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Compared pharmacology of human histamine H_3 and H_4 receptors: structure–activity relationships of histamine derivatives

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1 Various histamine derivatives were investigated at the human H_3 receptor (H_3R) and H_4 receptor (H_4R) stably expressed in human embryonic kidney (HEK)-293 cells using [^{125}I]iodoproxyfan and [^{3}H]histamine binding, respectively.

2 In Tris buffer, [^{3}H]histamine binding to membranes of HEK(hH_4R) cells was monophasic (K_D of 3.8 ± 0.8 nM). In phosphate buffer, the Hill coefficient was decreased ($n_H = 0.5 \pm 0.1$) and a large fraction of the binding was converted into a low-affinity component ($K_D = 67 \pm 27$ nM).

3 The inhibition of [^{3}H]histamine binding by two agonists, a protean agonist and five antagonists/inverse agonists confirms that the potency of many H_3R ligands is retained or only slightly reduced at the H_4R.

4 Histamine derivatives substituted with methyl groups in α, β or N^{α} position of the side chain retained a nanomolar potency at the H_3R, but their affinity was dramatically decreased at the H_4R. With relative potencies to histamine of 282 and 0.13% at the H_3R and H_4R, respectively, ($\pm$)-α,β-dimethylhistamine is a potent and selective H_3R agonist.

5 Chiral α-branched analogues exhibited a marked stereoselectivity at the H_3R and H_4R, the enantiomers with a configuration equivalent to L-histidine being preferred at both receptors.

6 The methylsubstitution of the imidazole ring was also studied. The relative potency to histamine of 4-methylhistamine (4-MeHA) at the H_4R (67%) was similar to that reported at H_2 receptors but, owing to its high affinity at the H_4R ($K_i = 7.0 \pm 1.2$ nM) and very low potency at H_1- and H_3-receptors, it can be considered as a potent and selective H_4R agonist.

7 On inhibition of forskolin-induced cAMP formation, all the compounds tested, including 4-MeHA, behaved as full agonists at both receptors. However, the maximal inhibition achieved at the H_4R ($\sim -30\%$) was much lower than at the H_3R ($\sim -80\%$). Thioperamide behaved as an inverse agonist at both receptors and increased cAMP formation with the same maximal effect ($\sim +25\%$).

8 In conclusion, although the pharmacological profiles of the human H_3R and H_4R overlap, the structure–activity relationships of histamine derivatives at both receptors strongly differ and lead to the identification of selective compounds.
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Abbreviations: EtHA, ethylhistamine; FSK, forskolin; HEK, human embryonic kidney; hH_3R, human H_3 receptor; hH_4R, human H_4 receptor; MAP kinase, mitogen-activated protein kinase; MeHA, methylhistamine

Introduction

The H_3 receptor (H_3R) was detected in the 1980s as an autoreceptor controlling histamine synthesis and release in the rat and human brain (Arrang *et al*., 1983; 1987; 1988). The cDNAs encoding the H_3R from various species, including human (Lovenberg *et al*., 1999) and rat (Lovenberg *et al*., 2000; Morisset *et al*., 2000; Drutel *et al*., 2001), were cloned recently. Screening of human libraries and genome databases by various groups led to the cloning and preliminary characterization of a receptor closely related to the H_3R, the H_4 receptor (H_4R) (Nakamura *et al*., 2000; Oda *et al*., 2000; Liu *et al*., 2001a; Morse *et al*., 2001; Nguyen *et al*., 2001; Zhu *et al*., 2001). The H_4R has about 40% sequence homogy to the H_3R (58% in transmembrane domains) and both receptors display similar genomic structures with two introns and three exons (Coge *et al*., 2001b; Tardivel-Lacombe *et al*., 2001). In addition, some reports suggest that the recombinant H_4R, like the H_3R (Lovenberg *et al*., 1999), couples to G_i/G_o proteins and inhibits forskolin-induced cAMP formation (Zhu *et al*., 2001). In contrast to these structural similarities, the expression patterns of both receptors strongly differ. Whereas the H_3R is predominantly localized in the brain, where it is present on many neuronal perikarya, dendrites and projections (Pillot *et al*., 2002), the H_4R is mainly expressed on hematopoietic cells (Oda *et al*., 2000; Liu *et al*., 2001a; Morse *et al*., 2001; Zhu

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et al., 2001). The presence of the H_4R on leukocytes and mast cells suggests that it plays an important role in immune responses and inflammation (Gantner *et al*., 2002; Buckland *et al*., 2003; Hofstra *et al*., 2003; Takeshita *et al*., 2003; Bell *et al*., 2004; Thurmond *et al*., 2004).

The pharmacological profiles of H_3Rs (Ligneau *et al*., 2000; Lovenberg *et al*., 2000) and H_4Rs (Liu *et al*., 2001b) show strong species differences, but early studies indicated that they overlap, several H_3R agonists or antagonists having appreciable activity at the H_4R (Hough, 2001). Few selective H_4R ligands are yet available (de Esch *et al*., 2005). The first potent and selective H_4R antagonist is a non-imidazole derivative that was designed recently and used for the characterization of the recombinant and native H_4R (Ling *et al*., 2004; Thurmond *et al*., 2004). Among agonists, the atypical neuroleptic clozapine has been shown to fully activate the H_4R, although it displays a moderate (submicromolar) affinity (Oda *et al*., 2000; Liu *et al*., 2001a, b; Buckland *et al*., 2003). The two methylcyanoguanidine derivatives of imifuramine, OUP-13 and OUP-16, also act as full agonists at the human H_4 receptor (hH_4R), but display a higher potency with a 40-fold selectivity over the human H_3 receptor (hH_3R) (Hashimoto *et al*., 2003).

In the present study, the potencies of various chiral and nonchiral histamine derivatives were determined at the recombinant human H_4R on inhibition of [^{3}H]histamine binding (in different experimental conditions). For comparison, and because most of these histamine derivatives had been previously studied only at the rat H_3 autoreceptor, their potencies have also been determined at the recombinant hH_3R on inhibition of [^{125}I]iodoproxyfan binding (Ligneau *et al*., 1994). In addition, some selected compounds have been studied at the human H_3R and H_4R on inhibition of forskolin-induced cAMP formation.

Methods

Cloning of the hH_3R and hH_4R cDNAs

The hH_3R was cloned by screening of a human striatum cDNA library as described (Ligneau *et al*., 2000). cDNAs corresponding to the full-length coding sequence of the human H_4 receptor (hH_4R) were cloned by PCR. Human bone marrow Marathon-Ready cDNAs (Clontech, Basingstoke, U.K.) were amplified for 40 cycles (94°C, 55°C and 72°C for 30 s each) using AmpliTaq Gold polymerase (Perkin-Elmer Life Sciences, Boston, MA, U.S.A.) and primers based on the N-terminal and C-terminal regions of the hH_4R sequence (forward primer: 5′-ATGCCAGATACTAATAGCACAATC AATTTATC-3′ and reverse primer: 5′-TTAAGAAGATACT GACCGACTGTGTTGT-3′). PCR products were electrophoresed on a 1% agarose gel, subcloned and sequenced.

Stable transfection of human embryonic kidney (HEK)-293 cells

cDNAs corresponding to the full-length coding sequences of the hH_3R and hH_4R were ligated into the mammalian expression vector pCIneo (Promega, Charbonnières, France). HEK-293 cells were transfected using PolyFect (Qiagen, Courtaboeuf, France). Stable transfectants were selected with 2 mg ml^{-1} of geneticin (G418, Invitrogen Gibco BRL, Cergy-Pontoise, France) and tested for [^{125}I]iodoproxyfan binding (hH_3R) (Ligneau *et al*., 1994) or for [^{3}H]histamine binding (hH_4R). Several clones named HEK(hH_3R) or HEK(hH_4R) were selected for further characterization and maintained in the presence of 0.2−1 mg ml^{-1} of G418.

Binding assays

Binding assays on the hH_3R were performed as described previously (Ligneau *et al*., 1994). Aliquots of membrane suspensions from HEK(hH_3R) cells (10−20 μg of protein) were incubated for 60 min at 25°C with 25 pM [^{125}I]iodoproxyfan alone or together with competing drugs in phosphate buffer (Na_2HPO_4/KH_2PO_4 50 mM, pH 7.5) (200 μl final volume). The nonspecific binding was determined using imetit (1 μM).

For binding assays on the hH_4R, HEK(hH_4R) cells were washed and homogenized with a Polytron in ice-cold Tris buffer (Tris-HCl 50 mM, pH 7.5). After centrifugation (12,000 × *g* for 30 min at 4°C), the pellet was suspended in 1 ml of the same ice-cold binding buffer. Aliquots of the membrane suspension (10−20 μg of protein) were incubated for 60 min at 25°C with [^{3}H]histamine alone or together with competing drugs (1 ml final volume). The nonspecific binding was determined using imetit (1 μM). In some saturation studies, the membrane fraction and binding assay was performed in phosphate buffer (Na_2HPO_4/KH_2PO_4 50 mM, pH 7.5).

cAMP accumulation

HEK(hH_3R) or HEK(hH_4R) cells were incubated for 10 min at 37°C with 1 μM forskolin and, when required, 10 μM of the various ligands. cAMP was extracted and measured by radioimmunoassay (Perkin-Elmer Life Sciences, Boston, MA, U.S.A.). Statistical evaluation of the results was performed by one-way ANOVA followed by Newman–Keuls test.

Analysis of data

The saturation and inhibition curves were analyzed with an iterative least-squares method derived from that of Parker & Waud (1971). Computer analysis was performed by nonlinear regression using a one-site cooperative model, except in some saturation experiments in which a significant improvement of the analysis was obtained by resolution of the data in two components with a two-site model. The method provided estimates for K_D and B_{max} values of [^{125}I]iodoproxyfan (hH_3R) and [^{3}H]histamine (hH_4R) and for IC_{50} values of competing drugs. K_i values of the latter were calculated from their IC_{50} values assuming a competitive antagonism and by using the relationship $K_i = IC_{50}/(1 + S/K_D)$, where S represents the concentration of the radioligand (25 pM [^{125}I]iodoproxyfan or 2 nM [^{3}H]histamine) and K_D its apparent dissociation constant (Cheng & Prussoff, 1973).

Radiochemicals and drugs

[^{125}I]Iodoproxyfan (2000 Ci mmol^{-1}) was prepared as described (Krause *et al*., 1997). [^{3}H]Histamine (51 Ci mmol^{-1}) was from Amersham Pharmacia Biotech (Les Ulis, France). Thioperamide and ciproxifan were from Bioprojet (Paris, France). Clobenpropit was from Tocris (Bristol, U.K.). Proxyfan, FUB

349 (Ligneau *et al*., 2000), FUB 465 (Morisset *et al*., 2000), N^{α}-ethylhistamine, β-methylhistamine (β-MeHA), α,α-, α,β- and β,β-dimethylhistamine (α,α-diMeHA, α,β-diMeHA and β,β-diMeHA), (*R*)- and (*S*)-α-methylhistamine (α-MeHA), (*R*)- and (*S*)-α-chloromethylhistamine (α-ChloroMeHA) and (*R*)- and (*S*)-α-hydroxymethylhistamine (α-HydroxyMeHA) were provided by W. Schunack (Freie Universität Berlin, Germany). Imetit, N^{α}-MeHA, N^{α},N^{α}-diMeHA, 2- and 4-MeHA were provided by C.R. Ganellin (University College, London, U.K.).

Results

Characterization of [^{3}H]histamine binding to the hH_4R

[^{3}H]histamine binding to membranes of HEK(hH_4R) cells in Tris-HCl buffer (50 mM, pH 7. 5) was saturable and analysis of the data using an one-site cooperative model indicated that the Hill coefficient was not significantly different from unity ($n_H = 0.9 \pm 0.1$), with a K_D value of 3.8 ± 0.8 nM and a B_{max} value of 2400 ± 200 fmol mg^{-1} protein. Scatchard analysis of saturation binding data also disclosed a single population of sites with a K_D value of 3.5 ± 0.4 nM (Figure 1a). In the presence of 100 μM GTPγS, no significant specific binding could be detected when the membranes were incubated in Tris-HCl buffer with 25 nM [^{3}H]histamine (data not shown).

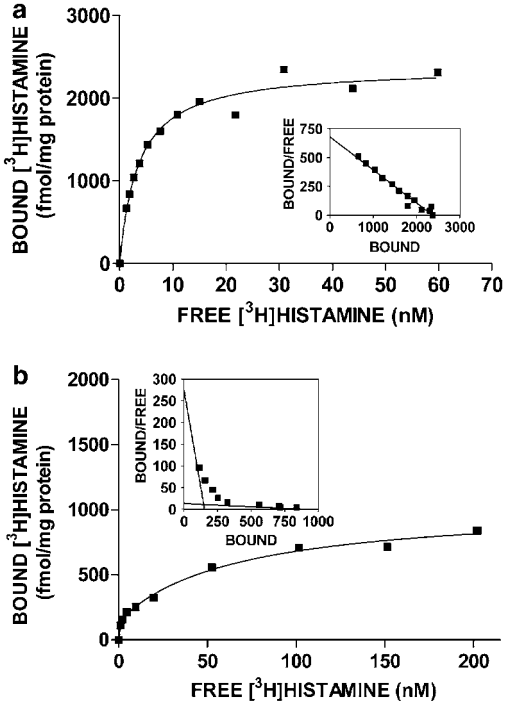


Figure 1 Saturation of [^{3}H]histamine binding to the recombinant hH_4R. Membranes of HEK(hH_4R) cells were incubated with [^{3}H]histamine in increasing concentrations for 60 min at 25°C in 50 mM Tris-HCl buffer, pH 7.5 (a) or 50 mM Na_2HPO_4/KH_2PO_4 buffer, pH 7.5 (b). Specific binding was defined as that inhibited by 1 μM imetit. The insets show the Scatchard transformation of the data. The lines in (b) were drawn after analysis of the data by nonlinear regression using a least-square curve fitting procedure for a two-site model. Each point represents the mean of 4–8 determinations from two separate experiments.

When the experiments were performed with the same membrane samples in phosphate buffer (50 mM Na_2HPO_4/KH_2PO_4, pH 7.5), analysis of the data using an one-site cooperative model indicated that the Hill coefficient was greatly decreased as compared with a Tris-HCl buffer ($n_H = 0.5 \pm 0.1$). The B_{max} was also significantly decreased and the data fitted significantly better to a two-site model analysis. The Scatchard plot could also be resolved in a high-affinity population of sites ($K_D = 0.56 \pm 0.12$ nM and $B_{max} = 154 \pm 64$ fmol mg^{-1} protein, i.e., 15% of maximal specific binding) and a low-affinity population with K_D and B_{max} values of 67 ± 27 nM and 883 ± 72 fmol mg^{-1} protein, respectively (Figure 1b). When the membranes were incubated in phosphate buffer with 25 nM [^{3}H]histamine in the presence of 100 μM GTPγS, a partial but significant decrease of specific binding was observed (-29 ± 5%), and this decrease corresponded to the density of the high affinity population of sites (-144 ± 28 fmol mg^{-1} protein) (data not shown).

Results from competition studies using Tris-HCl (50 mM, pH 7.5) buffer show that [^{3}H]histamine binding to membranes of HEK(hH_4R) cells was inhibited in a concentration-dependent manner by a range of H_3-receptor ligands. All of the compounds inhibited the binding with Hill coefficients not significantly different from unity, although the coefficient found for thioperamide in the one-site model tended to be slightly lower ($n_H = 0.82 \pm 0.03$). The deduced K_i values for each compound are given in Table 1. Unlabelled histamine inhibited the binding with a K_i value similar to its K_D value (4.7 ± 0.3 and 3.8 ± 0.8 nM, respectively). The affinities of the compounds at the hH_4R were also compared to their affinities that we recently reported at the hH_3R (Ligneau *et al*., 2000). The agonist imetit and the protean agonist proxyfan (Gbahou *et al*., 2003) were about two- and 13-fold less potent at the H_4R, respectively. Among the H_3-receptor antagonists/inverse agonists, thioperamide displayed similar potencies at human H_4Rs and H_3Rs, whereas clobenpropit, FUB 349, FUB 465 and ciproxifan were about 2–13-fold less potent at the H_4R (Table 1).

Potencies of N$^{\alpha}$-substituted histamine derivatives, and of 2- and 4-MeHA at the human H_3Rs and H_4Rs

The potencies of three N^{α}-substituted histamine derivatives at the hH_3R were determined by the [^{125}I]iodoproxyfan-binding assay (Ligneau *et al*., 1994) on membranes of HEK(hH_3R)

Table 1 Compared potencies of H_3-receptor ligands at the human H_4 and H_3 receptors (H_4Rs and H_3Rs)

Agent	*hH_4R*	*hH_3R*	*Selectivity (ratio)*
Histamine	4.7 ± 0.3	11 ± 2	H_4 (2.3)
Imetit	1.6 ± 0.1	0.7 ± 0.1	H_3 (2.3)
Proxyfan	34 ± 1	2.7 ± 0.1	H_3 (13)
Thioperamide	43 ± 3	60 ± 12	H_4 (1.4)
Ciproxifan	612 ± 32	46 ± 4	H_3 (13)
Clobenpropit	4.3 ± 0.2	2.4 ± 0.6	H_3 (1.8)
FUB 465	704 ± 74	188 ± 12	H_3 (3.7)
FUB 349	9.5 ± 0.2	2.1 ± 0.2	H_3 (4.5)

Values (K_i, nM) regarding the H_3 receptor are taken from Ligneau *et al*. (2000), except for imetit (Wulff *et al*., 2002), histamine and FUB 465.

cells stably expressing the receptor. Specific [^{125}I]iodoproxyfan binding was monophasic and saturable, and analysis by nonlinear regression using a one-site cooperative model led to a K_D value of 82 ± 12 pM, in close agreement with the value that we previously reported on membranes of CHO(hH_3R) cells (50 ± 7 pM, Ligneau *et al*., 2000). The inhibition curves for the agonists N^{α}-MeHA and N^{α},N^{α}-dimethylhistamine (N^{α},N^{α}-diMeHA), were found to be shallow, their pseudo-Hill coefficients being close to 0.7 (Figure 2). A similar observation could be made for the inhibition curve of histamine ((Ligneau *et al*., 2000) and Figure 2). The apparent K_i values that were deduced from the mean IC_{50} values are given in Table 2. The potencies of the agonists relative to histamine ($=100$) calculated from these K_i values indicated that N^{α}-MeHA and N^{α},N^{α}-diMeHA were about four- and two-fold more potent than histamine itself, respectively, whereas N^{α}-ethylhistamine

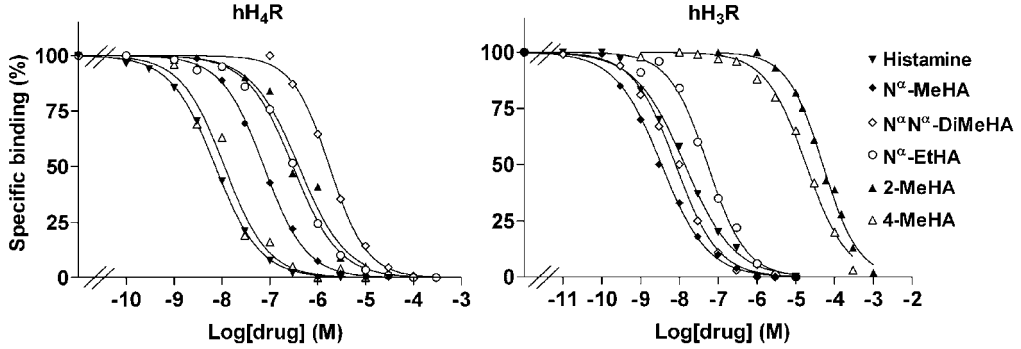
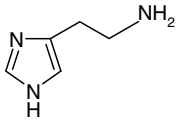
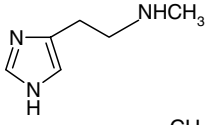
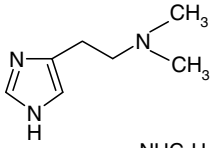
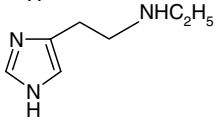
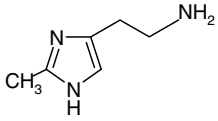
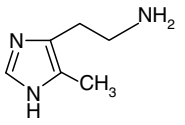


Figure 2 Effect of N^{α}-substituted histamine derivatives and of 2- and 4-MeHA on [^{125}I]iodoproxyfan binding to the hH_3R and on [^{3}H]histamine binding to the hH_4R. Membranes of HEK(hH_3R) cells were incubated with 25 pM [^{125}I]iodoproxyfan and drugs as described (Ligneau *et al*., 1994). Membranes of HEK(hH_4R) cells were incubated in Tris-HCl buffer with 2 nM [^{3}H]histamine and drugs in increasing concentrations. Each point represents the results from two independent experiments with triplicate determinations.

Table 2 Compared potencies of N^{α}-substituted histamine derivatives and of 2- and 4-methylhistamine at the human H_4 and H_3 receptors (H_4Rs and H_3Rs)

Compounds	*Structure*	*hH_4R*	*hH_3R*	*Selectivity (ratio)*
Histamine		4.7 ± 0.3 (100)	11 ± 2 (100)	H_4 (2.3)
N^{α}-methylhistamine		48 ± 2 (9.8)	2.7 ± 0.3 (407)	H_3 (18)
N^{α},N^{α}-dimethylhistamine		1178 ± 70 (0.4)	6.6 ± 1.0 (167)	H_3 (178)
N^{α}-ethylhistamine		192 ± 14 (2.4)	43 ± 4 (26)	H_3 (4.5)
2-methylhistamine		252 ± 45 (1.9)	42,020 $\pm$ 3600 (0.03)	H_4 (167)
4-methylhistamine		7.0 ± 1.2 (67)	18,960 $\pm$ 2140 (0.06)	H_4 (2709)

Values (K_i, nM) were derived from data shown in Figure 2. The relative potencies (indicated between brackets) were calculated as the ratio: (K_i value of histamine/K_i value of compound) $\times$ 100. The relative potencies of 4-methylhistamine at H_1- and H_2-receptors were of 0.2 and 43%, respectively (Black *et al*., 1972; Ganellin, 1982; Hill *et al*., 1997).

(N^{α}-EtHA) was about four-fold less potent than histamine at the hH_3R (Table 2). Both 2-MeHA and 4-MeHA displayed a much lower affinity with K_i values in the micromolar range leading to potencies relative to histamine less than 0.1% (Table 2).

The potencies of the histamine derivatives were then determined at the hH_4R by the [^{3}H]histamine-binding assay performed in 50 mM Tris-HCl buffer (pH 7.5) on membranes of HEK(hH_4R) cells. The three N^{α}-substituted compounds inhibited the binding with Hill coefficients not significantly different from unity and displayed a decreased potency relative to histamine at H_4Rs (Figure 2). N^{α}-MeHA was about 10-fold less potent than histamine. N^{α}-ethylhistamine was about four-fold less potent than its methyl analogue. N^{α},N^{α}-diMeHA inhibited [^{3}H]histamine binding with a very low (micromolar) affinity, leading to a potency relative to histamine of only 0.4% (Table 2). 2-MeHA was also much less potent than histamine with a relative potency of 1.9%. In contrast, 4-MeHA displayed a high affinity at the H_4R with a K_i value of 7.0 ± 1.2 nM leading to a potency relative of histamine of 67% (Table 2).

Potencies of non-chiral α- and/or β-methylated histamine derivatives at the human H_3Rs and H_4Rs

Substitution with methyl groups in α- and/or β-position of the side chain of the histamine molecule leads to compounds that displaced specific [^{125}I]iodoproxyfan binding to hH_3Rs ($n_H = 0.7$–0.8) with a high affinity (Figure 3). (±)-β-MeHA and (±)-α,β-diMeHA displayed a nanomolar affinity and were two- to three-fold more potent than histamine. The affinities of α,α-diMeHA and β,β-diMeHA were lower, these two derivatives being about three- to four-fold less potent than histamine (Table 3).

These histamine derivatives displaced specific [^{3}H]histamine binding to hH_4Rs with a low affinity and with Hill coefficients not significantly different from unity (Figure 3). (±)-β-MeHA displayed a potency relative to histamine of 4.3% on membranes of HEK(hH_4R) cells. The affinity of the dimethyl analogues was even lower with K_i values in the micromolar range leading to potencies relative to histamine below 0.5% (Table 3).

Potencies of chiral histamine derivatives at the human H_3Rs and H_4Rs

[^{125}I]iodoproxyfan binding to membranes of HEK(hH_3R) cells was inhibited in a concentration-dependent and stereoselective manner ($n_H = 0.6$–0.7) by the enantiomeric pairs of three chiral α-branched histamine derivatives, that is, α-MeHA, α-Chloro-MeHA and α-HydroxyMeHA. As expected, the binding of the compounds was stereoselective and the enantiomer with the same spatial configuration as L-histidine, that is, (*R*)-α-MeHA, (*S*)-α-ChloroMeHA and (*S*)-α-HydroxyMeHA, was in each case preferred at hH_3Rs (Table 4). The potent agonist (*R*)-α-MeHA displaced specific binding with a nanomolar affinity similar to that previously reported in the same binding test (Ligneau *et al*., 2000; Coge *et al*., 2001a; Uveges *et al*., 2002; Wulff *et al*., 2002). (*R*)-α-MeHA was 17-fold more potent than (*S*)-α-MeHA, the potencies relative to histamine of the two enantiomers being of 611 and 37%, respectively (Table 4). The enantiomers of α-ChloroMeHA and α-HydroxyMeHA displayed a lower affinity but a marked stereoselectivity was again observed with these two analogues. The isomer with the same relative configuration as L-histidine, and therefore (*R*)-α-MeHA, in their case, the S-isomer, was preferred at hH_3Rs. The K_i values obtained for each enantiomeric pair yielded a ratio *S*/*R* of 4.3 for the isomers of α-ChloroMeHA and 7.1 for the isomers of α-HydroxyMeHA at hH_3Rs (Table 4).

These chiral derivatives displayed a lower potency relative to histamine (below 4%) on [^{3}H]histamine binding to membranes of HEK(hH_4R) cells, but a marked difference in affinity was also observed between enantiomers. Although it was about 25-fold less potent than histamine, the affinity of (*R*)-α-MeHA was 17-fold higher than that displayed by (*S*)-α-MeHA (Table 4). A marked stereoselectivity was also observed with the two enantiomers of α-ChloroMeHA and again, the isomer corresponding to L-histidine, in that case the S-isomer, was the most potent at hH_4Rs (ratio *S*/*R* of 4.4). The potencies relative to histamine of the R- and S-enantiomers of α-HydroxyMeHA were not significantly different (0.13 and 0.08%), indicating that this chiral analogue binds to H_4Rs without any clear stereoselectivity (Table 4).

The affinities of the ligands at the hH_4R were also evaluated when [^{3}H]histamine binding to membranes of HEK(hH_4R)

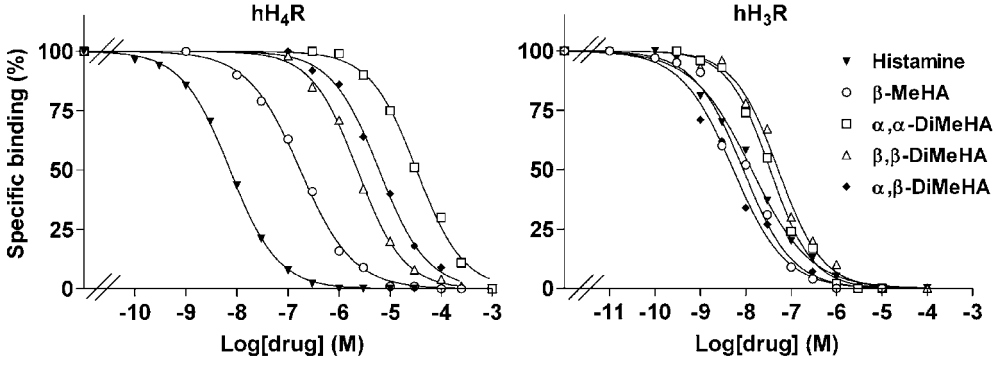
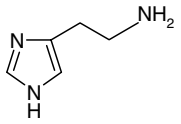
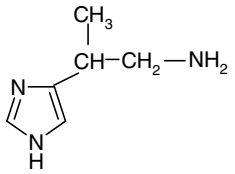
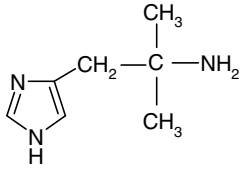
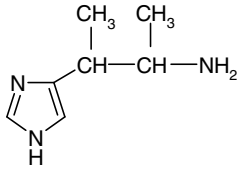
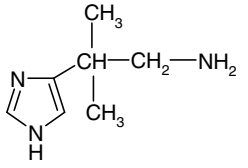


Figure 3 Effect of nonchiral α- and/or β-methylated histamine derivatives on [^{125}I]iodoproxyfan binding to the hH_3R and on [^{3}H]histamine binding to the hH_4R. Each point represents the results from two independent experiments with triplicate determinations.

Table 3 Compared potencies of nonchiral α- and/or β-methylated histamine derivatives at the human H_4 and H_3 receptors (H_4Rs and H_3Rs)

Compounds	*Structure*	*hH4R*	*hH3R*	*Selectivity (ratio)*
Histamine		4.7±0.3 (100)	11±2 (100)	H_4 (2.3)
(±)-β-methylhistamine		109±10 (4.3)	7.3±1.5 (151)	H_3 (15)
α,α-dimethylhistamine		19,230±2300 (0.02)	27±3 (40)	H_3 (712)
(±)-α,β-dimethylhistamine		3520±250 (0.13)	3.9±0.8 (282)	H_3 (903)
β,β-dimethyllhistamine		1416±95 (0.33)	40±12 (28)	H_3 (35)

Values (K_i, nM) were derived from data shown in Figure 3. The relative potencies (indicated between brackets) were calculated as the ratio: (K_i value of histamine/K_i value of compound) × 100. The relative potencies of (±)-α,β-dimethylhistamine at H_1- and H_2-receptors were 0.07 and 0.11%, respectively (Lipp *et al*., 1991; 1992a).

cells was performed in the phosphate buffer. When [^{3}H]histamine was added at a concentration (10 nM) selected to ensure a maximal specific binding at the high affinity site, without any significant binding to the low affinity site, the K_i values calculated from the obtained IC_{50} values indicated that the affinity of the H_3R ligands (see Table 1), histamine itself and the various histamine derivatives, was very similar to that found in Tris buffer, the K_i values being about 2–6 fold higher.

Effect of histamine derivatives on forskolin-induced cAMP accumulation in HEK(hH_3R) and HEK(hH_4R) cells

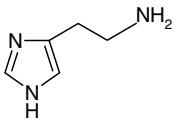
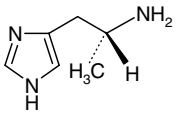
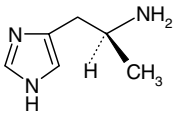
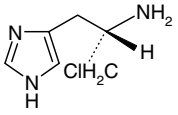
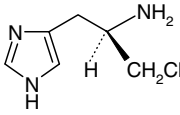
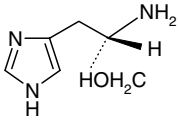
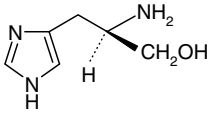
The functional properties of some of the various derivatives described above were investigated on forskolin-induced cAMP accumulation in the HEK-293 cells expressing the human H_3Rs and H_4Rs. In agreement with previous findings obtained in the same cells (Wulff *et al*., 2002; Takahashi *et al*., 2003), histamine itself used at a maximal concentration strongly inhibited cAMP formation in HEK(hH_3R) cells (−86±2%, $P<0.001$). N^{α}-MeHA, (*R*)-α-MeHA, β-MeHA, α,β-diMeHA (10 μM) all induced the same maximal inhibition of forskolin-induced cAMP accumulation, indicating that they all behave as full agonists at the hH_3R. In agreement with its low affinity at the H_3R, a very low inhibition (−18±4%) was induced by 4-MeHA used at the same concentration (10 μM) (Figure 4). Thioperamide, a standard inverse agonist, significantly increased cAMP formation in HEK(hH_3R) cells (+23±8%) (Figure 4).

Histamine also significantly inhibited forskolin-induced cAMP accumulation in HEK(hH_4R) cells. However, the maximal inhibition was much lower than that observed in HEK(hH_3R) cells (−34±3%) (Figure 4). Such a low inhibition was also observed with all the substituted derivatives tested. In addition, all the derivatives appeared to behave as full agonists at the hH_4R, exhibiting a maximal inhibitory effect similar to that of histamine (around −30%) (Figure 4). In contrast, thioperamide significantly increased cAMP formation in HEK(hH_4R) cells (+25±4%) (Figure 4).

Discussion

As frequently reported for ^{3}H-agonist binding to various G-protein-coupled receptors, the binding characteristics of [^{3}H]histamine to H_4Rs varied with the incubation medium,

Table 4 Compared potencies of chiral histamine derivatives at the human H_4 and H_3 receptors (H_4Rs and H_3Rs)

Compounds	*Structure*	*hH$_4$R*	*hH$_3$R*	*Selectivity (ratio)*
Histamine		4.7±0.3 (100)	11±2 (100)	H_4 (2.3)
(*R*)-α-methylhistamine		113±4 (4.1)	1.8±0.3 (611)	H_3 (63)
(*S*)-α-methylhistamine		1942±111 (0.24)	30±5 (37)	H_3 (65)
(*S*)-α-chloromethylhistamine		978±107 (0.48)	122±44 (9.0)	H_3 (8)
(*R*)-α-chloromethylhistamine		4088±410 (0.11)	513±187 (2.1)	H_3 (8)
(*S*)-α-hydroxymethylhistamine		5480±704 (0.08)	172±27 (6.4)	H_3 (32)
(*R*)-α-hydroxymethylhistamine		3677±548 (0.13)	1219±408 (0.9)	H_3 (3)

The relative potencies (indicated between brackets) were calculated as the ratio: (K_i value of histamine/K_i value of compound) × 100.

being markedly altered by the presence of monovalent cations. In Tris buffer, that is, in the absence of monovalent cations, the specific binding of [^{3}H]histamine to membranes of HEK(hH_4R) cells occurred with a nanomolar affinity to a single high affinity population of sites. When the experiments were performed with the same membranes in sodium/potassium phosphate buffer, the apparent maximal specific binding was decreased by ~50%, a finding probably resulting from the conversion by sodium ions of a fraction of the sites to low-affinity sites, no longer detectable in our conditions. Moreover, the remainder of the [^{3}H]histamine-binding sites that displayed an affinity high enough to be observed in sodium/potassium phosphate buffer was heterogeneous. The population displaying the highest affinity (in the nanomolar range) was similar to that found in Tris buffer. In agreement, the K_D value of [^{3}H]histamine and the K_i values of all competing ligands at this high affinity site were only 2–6-fold lower in phosphate buffer than in Tris buffer. In addition, in both conditions, the binding to this high affinity site was entirely abolished by GTPγS, indicating that it represents an active state of the receptor coupled to G proteins. The presence of monovalent cations in the phosphate buffer promoted the conversion of a large fraction of sites into a component displaying an ~120-fold lower affinity ($K_D = 67 \pm 24$ nM). This low-affinity site remained apparently unaltered by GTPγS and therefore presumably corresponded to an uncoupled state of the receptor.

This strong heterogeneity of [^{3}H]histamine binding at the hH_4R revealed in phosphate buffer probably accounts for the large differences found in the various estimations of the K_D value of [^{3}H]histamine at the high-affinity conformations (from 0.56 nM (the present study in sodium/potassium phosphate buffer) to 17.6 nM (Zhu *et al*., 2001)). The existence of these different conformations also presumably accounts for the lower K_D and K_i values of histamine in binding tests as compared to its EC_{50} value in some functional tests (Liu *et al*., 2001a; Oda *et al*., 2002). The modulation of ^{3}H-agonist binding to native H_3Rs by sodium and calcium ions (Arrang *et al*., 1990; Kilpatrick & Michel, 1991; Clark & Hill, 1995), as well as the shallow inhibition curves observed for various agonists at recombinant H_3Rs from various species (Ligneau *et al*., 2000; Morisset *et al*., 2001; Uveges *et al*., 2002; Rouleau *et al*., 2004) indicated a similar heterogeneity among agonist-binding sites at the H_3R. However, the pattern of ^{3}H-agonist binding to H_3 and H_4Rs is clearly different. The binding of both [^{3}H](*R*)-α-MeHA and [^{3}H]N^{α}-MeHA in sodium/potas-

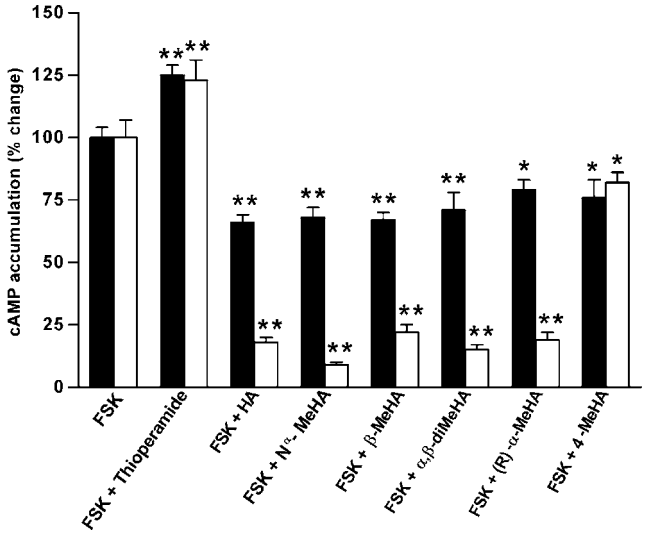


Figure 4 Effect of histamine derivatives on forskolin-induced cAMP accumulation in HEK(hH_3R) (white bars) and HEK(hH_4R) (black bars) cells. Cells were incubated with 1 μM forskolin (FSK) and, when required, drugs at a 10 μM final concentration. Results are expressed as the percent change of the FSK-evoked response which represented 7.5 ± 0.5 pmol (hH_3R) and 2.1 ± 0.1 pmol (hH_4R), and are the mean values from two to three (hH_3R) and four to nine (hH_4R) separate experiments with 4–10 determinations. $^*P<0.05$; $^{**}P<0.001$ vs FSK.

sium phosphate buffer apparently occurred to a single high-affinity H_3-receptor conformation and could therefore be successfully used for the pharmacological characterization of the H_3R (Arrang *et al*., 1990; Clark & Hill, 1995).

The inhibition of [^{3}H]histamine binding by various H_3-receptor ligands was also consistent with the labelling of a single conformation of H_4Rs in Tris buffer. Moreover, in agreement with previous studies, the inhibition induced by two H_3-receptor agonists (histamine and imetit), a H_3-receptor protean agonist (proxyfan) and five H_3-receptor antagonists/inverse agonists (thioperamide, ciproxifan, clobenpropit, FUB 349 and FUB 465) further showed that the pharmacological profiles of the human H_3Rs and H_4Rs strongly overlap (Hough, 2001). The potencies of the compounds at the hH_4R were consistent with those previously found in various cells (Liu *et al*., 2001a, b; Morse *et al*., 2001; Zhu *et al*., 2001; Oda *et al*., 2002; O'Reilly *et al*., 2002; Esbenshade *et al*., 2003) and, for most of them, were close to their potencies previously reported at the hH_3R (Lovenberg *et al*., 1999; 2000; West *et al*., 1999; Ligneau *et al*., 2000; Coge *et al*., 2001a; Ireland-Denny *et al*., 2001; Liu *et al*., 2001a; Wieland *et al*., 2001; O'Reilly *et al*., 2002; Uveges *et al*., 2002; Wulff *et al*., 2002; Esbenshade *et al*., 2003; Yao *et al*., 2003). For example, the potency of imetit at the hH_4R ($K_i = 1$–6 nM) was only five-fold lower than its mean potency at the hH_3R ($K_i = 0.2$–1.6 nM). The protean agonist proxyfan had a 10-fold lower potency at the hH_4R than at the hH_3R ($K_i = 34$ vs 2.7–5.0 nM). Among H_3-receptor antagonists/inverse agonists, the potency of clobenpropit at the hH_4R was in close agreement with that previously reported in various cells ($K_i = 5$–42 nM) and was only 2–10-fold lower than at the hH_3R ($K_i = 0.4$–5.7 nM). FUB 349 and FUB 465, two imidazole antagonists exhibiting a nanomolar and submicromolar affinity, respectively, at the hH_3R (this study and Ligneau *et al*., 2000) were also only four-fold less potent at the hH_4R. Ciproxifan exhibited only a micromolar potency at the hH_4R (K_i of 612 nM in the present study and 1.86 μM in (Esbenshade *et al*., 2003)), and tended therefore to be significantly less potent than at the hH_3R ($K_i = 46$–180 nM). In the studies mentioned above, a strong difference (up to 20-fold) was curiously found between the various determinations of the K_i value of thioperamide at hH_4Rs (27 to 519 nM), and it was also suggested that it may be less potent at hH_4Rs than at hH_3Rs (Hough, 2001). However, the potency found in the present study at H_4Rs ($K_i = 43 \pm 3$ nM) was similar to that previously found at the hH_3R ($K_i = 25$–200 nM). Taken together, the present results added to the preliminary pharmacology previously obtained with other imidazole compounds, confirm that the potency of many H_3R ligands is retained or only slightly reduced at the hH_4R.

The present study shows that methylsubstitution in the side chain of the histamine molecule in N^{α}, α, or β position is much less tolerated by the H_4R than by the H_3R. The corresponding methylsubstituted derivatives display a high agonist potency at H_3 autoreceptors regulating histamine release in the rat brain (Arrang *et al*., 1991; Lipp *et al*., 1991). In the present study, they also retained a high potency at the hH_3R but their potency at hH_4Rs stably expressed in the same cells, that is, HEK-293 cells, was dramatically decreased.

The negative influence of N^{α}-substitution at the H_4R became obvious when the methyl and dimethyl substituents were compared. In agreement with previous binding studies, N^{α}-MeHA exhibited a nanomolar affinity at the hH_3R and was about four-fold more potent than histamine, a relative potency in the same range as that found at the rat H_3R (Arrang *et al*., 1983; 1990). Its affinity at the hH_4R was about 20-fold lower, with a relative potency to histamine of about 10% and its maximal effect on the inhibition of cAMP formation confirmed that it acts as a full agonist. N^{α},N^{α}-diMeHA was also slightly more potent than histamine at the rat (Arrang *et al*., 1983) and hH_3Rs, but its affinity became about 200-fold lower at the hH_4R than at the H_3R, with a relative potency to histamine less than 1%.

Substitution with methyl groups in α- and/or β-position of the side chain leads to similar observations. The corresponding compounds retained a high potency at the H_3R, but their affinity was dramatically decreased at the H_4R, although they still behaved as full agonists on cAMP formation. ($\pm$)-β-MeHA, which was about two-fold more potent than histamine at the rat (Arrang *et al*., 1991; 1992; Lipp *et al*., 1991; 1992b) and hH_3R, was 15-fold less potent at the hH_4R than at the hH_3R. β,β-diMeHA displayed a micromolar affinity at the hH_4R, 35-fold lower than at the hH_3R. A high selectivity ratio was found with α,α-diMeHA which exhibited a negligible affinity at the hH_4R, 700-fold lower than at the hH_3R. Interestingly, α,α-diMeHA and β,β-diMeHA exhibited similar potencies at the hH_3R, whereas α,α-diMeHA was 75-fold more potent than β,β-diMeHA at the rat H_3 autoreceptor (Arrang *et al*., 1991; 1992; Lipp *et al*., 1991; 1995). This finding may result from the distinct pharmacological profiles of the rat and hH_3Rs (Arrang *et al*., 1987; 1988; West *et al*., 1999; Ligneau *et al*., 2000; Lovenberg *et al*., 2000; Ireland-Denny *et al*., 2001; Wulff *et al*., 2002; Yao *et al*., 2003). Alternatively, these two compounds may discriminate distinct conformations of the receptor in the functional assay (modulation of histamine release) and the [^{125}I]iodoproxyfan-binding assay, inasmuch as agonists, including α,α-diMeHA and β,β-diMeHA, inhibit the

binding with the Hill coefficients significantly lower than unity, revealing an heterogeneity among agonist-binding sites at the H_3R.

Among the α and/or β-substituted compounds tested, (±)-α,β-diMeHA was the most potent H_3R agonist, both in the rat (Lipp *et al*., 1991; 1992a) and human. It also displayed the highest selectivity for the H_3R, its affinity being 900-fold lower at the hH_4R. As it also displays a very low potency at H_1 and H_2 receptors (Lipp *et al*., 1991; 1992a), (±)-α,β-diMeHA can, therefore, be considered as a potent and highly selective H_3-receptor agonist.

As expected (Arrang *et al*., 1985; 1987; 1990), the binding of the chiral α-branched ligands at the hH_3R exhibited a pronounced stereoselectivity, and in all cases the enantiomer with a configuration equivalent to L-histidine was preferred, as already observed for the rat receptor (Arrang *et al*., 1985; Lipp *et al*., 1992b). (*R*)-α-MeHA, which exhibited an expected nanomolar affinity, was 17-fold more potent than (*S*)-α-MeHA, a ratio similar to that previously found at the human receptor (West *et al*., 1999; Ireland-Denny *et al*., 2001; Wulff *et al*., 2002), but lower than that reported at the rat receptor (Arrang *et al*., 1990; Ligneau *et al*., 1994; Wulff *et al*., 2002). Compared to the *R*-isomer, the higher potency of the *S*-isomer of α-ChloroMeHA and α-HydroxyMeHA, in that case the isomer with the relative configuration corresponding to L-histidine, and therefore to (*R*)-α-MeHA, also revealed a marked stereoselectivity with these two chiral analogues.

In spite of much lower affinities of the derivatives, a marked stereoselectivity was also observed with the chiral α-branched ligands at the hH_4R, and the enantiomers preferred were the same as those preferred at the hH_3R. In agreement with previous reports (Liu *et al*., 2001a, b; Morse *et al*., 2001; Zhu *et al*., 2001; Oda *et al*., 2002; O'Reilly *et al*., 2002), (*R*)-α-MeHA still acted as a full agonist on cAMP formation but was about 60-fold less potent at the hH_4R than at the H_3R, confirming that the methylation of the side chain of the histamine molecule is not well tolerated by the H_4R. In agreement with previous functional studies (Shin *et al*., 2002), it was more potent than (*S*)-α-MeHA. (*S*)-α-ChloroMeHA was also more potent than the corresponding *R*-isomer. In addition, the stereoselectivity ratios between the two isomers of both chiral derivatives were similar to those found at the hH_3R. However, the marked stereoselectivity found between the isomers of α-HydroxyMeHA at the hH_3R was no longer observed at the hH_4R, indicating a different degree of stereoselectivity of both receptors for some compounds.

In addition to the effect of methylsubstitution in the side chain of the histamine molecule, the effect of methylsubstitution of the imidazole ring at human H_3Rs and H_4Rs was also studied with 2- and 4-MeHA, two compounds known to display a relative selectivity for H_1 and H_2 receptors, respectively (Black *et al*., 1972; Ganellin, 1982; Hill *et al*., 1997). As expected from our previous studies at the rat H_3 autoreceptor (Arrang *et al*., 1983), both compounds displayed a very low affinity at the hH_3R. Although its affinity was about 150-fold higher at the hH_4R than at the H_3R, 2-MeHA remained much less potent than histamine at the hH_4R. In contrast, 4-MeHA, which acted as a full agonist on the inhibition of cAMP formation, displayed a potency similar to that of histamine at the hH_4R, with an affinity in the nanomolar range, 2700-fold higher than at the hH_3R. 4-MeHA is very poorly active at H_1 receptors but it has also long been considered as a relatively selective H_2-receptor agonist. Its potency has been reported to be about 50% that of histamine at H_2 receptors in various systems and species (Black *et al*., 1972; Ganellin, 1982; Hill *et al*., 1997). Therefore, its potency relative to histamine that we report here at H_4Rs (67%) is much higher than at H_1Rs and H_3Rs but is in the same range as that found at H_2 receptors, which may indicate a rather limited selectivity. However, the binding affinity of histamine at H_4Rs being several orders of magnitude higher than at either the H_1- or H_2 receptors (Hill *et al*., 1977; Arrang *et al*., 1990; Ruat *et al*., 1990), 4-MeHA is likely to activate H_4Rs at concentrations much lower than those required to activate the three other subclasses of histamine receptors. Therefore, although 4-MeHA acts as an agonist and the potency of agonists is well known to be dependent on the test system used, it can be considered as selective for H_4Rs. While this work was in progress, Lim *et al*. (2005) have also identified 4-MeHA as a potent agonist at hH_4Rs expressed in SK-N-MC cells, although its affinity was found to be lower ($K_i = 50$ nM).

Like the recombinant H_3R (Lovenberg *et al*., 1999; Morisset *et al*., 2000; Drutel *et al*., 2001), the recombinant H_4R has been reported to be coupled to $G_{i/o}$ proteins and inhibition of cAMP formation. However, in spite of a high expression level, our data show that the maximal inhibition achieved by various full agonists in HEK(hH_4R) cells was much lower than that obtained in HEK(hH_3R) cells, making quantification of the modulation of cAMP levels problematic. In agreement, in other studies, direct coupling of the H_4R to inhibition of adenylate cyclase could not be detected (Morse *et al*., 2001) or was also found to be weak (Nakamura *et al*., 2000; Oda *et al*., 2000; Liu *et al*., 2001a, b). This might suggest that inhibition of adenylate cyclase is not a primary transduction pathway of the H_4R or that it involves a low coupling efficacy of the receptor to the G proteins.

Recently, we showed that the recombinant rat and hH_3Rs expressed at moderate densities display constitutive activity (Morisset *et al*., 2000; Rouleau *et al*., 2002). This constitutive activity was clearly evidenced by the enhancement of cAMP accumulation induced in HEK(hH_3R) cells by thioperamide acting as an inverse agonist. Interestingly, in agreement with a previous study based upon [^{35}S]GTPγ[S] binding and MAP kinase activity (Morse *et al*., 2001), constitutive activity of the H_4R could also be easily detected by the enhancement of cAMP formation induced by thioperamide in HEK(hH_4R) cells. Consistent with the physiological relevance of the phenomenon, we demonstrated constitutive activity of the native rat and mouse H_3Rs and showed that it controls histaminergic neuron activity in rodent brain *in vivo* (Morisset *et al*., 2000; Rouleau *et al*., 2002). Whether native H_4Rs also display constitutive activity remains to be established. However, it is worth noting that, whereas the maximal effect of the agonists at the H_4R was much lower than at the H_3R, the intrinsic efficacy of thioperamide acting as an inverse agonist was similar at H_3Rs and H_4Rs, indicating a relatively high coupling efficacy of constitutively active conformations of the H_4R compared to that of ligand-selective active conformations (Gbahou *et al*., 2003).

In conclusion, the present data confirm that the affinity of many H_3R ligands, including standard agonists and antagonists, is retained or only slightly reduced at the hH_4R. However, the structure–activity relationships of histamine derivatives at human H_3Rs and H_4Rs strongly differ and lead

to the identification of selective compounds. Derivatives methylsubstituted in N^{α}, α- or β-position of the side chain retain a high affinity at the H_3R but their affinity at the H_4R is strongly decreased. Among them, (±)-α,β-diMeHA is a potent and selective H_3-receptor agonist, with a 900-fold lower affinity at H_4Rs and a very low potency at H_1 and H_2 receptors. In contrast, 4-MeHA displays a much higher affinity at hH_4Rs than at H_3Rs and can be considered as a potent and selective H_4-receptor agonist.

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References

ARRANG, J.M., DEVAUX, B., CHODKIEWICZ, J.P. & SCHWARTZ, J.C. (1988). H_3-receptors control histamine release in human brain. *J. Neurochem.,* **51,** 105–108.

ARRANG, J.M., GARBARG, M., LANCELOT, J.C., LECOMTE, J.M., POLLARD, H., ROBBA, M., SCHUNACK, W. & SCHWARTZ, J.C. (1987). Highly potent and selective ligands for histamine H_3-receptors. *Nature,* **327,** 117–123.

ARRANG, J.M., GARBARG, M. & SCHWARTZ, J.C. (1983). Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature,* **302,** 832–837.

ARRANG, J.M., GARBARG, M. & SCHWARTZ, J.C. (1992). H_3-Receptor and control of histamine release. In: *The Histamine Receptor*. ed. Schwartz, J.C. & Haas, H.L. pp. 145–159. New York: Wiley-Liss.

ARRANG, J.M., GARBARG, M., SCHWARTZ, J.C., LIPP, R., STARK, H., SCHUNACK, W. & LECOMTE, J.M. (1991). The histamine H_3-receptor: pharmacology, roles and clinical implications studied with agonists. *Agents Actions Suppl.,* **33,** 55–67.

ARRANG, J.M., ROY, J., MORGAT, J.L., SCHUNACK, W. & SCHWARTZ, J.C. (1990). Histamine H_3 receptor binding sites in rat brain membranes: modulations by guanine nucleotides and divalent cations. *Eur. J. Pharmacol.,* **188,** 219–227.

ARRANG, J.M., SCHWARTZ, J.C. & SCHUNACK, W. (1985). Stereoselectivity of the histamine H_3-presynaptic autoreceptor. *Eur. J. Pharmacol.,* **117,** 109–114.

BELL, J.K., MCQUEEN, D.S. & REES, J.L. (2004). Involvement of histamine H_4 and H_1 receptors in scratching induced by histamine receptor agonists in BalbC mice. *Br. J. Pharmacol.,* **142,** 374–380.

BLACK, J.W., DUNCAN, W.A., DURANT, C.J., GANELLIN, C.R. & PARSONS, E.M. (1972). Definition and antagonism of histamine H_2-receptors. *Nature,* **236,** 385–390.

BUCKLAND, K.F., WILLIAMS, T.J. & CONROY, D.M. (2003). Histamine induces cytoskeletal changes in human eosinophils *via* the H_4 receptor. *Br. J. Pharmacol.,* **140,** 1117–1127.

CHENG, Y. & PRUSSOFF, W.H. (1973). Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol.,* **22,** 3099–3108.

CLARK, E.A. & HILL, S.J. (1995). Differential effect of sodium ions and guanine nucleotides on the binding of thioperamide and clobenpropit to histamine H_3-receptors in rat cerebral cortical membranes. *Br. J. Pharmacol.,* **114,** 357–362.

COGE, F., GUENIN, S.P., AUDINOT, V., RENOUARD-TRY, A., BEAUVERGER, P., MACIA, C., OUVRY, C., NAGEL, N., RIQUE, H., BOUTIN, J.A. & GALIZZI, J.P. (2001a). Genomic organization and characterization of splice variants of the human histamine H_3 receptor. *Biochem. J.,* **355,** 279–288.

COGE, F., GUENIN, S.P., RIQUE, H., BOUTIN, J.A. & GALIZZI, J.P. (2001b). Structure and expression of the human histamine H_4-receptor gene. *Biochem. Biophys. Res. Commun.,* **284,** 301–309.

DE ESCH, I.J., THURMOND, R.L., JONGEJAN, A. & LEURS, R. (2005). The histamine H_4 receptor as a new therapeutic target for inflammation. *Trends Pharmacol. Sci.,* **26,** 462–469.

DRUTEL, G., PEITSARO, N., KARLSTEDT, K., WIELAND, K., SMIT, M.J., TIMMERMAN, H., PANULA, P. & LEURS, R. (2001). Identification of rat H_3 receptor isoforms with different brain expression and signaling properties. *Mol. Pharmacol.,* **59,** 1–8.

ESBENSHADE, T.A., KRUEGER, K.M., MILLER, T.R., KANG, C.H., DENNY, L.I., WITTE, D.G., YAO, B.B., FOX, G.B., FAGHIH, R., BENNANI, Y.L., WILLIAMS, M. & HANCOCK, A.A. (2003). Two novel and selective nonimidazole histamine H_3 receptor antagonists A-304121 and A-317920: I. *In vitro* pharmacological effects. *J. Pharmacol. Exp. Ther.,* **305,** 887–896.

GANELLIN, C.R. (1982). Chemistry and structure-activity relationships of drugs acting at histamine receptors. In: *Pharmacology of Histamine Receptors*. ed. Ganellin, C.R. & Parsons, M.E. pp. 10–102. Bristol, U.K.: Wright PSG.

GANTNER, F., SAKAI, K., TUSCHE, M.W., CRUIKSHANK, W.W., CENTER, D.M. & BACON, K. (2002). Histamine H_4 and H_2 receptors control histamine-induced interleukin-16 release from human CD8(+) T cells. *J. Pharmacol. Exp. Ther.,* **303,** 300–307.

GBAHOU, F., ROULEAU, A., MORISSET, S., PARMENTIER, R., CROCHET, S., LIN, J.S., LIGNEAU, X., TARDIVEL-LACOMBE, J., STARK, H., SCHUNACK, W., GANELLIN, C.R., SCHWARTZ, J.C. & ARRANG, J.M. (2003). Protean agonism at histamine H_3 receptors *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.,* **100,** 11086–11091.

HASHIMOTO, T., HARUSAWA, S., ARAKI, L., ZUIDERVELD, O.P., SMIT, M.J., IMAZU, T., TAKASHIMA, S., YAMAMOTO, Y., SAKAMOTO, Y., KURIHARA, T., LEURS, R., BAKKER, R.A. & YAMATODANI, A. (2003). A selective human H_4-receptor agonist:(−)-2-cyano-1-methyl-3-[(2*R*,5*R*)-5-[1*H*-imidazol-4(5)yl]tetrahydrofuran-2-y] methylguanidine. *J. Med. Chem.,* **46,** 3162–3165.

HILL, S.J., GANELLIN, C.R., TIMMERMAN, H., SCHWARTZ, J.C., SHANKLEY, N.P., YOUNG, J.M., SCHUNACK, W., LEVI, R. & HAAS, H.L. (1997). International Union of Pharmacology. XIII. Classification of histamine receptors. *Pharmacol. Rev.,* **49,** 253–278.

HILL, S.J., YOUNG, J.M. & MARRIAN, D.H. (1977). Specific binding of 3H-mepyramine to histamine H_1 receptors in intestinal smooth muscle. *Nature,* **270,** 361–363.

HOFSTRA, C.L., DESAI, P.J., THURMOND, R.L. & FUNG-LEUNG, W.P. (2003). Histamine H_4 receptor mediates chemotaxis and calcium mobilization of mast cells. *J. Pharmacol. Exp. Ther.,* **305,** 1212–1221.

HOUGH, L.B. (2001). Genomics meets histamine receptors: new subtypes, new receptors. *Mol. Pharmacol.,* **59,** 415–419.

IRELAND-DENNY, L., PARIHAR, A.S., MILLER, T.R., KANG, C.H., KRUEGER, K.M., ESBENSHADE, T.A. & HANCOCK, A.A. (2001). Species-related pharmacological heterogeneity of histamine H_3 receptors. *Eur. J. Pharmacol.,* **433,** 141–150.

KILPATRICK, G.J. & MICHEL, A.D. (1991). Characterisation of the binding of the histamine H_3 receptor agonist [^{3}H](*R*)-alpha methyl histamine to homogenates of rat and guinea-pig cortex. *Agents Actions Suppl.,* **33,** 69–75.

KRAUSE, M., STARK, H. & SCHUNACK, W. (1997). Iododestannylation : an improved synthesis of [^{125}I]iodoproxyfan, a specific radioligand of the histamine H_3 receptor. *J. Label. Comp. Radiopharm.,* **39,** 601–606.

LIGNEAU, X., GARBARG, M., VIZUETE, M.L., DIAZ, J., PURAND, K., STARK, H., SCHUNACK, W. & SCHWARTZ, J.C. (1994). [^{125}I]iodoproxyfan, a new antagonist to label and visualize cerebral histamine H_3 receptors. *J. Pharmacol. Exp. Ther.,* **271,** 452–459.

LIGNEAU, X., MORISSET, S., TARDIVEL-LACOMBE, J., GBAHOU, F., GANELLIN, C.R., STARK, H., SCHUNACK, W., SCHWARTZ, J.C. & ARRANG, J.M. (2000). Distinct pharmacology of rat and human histamine H_3 receptors: role of two amino acids in the third transmembrane domain. *Br. J. Pharmacol.,* **131,** 1247–1250.

LIM, H.D., VAN RIJN, R.M., LING, P., BAKKER, R.A., THURMOND, R.L. & LEURS, R. (2005). Evaluation of histamine H_1-, H_2-, and H_3-receptor ligands at the human histamine H_4 receptor: identification of 4-methylhistamine as the first potent and selective H_4 receptor agonist. *J. Pharmacol. Exp. Ther.,* **314,** 1310–1321.

LING, P., NGO, K., NGUYEN, S., THURMOND, R.L., EDWARDS, J.P., KARLSSON, L. & FUNG-LEUNG, W.P. (2004). Histamine H_4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br. J. Pharmacol.,* **142,** 161–171.

LIPP, R., ARRANG, J.M., BUSCHMANN, J., GARBARG, M., LUGER, P., SCHUNACK, W. & SCHWARTZ, J.C. (1991). Novel chiral H_3-receptor agonists. *Agents Actions Suppl.,* **33,** 277–282.

LIPP, R., ARRANG, J.M., GARBARG, M., LUGER, P., SCHWARTZ, J.C. & SCHUNACK, W. (1992a). Synthesis, absolute configuration, stereoselectivity, and receptor selectivity of (alpha *R*, beta *S*)-alpha,beta-dimethylhistamine, a novel high potent histamine H_3 receptor agonist. *J. Med. Chem.,* **35,** 4434–4441.

LIPP, R., STARK, H., ARRANG, J., GARBARG, M., SCHWARTZ, J. & SCHUNACK, W. (1995). Synthesis and histamine H_3-receptor agonist activity of mono- and dialkyl-substituted histamine derivatives. *Eur. J. Med. Chem.,* **30,** 219–225.

LIPP, R., STARK, H. & SCHUNACK, W. (1992b). Pharmacochemistry of H_3 receptors. In: *The Histamine Receptor*. ed. Schwartz, J.C. & Haas, H.L. pp. 57–72. New York: Wiley-Liss.

LIU, C., MA, X., JIANG, X., WILSON, S.J., HOFSTRA, C.L., BLEVITT, J., PYATI, J., LI, X., CHAI, W., CARRUTHERS, N. & LOVENBERG, T.W. (2001a). Cloning and pharmacological characterization of a fourth histamine receptor (H_4) expressed in bone marrow. *Mol. Pharmacol.,* **59,** 420–426.

LIU, C., WILSON, S.J., KUEI, C. & LOVENBERG, T.W. (2001b). Comparison of human, mouse, rat, and guinea pig histamine H_4 receptors reveals substantial pharmacological species variation. *J. Pharmacol. Exp. Ther.,* **299,** 121–130.

LOVENBERG, T.W., PYATI, J., CHANG, H., WILSON, S.J. & ERLANDER, M.G. (2000). Cloning of rat histamine H_3 receptor reveals distinct species pharmacological profiles. *J. Pharmacol. Exp. Ther.,* **293,** 771–778.

LOVENBERG, T.W., ROLAND, B.L., WILSON, S.J., JIANG, X., PYATI, J., HUVAR, A., JACKSON, M.R. & ERLANDER, M.G. (1999). Cloning and functional expression of the human histamine H_3 receptor. *Mol. Pharmacol.,* **55,** 1101–1107.

MORISSET, S., ROULEAU, A., LIGNEAU, X., GBAHOU, F., TARDIVEL-LACOMBE, J., STARK, H., SCHUNACK, W., GANELLIN, C.R., SCHWARTZ, J.C. & ARRANG, J.M. (2000). High constitutive activity of native H_3 receptors regulates histamine neurons in brain. *Nature,* **408,** 860–864.

MORISSET, S., SASSE, A., GBAHOU, F., HERON, A., LIGNEAU, X., TARDIVEL-LACOMBE, J., SCHWARTZ, J.C. & ARRANG, J.M. (2001). The rat H_3 receptor: gene organization and multiple isoforms. *Biochem. Biophys. Res. Commun.,* **280,** 75–80.

MORSE, K.L., BEHAN, J., LAZ, T.M., WEST JR., R.E., GREENFEDER, S.A., ANTHES, J.C., UMLAND, S., WAN, Y., HIPKIN, R.W., GONSIOREK, W., SHIN, N., GUSTAFSON, E.L., QIAO, X., WANG, S., HEDRICK, J.A., GREENE, J., BAYNE, M. & MONSMA JR., F.J. (2001). Cloning and characterization of a novel human histamine receptor. *J. Pharmacol. Exp. Ther.,* **296,** 1058–1066.

NAKAMURA, T., ITADANI, H., HIDAKA, Y., OHTA, M. & TANAKA, K. (2000). Molecular cloning and characterization of a new human histamine receptor, HH4R. *Biochem. Biophys. Res. Commun.,* **279,** 615–620.

NGUYEN, T., SHAPIRO, D.A., GEORGE, S.R., SETOLA, V., LEE, D.K., CHENG, R., RAUSER, L., LEE, S.P., LYNCH, K.R., ROTH, B.L. & O'DOWD, B.F. (2001). Discovery of a novel member of the histamine receptor family. *Mol. Pharmacol.,* **59,** 427–433.

ODA, T., MATSUMOTO, S., MASUHO, Y., TAKASAKI, J., MATSUMOTO, M., KAMOHARA, M., SAITO, T., OHISHI, T., SOGA, T., HIYAMA, H., MATSUSHIME, H. & FURUICHI, K. (2002). cDNA cloning and characterization of porcine histamine H_4 receptor. *Biochim. Biophys. Acta,* **1575,** 135–138.

ODA, T., MORIKAWA, N., SAITO, Y., MASUHO, Y. & MATSUMOTO, S. (2000). Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J. Biol. Chem.,* **275,** 36781–36786.

O'REILLY, M., ALPERT, R., JENKINSON, S., GLADUE, R.P., FOO, S., TRIM, S., PETER, B., TREVETHICK, M. & FIDOCK, M. (2002). Identification of a histamine H_4 receptor on human eosinophils – role in eosinophil chemotaxis. *J. Recept Signal Transduct. Res.,* **22,** 431–448.

PARKER, R.B. & WAUD, D.R. (1971). Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation. I. Agonists. *J. Pharmacol. Exp. Ther.,* **177,** 1–12.

PILLOT, C., HERON, A., COCHOIS, V., TARDIVEL-LACOMBE, J., LIGNEAU, X., SCHWARTZ, J.C. & ARRANG, J.M. (2002). A detailed mapping of the histamine H_3 receptor and its gene transcripts in rat brain. *Neuroscience,* **114,** 173–193.

ROULEAU, A., HERON, A., COCHOIS, V., PILLOT, C., SCHWARTZ, J.C. & ARRANG, J.M. (2004). Cloning and expression of the mouse histamine H_3 receptor: evidence for multiple isoforms. *J. Neurochem.,* **90,** 1331–1338.

ROULEAU, A., LIGNEAU, X., TARDIVEL-LACOMBE, J., MORISSET, S., GBAHOU, F., SCHWARTZ, J.C. & ARRANG, J.M. (2002). Histamine H_3-receptor-mediated [^{35}S]GTPγ[S] binding: evidence for constitutive activity of the recombinant and native rat and human H_3 receptors. *Br. J. Pharmacol.,* **135,** 383–392.

RUAT, M., TRAIFFORT, E., BOUTHENET, M.L., SCHWARTZ, J.C., HIRSCHFELD, J., BUSCHAUER, A. & SCHUNACK, W. (1990). Reversible and irreversible labeling and autoradiographic localization of the cerebral histamine H_2 receptor using [^{125}I]iodinated probes. *Proc. Natl. Acad. Sci. U.S.A.,* **87,** 1658–1662.

SHIN, N., COATES, E., MURGOLO, N.J., MORSE, K.L., BAYNE, M., STRADER, C.D. & MONSMA JR, F.J. (2002). Molecular modeling and site-specific mutagenesis of the histamine-binding site of the histamine H_4 receptor. *Mol. Pharmacol.,* **62,** 38–47.

TAKAHASHI, K., TOKITA, S. & KOTANI, H. (2003). Generation and characterization of highly constitutive active histamine H_3 receptors. *J. Pharmacol. Exp. Ther.,* **307,** 213–218.

TAKESHITA, K., SAKAI, K., BACON, K.B. & GANTNER, F. (2003). Critical role of histamine H_4 receptor in leukotriene B4 production and mast cell-dependent neutrophil recruitment induced by zymosan *in vivo*. *J. Pharmacol. Exp. Ther.,* **307,** 1072–1078.

TARDIVEL-LACOMBE, J., MORISSET, S., GBAHOU, F., SCHWARTZ, J.C. & ARRANG, J.M. (2001). Chromosomal mapping and organization of the human histamine H_3 receptor gene. *Neuroreport,* **12,** 321–324.

THURMOND, R.L., DESAI, P.J., DUNFORD, P.J., FUNG-LEUNG, W.P., HOFSTRA, C.L., JIANG, W., NGUYEN, S., RILEY, J.P., SUN, S., WILLIAMS, K.N., EDWARDS, J.P. & KARLSSON, L. (2004). A potent and selective histamine H_4 receptor antagonist with anti-inflammatory properties. *J. Pharmacol. Exp. Ther.,* **309,** 404–413.

UVEGES, A.J., KOWAL, D., ZHANG, Y., SPANGLER, T.B., DUNLOP, J., SEMUS, S. & JONES, P.G. (2002). The role of transmembrane helix 5 in agonist binding to the human H_3 receptor. *J. Pharmacol. Exp. Ther.,* **301,** 451–458.

WEST JR., R.E., WU, R.L., BILLAH, M.M., EGAN, R.W. & ANTHES, J.C. (1999). The profiles of human and primate [^{3}H]N^{α}-methylhistamine binding differ from that of rodents. *Eur. J. Pharmacol.,* **377,** 233–239.

WIELAND, K., BONGERS, G., YAMAMOTO, Y., HASHIMOTO, T., YAMATODANI, A., MENGE, W.M., TIMMERMAN, H., LOVENBERG, T.W. & LEURS, R. (2001). Constitutive activity of histamine H_3 receptors stably expressed in SK-N-MC cells: display of agonism and inverse agonism by H_3 antagonists. *J. Pharmacol. Exp. Ther.,* **299,** 908–914.

WULFF, B., HASTRUP, S & RIMVALL, K. (2002). Characteristics of recombinantly expressed rat and human histamine H_3 receptors. *Eur. J. Pharmacol.,* **453,** 33–41.

YAO, B., HUTCHINS, C.W., CARR, T.L., CASSAR, S., MASTERS, J.N., BENNANI, Y.L., ESBENSHADE, T.A. & HANCOCK, A.A. (2003). Molecular modeling and pharmacological analysis of species-related histamine H_3 receptor heterogeneity. *Neuropharmacology,* **44,** 773–786.

ZHU, Y., MICHALOVICH, D., WU, H., TAN, K.B., DYTKO, G.M., MANNAN, I.J., BOYCE, R., ALSTON, J., TIERNEY, L.A., LI, X., HERRITY, N.C., VAWTER, L., SARAU, H.M., AMES, R.S., DAVENPORT, C.M., HIEBLE, J.P., WILSON, S., BERGSMA, D.J. & FITZGERALD, L.R. (2001). Cloning, expression, and pharmacological characterization of a novel human histamine receptor. *Mol. Pharmacol.,* **59,** 434–441.

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