Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 523 (2005) 54–63
www.elsevier.com/locate/ejphar

Dose- and duration-dependent effects of betahistine dihydrochloride treatment on histamine turnover in the cat

Brahim Tighilet [a,*], Suzanne Trottier [b], Michel Lacour [a]

[a] UMR 6149 Université de Provence/CNRS "Neurobiologie Intégrative et Adaptative", Pôle 3C « Comportement, Cerveau, Cognition », Centre de St Charles, Case B, 3 Place Victor Hugo, 13331 Marseille Cedex 3, France
[b] INSERM U 573, Centre Paul Broca, 2 ter Rue d'Alésia, 75014 Paris, France

Received 15 July 2005; accepted 8 September 2005
Available online 14 October 2005

Abstract

Drugs interacting with the histaminergic system are currently used for vertigo treatment and it was shown in animal models that structural analogues of histamine like betahistine improved the recovery process after vestibular lesion. This study was aimed at determining the possible dose and duration effects of betahistine treatment on histamine turnover in normal adult cats, as judged by the level of messenger RNA for histidine decarboxylase (enzyme synthesizing histamine) in the tuberomammillary nuclei. Experiments were conducted on betahistine-treated cats receiving daily doses of 2, 5, 10, or 50 mg/kg during 1 week, 3 weeks, 2 months, or 3 months. The 1-week, 3-week, and 2- and 3-month treatments correspond to the acute, compensatory, and sustained compensatory stages of vestibular compensation, respectively. The lowest dose (2 mg/kg) given the longest time (3 months) was close to the dosage for vestibular defective patients. Data from the experimental groups were compared to control, untreated cats and to placebo-treated animals.

The results clearly show that betahistine dihydrochloride administered orally in the normal cat interferes with histamine turnover by increasing the basal expression level of histidine decarboxylase mRNA of neurons located in the tuberomammillary nuclei of the posterior hypothalamus. The effects were both dose- and time-dependent.

In conclusion, compensation of both static and dynamic deficits is subtended by long-term adaptive mechanisms that could be facilitated pharmacologically using betahistine dihydrochloride.

Keywords: Histidine decarboxylase; In situ hybridization; Betahistine dihydrochloride; Tuberomammillary nuclei; (Cat)

1. Introduction

Vestibular defective patients with invalidating vertigo can be successfully treated pharmacologically. Betahistine dihydrochloride is one of the drugs currently prescribed for the symptomatic treatment of vertigo, especially in Menière's patients (Rascol et al., 1995). This substance, a structural analogue of histamine, is a weak histamine H_1 receptor agonist and a more potent histamine H_3 receptor antagonist (Arrang et al., 1985; Timmerman, 1991). The efficacy of histamine results from vascular effects improving cochlear blood flow (Meyer et al., 1974; Laurikainen et al., 1993, 1998), but histamine acts also on central neural networks involved in the recovery process after vestibular loss (see Lacour and Sterkers, 2001, for a review).

Behavioral recovery after unilateral vestibular neurectomy in the cat is strongly accelerated under betahistine treatment (Tighilet et al., 1995). Immunohistochemical investigations with the same cat model suggested that the improvement in vestibular compensation is related to changes in histamine synthesis and release (Tighilet and Lacour, 1997). Histamine-containing perikarya are exclusively located in the tuberomammillary nuclei of the posterior hypothalamus (Pollard and Schwartz, 1987), and these histaminergic neurons send axonal projections to the whole vestibular nuclei complex in many species (Panula et al., 1989: rat; Airaksinen and Panula, 1988: guinea pig; Tighilet and Lacour, 1996: cat). The vestibular nuclei play a crucial role in the recovery process after vestibular lesion (Lacour et al., 1989; Smith and Curthoys,

* Corresponding author. Tel.: +33 0488576841; fax: +33 0488576818.
E-mail address: tighilet@up.univ-mrs.fr (B. Tighilet).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.09.017

1989) and they contain all types of histamine receptors (H_1, H_2 and H_3), as shown with ligand-binding (Bouthenet et al., 1988; Vizuete et al., 1997; Tighilet et al., 2002) and in situ hybridization methods (Ruat et al., 1991; Vizuete et al., 1997; Pillot et al., 2002; Rouleau et al., 2004). In situ hybridization experiments with histidine decarboxylase, the enzyme synthesizing histamine, pointed to an upregulation of histidine decarboxylase mRNA in the tuberomammillary nuclei of cats treated with histamine-like drugs (Tighilet et al., 2002). In addition, binding experiments showed a downregulation of histamine H_3 receptors in vestibular and tuberomammillary nuclei. It therefore seems plausible that betahistine dihydrochloride upregulates histamine turnover, very likely by blocking the presynaptic histamine H_3 receptors. This assumption is supported by the role of these receptors in mediating autoinhibition of brain histamine release (Arrang et al., 1983) and autoregulation of histamine synthesis (Arrang et al., 1987, 1992). In rat brain slices, agonists and antagonists of the histamine H_3 receptors reduce and enhance histamine release, respectively (Arrang et al., 1983, 1987; Garbarg et al., 1989). The effectiveness of betahistine in treating vertigo and vestibular disease may also be explained by histamine effects on central vestibular targets. Histamine depolarizes the medial vestibular nuclei cells in vitro in rats (Phelan et al., 1990; Inverarity et al., 1993) and guinea pigs (Sérafin et al., 1993; Wang and Dutia, 1995) and these nuclei are implicated in the vestibulo–ocular and vestibulo–spinal reflexes (Wilson and Melvill Jones, 1979; Lacour and Borel, 1993). Perfusion of the guinea pig vestibular nuclei on one side with histamine H_3 receptor agonists induces a stereotyped postural and oculomotor syndrome mimicking that found after unilateral vestibular lesion (Yabe et al., 1993). Betahistine was also reported to modify the peripheral activity of vestibular receptors (Botta et al., 2000; Valli, 2000; Soto et al., 2001).

As a rule, pharmacological investigations in animal models are initially performed with high doses of the drugs tested. In our unilateral vestibular neurectomized cat analyses, for example, betahistine dihydrochloride was administered at daily doses of 100 or 50 mg/kg for 3 weeks. The route of administration (oral solution) and the substance (methyl-amino-2 ethyl-pyridine dihydrochloride) were similar to those used in vestibular pathology, but the doses were up to 100 times higher. In addition, the treatment was shorter in cats (3 weeks) than in vestibular patients (several months).

The present study was aimed at determining the possible dose and duration effects of betahistine treatment on histamine turnover in normal adult cats, as judged by the level of histidine decarboxylase mRNA in the tuberomammillary nuclei. Experiments were conducted on betahistine-treated cats receiving daily doses of 2, 5, 10, or 50 mg/kg during 1 week, 3 weeks, 2 months, or 3 months. The lowest dose (2 mg/kg) given the longest time (3 months) was close to the dosage for vestibular defective patients. This dosage has anti-vertigo effects and improves vestibular compensation in double-blind, placebo-controlled studies (Oosterveld, 1984; Kingma et al., 1997; Constantinescu et al., 1996). The 1-week, 3-week, and 2- and 3-month treatments correspond to the acute, compensatory, and sustained compensatory stages of vestibular compensation, respectively (Lacour et al., 1989). Data from the experimental groups were compared to control, untreated cats and to placebo-treated animals.

2. Material and methods

Experiments were performed with 52 adult pigmented domestic cats (3–4 kg) obtained from the "Centre d'Elevage du Contigné", one of the approved sources. All experiments have been carried out in accordance with the U.K. Animals (scientific procedures) Act, 1986 and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC) or the national Institutes of Health guide for the care and use of laboratory animals (NIH publications No. 8023, revised 1978). These normal cats were housed under a constant 12-h light–dark cycle with free access to food and water.

2.1. Experimental protocol

The changes in histamine turnover induced by betahistine treatment were investigated by comparing control untreated cats ($N=4$) and placebo-treated cats ($N=4$) with betahistine-treated animals ($N=44$). The placebo group received oral administration of saline water (2 ml) for three months. The experimental cats were subdivided into 11 groups ($n=4$ per group) receiving the drug orally. These groups differed both by the daily dose (2, 5, 10, or 50 mg/kg) of betahistine treatment and by its duration (1 week, 3 weeks, 2 months, or 3 months) (see Table 1). The protocol was designed to determine the minimal dose and duration of treatment that modified the basal level of histidine decarboxylase mRNA relative to the controls.

Table 1
Experimental protocol

50 mg	1W	3W		
10 mg	1W	3W		3M
5 mg		3W	2M	3M
2 mg	1W	3W		3M

Synoptic table showing the 11 experimental groups of cats treated with betahistine dihydrochloride. Each group was made of four animals. Cats received of the drug orally at daily doses of 2, 5, 10, or 50 mg/kg for 1 week (1W), 3 weeks (3W), 2 months (2M), or 3 months (3M). The rationale was to determine the lowest dose and the shortest time that significantly increased the basal expression level of histidine decarboxylase mRNA in the tuberomammillary nuclei. The control group ($N=4$) and the placebo group ($N=4$) are not represented.

Table 2
Statistical analysis of the effects of betahistine treatment on histidine decarboxylase mRNA expression in the tuberomammillary nuclei

Source of variation	*df*	*F*	*P*
Side	1	0.17	0.67
Interindividual differences	51	1.15	0.32
Group (control+placebo/treated)	12	29.36	0.0001[a]
Dose (2, 5, 10, 50 mg/kg)	3	36.85	0.0001[a]
Treatment duration (1W, 3W, 2M, 3M)	3	13.12	0.0001[a]

Repeated-measure analysis of variance on the labeled surface (in pixel square) occupied by positively hybridized cell bodies in the tuberomammillary nuclei. Group, dose, and treatment duration are the main fixed effects providing the sources of variation among cats ([a]). No significant differences were seen between sides (left versus right) or between individuals. *df*: degree of freedom; *F*: Scheffé test; *P*: probability level.
[a] Significant differences between variables.

2.2. Tissue preparation

Cats of each group were deeply anesthetized with ketamine dihydrochoride (20 mg/kg, IM, Merial, Lyon, France) and killed by decapitation. After removal from the skull, their brains were cut into blocks containing the posterior hypothalamic nuclei and rapidly frozen with CO2 gas. Coronal sections (10-μm thick) were cut in a cryostat (Leica, Reuil-Malmaison, France) and thawed onto 'superfrost ++' glass slides (Fisher Scientific, Elancourt, France). They were fixed in paraformaldehyde 4% in 0.1 M phosphate buffer saline (PBS) (pH 7.2–7.4) for 45 min at +4 °C, rinsed three times in 0.1 M PBS, dehydrated in graded alcohol, air-dried for 30 min, and stored at −80 °C until being processed for in situ hybridization histochemistry.

2.3. In situ hybridization histochemistry

A DNA fragment encoding amino acids 503–639 of the human histidine decarboxylase gene sequence (Yamauchi et al., 1990; Zahnow et al., 1991) was selectively amplified by polymerase chain reaction from human genomic DNA and subcloned in pGEM-4Z (Promega, Charbonnieres, France). The subcloned DNA was submitted to limited restriction mappings to check its identity and to determine the orientation of the insert in the vector. Then, [^{33}P]-labeled antisense- and sense-strand RNA probes were prepared by in vitro transcription using a Riboprobe kit (Promega).

The sections were rinsed for 5 min in Tris (0.1 M, pH 7.5)–EDTA (0.5 M) buffer, treated with proteinase K (10 μg/ml buffer) at 37 °C for 10 min, and rinsed in 0. 1 M Tris buffer (2×5 min). They were washed in 2× standard saline citrate (SSC; 17.53 g/l NaCl, 8.82 g sodium citrate–2H_2O, pH 7.0) for 2×5 min, immersed in 0. 1 M triethanolamine, pH 8, for 5 min and then in 0.1 M triethanolamine +0.25% acetic anhydride for 10 min, and rinsed in 2× SSC (2×5 min). After being washed in glycine buffer (7 mg/ml in 0.1 M Tris buffer, pH 7.5) for 60 min, they were rinsed in 2× SSC, dehydrated in graded alcohols and air-dried for 30 min. The [^{33}P]-labeled cRNA probes (sense and antisense) were denatured by heating at 70 °C for 6 min, cooled in ice, and mixed to the hybridization buffer (50% deionized formamide, 10% dextran sulfate, 1× Denhart's solution, 2× SSC, 0.1% sodium pyrophosphate, 100 μg/ml yeast tRNA, 100 μg/ml denatured salmon sperm DNA). Each section was covered with 75 μl of diluted probe at the final concentration of 2×10^6 cpm, coverslipped with a sterile piece of Nescofilm, placed in a humidified box, and kept overnight at 58 °C. The following day, the sections were rinsed in 2× SSC (2×5 min), incubated in RNAse buffer (200 μg/ml in 2× SSC) for 60 min at 37 °C, and rinsed in 2× SSC (3×15 min). They were successively washed in 0.5× SSC for 30 min at 58 °C, in 0.1× SSC for 30 min at 60 °C, and in 0.1× SSC for 30 min at room temperature, and then they were dehydrated through 30%, 50%, 80%, and 95% ethanols containing 300 mM ammonium acetate and 100% ethanol and air-dried.

Autoradiography films (Hyperfilm β-max, Amersham, Orsay, France) were apposed to the sections and stored at 4 °C for 6 days. The films were developed for 6 min in Kodak D-19 (Eastman Kodak, Rochester, NY) and fixed in GBX (Eastman Kodak) for 15 min. Slides were dipped in Amersham LM 141 emulsion at 43 °C, and stored in a light-proof box (containing dessicator) at 4 °C for 6 to 10 days. They were developed, counterstained with hemalun (Merck, Fontenay-sous-Bois, France) or 0.1% cresyl violet, and mounted with Permount. Controls of hybridization histochemistry using the sense strand probe at the same final concentration gave no specific hybridization signal.

2.4. Data quantification

The tuberomammillary nuclei of the posterior hypothalamus were identified through Berman's stereotaxic atlas (Berman and Jones, 1982). Neurons expressing histidine decarboxylase mRNA were analyzed. To accurately quantify the surface occupied by positively hybridized tuberomammillary cell bodies, sections from control, placebo, and treated cats were processed concomitantly with the [^{33}P]-labeled histidine decarboxylase probe. The different tuberomammillary sections collected at similar stereotaxic levels from the different experimental groups were exposed on the same autoradiographic film. Seventy-five serial sections were quantified in the tuberomammillary nuclei of each animal; 15 sections were used

Table 3
Statistical analysis of the effects of betahistine treatment on the number of silver grains per histidine decarboxylase radiolabeled neurons in the tuberomammillary nuclei

Source of variation	*df*	*F*	P
Side	1	1.40	0.23
Interindividual differences	51	2.35	0.52
Group (control+placebo/treated)	12	90.78	0.0001[a]
Dose (2, 5, 10, 50 mg/kg)	3	146.39	0.0001[a]
Treatment duration (1W, 3W, 2M, 3M)	3	40.46	0.0001[a]

Repeated-measure analysis of variance of the number of grain per histidine decarboxylase radiolabeled neurons in the tuberomammillary nuclei. Group, dose, and treatment duration are the main fixed effects providing the sources of variation among cats ([a]). No significant differences were seen between sides (left versus right) or between individuals. *df*: degree of freedom; *F*: Scheffé test; *P*: probability level.
[a] Significant differences between variables.

Table 4
Statistical analysis of the effects of betahistine treatment on the number of histidine decarboxylase radiolabeled neurons in the tuberomammillary nuclei

Source of variation	*df*	*F*	P
Side	1	0.27	0.59
Interindividual differences	51	0.22	0.71
Group (control+placebo/treated)	12	0.008	0.92
Dose (2, 5, 10, 50 mg/kg)	3	0.59	0.61
Treatment duration (1W, 3W, 2M, 3M)	3	0.61	0.60

Repeated-measure analysis of variance of the number of histidine decarboxylase radiolabeled neurons in the tuberomammillary nuclei showing no significant variations neither on the group, nor on the duration or dose factor. No significant differences were seen between sides (left versus right) or between individuals. *df*: degree of freedom; *F*: Scheffé test; *P*: probability level.

for each of the five main levels examined (rostro-caudal planes of A12, A11, A10.2, A9.5, and A8.3). Autoradiographic signals were captured from the films through a high-resolution video camera (1024×1024 pixels) linked to a computer-image analyzer (NIH, Image 1,62b7). Because of the scattered distribution of tuberomammillary neurons containing histidine decarboxylase mRNA, we binalized, in the digitized images, a constant surface unit containing the tuberomammillary nuclei, after thresholding. The threshold value was identical for all the sections. Data analysis consisted in evaluating the labeled histidine decarboxylase mRNA surface expressed in pixel^2. Reproducibility was assessed by comparing the data collected independently by two researchers. They were blinded to the animal groups they analyzed with the image analysis system. The specific hybridization signal was measured in each section as a labeled surface and was automatically computed and evaluated thereafter as the mean (±S.E.M.) for each side, each cat, and each subgroup of cats (see Tighilet et al., 2002).

Quantification of histidine decarboxylase mRNA expression in tuberomammillary neurons was carried out at the cellular level on adjacent dipped sections. For each control and treated cats, we first counted the number of histidine decarboxylase radiolabeled neurons in the tuberomammillary nuclei on both sides (left/right). Positive [^{33}P]-radiolabeled neurons were defined as those displaying at least four silver grains around the nucleus. For each cat, we examined an average of 50 rostro-caudal sections. Grain counts over individual cells were than analyzed in the tuberomammillary nuclei of these rostro-caudal sections. The number of grains per cell was quantified using an image analyzer system (LUCIA G, Nikon, Champigny sur Marne, France).

2.5. Statistical analysis

Analysis of variance (ANOVA) was used to test 1) inter-individual differences and/or side effects and 2) the effects of betahistine treatment (controls versus treated cats), of the dose (2, 5, 10 versus 50 mg for a given duration) and of the duration (1 week, 3 weeks, 2 months versus 3 months for a given dose) on a) histidine decarboxylase mRNA expression (labeled surface) in the tuberomammillary nuclei, b) the number of histidine decarboxylase radiolabeled neurons in the tuberomammillary nuclei, c) the number of grains per cell in the tuberomammillary nuclei and to determine the interactions between these variables. ANOVA was followed by post hoc analysis with the Scheffe test and multicomparison Fisher's test (Stateview II software).

3. Results

A moderate expression of histidine decarboxylase mRNA was found in the tuberomammillary nuclei of the controls, confirming our previous results (Tighilet et al., 2002). A similar basal expression was recorded in the placebo-treated cats

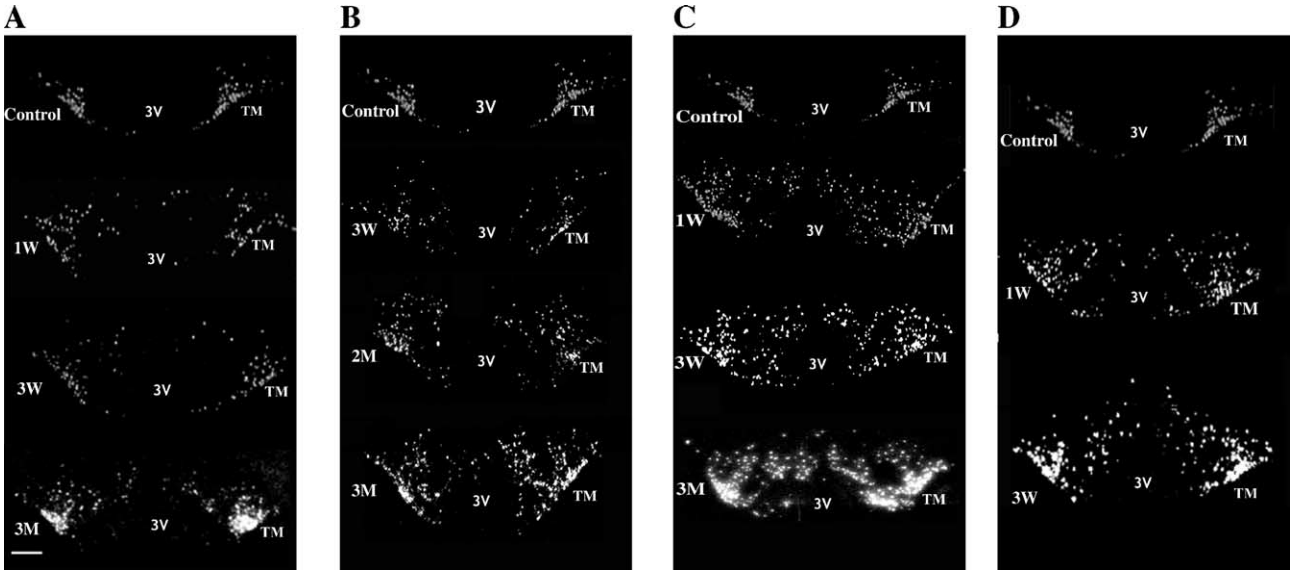

Fig. 1. (A–D) In situ hybridization with the histidine decarboxylase cRNA probe in coronal sections of the posterior hypothalamus. Illustrations of the typical labeling recorded after betahistine treatment at the daily dose of 2 mg/kg (A), 5 mg/kg (B), 10 mg/kg (C) and 50 mg/kg (D) in representative cats. The labeling in control untreated cats (Controls) is reported at the top of each panel for direct comparison. The experimental animals received betahistine either for one week (1W), three weeks (3W), two months (2M), or three months (3M). F: fornix; TM: tuberomammillary nuclei; 3V: third ventricle. Bar, 1 mm.

($P=0.91$). No significant differences in the expression of histidine decarboxylase mRNA were seen between the left and right sides, whatever the tested group, or between individuals for the control group, the placebo group, and the betahistine-treated groups (Table 2). Data from both sides and for all the cats of a given group were therefore pooled for the statistical evaluation. Placebo and control groups were also pooled and referred to as controls.

Repeated-measure analysis of variance showed that group (control+placebo versus treated cats), betahistine treatment duration (1W, 3W, 2M versus 3M), and dose of orally administered betahistine (2, 5, 10 versus 50 mg/kg) constituted the main fixed effects causing the variation among cats. A significant increase in both histidine decarboxylase mRNA expression (labeled surface in the tuberomammillary nuclei) and in the number of grains per labeled neurons in the tuberomammillary nuclei was seen for both the dose factor (F 36.85, F 146.39; $P<0.0001$, respectively) and the duration factor (F 13.12, F 40.46; $P<0.0001$, respectively). These effects were also corroborated by the significant interaction between the three variables (Tables 2 and 3). By contrast, the number of histidine decarboxylase positive neurons depended neither on the group, nor on the duration or dose factor (Table 4).

3.1. Expression of histidine decarboxylase mRNA: qualitative data

Fig. 1A–D shows autoradiography pictures of representative sections from the posterior hypothalamus area from cats submitted to betahistine dihydrochloride treatment for short durations (1 and 3 weeks) and long ones (2 and 3 months). This region was the sole central nervous system structure to express histidine decarboxylase mRNA, and the labeling was observed only with the [^{33}P]-labeled antisense-strand RNA probe. Each panel illustrates the effects of a single dose over the periods

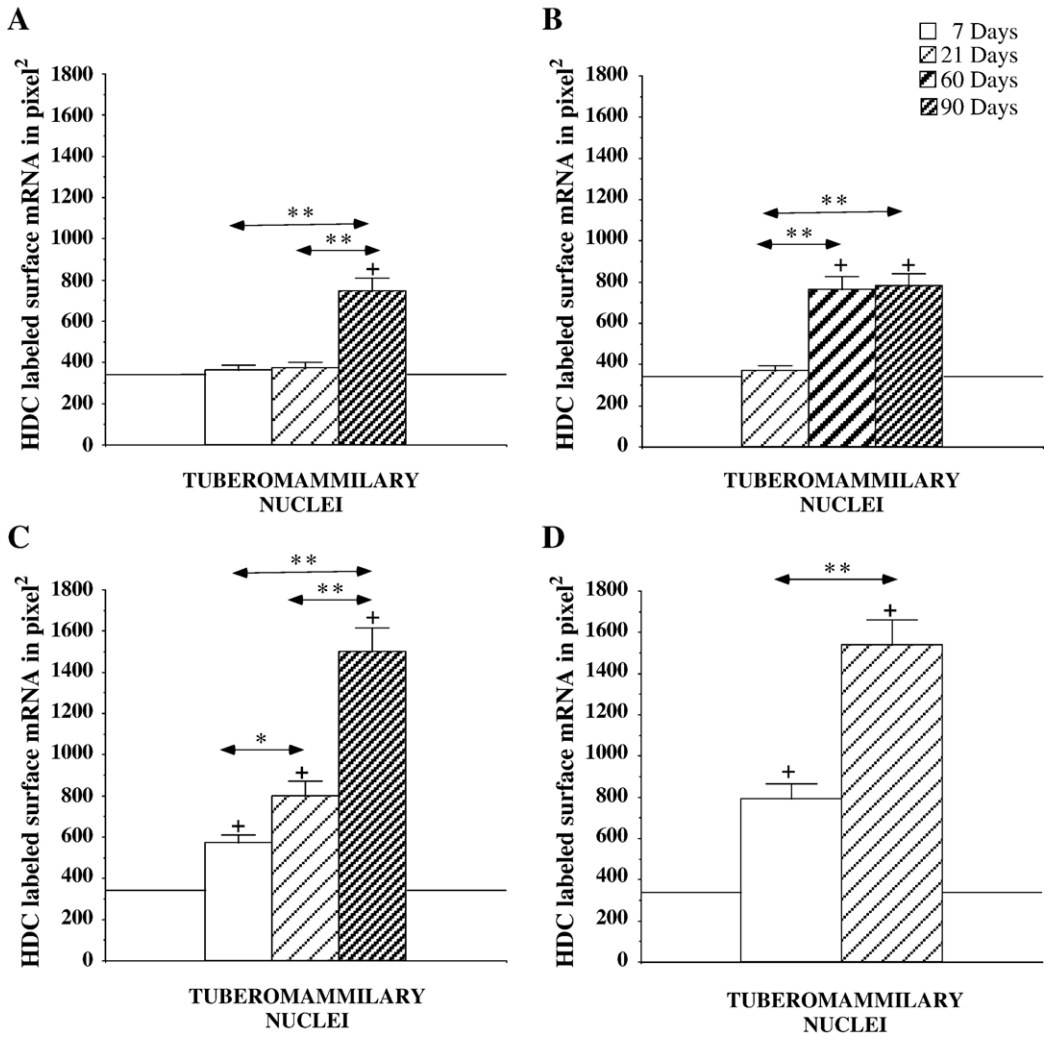

Fig. 2. (A–D) Quantification of the effects of betahistine treatment on histidine decarboxylase mRNA levels expressed in the tuberomammillary nuclei. Data are expressed as mean values (±S.E.M.) of the labeled surface expressed in pixel^2. Effects of betahistine treatment are shown for the 2 mg/kg (A), 5 mg/kg (B), 10 mg/kg (C), and 50 mg/kg (D) daily doses the cats received for 1 week (white histograms), 3 weeks, 2 months, or 3 months (hatched histograms). The horizontal line in each panel shows the mean basal expression level of histidine decarboxylase mRNA in the control and placebo groups of cats. Data for the left and right sides were pooled in each group of cats. *$P<0.001$; **$P<0.0001$ for comparisons between the experimental groups of cats. $^{+}P<0.0001$ versus controls. HDC: histidine decarboxylase.

tested. Fig. 1A shows labeling in the tuberomammillary nuclei of a representative cat treated at the daily dose of 2 mg/kg while Fig. 1B, C and D illustrates representative animals treated at daily doses of 5, 10 and 50 mg/kg, respectively. For a direct comparison, data from control untreated animals are reported at the top of each panel.

The betahistine-treated cats receiving the drug orally at a daily dose of 50 mg/kg for 1 or 3 weeks (Fig. 1D) exhibited strongly increased expression of histidine decarboxylase mRNA in their tuberomammillary nuclei, confirming our previous study (Tighilet et al., 2002). By contrast, the histidine decarboxylase mRNA expression did not change relative to the controls for low doses orally administered for short durations, that is, 1–3 weeks (2 mg/kg: Fig. 1A; 5 mg/kg: Fig. 1B). Increasing the duration of treatment induced more intense labeling, which depended on the dose given to the cats. Histidine decarboxylase mRNA expression in the tuberomammillary nuclei was slightly enhanced on both sides after 1 week treatment with the 10 mg/kg dose, more intensively after 3 weeks for the same dose, after 2 months with 5 mg/kg, and after 3 months with the lowest dose, 2 mg/kg. These qualitative data show histidine decarboxylase mRNA expression levels depend on the dose/duration interaction of betahistine treatment. They are confirmed by the quantitative analysis of the data.

3.2. Expression of histidine decarboxylase mRNA: quantitative data

The quantitative analysis of the surface occupied by tuberomammillary neurons expressing histidine decarboxylase mRNA is shown in Fig. 2A–D. The basal expression found in the controls was 341±24.1 pixels^2. The lowest dose (2 mg/kg) did not significantly modify the mean labeled surface for short betahistine treatment (1 and 3 weeks), but the longest duration, 3 months, significantly increased labeling (cf Fig. 2A). The mean value reached 746.5±88.6 pixels^2 after 3 months of treatment. For the 5 mg/kg dose, no significant changes were found at 3 weeks, but labeling was strongly increased after 2 and 3 months. The mean values are 763.5±90.7 and 780.2±87.8 pixels^2 for the 2 and 3 months of treatment, respectively ($P<0.0001$: cf Fig. 2B). The 10 mg/kg dose induced significant changes of the surface occupied by the tuberomammillary neurons expressing histidine decarboxylase mRNA for the 3-week treatment, and the amount of increase was in the same range as that for the 2 and 5 mg/kg doses given in long periods (798.9±101.9 pixels^2, $P<0.0001$). For the 10 mg/kg dose, labeling was twofold higher at 3 months than at 3 weeks (1498.4±165.5 pixels^2, $P<0.0001$: cf Fig. 2C). Note that the values for this dose and time duration (10 mg/kg for 3 months) are similar to those for a high dose given in a short period (50 mg/kg for 3 weeks: cf Fig. 2D).

3.3. Expression of histidine decarboxylase mRNA at the cellular level: qualitative data

Fig. 3 shows the dose and duration effects of betahistine treatment on histamine turnover in normal adults cats, as judged by the neuronal level of histidine decarboxylase mRNA in the tuberomammillary nuclei. This figure illustrates the increased number of silver grains per labeled neuron in the tuberomammillary nuclei of cats treated for 3 months at 2 mg/kg (C), 5 mg/kg (D), 10 mg/kg (E) and cats treated for three weeks at 50 mg/kg (F). For a direct comparison, data from a control untreated cat (A) and a placebo-treated cat 2 mg/kg (B) are illustrated.

3.4. Expression of histidine decarboxylase mRNA at the cellular level: quantitative data

The quantitative analysis of the intraneuronal level of histidine decarboxylase mRNA in the tuberomammillary nuclei is shown in Fig. 4B. The results correlate with the quantification of the histidine decarboxylase mRNA labeled surface occupied

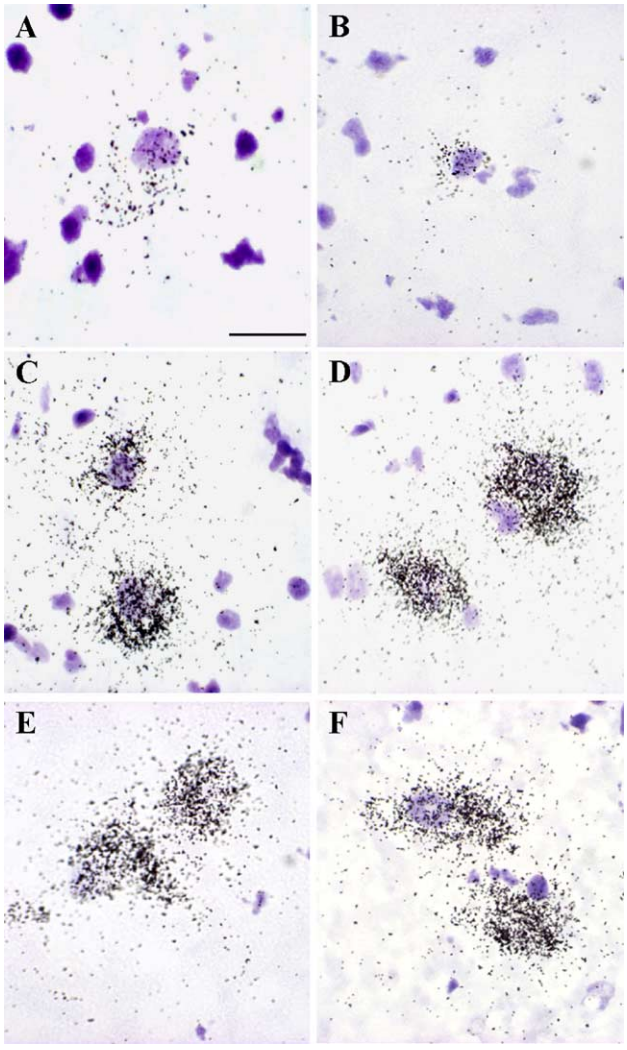

Fig. 3. (A–F) High magnification photomicrographs from emulsion autoradiograms showing hybridization of cRNA specific for histidine decarboxylase mRNAs to neurons in the tuberomammillary nucleus (TM). Illustration of the increased number of silver grains per labeled neuron in the tuberomammillary nuclei of cats treated for 3 months at 2 mg/kg (C), 5 mg/kg (D), 10 mg/kg (E) and cats treated for three weeks at 50 mg/kg (F). For a direct comparison, data from a control untreated cat (A) and a placebo-treated cat (B) are illustrated. Scale bar, 10 μm.

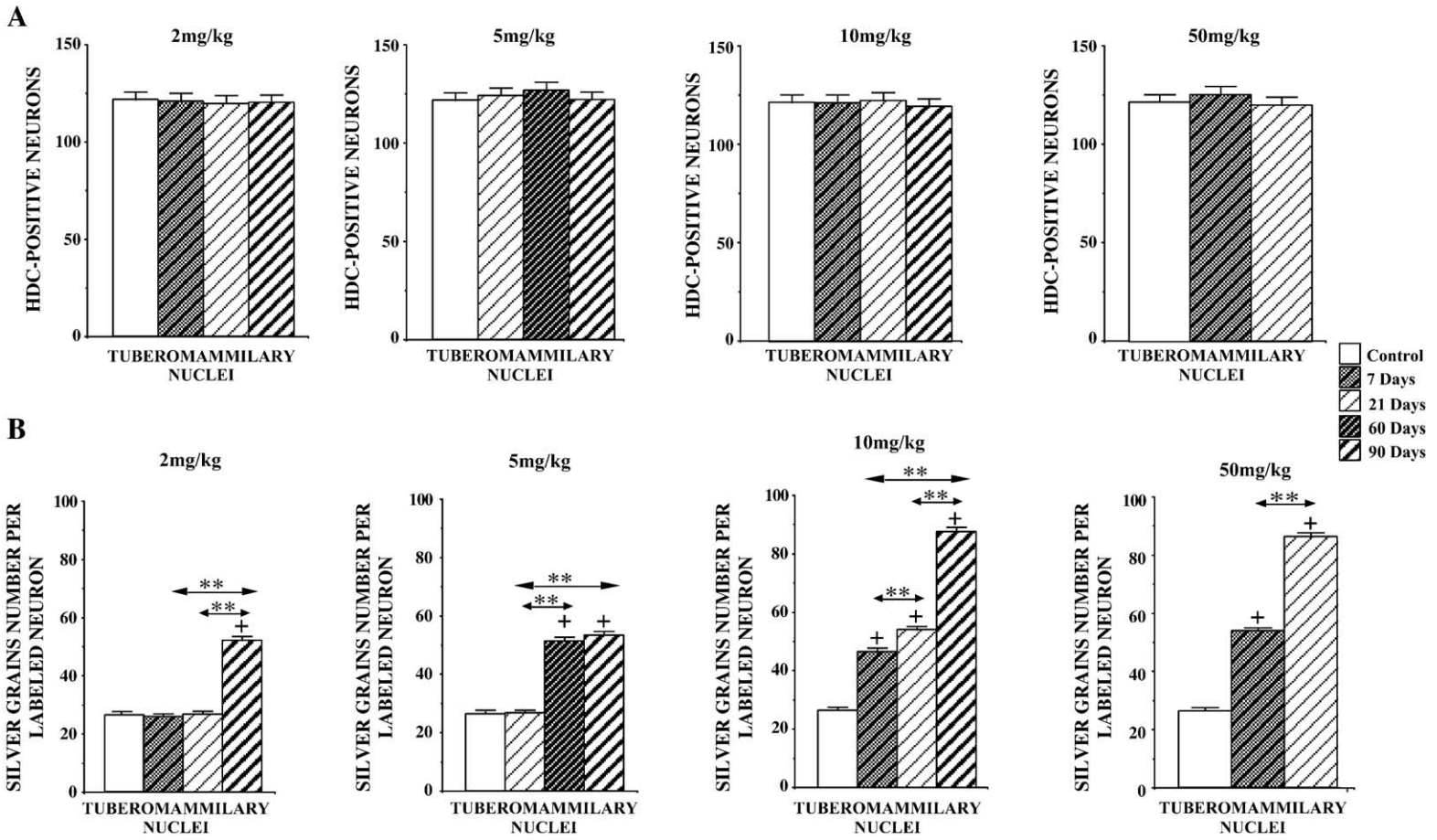

Fig. 4. (A–B) Quantification of the effects of betahistine treatment on the number of histidine decarboxylase radiolabeled neurons (A) and the number of silver grains per radiolabeled neurons (B) in the tuberomammillary nuclei. Data are expressed as mean values (±S.E.M.). Effects of betahistine treatment are shown for the 2, 5, 10, and 50 mg/kg daily doses the cats received for 1 week, 3 weeks, 2 months, or 3 months (hatched histograms). The white histograms show the mean values recorded in the control and placebo groups of cats. Data for the left and right sides were pooled in each group of cats. $^{**}P<0.0001$ for comparisons between the experimental groups of cats. $^{+}P<0.0001$ versus controls. HDC: histidine decarboxylase.

by tuberomammillary neurons illustrated in Fig. 2. The basal number of silver grains per labeled neuron in the control was 26.33±0.54. The lowest dose (2 mg/kg) did not significantly modify the mean number of grains per labeled neuron for short betahistine treatment (1 and 3 weeks), but the longest duration, 3 months, significantly increased labeling (cf Fig. 4B). The mean value reached 52.24±0.86 after 3 months of treatment. For the 5 mg/kg dose, no significant changes were found at 3 weeks, but labeling was strongly increased after 2 and 3 months. The mean values are 51.36±0.94 and 53.39±1.01 for the 2 and 3 months of treatment, respectively ($P<0.0001$: cf Fig. 4B). The 10 mg/kg dose induced significant changes of the number of silver grains per labeled neuron for the 3-week treatment, and the amount of increase was in the same range as that for the 2 and 5 mg/kg doses given in long periods (53.93±0.91, $P<0.0001$). For the 10 mg/kg dose, labeling was almost twofold higher at 3 months than at 3 weeks (87.48±1.58, $P<0.0001$: cf Fig. 4B). Note that the values for this dose and time duration (10 mg/kg for 3 months) are similar to those for a high dose given in a short period (50 mg/kg for 3 weeks: 86.26±1.50 cf Fig. 4B). The quantitative analysis of the number of histidine decarboxylase radiolabeled neurons in the tuberomammillary nuclei is shown in Fig. 4A. The mean number found in the controls was 121.79±3.95. This number remains unchanged whatever the dosage and the treatment duration, compared to the control (Fig. 4A).

Fig. 5 summarizes the whole of these quantitative data by grouping the treated groups of cats according to the effects on the surface occupied by the tuberomammillary nuclei neurons expressing histidine decarboxylase mRNA. Compared to controls, betahistine treatment induced either no change or it increased the labeling. In this latter case, basal expression level was up to fourfold higher. Indeed, small doses of the drug (2 or 5 mg/kg) given for short periods (1 or 3 weeks) had no effect. By contrast, small doses given for long periods (2 or 3 months) or higher doses (10 or 50 mg/kg) given for short periods doubled the effects found in the controls. As expected, high doses orally administered for long periods provided the strongest effects, with a basal expression level of histidine decarboxylase mRNA about four times that of the controls.

4. Discussion

The data reported in this study clearly show that betahistine dihydrochloride administered orally in the normal cat increases the basal expression level of histidine decarboxylase mRNA of neurons located in the tuberomammillary nuclei of the posterior hypothalamus. The effects were both dose- and time-dependent.

4.1. Betahistine and its interaction with the histaminergic system

Betahistine is a partial histamine H_1 receptor agonist and a more potent histamine H_3 receptor antagonist. Its interaction with the histaminergic system is now well established. The histamine H_3 receptor mediates autoinhibition of brain

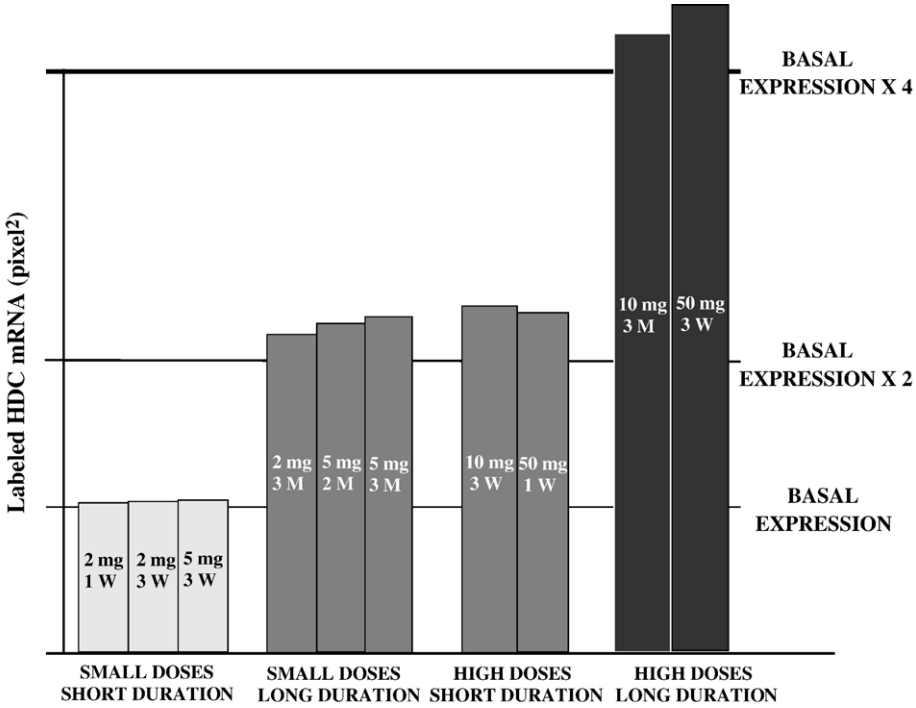

Fig. 5. Functional summary of the results. The figure pools the experimental groups of cats as a function of the importance of the effects of betahistine treatment on the histidine decarboxylase mRNA expression in the tuberomammillary nuclei neurons. Note that the basal expression level remained unchanged in the groups receiving low doses (2 and 5 mg/kg) for short periods (1 or 3 weeks). By contrast, the basal level is twofold higher than for the controls in both the groups receiving low doses for long periods (2 or 3 months) and those receiving high doses (10 and 50 mg/kg) for short periods. The greatest effects were seen in the two experimental groups treated with betahistine at high daily doses over long periods, for which the basal expression level of histidine decarboxylase mRNA was fourfold higher. HDC: histidine decarboxylase.

histamine release and autoregulation of histamine synthesis (Arrang et al., 1983, 1985; Schwartz et al., 1990; Arrang et al., 1992). By blocking the histamine H_3 autoreceptors, betahistine increases the synthesis and release of histamine in the tuberomammillary nuclei, as shown also with in situ hybridization and binding to histamine H_3 receptors methods in our cat model (Tighilet et al., 2002). Changes in the excitability of vestibular nuclei neurons were observed after betahistine injection both in vitro (Phelan et al., 1990; Sérafin et al., 1993; Wang and Dutia, 1995) and in vivo (Unemoto et al., 1982). In addition, local perfusion of the vestibular nuclei on one side with histamine H_3 receptor agonists reproduced the vestibular syndrome seen after unilateral vestibular loss (Yabe et al., 1993). All these arguments support the notion that improved vestibular compensation as previously reported (Tighilet et al., 1995) is due to changes in histamine synthesis and release (Tighilet and Lacour, 1997), at least partly, and that betahistine is therefore helpful for the treatment of vertigo (Lacour and Sterkers, 2001). It should be however reminded that the increase in the histidine decarboxylase mRNA levels observed in the present work not necessarily implicates an increase in the functional enzyme and in histamine synthesis. Changes in histidine decarboxylase activity depends on a variety of molecular processes other than changes in the level of the enzyme protein, e.g. availability of the precursor amino acid and/or calcium in a synthesis pool (Schwartz et al., 1970; Verdière et al., 1977). Furthermore, regulation of histamine release and/or synthesis at the somatodendritic end of tuberomammillary neurons and at the nerve terminals is not well known.

4.2. Clinical implications for vertigo treatment

The most drastic changes in histidine decarboxylase mRNA expression in the tuberomammillary nuclei were seen with high doses ($\geq$ 10 mg/kg) given 3 weeks to 3 months, since the basal expression level of histidine decarboxylase mRNA was fourfold higher than in controls.

From a clinical point of view betahistine is administered at a daily dose of 48 mg/day to have a rapid onset of action and a prolonged effect. What is particularly important is that low doses close in our cat model to ones given to humans, i.e. 2 or 5 mg/kg/day, led to significantly increased histidine decarboxylase mRNA expression only when the treatment was long. The basal level was twofold higher the control when the treatment was given for 3 months, 2 months and 3 weeks for the 2, 5 and 10 mg doses, respectively.

In clinical practice, betahistine is recommended for long-term treatment. This approach could be beneficial particularly for patients with unilateral vestibular loss. For such patients, vestibulo–spinal reflex compensation takes considerably longer than 2 months (Allum et al., 1988). In line with this, our team showed that head stabilization in space required 3 months to recover while shoulder and hip stabilization were not totally recovered in this time (Borel et al., 2002). Also, the horizontal optokinetic afternystagmus remained asymmetrical 4 years after unilateral vestibular loss (Brantberg et al., 1996), the horizontal vestibulo–ocular reflex (Halmagyi et al., 1990) and dynamic ocular counterrolling (Diamond and Markham, 1983) showed asymmetrical patterns several years after unilateral neurotomy, and impairments of walking performance during goal-directed locomotion were seen up to 3 months in patients submitted to similar curative surgery (Borel et al., 2004). Finally, the static components of the vestibular syndrome are also concerned since ocular cyclotorsion (Curthoys et al., 1991), torsional optokinetic nystagmus (Lopez et al., 2005), subjective visual vertical perception (Vibert and Häusler, 2000) as well as body roll–tilt perception (Dai et al., 1989) were never totally compensated after more than 3 months.

In conclusion, compensation of both static and dynamic deficits is subtended by long-term adaptive mechanisms that could be facilitated pharmacologically using betahistine dihydrochloride treatment or other medications able to accelerate centrally the rebalancing of the vestibular loss-induced asymmetries.

Acknowledgements

This work was supported from grants from Ministère de l'Enseignement Supérieur et de la Recherche and CNRS (UMR 6149 Université de Provence/CNRS) and by Solvay Pharma Laboratories (Suresnes, France) which provided betahistine.

References

Airaksinen, M.S., 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.

Allum, J.H.J., Keshner, E.A., Honegger, F., Pfaltz, C.R., 1988. Indicators of the influence a peripheral vestibular deficit has on vestibulo–spinal reflex responses controlling postural stability. Acta Oto-laryngol. 106, 252–263.

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

Arrang, J.M., Garbarg, M., Schwartz, J.C., 1992. H_3-receptor and control of histamine release. In: Schwartz, J.C., Haas, H.L. (Eds.), The Histamine Receptors. Wiley-Liss, New-York, pp. 145–160.

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.

Borel, L., Harlay, F., Magnan, J., Chays, A., Lacour, M., 2002. Deficits and recovery of head and trunk orientations and stabilization after unilateral vestibular loss. Brain 125, 880–894.

Borel, L., Harlay, F., Lopez, C., Magnan, J., Chays, A., Lacour, M., 2004. Walking performance of vestibular-defective patients before and after unilateral vestibular neurotomy. Behav. Brain Res. 150, 191–200.

Botta, L., Mira, E., Valli, S., Zucca, G., Perin, P., Benvenuti, C., Fossati, A., Valli, P., 2000. Effects of betahistine metabolites on frog ampullar receptors. Acta Oto-laryngol. 120, 25–27.

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 [I125] idobolpyramine. Neuroscience 26, 553–600.

Brantberg, K., Fransson, P.A., Bergenius, J., Tribukait, A., 1996. Tilt suppression, OKAN, and head-shaking nystagmus at long-term follow-up after unilateral vestibular neurectomy. J. Vest. Res. 6, 235–241.

Constantinescu, L., Schneider, D., Claussen, C.F., 1996. The influence of betahistine on the vestibular evoked potentials in patients with peripheral vestibular disorders. 3rd EUFOS, Bologna: Monduzzi Editore, pp. 95–98.

Curthoys, I.S., Dai, M.J., Halmagyi, G.M., 1991. Human ocular torsional position before and after unilateral vestibular neurectomy. Exp. Brain Res. 85, 218–225.

Dai, M.J., Curthoys, I.S., Hamlagyi, G.M., 1989. Linear acceleration perception in the roll plane before and after unilateral vestibular neurectomy. Exp. Brain Res. 77, 315–328.

Diamond, S.G., Markham, C.H., 1983. Ocular counterrolling as an indicator of vestibular otolith function. Neurology 33, 1460–1469.

Garbarg, M., 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., Curthoys, I.S., Cremer, P.D., Henderson, C.J., Todd, M.J., Staples, M.J., 1990. The human horizontal vestibulo–ocular reflex in response to high-acceleration stimulation before and after unilateral vestibular neurectomy. Exp. Brain Res. 81, 479–490.

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. (Lond.) 459. 466 pp.

Kingma, H., Bonink, M., Meulenbroeks, A., Konijnenberg, H., 1997. Dose-dependent effect of betahistine on vestibulo–ocular reflex: a double-blind, placebo controlled study in patients with paroxysmal vertigo. Acta Oto-laryngol. 117, 641–646.

Lacour, M., Borel, L., 1993. Vestibular control of posture and gait. Arch. Ital. Biol. 131, 81–104.

Lacour, M., Sterkers, O., 2001. Histamine and betahistine in the treatment of vertigo: elucidation of mechanisms of action. CNS Drugs 15, 853–870.

Lacour, M., Toupet, M., Denise, P., Christen, Y., 1989. Vestibular compensation. Facts, Theories and Clinical Perspectives. Elsevier, Paris. 308 pp.

Laurikainen, E.A., Miller, J.M., Quirk, W.S., Kallinen, J., Ren, T., Nuttall, A.L., Grenman, R., Virolainen, E., 1993. Betahistine-induced vascular effects in the rat cochlea. Am. J. Otol. 14, 24–30 (Jan.).

Laurikainen, E., Miller, J.M., Nuttall, A.L., Quirk, W.S., 1998. The vascular mechanism of action of betahistine in the inner ear of the guinea pig. Eur. Arch. Otorhinolaryngol. 255, 119–123.

Lopez, C., Borel, L., Magnan, J., Lacour, M., 2005. Torsional optokinetic nystagmus after unilateral vestibular loss: asymmetry and compensation. Brain 128, 1511–1524.

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, 280–289.

Oosterveld, W.J., 1984. Betahistine dihydrochloride in the treatment of vertigo of peripheral vestibular origin. A double-blind placebo-controlled study. J. Laryngol. Otol. 98, 37–41.

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.

Pillot, C., Heron, A., Cochois, V., Tardivel-Lacombe, J., Ligneau, X., Schwartz, J., Arrang, J., 2002. A detailed mapping of the histamine H_3 receptor and its gene transcripts in rat brain. Neuroscience 114, 173–193.

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

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.

Rouleau, A., Heron, A., Cochois, V., Pillot, C., Schwartz, J.C., Arrang, J.M., 2004. Cloning and expression of the mouse histamine H_3 receptor: evidence for multiple isoforms. J. Neurochem. 90, 1331–1338.

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.

Schwartz, J.C., Lampart, C., Rose, C., 1970. Properties and regional distribution of histidine decarboxylase in rat brain. J. Neurochem. 17, 1527–1534.

Schwartz, J.C., Arrang, J.M., Garbarg, M., Pollard, H., Ruat, M., 1990. A third histaminergic receptor subtype: characterisation, location and functions of the H3-receptor. Agents Actions 30, 13–23.

Sérafin, 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.

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

Soto, E., Chavez, H., Valli, P., Benvenuti, C., Vega, R., 2001. Betahistine produces post-synaptic inhibition of the excitability of the primary afferent neurons in the vestibular endorgans. Acta Oto-laryngol., Suppl. 545, 19–24.

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

Tighilet, B., Lacour, M., 1997. Histamine immunoreactivity changes in vestibular-lesioned and histaminergic-treated cats. Eur. J. Pharmacol. 330, 65–77.

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

Tighilet, B., Trottier, S., Mourre, C., Chotard, C., Lacour, M., 2002. Betahistine dihydrochloride interaction with the histaminergic system in the cat: neurochemical and molecular mechanisms. Eur. J. Pharmacol. 446, 63–73.

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

Unemoto, H., Sasa, M., Takaori, S., Ito, J., Matsuoka, I., 1982. Inhibitory effect of betahistine on polysynaptic neurons in the lateral vestibular nucleus. Arch. Oto-Rhino-Laryngol. 236, 229–236.

Valli, P., 2000. Betahistine reduces the resting firing rate of vestibular receptors in the frog. Acta Oto-laryngol., Suppl. 544, 8–10.

Verdière, M., Rose, C., Scwartz J, C., 1977. Turnover of cerebral histamine in a stressful situation. Brain Res. 129, 107–119.

Vibert, D., Häusler, R., 2000. Long-term evolution of subjective visual vertical after vestibular neurectomy and labyrinthectomy. Acta Oto-laryngol 120, 620–622.

Vizuete, M.L., Traiffort, E., Bouthenet, M.L., Ruat, M., Souil, E., Tardivel-Lacombe, J., Schwartz, J.C., 1997. Detailed mapping of the histamine H_2 receptor and its gene transcripts in guinea-pig brain. Neuroscience 80, 321–343.

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.

Wilson, V.J., Melvill Jones, G., 1979. Mammalian Vestibular Physiology. Plenum Press, New York.

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.

Yamauchi, K., Sato, R., Tanno, Y., Ohkawara, Y., Maeyama, K., Watanabe, T., Satoh, K., Yoshizawa, M., Shibahara, S., Takishima, T., 1990. Nucleotide sequence of the cDNA encoding L-histidine decarboxylase derived from human basophilic leukemia cell line, KU-812. Nucleic Acids Res. 18, 5891–5895.

Zahnow, C., Yi, H.F., McBride, O.W., Joseph, D.R., 1991. Cloning of the cDNA encoding human histidine decarboxylase from an erythroleukemia cell line and mapping of the gene locus to chromosome 15. DNA Sequence 1, 395–400.