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Characteristics of recombinantly expressed rat and human histamine H_3 receptors

Birgitte S. Wulff[a], Sven Hastrup[b], Karin Rimvall[a,*]

[a]Research and Development, Department of Molecular Pharmacology, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark
[b]Research and Development, Department of Molecular Genetics, Novo Nordisk A/S, Denmark

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Abstract

Human and rat histamine H_3 receptors were recombinantly expressed and characterized using receptor binding and a functional cAMP assay. Seven of nine agonists had similar affinities and potencies at the rat and human histamine H_3 receptor. *S*-α-methylhistamine had a significantly higher affinity and potency at the human than rat receptor, and for 4-[(1*R**,2*R**)-2-(5,5-dimethyl-1-hexynyl)cyclopropyl]-1*H*-imidazole (Perceptin®) the preference was the reverse. Only two of six antagonists had similar affinities and potencies at the human and the rat histamine H_3 receptor. Ciproxifan, thioperamide and (1*R**,2*R**)-*trans*-2-imidazol-4 ylcyclopropyl) (cyclohexylmethoxy) carboxamide (GT2394) had significantly higher affinities and potencies at the rat than at the human histamine H_3 receptor, while for *N*-(4-chlorobenzyl)-*N*-(7-pyrrolodin-1-ylheptyl)guanidine (JB98064) the preference was the reverse. All antagonists also showed potent inverse agonism properties. Iodoproxyfan, Perceptin®, proxyfan and GR175737, compounds previously described as histamine H_3 receptor antagonists, acted as full or partial agonists at both the rat and the human histamine H_3 receptor.

Keywords: Histamine H_3 receptor; (Rat); (Human); ^{125}Iodoproxyfan; cAMP; Inverse agonism

1. Introduction

The monoamine histamine plays a role in a variety of biological processes ranging from effects on neurotransmission in the central and peripheral nervous systems (CNS and PNS), to peripheral effects on for example inflammatory conditions, gastric acid secretion and smooth muscle contraction. According to current knowledge, histamine mediates all its actions through four distinct histamine receptors, the histamine H_1, H_2, H_3 and H_4 receptors. All four histamine receptors have been identified as G-protein coupled receptors and, while the human histamine H_3 receptor shows less than 30% homology to the human histamine H_1 and H_2 receptors, it has more than 40% homology to the human histamine H_4 receptor (58% in the transmembrane region) (Hough, 2001). Each of the four histamine receptors possess a unique pharmacology (Hough, 2001; Liu et al., 2001), and for the histamine H_1, H_2 and H_3 receptors many selective ligands are available (van der Goot and Timmerman, 2000).

The histamine H_3 receptor was identified pharmacologically, as a presynaptic receptor that influenced neurotransmission in the rat CNS, already in 1983 (Arrang et al.). Subsequently, it was also characterized pharmacologically in the rodent PNS (e.g. Imamura et al., 1995; Yamasaki et al., 2001; Coruzzi et al., 2000), but the molecular cloning of this receptor was not successful until 1999 (Lovenberg et al.). During the 16 years between the first pharmacological description of the histamine H_3 receptor in 1983 and its final cloning in 1999, many histamine H_3 receptor selective agonists and antagonists were identified (see van der Goot and Timmerman, 2000, for an overview), and the receptor's biochemical and functional characteristics were extensively investigated. It was shown that the histamine-induced activation of the presynaptic histamine H_3 autoreceptor causes a decreased synthesis of presynaptic histamine as well as a decreased release of this transmitter (e.g. Arrang et al., 1983, 1987). In addition, the histaminergic activation of histamine H_3 heteroreceptors, situated on presynaptic terminals of for example noradrenergic, cholinergic,

* Corresponding author. Tel.: +45-44-434-757; fax: +45-44-663-939.
E-mail address: KRim@novonordisk.com (K. Rimvall).

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dopaminergic and serotonergic neurons, were demonstrated to inhibit the release also of these neurotransmitters (Schlicker et al., 1988, 1989, 1993; Blandina et al., 1996; Arrang et al., 1995). There is a wide variety of human disorders in which a histamine H_3 receptor antagonist or a histamine H_3 receptor agonist are suggested to be of benefit, and several reviews dealing with this issue have recently appeared (e.g. Tozer and Kalindjian, 2000; Leurs et al., 1998a; Hill et al., 1997).

Currently available data show that most histamine H_3 receptor ligands have similar binding affinities for the cloned human histamine H_3 receptor and for the receptor specifically labeled by a radioactive histamine H_3 receptor agonists in rat cortex homogenates. However, differences in both affinity and potency of some histamine H_3 receptor antagonist at the recombinantly expressed rat and human histamine H_3 receptors, respectively, have been demonstrated (Lovenberg et al., 2000; Ligneau et al., 2000). These descriptions of species differences, together with previous data from receptor binding studies on rodent, monkey and human brain homogenates (West et al., 1990, 1999; Cumming and Gjedde, 1994) that also suggested a histamine H_3 receptor heterogeneity in the brain (see Hill et al., 1997, for review), have again focused much interest on the issues of species differences and of the possible existence of histamine H_3 receptor subtypes. To date, no bona fide, genetic histamine H_3 receptor subtypes have been identified, but several histamine H_3 receptor splice variants have been found to be expressed in the guinea-pig brain (histamine H_3L and H_3S receptors; Tardivel-Lacombe et al., 2000) and in the rat brain (histamine H_3A, H_3B and H_3C receptors; Drutel et al., 2001; Morisset et al., 2001). The splice variants all differ in the third cytoplasmic loop and there are data suggesting that they have different distributions in the rat CNS and that they differ in their intracellular coupling patterns (Drutel et al., 2001). It was not possible to detect splice variants of the central human histamine H_3 receptor (Liu et al., 2000) until recently, when three studies appeared that indicate that also the human histamine H_3 receptor can be alternatively spliced (Wellendorph et al., 2000; Cogé et al., 2001; Tardivel-Lacombe et al., 2001). Some of these human splice variants appear to differ with regards to their signal transduction capabilities (Cogé et al., 2001).

In the study presented here, histamine H_3 receptors, cloned from hypothalamic rat and human cDNA libraries and expressed in human embryonic kidney (HEK) 293 cells, have been used to further investigate the pharmacology of histamine H_3 receptors and the issue of species differences. Receptor affinities as well as agonist and antagonist potencies for a large number of known histamine H_3 receptor compounds have been determined and our results confirm and extend the results from other laboratories in that we clearly demonstrate species differences in the action of some, but not all, histamine H_3 receptor ligands. Furthermore, we show that some compounds, previously demonstrated to be histamine H_3 receptor antagonists act as agonists, and that all antagonists tested by us also possess inverse agonism characteristics.

2. Materials and methods

2.1. Compounds

Iodoproxyfan (Stark et al., 1996; Ligneau et al., 1994), 3-(4-chlorobenzyl)-5-[2-(1*H*-imidazol-4-yl)ethyl]-1,2,4-oxadiazole (GR175737; Clitherow et al., 1996), proxyfan (Morisset et al., 2000) and *N*-(4-chlorobenzyl)-*N*-(7-pyrrolodin-1-ylheptyl)guanidine (JB98064; Linney et al., 2000; Harper et al., 2000) were synthesized at Novo Nordisk. Iodoproxyfan was radioactively labeled (^{125}Iodoproxyfan) at Novo Nordisk (2000 Ci/mmol). Ciproxifan, known histamine H_3 receptor antagonist (Ligneau et al., 1998), was received from Boehringer Ingelheim (Biberach, Germany). The histamine H_3 receptor ligands 4-[(1*R**,2*R**)-2-(5,5-dimethyl-1-hexynyl)cyclopropyl]-1*H*-imidazole (Perceptin®; GT2331; Tedford et al., 1998) and (1*R**,2*R**)-*trans*-2-imidazol-4 ylcyclopropyl) (cyclohexylmethoxy) carboxamide (GT2394; Yates et al., 2000) were purchased from Epsilon Chimie, Brest, France. The following histamine H_3 receptor compounds were purchased from commercial sources (Tocris and Sigma): histamine, *R*-α-methylhistamine, *S*-α-methylhistamine, imetit, immepip, clobenpropit, iodophenpropit and thioperamide.

2.2. Cloning and expression of the human and the rat histamine H_3 receptors

The DNA coding for the human and rat histamine H_3 receptors were cloned by polymerase chain reaction on human hypothalamic and rat brain cDNA (Clontech). The sequence information was based on the GenBank sequences AF140538 (Lovenberg et al., 1999) and AF237919 (Lovenberg et al., 2000) and confirmed by the Novo Nordisk Sequencing Unit. The DNAs were inserted into the mammalian cell expression vector pcDNA3.1 (Invitrogen) and cells, stably expressing the histamine H_3 receptors, were generated by transfecting the histamine H_3 receptor expression vector into HEK 293 cells and using G418 to select for stable histamine H_3 receptor expressing clones. The cells were cultured in Dulbecco's Minimal Essential Medium with glutamax, 10% fetal calf serum, 1% penicillin and streptomycin mixture and 1 mg/ml G418 at 37 °C and 5% CO_2.

2.3. Receptor binding

Cultured, confluent HEK293 cells, expressing either the human or the rat histamine H_3 receptor, were harvested by flushing with phosphate buffered saline (PBS). After an initial homogenization and centrifugation step, the

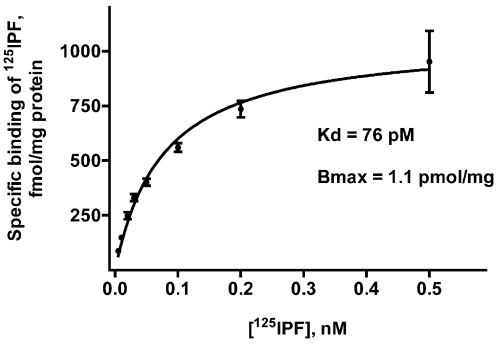

Fig. 1. Specific binding of ^{125}Iodoproxyfan to membranes of the human histamine H_3 receptor expressed in HEK293 cells. Symbols represent means ± S.E.M. of data points from triplicate determinations. The K_d and B_{max} values determined by nonlinear regression analysis were, in this particular experiment, 76 pM and 1060 fmol/mg protein, respectively.

membrane pellet was resuspended in Hepes buffer (20 mM Hepes, 5 mM $MgCl_2$, pH 7.1) and homogenized again. After two additional centrifugation steps, the final membrane pellet was resuspended in Hepes buffer and the protein concentrations were determined. Membranes were incubated for 1 h, at room temperature in plastic tubes, in Hepes buffer (total volume, 500 μl) and in the presence of 30 pM ^{125}Iodoproxyfan and increasing concentrations of test compounds. Cold iodoproxyfan (10 μM) was used to define nonspecific binding. Preliminary experiments revealed that 1 h incubation was sufficient to reach equilibrium and that 5 μg protein per 500 μl assay volume was a suitable amount of protein to use. The reaction was terminated by rapid filtering through GF/B filters, pretreated for 1 h with 0.5% polyethyleneimine and subsequent washing with ice-cold NaCl. The radioactivity retained on the filters was measured with a Cobra II auto gamma counter. Saturation experiments were performed with increasing concentrations of ^{125}Iodoproxyfan. K_d-, B_{max}- and IC_{50}-values were determined using nonlinear regression analysis (GraphPad, Prism). K_i-values were calculated according to the Cheng–Prusoff equation (Cheng and Prusoff, 1973).

2.4. Functional assay—cAMP accumulation

cAMP accumulation was measured using the Flash Plate® cAMP assay (NEN™ Life Science Products). The assay was generally performed as described by the manufacturer. The histamine H_3 receptor expressing cells were washed once with PBS and harvested using versene (GIBCO-BRL). PBS was added and the cells were centrifuged for 5 min at 188 × *g*. The cell pellet was resuspended in stimulation buffer at a concentration of 1×10^6 cells/ml. Fifty-microliter cell suspension was added to each Flash Plate well which also contained 25 μl 40-μM isoprenaline, to stimulate cAMP generation, and 25 μl test compound (either agonist alone or agonist and antagonist in combination). The final volume in each well was 100 μl. Test compounds were dissolved in dimethyl sulfoxide and diluted in H_2O. The mixture was shaken for 5 min, and allowed to stand for 25 min at room temperature. The reaction was stopped with 100 μl "Detection Mix" per well. The plates were sealed with plastic, shaken for 30 min, allowed to stand overnight, and finally the samples were counted in the Cobra II auto gamma topcounter. EC_{50} values were calculated by nonlinear regression analysis of dose response curves (six points minimum) using GraphPad Prism.

3. Results

3.1. Receptor binding

Specific binding of ^{125}Iodoproxyfan was always < 10% of total binding, and no specific binding to non-transfected HEK293-cells could be detected. ^{125}Iodoproxyfan bound specifically to one single binding site, as determined using nonlinear regression analysis. One representative experiment is shown in Fig. 1. The K_d values determined at the human histamine H_3 receptor expressed in HEK293 cells in three independent experiment were 60 ± 9.9 pM and the B_{max} values were 950 ± 160 fmol/mg protein. Similar, but

Table 1A

Compound	Human histamine H_3 receptor		Rat histamine H_3 receptor	
	K_i (nM)	EC_{50} (nM)	K_i (nM)	EC_{50} (nM)
Histamine	7.4 ± 0.53 (3)	2.1 ± 0.80 (3)	nt	2.2 ± 0.99 (3)
R-α-methyl-histamine	4.1 ± 1.4 (5)	0.48 ± 0.16 (10)	4.0 ± 0.46 (3)	0.44 ± 0.19 (4)
S-α-methyl-histamine	25 ± 3.6 (4)	6.7 ± 0.81 (3)	250 ± 32 (4)[a]	16 ± 0.5 (3)[a]
Imetit	0.74 ± 0.13 (3)	0.18 ± 0.032 (3)	0.97 ± 0 (3)	0.14 ± 0.044 (3)
Immepip	0.62 ± 0.062 (3)	0.12 ± 0.012 (3)	1.6 ± 0.16 (3)[a]	0.12 ± 0.046 (3)
GR175737[b]	2.6 ± 0.23 (3)	7.8 ± 5.2 (3)	4.6 ± 0.46 (3)	4.0 ± 1.0 (3)
Proxyfan	5.0 ± 1.5 (3)	5.5 ± 1.5 (3)	10 ± 1.0 (3)	3.6 ± 1.3 (3)
Iodoproxyfan	0.10 ± 0.008 (20)	0.14 ± 0.026 (4)	0.28 ± 0.026 (4)	0.84 ± 0.33 (3)[a]
Perceptin®	2.4 ± 0.47 (3)	7.9 ± 1.1 (3)	0.32 ± 0.029 (3)[a]	1.4 ± 0.67 (4)[a]

Significance of difference to EC_{50}/K_i values at human histamine H_3 receptor (Mann–Whitney).

[a] $P < 0.05$.

[b] Partial agonism: 62 ± 8.0% (human histamine H_3) and 40 ± 7.3% (rat histamine H_3).

Table 1B

Compound	Human histamine H_3 receptor			Rat histamine H_3 receptor		
	K_i (nM)	EC_{50} (inverse agonism) (nM)	K_b (nM)	K_i (nM)	EC_{50} (inverse agonism) (nM)	K_b (nM)
Clobenpropit	1.8 ± 0.24 (3)	1.1 ± 0.12 (3)	0.91 ± 0.19 (3)	1.2 ± 0.029 (3)	1.4 ± 0.60 (4)	0.50 ± 0.30 (3)
Iodophenpropit	1.9 ± 0.18 (3)	3.3 ± 1.8 (3)	1.2 ± 0.37 (3)	0.79 ± 0.047 (3)	1.8 ± 0.26 (4)	1.5 ± 0.63 (3)
Thioperamide	56 ± 15 (3)	15 ± 2.3 (3)	31 ± 13 (3)	19 ± 1.6 (4)[a]	2.5 ± 0.46 (4)[a]	1.1 ± 0.40 (3)[a]
Ciproxifan	180 ± 36 (5)	116 ± 40 (4)	80 ± 19 (3)	2.6 ± 0.44 (3)[a]	0.77 ± 0.47 (3)[a]	0.25 ± 0.11 (3)[a]
GT2394	410 ± 32 (3)	165 ± 32 (3)	155 ± 3.5 (3)	22 ± 1.9 (3)[a]	not determined	13 ± 2.0 (3)[a]
JB98064	47 ± 8.0 (3)	1.6 ± 0.76 (6)	0.91 ± 0.26 (3)	97 ± 11 (3)[a]	not determined	9.2 ± 3.4 (3)[a]

Significance of difference to $EC_{50}/K_b/K_i$ value at human H_3 receptor (Mann–Whitney).
[a] $P < 0.05$.

somewhat higher, binding values were determined for the rat histamine H_3 receptor expressed in HEK293 cells ($K_d = 220 \pm 51$ pM and $B_{max} = 2600 \pm 98$ fmol/mg protein in three separate experiments). These binding constants agree quite well with those determined in rat cortex (not shown) and by others working with recombinantly expressed histamine H_3 receptors (e.g. Lovenberg et al., 1999, 2000).

K_i values were determined for a number of test compounds in displacement studies using 30 pM of ^{125}Iodoproxyfan and membranes from the human or rat histamine H_3 receptor, respectively. For agonists (Table 1A), the K_i values for the tested compounds were generally of the same order of magnitude at the human and at the rat histamine H_3 receptor and also agreed with K_i values previously determined in rat cortex homogenates (not shown) and with values reported in the literature (e.g. Lovenberg et al., 1999, 2000). However, for Perceptin® and *S*-α-methylhistamine significant differences in the affinities for the rat and human histamine H_3 receptors, respectively, were observed. Perceptin® showed a ~10-fold higher affinity for the rat receptor compared to the human receptor. As would be expected, *S*-α-methylhistamine, the stereoisomer of the histamine H_3 receptor agonist *R*-α-methylhistamine, had significantly lower affinity for both the rat and the human histamine H_3 receptor than *R*-α-methylhistamine had. However, while the difference in affinity at the rat receptor for the two stereoisomers was almost 60-fold, the difference at the human receptor was only 6-fold.

For antagonists (Table 1B), the K_i values for clobenpropit and iodophenpropit were of the same order of magnitude at the human and at the rat histamine H_3 receptor. For the antagonists thioperamide, ciproxifan, GT2394 and JB98064, differences in affinities for the human and the rat histamine H_3 receptors, respectively, were observed. Ciproxifan displaced ^{125}Iodoproxyfan with K_i values in the low nM-range in cells expressing the rat-cloned receptor. However, its K_i value in the human histamine H_3 receptor expressing cells was considerably higher. The same was true for the compounds GT2394 and thioperamide. The compound JB98064 had a minor, but significant, higher affinity for the human histamine H_3 receptor than for the rat histamine H_3 receptor.

3.2. Functional testing—agonism

In Fig. 2A,B representative dose-response curves for the action of nine agonists at the human histamine H_3 receptor are shown, and in Table 1A, the potencies, i.e. the EC_{50} values, of these nine agonists at the human as well as the rat histamine H_3 receptor are summarized. Histamine, *R*-α-methylhistamine, imetit, immepip, GR175737 and proxyfan inhibited isoprenaline-induced cAMP accumulation in cells expressing either the human or the rat histamine H_3 receptor and with EC_{50} values that were essentially identical in the

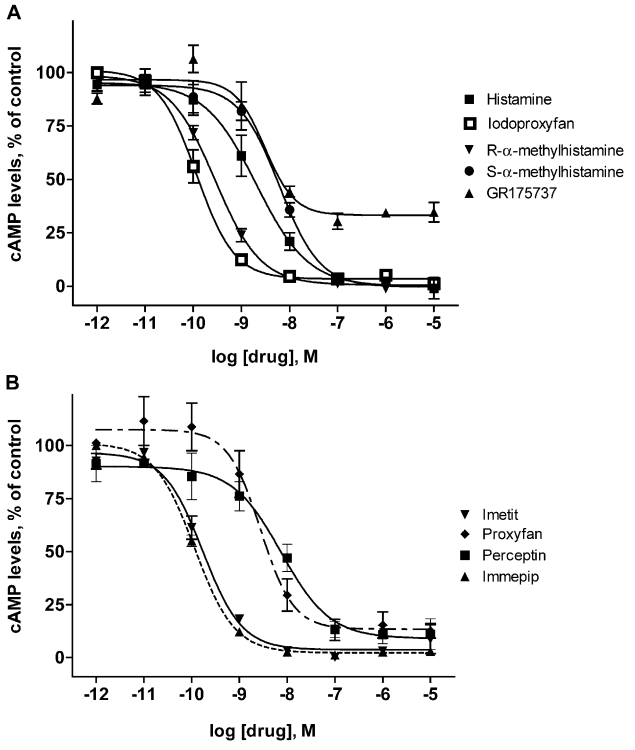

Fig. 2. Nine histamine H_3 receptor agonists decrease the isoprenaline-induced cAMP levels in HEK293 cells expressing the human histamine H_3 receptor. To enhance readability, the figure is in two parts. EC_{50} values were determined by nonlinear regression analysis and are summarized in Table 1A. Symbols represent means ± S.E.M. of data points from duplicate determinations in at least three independent experiments.

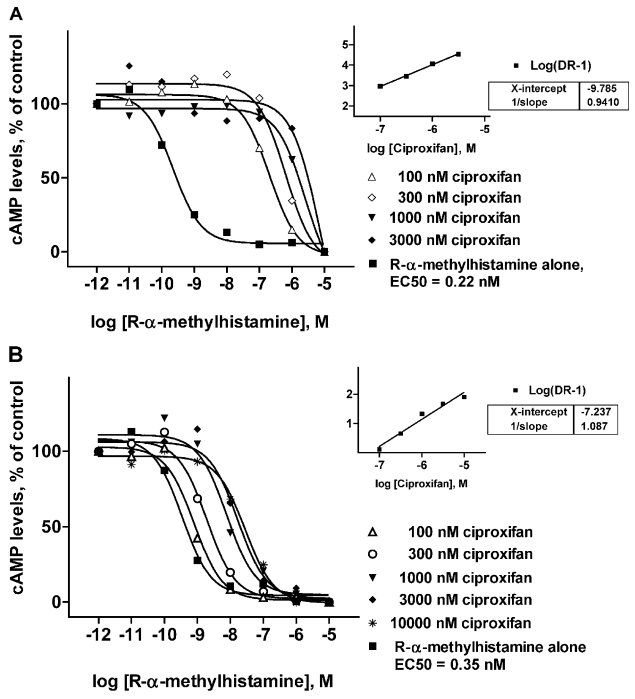

Fig. 3. Schild plots demonstrating the determination of the antagonist potency of ciproxifan at the rat histamine H_3 (A) and the human histamine H_3 (B) receptor expressed in HEK293 cells. Dose-response curves for the *R*-α-methylhistamine-induced inhibition of isoprenaline-induced cAMP accumulation were generated in both cell lines in the presence of increasing concentrations of ciproxifan. The log of the (dose ratio − 1) at each concentration of antagonist was plotted versus the log of the ciproxifan concentration. K_b values were determined from the *x*-intercept on the linear, Schild regression plot (insets) and were in these particular experiments 0.16 nM at the rat receptor and 58 nM at the human receptor. *R*-α-methylhistamine has similar EC_{50} values at the rat and the human receptor (0.22 and 0.35 nM, respectively). The data points represent means ± S.E.M. of duplicate determinations from one representative experiment.

two species. With the exception of GR175737, they were all full agonists as defined using 10 μM *R*-α-methylhistamine. The differences in affinities to the rat and human receptors that were observed for Perceptin® and *S*-α-methylhistamine were also seen in the functional test. Perceptin® had ~6-fold lower agonist potency at the human than at the rat receptor. *S*-α-methylhistamine had lower agonist potency, both at the rat and human histamine H_3 receptor, than *R*-α-methylhistamine had. However, the difference in EC_{50} values was less pronounced at the human than at the rat receptor.

GR175737, proxyfan, iodoproxyfan and Perceptin® are compounds described in the literature as histamine H_3 receptor antagonists (Ligneau et al., 1994; Stark et al, 1996; Tedford et al., 1998; Morisset et al., 2000; Clitherow et al., 1996). In our study they acted as potent, full (proxyfan, iodoproxyfan and Perceptin®) and partial (GR175737) agonists at both the rat and the human histamine H_3 receptor (Fig. 2; Table 1A).

3.3. Functional testing—antagonism

The antagonist potencies of six histamine H_3 receptor compounds, i.e. their ability to inhibit *R*-α-methylhistamine-induced inhibition of cAMP-accumulation in HEK293 cells expressing either the rat or the human histamine H_3 receptor, were tested by generating Schild plots. In Table 1B, the K_b values for the six histamine H_3 receptor antagonists available to us are shown. Corresponding to the K_i values generated using receptor binding, clobenpropit and iodophenpropit had similar K_b values at both the rat and the human histamine H_3 receptor. Thioperamide, ciproxifan and GT2394 had higher potencies at the rat receptor than at the human histamine H_3 receptor. JB98064 had a 10-fold lower potency at the rat compared to the human H_3 receptor.

In Fig. 3A, the ability of increasing doses of ciproxifan to cause a rightward shift in the dose-response curve of *R*-α-methylhistamine's inhibitory effects on cAMP accumulation in cells expressing the rat histamine H_3 receptor is shown. In this particular experiment, the K_b value was 0.16 nM. In Fig. 3B, ciproxifan's potency at the human histamine H_3 receptor is clearly shown to be considerably lower (K_b in this experiment = 58 nM).

All of the six histamine H_3 receptor antagonists tested also demonstrate inverse agonism both at the rat (not shown) and at the human histamine H_3 receptor (Fig. 4). Generally, there was a good agreement between the compounds' potencies as competitive antagonists (i.e. their K_b values) and their potencies as inverse agonists (i.e. their inverse agonism—EC_{50} values) both at the rat and at the human histamine H_3 receptors (Table 1B). None of all the compounds tested were neutral antagonists in these receptor systems.

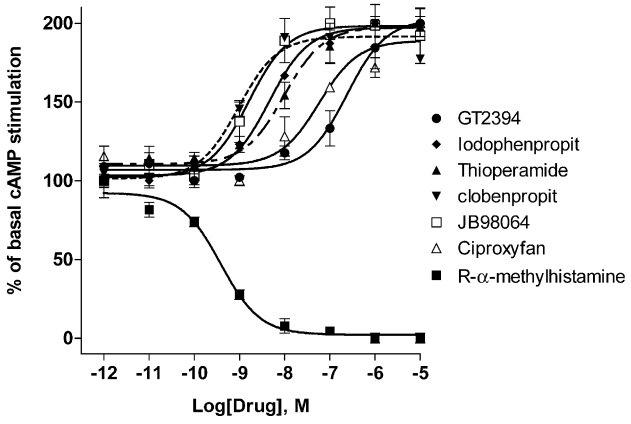

Fig. 4. The histamine H_3 receptor antagonists thioperamide, clobenpropit, ciproxifan, iodophenpropit, JB98064 and GT2394, all demonstrate inverse agonism at the human histamine H_3 receptor expressed in HEK293 cells by increasing cAMP levels in the absence of an agonist. As expected, the agonist *R*-α-methylhistamine decreases the cAMP levels. The data points represent means ± S.E.M. of duplicate determinations in a representative experiment with each compound.

4. Discussion

The data presented here, confirm and extend results from other laboratories investigating the pharmacology of recombinantly expressed histamine H_3 receptors from human and rat.

We have clearly demonstrated that there are substantial species differences in both histamine H_3 receptor affinity and antagonist potency of four of the six histamine H_3 receptor antagonists tested, i.e. thioperamide, ciproxifan, GT2394 and JB98064. Three of these four antagonists had higher affinity and potency at the rat than at the human receptor. For JB98064 the situation was the reverse—it had higher affinity and potency at the human receptor. That clobenpropit and iodophenpropit with small chloro- and iodo-atoms in their side chains are less bulky molecules than ciproxifan and thioperamide that possess a cyclopropylcarbonyl (ciproxifan) and a cycloexane ring (thioperamide) may be part of the explanation for the differences in affinities and potencies at the rat and human histamine H_3 receptors, respectively. Receptor modelling with docking of the molecules will have to be done in order to substantiate this.

Using site-directed mutagenesis, Ligneau et al. (2000) recently demonstrated that the two amino acids in transmembrane region 3 close to the conserved aspartic acid, that are different in the rat and human sequences, i.e. Thr(h)/Ala(r)^{119} and Ala(h)/Val(r)^{122}, are responsible for differences in histamine H_3 receptor binding affinity of ciproxifan in the two species. Using our functional assay, we have now also demonstrated that ciproxifan was more than100-fold more potent in increasing cAMP-levels at the rat receptor than at the human receptor (K_b values of 0.25 and 80 nM, respectively, and EC_{50} inverse agonism values of 0.77 and 116 nM, respectively; Table 1B; see also Fig. 3). Similarly, Lovenberg et al. (2000) observed considerably lower binding affinity and antagonist potency of thioperamide at the human compared to the rat receptor, while clobenpropit was equally potent at both specie's receptors. These data are also in agreement with ours.

That also two agonists, i.e. *S*-α-methylhistamine and Perceptin®, can actually discriminate between the rat and human histamine H_3 receptor is a new finding. *S*-α-methylhistamine appeared to have a higher affinity for the human than for the rat histamine H_3 receptor, while Perceptin® showed the opposite. These species differences were also demonstrated using the functional assays. It is unknown whether the two amino acid substitutions at position 119 and 122 are responsible also for the species differences demonstrated by these agonists. This will have to be tested in future studies.

Although the compounds that bound with high affinity to the histamine H_3 receptors were also highly potent at the receptor and the compounds with low affinity were not as potent, the absolute K_i values from binding experiments and the absolute K_b and EC_{50} values from the functional experiments did not always agree very well. This was particularly evident for JB98064: here K_i values at the human histamine H_3 and rat histamine H_3 receptor were 47 and 97 nM, respectively, whereas the K_b values were 0.91 and 9.2 nM. However, when comparing potency values from functional experiments with K_i values from displacement experiments, it should be kept in mind that we are not only working with whole-cells and membranes, respectively, but the ligand we used in the displacement experiments is an agonist (^{125}Iodoproxyfan). That the agonist/antagonist status of the ligand is of importance when establishing correlations between affinities and potencies has previously been described in other receptor systems (e.g. Holst et al., 2002). Using a radioactively labeled, iodinated analog of the histamine H_3 receptor antagonist JB98064 as a ligand in binding experiments, we have recently demonstrated that this is an important aspect also with regards to histamine H_3 receptors (Kolling et al., 2002; Rimvall et al., in preparation).

That G-protein coupled receptors can signal without agonist stimulation has been shown in several recombinant receptor systems as well as in some in vivo systems (Leurs et al., 1998b, 2000; Milligan et al., 1995; Bakker et al., 2000; Wieland et al., 2001). Such spontaneous or constitutive G-protein coupled receptor activity was also observed in our HEK293 cells expressing the human or the rat histamine H_3 receptor: in the absence of any drugs, the isoprenaline-induced cAMP levels were much lower in the histamine H_3 receptor expressing cells than in the non-transfected HEK293 cells. Since the histamine H_3 receptor is G_i coupled, the histamine H_3 receptor agonists tested in our functional assays of course decreased the isoprenaline-induced cAMP levels even further. Interestingly, we also show that when a competitive histamine H_3 receptor antagonists is added to the histamine H_3 receptor expressing cells together with only isoprenaline, they induce an increase in cellular cAMP levels above the levels induced by isoprenaline alone (Fig. 4). That means that they act as inverse agonists at both the human and the rat histamine H_3 receptor. The compounds' potencies as inverse agonists are of the same rank order as their competitive antagonist potencies (K_b values) at the histamine H_3 receptor. In fact, the compounds' potencies as inverse agonists agree nicely, also in absolute values, to their K_b values (Table 1B). Our finding that the competitive histamine H_3 receptor antagonists also act as inverse agonists agree with and extend findings from other laboratories (Morisset et al., 2000; Wieland et al., 2001; Leurs et al., 2001; Rouleau et al., 2002).

It is well known, that the inverse agonist efficacy of a compound depends on the specific cell line used for expressing the receptor as well as on the expression levels (Morisset et al., 2000; Leurs et al., 1998b, 2000; Milligan et al., 1995). However, that histamine H_3 receptor antagonists' inverse agonism-properties is not only a cell line phenomenon but might actually have relevance in vivo, was recently demonstrated by Morisset et al. (2000). These authors

showed that proxyfan acts a neutral antagonist in their cellular systems as well as in their more physiological systems, and subsequently they used this compound to inhibit both the actions of histamine H_3 receptor agonists and histamine H_3 receptor inverse agonists in vitro as well as in vivo. However, Morisset et al. (2000) also mentioned that the pharmacological profile of proxyfan depended on the test system and that proxyfan could act as a partial inverse agonist (arachidonic acid release) as well as a partial agonist (cAMP decrease) in Chinese hamster ovary (CHO) cells with high histamine H_3 receptor expression levels. In our cAMP assay, proxyfan behaved as a full histamine H_3 receptor agonist both at the rat and human histamine H_3 receptor. This might be explained by the relatively high expression levels in our cell lines (950 fmol/mg protein in the cells expressing the human histamine H_3 receptor). The fact that we used HEK293 cells and not CHO cells, may also have played a role, since it is known that these two cell lines contain very different levels of various G-proteins (Dr. S. Møller-Knudsen, personal communication). These observations highlight the difficulties associated with these type of studies and in translating cell line data into in vivo functionality.

We found iodoproxyfan to be a full agonist at both the rat and the human histamine H_3 receptor (Table 1A and Fig. 2) and this observation agrees with findings of Lovenberg et al. (2000) with the chlorinated analog chloroproxyfan. Iodoproxyfan, which has been considered to be a histamine H_3 receptor antagonist based on its action on neurotransmitter release from rat synaptosomes (Stark et al., 1996), has previously been demonstrated to act as an agonist in the mouse brain preparation and the guinea-pig ileum preparation (Schlicker et al., 1996; Hill et al., 1997; Sasse et al., 1999). Also GR173757 has been described as histamine H_3 receptor antagonists on the basis of experiments with guinea-pig muscle strips and rat drinking test (Clitherow et al., 1996). We found it to be a partial histamine H_3 receptor agonist and of course it should be kept in mind that a partial agonist in one system definitely can act as a functional antagonist in another histamine H_3 receptor system. Finally, Perceptin® and proxyfan, which also previously have been described as histamine H_3 receptor antagonists on the basis of experiments with guinea-pig heart and jejunum tissue preparations (Perceptin®; Tedford et al., 1998) and effects on for example histamine release from rat brain synaptosomes (proxyfan; Morisset et al., 2000), possess full agonist properties at the human and rat histamine H_3 receptor expressed in HEK293 cells. There are no data available suggesting that Perceptin® has partial agonist activity in other systems, whereas, as already mentioned above, proxyfan can show partial agonism in CHO cells with high histamine H_3 receptor expression levels. Both these compounds have, however, been shown to act as neutral antagonists. For proxyfan, this was demonstrated by Morisset et al. (2000; see discussion above). For Perceptin® it was demonstrated using a rat cortex membrane [^{3}H]-*N*-α-methylhistamine H_3 receptor-binding assay. In this study, only thioperamide's and not Perceptin®'s K_i values increased by adding Na^+-ions or GTPγS to the incubation medium (Tedford et al., 1999) and these observations, together with similar data for thioperamide published by Clark and Hill (1995), were interpreted as thioperamide possessing inverse agonism properties while Perceptin acted as a neutral antagonist.

In conclusion, the recent cloning of the human and the rat histamine H_3 receptors has revealed some substantial species differences in affinity and potency of a number of histamine H_3 receptor ligands. Whether the two amino acid substitutions in positions 119 and 121 are responsible for the species differences displayed by all these compounds, antagonists as well as agonists, or whether any of the other amino acid differences in other parts of the receptor molecule play a role will have to be investigated further using site-directed mutagenesis studies. In addition, the use of cell lines have also revealed that some compounds, previously described as histamine H_3 receptor antagonists based on various functional tissue and in vivo assays, appear to be agonists (full or partial) and that all competitive antagonists we have tested also possess inverse agonism properties. Whether these in vitro findings actually have relevance in more intact cellular systems will have to be investigated further.

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