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Species-related pharmacological heterogeneity of histamine H_3 receptors

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Abstract

We compared radioligand binding and functional data for histamine H_3 receptor ligands across different tissues or species to evaluate the basis for pharmacological evidence of receptor heterogeneity previously reported. Agonist binding affinities showed correlation coefficients near unity in comparing human, dog, rat, and guinea pig cerebral cortical histamine H_3 receptors. Antagonist binding affinities revealed lower correlations for human compared to dog, rat, or guinea pig, suggesting species-based pharmacological differences. The functional potencies of histamine H_3 receptor antagonists in field-stimulated guinea pig ileum were highly correlated to binding affinities for guinea pig, dog, and, to a lesser extent, rat cerebral cortex. However, antagonist binding affinity at human cerebral cortex did not correlate well with guinea pig ileum functional potency. These results suggest significant interspecies histamine H_3 receptor heterogeneity, consistent with recent receptor gene sequence data. Therefore, genetic heterogeneity, rather than peripheral and central histamine H_3 receptor diversity, is responsible for the pharmacological differences observed.

Keywords: Histamine H_3 receptor; Receptor heterogeneity; Radioligand binding; Ileum, guinea pig; Electrical field stimulation

1. Introduction

Histamine H_3 receptors are members of the G-protein coupled receptor family (Arrang et al., 1990; Lovenberg et al., 1999) involved in the regulation of neurotransmitter release in both central and peripheral neurons (Timmerman, 1990; Ishikawa and Sperelakis, 1987; Arrang et al., 1983). The cDNA for the human histamine H_3 receptor encodes a 445 amino acid protein that, when recombinantly expressed, couples to inhibition of adenylate cyclase presumably through $G_{\alpha i}$ (Lovenberg et al., 1999). The histamine H_3 receptor mRNA is highly expressed in central nervous tissues consistent with experimental findings such as autoradiographic (Arrang et al., 1987; Lovenberg et al., 1999), radioligand binding (Tedford et al., 1998; Harper et al., 1999b) and functional (Coruzzi et al., 1991; Leurs et al., 1996) data describing the role of histamine H_3 receptors in regulating central neurotransmitter release. Stimulation of presynaptic histamine H_3 receptors inhibits the release of neurotransmitters such as histamine (Arrang et al., 1983), norepinephrine (Schlicker et al., 1988), serotonin (Fink et al., 1990) and acetylcholine (Clapham and Kilpatrick, 1992; Lee and Parsons, 2000) from brain slices, synaptosomes and various peripheral tissue preparations. Histamine H_3 receptor ligands have also been shown to modulate the release of non-adrenergic, non-cholinergic neurotransmitters including substance P from isolated guinea pig ileum segments (Menkveld and Timmerman, 1990; Taylor and Kilpatrick, 1992). The potential therapeutic roles of histamine H_3 receptor antagonists in the central nervous system have been tested in models of learning and memory impairment, attention-deficit hyperactivity disorder, obesity and epilepsy (for review, see Leurs et al., 1998). Studies of the regulation of inflammatory processes (Rouleau et al., 1997), gastroprotection (McLeod et al., 1998), and cardiovascular function (Levi and Smith, 2000) suggest several therapeutic possibilities for peripherally acting histamine H_3 receptor agonists.

The existence of histamine H_3 receptor heterogeneity has been postulated from binding and functional assays using a limited number of selective histamine H_3 receptor ligands. For example, West et al. (1990a) suggested that histamine H_3 receptor could be classified into H_{3A} (high affinity)- and H_{3B} (low affinity)-receptors based upon the biphasic displacement of [^{3}H]*N*-α-methylhistamine binding by thioperamide and burimamide in rat brain membranes. Comparing the binding affinities of histamine H_3 receptor ligands in

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guinea pig cerebral cortex and ileum longitudinal muscle myenteric plexus using [^{3}H]*R*-α-methylhistamine showed only a few histamine homologues able to discriminate pharmacologically between the two tissue histamine H_3 receptors (Harper et al., 1999a). Studies examining the functional effects of histamine H_3 receptor antagonists on neurotransmitter release in mouse brain cortex and guinea pig ileum also suggested receptor heterogeneity (Schlicker et al., 1994). Other alternative explanations for the apparent histamine H_3 receptor heterogeneity could include species homologues of the histamine H_3 receptor (Ligneau et al., 2000; Lovenberg et al., 2000; Tardivel-Lacombe et al., 2000; West et al., 1999), differences in receptor coupling to second messenger systems by receptor isoforms (Drutel et al., 2001), or the use of different assay conditions. We have compared the binding affinities of histamine H_3 receptor ligands in human, dog, rat and guinea pig cerebral cortex to potencies of these ligands in the guinea pig ileum electric field stimulated model to ascertain if receptor heterogeneity or species differences could contribute to the variety of results obtained in similar studies of the histamine H_3 receptor.

2. Materials and methods

2.1. Chemicals

The following histaminergic compounds were purchased from Sigma RBI (St. Louis, MO): histamine, mepyramine, ranitidine, cimetidine, *R*(−)-α-methylhistamine, *S*(+)-α-methylhistamine, *N*-α-methylhistamine, 3-(1*H*-imidazol-4-yl)propyl-di(*p*-fluorophenyl)-methyl ether hydrochloride (FUB 322), burimamide, dimaprit, thioperamide, and iodophenpropit. Imetit, immepip, clobenpropit, and 5-bromo-6-(2-imidazolin-2-ylamino)quinoxaline (UK14304) were purchased from Tocris Cookson (Ballwin, MO). Aprotinin, leupeptin and pepstatin were obtained from Roche Molecular Biochemicals (Indianapolis, IN). 4-(1-Cyclohexylpentanoyl-4-piperidyl) 1*H*-imidazole (GT-2016) and cyclopropyl-(4-(3-(1*H*-imidazol-4-yl)propyloxy)phenyl) ketone (ciproxifan) were synthesized at Abbott Laboratories. [^{3}H]*N*-α-methylhistamine (80–90 Ci/mmol) was obtained from DuPont-NEN (Boston, MA). All other chemicals, including those for the Krebs–Henseleit bicarbonate buffer, were purchased from Sigma. Human cerebral cortex was obtained from autopsy, frozen at −80 °C, and membrane preparations implemented by Analytical Biological Services (Wilmington, DE).

2.2. Radioligand binding assays

Cell membranes from human, dog, rat or guinea pig cerebral cortex expressing histamine H_3 receptors were homogenized in cold 50 mM Tris–HCl/5 mM EDTA buffer (pH = 7.4) containing 1 mM benzamidine, 2 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml pepstatin. The homogenate was centrifuged at 40,000 × *g* for 20 min at 4 °C. This step was repeated and the resulting pellet was resuspended in Tris/EDTA buffer in a final volume of five times the wet weight of the tissue. Aliquots were frozen at −70 °C until needed. On the day of assay, membranes were thawed and diluted with 50 mM Tris/5 mM EDTA buffer to provide a concentration of 0.1 (rat, guinea pig, dog)- and 0.6 (human)-mg protein/tube. For competition assays, [^{3}H]*N*-α-methylhistamine (from 0.5 to 1.0 nM) was incubated with cell membranes either alone or in the presence of competing histaminergic ligands at 25 °C for 30–45 min. Non-specific binding was defined with thioperamide (10 μM). Radioligand binding was terminated by filtering through GF/B filters (Whatman® or Packard Filtermate/Unifilter®) followed by four rinses with 50 mM Tris–HCl buffer (pH = 7.4). The amount of bound radiolabel was determined by liquid scintillation counting. Data analysis was as previously described (Hancock and Esbenshade, 2000).

2.3. Guinea pig ileum electrical field stimulation histamine H_3 receptor functional model

The method of Trzeciakowski (1987) was modified to facilitate the examination of functional responses mediated by histamine H_3 receptors. A 20-cm section of ileum, obtained approximately 10 cm proximal to the ileocecal junction, was removed from young male guinea pigs (150–250 g) and sectioned into 2 cm segments, cleaned and placed in warm (37 °C) Krebs–Henseleit bicarbonate buffer containing 2.5 mM $CaCl_2$, 1 μM mepyramine and 10 μM ranitidine and 10 μM EDTA. One end of the segment was then mounted onto a stationary rod containing parallel platinum electro-

Table 1
Comparison of mean pK_i values of histamine ligands at human, rat, dog, and guinea pig cerebral cortical histamine H_3 receptors

Compound	Species source of histamine H_3 receptors			
	Human	Rat	Dog	Guinea pig
S(+)-α-methylhistamine	8.33 ± 0.18	7.33 ± 0.06	7.79 ± 0.11	7.63 ± 0.18
Histamine	8.59 ± 0.08	8.29 ± 0.03	8.50 ± 0.08	8.18 ± 0.15
R-α-methylhistamine	9.15 ± 0.06	8.71 ± 0.08	8.93 ± 0.19	8.63 ± 0.25
N-α-methylhistamine	9.41 ± 0.06	8.78 ± 0.13	9.15 ± 0.09	8.67 ± 0.27
Immepip	9.58 ± 0.06	8.99 ± 0.20	9.25 ± 0.06	8.68 ± 0.34
Imetit	9.67 ± 0.06	9.46 ± 0.10	9.31 ± 0.06	8.99 ± 0.25
GT-2016	6.30 ± 0.12	7.56 ± 0.08	7.39 ± 0.13	7.51 ± 0.13
Dimaprit	6.66 ± 0.17	6.45 ± 0.04	6.25 ± 0.05	6.30 ± 0.13
Ciproxifan	6.99 ± 0.08	9.15 ± 0.05	8.20 ± 0.08	8.70 ± 0.08
Thioperamide	7.13 ± 0.06	8.10 ± 0.06	8.17 ± 0.15	8.34 ± 0.12
FUB 322	7.48 ± 0.14	8.12 ± 0.08	8.06 ± 0.12	8.42 ± 0.21
Burimamide	8.34 ± 0.21	8.18 ± 0.15	8.42 ± 0.13	8.27 ± 0.05
Iodophenpropit	8.45 ± 0.14	9.23 ± 0.10	9.59 ± 0.09	9.39 ± 0.21
Clobenpropit	9.08 ± 0.13	9.38 ± 0.08	10.09 ± 0.13	9.65 ± 0.22

Histamine H_3 receptor competition binding assays were preformed using [^{3}H]-*N*-α-methylhistamine as the radioligand and thioperamide (10 μM) to define non-specific binding. Data are expressed as mean pK_i values ± S.E.M. for $n \geq 3$.
FUB 322 is an abbreviation for 3-(*1*H-imidazol-4-yl)propyl-di(*p*-fluorophenyl)-methyl ether hydrochloride.

des aligned on each side of the tissue, and the other end connected to a Grass FT03 transducer at a basal preload tension of 1 g. After a 10-min equilibrium period in heated (37 °C) 10 ml organ baths, the tissues were electrically stimulated (supramaximal voltage ~15 V, 0.1 Hz frequency, 0.5 ms duration) and rinsed every 10 min for 1 h. The intensity of the stimulus was then decreased every 5 min, initially by 2 V (smaller decrements in voltage as the threshold was approached) until the threshold voltage for electrical field stimulated contraction could be established. The experiment was then run at a test voltage of 1.5 times the observed threshold voltage. The tissues were stimulated for an additional 30 min (without rinsing) at the test voltage (generally, 7–8 V) before the control agonist (*R*-α-methylhistamine) dose–response analysis was begun. Antagonists (or vehicle) were added at various concentrations to the tissue baths during this period. Agonists were tested by cumulative addition of semi-logarithmically increasing doses to the baths. Each agonist was run in parallel and compared with the effects of *R*-α-methylhistamine on electrical field stimulation contraction. Due to histamine H_3 receptor desensitization (Perez-García et al., 1998), only one control agonist

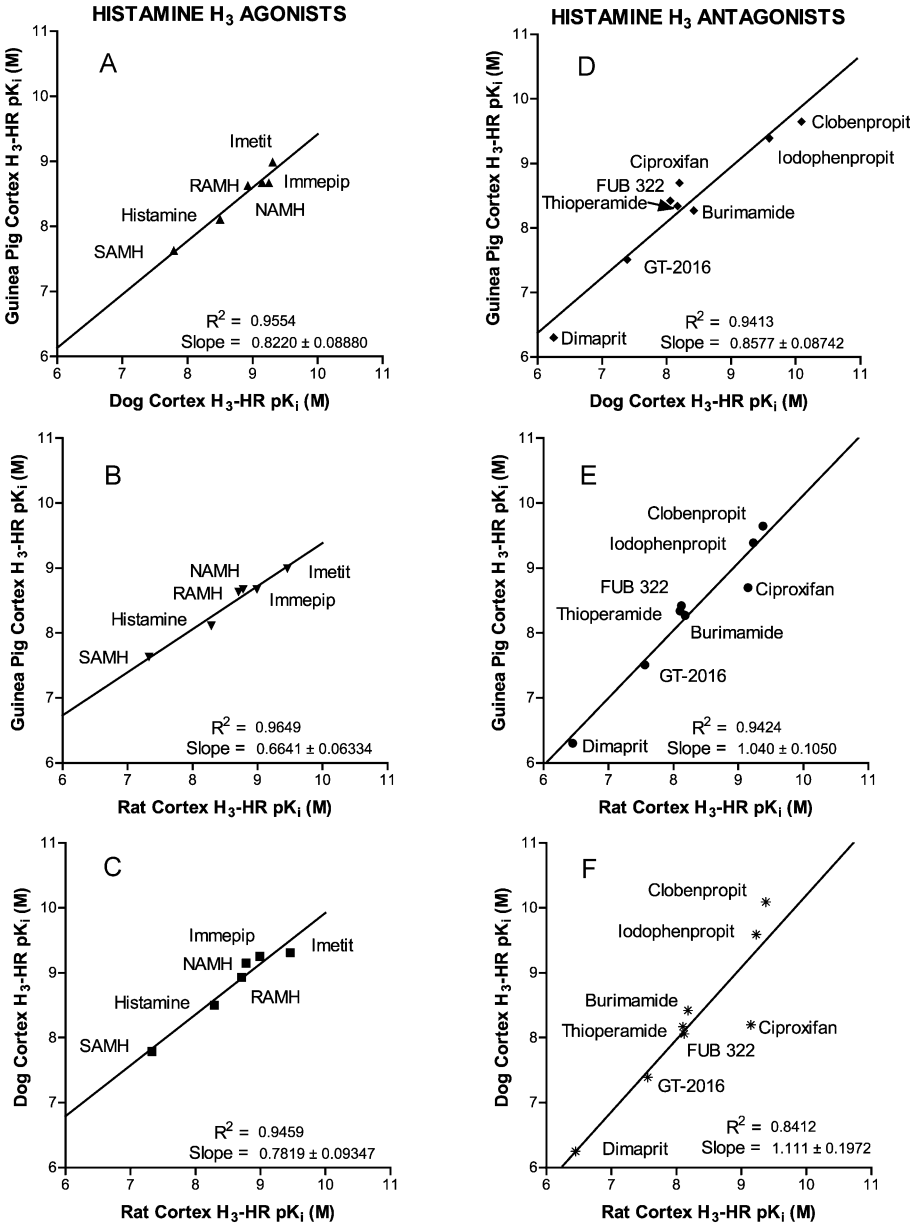

Fig. 1. Linear correlation of the potencies of selective histamine H_3 receptor agonists and antagonists for dog versus guinea pig (A and D) rat versus guinea pig (B and E) and dog versus rat (C and F) cerebral cortical histamine H_3 receptors. Data expressed as pK_i values for $n \geq 4$. SAMH, RAMH, NAMH and FUB 322 are abbreviations for *S*(+)-α-methylhistamine, *R*-α-methylhistamine, *N*-α-methylhistamine and 3-(1*H*-imidazol-4-yl)propyl-di(*p*-fluorophenyl)-methyl ether hydrochloride, respectively.

concentration curve was run on each ileal segment. Using ileal segments from young guinea pigs also minimized desensitization. The concentration of the agonist necessary to cause a 50% inhibition in the electrical field stimulation contraction (EC_{50}) was calculated using an Excel-based program, AGANTG (Zielinski and Buckner, 1998), and expressed as the negative logarithm (pD_2). The potency of the antagonists (pA_2) to inhibit the *R*-α-methylhistamine response was calculated according to the method of Schild (1947) using AGANTG. These studies were carried out in accordance with guidelines outlined by the Animal Welfare Act, the Association for Assessment and Accreditation of Laboratory Animals (AAALAC) and the Institutional Animal Care and Use Committee of Abbott Laboratories.

3. Results

3.1. Radioligand binding experiments

Saturation binding isotherms generated from experiments utilizing at least 12 concentrations of [^{3}H]*N*-α-methylhist-

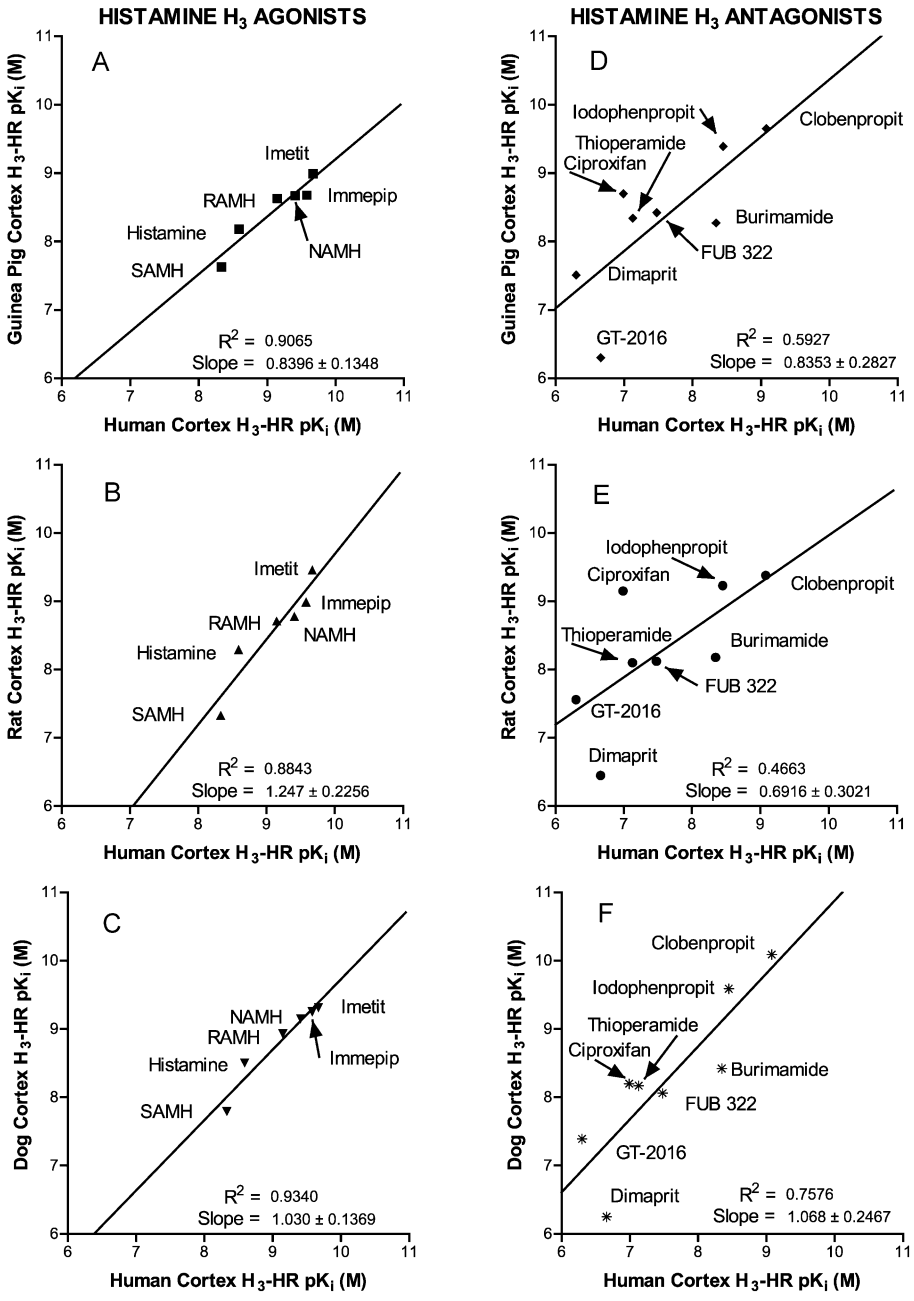

Fig. 2. Linear correlation of the potencies of selective histamine H_3 receptor agonists and antagonists for human versus guinea pig (A and D) human versus rat, (B and E) and human versus dog (C and F) cerebral cortical histamine H_3 receptors. Data expressed as pK_i values for $n \geq 4$. Abbreviations are as given in the legend to Fig. 1.

amine (0.018–5 nM) were performed on membranes prepared from human, dog, rat and guinea pig cerebral cortices. Linear Scatchard plots and data analysis (GraphPad Prism) were consistent with binding to a single high affinity site. pK_d and B_{max} values (fmol/mg protein) for histamine H_3 receptor in these tissues were as follows: human 9.52 ± 0.03, 13.3 ± 0.6; dog 9.16 ± 0.06, 51.5 ± 1.6; rat 9.31 ± 0.03, 106 ± 5.4; guinea pig 9.38 ± 0.03, 124 ± 9.3, respectively (data not shown). Competition assays were performed using selective histamine H_3 receptor ligands. These compounds have pK_i values between 4 and 6 at cloned human histamine H_1 and human histamine H_2 receptors (data not shown, see Hancock and Esbenshade, 2000) with the exception of FUB 322 (pK_i = 6.9) and iodophenpropit (pK_i = 6.1) at the human H_1-receptor. Theses ligands all gave monophasic displacement of the radioligand with Hill slopes close to unity and had sub micromolar K_i values at histamine H_3 receptors (Table 1).

Comparisons of the binding affinities of these selective histamine H_3 receptor ligands at human, dog, rat and guinea pig cerebral cortex preparations were done using linear regression correlation analysis (GraphPad Prism). Analyzing the binding affinities of the ligands at guinea pig, rat and dog cerebral cortex clearly show that the agonists did not differentiate between the histamine H_3 receptor of these species (Fig. 1A, B and C) since the correlation coefficients for each cross-species comparison were approximately 0.94 or higher. Antagonist binding affinities were also highly correlated when comparing the guinea pig cortex histamine H_3 receptor to dog or rat cerebral cortical histamine H_3 receptors where correlation coefficients for these cross-species comparisons were approximately 0.94 (Fig. 1D and E). The lower coefficient of correlation (R^2 = 0.84) for the dog and rat cortex histamine H_3 receptor comparison was primarily due to ciproxifan, which had higher affinity for the rat cortex histamine H_3 receptor than dog cerebral cortical histamine H_3 receptors (Fig. 1F).

When comparing the binding affinities of the agonists at the human cerebral cortical histamine H_3 receptor to other species, the coefficients of correlation ranged from R^2 = 0.88 to 0.93 for guinea pig, rat and dog versus human histamine H_3 receptors (Fig. 2A, B and C). The slightly lower correlation of human histamine H_3 receptors to the other species using this limited set of commercially available agonists was influenced by the higher affinity of *S*(+)-α-methylhistamine for the human receptor as compared to the other species (Fig. 2). The comparisons of the antagonist binding affinities at human cerebral cortical histamine H_3 receptors to the other three species show that a number of compounds are clearly able to discriminate between the human cortical histamine H_3 receptors and that of the other species studied (Table 1, Fig. 2D, E and F). Correlation coefficients comparing H_3 receptor antagonist binding for human cortex to the other species showed the greatest diversity against the rat (R^2 = 0.47, Fig. 2E), but also substantial differences between human and guinea pig (R^2 = 0.59, Fig. 2D) or dog (R^2 = 0.76, Fig. 2F).

3.2. Guinea pig ileum histamine H_3 receptor functional model

At a supramaximal voltage (15 V, 0.1 Hz for 0.5 ms duration), the maximal *R*-α-methylhistamine response was 30% inhibition of electrical field stimulation-induced contraction. At supramaximal voltage, activation of preganglionic fibers can occur which may contribute to the apparent histamine H_3 receptor resistant electrical field stimulation contraction (Trzeciakowski, 1987). Similar moderate efficacy by *R*-α-methylhistamine to inhibit twitch responses was observed by Lee and Parsons (2000), using near maximal voltage. Therefore, the threshold stimulation voltage at which electrical field stimulation contractions occur was observed for each guinea pig ileal section and the test voltage determined at which the maximal histamine H_3 receptor agonist response ranged from 50% to 100% inhibition of the electrical field stimulation contraction. Using the same experimental protocol, we found that alpha-$_2$ adrenergic receptor stimulation using the selective agonist UK-14304 was able to completely block the supramaximal and the test voltage electrical field stimulation contraction (pD_2 = 8.0, n = 4; data not shown), similar to data previously reported in guinea pig duodenum (Poli et al., 1997). The potent agonist *R*-α-methylhistamine gave the most reproducible and efficacious histamine H_3 receptor response of all the histamine H_3 receptor-selective ligands tested (Table 2) and was, therefore, used as the control agonist. Comparable high efficacy inhibition of twitch responses by *R*-α-methylhistamine at submaximal voltages has also been noted

Table 2
Mean pA_2 and pD_2 values of histamine ligands at the guinea pig ileum histamine H_3 receptor functional model

Agonist	pD_2 ± S.E.M.	%Max. response ± S.E.M.
S(+)-α-methylhistamine	6.21 ± 0.15	87.3 ± 7.0
Histamine	6.46 ± 0.18	45.8 ± 15.3
R-α-methylhistamine	7.60 ± 0.05	82.6 ± 2.2
N-α-methylhistamine	7.17 ± 0.07	67.3 ± 6.0
Immepip	8.35 ± 0.08	85.7 ± 7.3
Imetit	7.80 ± 0.10	57.8 ± 7.5
Antagonist	**pA_2 ± S.E.M.**	**Slope ± S.E.M.**
GT-2016	6.92 ± 0.40	0.94 ± 0.11
Dimaprit	6.16 ± 0.10	0.79 ± 0.09
Ciproxifan	8.12 ± 0.56	0.81 ± 0.08
Thioperamide	8.44 ± 0.45	0.77 ± 0.05
FUB 322	7.62 ± 0.18	1.03 ± 0.09
Burimamide	8.05 ± 0.68	0.63 ± 0.25
Iodophenpropit	9.14 ± 0.61	1.15 ± 0.13
Clobenpropit	9.66 ± 0.49	1.00 ± 0.06

Drugs were tested for effects on electrical field stimulation contraction of guinea pig ileal segments (approximately 7–8 V, 0.5 ms, 0.1 Hz). Histamine H_3 receptor antagonist Schild analysis data are expressed as pA_2 value and slope. Histamine H_3 receptor agonist data are expressed as pD_2 value and %maximum response efficacy value, where complete inhibition of electrical field stimulation contraction is equal to the maximum response ($n \geq 4$). FUB 322 is an abbreviation for 3-(1*H*-imidazol-4-yl)propyl-di(*p*-fluorophenyl)-methyl ether hydrochloride.

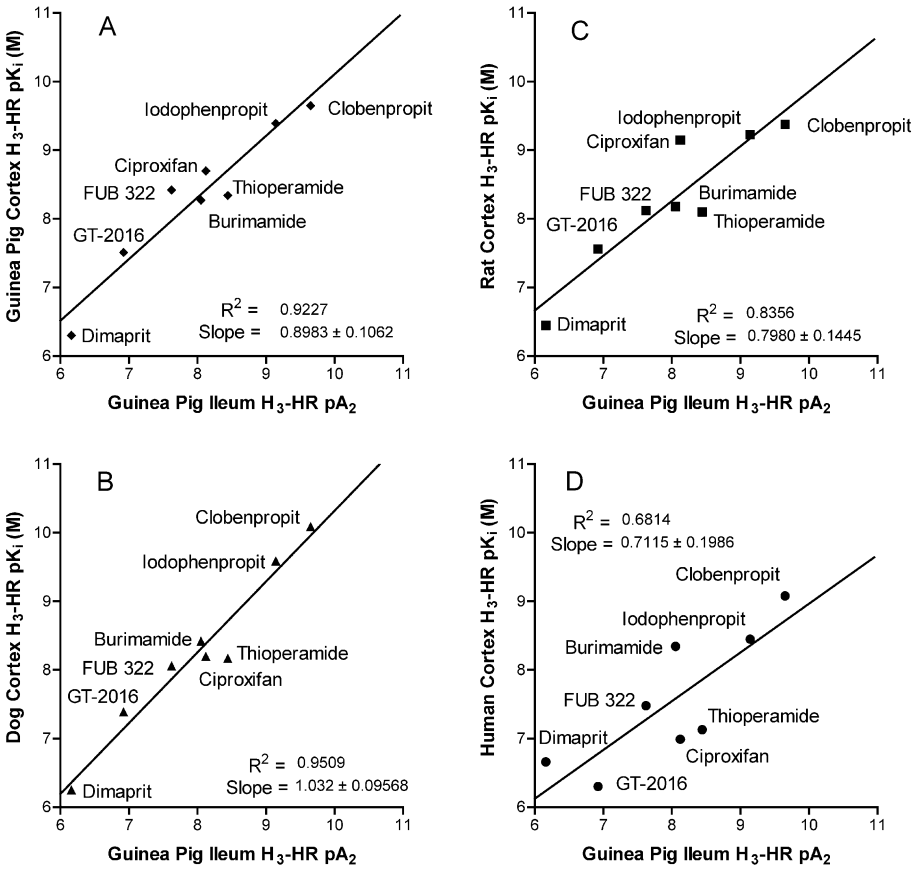

Fig. 3. Linear correlation of the binding affinities of the histamine H_3 receptor antagonists at human, guinea pig, dog and rat cerebral cortical membranes compared with functional potencies at the guinea pig ileum. Data expressed as pK_i and pA_2 values for $n \geq 3$. Abbreviations are as given in the legend to Fig. 1.

(Barocelli et al., 1993). Schild analyses of antagonist reversal of *R*-α-methylhistamine-induced responses were consistent with competitive blockade, with slopes not different from unity, with the noted exception of burimamide (Table 2).

Comparing agonist potencies (pD_2 values) in guinea pig ileum to their respective radioligand binding affinities (pK_i) in cortical tissue showed high correlations (0.84–0.94) across all species (data not shown). These data are consistent with the lack of discriminatory power of these agonists shown above in radioligand binding comparisons (Figs. 1A, B and C and 2A, B and C, Table 1). Notably, agonist affinities in radioligand binding were as much as 100-fold greater than their corresponding potencies in the functional assay. Such discrepancies between agonist potency in functional assays compared to agonist affinities in radioligand binding assays have been previously observed (Zernig et al., 1995; Tian et al., 1996). Tian et al. (1996) suggested that agonist potencies in functional models generally lie between the binding pK_i values for high and low affinity states of receptors. While our assay conditions did not reveal any low affinity binding state for agonists, this may nevertheless be a contributing factor to these potency differences. Another possibility is that high presynaptic receptor occupancy by a histamine H_3 receptor agonist may be required to reduce neurotransmitter release.

For antagonists, comparison of the guinea pig ileum pA_2 values against pK_i values obtained in guinea pig, dog, rat and human cerebral cortex radioligand binding assays showed species variability resulting from the difference in rank order of binding affinities (Fig. 3). The histamine H_3 receptor antagonist binding affinities (pK_i) for the guinea pig cerebral cortex were highly correlated ($R^2 = 0.93$, slope = 0.89) with pA_2 values obtained in the guinea pig ileum histamine H_3 receptor functional model (Fig. 3A). The dog cerebral cortex binding affinities also correlated well ($R^2 = 0.95$, slope = 1.03) with the pA_2 values in the guinea pig ileum model (Fig. 3B). The rat cerebral cortical histamine H_3 receptor pK_i values correlated to a lesser extent ($R^2 = 0.84$, slope = 0.79) to antagonist pA_2 values in the guinea pig ileum histamine H_3 receptor functional model (Fig. 3C), while human cerebral cortical histamine H_3 receptor binding gave the lowest correlation ($R^2 = 0.68$ with a shallow slope of 0.71) to pA_2 values obtained in the guinea pig ileum (Fig. 3D).

4. Discussion

Radioligand binding assays with potent and selective histamine H_3 receptor ligands were used to compare com-

pound potencies and determine the extent of receptor heterogeneity within the central nervous system across species. Despite the availability of the gene sequences for several histamine H_3 receptors, not all of these receptors have been expressed successfully in stable cell lines, including two of the species (guinea pig and dog) that are fundamental to the comparisons integral to the present studies. In addition, the potential for pharmacological variability between full length and truncated forms of these receptors (see below) led us to begin our studies with native receptors in simple systems that might avoid artifactual differences between cell lines, expression levels or signal transduction pathways and the effects these might have on receptor–ligand interactions. Therefore, our studies focused on cortical homogenate binding from the four species, and compared ligand potencies to those obtained in a functional assay in the guinea pig ileum.

All agonists displayed rank orders of binding affinities for the histamine H_3 receptors that were similar across the four species tested. In particular, [^{3}H]*N*-α-methylhistamine did not discriminate across species, facilitating the radioligand binding assay cross-comparisons.

The pK_i values of the antagonists tested on rat, dog and guinea pig cerebral cortical preparations were also very similar. However, many antagonists were less potent at the human histamine H_3 receptor than at dog, rat or guinea pig histamine H_3 receptors, with ciproxifan having a K_i that was 100-fold weaker at the human receptor than at the rat histamine H_3 receptor (Table 1). The lower binding affinity of the antagonists does not appear to be due to the increased tissue concentration in the human histamine H_3 receptor assays necessitated by the low receptor density since, when the human cerebral cortical tissue concentration was diluted 10-fold (by a corresponding increase in the assay incubation volume while keeping the number of receptors comparable), similar pK_i values were obtained (data not shown). Also, the observation that some compounds, in particular the agonists, did not exhibit differential affinities at the human compared to the other species is consistent with no systematic or artifactual alteration in compound affinity at the human receptor.

It has been reported that thioperamide and burimamide show lower binding potency in monkey and human brain as compared to rodent (West et al., 1999). Cloning of the rat histamine H_3 receptor allowed direct comparison with the cloned human histamine H_3 receptor, which also showed a similar difference in affinity of thioperamide between the two species (Ligneau et al., 2000; Lovenberg et al., 2000). In our studies, thioperamide also showed lower affinity for human, compared to dog, rat and guinea pig cerebral cortical histamine H_3 receptor membrane preparations. Burimamide has been shown to be better characterized by a two-site curve-fit for inhibiting [^{3}H]*N*-α-methylhistamine binding in human cerebral cortical membrane preparations (West et al., 1999). However, we observed only a one-site curve-fit for burimamide when using 50 mM Tris buffer containing 5 mM EDTA. Using our assay conditions, we found that burimamide did not discriminate between human and rat cerebral cortical histamine H_3 receptors. In their report, Lovenberg et al. (2000) observed that clobenpropit did not distinguish between cloned human and rat histamine H_3 receptors, whereas in our hands, clobenpropit had 4-fold higher affinity for the rat cerebral cortical histamine H_3 receptor membrane preparation over the human. We have also shown that the histamine H_3 receptor antagonists ciproxifan, GT-2016, iodophenpropit, and FUB 322 have lower affinity for human cerebral cortical histamine H_3 receptors than for dog, rat or guinea pig histamine H_3 receptors. These results give further evidence that some antagonists may differentiate between dog, rat and guinea pig cerebral cortical histamine H_3 receptors compared to human cerebral cortical histamine H_3 receptors.

Other investigators have also recently described one or more compounds that exhibit pharmacological differences in diverse histamine H_3 receptor assays. Among the more interesting is impentamine, a histamine analog with a five-carbon linker between the imidazole and the amine, unlike histamine, which has only two carbon atoms. Leurs et al. (1996) showed impentamine to have partial agonist activity in studies of neurotransmitter release in the mouse brain, and to display both high and low affinity components in histamine H_3 receptor competition radioligand binding assays to rat cortical homogenates. Similarly, Harper et al. (1999a) found impentamine to exhibit heterogeneous displacement of radioligand bound to guinea pig cortical histamine H_3 receptors and to have higher affinity in cortical tissue than at histamine H_3 receptors in homogenates of guinea pig myenteric plexus. Similar discrepancies were also observed with several other histamine analogs by Harper et al. (1999a). In contrast, although all of the compounds evaluated in the present studies are imidazole based, they demonstrate additional structural diversity beyond straight-chain histamine analogs like impentamine, thus providing additional opportunity to expose pharmacological diversity among histamine H_3 receptors.

The electrical field stimulation guinea pig ileum functional model has been widely used to measure ligand potency at the histamine H_3 receptor. Unlike functional assays using tritiated neurotransmitter release from rodent brain slices (Alves-Rodrigues et al., 1996; Clapham and Kilpatrick, 1992; Schlicker et al., 1994) or from human myocardium (Hatta et al., 1997) where histamine H_3 receptor agonists inhibited from 30% to 50% of the stimulated neurotransmitter release, our modification of the guinea pig ileum model gives a robust response to histamine H_3 receptor activation with a typical agonist effect between 60% and 95% inhibition of the electrical field stimulation contraction. Furthermore, *R*-α-methylhistamine elicited consistent and reproducible maximal inhibition of 85–95%. In our hands, the histamine H_3 receptor antagonists examined were competitive in nature, with Schild plot slopes not different from unity and with no reduction in maximum

agonist response, excepting burimamide which exhibited a slope of 0.63 due to a major concentration-dependent reduction in maximum *R*-α-methylhistamine response (data not shown). This blunting of the histamine H_3 receptor-agonist response to electrical field stimulation in the guinea pig ileum by burimamide suggests this compound may be binding to the receptor in an insurmountable manner. Such insurmountable binding could contribute to the two-site best-fit analysis in rodent and primate brain that was observed in some experimental conditions (West et al., 1999; Alves-Rodrigues et al., 1996; Arrang et al., 1990). Alternatively, burimamide may have partial agonist effects at histamine H_3 receptors, since the compound exhibits partial inhibition of the ileal twitch response in a dose-dependent manner and only partially reverses the established inhibition of twitch responses caused by pre-incubation with *R*-α-methylhistamine (Denny and Miller, unpublished observations).

Comparison of histamine H_3 receptor antagonist functional potencies and radioligand binding affinities showed compelling regression correlations across several species. The guinea pig cerebral cortical histamine H_3 receptor antagonist binding affinities had excellent correlation with the guinea pig ileum histamine H_3 receptor functional potencies. The high correlation between cerebral cortical and ileal histamine H_3 receptors suggests that these receptors are the same. The antagonist binding affinities in dog cerebral cortical membrane preparations paralleled the guinea pig ileum functional data, suggesting that these histamine H_3 receptors may be quite similar. Although exhibiting a lower correlation, the rat cerebral cortex antagonist binding affinities still correlated well with the guinea pig ileum histamine H_3 receptor functional potency. These data are supported by the correlation seen in comparing the binding affinities between rat and guinea pig cerebral cortex histamine H_3 receptors and suggest minor species differences in the pharmacology of histamine H_3 receptor in rat compared to guinea pig. Comparing the human cerebral cortical histamine H_3 receptor antagonist binding affinities to the functional potency in guinea pig ileum provides the lowest correlation value. This difference between binding affinity and functional potency parallels the species difference seen in correlation graphs comparing antagonist binding affinities for the guinea pig versus human cerebral cortex histamine H_3 receptors.

Evidence for the existence of histamine H_3 receptor subtypes has generally been based on the comparison of results from competition and kinetic radioligand binding or functional assays. Using radioligand binding studies with rat brains, West et al. (1990a,b) provided evidence for low and high affinity binding sites, for two sites with different on-and-off rates, and for differential guanine nucleotide sensitivity of the two subtypes of histamine H_3 receptor. In our hands, binding assays performed on rat, guinea pig, dog or human cerebral cortex using 50 mM Tris–HCl with 5 mM EDTA buffer gave indication of only the high affinity binding site. In direct, parallel radioligand binding assays utilizing [^{3}H]*N*-α-methylhistamine, the results clearly established that imidazole-based antagonists can discriminate between dog, rat or guinea pig versus human cerebral cortical histamine H_3 receptors, a result supported by West et al. (1999). Evidence for receptor heterogeneity has also involved comparing functional models to binding assays. Different homologues of histamine or iodoproxyfan were able to discriminate between the guinea pig jejunum and mouse brain cortex histamine H_3 receptors (Leurs et al., 1996; Schlicker et al., 1996). We did not find significant differences between the functional potencies at the guinea pig ileum and the binding affinities at guinea pig cerebral cortex using the available histamine H_3 receptor agonists and antagonists. We did observe a slight difference between rat cerebral cortical histamine H_3 receptor antagonist binding affinity and functional potency in the guinea pig ileum that may be due to species differences that may also occur between mouse and rat compared to guinea pig histamine H_3 receptors.

Within the past year, histamine H_3 receptors have been cloned from several species. Although the receptors are highly homologous (94% amino acid identity for the rat and human histamine H_3 receptors, with even higher identity in putative transmembrane spanning domains), key amino acid differences are a probable factor in the pharmacological differences between species. The rat and human receptors differ, in particular, in transmembrane domain 3 at amino acids 119 and 122, where alanine and valine in the rat are replaced by threonine and alanine in the human receptor, respectively (Lovenberg et al., 1999, 2000; Ligneau et al., 2000). These two amino acids are close to the key aspartate, conserved in all aminergic receptors (Ligneau et al., 2000) and are located within one turn of each other on the putative alpha helix of transmembrane spanning domain 3. Mutation analysis has revealed that altering one or both of these residues alters the affinity of both thioperamide and ciproxifan from higher affinity for the rat receptor to lower affinity for the human receptor, and that single mutations cause intermediate changes in potency for these antagonists (Ligneau et al., 2000). A recent report on the cloning of the guinea pig receptor (Tardivel-Lacombe et al., 2000) shows that its amino acid sequence differs from both rat and human at these two sites. At amino acid 119, the guinea pig and human have the larger threonine residue, rather than alanine. In contrast, amino acid 122 is a valine in guinea pig and rat, but alanine in human. Published data indicate that, at these key amino acids, the mouse receptor is identical to the rat (Goodearl et al., 1999), whereas we have recently determined that the dog sequence is identical to the guinea pig at this portion of the receptor (Witte et al., unpublished observations).

The results of the aforementioned gene sequencing studies now shed considerable light on the diversity of pharmacology that we and other investigators have observed in a variety of models of histamine H_3 receptor

function. The high correlation we found between the potency of histamine H_3 receptor ligands at the guinea pig and dog receptors is probably a function of their identical amino acid sequences in transmembrane domain 3, since the key amino acid residues that are important for determining receptor affinity for many ligands are identical for these species. Similarly, the rat and mouse proteins are identical at these sites in transmembrane domain 3, and would be expected to have comparable affinities for most histamine H_3 ligands, if, as proposed (Ligneau et al., 2000), amino acids 119 and 122 are key to determining receptor affinity. Notably, the human receptor is different from all the above species at one or both of these key amino acids, and the pharmacology of many compounds should be expected to differ in potency depending on the relative importance of either amino acids 119 or 122 to the binding of any particular compound. These considerations could be of increased importance for novel non-imidazole based antagonists of the histamine H_3 receptor, which may interact with different binding domains of the receptor. Moreover, these results suggest additional caution will be required in evaluating either safety pharmacology or toxicological data with histamine H_3 receptor ligands across species, since the affinity of such compounds can be expected to change from one species to another.

An additional source of pharmacological variation among histamine H_3 receptors may derive from multiple second messenger signaling systems in different tissues, as well as the ability of splice variants of some histamine H_3 receptors to modulate signal transduction (Drutel et al., 2001). Three functional isoforms of the rat histamine H_3 receptor have been reported, based on various deletions in cytoplasmic loop 3. A splice variant of the guinea pig histamine H_3 receptor has also been observed (Tardivel-Lacombe et al., 2000; Cassar, 2000). A similar splice site occurs in the human gene sequence. A recent report failed to detect expression of the splice variant in human tissues (Liu et al., 2000), although we have observed that a short form of the receptor does exist in human tissues (Yao et al., unpublished observations). Thus, additional reasons for complex pharmacological effects, particularly for agonists and their ability to couple receptors to signal transduction cascades, may exist in different tissues as well as different species, and the ability of selective histamine H_3 receptor antagonists to reverse these effects will depend on the inherent potency, efficacy and coupling efficiency of agonists for this complex receptor in various systems.

In summary, pharmacological similarities and differences for a number of selective histamine H_3 receptor antagonists in several species are consistent with recently emerging molecular biological data on the amino acid sequences of these homologous receptors. Historical data showing evidence of receptor heterogeneity in various systems may now be reevaluated in the context of either the peptide sequence information of these receptors or in the context of the potential differential pharmacology of splice variants of these receptors.

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