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Pharmacological and behavioral properties of A-349821, a selective and potent human histamine H_3 receptor antagonist

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Abstract

Histamine H_3 receptors regulate the release of a variety of central neurotransmitters involved in cognitive processes. A-349821 ((4′-(3-((*R,R*)2,5-dimethyl-pyrrolidin-1-yl)-propoxy)-biphenyl-4-yl)-morpholin-4-yl-methanone) is a novel, non-imidazole H_3 receptor ligand, displaying high affinity for recombinant rat and human H_3 receptors, with pK_i values of 9.4 and 8.8, respectively, and high selectivity for the H_3 receptor versus H_1, H_2, and H_4 histamine receptors. A-349821 is a potent H_3 receptor antagonist in a variety of models using recombinant human and rat receptors, reversing agonist induced changes in cyclic AMP formation ($pK_b = 8.2$ and $pK_b = 8.1$, respectively), [^{35}S]-GTPγS binding ($pK_b = 9.3$ and $pK_b = 8.6$, respectively) and calcium levels (human $pK_b = 8.3$). In native systems, A-349821 competitively reversed agonist induced inhibition of electric field stimulated guinea-pig ileum ($pA_2 = 9.5$) and histamine-mediated inhibition of [^{3}H]-histamine release from rat brain cortical synaptosomes ($pK_b = 9.2$). Additionally, A-349821 inhibited constitutive GTPγS binding at both rat and human H_3 receptors with respective pEC_{50} values of 9.1 and 8.6, demonstrating potent inverse agonist properties. In behavioral studies, A-349821 (0.4 mg/kg—4 mg/kg) potently blocked (*R*)-α-methylhistamine-induced dipsogenia in mice. The compound also enhanced cognitive activity in a five-trial inhibitory avoidance model in spontaneously hypertensive rat (SHR) pups at doses of 1–10 mg/kg, with the 1 mg/kg dose showing comparable efficacy to a fully efficacious dose of ciproxifan (3 mg/kg). These doses of A-349821 were without effect on spontaneous locomotor activity. Thus, A-349821 is a novel, selective non-imidazole H_3 antagonist/inverse agonist with balanced high potency across species and favorable cognition enhancing effects in rats.

Keywords: Histamine H_3 receptor; Cognition; Inverse agonist; Dipsogenia; GTPγS binding; Neurotransmitter release

1. Introduction

Histamine H_3 receptors are G-protein coupled receptors originally described as central histamine modulating auto-receptors [1] and later as heteroreceptors regulating release of other neurotransmitters involved in vigilance, attention, and cognition enhancement [2]. Thus, H_3 receptor antagonists may be potential therapeutic agents for attention deficit/hyperactivity disorder, Alzheimer's disease, mild cognitive impairment, or schizophrenia [3]. Drug discovery efforts to find potential therapeutic H_3 antagonists have been impacted by unique H_3 receptor properties that may influence antagonist activity including (1) existence of differentially expressed splice isoforms in the brain [4,5], (2) agonist potency differences at various isoforms [6], (3) differential isoform regulation of signaling [5,7], (4) constitutive activity of native and cloned H_3 receptors [6,8,9], and (5) distinct pharmacological species differences that may be manifested in each of the first four aspects of H_3 receptor pharmacology [10]. It has been

Abbreviations: cAMP, cyclic 3′,5′-adenosine monophosphate; FLIPR, fluorometric imaging plate reader; HEK, human embryonic kidney; TE, 50 mM Tris/5 mM EDTA; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethane sulfonic acid; DPBS, Dulbecco's phosphate buffered saline; SHR, spontaneous hypertensive rat; EFS, electric field stimulated; BSA, bovine serum albumin; S.E.M., standard error of the mean

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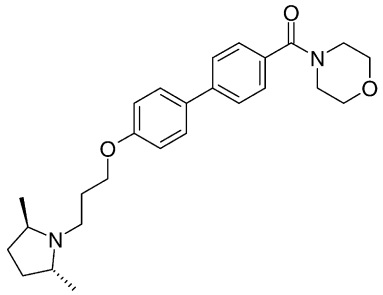

Fig. 1. Chemical structure of A-349821 ((4′-(3-((*R*,*R*)2,5-dimethyl-pyrrolidin-1-yl)-propoxy)-biphenyl-4-yl)-morpholin-4-yl-methanone).

suggested that H_3 receptor inverse agonists may be most desirable for the treatment of cognitive deficits because of H_3 receptor constitutive activity [11], although no H_3 receptor therapeutic agents have been approved for clinical use.

Most early H_3 receptor antagonists were imidazole derivatives like thioperamide [12], ciproxifan [13], clobenpropit [14], and GT-2331 [15] that, as a class, improve cognitive performance across many rodent behavioral models including those for attention [13], inhibitory (passive) avoidance responses [16–18], short-term memory [18], social recognition [19,20] and place recognition [21,22]. While effective tools, imidazole H_3 receptor antagonists suffer human therapeutic drawbacks including (1) generally lower human H_3 receptor affinity compared to rat [23–26], (2) limited selectivity versus other drug targets including serotonin $5HT_3$ [27], α_2-adrenergic, and histamine H_4 [28,29] receptors, and (3) potential interactions with cytochrome P_{450} enzymes [30–33]. Thus, it is desirable to synthesize potent and selective non-imidazole H_3 receptor antagonists as potential therapeutic agents in man.

We recently described the pharmacological properties of two novel, non-imidazole H_3 receptor antagonists, A-304121 and A-317920 [20,29]. While both are valuable tools for probing H_3 receptor cognitive-enhancing properties in rats, they exhibit weaker binding affinity for the human H_3 receptor. Herein, we describe the pharmacological and behavioral profile of A-349821 (Fig. 1) [34], a non-imidazole, procognitive H_3 receptor antagonist with high affinity and selectivity for both rat and human H_3 receptors.

2. Methods

2.1. Chemicals

A-349821 ((4′-(3-((*R*,*R*)2,5-dimethyl-pyrrolidin-1-yl)-propoxy)-biphenyl-4-yl)-morpholin-4-yl-methanone) (Fig. 1) [34], A-304121 ((4-(3-((2*R*)-2-aminopropanoyl-1-piperazinyl)propoxy)phenyl)(cyclopropyl)methanone), A-317920 (N-((1*R*)-2-(4-(3-(4-(cyclopropylcarbonyl)phenoxy)propyl)-1-piperazinyl)-1-methyl-2-oxoethyl-)-2-furamide) and ciproxifan were synthesized at Abbott Laboratories. [^{3}H]-*N*-α-methylhistamine, 45–90 Ci/mmol, [^{3}H]-pyrilamine, 20–30 Ci/mmol, [^{3}H]-tiotidine, 70–90 Ci/mmol, [^{3}H]-histidine, 40–60 Ci/mmol, [^{3}H]-rauwolscine, 75 Ci/mmol, and [^{35}S]-GTPγS, 1250 Ci/mmol, and Microscint 20 were obtained from Perkin Elmer Life Science Products and [^{3}H]-histamine, 30–60 Ci/mmol, and [^{3}H]-LY-278584 (1-methyl-*N*-(8-methyl-8-azabicyclo [3.2.1]-oct-3-yl)-1H-indazole-3-carboxamide), 60-85 Ci/mmol, were from Amersham Biosciences. (*R*)-α-methylhistamine and clobenpropit were purchased from Tocris, and 3-isobutyl-1-methylxanthine, thioperamide, and methylphenidate were purchased from Sigma.

2.2. Animals

Animals for in vitro experiments were housed in facilities approved by Association for the Assessment and Accreditation of Laboratory Animal Care at Abbott Laboratories in a temperature-regulated environment with lights on between 6:00 and 18:00 h. Male, Sprague-Dawley rats (weighing 200–250 g on arrival) and male, Hartley guinea pigs (weighing 150–200 g on arrival), were supplied by Charles River. Male, beagle dogs were obtained from Marshall Farms. The animals were acclimated to laboratory conditions for at least 1 week before testing. All in-house testing was conducted according to protocols approved by Abbott's Institutional Animal Care and Use Committee and National Institutes of Health Guide for Care and Use of Laboratory Animals guidelines.

For in vivo studies, male CD-1 mice were obtained from Charles River Laboratories at approximate post-natal day 70 (P 70) for dipsogenia studies and at 20–25 g body weight for general observation studies and maintained at Abbott facilities for 10 days prior to testing. Mice were housed up to 10 per large colony cage (52 cm × 28 cm × 20 cm) in a dedicated quiet room under conditions of 12 h lights on/12 h lights off (on at 06:00), with food and water available ad libitum. Nestlets were provided on cage/bedding change days to reduce territorial fighting. All testing occurred during the light phase.

Male SHR pups for five-trial inhibitory avoidance studies were obtained from Harlan at post-natal day 7 (P 7) and housed in Abbott facilities until use on days P 20–24 (body weights ranged from 35 to 50 g). Pups were housed up to 12 per cage (average of 2 litters) and fostered with Long-Evans lactating females (2 per cage). All rats were housed in a quiet room under conditions of 12 h lights on/12 h lights off (on at 06:00), with food and water available ad libitum. All testing occurred during the light phase.

2.3. H_3 receptor cloning and cell membrane preparation

The full-length (445 amino acids) human histamine H_3 receptor gene [7,26] was cloned using human thalamus

poly-A RNA (Clontech) and the full-length rat histamine H_3 receptor cDNA was obtained from rat thalamus RNA using RT-PCR methods as previously described [29]. Both genes were subcloned into the pCIneo expression vector and stably expressed in HEK and C6 cells as described previously [29].

For membrane preparations, cells were harvested and homogenized in TE buffer (50 mM Tris–HCl, 5 mM EDTA, pH 7.4) using a polytron at 20,000 rpm for 2 × 10 s bursts in the presence of protease inhibitors (1 mM benzamidine, 2 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml pepstatin, Sigma) followed by centrifugation at 40,000 × *g*. The homogenization and centrifugation steps were repeated as described above to further purify the membrane pellets. Final membrane preparations were obtained by re-homogenizing the pellets in 6.25 volumes (weight/volume) of TE buffer and were frozen at −70 °C until used.

Membranes expressing native H_3 receptors from human (Analytical Biological Services), rat (Pelfreez), dog, or guinea pig brain cerebral cortices were prepared by homogenization in cold TE buffer containing protease inhibitors. The homogenate was centrifuged at 40,000 × *g* for 20 min at 4 °C, the pellet resuspended by homogenization, centrifuged as in the previous step and the resulting pellet resuspended in TE buffer in 6.25 volumes (wet weight/volume). Aliquots were frozen at −70 °C until needed.

Clonal cells stably expressing the human histamine H_1 receptor [35] or H_2 receptor [36] in HEK cells were harvested and homogenized in TE buffer as described above and final membrane preparations were obtained by re-homogenizing the pellets in 6.25 volumes (weight/volume) of TE buffer and frozen at −70 °C until used. Membranes from HEK cells transiently expressing the human histamine H_4 receptor [28] subcloned into pCIneo were prepared as described above for the H_3 receptor.

2.4. Radioligand binding assays

Membrane preparations were incubated with [^{3}H]-*N*-α-methylhistamine (0.5–1.0 nM) in the presence or absence of increasing concentrations (from 5 to 11 concentrations over a five log unit range) of ligands for H_3 receptor competition binding. The binding incubations were in a final volume of 0.5 ml TE buffer at 25 °C and were terminated after 30 min. Thioperamide (30 μM) was used to define non-specific binding. Radioligand binding assays for cloned human histamine H_1, H_2, and H_4 receptors were performed as described [29,37] using [^{3}H]-mepyramine, [^{3}H]-tiotidine, and [^{3}H]-histamine, respectively. Radioligand binding assays for the cloned human α_{2a}- and α_{2c}-receptors expressed were performed using [^{3}H]-rauwolscine and rat frontal cortex $5HT_3$-serotonin binding assays were performed using [^{3}H]-LY278584 as previously described [29]. All binding reactions were terminated by filtration under vacuum onto polyethylenimine (0.3%) pre-soaked Unifilters (Perkin Elmer Life Sciences) or Whatman GF/B filters (for human cortex H_3 receptor, human H_4 receptor, and rat $5HT_3$ receptor) followed by three brief washes with 2 ml of ice-cold TE buffer. Bound radiolabel was determined by liquid scintillation counting.

For all of the radioligand competition binding assays, IC_{50} values and Hill slopes were determined by Hill transformation of the data as previously described [37] and pK_i values were determined by the Cheng–Prusoff equation [38]. Data are presented as the mean $pK_i \pm$ standard error of the mean. For compounds where the Hill slope was less than 0.8, the data were reanalyzed using GraphPad Prism and the best fit to a one- or two-site binding curve determined.

2.5. Adenylate cyclase assay

The reversal of 30 nM (human) or 300 nM (rat) (*R*)-α-methylhistamine-mediated inhibition of 10 μM forskolin-stimulated increases in cAMP accumulation after 20 min in the presence of 3-isobutyl-1-methylxanthine (10 mM) was determined by scintillation proximity assay (Amersham Biosciences) in C6 cells or HEK cells stably expressing the full-length human or rat H_3 receptor as previously described [29]. Data were normalized to the amount of cAMP produced in control wells and are expressed as a percentage of the forskolin-stimulated cAMP response. Experiments were run in triplicate and data were analyzed using GraphPad Prism to obtain IC_{50} values and Hill slopes. pK_b values were determined by the generalized Cheng–Prusoff equation [38,39] which are presented as the mean ± S.E.M.

2.6. Measurement of intracellular calcium levels

The ability of H_3 receptor ligands to block (*R*)-α-methylhistamine stimulated increases in intracellular calcium levels by the full-length human H_3 receptor was determined in a stable HEK cell line co-expressing the receptor and $G\alpha_{q/i5}$ [40] using a 96-well format fluorometric imaging plate reader (FLIPR, Molecular Devices), as previously described [29]. Peak response values were expressed as a percentage of the reference peak response for 30 nM (*R*)-α-methylhistamine in the absence of H_3 receptor antagonists. Experiments were performed in duplicate and data were analyzed using GraphPad Prism to obtain IC_{50} values and Hill slopes. The generalized Cheng–Prusoff equation [38,39] was used to determine pK_b values which are presented as the mean ± S.E.M.

2.7. Electric field stimulated (EFS) guinea-pig ileum

The reversal of (*R*)-α-methylhistamine-mediated inhibition of EFS (test voltage ~7–8 V, 0.1 Hz frequency, 0.5 ms duration) elicited twitches of guinea-pig ileum by H_3 receptor antagonists was determined as previously described [25]. The concentration of (*R*)-α-methylhista-

mine necessary to cause a 50% inhibition in the stimulated contraction (EC_{50}) was calculated using an Excel-based program, AGANTG [41], and expressed as the negative logarithm (pD_2). H_3 receptor antagonists were tested by adding various concentrations to the tissue baths 30 min prior to the generation of (*R*)-α-methylhistamine cumulative concentration response curves. The potency of the antagonists (pA_2) to inhibit the (*R*)-α-methylhistamine response was calculated according to the method of Schild [42] using AGANTG.

2.8. Rat cerebral cortical histamine release assay

The reversal by H_3 receptor antagonists of histamine-mediated inhibition of [^{3}H]-histamine release from rat cerebral cortical synaptosomes evoked by potassium (15 mM) was determined essentially as previously described [29,43]. Basal release values (obtained in buffer without additional potassium) were subtracted from each sample and the data expressed as a percentage of the maximum potassium-stimulated release for each assay. Data were analyzed from duplicate experiments using GraphPad Prism to obtain IC_{50} values and Hill slopes. pK_b values were determined by the generalized Cheng–Prusoff equation [38,39] which are presented as the mean ± S.E.M.

2.9. [^{35}S]-GTPγS binding assays

Membranes from HEK cells expressing the human H_3 receptor or from C6 cells expressing the rat H_3 receptor were prepared by homogenization in cold buffer containing 50 mM Tris–HCl (pH 7.4), 5 mM EDTA, 10 mM $MgCl_2$, 1 mM benzamidine, 2 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml pepstatin. The homogenate was centrifuged two times at 40,000 × *g* for 20 min at 4 °C and the resulting pellet was resuspended in buffer containing 50 mM Tris–HCl (pH 7.4), 5 mM EDTA, and 10 mM $MgCl_2$. After homogenization, glycerol and bovine serum albumin (BSA) were added to a final concentration of 10% glycerol and 1% BSA prior to freezing the membranes. For assays to determine inverse agonist activity, membranes were diluted in GTPγS assay buffer (25 mM HEPES, 2.5 mM $MgCl_2$, and 75 mM NaCl, pH 7.4) and 10 μg of membrane protein was incubated in a 96-well deep-well block in the presence of 5.0 μM unlabeled GDP, approximately 0.5 nM of [^{35}S]-GTPγS, and various concentrations of H_3 receptor antagonists. Samples were subsequently incubated at 37 °C for 20 min. For assays to determine antagonist activity, (*R*)-α-methylhistamine (30 nM for human and 300 nM for rat H_3 receptor, respectively), was added in addition to the assay components described above and the samples were incubated at 37 °C for 5 min. The assays were terminated by the addition of cold buffer (50 mM Tris–HCl, 75 mM NaCl, and 2.5 mM $MgCl_2$, pH 7.6) and subsequent harvesting onto a Packard Unifilter 96-well GF/B plate (Perkin Elmer Life Sciences). After extensive washing, the plates were dried, Microscint 20 was added to the samples, and the amount of bound [^{35}S]-GTPγS was determined utilizing the Topcount (Perkin Elmer Life Sciences). The percentage of [^{35}S]-GTPγS bound in each sample was calculated as a percentage of that bound to control samples incubated in the absence of histamine H_3 ligands. Triplicate determinations were obtained at each concentration and the data were analyzed using GraphPad Prism (San Diego, CA) to obtain EC_{50} or IC_{50} values and Hill slopes. pK_b values were calculated using the generalized Cheng–Prusoff equation [38,39]. The mean ± S.E.M. of at least three independent experiments is reported.

2.10. Dipsogenia model

Mice were tested in a dipsogenia model as previously described [44]. Briefly, each mouse was injected i.p. with saline or one of the H_3 receptor antagonists. After 5 min, saline or (*R*)-α-methylhistamine (30 mg/kg), was injected i.p. on the opposite side of the abdomen, and the animals returned to their home cages. Following a period of 30 min, mice were then placed separately into smaller cages with access to water via a modified sipper. The mice were left alone for a further 30 min after which they were removed to their home cages once more and the amount of water consumed was recorded to the nearest 0.01 ml. Sixteen animals were treated at a time for a total of 12–16 mice per group. Data were normalized as a percentage of the maximal (*R*)-α-methylhistamine response and represent the mean ± S.E.M.

2.11. Five-trial inhibitory avoidance

SHR pups were trained in an inhibitory avoidance response with five acquisition trials as previously described [17]. Briefly, the animals were trained to avoid a mild footshock (0.1 mA, 1 s duration) delivered when the pup transferred from a brightly lit to a darkened compartment of a computer-controlled Gemini inhibitory avoidance system (San Diego Instruments). After the first trial, the pup was removed and returned to its home cage and littermates and the transfer latency noted. One minute later, the same pup was once again placed in the brightly lit compartment and the training process repeated for a total of five trials. A criterion time of 1 min applied for the first trial and 3 min for each of the four subsequent trials. Ciproxifan (3 mg/kg—internal positive control), A-349821 (1, 3, 10 mg/kg) or saline vehicle were injected s.c. 30 min prior to the first trial. An oscilloscope (Hitachi V-212, 20 MHz) and a 100 kΩ resistor were used frequently to ensure correct calibration of the equipment in producing this relatively mild footshock. Pups were not habituated to the avoidance apparatus before the first trial to avoid potentially confounding latent inhibitory effects. Each drug treatment was equally represented among each

litter ($n = 12$ per group) and the investigator was normally blinded to treatment. Separate groups of pups ($6 < n < 12$ per group) were used to control for footshock sensitivity. In these experiments, pups were treated with drug as before, but were exposed to an inescapable footshock that was increased gradually from 0.05 to 0.4 mA and back to 0.05 mA over a period of 30 s. The currents at which vocalization first occurred, termed 'iMax' and then ceased, termed 'iMin' were noted.

2.12. Spontaneous locomotor activity

To assess possible stimulant-like drug effects on spontaneous locomotor activity, non-habituated SHR pups were placed separately into one of 16 acrylic open field environments (42 cm L × 42 cm W × 40 cm H; Piper Plastics) situated inside Versamax/Digiscan monitors, each equipped with 32 horizontal and 16 vertical infrared sensors (Accuscan Instruments) in a darkened room. After habituating to the test room in their home cages for 30 min, SHR pups were injected s.c. with methylphenidate, ciproxifan, or A-349821 (1, 3, and 10 mg/kg for each) or saline vehicle and allowed to habituate an additional 30 min to the test room in order to duplicate, with the exception of the test chamber, the conditions used in the five-trial inhibitory avoidance test. Spontaneous locomotor activity was then monitored for 30 min at 1 min intervals.

2.13. General observation test

Adult mice were separated into groups of three and placed into observation cages (23 cm × 21 cm × 20 cm). Baseline core (rectal) body temperature was recorded with a rapid read digital thermometer (Model BAT-12, Physitemp Instruments Inc.). In separate experiments, mice were then injected with vehicle or A-349821 (12, 35, 118, 355 mg/kg i.p.). All mice were continuously observed for adverse behavior such as changes in activity levels, piloerection, ptosis and seizure activity for the first hour and then intermittently at 2, 3, 6 and 24 h following drug administration. Body temperature was recorded 0.25, 0.5, 1, 2, 3, 6 and 24 h following drug administration.

2.14. Behavioral statistical analyses

Dipsogenia data were assessed for significance using a one-way analysis of variance (ANOVA) followed by Tukey's pairwise comparison post hoc tests. Spontaneous locomotor activity data were analyzed using one-way ANOVAs, with time as a repeated measure, followed by Tukey's post hoc tests. Non-parametric Kruskal–Wallis and individual Mann–Whitney *U* tests were used to compare performance in the five-trial inhibitory avoidance test. A P value <0.05 was considered significant for all post hoc tests. All analyses were performed using Statview 5.0 (SAS Institute Inc.).

3. Results

3.1. Histamine H_3 receptor binding

Binding affinities for A-349821, A-304121, A-317920, thioperamide, and ciproxifan for H_3 receptors were determined by displacement of specific [^{3}H]-*N*-α-methylhistamine binding in membranes prepared from C6 cells expressing the full-length recombinant rat and human H_3 receptors as well as brain cortical membranes prepared from rat, human, dog, and guinea pig (Table 1). All five compounds potently bound with pK_i values greater than 8.0 to both the recombinant rat H_3 as well as rat brain cortical membrane H_3 receptors with only a one order of magnitude separation in potencies. However, only A-349821 demonstrated potent binding to human H_3 receptors (p$K_i = 9.4$ for both recombinant and brain cortical H_3 receptors) with A-304121, A-317920, thioperamide, and ciproxifan all demonstrating markedly lower potencies (pK_is < 7.2) at both the recombinant and human cortical H_3 receptors, as previously reported [29]. The overall binding affinities of the compounds to dog and guinea pig H_3 receptors in brain cortical membranes were intermediate to those for rat and human H_3 receptors with A-349821 being the most potent at both guinea pig (p$K_i = 9.3$) and dog (p$K_i = 8.9$) brain cortical H_3 receptors. All the compounds recognized a single H_3 receptor binding site in each membrane preparation as evidenced by Hill slopes that approached unity for all of the antagonist displacement curves.

3.2. Histamine and biogenic amine receptor binding selectivity profile

The binding affinities of A-349821, A-304121, A-317920, thioperamide, and ciproxifan were determined at human H_1, H_2, and H_4 histamine receptors as well as human α_{2A}- and α_{2C}-adrenergic receptors and 5HT$_3$-serotonergic receptors (Table 2). A-349821 exhibited low binding affinity for the human H_1 receptor (p$K_i = 5.6$) and no binding at concentrations up to 10 μM (p$K_i < 5$) for H_2 and H_4 receptors. Similarly, A-317920 had no affinity for either the H_2 or H_4 receptors (p$K_i < 5$) and low affinity for the H_1 receptor (p$K_i = 5.4$), whereas A-304121 exhibited no binding at the maximal tested concentrations (p$K_i <$ 5) for H_1, H_2, or H_4 receptors [29]. Thioperamide and ciproxifan demonstrated no affinity for either the H_1 or H_2 receptor (pK_i values < 5), but thioperamide did exhibit H_4 receptor binding affinity (p$K_i = 7.3$) whereas ciproxifan exhibited a lower potency (pK_i value = 5.7) at H_4 receptors [29]. A-349821 had low affinity (pK_i value = 5.8) for the α_{2A}-adrenergic and 5HT$_3$ receptor binding sites (pK_i value < 5) and over 100-fold lower affinity (pK_i values = 7.3) for the α_{2C}-adrenergic receptor in comparison to the human H_3 receptor. Ciproxifan and thioperamide exhibited little to no selectivity for the human H_3 receptor versus the α_{2A}- (pK_i values = 7.4 and 6.9, respectively), and α_{2C}-adrenergic

Table 1
Comparison of A-349821, A-304121, A-317920, thioperamide and ciproxifan binding at histamine H_3 receptors

Receptor	Mean pK_i ± S.E.M. (mean n_H[a] ± S.E.M.)				
	A-349821	A-304121	A-317920	Thioperamide	Ciproxifan
Human H_3	9.39 ± 0.08 (0.9 ± 0.03)	6.12 ± 0.08 (0.9 ± 0.06)	7.03 ± 0.04 (0.89 ± 0.03)	7.14 ± 0.06 (0.93+ 0.04)	7.20 ± 0.05 (0.84+ 0.04)
Rat H_3	8.78 ± 0.12 (0.93 ± 0.06)	8.60 ± 0.1 (0.88 ± 0.1)	9.15 + 0.08 (0.86 ± 0.02)	8.44 + 0.07 (0.84+ 0.06)	9.29 ± 0.09 (0.88+ 0.02)
Human brain cortex H_3	9.37 ± 0.08 (0.77 ± 0.06)[b]	6.09 ± 0.12 (0.94 ± 0.08)	6.93 + 0.08 (0.92 ± 0.07)	7.18 + 0.008 (1.01+ 0.09)	7.05 ± 0.06 (1.06+ 0.07)
Rat brain cortex H_3	8.84 ± 0.07 (0.87 ± 0.04)	8.90 ± 0.05 (0.82 ± 0.03)	9.14 + 0.04 (0.92 ± 0.03)	8.15 ± 0.06 (0.90+ 0.04)	9.20 ± 0.04 (0.90+ 0.03)
G.P. brain cortex H_3	9.26 ± 0.13 (0.71 ± 0.07)[b]	7.76 ± 0.08 (0.75 ± 0.04)[b]	8.62 + 0.11 (0.93 ± 0.09)	8.34 ± 0.11 (1.10+ 0.08)	8.76 ± 0.07 (0.89+ 0.05)
Dog brain cortex H_3	8.92 ± 0.10 (0.83 ± 0.07)	7.33 ± 0.09 (0.76 ± 0.06)[b]	8.37 + 0.15 (0.77 ± 0.04)[b]	8.17 ± 0.12 (0.83+ 0.05)	8.24 ± 0.06 (0.88+ 0.02)

[a] n_H is the Hill slope, $n = 3$–31 independent experiments performed in duplicate.
[b] Inhibition curve best fit to one-site model when compared to two-site model (GraphPad Prism).

Table 2
Comparison of A-349821, A-304121, A-317920, thioperamide and ciproxifan binding at histamine H_1, H_2, and H_4 and other biogenic amine receptors

Receptor	Mean pK_i ± S.E.M. (mean n_H[a] ± S.E.M.)				
	A-349821	A-304121	A-317920	Thioperamide	Ciproxifan
Human H_1	5.63 ± 0.04 (0.85 ± 0.12)	<5	5.40 ± 0.15 (0.99 ± 0.14)	<5	<5
Human H_2	<5	<5	< 5	<5	<5
Human H_4	<5	<5	<5	7.32 ± 0.25 (0.62 ± 0.01)	5.73 ± 0.09 (0.81 ± 0.19)
Human α_{2A}-adrenergic	5.79 ± 0.06 (1.23 ± 0.09)	<5	<5	6.90 ± 0.08 (0.99 ± 0.06)	7.37 ± 0.07 (1.03 ± 0.06)
Human α_{2C}-adrenergic	7.3 ± 0.13 (1.06 ± 0.12)	5.54 ± 0.16 (0.97 ± 0.06)	5.62 ± 0.27 (1.02 ± 0.16)	6.46 ± 0.11 (0.84 ± 0.13)	7.20 ± 0.13 (0.89 ± 0.07)
Rat $5HT_3$-serotonergic	<5	<5	<5	5.64 ± 0.13 (0.84+ 0.16)	6.52 ± 0.19 (0.66 ± 0.16)

[a] n_H is the Hill slope, $n = 3$–6 independent experiments performed in duplicate.

(pK_i values = 7.2 and 6.5, respectively) receptors and less than 100-fold selectivity when compared to binding affinities for the serotonin $5HT_3$-receptor (pK_i values = 6.5 and 5.6, respectively) [29].

3.3. Functional antagonism at recombinant H_3 receptors

A-349821 inhibited (*R*)-α-methylhistamine-mediated reversal of forskolin-stimulated cAMP accumulation in both C6 cells expressing the full-length human (Fig. 2, top panel) and rat (Fig. 2, bottom panel) H_3 receptors in an equipotent and concentration-dependent manner with pK_b values of 8.2 and 8.1 (Table 3), respectively, in contrast to the more potent inhibition of rat H_3 receptor-mediated adenylate cyclase responses by A-304121, A-317920, thioperamide, and ciproxifan previously reported [29]. The competitive antagonist property of A-349821 was revealed by its ability to elicit concentration-dependent parallel, rightward shifts of the concentration response curves of (*R*)-α-methylhistamine in the same assay system for both the human H_3 (Fig. 3, top panel) and rat H_3 (Fig. 3, bottom panel) receptors with Schild analysis revealing pA_2 values of 8.7 and slopes approaching unity for both receptors. A similar pharmacological profile was also seen for the inhibition of (*R*)-α-methylhistamine-activated GTPγS binding at the human and rat H_3 receptors for A-349821 (pK_b values = 9.3 and 8.6, respectively, Table 3) with, again, A-304121, A-317920, thioperamide, and ciproxifan exhibiting lower potency for the human H_3 receptor [29]. Additionally, A-349821 inhibited (*R*)-α-methylhistamine-stimulated increases in intracellular calcium in HEK cells co-expressing the full-length human H_3 receptor with the chimeric $G\alpha_{qi5}$-protein with a pK_b value of 8.3 (Table 3), over 10-fold more potent than the other H_3 receptor antagonists tested.

3.4. Models of neurotransmitter release

Activation of H_3 receptors by (*R*)-α-methylhistamine concentration-dependently inhibits electrically evoked release of acetylcholine from nerve terminals that elicit

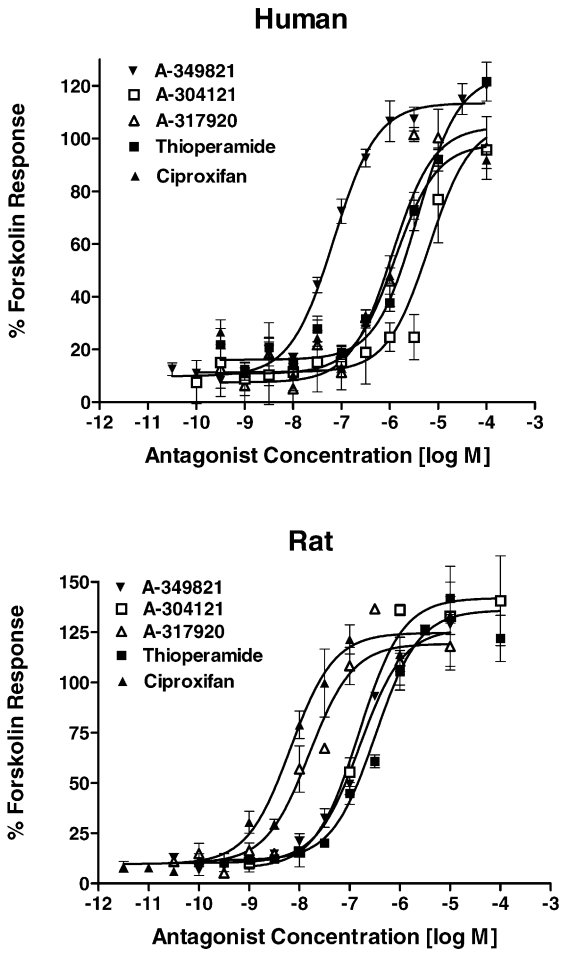

Fig. 2. A-349821 blocks (*R*)-α-methylhistamine reversal of forskolin-stimulated cAMP formation in a concentration-dependent manner in C6 cells stably expressing the full-length human (top panel) and rat (bottom panel) histamine H_3 receptors. Cells were incubated with increasing concentrations of the H_3 receptor antagonists prior to the addition of 30 nM (human) or 300 nM (rat) (*R*)-α-methylhistamine and 10 μM forskolin, and cAMP formation was determined as described in Section 2. Data are expressed as a percentage of the maximal forskolin-stimulated cAMP response in the absence of H_3 receptor antagonist and (*R*)-α-methylhistamine. Results for A-349821, A-304121, A-317920, thioperamide, and ciproxifan represent the mean ± S.E.M. of 3–9 concentration response assays performed in triplicate.

Table 3
Comparison of antagonist potencies of A-349821, A-304121, A-317920, ciproxifan, and thioperamide in histamine H_3 receptor functional assays

Functional assay[a]	Mean pK_b or pA_2 (±S.E.M.) [*n*]				
	A-349821	A-304121	A-317920	Thioperamide	Ciproxifan
Human H_3 cyclase	8.24 ± 0.10 [7]	5.26 ± 0.05 [6]	6.52 ± 0.11 [6]	6.10 ± 0.12 [6]	6.59 ± 0.04 [9]
Human H_3 GTPγS	9.26 ± 0.02 [4]	6.16 ± 0.19 [3]	7.08 ± 0.09 [4]	7.39 ± 0.04 [4]	7.07 ± 0.13 [4]
Rat H_3 cyclase	8.08 ± 0.10 [3]	8.03 ± 0.12 [4]	9.13 ± 0.29 [3]	7.61 ± 0.14 [3]	9.20 ± 0.10 [4]
Rat H_3 GTPγS	8.62 ± 0.11 [4]	7.83 ± 0.14 [5]	8.53 ± 0.10 [5]	8.13 ± 0.14 [5]	8.78 ± 0.12 [5]
Human H_3 FLIPR	8.27 ± 0.12 [8]	5.95 ± 0.10 [5]	7.26 ± 0.14 [5]	6.82 ± 0.06 [11]	6.84 ± 0.08 [12]
EFS guinea-pig ileum	9.47 ± 0.56 [31]	7.11 ± 0.29 [17]	8.25 ± 0.05 [15]	8.44 ± 0.49 [20]	8.12 ± 0.56 [21]
Rat synaptosomes [^{3}H]-histamine release	9.24 ± 0.16 [2]	8.64 ± 0.18 [3]	9.30 ± 0.08 [2]	8.58 ± 0.21 [3]	9.12 ± 0.11 [6]

[a] All antagonist potencies shown represent the mean ± S.E.M. of pK_b determinations for each of the assays except for the EFS guinea-pig ileum model where the values represent the mean ± S.E.M. of pA_2 determinations.

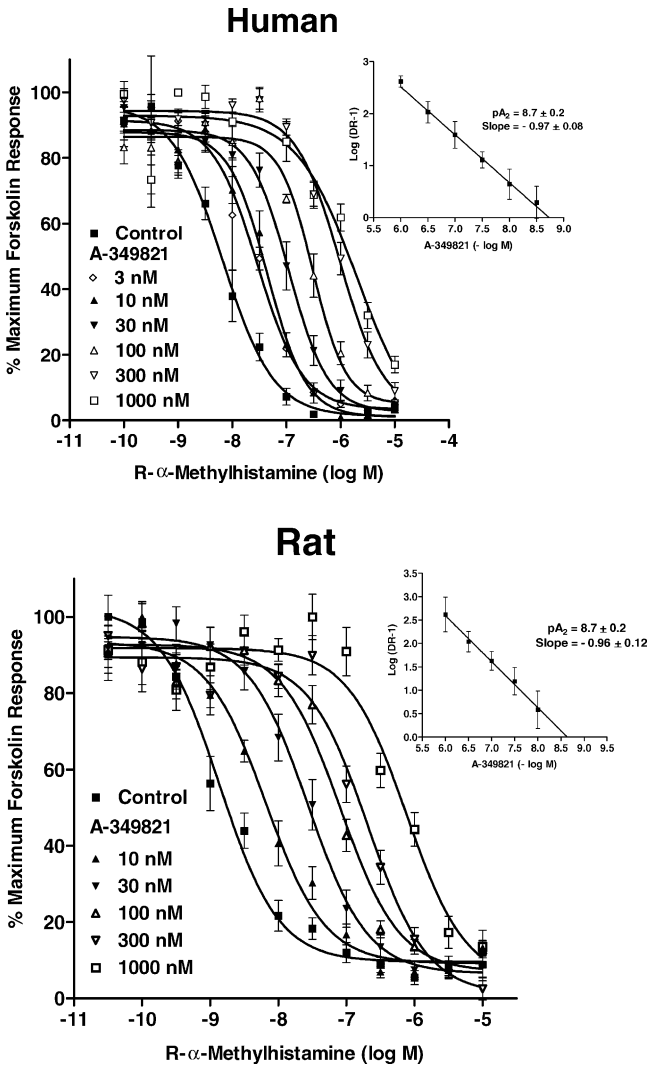

Fig. 3. Schild analysis of A-349821 competitive antagonism of (*R*)-α-methylhistamine-mediated reversal of forskolin-stimulated cAMP accumulation. Tissues were incubated with varying concentrations of A-349821 for 2 min prior to generating the (*R*)-α-methylhistamine concentration response curves as described in Section 2. The top (human H_3 receptor) and bottom (rat H_3 receptor) panels show the dextral shifts in the (*R*)-α-methylhistamine concentration response assays resulting from increasing concentrations of A-349821 with the Schild transformation shown in the respective insets. Data for the (*R*)-α-methylhistamine concentration response curves are expressed as a percentage of the maximal forskolin-stimulated cAMP response in the absence of H_3 receptor antagonist and (*R*)-α-methylhistamine. Results represent the mean ± S.E.M. of three (*R*)-α-methylhistamine concentration response assays for each concentration of A-349821.

contraction of guinea-pig ileum, thus serving as a useful model for presynaptic H_3 receptor modulation of neurotransmitter release [25]. Increasing concentrations of A-349821 caused parallel, rightward shifts of the concentration response curves for (*R*)-α-methylhistamine-mediated reversal of electric field stimulated contractions of guinea-pig ileum revealing a potent pA_2 value of 9.5 with a slope of −0.91 (Table 3), consistent with competitive antagonist activity. The rank order of potency for all of the H_3 receptor antagonists tested in this model was A-349821 > thioperamide = A-317920 = ciproxifan > A-304121.

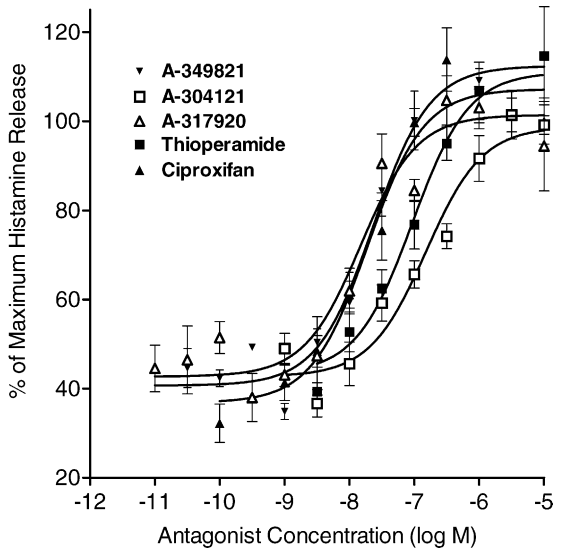

Fig. 4. A-349821 blocks histamine reversal of potassium-stimulated [^{3}H]-histamine release in a concentration-dependent manner in rat brain cortical synaptosomes. Synaptosomes were incubated with increasing concentrations of the H_3 receptor antagonists prior to the addition of 1 μM histamine and 15 mM potassium and [^{3}H]-histamine release was determined as described in Section 2. Data are expressed as a percentage of the maximal potassium-stimulated [^{3}H]-histamine release in the absence of H_3 receptor antagonist and histamine. Results represent the mean ± S.E.M. of 2–6 concentration response assays performed in triplicate.

Activation of H_3 receptors inhibits the release of [^{3}H]-histamine caused by potassium-stimulated depolarization in the rat brain cortical synaptosome model of neurotransmitter release. A-349821 potently antagonized the histamine-mediated reversal of [^{3}H]-histamine release from rat synaptosomes (Fig. 4) in a concentration-dependent manner with a pK_b value of 9.2 (Table 3). All of the H_3 receptor antagonists tested in this model were potent with pK_b values greater than 8.5.

3.5. Inverse agonism: [^{35}S]-GTPγS binding

Basal [^{35}S]-GTPγS binding in membranes from HEK cells expressing the full-length human H_3 receptor was reduced in a concentration-dependent manner by A-349821 (Fig. 5, top) with a pEC_{50} value of 9.1 and a maximal inhibition of 23% from basal (Table 4). The other tested H_3 receptor antagonists were much less potent (Table 4) with a rank order of potency of A-349821 > thioperamide > ciproxifan = A-317920 > A-304121 and also exhibited lower efficacy in reversing basal GTPγS binding levels with a rank order of intrinsic inverse agonist activity of A-349821 = A-317920 > A-304121 > thioperamide = ciproxifan. A-349821 also potently inhibited basal [^{35}S]-GTPγS binding by full-length rat H_3 receptors in a concentration-dependent manner (Fig. 5, bottom) with a pEC_{50} value of 8.6 and a maximal inhibition of 15% from basal (Table 4). A-349821, A-317920, and ciproxifan were approximately equipotent at the rat H_3 receptor and more potent than thioperamide and A-304121; however, all H_3

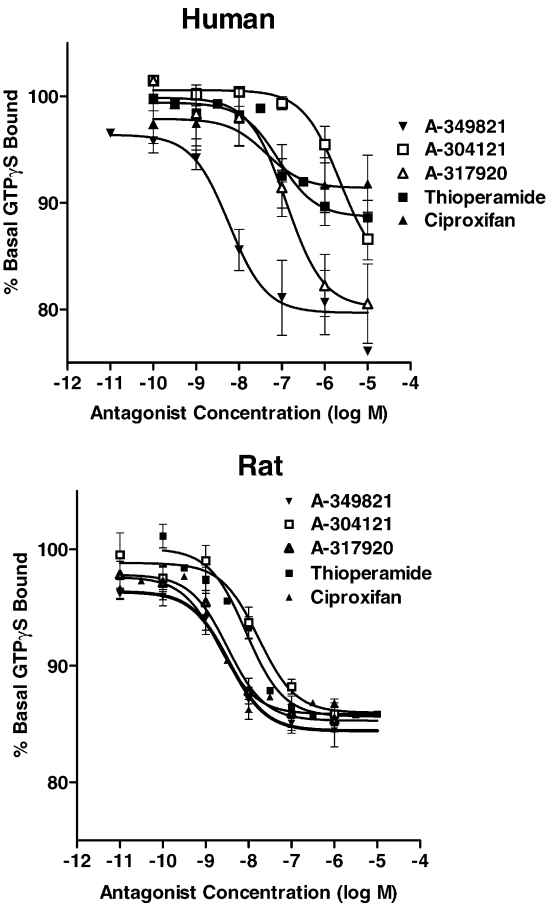

Fig. 5. A-349821 decreases basal [^{35}S]-GTPγS binding in a concentration-dependent manner in membranes from cells stably expressing the full-length human (top panel) and rat (bottom panel) histamine H_3 receptors. Membranes were incubated with increasing concentrations of the H_3 receptor antagonists and [^{35}S]-GTPγS binding determined as described in Section 2. Data are expressed as a percentage of the basal [^{35}S]-GTPγS binding in the absence of H_3 receptor antagonist. Results represent the mean ± S.E.M. of 4–10 concentration response assays performed in triplicate.

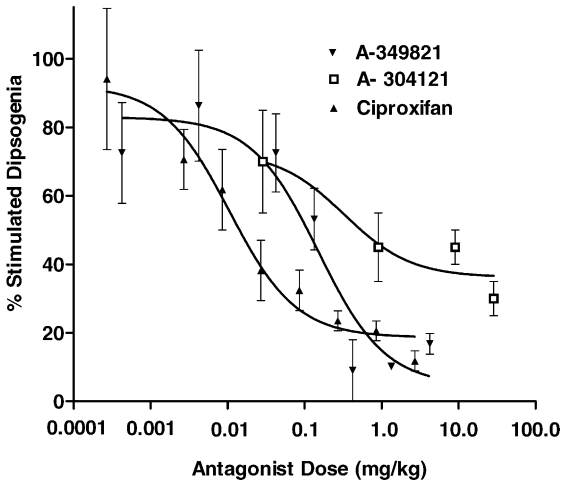

Fig. 6. Dose-dependent attenuation of (*R*)-α-methylhistamine-induced dipsogenia in mice by A-349821, A-304121, and ciproxifan. Mice were dosed with antagonist 5 min before (*R*)-α-methylhistamine and water intake was recorded 30 min later for an additional 30 min period. (*R*)-α-methylhistamine (30 mg/kg) induced a pronounced dipsogenia response in vehicle-treated mice, increasing water consumption (0.29 ± 0.04 ml) about six-fold over basal (0.05 ± 0.01 ml) levels ($F(5,57) = 8.939$, $P < 0.0001$). Results represent the mean ± S.E.M. Significant ($P < 0.05$) attenuations of the (*R*)-α-methylhistamine response were achieved for A-349821 (>0.1 mg/kg), A-304121 (>0.9 mg/kg), and ciproxifan (>0.03 mg/kg).

receptor antagonists tested had equivalent intrinsic inverse agonist activity at the rat receptor (Table 4).

3.6. Dipsogenia model

A-349821 completely blocked dipsogenia induced by (*R*)-α-methylhistamine in a dose-dependent manner, reaching significance at 0.1 mg/kg (Fig. 6), with a fully efficacious dose achieved at 0.4 mg/kg. Administered alone, A-349821 had no effect on basal water intake when tested at 4 mg/kg (data not shown). Ciproxifan was slightly more potent than A-349821 in this model with a significant effect observed at 0.03 mg/kg whereas A-304121 was less potent with a significant effect observed at 0.9 mg/kg (Fig. 6). Ciproxifan was also fully efficacious in reversing (*R*)-α-methylhistamine-induced dipsogenia and did not affect basal water intake when administered alone [44].

3.7. Five trial inhibitory avoidance response

Control SHR pups treated with vehicle only gradually acquired this task over the five trials as can be seen by the increase in transfer latencies from the lighted to the dark compartment (Fig. 7, top and middle panels). SHR pups treated with A-349821 dose-dependently (1–10 mg/kg)

Table 4
Inverse agonist properties of A-349821, A-304121, A-317920, ciproxifan, and thioperamide at human and rat histamine H_3 receptors as determined by reversing basal [^{35}S]-GTPγS binding

Compound	Human H_3 receptor			Rat H_3 receptor		
	Potency pEC_{50} (mean ± S.E.M.)	Efficacy maximum inhibition (mean ± S.E.M.)	Intrinsic activity[a]	Potency pEC_{50} (mean ± S.E.M.)	Efficacy maximum inhibition (mean ± S.E.M.)	Intrinsic activity[a]
A-349821	9.09 ± 0.08	22.9 ± 1.9	−1.0	8.57 ± 0.09	14.8 ± 1.5	−1.0
A-304121	5.75 ± 0.06	15.5 ± 2.2	−0.7	7.67 ± 0.07	14.7 ± 1.0	−1.0
A-317920	6.83 ± 0.09	21.3 ± 3.1	−0.9	8.66 ± 0.06	14.0 ± 1.6	−0.9
Ciproxifan	7.04 ± 0.17	8.3 ± 2.0	−0.4	8.87 ± 0.16	13.7 ± 0.8	−0.9
Thioperamide	7.41 ± 0.12	11.4 ± 1.1	−0.5	8.06 ± 0.1	14.2 ± 0.5	−1.0

[a] Intrinsic activity is defined as the ratio of the efficacy of each compound relative to A-349821. $n = 4$–10 independent experiments performed in triplicate.

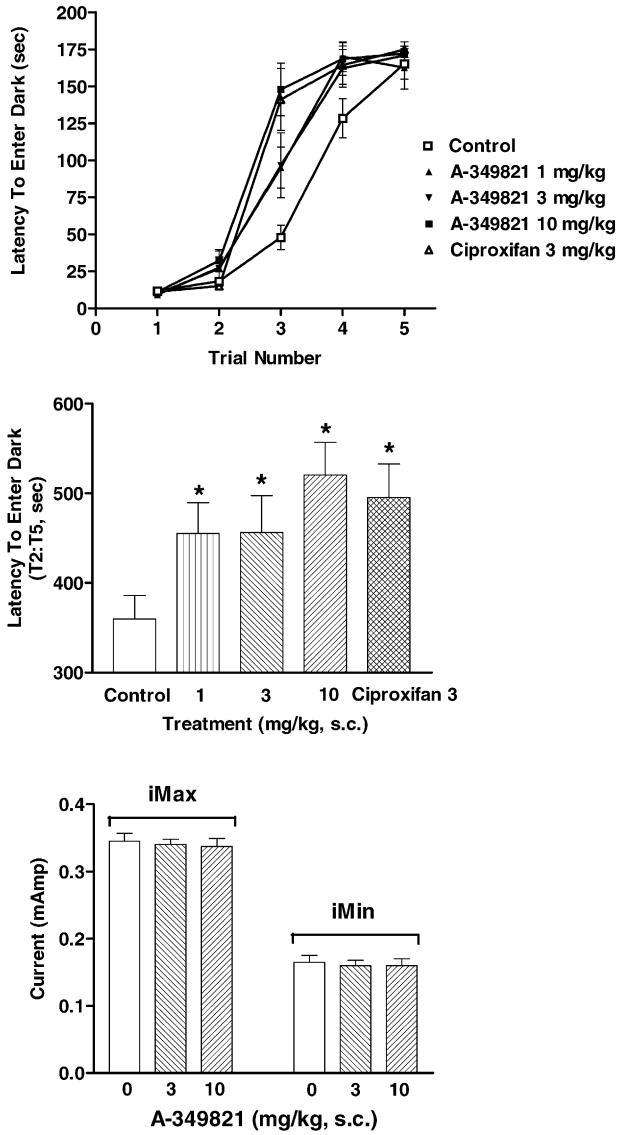

Fig. 7. Dose-dependent enhancement of acquisition of a five-trial inhibitory avoidance response (latency to enter dark chamber) in rat pups with A-349821 across individual trials (top panel) and in combined trials 2–5 (T2:T5, middle panel). SHR pups were dosed with H_3 receptor antagonist 30 min prior to the first trial. Data are represented by mean ± S.E.M. ($^*P < 0.05$ with respect to control response). A-349821 showed no effect on sensitivity to footshock (bottom panel). SHR pups were dosed with antagonist 30 min prior to inescapable exposure to increasing currents (iMax) and subsequent vocalization, followed by decreasing currents (iMin) and subsequent cessation of vocalization. Data are represented by mean ± S.E.M.

exhibited significantly prolonged transfer latencies in trials 2–5 indicative of improved acquisition of the task, similar to that seen with 3 mg/kg ciproxifan used as an internal control (Fig. 7, top and middle panels) and to A-304121 (10 mg/kg) and A-317920 (3 mg/kg) shown previously [20]. This effect was most evident on trials 3, 4 and 5, when compared with vehicle-treated controls. The absence of effect of A-349821 on transfer latencies on the first trial is indicative of a lack of non-specific effects on locomotor activity. Importantly, sensitivity to footshock was not

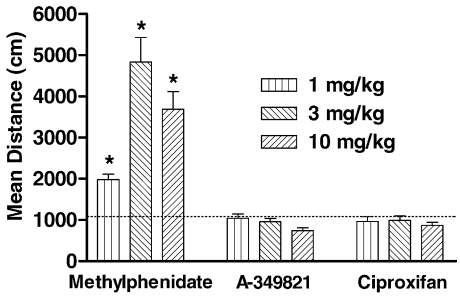

Fig. 8. Effects of A-349821, ciproxifan, and methylphenidate on spontaneous locomotor activity in SHR pups. Drugs were administered 30 min before rat pups were placed into the activity monitors and distance traveled was recorded for a further 30 min. Data are represented by mean ± S.E.M. [$^*P < 0.05$ with respect to control vehicle response of 1049 + 260 cm (dotted line), Tukey's post hoc test].

affected by A-349821 for iMax or iMin (Fig. 7, bottom panel) as assessed in separate, control experiments indicating the improved acquisition of the task was not due to enhanced sensitivity to the footshock.

3.8. Spontaneous locomotor activity

A-349821, at doses from 1 to 10 mg/kg, did not significantly affect locomotor activity (Fig. 8) in SHR pups over a 30 min observation period. Similarly, ciproxifan did not alter locomotor activity when tested over the same dose range. In contrast, methylphenidate induced a dose-dependent increase in locomotor activity in SHR pups (Fig. 8), resulting in a significant ($P < 0.05$) treatment effect at each of the doses tested (1, 3, and 10 mg/kg).

3.9. General observation test

The behavioral profile of A-349821 was also examined following i.p. administration in a general observation test in mice with the first noticeable effects seen at 35 mg/kg. These effects included transient hypothermia (less than 1 h) and hypoactivity. At the highest tested dose (118 mg/kg), A-349821 caused seizures and lethality within 15 min.

4. Discussion

The novel, non-imidazole compound A-349821 is a potent H_3 receptor antagonist with cognition enhancing properties. Unlike many imidazole H_3 receptor antagonists such as thioperamide and ciproxifan as well as other previously described non-imidazole H_3 receptor antagonists such as A-304121 and A-317920 that demonstrate high affinity for only the rat receptor, A-349821 potently and selectively binds to both the rat and human H_3 receptor. A-349821 is over 100-fold more potent than A-304121, A-317920, thioperamide, and ciproxifan at the human H_3 receptor, exhibiting subnanomolar affinity at this site while also maintaining low nanomolar affinity for the rat H_3

receptor similar to other H_3 antagonists. While A-304121, A-317920, thioperamide, and ciproxifan are all at least 20-fold more potent at the rat H_3 receptor than human, A-349821 is slightly more potent (four-fold) at the human H_3 receptor. Additionally, A-349821 is over 1000-fold selective for the human histamine H_3 receptor versus the other three human histamine receptor subtypes. In contrast, the imidazole H_3 antagonist thioperamide binds the human H_4 receptor with the same affinity as the human H_3 receptor and ciproxifan is only 30-fold selective for the human H_3 receptor in comparison to its affinity for the H_4 receptor. Additionally, A-349821 is over 100-fold selective for the human H_3 receptor compared to over 80 rodent and human G-protein coupled receptors and ligand activated ion channels (data not shown) including those for the biogenic amine serotonin $5HT_3$ and α_2-adrenergic receptors that generally show appreciable binding to imidazole H_3 receptor antagonists [29].

A-349821 displays competitive antagonist properties when examined in a variety of tissue and cell-based functional assays including the inhibition of recombinant rat and human H_3 receptor mediated signaling pathways as well as antagonism in models of H_3 receptor mediated neurotransmitter release. A-349821 was over 40-fold more potent than the imidazole H_3 antagonists thioperamide and ciproxifan as well as the non-imidazole compounds A-304121 and A-317920 in competitively inhibiting (*R*)-α-methylhistamine-induced reversal of forskolin-stimulated cAMP accumulation in C6 cells expressing the human H_3 receptor, whereas it was about 10-fold less potent than A-317920 and ciproxifan at the rat H_3 receptor. A similar profile was seen for A-349821 mediated inhibition of (*R*)-α-methylhistamine stimulated GTPγS binding, demonstrating over 70-fold higher affinity than the comparative antagonists at the human H_3 receptor but equipotency at the rat H_3 receptor. Likewise, when comparing antagonist potencies in blocking (*R*)-α-methylhistamine-induced increases in intracellular calcium using FLIPR, A-349821 is over 100-fold more potent at the human H_3 receptor than any of the more rat-selective H_3 antagonists A-304121, A-317920, thioperamide, and ciproxifan. Across all three functional assays, A-349821 antagonist potency tends to be slightly higher (up to four-fold, similar to that seen in radioligand binding) at the human than the rat H_3 receptor. In assays examining native H_3 receptors, A-349821 exhibited potent, competitive H_3 antagonist properties, reversing histamine-mediated inhibition of potassium-evoked [^{3}H]-histamine release in the rat brain synaptosome model as well as antagonizing (*R*)-α-methylhistamine-mediated inhibition of electric field stimulated contractions in the classic guinea-pig ileum H_3 receptor model.

Many H_3 receptor antagonists, including thioperamide, ciproxifan, A-304121, and A-317920, are also inverse agonists, capable of reversing basal H_3 receptor constitutive activity, be it measured by [^{35}S]-GTPγS binding and/or enhancing cAMP formation [6,8,29]. Similarly, A-349821 is also a potent inverse agonist at the human H_3 receptor, demonstrating over 100-fold higher potency than the other comparative H_3 antagonists, in line with the binding potencies. A-349821 also appears to be more efficacious as a human H_3 receptor inverse agonist than A-304121 and the two imidazole H_3 receptor antagonists tested. In contrast, A-349821 was approximately equipotent and equally efficacious as an inverse agonist with the other H_3 antagonists tested at the rat H_3 receptor. Whether this represents inherent species differences in the ligand affinities, constitutive activities of the H_3 receptors, primary protein structures, conformational changes induced by the ligands, or other factors remains to be determined. Not only have H_3 receptors been shown to exhibit constitutive activity in recombinant systems, they also exhibit constitutive activity in native in vitro and in vivo systems, with inverse agonists enhancing neurotransmitter release [8,11]. Thus, it may be important to design compounds that not only potently bind and antagonize agonist activity, but are also potent and efficacious H_3 receptor inverse agonists in order to obtain a highly effective therapeutic agent with human CNS activity [11], although this remains yet to be proven in the clinic.

A number of centrally mediated functions such as food and water intake as well as learning and memory are regulated by H_3 receptors in rodent behavioral models and can be modulated by H_3 receptor agonists and antagonists. A-349821 dose-dependently blocks the effects of (*R*)-α-methylhistamine at central H_3 receptors in a previously characterized H_3 receptor dependent mouse dipsogenic response model [44,45], where antagonists potent at the rat H_3 receptor, which has 98% amino acid identity with the murine H_3 receptor [46], inhibit agonist induced dipsogenia. Like ciproxifan, A-349821 completely reversed the effect of (*R*)-α-methylhistamine, albeit with lower potency, and improved upon both the potency and efficacy of our previous rat-selective non-imidazole H_3 receptor antagonist, A-304121. Basal water intake was not affected by A-349821. These results demonstrate that A-349821 can adequately penetrate the CNS and can serve as a functional antagonist of murine central H_3 receptors.

A-349821 enhanced performance of SHR pups in a trial- and dose-dependent manner relative to vehicle-treated controls in a five-trial, repeated acquisition, inhibitory avoidance task with a maximal response that compared favorably with ciproxifan (3 mg/kg). SHR pups show a naturally slower learning curve over the five trials used in this model when compared to age, size, and sex-matched pups from other strains [17]. The SHR is a key animal model that exhibits genetically induced impairments in sustained and non-selective attention as well as increased activity and impulsivity in novel surroundings, behavioral characteristics associated with ADHD, and proposed to result from hypofunctioning dopaminergic activity [47]. The predictive utility of this model has been shown for the stimulant methylphenidate and the nicotinic agonist ABT-

418 [17], both of which demonstrate clinical efficacy in ADHD. Thus, this model may serve as a predictive preclinical model for H_3 receptor antagonists such as A-349821 for the treatment of cognitive deficits associated with ADHD and other neuropsychiatric disorders.

In order to distinguish the behavioral profile of the H_3 antagonist A-349821 from that of the stimulant methylphenidate, non-habituated SHR pups treated with these compounds were assessed for locomotor activity in open field arenas by measuring the distance traveled over 30 min. The stimulant properties of methylphenidate were pronounced, as expected, with significant increases in locomotor activity seen at all three tested doses (1, 3, 10 mg/kg). In contrast, A-349821 did not exhibit stimulant-like activity since treatment of the SHR pups did not increase locomotor activity at any of the tested doses (1, 3, 10 mg/kg) and perhaps showed a slight tendency towards a decrease in locomotor activity at the highest dose, 10 mg/kg. Ciproxifan and A-304121, another non-imidazole H_3 antagonist tested in this model using SHR pups, also had no effect on locomotor activity (data not shown), consistent with findings for these compounds in mice [20]. Interestingly, blockade of H_3 receptors does not induce locomotor sensitization upon repeated administration, unlike stimulants, nor does the effect cross sensitize with stimulants [48]. These characteristics further support the potential clinical differentiation between stimulants and H_3 receptor antagonists as potential therapies for cognitive dysfunctions as in ADHD. Based on the efficacious dose (1 mg/kg) in the cognition model, a therapeutic ratio of over 100 is obtained with respect to serious adverse effects in the general observation test (118 mg/kg), greater than that for the imidazole H_3 receptor antagonists thioperamide, ciproxifan, and GT-2331, as well as the previously described non-imidazole H_3 receptor antagonists A-304121 and A-317920 [20].

The present study identifies A-349821 as a novel, non-imidazole H_3 receptor competitive antagonist/inverse agonist that is potent at both the rat and human H_3 receptor, is highly selective, and can modulate neurotransmitter release in several in vitro models. Functionally, A-349821 can also penetrate the CNS to block central H_3 receptors, eliciting cognition enhancing activity at doses well below those that cause serious central effects and without stimulant activity. Although H_3 receptor antagonists have been proposed as potential therapeutic agents for a variety of cognitive disorders including attention deficit/hyperactivity disorder, Alzheimer's disease, and schizophrenia, no potent, selective, and safe H_3 receptor antagonist has yet been tested for efficacy in man; thus it remains to be seen whether such an approach will be effective for the treatment of such disorders. Our drug discovery effort has focused on the synthesis of non-imidazole H_3 receptor antagonists, exemplified by A-349821 [34], that demonstrate potent, balanced affinities for human and rat H_3 receptors, inverse agonist activity, and cognition enhancing effects with a wide therapeutic window in order to develop potent, selective and efficacious human H_3 receptor antagonists that will be important therapeutic agents in the treatment of a variety of neuropsychiatric disorders.

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