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Dynamics of histamine H_3 receptor antagonists on brain histamine metabolism: do all histamine H_3 receptor antagonists act at a single site?

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Abstract

Thioperamide, the prototypical histamine H_3 receptor antagonist, acts at the brain histamine H_3 autoreceptor to promote the release and metabolism of neuronal histamine, resulting in higher brain levels of the metabolite tele-methylhistamine. However, unlike thioperamide, several new histamine H_3 receptor antagonists enter the central nervous system (CNS), block brain histamine H_3 receptors and increase histamine release without increasing brain tele-methylhistamine levels. Experiments were performed presently in an attempt to understand these results. Consistent with previous findings, thioperamide significantly increased the content and synthesis rate of tele-methylhistamine in mouse and rat brain. In contrast, the histamine H_3 receptor antagonists GT-2227 (4-(6-cyclohexylhex-*cis*-3-enyl)imidazole) and clobenpropit did not affect tele-methylhistamine synthesis rate in mouse whole brain. The histamine H_3 receptor ligand GT-2016 (5-cyclohexyl-1-(4-imidazol-4-ylpiperidyl)pentan-1-one) had no effect on tele-methylhistamine levels in any rat brain region and *decreased* tele-methylhistamine synthesis rates in the mouse whole brain. To examine the possibility that these histamine H_3 receptor antagonists might prevent the methylation of newly released histamine, they were co-administered with thioperamide to determine their effects on the thioperamide-induced stimulation of tele-methylhistamine synthesis. GT-2016 significantly reduced the thioperamide-induced activation of tele-methylhistamine synthesis in mouse whole brain and in several regions of rat brain. Although further clarification is needed, these results suggest that some histamine H_3 receptor antagonists may promote the release of neuronal histamine, but also act to reduce histamine methylation in vivo by an unknown mechanism.

Keywords: Histamine; Histamine H_3 receptor; Histamine turnover; Thioperamide; Histamine *N*-methyltransferase; Methylation

1. Introduction

The histaminergic system, comprised of neurons in the posterior hypothalamus which project throughout the brain, may play a role in many brain processes, including cognition, epilepsy, sleep–wake cycles, obesity, and Alzheimer's disease (Hough, 1999). Understanding the mechanisms that control histamine dynamics in the brain may therefore be of therapeutic value (Leurs et al., 1998).

Of the four histamine receptors in the central nervous system (CNS) that have been cloned (histamine H_1, H_2, H_3, and H_4 receptors (Hill et al., 1997; Leurs et al., 1995a; Lovenberg et al., 1999; Nakamura et al., 2000)), the histamine H_3 receptor may be expressed both post-synaptically (as a heteroreceptor) and pre-synaptically (as an autoreceptor). In 1987, the histamine H_3 receptor antagonist thioperamide was introduced (Arrang et al., 1987). Thioperamide acts at the brain histamine H_3 autoreceptor to promote the release of histamine both in vivo (Mochizuki et al., 1991; Itoh et al., 1991; Barke and Hough, 1994; Jansen et al., 1998) as well as in vitro (Arrang et al., 1987; Ligneau et al., 1998). Released histamine is then inactivated by histamine *N*-methyltransferase to form tele-methylhistamine. Hence, the thioperamide-induced increase in histamine release in vivo leads to higher brain levels of tele-methylhistamine (Yates et al., 1999; Oishi et al., 1989; Ligneau et al., 1998).

The last decade has produced many new, potent histamine H_3 receptor antagonists, which, unlike thioperamide, do not increase brain tele-methylhistamine levels. In fact, compounds reported to have potent histamine-releasing properties in vitro were assumed to have poor bioavailibility if they did not increase brain tele-methylhistamine levels in vivo (Ganellin et al., 1996, 1998). However, several new histamine H_3 receptor antagonists,

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such as clobenpropit (Van der Goot et al., 1992), GT-2016 (5-cyclohexyl-1-(4-imidazol-4-ylpiperidyl)pentan-1-one) (Tedford et al., 1995), and GT-2227 (4-(6-cyclohexylhex-*cis*-3-enyl)imidazole) (Tedford et al., 1998) were shown to enter the CNS, bind to brain histamine H_3 receptors (Mochizuki et al., 1996; Ligneau et al., 1998; Tedford et al., 1995; Yates et al., 1999), and promote the release of histamine (Jansen et al., 1998; Tedford et al., 1995). Contrary to thioperamide, however, GT-2016 and GT-2227 do not increase brain tele-methylhistamine levels (Yates et al., 1999). Studies of the effect of clobenpropit on tele-methylhistamine levels have reached different conclusions which may be due to differences in the route of drug administration (i.p. vs. p.o.) and/or assay methods (radioimmunoassay vs. gas chromatography-mass spectroscopy (GCMS)) (Yates et al., 1999; Ligneau et al., 1998). Thus, it seems that all brain-penetrating histamine H_3 receptor antagonists do not increase brain tele-methylhistamine levels, but the reasons for this remain unclear. Presently, we have extended studies of the effects of several histamine H_3 receptor antagonists on brain histamine metabolism. We hypothesized that if GT-2016, GT-2227, and clobenpropit act at a site in addition to the histamine H_3 autoreceptor to prevent an increase in tele-methylhistamine levels, then these histamine H_3 receptor antagonists would also inhibit the increase in tele-methylhistamine levels observed after thioperamide administration.

2. Materials and methods

2.1. Animals

Male Swiss–Webster mice (30–45 g, Taconic Farms, Germantown, NY) were housed in groups of five or six and male Sprague–Dawley rats (200–300 g, Taconic Farms) were housed in groups of three or four with food and water freely available. They were maintained on a 12-h light–dark cycle (lights on at 7:00 a.m., lights off at 7:00 p.m.). All experiments were conducted between 9:00 a.m. and 3:00 p.m. All animal procedures were approved by the Institutional Animal Care and Use Committee of Albany Medical College.

2.2. Animal treatments

Subjects received various combinations of saline, thioperamide (base), GT-2016 (maleate salt), GT-2227 (maleate salt), or clobenpropit (hydrobromide salt) by i.p. injection. In some experiments, pargyline hydrochloride was simultaneously administered by a separate i.p. injection, along with the histamine H_3 receptor antagonists in order to completely inhibit tele-methylhistamine metabolism by monoamine oxidase and estimate tele-methylhistamine synthesis rates (Hough et al., 1984). Gliatech (Cleveland, OH) kindly provided all histamine H_3 receptor antagonists, with the exception of clobenpropit, which was kindly provided by Dr. H. Timmerman (Vrije Universiteit, Amsterdam).

Rats and mice were euthanized with pentobarbital (80 and 100 mg/kg, i.p., respectively) 55 and 85 min, respectively, post-injection and decapitated 5 min later. Brains were rapidly removed and placed on ice. Rat brains were dissected into the cerebral cortex, hypothalamus, midbrain, thalamus, caudate, hippocampus, brain stem, and brain remainder. Rat brain regions and mouse whole brains were homogenized in three to six volumes of 0.4 N $HClO_4$ and frozen (-20 °C) until assayed. Prior to analysis, samples were thawed and centrifuged at $20{,}000 \times g$ for 20 min.

2.3. GCMS measurements of tele-methylhistamine

Supernatant fractions were assayed for tele-methylhistamine as described previously (Hough et al., 1981), except that trideuteromethylhistamine was used as the internal standard. Briefly, fractions were made alkaline, extracted with *n*-butanol-chloroform (1:1), back-extracted with HCl (0.01 N) and evaporated to dryness. Residues were derivatized with heptafluorobutyric anhydride and pyridine. Derivatives were extracted into toluene and assayed by selected ion monitoring of m/e 304 and 307 for tele-methylhistamine and its internal standard, respectively. Ions 517 and 520 were also monitored as confirming ions. Gas chromatography was performed with an HP6890 GC operating in splitless mode with a temperature-programmed DB-5MS column (30 m, 0.25 mm i.d., 0.1 μm film thickness, helium). Electron impact mass spectra were obtained with an HP5973 mass selective detector at -70 eV.

2.4. Data analysis

Data were analyzed by analysis of variance (ANOVA) followed by Newman–Keuls post-hoc analyses, where appropriate, with Statistica scientific software (StatSoft, Tulsa, OK).

3. Results

Pargyline, a monoamine oxidase inhibitor, significantly raised mouse whole brain levels of tele-methylhistamine from 120 to 220 ng/g 90 min after administration, indicating a rate of tele-methylhistamine synthesis of 0.52 nmol/g h (Fig. 1). Thioperamide increased tele-methylhistamine steady-state levels from 120 to 158 ng/g over the same interval. Thioperamide also increased the overall rate of tele-methylhistamine synthesis from 0.52 to 0.77 nmol/g h as measured after co-administration with pargyline.

GT-2016 (10 or 30 mg/kg) had no effect on steady-state tele-methylhistamine levels in mouse whole brain (Fig. 2). In the presence of pargyline, GT-2016 significantly *de-*

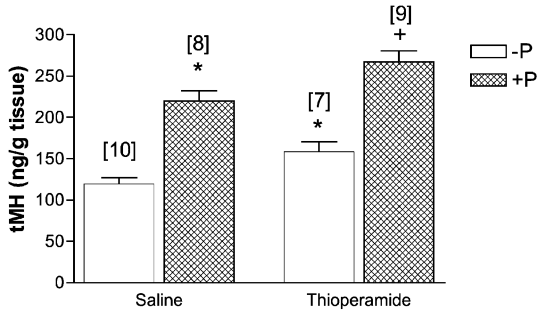

Fig. 1. Effects of pargyline and thioperamide on tele-methylhistamine levels in the mouse whole brain. Saline or thioperamide (10 mg/kg i.p.), along with a second injection of either saline or pargyline (±P, 65 mg/kg i.p.), were administered 90 min before animals were decapitated. Tele-methylhistamine levels are expressed in ng/g of wet tissue (mean ± S.E.M. for *n* values shown). Separate saline/saline treatment groups from experiments shown here and in Figs. 2 and 3 gave tele-methylhistamine values not different from each other and were pooled. These pooled data are shown here and in Figs. 2 and 3. $^{*}P < 0.001$ indicates a significant increase in tele-methylhistamine levels as compared with the saline (−P) treatment group. $+P < 0.01$ indicate a significant thioperamide-induced increase in tele-methylhistamine levels vs. the saline (+P) treatment group.

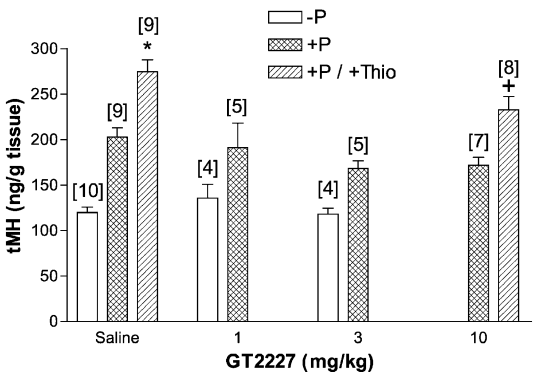

Fig. 3. Effects of GT-2227 on tele-methylhistamine levels in the presence and absence of pargyline and on thioperamide-induced stimulation of tele-methylhistamine synthesis in the mouse whole brain. Saline or GT-2227, ±thioperamide (Thio, 10 mg/kg i.p.), and ±pargyline (±P, 65 mg/kg i.p.) were administered 90 min before animals were decapitated. Tele-methylhistamine levels are expressed in ng/g of wet tissue (mean ± S.E.M. for *n* values shown). $^{*}P < 0.001$ indicates significant difference from the saline (+P) group. $+P < 0.05$ indicates significant difference from the saline (+P/+Thio) group.

creased mouse brain tele-methylhistamine levels (Fig. 2). Also in the presence of pargyline, GT-2016 (30 mg/kg) significantly antagonized thioperamide's stimulating action on tele-methylhistamine levels in mouse brain. GT-2016 had no effect on tele-methylhistamine levels in the presence of pargyline in rat regional brain studies (see below).

GT-2227 (1 or 3 mg/kg) had no effect on tele-methylhistamine steady-state levels in mouse whole brain (Fig. 3). This drug in doses up to 10 mg/kg also had no significant effect on tele-methylhistamine levels in the presence of pargyline. However, in the presence of thioperamide, GT-2227 (10 mg/kg) significantly reduced tele-methylhistamine synthesis in mouse brain.

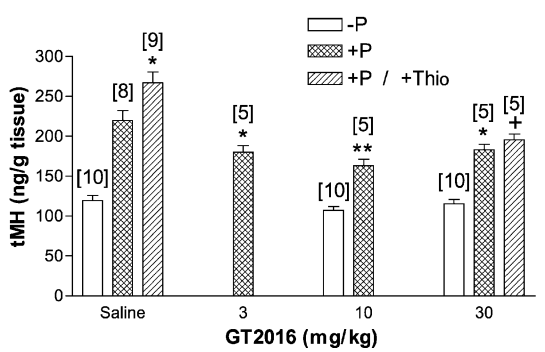

Fig. 2. Effects of GT-2016 on tele-methylhistamine levels in the presence and absence of pargyline and on thioperamide-induced stimulation of tele-methylhistamine synthesis in the mouse whole brain. Saline or GT-2016, ±thioperamide (Thio, 10 mg/kg i.p.), and ±pargyline (±P, 65 mg/kg i.p.) were administered 90 min before animals were decapitated. Tele-methylhistamine levels are expressed in ng/g of wet tissue (mean ± S.E.M. for *n* values shown). $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant differences from the saline (+P) group. $+P < 0.01$ indicates significant difference from the saline (+P/+Thio) group.

Clobenpropit had no effect on tele-methylhistamine levels in the presence of pargyline over a large range of doses (0.01–10 mg/kg) in mouse whole brain (Fig. 4). In contrast to GT-2016 and GT-2227, clobenpropit (10 mg/kg) also had no effect on tele-methylhistamine synthesis in the presence of thioperamide.

Rat regional studies quantified the effect of GT-2016 and thioperamide administered alone, and together, on tele-methylhistamine synthesis (Table 1). A three-factor ANOVA (thioperamide, GT-2016, brain region) revealed main effects of thioperamide ($P < 0.001$), GT-2016 ($P < 0.01$), and region ($P < 0.001$). In addition, significant

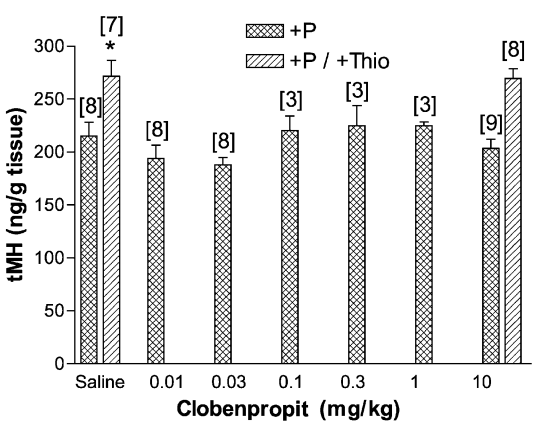

Fig. 4. Effects of clobenpropit on tele-methylhistamine levels in the presence of pargyline and on thioperamide-induced stimulation of tele-methylhistamine synthesis in the mouse whole brain. All subjects received pargyline (65 mg/kg i.p.), along with clobenpropit, ±thioperamide (Thio, 10 mg/kg i.p.), and were decapitated 90 min later. Tele-methylhistamine levels are expressed in ng/g of wet tissue (mean ± S.E.M. for *n* values shown). $^{*}P < 0.01$, indicates significant difference from the saline (+P) group.

Table 1
Interactions of the H_3 antagonists GT-2016 and thioperamide on tele-methylhistamine synthesis in regions of the rat brain

	Saline	GT-2016	Thioperamide	GT-2016 + Thio	GT-2016 + Thio %Inhibition[a]
Pons–medulla–cerebellum	15 ± 2 (6)	16 ± 4 (7)	33 ± 7 (5)[b]	15 ± 2 (4)[c]	100%
Hypothalamus	298 ± 25 (7)	316 ± 57 (7)	564 ± 115 (7)[b]	341 ± 15 (7)[c]	84%
Thalamus	124 ± 16 (8)	109 ± 8 (8)	169 ± 16 (8)[b]	138 ± 10 (8)	69%
Caudate	115 ± 15 (8)	99 ± 9 (8)	154 ± 9 (7)[b]	133 ± 9 (8)	54%
Remainder	76 ± 7 (8)	67 ± 5 (8)	109 ± 5 (8)[b]	91 ± 10 (8)	55%
Midbrain	57 ± 3 (7)	56 ± 5 (8)	115 ± 13 (8)[b]	85 ± 7 (8)[c]	52%
Hippocampus	62 ± 5 (8)	53 ± 8 (8)	98 ± 13 (8)[b]	86 ± 12 (8)	33%
Cerebral Cortex	94 ± 4 (12)	79 ± 7 (12)	131 ± 11 (12)[b]	121 ± 10 (12)	27%

All treatment groups received pargyline (75 mg/kg, i.p.). In addition, each treatment group received either saline, GT-2016 (10 mg/kg, i.p.), thioperamide (10 mg/kg, i.p.), or both GT-2016 and thioperamide. All injections were administered 60 min before the animals were decapitated. Tele-methylhistamine levels are expressed in ng/g of wet tissue (mean ± S.E.M. for n value shown in parenthesis).

[a] Percent inhibition was derived from the equation:

$$\%\text{Inhib} = \left(1 - \left[(\text{tele-methylhistamine}_{\text{GT+Thio}} - \text{tele-methylhistamine}_{\text{saline}})/(\text{tele-methylhistamine}_{\text{Thio}} - \text{tele-methylhistamine}_{\text{saline}})\right]\right) \times 100\%.$$

[b] $P < 0.05$, indicates a significant difference from the saline treatment group.

[c] $P < 0.01$, indicates a significant difference from the thioperamide treatment group.

interaction terms were found for thioperamide by region ($P < 0.001$), thioperamide by GT-2016 ($P < 0.05$) and thioperamide by GT-2016 by region ($P < 0.01$). Thioperamide significantly raised tele-methylhistamine synthesis rates in all brain regions. GT-2016 (10 mg/kg) had no effect on tele-methylhistamine levels in any rat brain region studied. However, when GT-2016 was co-administered with thioperamide, it significantly inhibited thioperamide-induced elevations in tele-methylhistamine levels in the hypothalamus, midbrain, and medulla–pons–cerebellum.

4. Discussion

4.1. Actions of thioperamide on histaminergic dynamics

A detailed understanding of thioperamide's action on the histaminergic system is necessary for interpreting the present findings. It has been generally accepted that neuronal histamine tonically stimulates the histamine H_3 autoreceptor, such that steady-state extracellular histamine inhibits its own release (Arrang et al., 1983). According to this view, thioperamide acts as a "neutral" histamine H_3 receptor antagonist, interfering with histamine's autoinhibitory action, thereby increasing histamine release. Increases in neuronal histamine release are accompanied by increased synthesis of tele-methylhistamine because neuronal histamine is primarily inactivated by methylation, and tele-methylhistamine levels are closely related to histaminergic activity (Hough et al., 1984). It is clear that thioperamide enters the CNS, binds to histamine H_3 receptors (Ligneau et al., 1998), increases histamine release (Mochizuki et al., 1991; Itoh et al., 1991; Jansen et al., 1998), and raises brain levels of tele-methylhistamine in rats and mice (Ligneau et al., 1998; Oishi et al., 1989; Garbarg et al., 1989). The present effects of thioperamide on steady-state tele-methylhistamine levels and on tele-methylhistamine synthesis rate (Fig. 1, Table 1) are in good agreement with previous findings in mouse (Oishi et al., 1989) and rat (Itoh et al., 1991) brain.

If the above model for thioperamide is correct, then all neutral antagonists of the histamine H_3 receptor should increase histamine release and metabolism. However, this is not the case since Morisset et al. (2000) recently described the existence of a neutral histamine H_3 receptor antagonist capable of entering the brain, blocking thioperamide's effects, but lacking the ability to increase histamine release or metabolism. These findings strongly suggest that normal extracellular histamine levels in the brain are not contributing to the control of histamine release and that thioperamide's stimulation of histamine release and metabolism arise from an inverse agonist action at the histamine H_3 autoreceptor.

4.2. Effects of GT-2016, GT-2227, and clobenpropit on histaminergic dynamics

The histamine H_3 receptor ligands GT-2016, GT-2227, and clobenpropit have been shown to enter the CNS and to bind to histamine H_3 receptors. These compounds have ED_{50} values of 12, 0.6, and 3.9 mg/kg, i.p., respectively, in ex vivo histamine H_3 receptor binding studies of rat cortex (Yates et al., 1999). Also consistent with histamine H_3 receptor antagonist actions, GT-2227 blocked both the (R)-α-methylhistamine-induced inhibition of neurogenic contractions of guinea-pig jejunum, and the inhibitory effects of the histamine H_3 receptor agonist GT-2203 on

norepinephrine release from guinea-pig heart synaptosomes (Tedford et al., 1998). More importantly, clobenpropit and GT-2016 have both been shown to increase extracellular histamine levels as studied by microdialysis (Jansen et al., 1998; Tedford et al., 1995). Additionally, very low doses (1 mg/kg i.p.) of clobenpropit have been shown to decrease histamine levels and increase histidine decarboxylase activity in mouse brain, indicative of a histamine H_3 receptor antagonist action (Yokoyama et al., 1994). GT-2227 also increased extracellular histamine levels in rat brain as measured by microdialysis (unpublished results). Thus, clobenpropit, GT-2227, and GT-2016, like thioperamide, seem to behave as inverse histamine H_3 receptor agonists to stimulate the release of histamine (Morisset et al., 2000).

Previous results found that, unlike thioperamide, the histamine H_3 receptor antagonists GT-2016, GT-2227, and clobenpropit have no effects on tele-methylhistamine content or synthesis in rat cerebral cortex (Yates et al., 1999). These findings have been presently confirmed for GT-2016 in the rat cortex and extended to include seven other rat brain regions (Table 1). In the mouse whole brain, GT-2016 had no effect on steady-state tele-methylhistamine levels and actually lowered the rate of tele-methylhistamine synthesis (Fig. 2). Also in the mouse whole brain, GT-2227 had no effect on tele-methylhistamine steady-state levels and neither GT-2227 nor clobenpropit had an effect on tele-methylhistamine synthesis at the indicated doses (Figs. 3 and 4). Contrary to present results, clobenpropit (10 and 30 mg/kg, p.o.), was reported by Ligneau et al. (1998) to increase tele-methylhistamine levels in mouse brain as measured by radio-immunoassay. Yates et al. (1999) found a similar result in the rat cortex when tele-methylhistamine was measured by radio-immunoassay, but this effect was not confirmed when the same samples were analyzed by gas chromatography-mass spectrometry. The former method (but not the latter) can yield artifactual increases in apparent tele-methylhistamine levels due to antibody cross-reactivity with some histamine H_3 receptor compounds (Yates et al., 1999). Hence, these newer histamine H_3 receptor antagonists resemble thioperamide in their ability to bind to brain histamine H_3 receptors and to stimulate neuronal histamine release, but they do not have thioperamide-like actions on brain histamine metabolism.

4.3. Inhibition of thioperamide activity by other histamine H_3 receptor antagonists

Present studies have further characterized the inability of GT-2016, GT-2227, and clobenpropit to increase brain histamine metabolism. However, since these compounds increase extracellular histamine levels, but not tissue tele-methylhistamine levels, it seemed plausible that they might be some how preventing histamine metabolism in vivo. To test this possibility, these drugs were administered along with thioperamide. GT-2016 significantly inhibited thioperamide's ability to raise tele-methylhistamine levels in the mouse (Fig. 2) and the rat (Table 1). Interpretation of the GT-2227 results (Fig. 3) seems less clear. Although tele-methylhistamine levels were significantly reduced in the presence of GT-2227, thioperamide, and pargyline (as compared with thioperamide/pargyline alone), there was a tendency ($P < 0.07$) for GT-2227 (10 mg/kg) to lower tele-methylhistamine levels in the presence of pargyline even when thioperamide was absent. Clobenpropit, a compound which did not affect tele-methylhistamine levels over a wide range of doses (0.01–10 mg/kg), had no inhibitory effect on the thioperamide-induced activation of tele-methylhistamine synthesis (Fig. 4). Many studies (Barnes et al., 1993; Yokoyama et al., 1994; Jansen et al., 1998; Yates et al., 1999), but not all studies (Mochizuki et al., 1996; Ligneau et al., 1998), suggest that the doses of clobenpropit tested presently were adequate for blocking histamine H_3 receptors.

Several explanations can be considered to account for the ability of GT-2016 and GT-2227 to lower tele-methylhistamine synthesis. Based on the observation that GT-2016 lowered histamine turnover in the mouse whole brain, it could be argued that GT-2016 and GT-2227 form metabolites in vivo that are agonists at the histamine H_3 receptor. For example, it has been shown that a significant portion of GT-2016 is metabolized to GT-2035 (nor-immepip) (Yates et al., 1999), a compound which resembles the structure of known histamine H_3 receptor agonists. However, GT-2035 has been previously described as a weak histamine H_3 receptor antagonist ($pA_2 = 5.7$) (Leurs et al., 1995b), not an agonist. In addition, due to differences in structure, GT-2227 cannot be metabolized to form GT-2035-like compounds. Finally, although GT-2016 lowered tele-methylhistamine synthesis in the mouse (an agonist-like action), this compound had no effect on tele-methylhistamine steady-state levels. In contrast, histamine H_3 receptor agonists (e.g. (*R*)-α-methylhistamine) are known to reduce both tele-methylhistamine synthesis and tele-methylhistamine steady-state levels (Oishi et al., 1989). Therefore, it seems unlikely that GT-2016 and GT-2227 form metabolites with histamine H_3 receptor agonist properties.

Another explanation of the ability of GT-2016 and GT-2227 to lower tele-methylhistamine synthesis might be that these drugs inhibit histamine *N*-methyltransferase. Inhibition of histamine *N*-methyltransferase would lead to a general reduction of tele-methylhistamine levels and would also indirectly inhibit thioperamide's ability to raise tele-methylhistamine levels. However, GT-2016 does not significantly inhibit histamine *N*-methyltransferase at concentrations up to 10 μM (Tedford et al., 1995). In addition, unpublished studies in our lab show that GT-2016 (30 μM) only inhibited histamine *N*-methyltransferase from crude rat brain by 7%. GT-2227 (100 μM) had no effect

on mouse brain histamine *N*-methyltransferase. The GT-2016 metabolite, GT-2035 (1 μM) inhibited histamine *N*-methyltransferase by 10%. In contrast, histamine *N*-methyltransferase inhibitors like metoprine (5 mg/kg, i.p.) lead to large decreases in tele-methylhistamine levels (Hough et al., 1986) and inhibit histamine *N*-methyltransferase by 70% at 1 μM in vitro (Duch et al., 1979). Therefore, it seems unlikely that GT-2016 and GT-2227, compounds that do not decrease tele-methylhistamine steady-state levels in vivo, lower tele-methylhistamine synthesis levels by the inhibition of histamine *N*-methyltransferase.

The most conservative hypothesis to explain the antagonism of thioperamide by GT-2016 and the general lowering of tele-methylhistamine synthesis by both GT-2016 and GT-2227 is that all of the observed effects of these compounds occur by actions at the histamine H_3 receptor. However, this is not likely to be the case. If it is postulated that these drugs antagonize the inverse agonist action of thioperamide because they are neutral histamine H_3 receptor antagonists, then they *should not* increase histamine release when given alone. However, both GT-2016 (Tedford et al., 1995) and GT-2227 (unpublished) increased brain extracellular histamine levels in rats, making it unlikely that the compounds behave as neutral histamine H_3 receptor antagonists at the doses presently studied.

The doses used in the present rat regional study (Table 1) also make it very unlikely that GT-2016 antagonizes thioperamide by an action at the histamine H_3 receptor. In fact, this experiment was designed so that thioperamide would preferentially bind the histamine H_3 receptor in all cases. Specifically, a low dose of GT-2016 (10 mg/kg, compared with a histamine H_3 receptor occupancy ED_{50} of 15 mg/kg) was administered along with a high dose of thioperamide (10 mg/kg, compared with a histamine H_3 receptor occupancy ED_{50} of 1.5 mg/kg) (Yates et al., 1999). Occupancy calculations (Kenakin, 1997) suggest that combining these doses of drugs would reduce thioperamide's histamine H_3 receptor binding from 87% to 80%, whereas GT-2016 binding would be reduced from 40% to 8%. Hence, if both compounds were acting exclusively at the histamine H_3 receptor, then the combined dosing regimen should not have significantly reduced the effect of thioperamide in the rat brain. However, this low dose of GT-2016 reduced the thioperamide-induced increase in tele-methylhistamine synthesis in the rat medulla–pons–cerebellum, hypothalamus, and midbrain by 52–100% (Table 1), suggesting that GT-2016 prevents histamine methylation by acting at a site other than the histamine H_3 receptor. The statistical significance of the three-way interaction term in the ANOVA of the data in Table 1 suggests that the regional differences in the ability of GT-2016 to inhibit thioperamide are not a result of chance, but rather reflect genuine differences across brain regions. This result may also add to the evidence for a non-histamine H_3 receptor site of action.

4.4. Inhibition of histamine metabolism by histamine H_3 receptor antagonists

The precise mechanism of histamine inactivation in the brain is still unknown. Although histamine methylation has been implicated in this process, few details are known (Nishibori et al., 2000; Huszti et al., 1990). A histamine transporter may exist in astrocytes (Huszti, 1998), but it has not been found in neurons (Hoffman et al., 1998). This transporter has not been cloned, and the relationship between histamine transport and methylation has not been clarified. This gap in the understanding of histamine inactivation allows for the possibility that histamine H_3 receptor ligands such as GT-2016 and GT-2227 may antagonize a crucial step between histamine release and its metabolism by histamine *N*-methyltransferase. If this proves true, it would not only explain the inability of these compounds to raise tele-methylhistamine levels in rat and mouse brain, but also the ability of GT-2016 to inhibit the thioperamide-induced activation of histamine metabolism. Furthermore, the present observation that the high doses of GT-2016 and GT-2227 administered in mice *lowered* tele-methylhistamine synthesis would make sense if these compounds had an action (in addition to their histamine H_3 receptor affinity) that led to the inhibition of histamine metabolism.

Although the present data permit the argument that the actions of these compounds are exclusive of the histamine H_3 receptor, it should be pointed out that multiple forms of this receptor have recently been described (Drutel et al., 2001). Pharmacological and/or brain regional differences in these subtypes might also help to explain the present results.

In conclusion, the histamine H_3 receptor ligands GT-2016 and GT-2227 act at the brain histamine H_3 autoreceptor to stimulate histamine release, but not histamine metabolism; the ability of both of these compounds to lower tele-methylhistamine synthesis rates and for GT-2016's ability to antagonize thioperamide's activation of the histamine system may account for these observations. The mechanisms by which these drugs reduce histamine methylation in vivo remains unclear, but may be related to the existence of histamine H_3 receptor subtypes, or to additional non-histamine H_3 receptor actions on the histaminergic system.

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References

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., Lancelot, J.C., Lecomte, J.M., Pollard, H., Robba, M., Schunack, W., Schwartz, J.-C., 1987. Highly potent and selective ligands for histamine H_3-receptors. Nature 327, 117–122.

Barke, K.E., Hough, L.B., 1994. Characterization of basal and morphine-induced histamine release in the rat periaqueductal gray. J. Neurochem. 63, 238–244.

Barnes, J.C., Brown, J.D., Clarke, N.P., Clapham, J., Evans, D.J., O'Shaughnessy, C.T., 1993. Pharmacological activity of VUF 9153, an isothiourea histamine H_3 receptor antagonist. Eur. J. Pharmacol. 250, 147–152.

Drutel, G., Peitsaro, N., Karlstedt, K., Wieland, K., Smit, M.J., Timmerman, H., Panula, P., Leurs, R., 2001. Identification of rat H(3) receptor isoforms with different brain expression and signaling properties. Mol. Pharmacol. 59, 1–8.

Duch, D.S., Bowers, S., Edelstein, M., Nichol, C.A., 1979. Histamine: elevation of brain levels by inhibition of histamine *N*-methyl transferase. In: Usdin, E., Borchardt, R.T., Creveling, C.R. (Eds.), Transmethylation. Elsevier/North-Holland, New York, pp. 287–295.

Ganellin, C.R., Fkyerat, A., Bang-Andersen, B., Athmani, S., Tertiuk, W., Garbarg, M., Ligneau, X., Schwartz, J.-C., 1996. A novel series of (phenoxyalkyl)imidazoles as potent H_3-receptor histamine antagonists. J. Med. Chem. 39, 3806–3813.

Ganellin, C.R., Leurquin, F., Piripitsi, A., Arrang, J.M., Garbarg, M., Ligneau, X., Schunack, W., Schwartz, J.-C., 1998. Synthesis of potent non-imidazole histamine H_3-receptor antagonists. Arch. Pharm. (Weinheim, Ger.) 331, 395–404.

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.

Hill, S.J., Ganellin, C.R., Timmerman, H., Schwartz, J.-C., Shankley, N.P., Young, J.M., Schunack, W., Levi, R., Haas, H.L., 1997. International union of pharmacology: XIII. Classification of histamine receptors. Pharmacol Rev. 49, 253–278.

Hoffman, B.J., Hansson, S.R., Mezey, E., Palkovits, M., 1998. Localization and dynamic regulation of biogenic amine transporters in the mammalian central nervous system. Front. Neuroendocrinol. 19, 187–231.

Hough, L.B., 1999. Histamine. In: Siegel, G.J., Agranoff, B.W., Albers, R.W., Fisher, S.K., Uhler, M.D. (Eds.), Basic Neurochemistry. Lippincott-Raven, Philadelphia, pp. 293–313.

Hough, L.B., Khandelwal, J.K., Morrishow, A.M., Green, J.P., 1981. An improved GCMS method to measure tele-methylhistamine. J. Pharmacol. Methods 5, 143–148.

Hough, L.B., Khandelwal, J.K., Green, J.P., 1984. Histamine turnover in regions of rat brain. Brain Res. 291, 103–109.

Hough, L.B., Khandelwal, J.K., Green, J.P., 1986. Inhibition of brain histamine metabolism by metoprine. Biochem. Pharmacol. 35, 307–310.

Huszti, Z., 1998. Carrier-mediated high affinity uptake system for histamine in astroglial and cerebral endothelial cells. J. Neurosci. Res. 51, 551–558.

Huszti, Z., Rimanoczy, A., Juhasz, A., Magyar, K., 1990. Uptake, metabolism, and release of [^{3}H]-histamine by glial cells in primary cultures of chicken cerebral hemispheres. Glia 3, 159–168.

Itoh, Y., Oishi, R., Nishibori, M., Saeki, K., 1991. Characterization of histamine release from the rat hypothalamus as measured by in vivo microdialysis. J. Neurochem. 56, 769–774.

Jansen, F.P., Mochizuki, T., Yamamoto, Y., Timmerman, H., Yamatodani, A., 1998. In vivo modulation of rat hypothalamic histamine release by the histamine H_3 receptor ligands, immepip and clobenpropit. Effects of intrahypothalamic and peripheral application. Eur. J. Pharmacol. 362, 149–155.

Kenakin, T., 1997. Pharmacologic Analysis of Drug–Receptor Interaction. 3rd edn. Lippincott-Raven, Philadelphia.

Leurs, R., Smit, M.J., Timmerman, H., 1995a. Molecular pharmacological aspects of histamine receptors. Pharmacol. Ther. 66, 413–463.

Leurs, R., Vollinga, R.C., Timmerman, H., 1995b. The medicinal chemistry and therapeutic potentials of ligands of the histamine H_3 receptor. Prog. Drug Res. 45, 107–165.

Leurs, R., Blandina, P., Tedford, C., Timmerman, H., 1998. Therapeutic potential of histamine H_3 receptor agonists and antagonists. Trends Pharmacol. Sci. 19, 177–183.

Ligneau, X., Lin, J., Vanni-Mercier, G., Jouvet, M., Muir, J.L., Ganellin, C.R., Stark, H., Elz, S., Schunack, W., Schwartz, J.-C., 1998. Neurochemical and behavioral effects of ciproxifan. A potent histamine H_3-receptor antagonist. J. Pharmacol. Exp. Ther. 287, 658–666.

Lovenberg, T.W., Roland, B.L., Wilson, S.J., Jiang, X., Pyati, J., Huvar, A., Jackson, M.R., Erlander, M.G., 1999. Cloning and functional expression of the human histamine H_3 receptor. Mol. Pharmacol. 55, 1101–1107.

Mochizuki, T., Yamatodani, A., Okakura, K., Takemura, M., Inagaki, N., Wada, H., 1991. In vivo release of neuronal histamine in the hypothalamus of rats measured by microdialysis. Naunyn-Schmiedeberg's Arch. Pharmacol. 343, 190–195.

Mochizuki, T., Jansen, F.P., Leurs, R., Windhorst, A.D., Yamatodani, A., Maeyama, K., Timmerman, H., 1996. Brain penetration of the histamine H_3 receptor antagonists thioperamide and clobenpropit in rat and mouse, determined with ex vivo [^{125}I]iodophenpropit binding. Brain Res. 743, 178–183.

Morisset, S., Rouleau, A., Ligneau, X., Gbahou, F., Tardivel-Lacombe, J., Stark, H., Schunack, W., Ganellin, C.R., Schwartz, J.-C., Arrang, J.-M., 2000. High constitutive activity of native H_3 receptors regulates histamine neurons in brain. Nature 408, 860–864.

Nakamura, T., Itadani, H., Hidaka, Y., Ohta, M., Tanaka, K., 2000. Molecular cloning and characterization of a new human histamine receptor, HH4R. Biochem. Biophys. Res. Commun. 279, 615–620.

Nishibori, M., Tahara, A., Sawada, K., Sakiyama, J., Nakaya, N., Saeki, K., 2000. Neuronal and vascular localization of histamine *N*-methyltransferase in the bovine central nervous system. Eur. J. Neurosci. 12, 415–424.

Oishi, R., Itoh, Y., Nishibori, M., Saeki, K., 1989. Effects of the histamine H_3-agonist (*R*)-α-methylhistamine and the antagonist thioperamide on histamine metabolism in the mouse and rat brain. J. Neurochem. 52, 1388–1392.

Tedford, C.E., Yates, S.L., Pawlowski, G.P., Nalwalk, J.W., Hough, L.B., Khan, M.A., Phillips, J.G., Durant, G.J., Frederisckson, C.A., 1995. Pharmacological characterization of GT-2016, a non-thiourea-containing histamine H_3 receptor antagonist: in vitro and in vivo studies. JPET 275, 598–604.

Tedford, C.E., Hoffmann, M., Seyedi, N., Maruyama, R., Levi, R., Yates, S.L., Ali, S.M., Phillips, J.G., 1998. High antagonist potency of GT-2227 and GT-2331, new histamine H_3 receptor antagonists, in two functional models. Eur. J. Pharmacol. 351, 307–311.

Van der Goot, H., Schepers, M.J.P., Sterk, G.J., Timmerman, H., 1992. Isothiourea analogues of histamine as potent agonists or antagonists of the histamine H_3-receptor. Eur. J. Med. Chem. 27, 511–517.

Yates, S.L., Tedford, C.E., Gregory, R., Pawlowski, G.P., Handley, M.K., Boyd, D.L., Hough, L.B., 1999. Effects of selected histamine H_3 receptor antagonists on tele-methylhistamine levels in rat cerebral cortex. Biochem. Pharmacol 57, 1059–1066.

Yokoyama, H., Onodera, K., Maeyama, K., Sakurai, E., Iinuma, K., Leurs, R., Timmerman, H., Watanabe, T., 1994. Clobenpropit (VUF-9153), a new histamine H_3 receptor antagonist, inhibits electrically induced convulsions in mice. Eur. J. Pharmacol. 260, 23–28.