Effects of Betahistine at Histamine H₃ Receptors: Mixed Inverse Agonism/Agonism In Vitro and Partial Inverse Agonism In Vivo

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ABSTRACT

We previously suggested that therapeutic effects of betahistine in vestibular disorders result from its antagonist properties at histamine H₃ receptors (H₃Rs). However, H₃Rs exhibit constitutive activity, and most H₃R antagonists act as inverse agonists. Here, we have investigated the effects of betahistine at recombinant H₃R isoforms. On inhibition of cAMP formation by betahistine in rat and human, we then investigated the effects of betahistine on histamine neuron activity by measuring "tele-methylhistamine" (t-MeHA) levels in the brains of mice. Its acute intraperitoneal administration increased t-MeHA levels with an ED₅₀ of 0.4 mg/kg, indicating inverse agonism. At higher doses, t-MeHA levels gradually returned to basal levels, a profile probably resulting from agonism. After acute oral administration, betahistine increased t-MeHA levels with an ED₅₀ of 2 mg/kg, a rightward shift probably caused by almost complete first-pass metabolism. In each case, the maximal effect of betahistine was lower than that of ciproxifan, indicating partial inverse agonism. After an oral 8-day treatment, the only effective dose of betahistine was 30 mg/kg, indicating that a tolerance had developed. These data strongly suggest that therapeutic effects of betahistine result from an enhancement of histamine neuron activity induced by inverse agonism at H₃ autoreceptors.

Introduction

The histamine H₃ receptor (H₃R) initially was characterized as an autoreceptor regulating histamine release in brain (Arrang et al., 1983). Its coupling to Gₛ proteins was confirmed by its cloning in human (Lovenberg et al., 1999). Activation of recombinant H₃Rs inhibits adenylate cyclase (Lovenberg et al., 1999) and activates phospholipase A₂ (Morisset et al., 2000a). In different species, including human, various functional isoforms are generated by deletion of a pseudo-intron, variable in length and located in the third intracellular loop of the H₃R (Hancock et al., 2003).

Recombinant H₃Rs exhibit high constitutive activity, and most antagonists act in fact as inverse agonists on various responses (Arrang et al., 2007). Prototypic antagonists themselves, such as thioperamide or ciproxifan (CPX), act as inverse agonists and abrogate constitutive activity (Morisset et al., 2000a). Moreover, constitutive activity of the recombinant H₃R depends on species, isoforms, cell lines, and signaling pathways (Arrang et al., 2007), but all of the data are consistent with high constitutive activity of the rat H₃R (rH₃R) and human H₃R (hH₃R).

Consistent with the physiological relevance of the phenomenon, we demonstrated high constitutive activity of native H₃Rs in rodent brain in vitro (Morisset et al., 2000a; Rouleau et al., 2002). Moreover, in vivo, H₃R-inverse agonists enhance "tele-methylhistamine" (t-MeHA) levels, a reliable index of histamine neuron activity, by abrogating the brake triggered by constitutive activity of H₃ autoreceptors (Morisset et al., 2000a; Gbahou et al., 2003). The recombinant human H₃Rs expressed at moderate densities also display constitutive activity, suggesting it is present in human brain (Rouleau et al., 2002).

Betahistine is an orally active drug that has been extensively used in the symptomatic treatment of vestibular dis-
orders such as Meniere’s disease (Canty and Valentine, 1981; Oosterveld, 1984). However, the molecular mechanisms underlying its therapeutic effects remain unclear, inasmuch as its first metabolite may also display some activity (Fossati et al., 2001). We have reported previously in the rat that betahistine behaves as a H3R antagonist with moderate potency at autoreceptors modulating histamine release in vitro (Arrang et al., 1985), and we suggested that this property, coupled to moderate H3 receptor agonist activity, might account for the beneficial effects of the compound in the treatment of vertigo. In agreement, the systemic administration of tiotep- amide results in a strong vestibulopelagic effect and strongly depresses the horizontal vestibular-ocular reflex gain (Yabe et al., 1993). It also accelerates the recovery of behavioral functions in the cat after unilateral vestibular neuroectomy (Tighilet et al., 2006, 2007).

The antagonist property of betahistine that we initially reported at H3 autoreceptors (Arrang et al., 1985) was established on histamine release induced by a low stimulus, that is, when the H3 autoreceptor displayed no apparent constitutive activity (Morisset et al., 2000a). However, the improvement of vestibular compensation induced in the cat by betahistine was accompanied by an enhancement of histidine decarboxylase mRNA expression in histamine perikarya (Tighilet et al., 2007), thereby suggesting that betahistine was in fact acting as an H3R inverse agonist.

In the present study, we first investigated the in vitro activity of betahistine at recombinant rH3(413)R and rH3(445)R isoforms mediating inhibition of cAMP formation and [3H]arachidonic acid release in CHO cells. The H3R displays constitutive activity on these two functional responses (Morisset et al., 2000a; Rouleau et al., 2002); therefore, the whole spectrum of drug activity, ranging from agonism to inverse agonism, can be explored. In addition, because H3Rs show important species-related pharmacological differences (Leurs et al., 2005), and betahistine had not yet been studied at human H3Rs, we compared the profiles of betahistine at recombinant human and rat H3(445)Rs in the same tests. We have investigated the in vivo activity of betahistine on histamine neuron activity, by measuring t-MeHA levels, a reliable index of this activity, in the brains of mice after acute intraperitoneal administration and acute or repeated oral administration.

Materials and Methods

Stable Transfection of CHO-K1 Cells. The clones used in this study were obtained as described previously (Rouleau et al., 2002). cDNA inserts corresponding to the full-length coding sequences of the H3R, and hH3R isoforms were ligated into the mammalian expression vector pCIneo (Promega, Charbonnières, France). CHO-K1 cells were transfected by using SuperFect (QIAGEN S.A., Courtaboeuf, France). Stable transfectants were selected with 2 mM Nut mix F-12 containing 0.2% bovine serum albumin (BSA). After washings, cells were incubated for 30 min with 2 μM C[125I]iodoproxyfan ([125I]iodoproxyfan alone (total binding) or together with betahistine at increasing concentrations (200 μM, final volume). The nonspecific binding was determined in the presence of the selective H3R agonist imetit (1 μM). The specific binding was calculated from the difference between the total binding and nonspecific binding and represented ~80% of the total binding.

cAMP Formation. CHO(rH3(445)R), CHO(rH3(413)R), and CHO(hH3(445)R) cells expressing 200 to 700 fmol/mg protein were incubated in 96-well plates for 10 min at 37°C with 3 μM forskolin (FSK), the adenylyl cyclase activator, and when required, betahistine at increasing concentrations in Dulbecco’s modified Eagle’s medium-Nut mix F-12 (Invitrogen) containing 0.1 M isobutyl-methyl-xantine. The effects of betahistine were compared with those of histamine (10 μM), the natural agonist, and tiotepamide (10 μM), a potent and selective inverse agonist (Morisset et al., 2000a). cAMP was extracted and measured by radioimmunoassay.

[3H]Arachidonic Acid Release. CHO(rH3(445)R), CHO(rH3(413)R), and CHO(hH3(445)R) cells expressing 200 to 700 fmol/mg protein were incubated for 2 h at 37°C with 0.5 μM of [3H]arachidonic acid in Dulbecco’s modified Eagle’s medium-Nut mix F-12 containing 0.2% bovine serum albumin. After washings, cells were incubated for 30 min with 2 μM C125I-iodoproxyfan alone (total binding) or together with betahistine at increasing concentrations, histamine, or tiotepamide (10 μM). [3H]Arachidonic acid release was determined by liquid scintillation counting.

Determination of t-Methylhistamine Levels in Brain.

Drugs were dissolved in 1% methylcellulose or saline solution (0.9% w/v) NaCl for oral and intraperitoneal administration, respectively, to male Swiss mice (18–20 g) (Iffa-Credo, L’Arbresle, France). After treatment, animals were sacrificed by decapitation. The total brain was dissected out and homogenized in 10 volumes (w/v) of ice-cold perchloric acid (0.4 N). The clear supernatant was stored at –20°C immediately after centrifugation (4,000 g for 20 min). t-MeHA levels were determined by using an enzyme immunoassay derived from a radioimmunoassay described previously (Garbarg et al., 1989a, 1992). In brief, t-MeHA of the sample was derivatized with p-benzoquinone (BZQ) (2.5 mg/ml). The reaction was allowed to proceed at pH 7.9 for 3 h, then 2 M glycine was added to eliminate the excess of BZQ. The derivatized extract was mixed with t-MeHA-BZQ-Leu-Tyr-acetylcholinesterase as a tracer and an antiserum raised in rabbits against the tracer. The reaction was allowed to proceed at 37°C and the optical density was measured with a Dynatech MR5000 (Dynex Technologies, Chantilly, VA) at 405 nm. The limit of the detection was 5 pg of t-MeHA.

Analysis of Data. In binding experiments, IC50 values of beta-histine were determined with an iterative least-squares method derived from that of Parker and Waud (1971). The apparent affinity (Ki values) of betahistine were calculated from its IC50 values, assuming a competitive antagonism and by using the relationship (Cheng and Prusoff, 1973) Ki = IC50/1 + (S/Ks), where S represents the concentration (20 μM) and Ks is the apparent dissociation constant of [125I]iodoproxyfan at the rH3(445)R (85 ± 4 pM) and hH3(445)R (82 ± 3 pM).

In functional assays, EC50 values of betahistine corresponding to stimulatory and inhibitory phases of its biphasic effects were evaluated by nonlinear regression with Prism software (GraphPad Software, Inc., San Diego, CA). The following equation was fitted to the bell-shaped concentration-response curves: Y = Dip + Sec-
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Fig. 1. Inhibition of specific $[^{3}H]$iodoproyxfan binding to human and rat $H_3$ receptors by betahistine. Membranes of CHO(rH$_3^{(445)R}$), CHO (rH$_3^{(413)R}$), and CHO(hH$_3^{(445)R}$) cells were incubated with 20 pM $[^{3}H]$iodoproyxfan and betahistine in increasing concentrations. Each point represents the mean value from three different experiments with triplicate determinations.

TABLE 1

<table>
<thead>
<tr>
<th>Test System</th>
<th>rH$_3^{(445)R}$</th>
<th>rH$_3^{(413)R}$</th>
<th>hH$_3^{(445)R}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[^{3}H]$iodoproyxfan binding ($K_i$)</td>
<td>1.4 ± 0.1 μM</td>
<td>9 ± 2 μM*</td>
<td>2.5 ± 0.3 μM</td>
</tr>
<tr>
<td>cAMP accumulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inverse agonist (EC$_{50}$)</td>
<td>0.1 ± 0.1 nM (+32 ±7%)</td>
<td>0.05 ± 0.15 nM (+17 ±4%)</td>
<td>0.3 ± 1.4 nM (+15 ±4%)</td>
</tr>
<tr>
<td>Agonist (EC$_{50}$)</td>
<td>0.1 ± 0.1 μM (−21 ±3%)</td>
<td>≥15 ± 1 μM (−73 ±2%)</td>
<td>≥48 ± 2 μM (−70 ±3%)</td>
</tr>
<tr>
<td>$[^{3}H]$arachidonic acid release</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inverse agonist (EC$_{50}$)</td>
<td>0.1 ± 0.1 nM (−47 ±5%)</td>
<td>0.06 ± 0.23 nM (−16 ±5%)</td>
<td>0.6 ± 0.3 nM (−25 ±3%)</td>
</tr>
<tr>
<td>Agonist (EC$_{50}$)</td>
<td>7 ± 1 μM (+41 ±11%)</td>
<td>4 ± 1 μM (+51 ±7%)</td>
<td>≥65 ± 20 μM (±80 ±31%)</td>
</tr>
</tbody>
</table>

* IC$_{50}$ ($n_H = 0.5 ± 0.1$).
biphasic response was observed at the rat H₃(445)R in the presence of increasing concentrations of betahistine. At low concentrations, betahistine progressively reduced A23187-evoked [³H]arachidonic acid release (EC₅₀ = 0.1 ± 0.1 nM) with a maximal effect, observed up to 30 nM, similar to that of thioperamide (−47 ± 5 and −44 ± 4%, respectively; p < 0.001 versus A23187 alone). At concentrations higher than 30 nM, betahistine progressively enhanced the release with an EC₅₀ value of 7 ± 1 μM and full agonist activity, its maximal effect being similar to that of histamine (+41 ± 11% versus +48 ± 6%; p < 0.01 versus A23187 alone) (Fig. 2, C and D; Table 1). At this rat isoform, betahistine therefore had the same inverse agonist potency on cAMP formation and [³H]arachidonic acid release (EC₅₀ of 0.1 ± 0.1 and 0.1 ± 0.1 nM, respectively), whereas its agonist potency, like that of histamine, was lower on [³H]arachidonic acid release (EC₅₀ of 7 ± 1 μM versus 0.1 ± 0.1 μM; Table 1).

In CHO cells expressing the short rat H₃(413)R isoform, the same biphasic patterns were observed, both on cAMP accumulation and [³H]arachidonic acid release (Fig. 3). Betahistine increased in a dose-dependent manner the concentration of cAMP accumulation (EC₅₀ = 0.05 ± 0.15 nM) to reach a maximal effect, observed up to 1 nM, similar to that of thioperamide (+17 ± 4% versus +22 ± 2%, p < 0.05 versus FSK alone) (Fig. 3, A and B; Table 1). At concentrations higher than 1 nM betahistine behaved as histamine and inhibited cAMP accumulation by 73% at the highest concentration tested (300 μM). An EC₅₀ value of 15 ± 1 μM was obtained for betahistine acting as an agonist at CHO(rH₃(445)R) cells, assuming that the drug was acting as a full agonist (Table 1). This agonist effect was again entirely blocked by 10 μM thioperamide (3.9 ± 0.7 pmol cAMP versus 11.9 ± 1.2 pmol cAMP, respectively; p < 0.001).

On [³H]arachidonic acid release from CHO(rH₃(445)R) cells betahistine displayed a similar biphasic effect. It behaved as a full inverse agonist up to 3 nM (−16 ± 5% versus −20 ± 2% for thioperamide; p < 0.05 versus A23187 alone). Its agonist effect reached a plateau corresponding to 51% of the maximal effect of histamine at 100 μM, suggesting that betahistine was acting as a partial agonist (Fig. 3, C and D). EC₅₀ values
of 0.06 ± 0.03 nM and 4 ± 1 µM were obtained on this response at CHO(rH3(413)R) cells for betahistine acting as an inverse agonist and partial agonist, respectively (Table 1). At this short rat isoform, betahistine had a similar potency on cAMP formation and [3H]arachidonic acid release, not only as an inverse agonist (EC50 of 0.05 ± 0.15 and 4 ± 1 nM, respectively), but also as an agonist (EC50 ≥ 15 ± 1 and 4 ± 1 µM, respectively), in contrast to histamine itself, which remained less potent on [3H]arachidonic acid release than on cAMP formation at this isoform (EC50 of 110 ± 32 nM versus 15 ± 4 nM; not shown).

Functional Properties of Betahistine at the Human H3(445)R Isoform. Histamine at a maximal concentration (1–10 nM) significantly inhibited cAMP formation induced by 3 µM FSK in CHO(hH3(445)R) cells (−91 ± 1%; n = 9 experiments). In contrast, thioperamide (10 µM) enhanced FSK-induced-cAMP accumulation in CHO(hH3(445)R) cells (+20 ± 4%; n = 9 experiments), revealing the constitutive activity of the human H3(445)R (Fig. 4). Increasing concentrations of betahistine promoted the biphasic dose–response curve shown at the recombinant rat H3 isoforms (Fig. 4, A and B). When added at low concentrations, betahistine mimicked the effect of thioperamide, thereby behaving as an apparent inverse agonist, and progressively enhanced cAMP formation (EC50 = 0.3 ± 1.4 nM) with a maximal effect, observed up to 0.1 µM, similar to that of thioperamide (+15 ± 4% versus +20 ± 4%; p < 0.05 versus FSK alone). In contrast, at concentrations higher than 0.1 µM the effect of betahistine was similar to that observed for histamine, with a progressive inhibition of cAMP formation that reached 70% at the highest concentration tested (300 µM) (Fig. 4B). An EC50 value of 48 ± 2 µM was obtained, assuming that betahistine was acting as a full agonist (Table 1). As expected, this agonist effect was entirely blocked by 10 µM thioperamide, because thioperamide tested against betahistine increased cAMP formation with an amplitude (+22%) similar to that observed when it was used alone (Fig. 5A).

The effects of betahistine on cAMP formation were not observed in CHO cells transfected with the empty vector (mock cells) (Fig. 5B). In another set of experiments, the effects of betahistine on cAMP formation were studied at its highest doses as inverse agonist (1 nM) and agonist (100 µM)
without or with PTX. In the absence of PTX the biphasic effect of betahistine was observed (Fig. 5C), but both the inverse agonist and the agonist effects of betahistine were completely abolished in the presence of PTX (Fig. 5D).

On [3H]arachidonic acid release, histamine used as an agonist at a maximal concentration (1–10 μM) strongly enhanced [3H]arachidonic acid release evoked by the Ca²⁺ ionophore A23187 from CHO(hH3(445)R) cells (4452 ± 278 dpm; n = 19 experiments). In contrast, the inverse agonist thioperamide (10 μM) significantly decreased A23187-evoked [3H]arachidonic acid release (−31 ± 3%; n = 21 experiments), revealing constitutive activity of the human H₃R in this test system. Increasing concentrations of betahistine promoted a biphasic dose–response curve in CHO(hH3(445)R) cells (Fig. 4, A and B; Table 1). When added at low concentrations, betahistine behaved as an apparent inverse agonist and progressively decreased [3H]arachidonic acid release (EC₅₀ = 0.1 ± 0.3 nM) with a maximal effect, observed up to 30 nM, similar to that of thioperamide (−25 ± 3% versus −31 ± 3%; p < 0.01 versus A23187 alone). In contrast, at concentrations higher than 30 nM, the effect of betahistine on CHO(hH3(445)R) cells was similar to that observed for histamine, with a progressive increase in A23187-evoked [3H]arachidonic acid release that reached 180 ± 31% at the highest concentration tested (300 μM). An EC₅₀ value ≥65 ± 20 μM was obtained for betahistine acting as an agonist, assuming that the drug was acting as a full agonist. The stimulation induced by betahistine was entirely blocked by a maximal dose of thioperamide, acting as an agonist ciproxifan, used at the maximal dose of 3 mg/kg as a control, enhanced t-MeHA levels by 89% (31% at the highest concentration tested) in a dose-dependent manner (Fig. 6). At low doses, t-MeHA levels progressively increased (ED₅₀ = 0.4 ± 0.1 mg/kg) to reach a peak of ~30% (p < 0.05) after administration of 1 mg/kg. After intraperitoneal administration of higher doses of betahistine, the enhancement became gradually lower with t-MeHA levels returning to control values at 10 to 30 mg/kg (Fig. 6).
A single oral administration of ciproxifan (3 mg/kg) enhanced t-MeHA levels by 117 ± 28% (215 ± 22 versus 99 ± 17 ng/g; n = 8 mice). The acute oral administration of beta-histine increased t-MeHA levels in a dose-dependent manner (Fig. 7) with an ED$_{50}$ of 2 ± 1 mg/kg and a maximal effect of ~35% reached at 30 mg/kg.

Repeated oral administration of ciproxifan (3 mg/kg/day for 8 days) was followed 90 min after the last administration by a significant increase (+55%) of brain t-MeHA levels (Fig. 7). Repeated oral administration of the standard agonist (R)-α-MeHA (3 mg/kg/day for 8 days) did not change t-MeHA levels. Repeated oral administration of beta-histine at 3 mg/kg/day for 8 days had no significant effect, but beta-histine at 30 mg/kg/day for 8 days significantly increased t-MeHA levels by 37 ± 13% (Fig. 7).

**Discussion**

At recombinant H3Rs beta-histine behaved not only as a potent inverse agonist, but also as an agonist at higher concentrations. These inverse agonist and agonist effects both were mediated by H3Rs. They were found on a negatively (cAMP accumulation) and positively ([3H]arachidonic acid release) coupled response. They were not detected in mock cells, but both were found in all rat and human isoforms. They were both suppressed by PTX, in agreement with coupling of H3Rs to G$_i/o$ proteins. This wide spectrum of activity did not result from protean agonism (Kenakin, 1995), because, in contrast to proxifyan (Gbahou et al., 2003), it occurred at the same level of constitutive activity. It did not result from a switch in coupling from G$_i$ to G$_o$ proteins, such as that reported with agonists at muscarinic receptors (Michal et al., 2001), because opposite effects of beta-histidine would have been observed. Moreover, their PTX sensitivity shows that the two responses are mediated by G$_i/0$ proteins. Further studies are required to know which G$_i/0$ subunits are involved, inasmuch as the hH3 445 R couples similarly to different G$_i/0$ subunits (Schnell et al., 2010).

The multistate model of G protein-coupled receptors (Kenakin, 1995; Gbahou et al., 2003; Arrang et al., 2007) more likely accounts for these biphasic effects. Beta-histidine would first stabilize inactive conformations, thereby inducing inverse agonism. At higher concentrations, beta-histidine would...
iodoproxyfan. Nevertheless, betahistine displaced high affinity for betahistine are not expected to be labeled by zomo et al., 2007). Therefore, inactive conformations with [125I]iodoproxyfan (Witte et al., 2006; Yao et al., 2006; Mez-

H3R (Krueger et al., 2005; Schnell et al., 2010), a selective ligand selectivity. Conformations selective for a given ago-

1990). Moreover, the mean IC50 of betahistine at this coupled agonist states of this short isoform (Arrang et al.,

3 mg/kg of betahistine was surpris-

Fig. 6. t-MeHA levels in mouse brain after acute intraperitoneal admin-

Fig. 7. Changes in t-MeHA levels induced in mouse brain by acute (left) or repeated (right) oral administration of betahistine. Mice were treated acutely or for 8 days with saline, 3 mg/kg CPX, (R)-α-MeHA, or betahistine at increasing doses. Animals were sacrificed 90 min after the acute or last administration. Means ± S.E.M. of values from six to eight (acute) and 10 to 20 (repeated) animals. *, p < 0.05 versus saline.
being reduced by half after 8-day oral administration. After 8
days, the increase induced by oral administration of betahistine
was only found at the high dose of 30 mg/kg, suggesting either
that a tolerance had developed for betahistine as an inverse
agonist or that betahistine at such a high dose was acting as an agonist. Theoretically, repeated administration
of an agonist may desensitize autoreceptors, relieve the
brake, and lead to an enhancement of t-MeHA levels. (R)-α-
methylhistamine itself induced a desensitization, because its
repeated administration failed to decrease histamine neuron
activity. However, it also failed to enhance t-MeHA levels,
confirming that the increase induced by repeated administra-
tion of betahistine at 30 mg/kg was not caused by desen-
sitization of autoreceptors by its agonist effect. Therefore,
metabolism and tolerance made a high dose necessary to
induce inverse agonism after repeated oral administration of
betahistine.

Overall, our data show that betahistine acts as an inverse
agonist to increase histamine neuron activity. In agreement,
Tighilet et al. (2002, 2005) reported that betahistine given
orally to cats increased histidine decarboxylase mRNA ex-
pression in the tuberomammillary nucleus. Moreover, in
mice (the present study) and cats, the increase in histamine
neuron activity was observed after a 1- to 3-week treatment,
i.e., when lesioned animals treated by betahistine recover
their vestibular functions (Tighilet et al., 2007).

The present study confirms that human H3Rs display con-
stitutive activity (Rouleau et al., 2002). However, whether
inverse agonism and enhancement of histamine neuron activity
account, even partially, for the therapeutic effects of betahistine
requires further investigations, including positron emission to-
mography studies. After 1-week oral administration, the effec-
tive doses of betahistine in mice were higher than the ther-
apogetic dose (2 mg/kg) given, generally twice a day, to
vestibular-defective patients. However, the dose–response
curve of betahistine on histamine neuron activity in lesioned
animals is unknown. Its ED50 may be lower than in controls,
because imbalanced vestibular activity itself activates hista-
mine neurons (Tighilet et al., 2006, 2007) and the effect of the
drug may synergize with this compensatory activation. More-
over, in clinical practice, this therapeutic dose of 2 mg/kg
is given for several months. In agreement with our findings,
Tighilet et al. (2006, 2007) reported that this dose had no
effect on histidine decarboxylase mRNA expression in control
cats after a 1- or 3-week treatment. However, the same low
dose acquired an enhancing effect after 3 months (Tighilet et
al., 2005). This suggests either that betahistine progressively
accumulates into the brain or simply that a low dose given
over a long period of time yields the therapeutic effect. Its
activation of histamine neurons indicates that betahistine
enters the rodent brain after systemic administration, even
though its effective doses in vivo are rather high compared
with its subnanomolar inverse agonist potency in vitro. In
addition, the effect of betahistine may be enhanced and pro-
longed in time by its first metabolite [(2–2-aminoethyl)-pyri-
dine], which has the same affinity at rodent H3R binding
sites (Fossati et al., 2001). Although we did not investigate
the functional properties of this metabolite, it should be
emphasized that its presence, presumably predominant after
oral and repeated administration, renders even more com-
plex the interpretation of in vivo and therapeutic effects of
betahistine.

In conclusion, betahistine interacts in vitro with H3Rs as a
potent inverse agonist, a moderate antagonist (Arrang et
al., 1985), and a weak agonist. It acts in vivo as a partial inverse
agonist to enhance histamine neuron activity, an effect prob-
ably involved in therapeutics. Therefore, although H3 hetero-
receptors present on other neurons regulating vestibular
functions may also be involved (Lozada et al., 2004; Chavez
et al., 2005; Bergquist et al., 2006), this study strongly sug-
gests that H3 autoreceptors regulating histamine neurons in
human brain constitute a major target under betahistine
treatment.

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the functional properties of this metabolite, it should be
emphasized that its presence, presumably predominant after
oral and repeated administration, renders even more com-
plex the interpretation of in vivo and therapeutic effects of
betahistine.


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