2. Pharmacological treatment-induced changes of histamine immunoreactivity

The effects of betahistine treatment on histamine immunoreactivity were investigated in both vestibular lesioned and control cats. The former received oral administration of the drug during the first postoperative month (50 mg/kg per day) whereas the latter were treated during 1 week only, with the same dose. In addition, 2 controls and 2 lesioned cats killed 1 week postlesion were submitted to thioperamide treatment (3.5 mg/kg per day during 1 week) to compare the influence of a pure histamine H_3 receptor antagonist (thioperamide) to that of both a partial histamine H_1 receptor agonist and an histamine H_3 receptor antagonist (betahistine) on histamine immunoreactivity.

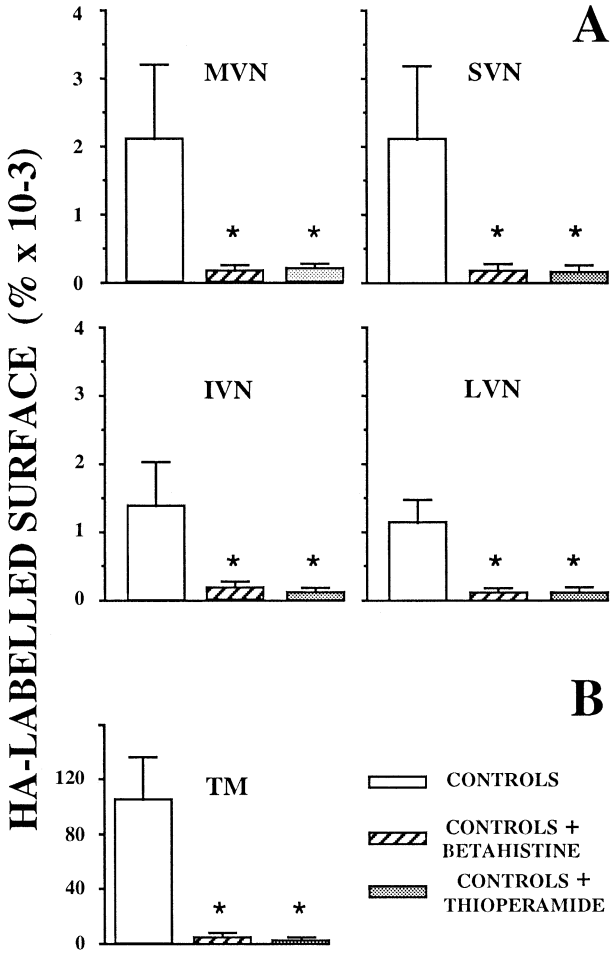
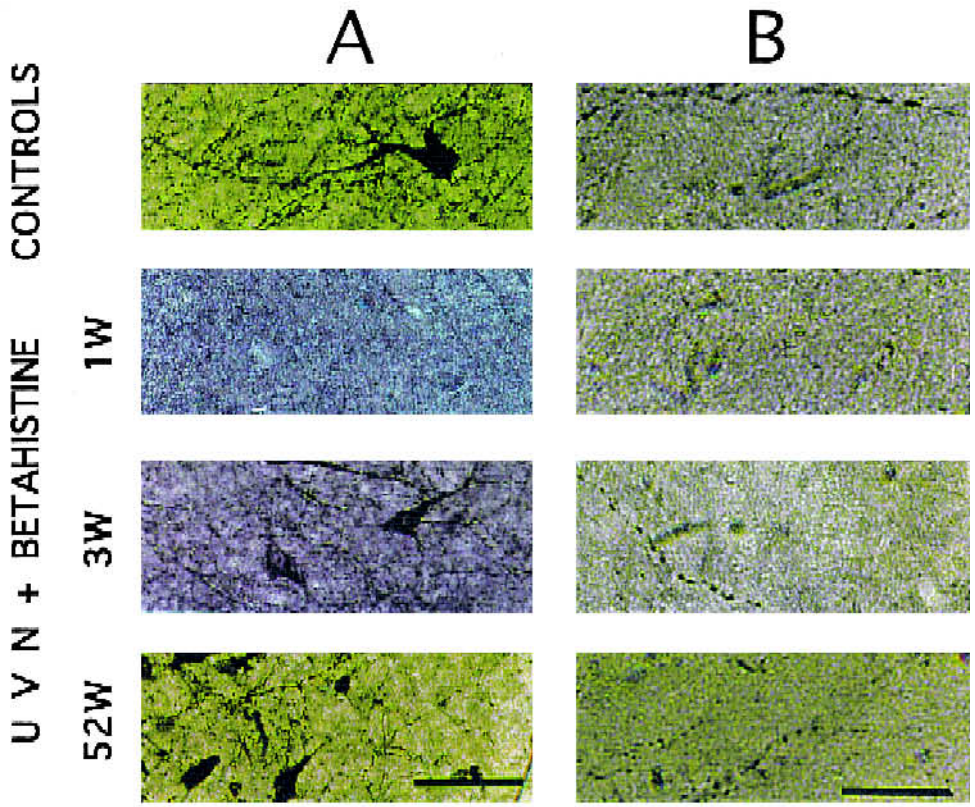
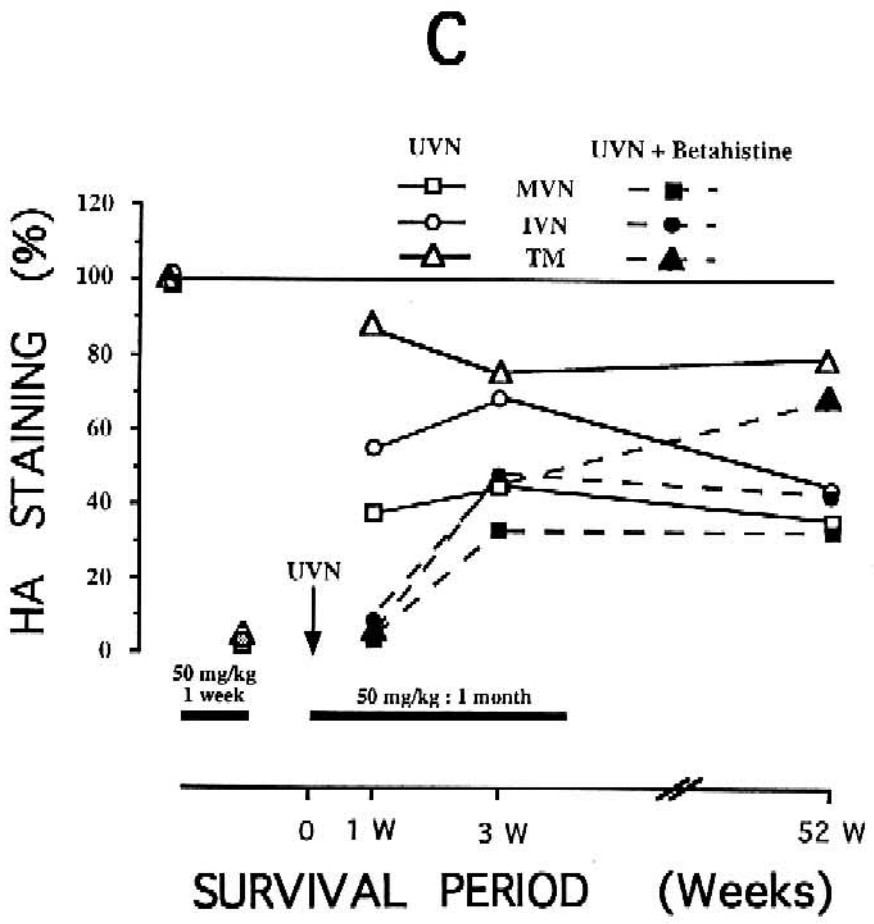


Fig. 5. Quantitative evaluation of the effects of treatment with histaminergic substances on histamine immunoreactivity in the posterior hypothalamus and vestibular nuclei of control cats. Histograms showing the mean results (±S.D.: vertical bars) in control cats ($n = 4$) submitted to 1-week betahistine (hatched histograms: $n = 2$) or thioperamide (dotted histograms: $n = 2$) treatment. The histamine-labeled surface is expressed on the ordinates in percent of the total tissue surface after blood vessels and stained neurons had been excluded. The effects of the treatments are shown in A for the medial (MVN), superior (SVN), inferior (IVN) and lateral (LVN) vestibular nuclei, and in B for the tuberomammillary (TM) nucleus. Data from both ipsilateral and contralateral sides are averaged. * Significant differences ($P < 0.001$) with respect to the controls (open histograms: $n = 4$).

Fig. 6. Effects of combined vestibular lesion and histaminergic treatment on histamine immunoreactivity in the cat posterior hypothalamus and vestibular nuclei. (A and B) Photomicrograph montages of 20 μm frontal brain sections in the ipsilateral tuberomammillary nucleus (A: bar = 75 μm) and medial vestibular nucleus (B: bar = 30 μm). From top to bottom: illustration of the histaminergic staining in 1 control cat (controls) and in 3 unilateral vestibular neurectomized (UVN) cats, submitted to 1-month postoperative betahistine treatment (50 mg/kg per day) and killed either 1 week (1 W), 3 weeks (3 W) or 1 year (52 W) after the vestibular lesion. C: Quantitative evaluation of the data. Changes of histamine immunoreactivity after betahistine treatment in control cats (50 mg/kg per day during 1 week: $n = 2$) and UVN animals (50 mg/kg per day during 1 month: $n = 9$) are expressed for the medial (MVN: squares) and inferior (IVN: circles) vestibular nuclei and for the tuberomammillary (TM: triangles) nucleus. Histamine staining is expressed on the ordinates in percent of the controls (untreated and unlesioned cats: level 100%; $n = 4$) as a function of the survival period in weeks (abscissae). Mean results from both the ipsilateral and contralateral sides in the untreated ($n = 9$: 3 cats per postoperative period) and treated ($n = 9$: 3 cats per postoperative time) UVN cats are shown as open and filled symbols, respectively. The effects of the betahistine treatment alone in control unlesioned cats are reported for comparison.


A
B
CONTROLS
U V N + BETAHISTINE
1W
3W
52W


C
UVN
UVN + Betahistine
MVN
IVN
TM
HA STAINING (%)
120
100
80
60
40
20
0
UVN
50 mg/kg
1 week
50 mg/kg : 1 month
0
1 W
3 W
52 W
SURVIVAL PERIOD (Weeks)

In the control cats, betahistine treatment led to a near-total lack of histamine immunoreactivity in all the vestibular nuclei as well as in the tuberomammillary nuclei. Similar findings were found under thioperamide treatment. Photomicrographs of representative sections from one betahistine-treated control cat and one thioperamide-treated control cat illustrate this strong histamine staining reduction in the tuberomammillary nucleus (Fig. 4A and B) and in the medial vestibular nucleus (Fig. 4C). Labeled cells in the tuberomammillary area of intact cats were darkly stained fusiform neurons with long stained dendrites. In contrast, the histamine-containing neurons exhibited very low staining density after betahistine or thioperamide treatment and the total number of stained neurons was significantly reduced. In controls, individual labeled axons showed very little branching beaded with smaller darkly stained swellings in the vestibular nuclei (0.8 μm on average) than in the tuberomammillary nucleus (1.6 μm on average). These histaminergic fibers and varicosities were nearly absent in the treated cats. Quantitative evaluation of the effects of betahistine and thioperamide on the histamine immunoreactive surface measurements is represented for each of the four main vestibular nuclei (Fig. 5A) and for the tuberomammillary nucleus (Fig. 5B). Both pharmacological substances produced a very strong reduction of histamine immunoreactivity in the vestibular nuclei, ranging from 87% to 92.4% with betahistine and from 90.4% to 92.5% with thioperamide, depending on the nuclei themselves (non-significant differences between the vestibular nuclei). A similar decrease in histamine immunoreactivity was found in the tuberomammillary area after thioperamide (98.9%) or betahistine (95.6%) treatment.

In the UVN cats, the 1-month treatment with betahistine induced modifications of the histamine immunoreactive surface in the vestibular and tuberomammillary nuclei that were a function of the postoperative time. The subgroup of cats killed 1 week after vestibular lesion showed histamine staining in both structures that was similar to that found in the treated control cats. On light microscopy brain sections exhibited the near-complete lack of staining reported above for the treated control cats, and the statistical evaluation of the data pointed to a similar percentage reduction of histamine immunoreactive surface with reference to the untreated control animals. Thioperamide treatment of 2 lesioned cats led to similar qualitative and quantitative findings 1 week after surgery. In contrast, histamine immunoreactive surface measurements for the cats killed 3 weeks after vestibular lesion were similar to those for the untreated lesioned cats, although these cats were still under betahistine treatment at this survival period. This observation indicates that histamine immunoreactive surface is significantly higher on both sides in this subpopulation of betahistine-treated lesioned cats compared to the subgroups of betahistine- or thioperamide-treated control cats, and that their histamine immunoreactivity remains significantly lower than that of the untreated controls. No significant differences were seen between the 2 subgroups of betahistine-treated and untreated lesioned cats 1 year after vestibular lesion. The percentage reduction of the histamine immunoreactive surface was similar in both subpopulations at this late stage, perhaps because the betahistine-treated lesioned cats tested at this survival period had not been treated for 11 months and thus likely constituted a subgroup close to the untreated lesioned subpopulation at the same survival period. These qualitative observations are illustrated for the tuberomammillary nucleus (Fig. 6A) and for the medial vestibular nucleus (Fig. 6B), and the quantitative measurements of histamine immunoreactivity are reported for the tuberomammillary, the medial and inferior vestibular nuclei (Fig. 6C).

4. Discussion

This study shows that histamine immunoreactivity in the cat brain was strongly reduced in both the tuberomammillary nucleus and in the 4 main vestibular nuclei on both sides after either unilateral vestibular neurectomy or pharmacological treatment with substances interfering with the histaminergic system (betahistine and thioperamide) compared to control (untreated and unlesioned) cats. The mean reduction of histamine staining was greater in the vestibular nuclei complex than in the tuberomammillary area in the untreated lesioned cats. This reduction was observed bilaterally and the decrease in histamine immunoreactivity remained roughly unchanged with time in the 3 subgroups of untreated lesioned cats examined 1 week, 3 weeks and 1 year after vestibular lesion. The reduction of the histamine immunoreactive surface was consistently accentuated for both the tuberomammillary area and the vestibular nuclei in the subgroups of lesioned cats that were submitted postoperatively to betahistine or thioperamide treatment for 1 week. In these subpopulations of treated lesioned cats, histamine staining was near-completely lacking and the mean histamine immunoreactive surface was similar to that found in the unlesioned control cats submitted to betahistine or thioperamide treatment for the same time. In the other 2 subpopulations of betahistine-treated lesioned cats examined 3 weeks and 1 year after vestibular lesion, histamine immunoreactivity was decreased in the same proportion as in the untreated lesioned animals at the same survival periods.

4.1. Effects of betahistine or thioperamide treatment on histamine immunoreactivity in the control cats

The material used was immunostained according to methods previously developed in the cat to label histamine immunoreactive cells, axons and terminal-like fibers (Panula et al., 1988; Lin et al., 1993). The procedure combined a highly sensitive polyclonal antibody for histamine with

biotinylated IgG and streptavidin antibodies, and the peroxidase reaction was intensified by nickel ammonium sulphate (Tighilet and Lacour, 1996). Under these experimental conditions, specific labeling of histamine-containing neurons (tuberomammillary nucleus) and fibers (tuberomammillary and vestibular nuclei) was observed. Histaminergic neurons and axonal fibers closely resembled those encountered in the tuberomammillary area and in other structures of the cat brain (Lin et al., 1986; Yoshimoto et al., 1989; Manning and Uhlrich, 1993).

In control cats submitted to 1-week treatment with betahistine or thioperamide, histamine-containing neurons in the tuberomammillary area bilaterally showed very low staining density compared to untreated control animals. In addition, a near-total lack of histamine-containing fibers and varicosities was observed in both the tuberomammillary and vestibular nuclei. These findings support the general statement that the tuberomammillary area is very likely the sole source of histaminergic neurons in the cat (Lin et al., 1986, 1993; Yoshimoto et al., 1989; Manning and Uhlrich, 1993) that project bilaterally into various brain structures, including the vestibular nuclei (Schwartz et al., 1991; Takeda et al., 1987; Tighilet and Lacour, 1996). Our results also indicate that betahistine as well as thioperamide induce histamine depletion from the synaptic vesicles in the nerve terminals. Since auto-inhibition of brain histamine release is mediated through the presynaptic histamine H_3 receptors (Arrang et al., 1983), blocking this receptor type should modify the histamine turnover. For rat brain slices, it was demonstrated that selective histamine H_3 receptor ligands led to such changes and that pure histamine H_3 receptor antagonists like thioperamide increased histamine synthesis in and release from cerebral neurons (Arrang et al., 1983, 1987; Garbarg et al., 1989). We therefore suppose that the decrease in histamine staining in the tuberomammillary area and in the vestibular nuclei after thioperamide treatment reflects an increased release of histamine from nerve terminals. This hypothesis can be applied to betahistine, an histamine-like substance which acts as both a weak histamine H_1 receptor agonist and an active histamine H_3 receptor antagonist (Arrang et al., 1985; Hill, 1990; Timmerman, 1991). The similar reduction of the histamine immunoreactive surface in the tuberomammillary and vestibular nuclei of control cats submitted to thioperamide or betahistine treatment is indicative of a strong effect of betahistine on the presynaptic histamine H_3 receptors.

4.2. Effects of unilateral vestibular lesion on histamine immunoreactivity

The histamine immunoreactive surface was significantly reduced (50% on average) in the vestibular nuclei for all the vestibular lesioned cats, whatever the survival period (1 week, 3 weeks or 1 year). The decrease in histamine immunoreactivity was smaller, and not significant, in the tuberomammillary nucleus at 1 week but it was significant during the compensatory stage, up to 1 year. These findings point to durable modifications of the histamine turnover that are not compensated with time in the lesioned cats. According to our hypothesis that histamine release from nerve terminals is increased after thioperamide or betahistine treatment, the decrease in histamine staining after vestibular lesion should also result from increased histamine release.

Interestingly, this was supported by a recent study in the rat through brain microdialysis coupled with liquid chromatography fluorometry (Horii et al., 1993). The authors demonstrated that electrical stimulation of the inner ear as well as unilateral activation or inhibition of the horizontal semi-circular canal by middle ear irrigation with 45°C or ice water, respectively, produced a 200% increase in histamine release from the anterior hypothalamic area on both sides. These results strongly suggest that the vestibular imbalance induced in the vestibular nuclei by the unilateral activation or inhibition of the peripheral labyrinth activates histaminergic neurons projecting onto these nuclei. Vestibular imbalance is also centrally generated in the vestibular nuclei after unilateral vestibular nerve section or hemilabyrinthectomy in animal models (see Lacour et al., 1989; Smith and Curthoys, 1989) as well as in acute unilateral vestibular defective patients exhibiting pathological vertigo (Brandt, 1991). Several indirect pathways linking the vestibular nuclei to the hypothalamus have been described: through the nucleus prepositus hypoglossi (Ericson et al., 1991), the reticular formation (Pompeiano, 1977) or the fastigial nucleus (Walberg et al., 1962; Harper and Heath, 1973). These vestibulo-hypothalamo-vestibular loops are likely activated during the acute stage (1 week) of vestibular compensation since asymmetrical resting discharges of the vestibular cells on both sides were reported in cats for the medial (Shimazu and Precht, 1976) and for the lateral (Zennou-Azogui et al., 1993) vestibular nuclei. Activation of these loops could explain the bilateral reduction of the histamine immunoreactive surface in the tuberomammillary and vestibular nuclei. Electrophysiological investigations during recovery in the same species still revealed asymmetrical spontaneous firing rates between the bilateral vestibular nuclei at the latest stages corresponding to the so-called behaviorally compensated stage, even though vestibular imbalance was smaller than that observed during the acute stage (Shimazu and Precht, 1976; Smith and Curthoys, 1989; Zennou-Azogui et al., 1993). This permanent central asymmetry for both static and dynamic vestibular responses could explain the permanent decrease in histamine staining we observed.

4.3. Effects of combined vestibular lesion and pharmacological treatment on histamine immunoreactivity

After 1 week, histamine staining in the vestibular lesioned cats was drastically lowered in the betahistine and

thioperamide subgroups compared to the untreated lesioned cats. The mean value of the histamine immunoreactive surface, close to zero (noise level), was similar to that found for the unlesioned treated cats, indicating a prominent effect of the pharmacological treatment on histamine immunoreactivity. In contrast, both treated and untreated lesioned cats exhibited a similar level of histamine staining (50% of the controls on average) after 3 weeks and 1 year. This could be expected for the compensated cats one year after surgery because the betahistine treatment had been stopped for a long time (11 months) in this subpopulation. Histamine staining changes must therefore be attributed to the vestibular lesion only, for both the treated and untreated lesioned subgroups of compensated cats. The absence of significant differences 3 weeks after unilateral vestibular neurectomy between the treated and untreated cats was surprising since the betahistine-treated subgroup of cats still received daily oral administration of the drug at this time. Assuming that a decrease in histamine immunoreactive surface reflects increased histamine synthesis and release from nerve terminals because of histamine H_3 receptor blockage, we suggest that long-term betahistine treatment would lead to reactive or compensatory up-regulation of this receptor subtype. This hypothesis is supported by in situ hybridization experiments showing that blockade of dopamine transmission during 2–3 weeks with haloperidol, a dopamine receptor antagonist, increased dopamine receptor mRNA in rat forebrain (Le Moine et al., 1990).

4.4. Conclusion

Taken together, these findings strongly suggest that histaminergic treatments (thioperamide, betahistine) and unilateral vestibular lesion induce histamine immunoreactivity changes in the vestibular and tuberomammillary nuclei in the cat, which very likely result from increased histamine turnover and release. For strengthening this conclusion, additional experiments based on histidine decarboxylase immunoreactivity changes are required. Our results also support the role of histamine in vestibular recovery that could be due to either aspecific action (vigilance level increase, sensorimotor activity improvement) or more specific action on the vestibular nuclei neurons and the different types of histamine receptors. Both actions could contribute to accelerate the vestibular compensation process.

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References

Aantaa, E., 1991. Treatment of acute vestibular vertigo. Acta Oto-Laryngol. 479, 44–47.

Airaksinen, M., Panula, P., 1988. The histaminergic system in the guinea pig central nervous system: an immunocytochemical mapping study using an antiserum against histamine. J. Comp. Neurol. 273, 163–186.

Arrang, J.M., Garbarg, M., Schwartz, J.C., 1983. Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. Nature 302, 832–837.

Arrang, J.M., Garbag, M., Quach, T.T., Dam Tuong, M., Yaramian, E., Schwartz, J.C., 1985. Actions of betahistine at histamine receptors in the brain. Eur. J. Pharmacol. 111, 73–84.

Arrang, J.M., Garbarg, M., Schwartz, J.C., 1987. Autoregulation of histamine synthesis in brain mediated by presynaptic H_3 receptors. Neuroscience 23, 149–157.

Berman, A.L., 1968. The Brain Stem of the Cats, a Cytoarchitectonic Atlas with Stereotaxic Coordinates. Wisconsin University Press, Madison, WI.

Berman, A.L., Jones, E.G., 1982. The Thalamus and Basal Telencephalon of the Cat. A Cytoarchitectonic Atlas with Stereotaxic Coordinates. Wisconsin University Press, Madison, WI.

Bertrand, R.A., 1982. Long-term evaluation of the treatment of Ménière's disease with betahistine HCl. Adv. Otorhinolaryngol. 28, 104–110.

Bouthenet, M.L., Ruat, M., Sales, N., Garbarg, M., Schwartz, J.C., 1988. A detailed mapping of histamine H_1 receptors in guinea-pig central nervous system established by autoradiography with (I^{125})idobolpyramine. Neuroscience 26, 553–600.

Brandt, T., 1991. Vertigo: Its Multisensory Syndromes. Springer-Verlag, London.

Canty, P., Valentine, J., Papworth, S.J., 1981. Betahistine in peripheral vertigo: a double blind placebo-controlled, cross-over study of Serc versus placebo. J. Laryngol. Otol. 95, 687–692.

De Waele, C., Serafin, M., Khateb, A., Vibert, N., Yabe, T., Arrang, J.M., Mülhethaler, M., Vidal, P.P., 1992. An in vivo and in vitro study of the vestibular nuclei histaminergic receptors in the guinea pig. Ann. NY Acad. Sci. 656, 550–565.

De Waele, C., Mühlethaler, M., Vidal, P.P., 1995. Neurochemistry of the central vestibular pathways. Brain Res. Rev. 20, 24–46.

Ericson, H., Blomqvist, A., Köhler, C., 1991. Origin of neuronal inputs to the region of the tuberomammillary nucleus of the rat brain. J. Comp. Neurol. 311, 45–64.

Frew, I.J.C., Menon, G.N., 1976. Betahistine dihydrochloride in Ménière's disease. Postgrad. Med. J. 52, 501–503.

Garbarg, M., Trung Tuong, M.D., Gros, C., Schwartz, J.C., 1989. Effects of histamine H_3 receptor ligands on various biochemical indices of histaminergic neuron activity in rat brain. Eur. J. Pharmacol. 164, 1–11.

Halmagyi, G.M., 1992. Vertigo and vestibular disorders. In: Eadie, J. (Ed.), Drug Therapy in Neurology. Churchill Livingstone, Edinburgh, pp. 383–394.

Harper, J.W., Heath, R.G., 1973. Anatomic connections of the fastigial nucleus to rostral forebrain in the cat. Exp. Neurol. 39, 285–292.

Hill, S.J., 1990. Distribution, properties, and functional characteristics of three classes of histamine receptor. Pharmacol. Rev. 42, 45–83.

Horii, A., Takeda, N., Matsunaga, T., Yamatodani, A., Mochizuki, T., Okakura-Machizuki, K., Wada, H., 1993. Effect of unilateral vestibular stimulation on histamine release from the hypothalamus of rats in vivo. J. Neurophysiol. 70, 1822–1826.

Inverarity, D.J., Johnston, A.R., McQueen, D.S., Dutia, M.B., 1993. Effects of histamine on rat medial vestibular nucleus neurones in vitro. J. Physiol. 459, 466P.

Kirsten, E.B., Sharma, J.N., 1976. Microiontophoresis of acetylcholine, histamine and their antagonists on neurons in the medial and lateral vestibular nuclei of the cat. Neuropharmacology 15, 743–753.

Lacour, M., Toupet, M., Denise, P., Christen, Y., 1989. Vestibular

Compensation: Facts, Theories and Clinical Perspectives. Elsevier, Paris.

Le Moine, C., Normand, E., Guitteny, A.F., Fouque, B., Teoule, R., Block, B., 1990. Dopamine receptor gene expression by enkephalin neurons in rat forebrain. Proc. Natl. Acad. Sci. USA 87, 230–234.

Lin, J.S., Sakai, K., Jouvet, M., 1986. Role of hypothalamic histaminergic systems in the regulation of vigilance states in cats. C.R. Acad. Sci. 303, 469–474.

Lin, J.S., Sakai, K., Vanni-Mercier, G., Arrang, J.M., Gasberg, M., Schwartz, J.C., Jouvet, M., 1990. Involvement of histaminergic neurons in arousal mechanisms demonstrated with H_3 receptor ligands in the cat. Brain Res. 523, 325–330.

Lin, J.S., Kitahama, K., Fort, P., Panula, P., Denney, R.M., Jouvet, M., 1993. Histaminergic system in the cat hypothalamus with reference to type B monoamine oxydase. J. Comp. Neurol. 330, 405–420.

Manning, K.A., Uhlrich, D.J., 1993. The distribution of histaminergic axons in the superior colliculus of the cat. Neuroscience 55, 1075–1084.

Meyer, J.S., Mathew, N.T., Hartmann, A., Rivera, V.M., 1974. Orally administrated betahistine and regional cerebral blood flow in cerebrovascular disease. J. Clin. Pharmacol. 14, 280–285.

Oosterveld, W.J., Blijleven, W., Van Elferen, L.W.M., 1989. Betahistine versus placebo in paroxysmal vertigo: a double-blind trial. J.D.R. 14, 4.

Panula, P., Häppölâ, O., Airskinen, M.S., Auvinen, S., Virkamäki, A., 1988. Carbodiimide as a tissue fixative in histamine immunohistochemistry and its application in developmental neurobiology. J. Histochem. Cytochem. 36, 259–269.

Panula, P., Pirvola, U., Auvinen, S., Atraksinen, M.S., 1989. Histamine-immunoreactive fibers in the rat brain. Neuroscience 22, 585–610.

Phelan, K.D., Nakamura, J., Gallagher, J.P., 1990. Histamine depolarizes rat medial vestibular nucleus neurons recorded intracellularly in vitro. Neurosci. Lett. 109, 287–292.

Pollard, H., Schwartz, J.C., 1987. Histamine neuronal pathways and their function. Trends Neurosci. 10, 86–89.

Pollard, H., Moreau, J., Arrang, J.M., Schwartz, J.C., 1993. A detailed autoradiographic mapping of histamine H_3 receptors in rat brain areas. Neuroscience 52, 169–189.

Pompeiano, O., 1977. Macular influences of somatosensory transmission through the spino-cerebellar pathway. J. Physiol. (London) 73, 387–400.

Prell, G.D., Green, J.P., 1986. Histamine as a neuroregulator. Annu. Rev. Neurosci. 9, 209–254.

Rascol, O., Hain, T.C., Brefel, C., Benazet, M., Clanet, M., Montastruc, J.L., 1995. Antivertigo medications and drug-induced vertigo: a pharmacological review. Drugs 5, 777–791.

Ruat, M., Traiffort, E., Arrang, J.M., Leurs, R., Schwartz, J.C., 1991. Cloning and tissue expression of a rat histamine H_2 receptor gene. Biochem. Biophys. Res. Commun. 179, 1470–1478.

Satayavidad, J., Kirsten, E.B., 1977. Iontophoretic studies of histamine and histamine antagonists in the feline vestibular nuclei. Eur. J. Pharmacol. 41, 17–26.

Schwartz, J.C., Pollard, H., Quach, T.T., 1980. Histamine as a neurotransmitter in mammalian brain: neurochemical evidence. J. Neurochem. 35, 26–33.

Schwartz, J.C., Arrang, J.M., Garbarg, M., Pollard, H., Ruat, M., 1991. Histaminergic transmission in the mammalian brain. Physiol. Rev. 71, 1–51.

Serafin, M., Khateb, A., Vibert, N., Vidal, P.P., Mühlethaler, M., 1993. Medial vestibular nucleus in the guinea-pig: histaminergic receptors I. An in vitro study. Exp. Brain Res. 93, 242–248.

Shimazu, H., Precht, W., 1976. Inhibition of central vestibular neurons from the contralateral labyrinth and its mediating pathway. J. Neurophysiol. 29, 467–492.

Smith, P.F., Curthoys, I.S., 1989. Mechanisms of recovery following unilateral labyrinthectomy: a review. Brain Res. Rev. 14, 155–180.

Smith, P.F., Darlington, C.L., 1991. Neurochemical mechanisms of recovery from peripheral vestibular lesions (vestibular compensation). Brain Res. Rev. 16, 117–133.

Steinbusch, H.W.M., Mulder, A.H., 1986. Localization and projections of histamine immunoreactive neurons in the central nervous system of the rat. In: Gannelin, C.R., Schwartz, J.C. (Eds.), Frontiers in Histamine Research. Plenum Press, Oxford, pp. 119–130.

Takeda, N., Morita, M., Kubo, T., Yamatodani, A., Watanabe, T., Tohyama, M., Wada Matsunaga, T., 1987. Histaminergic projection from the posterior hypothalamus to the medial vestibular nucleus of rats and its relation to motion sickness. In: Graham, M.D., Kemink, J.L. (Eds.), The Vestibular System: Neurophysiologic and Clinical Research. Raven Press, New York, NY, pp. 601–617.

Tighilet, B., Lacour, M., 1995. Betahistine dihydrochloride treatment facilitates vestibular compensation in the cat. J. Vestibular Res. 5, 53–66.

Tighilet, B., Lacour, M., 1996. Distribution of histamine axonal fibres in the vestibular nuclei of the cat. NeuroReport 7, 873–878.

Timmerman, H., 1991. Histamine agonists and antagonists. Acta Oto-Laryngol. 479, 5–11.

Walberg, F., Pompeiano, O., Westrum, L.E., Hauglie-Aanssen, E., 1962. Fastigioreticular fibers in the cat. An experimental study with silver methods. J. Comp. Neurol. 119, 187–199.

Wang, J.J., Dutia, M.B., 1995. Effects of histamine and betahistine on rat medial vestibular nucleus neurons: possible mechanism of action of anti-histaminergic drugs in vertigo and motion sickness. Exp. Brain Res. 105, 18–24.

Xerri, C., Lacour, M., 1981. Compensation des déficits posturaux et cinétiques après neurectomie vestibulaire unilatérale chez le chat. Rôle de l'activité sensorimotrice. Acta Oto-Laryngol. 90, 414–420.

Yabe, T., De Waele, C., Serafin, M., Vibert, N., Arrang, J.M., Mühlethaler, M., Vidal, P.P., 1993. Medial vestibular nucleus in the guinea-pig: histaminergic receptors II. An in vivo study. Exp. Brain Res. 93, 249–258.

Yoshimoto, Y., Sakai, K., Panula, P., Salvert, D., Jouvet, M., 1989. Cells of origin of histaminergic afferents to the cat median eminence. Brain Res. 504, 149–153.

Zennou-Azogui, Y., Borel, L., Lacour, M., Ez-Zaher, L., Ouaknine, M., 1993. Recovery of head postural control following unilateral vestibular neurectomy in the cat. Neck muscle activity and neuronal correlates in Deiters' nuclei. Acta Oto-Laryngol. 509, 1–19.