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Betahistine dihydrochloride interaction with the histaminergic system in the cat: neurochemical and molecular mechanisms

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Abstract

Drugs interfering with the histaminergic system facilitate behavioral recovery after vestibular lesion, likely by increasing histamine turnover and release. The effects of betahistine (structural analogue of histamine) on the histaminergic system were tested by quantifying messenger RNA for histidine decarboxylase (enzyme synthesizing histamine) by in situ hybridization and binding to histamine H_3 receptors (mediating, namely, histamine autoinhibition) using a histamine H_3 receptor agonist ([^{3}H]*N*-α-methylhistamine) and radioautography methods. Experiments were done in brain sections of control cats ($N=6$) and cats treated with betahistine for 1 ($N=6$) or 3 ($N=6$) weeks. Betahistine treatment induced symmetrical changes with up-regulation of histidine decarboxylase mRNA in the tuberomammillary nucleus and reduction of [^{3}H]*N*-α-methylhistamine labeling in both the tuberomammillary nucleus, the vestibular nuclei complex and nuclei of the inferior olive. These findings suggest that betahistine upregulates histamine turnover and release, very likely by blocking presynaptic histamine H_3 receptors, and induces histamine H_3 receptor downregulation. This action on the histaminergic system could explain the effectiveness of betahistine in the treatment of vertigo and vestibular disease.

Keywords: Histidine decarboxylase; Histamine H_3 receptor; Betahistine dihydrochloride; In situ hybridization; Radioautography; Vestibular nuclei; Tuberomammillary nuclei; (Cat)

1. Introduction

Among the drugs currently prescribed for the symptomatic treatment of Ménière's disease and vertigo in humans, those interfering with the histaminergic system play a key role (Fisher, 1991; Rascol et al., 1995). Both histamine H_1 receptor agonists (histamine and structural analogues) and antagonists (mepyramine and diphenhydramine) seem effective in the treatment of vertigos very likely by calling different mechanisms of action into play: Ca^{2+} channel blockade (flunarizine), sedation (mepyramine, diphenhydramine) or microcirculation improvement (histamine and structural analogues).

Betahistine dihydrochloride (betahistine) is a histamine-like drug that acts as both a partial histamine H_1 receptor agonist and a histamine H_3 receptor antagonist (Arrang et al., 1985; Timmerman, 1991). It is generally assumed that the efficacy of betahistine results from vascular effects (Meyer et al., 1974), inducing an increase in cochlear blood flow as shown in the rat (Laurikainen et al., 1993), the guinea pig (Laurikainen et al., 1998) and the dog (Anderson and Kubicek, 1971). Because betahistine was also found to be effective in vestibular syndromes unrelated to vascular insufficiency, other effects on the central nervous system have been suspected. It was indeed reported in double-blind, placebo-controlled clinical investigations that this structural analogue of histamine was effective in peripheral vestibular disorders (Canty et al., 1981; Oosterveld, 1984) and in Menière's disease (Frew and Menon, 1976; Bertrand, 1982). A betahistine-induced improvement of vestibular compensation (i.e., reduction of the vestibular deficits with time after vestibular lesion) was also reported in animal models (Tighilet et al., 1995), and its beneficial effects on the recovery process were supposed to result from a central action of histamine neurotransmission. Indeed, unilateral vestibular neurectomized cats treated for 3 weeks (50 mg/kg/day) exhibited a strong improvement of static and dynamic equilibrium functions (Tighilet et al., 1995), lead-

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ing to a time benefit of 2 weeks compared to untreated control cats. In addition, immunohistochemical investigations performed in cats treated subacutely (1 week) or more chronically (3 weeks) showed a strong bilateral decrease in histamine immunoreactivity in both the tuberomammillary and vestibular nuclei (Tighilet and Lacour, 1997).

It is generally admitted that the brain stem vestibular nuclei play a crucial role in the recovery process after vestibular lesion (Lacour et al., 1989; Smith and Curthoys, 1989), at least in terms of compensation for the static vestibular deficits. Interestingly, it was demonstrated that the tuberomammillary nuclei of the posterior hypothalamus, where histamine-containing perikarya are exclusively located (Pollard and Schwartz, 1987), send histaminergic 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 contain all types of histamine receptors (postsynaptic H_1 and H_2 and presynaptic H_3 receptors), as shown with ligand binding (Bouthenet et al., 1988), in situ hybridization methods (Ruat et al., 1991) and behavioral investigations using vestibular nuclei perfusion of histamine receptor ligands (De Waele et al., 1992; Yabe et al., 1993). Histamine depolarizes the medial vestibular nucleus cells in vitro (Phelan et al., 1990; Sérafin et al., 1993), very likely through histamine H_1 (Inverarity et al., 1993) and/or H_2 (Sérafin et al., 1993; Wang and Dutia, 1995) receptors, and histamine was found to be released in vivo after vestibular stimulation (Horii et al., 1993). Local perfusion of the vestibular nuclei on one side with histamine H_2 receptor antagonists or histamine H_3 receptor agonists induces a stereotyped postural and oculomotor syndrome in the guinea pig that mimics that observed after unilateral lesion of the peripheral vestibular system (Yabe et al., 1993). In addition, histamine immunoreactivity is drastically reduced in the vestibular and tuberomammillary nuclei of normal cats treated with histamine H_3 receptor antagonists (betahistine, thioperamide), very likely as a consequence of histamine depletion due to an increase in histamine synthesis and release (Tighilet and Lacour, 1997). Since the histamine H_3 autoreceptors regulate brain histamine release and synthesis (Arrang et al., 1983, 1992), the antagonistic properties of betahistine on this class of presynaptic autoreceptors should lead to an increase in histamine turnover.

The hypothesis that betahistine increases histamine synthesis by blocking the histamine H_3 autoreceptors was investigated in this study. The betahistine treatment-induced changes in histamine synthesis and histamine H_3 receptor binding were evaluated in the cat by comparing untreated cats to animals given oral betahistine at the daily dose of 50 mg/kg for 1 or 3 weeks. This treatment protocol was used for direct comparison with our previous behavioral (Tighilet et al., 1995) and immunohistochemical (Tighilet and Lacour, 1997) investigations in our cat model. The regulation of histidine decarboxylase, the enzyme that synthezises histamine, was analysed in the tuberomammillary nuclei by using in situ hybridization histochemistry, whereas the binding to histamine H_3 receptors was quantified in the tuberomammillary and vestibular nuclei by receptor radioautography. In addition, [^{3}H]*N*-α-methylhistamine binding assays were performed in control as well as betahistine-treated cats in order to analyse the affinity of the histamine H_3 receptor for this radioligand and that of betahistine for the histamine H_3 receptor in the cat.

2. Methods

Experiments were performed with 18 adult pigmented domestic cats (3–4 kg) obtained from the Centre d'Élevage du Contigné, one of the French approved sources. The principles of laboratory animal care and procedures used followed the guidelines of the Ministère de l'Agriculture. These normal cats were housed under a constant 12-h light–dark cycle.

2.1. Experimental protocol

The betahistine treatment-induced changes of histamine turnover and histamine H_3 receptor binding were investigated by comparing control untreated cats ($n=6$) with betahistine-treated animals receiving the drug orally at a daily dose of 50 mg/kg for 1 ($n=6$) or 3 ($n=6$) weeks. These subacute and chronic treatments were chosen for direct comparison with previous behavioral investigations showing a strong improvement of the recovery process in our cat model of vestibular compensation (Tighilet et al., 1995). The two survival time periods, i.e. 1 and 3 weeks, were taken as two key points in the recovery process that correspond to the acute and compensatory stages of vestibular compensation in the cats, respectively (cf. Lacour et al., 1989). The betahistine dihydrochloride used in these experiments was similar to that used in the commercial preparation (Serc) and was provided by Solvay Pharma (Suresnes, France). The orally administered drug was composed of methyl-amino-2 ethyl-pyridine dihydrochloride in oral solution (50 mg/ml). In all cases, the treated cats were killed 24 h after the last administration of betahistine. This time period between the end of treatment and killing of the animals was chosen to exclude residual betahistine in the brain and its possible interference with [^{3}H]*N*-α-methylhistamine radioligand binding.

2.2. In situ hybridization histochemistry

A DNA fragment encoding aminoacids 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). The subcloned DNA was submitted to limited restriction mappings to check its identity and to determine the orientation of the insert inside

the vector. Then, ^{33}P-labeled antisense- and sense-strand RNA probes were prepared by in vitro transcription using a Riboprobe kit (Promega).

After cryostat sectioning of fresh frozen tissue, sections (10 μm thick) 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 and dehydrated in graded alcohol, air-dried for 30 min and stored at −80 °C until being processed for in situ hybridization histochemistry.

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 × SCC (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 alcohol, and air-dried for 30 min. The ^{33}P-labeled cRNA probes (sense and anti-sense) were denaturated 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 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, in 0.1 × SSC for 30 min at room temperature, dehydrated in 0.3 M alcohol containing 0.3 M ammonium acetate and airdried.

Autoradiography films (Hyperfilm β-max, Amersham) were apposed to the sections and stored at +4 °C for 6 days. The films were developed for 6 min in D-19 (Kodak) and fixed for 15 min in GBX (Kodak). Controls of hybridization histochemistry using the sense strand probe at the same final concentration gave no specific hybridization signal.

2.3. [^{3}H]*N*-α-methylhistamine autoradiography

The binding of [^{3}H]*N*-α-methylhistamine (80 Ci/mmol, NEN™ Life Science Products) to histamine H_3 receptors was performed with tissue sections as previously described (Cumming et al., 1994). The brain sections (10-μm thick from fresh frozen tissue) were incubated with 4 nM [^{3}H]*N*-α-methylhistamine at 4 °C in a 150 mM sodium phosphate buffer, pH 7.4, containing 2 mM magnesium chloride and 100 μM dithiothreitol (Sigma). The non-specific binding component was measured by adding a large excess of thioperamide (2 μM, Tocris Cookson) 30 min before adding [^{3}H]*N*-α-methylhistamine. After a 45-min incubation, the sections were rinsed three times (each wash lasting 20 s) in a 4 °C buffer, then rinsed once in 4 °C water for 3 s. The slices were dried with a stream of cold air and exposed to tritium-sensitive film ([^{3}H]Hyperfilm, Amersham). After 9 months of exposure at −80 °C, the films were processed in Kodak industrex developer at room temperature for 2 min, fixed and then washed. Azur II-stained sections were used for reference.

2.4. [^{3}H]*N*-α-methylhistamine binding assays

To analyze the affinity of the histamine H_3 receptor for [^{3}H]*N*-α-methylhistamine in betahistine-treated and non-treated groups of cats, and the affinity of betahistine for the histamine H_3 receptor, we performed competition experiments. Sections of hypothalamus of control, 1- and 3-week treated cats were homogenized with a Potter homogenizer in 50 mM Tris buffer at pH 7.5, and then the homogenate was centrifuged at 1000 × *g* for 5 min. Protein was determined according to Bradford (1976).

Hypothalamus homogenate (250 μg of protein) was incubated with increasing concentrations of thioperamide or betahistine in the same autoradiographic binding buffer (150 mM sodium phosphate buffer, pH 7.4, containing 2 mM magnesium chloride, and 100 μM dithiothreitol) for 45 min at room temperature in the presence of 4 nM of [^{3}H]*N*-α-methylhistamine. After incubation, 250 μl aliquots were filtered, using a cell harvester, over glass-fiber filters (Whatman, GF/B) pre-soaked in 0.3% polyethylamine. They were rapidly washed three times with 4 ml of the same buffer. The radioactivity retained by the filters was counted in a beta scintillation analyzer (Packard). Curves were fit to the data with Prism nonlinear least-squares curve-fitting program (GraphPad software, San Diego, US). One site fits were tested.

2.5. Data quantification

2.5.1. Expression of histidine decarboxylase mRNA in the tuberomammillary nuclei

The brainstem and posterior hypothalamic nuclei were identified with Berman's stereotaxic atlas (Berman, 1968; Berman and Jones, 1982). Neurons expressing histidine decarboxylase mRNA were analyzed. In order to perform a valid quantitative analysis of the surface occupied by positively hybridized tuberomammillary cell bodies, sections from control and treated cats were processed in a unique experiment with the ^{33}P-labeled histidine decarboxylase probe. The different tuberomammillary sections collected at similar stereotaxic levels from the three experimental groups were exposed on the same autoradiographic film. Twenty serial sections were quantified in the tuberomammillary nuclei of each animal, five sections

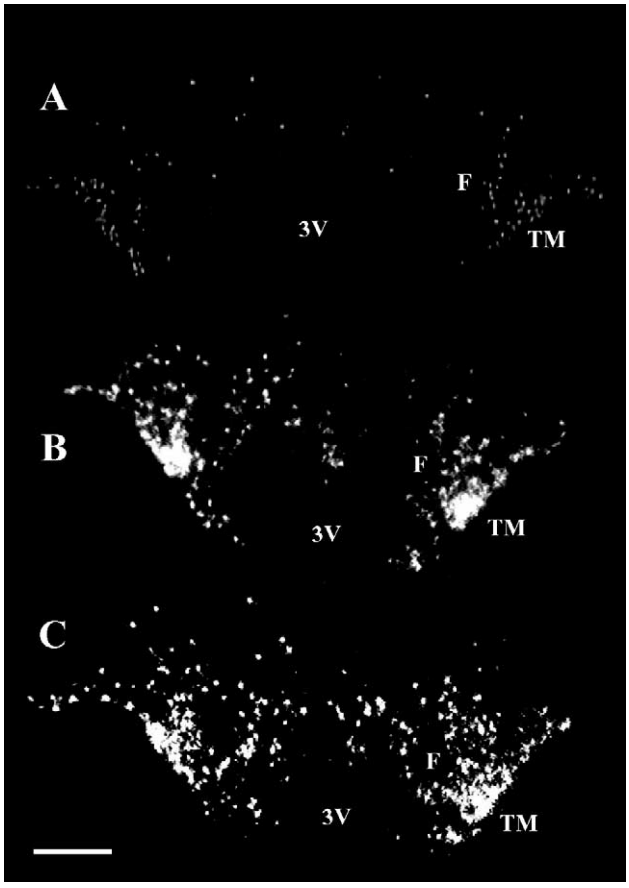

Fig. 1. (A–C) In situ hybridization pictures obtained with the histidine decarboxylase cRNA probe in coronal sections of the posterior hypothalamus. Illustration of the typical labeling recorded in a representative control cat (A) and in two experimental animals receiving betahistine treatment for 1 (B) or 3 (C) weeks at the daily dose of 50 mg/kg. Note that betahistine treatment induced a strong increase in histidine decarboxylase mRNA expression in the tuberomammillary nuclei on both sides. F: fornix; TM: tuberomammillary nucleus; 3V: third ventricle. Bar, 1 mm.

being used for each of the four main examined levels (rostrocaudal planes of A12, 11, 9.5 and 8.3). 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 histidine decarboxylase mRNA-containing tuberomammillary neurons, we chose to binalize 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 of evaluating the labeled histidine decarboxylase mRNA surface expressed in pixel^2. Reproducibility was assessed by comparing the data collected independently by two different experimenters. They were blinded to the animal groups that they analyzed with the image analysis system. The specific hybridization signal was measured in each section as the 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.

2.5.2. Histamine H_3 receptor binding quantification

The autoradiograms of the binding to histamine H_3 receptors were analyzed and quantified using NIH Image software. [^{3}H] Plastic standards (Amersham) were used to calibrate ^{3}H concentrations. Receptor density is expressed in fmol/mg of protein. Receptor density was evaluated for both the vestibular and the tuberomammillary nuclei. A mean receptor density value was calculated for each nucleus from 60 serial sections. The specific binding value was determined as the difference between total and non-specific binding components for a given area and was evaluated as the mean ± S.E.M. [^{3}H]*N*-α-methylhistamine binding site density was evaluated in brainstem structures including each of the four main vestibular nuclei (medial, inferior, superior and lateral, respectively), the three subdivisions of the inferior olive (medial, dorsal and posterior, respectively), the nucleus prepositus hypoglossi and the posterior hypothalamic nuclei. These latter structures included the tuberomammillary nucleus, the medial mammillary nucleus and the dorsal, lateral and posterior hypothalamic areas.

2.5.3. Statistical analysis

Statistical evaluation of the data was done by analysis of variance (super-ANOVA) to test interindividual differences as well as side (left vs. right), structure (each of the four main vestibular nuclei and the tuberomammillary nuclei), treatment (betahistine-treated cats vs. controls) and duration of the treatment (1 week vs. 3 weeks) effects on histidine decarboxylase mRNA regulation and histamine H_3 receptor binding. Super-ANOVA was followed by post hoc analysis

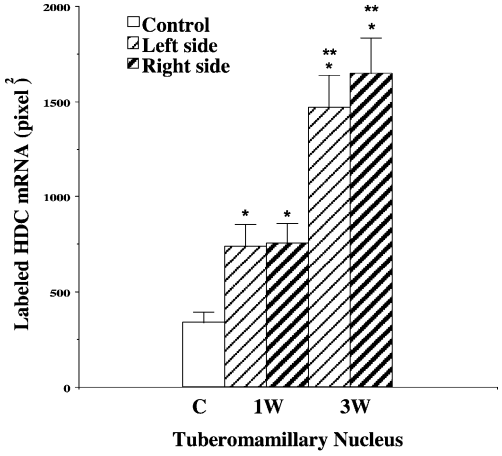

Fig. 2. Effects of betahistine treatment on histidine decarboxylase mRNA levels expressed in the tuberomammillary nuclei. Data are expressed as mean values (± S.E.M) of the labeled surface expressed in pixel^2 for the untreated (N=3) and treated cats (N=6). Both sides were pooled for the controls (open histograms) while the values recorded on the left (thin-hatched histograms) and right (thick-hatched histograms) sides are given separately for the subgroups of cats receiving betahistine treatment for 1 or 3 weeks. * P<0.0001 vs. control, ** P<0.0001 vs. 1 week treated cats.

with the Scheffe test and the multicomparison Fisher's test (Statview II software).

3. Results

As a rule, we observed a moderate expression of mRNA for histidine decarboxylase in the tuberomammillary nuclei and a relatively high [^{3}H]*N*-α-methylhistamine binding density was found in both the tuberomammillary and vestibular nuclei of the control cats. No significant differences were seen between the left and the right sides and no significant interindividual differences were found.

Strong changes were found in the betahistine-treated cats compared to the controls for both histidine decarboxylase mRNA expression and the density of histamine H_3 receptors. Repeated-measure analysis of variance demonstrated that group (controls vs. treated cats) and treatment duration (1 week vs. 3 weeks) constituted the main fixed causing the variation among animals. This was corroborated by the significant interaction between these two variables ($P < 0.0001$). Significant differences were found neither for the side (left vs. right) nor for the different animals tested.

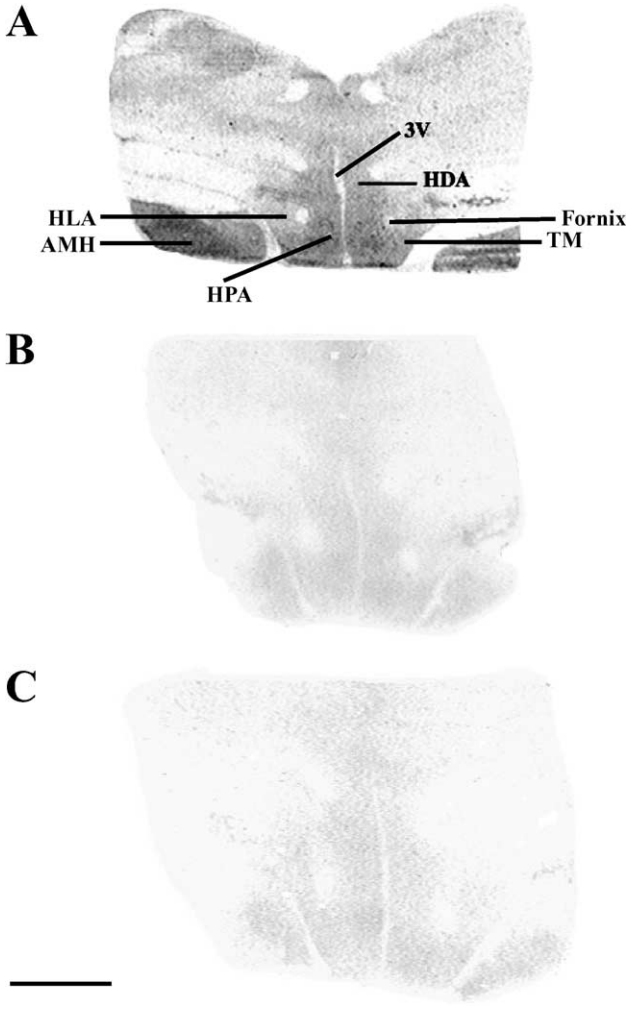

Fig. 3. (A–C) [^{3}H]*N*-α-methylhistamine binding sites in the cat posterior hypothalamus. Coronal sections from three representative control and betahistine-treated cats showing a drastic decrease in histamine H_3 receptor binding sites in the different structures of the posterior hypothalamus after 1 week (B) or 3 weeks (C) of treatment, as compared to controls (A). AMH: amygdalo-hippocampal area; HLA: lateral hypothalamic area; HPA: posterior hypothalamic area; HDA: dorsal hypothalamic area; TM: tuberomammillary nucleus; 3V: third ventricle. Bar: 1 mm.

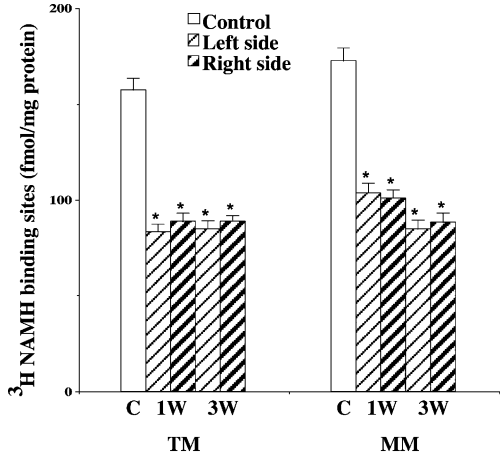

Fig. 4. Density of [^{3}H]*N*-α-methylhistamine binding sites in the cat posterior hypothalamus. The [^{3}H]*N*-α-methylhistamine binding to histamine H_3 receptors is expressed in fmol/mg protein (ordinates) as the mean ± standard error of the mean. Data from the tuberomammillary (TM) and medial mammillary (MM) nuclei are given as the average value for the right and left structures in the controls ($N = 3$) (open histograms), while they are provided separately for each side [right (thick-hatched histograms) vs. left (thin-hatched histograms)] for the cats receiving betahistine treatment for 1 ($N = 3$) or 3 weeks ($N = 3$). * $P < 0.0001$ vs. controls.

3.1. Expression of histidine decarboxylase mRNA in the tuberomammillary region

Fig. 1 shows autoradiography pictures of representative sections from the posterior hypothalamus area from three different animals either untreated (controls: Fig. 1A) or submitted to 1 (Fig. 1B) or 3 (Fig. 1C) weeks betahistine treatment. This region was the sole central nervous system structure to express histidine decarboxylase mRNA and the labeling was seen only with the ^{33}P-labeled antisense-strand RNA probe. Compared to the controls that exhibited a moderate expression of mRNA, betahistine treatment induced a strong and bilateral increase in the labeled surface in the tuberomammillary nuclei. This up-regulation was seen for the two durations of treatment that we examined.

The quantitative analysis of the surface occupied by tuberomammillary neurons expressing histidine decarboxylase mRNA is shown in Fig. 2. The labeled surface was 340.9 ± 51.1 pixels^2 on average in the tuberomammillary nuclei of the normal cats (325.6 ± 51.9 and 356.3 ± 51.8 on the left and right sides, respectively: NS). In the subgroup of cats treated for 1 week at the daily dose of 50 mg/kg, there was an increase in the labeled surface compared to that in

the controls; the difference between both sides was not significant. In the subgroup of cats treated for 3 weeks at the same daily dose of betahistine, the increase in the labeled surface was higher when compared both to the controls and to the 1 week treated group of cats. However, the mean values recorded on the left and right sides were not significantly different.

3.2. Histamine H_3 receptor binding sites in the cat posterior hypothalamus

Fig. 3 shows typical autoradiograms of frontal sections of the posterior hypothalamus from three representative animals either untreated (controls: Fig. 3A) or treated with betahistine for 1 (Fig. 3B) or 3 (Fig. 3C) weeks. The binding of the agonist [^{3}H]*N*-α-methylhistamine to histamine H_3 receptors is shown for the controls (3A) as dark stained structures. A high binding density was seen in different areas including the posterior, lateral and dorsal hypothalamic areas as well as in the tuberomammillary and the medial mammillary nuclei. The binding density was strongly decreased in all these structures in both subgroups of cats receiving the 50 mg/kg daily dose of betahistine for 1 (Fig. 3B) or 3 (Fig. 3C) weeks.

As a rule, the binding density of the agonist [^{3}H]*N*-α-methylhistamine to histamine H_3 receptors was reduced by 40% to 60%, depending on the subnuclei of the posterior hypothalamus. The data are plotted in Fig. 4 for the tuberomammillary and the medial mammillary nuclei areas that exhibited the highest density in the controls, and for the two subgroups of betahistine-treated cats. In the controls, the averaged values were 172.6 ± 6.9 and 157.5 ± 6.3 fmol/mg of protein in the medial mammillary and tuberomammillary nuclei, respectively. These values were bilaterally decreased in both structures after betahistine treatment for 1 week or 3 weeks. The left and right sides did not show any difference.

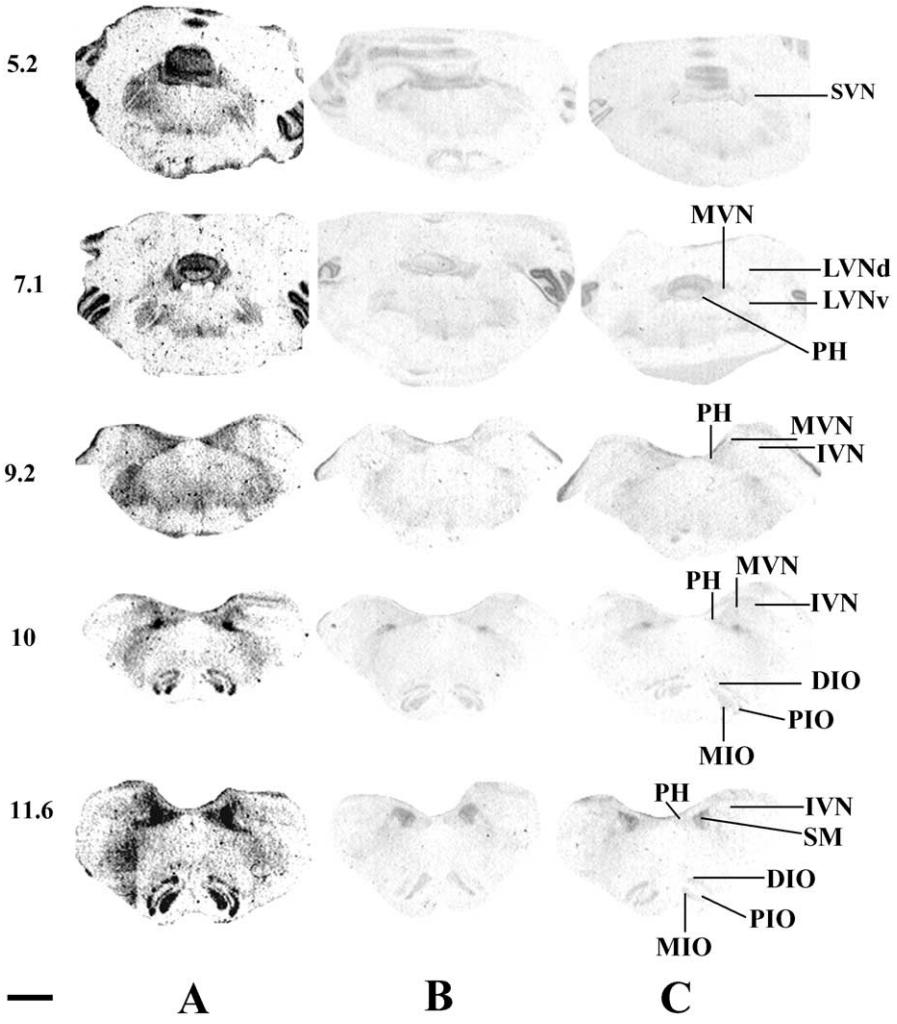

Fig. 5. (A–C) [^{3}H]*N*-α-methylhistamine binding sites in the cat brainstem. Coronal sections from three representative control and betahistine-treated cats showing a drastic decrease in histamine H_3 receptor binding in the different structures of the brainstem after 1 (B) or 3 weeks (C) of treatment, as compared to the controls (A). Illustrations are given for serial sections collected from the rostral (5.2) to the caudal (12.1) parts of the brainstem. IVN: inferior vestibular nucleus; LVNd and v: lateral vestibular nucleus, dorsal and ventral parts; MVN: medial vestibular nucleus; SVN: superior vestibular nucleus; PH: prepositus hypoglossi; DIO, MIO and PIO: dorsal, medial and posterior parts of the inferior olive, respectively; SM: medial part of the solitary tract. Bar: 1 mm.

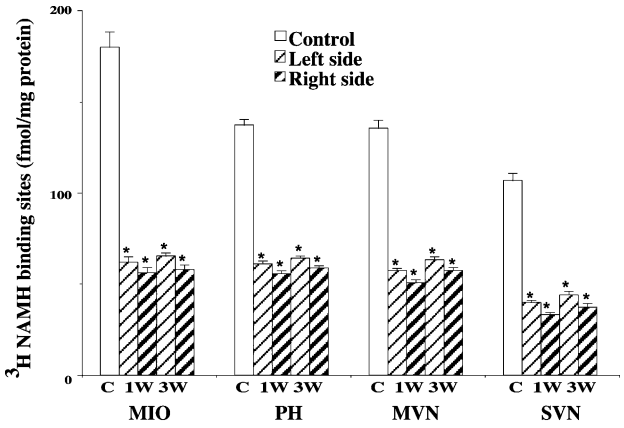

Fig. 6. Density of [^{3}H]*N*-α-methylhistamine binding sites in the cat brainstem. Changes in histamine H_3 receptor binding in the cat brain stem after administration of betahistine for 1 week or 3 weeks. Quantitative analysis is expressed as means and standard errors of fmol of [^{3}H]*N*-α-methylhistamine specifically bound per mg of protein from autoradiograms taken from three animals. Data from the medial (MVN) and superior (SVN) vestibular nuclei, the prepositus hypoglossi (PH), and the medial part of the inferior olive (MIO) are given as the average value of the right and left structures for the controls (open histograms) ($N=3$), while they are provided separately for each side [right (thick-hatched histograms) vs. left (thin-hatched histograms)] for the cats receiving betahistine treatment for 1 ($N=3$) or 3 weeks ($N=3$). * $P<0.0001$ vs. controls.

Furthermore, no significant differences were found between the subgroups of cats under betahistine treatment for 1 or 3 weeks.

3.3. Histamine H_3 receptor binding sites in the cat brain stem

Similar findings were recorded in the vestibular nuclei and associated brain stem structures. Fig. 5 illustrates the spatial distribution of binding density in representative serial frontal sections collected from the rostral (5.2) to the caudal (12.1) parts of the brainstem in a control cat (Fig. 5A) and in two representative cats treated with betahistine for either 1 (Fig. 5B) or 3 (Fig. 5C) weeks. In the control cats, strong labeling was observed in the different subnuclei of the inferior olive, including the medial, the dorsal and the posterior parts, in the prepositus hypoglossi and in the four main vestibular nuclei. Among the vestibular nuclei, the medial vestibular nucleus and the superior vestibular nucleus exhibited a stronger staining than the inferior vestibular nucleus and the lateral vestibular nucleus, with a binding density as high as for the posterior hypothalamus, the prepositus hypoglossi, the medial, dorsal and posterior parts of the inferior olive. The binding density of the agonist [^{3}H]*N*-α-methylhistamine to histamine H_3 receptors was strongly reduced in all these brain stem structures after 1 (Fig. 5B) or 3 (Fig. 5C) weeks of betahistine treatment, compared to the controls.

The data are quantified for the medial part of the inferior olive, the prepositus hypoglossi, the medial vestibular nucleus and the superior vestibular nucleus in Fig. 6. The bilateral reduction of histamine H_3 receptor binding sites in the treated cats was higher in these brain stem regions than in the posterior hypothalamus, with mean reductions ranging from 50% to 70%, depending on the structures. [^{3}H]*N*-α-methylhistamine binding was reduced by 64–68% in the medial part of the inferior olive, by 54–62% in the medial vestibular nucleus, by 59–69% in the superior vestibular nucleus and by 54–60% in the prepositus hypoglossi (Fig. 6). No significant influence of the side (right vs. left) and of the duration of betahistine treatment (1 week vs. 3 weeks) was found (Fig. 6).

3.4. Competition between [^{3}H]N-α-methylhistamine and thioperamide or betahistine on homogenate of control and treated cat hypothalamus

Table 1 shows the specific binding of [^{3}H]*N*-α-methylhistamine (4 nM) in the hypothalamus homogenate of the three groups of cats. The [^{3}H]*N*-α-methylhistamine specific binding observed in the betahistine-treated groups was significantly decreased with respect to that of the control group. The reduction was in the same range as that observed in the autoradiographic study. It averaged 66% and 68% of the controls for the cats treated with betahistine for 1 week and 3 weeks, respectively.

Increasing concentrations of thioperamide gradually inhibited [^{3}H]*N*-α-methylhistamine specific binding in hypothalamus homogenates (Fig. 7A). Under our experimental binding conditions, which were similar to those of the autoradiographic procedures with the presence of sodium ions, the concentration of thioperamide inducing 50% inhibition of [^{3}H]*N*-α-methylhistamine binding was $IC_{50}=2.89\pm0.54$ nM in control cats ($N=3$), 1.74 ± 0.53 nM in cats treated with betahistine for 1 week ($N=3$) and 6.11 ± 0.64 nM in cats treated with betahistine for 3 weeks ($N=3$). Thioperamide was able to inhibit completely the binding of [^{3}H]*N*-α-methylhistamine to cat hypothalamus (Fig. 7A), as observed in the autoradiograms. The IC_{50} results showed no changes in the affinity of thioperamide for H_3 receptors in competition with [^{3}H]*N*-α-methylhistamine, i.e. no change in IC_{50} value of the radioligand.

Fig. 7B presents the betahistine inhibition of [^{3}H]*N*-α-methylhistamine binding in control cat hypothalamus. Like thioperamide, betahistine interacts with H_3 receptor because

Table 1
[^{3}H]*N*-α-methylhistamine binding in the betahistine-treated vs. control cats

Cat group	Mean ± S.E.M.
Control	40.7 ± 11.09
1 week betahistine treatment	13.8 ± 0.89[a]
3 weeks betahistine treatment	13.1 ± 0.46[a]

[^{3}H]*N*-α-methylhistamine (4 nM) was incubated with hypothalamus homogenates (250 μg protein) in the same autoradiography binding buffer for each group of cats. The [^{3}H]*N*-α-methylhistamine binding is expressed in fmol/mg of protein.

[a] $P<0.05$, Student's *t*-test, comparison with control animals.

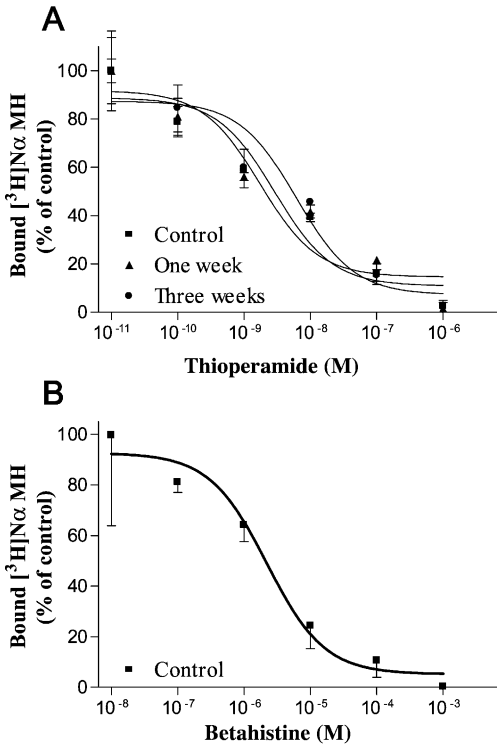

Fig. 7. (A–B) Binding of [^{3}H]*N*-α-methylhistamine. Competition curves obtained from binding experiments involving [^{3}H]*N*-α-methylhistamine and increasing concentrations of thioperamide (A) and betahistine (B) in hypothalamus homogenates from control cats and cats treated with betahistine for 1 or 3 weeks.

this ligand competed with [^{3}H]*N*-α-methylhistamine. One-site fitting gave an IC_{50} of 2.20 ± 0.46 μM ($N = 3$). Betahistine was less potent than thioperamide in control cat hypothalamus.

4. Discussion

This study in the cat clearly demonstrates that oral administration of betahistine for 1 or 3 weeks induces a marked increase in histidine decarboxylase mRNA expression, and a strong decrease in the binding density of the agonist [^{3}H]*N*-α-methylhistamine to histamine H_3 receptors. Taken together, these data indicate that treatment with a histamine analogue such as betahistine increases histamine synthesis, very likely through blockade of histamine H_3 autoreceptors.

Histaminergic cell bodies are exclusively located in the tuberomammillary nuclei of the posterior hypothalamus and send widespread projections to numerous brain areas (Panula et al., 1984; Pollard and Schwartz., 1987; Airaksinen et al., 1992). This study indicates also that perikarya expressing histidine decarboxylase mRNA are found in the tuberomammillary nuclei only, their distribution being similar to that of histamine immunoreactive neurons previously reported in the cat (Lin et al., 1993; Tighilet and Lacour, 1996). The histamine H_3 receptor distribution has been identified by autoradiographic studies in various species (Pollard et al., 1993; Cumming et al., 1991: rat; Cumming et al., 1994: guinea pig; Cumming et al., 1994; Janssen et al., 2000: mouse; West et al., 1999: primate and human). These studies showed a wide and heterogeneous distribution of histamine H_3 binding sites in various brain areas: cerebral cortex, hippocampal formation, striatum, nucleus accumbens, globus pallidus, thalamus, substantia nigra, hypothalamus, tuberomammillary nucleus and lower brain stem areas including the nucleus of the solitary tract (for review, see Schwartz et al., 1991). Our autoradiographic investigation confirms such a heterogeneous distribution in the cat brain stem (inferior olive, prepositus hypoglossi, vestibular nuclei) and diencephalon (posterior hypothalamus).

Betahistine administration for 1 or 3 weeks induced an up-regulation of histidine decarboxylase mRNA expression, as shown by the strong enhancement of mRNA labeling in the tuberomammillary nuclei. This up-regulation strongly suggests that histamine synthesis and release are increased under betahistine treatment. The role of the histamine H_3 receptor in mediating autoinhibition of brain histamine release (Arrang et al., 1983) and autoregulation of histamine synthesis (Arrang et al., 1987a, 1992) is now well established. Indeed, selective histamine H_3 receptor ligands modify the histamine turnover in rat brain slices, with agonists reducing and antagonists enhancing histamine release (Arrang et al., 1983, 1987a; Garbarg et al., 1989).

Betahistine administration led to a strong reduction in [^{3}H]*N*-α-methylhistamine binding in all the subcortical regions examined in our study. The reduction averaged 50% to 60%, depending on the structure investigated. This reduction can be caused by a decreased expression of the histamine H_3 receptor or by a change in the affinity of the radioligand for the H_3 receptor. Our findings in competition studies with thioperamide did not show significant changes in IC_{50} values for the [^{3}H]*N*-α-methylhistamine radioligand in the betahistine-treated groups of cats compared to the untreated group. Since betahistine treatment did not seem to affect the affinity of [^{3}H]*N*-α-methylhistamine for histamine H_3 receptors under our experimental conditions, the observed reduction of autoradiographic binding very likely results from a downregulation of the histamine H_3 receptor. The high endogenous histamine release due to betahistine administration at a high dose could lead to desensitization of the histamine H_3 receptor, its internalization and degradation. These molecular mechanisms have been demonstrated in the guinea pig ileum for the histamine H_3 receptor (Perez-Garcia et al., 1998), in a specific cell line for the histamine H_2 receptor (Lemos Legnazzi et al., 2000; Fukushima et al., 1997; Smit et al., 1995) as well as for other neurochemical systems involving the opioid receptors (Gastar, 2000).

A second possibility is that betahistine causes a decrease in [^{3}H]*N*-α-methylhistamine binding solely due to the fact that residual compound remains in the tissue. This possibility, however, can be excluded since residual betahistine in

the brain tissue is presumably not present because of (i) the long time (24 h) elapsed between the last drug administration and killing of the animals, (ii) the short half-life of the drug (1 h) and (iii) the likely dissociation during membrane preparation of any residual betahistine from the histamine H_3 receptor for which it displays modest micromolar affinity.

Since the histamine H_3 receptor has been recently cloned (Lovenberg et al., 1999, 2000; Tardivel-Lacombe et al., 2000), we can now look at the receptor message with in situ hybridization techniques, to investigate the plasticity of this receptor subtype. These complementary investigations should clarify the hypotheses we present in the present study on the mechanisms of action of betahistine. That the plasticity of the histamine H_3 receptor could depend on the dose and duration of betahistine treatment is also considered in this next step.

It has been reported previously that thioperamide and betahistine act as antagonists (and/or inverse agonists, see below) at the H_3 receptor level on the basis of pharmacological experiments with rodent brain tissue (Arrang et al., 1985, 1987b). In view of the known histamine H_3 species differences, we determined the IC_{50} value of thioperamide and betahistine for the cat histamine H_3 receptor. In the nanomolar and micromolar concentration range, thioperamide and betahistine, respectively, had a similar effect on [^{3}H]*N*-α-methylhistamine binding in cats as in rodents (West et al., 1990; Fossati et al., 2001).

However, recent findings with recombinant rat and human histamine H_3 receptors (Morisset et al., 2000; Wieland et al., 2001) indicate that ligands previously identified as antagonists should be reclassified either into neutral antagonists or inverse agonists. Morisset et al. (2000) have suggested that enhancement of histamine turnover in rodent brain is related to inverse agonism rather than neutral antagonism at the histamine H_3 receptor. Furthermore, improvement of vestibular compensation in the guinea pig was reported with thioperamide (Yabe et al., 1993), an effect that could be due to its inverse agonist properties. We are planning experiments with neutral antagonists and inverse agonists to establish whether the improvement of vestibular compensation in the cat is due to the neutral antagonist or inverse agonist properties of compounds.

Histamine has been largely used in the past for the treatment of vertigo and disturbances of the inner ear are assumed to be of vascular origin (Fisher, 1991). Betahistine is a structural analogue of histamine, the efficacy of which has been reported also in vestibular syndromes unrelated to vascular insufficiency such as peripheral vestibular disorders (Canty et al., 1981; Oosterveld, 1984) and Menière's disease (Frew and Menon, 1976; Bertrand, 1982). We have recently demonstrated that behavioral recovery after unilateral vestibular neurectomy in our cat model is strongly accelerated by betahistine (Tighilet and Lacour, 1995) and suggested that the improvement in vestibular compensation could be related to changes in histamine synthesis and release (Tighilet et al., 1995). The histaminergic system could contribute to the recovery process either by specific mechanisms restoring balanced activity of the vestibular nuclei cells on both sides (Lacour et al., 1989) or by unspecific mechanisms related to vascular and/or arousal effects (Tighilet and Lacour, 1997). This study gives evidence that betahistine upregulates mRNA for histidine decarboxylase, very likely through an action on the histamine H_3 receptor. It corroborates recent findings confirming the role of the histaminergic system in vestibular compensation in the unilateral labyrinthectomized rat (Pan et al., 1998). The remaining questions to be resolved in the near future concern both the plasticity of the histamine H_3 receptor after chronic treatment with betahistine and the real nature of this drug (H_3 antagonist or H_3 inverse agonist?).

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