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Short communication

High antagonist potency of GT-2227 and GT-2331, new histamine H_3 receptor antagonists, in two functional models

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Abstract

GT-2227 (4-(6-cyclohexylhex-*cis*-3-enyl)imidazole) and GT-2331 ((1*R*,2*R*)-4-(2-(5,5-dimethylhex-1-ynyl)cyclopropyl)imidazole) were developed as new potent histamine H_3 receptor antagonists. The functional activity of these ligands on the histamine H_3 receptor-mediated inhibition of neurogenic contraction of the guinea-pig jejunum and histamine H_3 receptor-mediated inhibition of norepinephrine release from guinea-pig heart synaptosomes were investigated. GT-2227 and GT-2331 both antagonized the inhibitory effects of (*R*)-α-methylhistamine on the contraction induced by electrical field stimulation in the guinea-pig jejunum with pA_2 values of 7.9 ± 0.1 and 8.5 ± 0.03, respectively. In addition, GT-2227 and GT-2331 antagonized the inhibition of norepinephrine release in cardiac synaptosomes by GT-2203 ((1*R*,2*R*)-*trans*-2-(1*H*-imidazol-4-yl)cyclopropylamine), a histamine H_3 receptor agonist. The current results demonstrate the antagonist activity for both GT-2227 and GT-2331 in two functional assays for histamine H_3 receptors.

Keywords: GT-2227; GT-2331; Histamine H_3 receptor; Jejunum; Norepinephrine; Heart

1. Introduction

Over the last several years, many reports have been published on the identification, localization and pharmacological properties of the histamine H_3 receptor (Arrang et al., 1987; Korte et al., 1990; Timmerman, 1990; Pollard et al., 1993; Onodera et al., 1994). Various laboratories have been keenly interested in the development of selective agents for this histamine receptor subtype, and selective histamine H_3 antagonists such as GT-2016, thioperamide, clobenpropit, and iodoproxyfan have been described (Arrang et al., 1987; Van Der Goot et al., 1992; Tedford et al., 1995; Stark et al., 1996). More recently, we have described a series of potent 1*H*-4-substituted imidazole H_3 ligands demonstrating the utility of non-polar olefin and acetylene spacer groups (Yates et al., 1998; Ali et al., 1998a,b). We also described the incorporation of an enantiomerically pure cyclopropane nucleus to optimize both stereochemical and conformational preferences of the H_3 receptor (Ali et al., 1998a). From those studies, two new prototype histamine H_3 receptor ligands, GT-2227 and GT-2331 were identified for further pharmacological characterization. The present studies provide functional data supporting the identification of GT-2227 and GT-2331 as new potent histamine H_3 receptor antagonists.

2. Materials and methods

2.1. Chemicals

GT-2227 (4-(6-cyclohexylhex-*cis*-3-enyl)imidazole), GT-2331 ((1*R*,2*R*)-4-(2-(5,5-dimethylhex-1-ynyl)cyclopropyl)imidazole) and GT-2203 ((1*R*,2*R*)-*trans*-2-(1*H*-imidazol-4-yl)cyclopropylamine) were developed and synthesized by Gliatech chemists. Acetylcholine chloride and (*R*)-α-methylhistamine dihydrogen maleate were obtained from Research Biochemical International (Natick, MA, USA). Triprolidine dihydrochloride was purchased from Sigma (St. Louis, MO, USA).

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2.2. Histamine H_3 receptor mediated inhibition of neurogenic contractions of the guinea-pig jejunum

The in vitro histamine H_3 receptor activity at the guinea-pig jejunum was determined as described by Vollinga et al. (1992). Male Dunkin–Hartley guinea-pigs (350–400 g, Harlan CPB, Zeist, Netherlands) were killed by cervical dislocation and the intestine was rapidly removed and kept in oxygenated (95% O_2/5% CO_2) Krebs buffer (composition in mM: NaCl 118, KCl 5.6, $MgSO_4$ 1.18, $CaCl_2$ 2.5, NaH_2PO_4 1.28, $NaHCO_3$ 25 and glucose 5.5), containing 0.1 μM triprolidine. Whole jejunum segments (ca. 2 cm) were mounted between two platinum electrodes (4 mm apart) in warm (37°C) Krebs buffer under a load of 1 g. After 60 min of equilibration the muscle was stimulated maximally (ca. 15 V) with a frequency of 0.1 Hz and a duration of 0.5 ms with rectangular wave electrical pulses (Grass Stimulator S-88, Grass Instruments, Quincy, MA, USA). Contractions were recorded isotonically (Hugo Sachs TL-2/HF-modem, Hugo Sachs Elektronik, Hugstetten, Germany). After 30 min of stimulation, a cumulative dose response curve for the histamine H_3 receptor agonist (*R*)-α-methylhistamine was recorded. Antagonists were preincubated for 15 min during the stimulation before the preparations were challenged again with (*R*)-α-methylhistamine. Maximally four dose response curves were recorded on one preparation.

In addition, in separate experiments the direct effects of histamine H_3 antagonists on contractile response to acetylcholine were investigated. Cumulative dose response curves for the acetylcholine-mediated contractions of the non-stimulated guinea-pig jejunum were determined isotonically under a load of 1 g. Antagonists were preincubated for 15 min before a new dose response curve for acetylcholine was recorded.

2.3. Norepinephrine release from guinea-pig heart synaptosomes

Guinea-pig heart synaptosomes were isolated as previously described (Seyedi et al., 1997). Minced tissue was digested with 40–75 mg collagenase (Type II, Worthington Biochemicals) per 10 ml HEPES-buffered saline solution (HBS) per gram wet heart weight for 1 h at 37°C. After low speed centrifugation (10 min at $120 \times g$ at 4°C), the resulting pellet was suspended in 10 vol. of 0.32 M sucrose and homogenized with a Teflon/glass homogenizer. The homogenate was spun at $650 \times g$ for 10 min at 4°C and the pellet rehomogenized and respun. The pellet was discarded and the supernatants from the last two spins were combined and equally subdivided into 10–12 tubes. Each tube was centrifuged for 20 min at $20\,000 \times g$ at 4°C. Each pellet, was resuspended in HBS to a final volume of 500 μl in the presence or absence of pharmacological agents (e.g., histamine H_3 receptor agonists and antagonists). Treated samples were incubated in a water bath at 37°C with a given agent for 20 min and then with 30 mM K^+ for 5 min. When antagonists were used, samples were incubated with the antagonist for 20 min prior to incubation with the agonist. Controls were incubated for an equivalent length of time without drugs. At the end of the incubation period each sample was recentrifuged for 20 min ($20\,000 \times g$ at 4°C). The supernatant was assayed for norepinephrine content by high pressure liquid chromatography with electrochemical detection. The pellet was assayed for protein content, by a modified Lowry procedure (Imamura et al., 1996).

2.4. Statistics

All values are reported as mean ± S.E.M. in text. For the guinea pig jejunum studies, pA_2 values and Schild slopes were calculated based on 3–4 dose-response curves on at least 3 separate preparations. In the norepinephrine release studies, data was analysed by ANOVA followed by post-hoc Dunnett's test, ($P < 0.05$).

3. Results

3.1. Histamine H_3 receptor mediated inhibition of neurogenic contractions of the guinea-pig jejunum

(*R*)-α-methylhistamine inhibited the neurogenic contractions of the guinea-pig jejunum with a pD_2 value of 8.1 ± 0.07 ($n = 6$) (Fig. 1). Increasing concentrations of GT-2227 produced a right-ward parallel shift in the dose-response curve for (*R*)-α-methylhistamine (Fig. 1A). The pA_2 value determined for GT-2227 was 7.9 ± 0.1 and a Schild slope of 1.04 ± 0.08 ($n = 16$) was obtained. At higher concentrations of GT-2227, the maximal effects of (*R*)-α-methylhistamine were reduced. Increasing concentrations of GT-2331 also produced a right-ward parallel shift in the dose-response curve for (*R*)-α-methylhistamine (Fig. 1B). The pA_2 value determined for GT-2331 was 8.5 ± 0.03 and a Schild slope of 1.1 ± 0.02 ($n = 20$) was obtained. GT-2331 also reduced the neurogenic twitch response at concentrations higher than 100 nM. Separate studies were conducted to evaluate the inhibitory effects of high concentrations of GT-2331 on direct contractile responses to acetylcholine ($pD_2 = 6.89 \pm 0.08$, $n = 3$). Inhibition of acetylcholine-induced contractions by GT-2331 was only seen at high concentrations (i.e., 10–30 μM) yielding an apparent pA_2 value of 5.24 ($n = 2$). The inhibition of the neurogenic contractions observed at the 10 μM concentration, was also unaffected by thioperamide, suggesting no histamine H_3 partial agonist activity (data not shown).

3.2. Norepinephrine release from guinea-pig heart synaptosomes

Release of norepinephrine from guinea-pig heart synaptosomes was measured following depolarization with

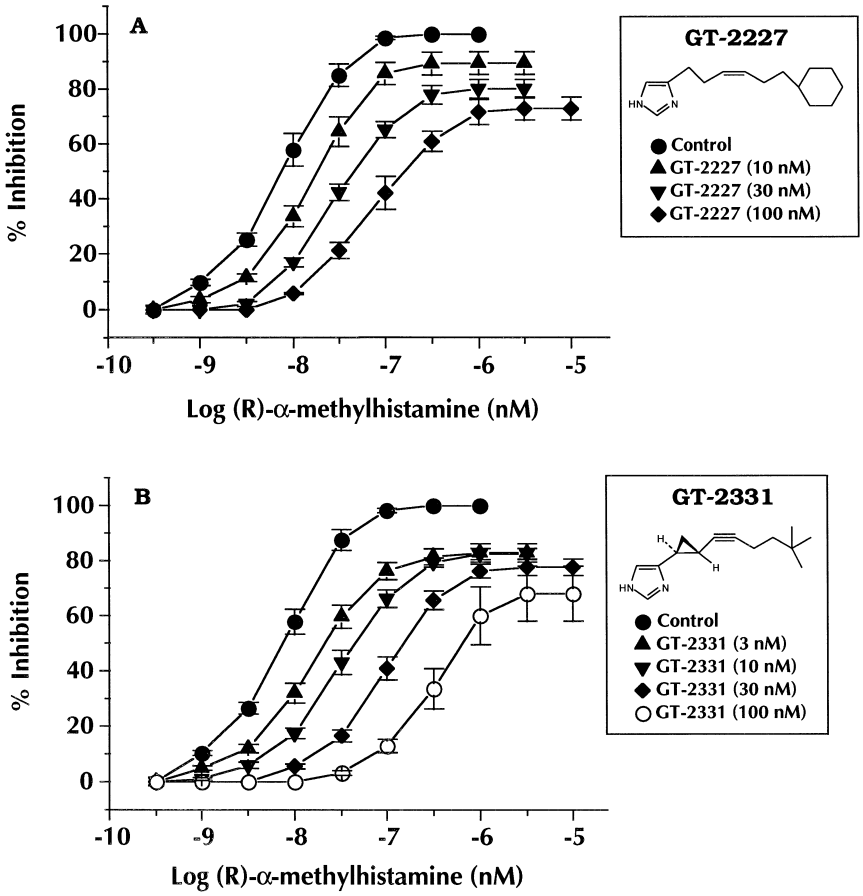

Fig. 1. Effects of GT-2227 (A) and GT-2331 (B) on (*R*)-α-methylhistamine inhibition of neurogenic contractions of the guinea-pig jejunum. Cumulative dose response curves for the H_3 receptor agonist (*R*)-α-methylhistamine were initially recorded (control). Control (-●-), GT-2227 (10 -▲- ; 30 -▼-; and 100 -◆- nM) or GT-2331 (3 -▲-; 10 -▼-; 30 -◆-; 100 -○- nM) were preincubated for 15 min during the stimulation before the preparations were challenged again with (*R*)-α-methylhistamine. Maximally four dose response curves were recorded at one preparation.

30 mM K^+ (Fig. 2). Norepinephrine release was increased 25.3% above basal levels. Incubation with GT-2203, the active cyclopropyl amine H_3 agonist isomer (Khan et al., 1997) attenuated the K^+-evoked norepinephrine release

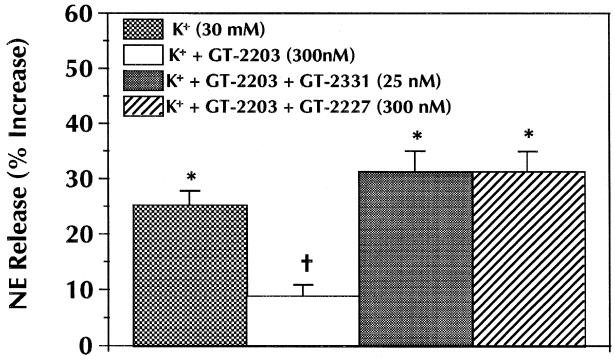

Fig. 2. Effects of GT-2227 (300 nM) and GT-2331 (25 nM) on GT-2203-mediated inhibition of NE release from guinea-pig heart synaptosomes. The H_3 agonist, GT-2203 (300 nM) was incubated alone or in the presence of the H_3 antagonists. Bars represent mean values ($\pm$S.E.M.; $n = 12$). Basal NE levels were 1.21 ± 0.09 pmol/mg protein. $*$Indicates significant difference ($P < 0.05$) from basal NE level. †Indicates significant difference ($P < 0.05$) from 30 mM K^+-evoked NE release by ANOVA followed by post-hoc Dunnett's test.

(not significantly different from basal). GT-2227 (300 nM) and GT-2331 (25 nM) blocked the effects on norepinephrine release by the histamine H_3 agonist, GT-2203, further demonstrating their antagonist properties at the histamine H_3 receptor.

4. Discussion

Recently, structure activity relationships were identified from which new potent histamine H_3 receptor ligands could be developed (Yates et al., 1998; Ali et al., 1998a,b). By utilizing olefin and acetylene spacers, imidazole analogs were identified which maintained the appropriate conformational and spatial orientations required for high affinity histamine H_3 receptor-ligand interactions (Yates et al., 1998; Ali et al., 1998a,b). GT-2227 and GT-2331 were developed with high binding affinity for the histamine H_3 receptor in vitro (pK_i values of 8.4 and 9.9, respectively; Yates et al., 1998; Ali et al., 1998a,b). The development of these specific ligands provides for some unique structural properties to further evaluate histamine H_3 receptor-ligand

interactions. Both compounds maintain only the imidazole head with the removal of all subsequent heteroatoms, thus minimizing hydrogen and ionic interactions distal to the imidazole head at the receptor site.

The present findings demonstrated in two functional assays the histamine H_3 receptor antagonist properties of these prototype compounds. Both compounds induced parallel shifts in the dose–response curve for (*R*)-α-methylhistamine in the isolated guinea-pig jejunum preparation demonstrating histamine H_3 antagonist activity. Separate results demonstrate that both compounds block the modulatory effects of a histamine H_3 agonist on norepinephrine release in guinea-pig cardiac synaptosomes.

The present findings are supportive of a selective interaction at the histamine H_3 receptor for GT-2227 and GT-2331. Recently, both GT-2227 (Yates et al., 1998) and GT-2331 (unpublished data) were screened against a large battery of receptors, hormones, and enzymes and minimal interactions were seen at concentrations of 1 μM confirming the compounds' high selectivity for the histamine H_3 receptor. Some direct-inhibitory effects were seen in our studies but only at high concentrations and were not histamine H_3 receptor related (thioperamide-insensitive). Moreover, as demonstrated by the acetylcholine-induced contractile studies with GT-2331 (apparent $pA_2 = 5.24$) the direct inhibitory effects were $>$ 1000-fold higher than that required for histamine H_3 receptor blockade. Direct inhibitory effects in guinea-pig bioassays have been previously described at high concentrations for other histamine H_3 antagonists including clobenpropit (Barnes et al., 1993) and iodoproxyfan (Watt et al., 1997) and maybe class-related. In addition, iodoproxyfan studies suggest some partial histamine H_3 agonist activity not seen with GT-2227 and GT-2331 in the current studies (Watt et al., 1997).

Overall, the histamine H_3 receptor affinity for GT-2227 in the guinea-pig jejunum assay is consistent with the histamine H_3 receptor binding affinity from the rat cortex ($pA_2 = 7.9$ vs. $pK_i = 8.4$, Yates et al., 1998). However, differences were apparent with GT-2331 between the two assays ($pA_2 = 8.5$ vs. $pK_i = 9.9$ Ali et al., 1998a). Discrepancies have been reported by other investigators with selected histamine H_3 compounds and differences between central and peripheral histamine H_3 receptors have been previously suggested (Barocelli et al., 1993; Leurs et al., 1996; Harper et al., 1997; Schlicker et al., 1996). In contrast, GT-2227 and GT-2331 both antagonized the effects of GT-2203 in the guinea-pig cardiac synaptosomes at concentrations that were in agreement with the relative differences ($\sim 10\times$) in affinity seen with the rat cortical histamine H_3 receptor. A further detailed evaluation of several structural classes of H_3 antagonists across assays might allow determination of true receptor, species, tissue or assay differences.

In summary, the functional activity of two new potent histamine H_3 receptor antagonists have been described. Additional in vitro and in vivo pharmacological characterization of these new histamine H_3 antagonists is ongoing. Most importantly, the identification of these prototype new histamine H_3 ligands provide for two unique classes of compounds and will help provide better understanding of ligand–histamine H_3 receptor interactions.

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