Novel histamine H₃ receptor antagonists: affinities in an H₃ receptor binding assay and potencies in two functional H₃ receptor models

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1 We determined the affinities of ten novel H₃ receptor antagonists in an H₃ receptor binding assay and their potencies in two functional H₃ receptor models. The novel compounds differ from histamine in that the aminoethyl side chain is replaced by a propyl or butyl chain linked to a polar group (amide, thioamide, ester, guanidine, guanidine ester or urea) which, in turn, is connected to a hexocyclic ring or to an alicyclic ring containing an alkyl residue.

2 The specific binding of [³H]-N^α-methylhistamine to rat brain cortex membranes was monophasically displaced by each of the ten compounds at pK_i values ranging from 7.56 to 8.68.

3 Inhibition by histamine of the electrically evoked tritium overflow from mouse brain cortex slices preincubated with [³H]-noradrenaline was antagonized by the ten compounds and the concentration-response curve was shifted to the right with apparent pA₂ values ranging from 7.07 to 9.20.

4 The electrically induced contraction in guinea-pig ileum strips (which was abolished by atropine) was inhibited by the H₃ receptor agonists R-(−)-α-methylhistamine (pEC₅₀ 7.76), N^α-methylhistamine (7.90) and imetit (8.18). The concentration-response curve of R-(−)-α-methylhistamine was shifted to the right by thioperamide (apparent pA₂ 8.79) and by the ten novel compounds (range of pA₂ values 6.64–8.81).

5 The affinities and potencies were compared by linear regression analysis. This analysis was extended to thioperamide, the standard H₃ receptor antagonist, which is also capable of differentiating between H_{3A} and H_{3B} sites. Comparison of the apparent pA₂ values in the two functional H₃ receptor models yielded a regression coefficient of 0.77 (*P* < 0.02). When the pA₂ of the drugs in the mouse brain cortex were compared to the pK_i for H₃ sites (ten novel compounds) and for H_{3A} sites (thioperamide), a significant correlation (*r* = 0.87; *P* < 0.001) was obtained. There was, however, no significant correlation when the pK_i of thioperamide for H_{3B} sites was used instead (*r* = 0.52). In a similar manner, comparison of the pA₂ in the guinea-pig ileum with the pK_i in the binding assay yielded a significant correlation (*r* = 0.70, *P* < 0.05) only when the pK_i of thioperamide for H_{3A} sites was used but not when its pK_i for H_{3B} sites was considered (*r* = 0.17, NS).

6 On the basis of these results, structure-activity relationships for the novel H₃ receptor antagonists, and the nature of the H₃ receptors in the guinea-pig ileum and mouse brain, are considered.

Keywords: [³H]-N^α-methylhistamine binding; rat brain cortex membranes; noradrenaline release; mouse brain cortex slices; guinea-pig ileum strips; H₃ receptor subtypes; presynaptic receptors; histamine; R-(−)-α-methylhistamine; thioperamide

Introduction

A new type of histamine receptor, termed H₃, has been characterized by Arrang *et al.* (1983), at which impromidine (a partial H₂ receptor agonist) and burimamide (an H₂ receptor antagonist) proved to be potent antagonists. Subsequently, Arrang *et al.* (1987) showed that thioperamide is superior to impromidine and burimamide with respect to its antagonistic potency and, more importantly, its selectivity towards H₃ receptors. Subsequently, a variety of H₃ receptor antagonists have been synthesized (for review, see Leurs & Timmerman, 1992; Lipp *et al.*, 1992) and the structural requirements necessary for an H₃ receptor antagonist have been defined (Lipp *et al.*, 1992). Thus, an antagonistic effect at H₃ receptors can be expected for a molecule which consists of a nitrogen-containing heterocyclic ring (most favourably an imidazole) connected to a polar group via an alkyl chain. The affinity is increased by a lipophilic residue linked to the polar group by a spacer. Numerous H₃ receptor antagonists have recently been synthesized by some of us according to this construction pattern (Schwartz *et al.*, 1992) and ten of these compounds will be considered in the present study.

We determined the affinities of these compounds for H₃ receptors in a radioligand binding study and their antagonis-

tic potencies in two functional H₃ receptor models. [³H]-N^α-methylhistamine was used for the binding studies. The specific binding of this ligand to rat brain membranes is displaced biphasically by thioperamide and burimamide; this finding led West *et al.* (1990) to propose the existence of H₃ receptor subtypes, termed H_{3A} and H_{3B}. According to this classification, the H₃ receptor involved in the inhibitory effect of histamine on noradrenaline release in the mouse brain cortex can be classified as H_{3A} (Schlicker *et al.*, 1992). Combining both experimental approaches with the H₃ receptor model in the guinea-pig ileum, the functional H₃ receptor model used most frequently (for review, see Vollinga *et al.*, 1992), we tried to obtain further evidence in favour of the occurrence of H₃ receptor subtypes. Another aim of the present study was to demonstrate structure-activity relationships for the novel H₃ receptor antagonists.

Methods

Binding studies

Binding experiments were carried out according to West *et al.* (1990) with the modifications described by Kathmann *et*

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al. (1993). Cerebral cortices obtained from male Wistar rats were homogenized (Potter-Elvehjem; 10 up and down strokes, in a period of 1 min) in 25 volumes of ice-cold Tris-HCl buffer (Tris 50 mmol l⁻¹, pH 7.5; EDTA 5 mmol l⁻¹; sucrose 10.27%) and centrifuged at 1000 g for 10 min (4°C). The supernatant was centrifuged at 35000 g for 10 min and the pellet was resuspended in 10 volumes of Tris-HCl buffer and frozen at -80°C.

The binding assay was performed in Tris-HCl buffer (Tris 50 mmol l⁻¹, pH 7.5; EDTA 5 mmol l⁻¹) in a final volume of 0.5 ml containing 0.2–0.3 mg protein. [³H]-N^α-methylhistamine (specific activity 80 Ci mmol⁻¹) was used at a concentration of 0.2 nmol l⁻¹. The incubation (30°C) was terminated after 40 min by filtration through polyethyleneimine (0.3%)-pretreated Whatman GF/C filters. Each filter was placed in a counting vial and 6 ml of the commercially available scintillant Ready Gel (Beckman, Fullerton, CA, U.S.A.) was added. The radioactivity of the filters was determined in a Beckman LS 6000TA counter.

Non-specific binding (7% of total binding) was determined in the presence of R-(−)-α-methylhistamine (R-αMH) 2 μmol l⁻¹. Protein was assayed according to the method described by Bradford (1976). Data were analysed using the programmes LIGAND (McPherson *et al.*, 1983) and GraphPadInPlot (GPPI; GraphPad Software, San Diego, CA, U.S.A.).

Superfusion studies

Superfusion experiments were performed according to Schlückler *et al.* (1992). Briefly, cerebrocortical slices from male NMRI mice were incubated for 60 min at 37°C with physiological salt solution (PSS; for composition, see Kathmann *et al.*, 1993) containing [³H]-noradrenaline 25 nmol l⁻¹ (specific activity 52.3 Ci mmol⁻¹) and subsequently superfused with PSS (37°C; 110 min) containing desipramine 1 μmol l⁻¹ plus rauwolscine 1 μmol l⁻¹. The superfusate was collected in 5-min samples. Tritium overflow was evoked by two 2-min periods of electrical field stimulation (36 rectangular pulses of 50 mA and 2 ms; 0.3 Hz) 40 (S₁) and 90 min (S₂) after onset of superfusion. Histamine was added to the PSS from 62 min of superfusion onward and the histamine receptor antagonist under study was present in the PSS throughout superfusion. At the end of superfusion, the slices were solubilized with 0.5 ml Soluene (Packard, Meriden, CT, U.S.A.) and 7 ml scintillant (containing 6 g PPO and 0.1 g POPOP in 1 l toluene) was added. The vials containing the superfusate samples were supplemented with 6 ml Ready Gel. The radioactivity of the solubilized slices and superfusate samples was determined in a Beckman LS 6000TA counter.

Stimulation-evoked tritium overflow was calculated by subtraction of the basal from the total tritium efflux during the period of stimulation and the subsequent 13 min and was expressed as percentage of the tritium in the slices at the onset of stimulation; basal efflux was assumed to decline linearly from the 5-min period before to that 15–20 min after onset of stimulation. To determine the effect of histamine (in the presence or absence of antagonists) on the evoked overflow, the ratio of the overflow evoked by S₂ over that evoked by S₁ was calculated (S₂/S₁). For quantification of the effects of histamine receptor antagonists on the evoked overflow, the tritium overflow evoked by S₁ was compared to the corresponding value obtained in the absence of the antagonist.

Organ bath studies

Longitudinal muscle strips (ca. 2 cm) were prepared from the small intestine, 20–50 cm proximal to the ileocaecal valve, of female guinea-pigs (350–450 g). The muscle strips were mounted between two platinum electrodes (4 mm apart) in 20 ml Krebs buffer (composition in mmol l⁻¹: NaCl 118, KCl 5.6, MgSO₄ 1.18, CaCl₂ 2.5, NaH₂PO₄ 1.28, NaHCO₃ 25 and glucose 5.5), connected to an isometric transducer, con-

tinuously gassed with carbogen (95% O₂:5% CO₂) and maintained at 37°C. After equilibration of the muscle segments for 1 h with washings every 10 min, they were stimulated continuously with rectangular pulses of 15 V and 0.5 ms at a frequency of 0.1 Hz. After 30 min of stimulation, cumulative concentration-response curves with half-logarithmic increments of R-αMH were recorded until no change in response was found. Subsequently, the preparations were washed 3 times every 10 min without any stimulation. The antagonist under study was allowed to equilibrate with the tissue for 20–30 min before the re-determination of the concentration-response curve of R-αMH. Up to three concentration-response curves could be constructed in one preparation. Mepyramine 1 μmol l⁻¹ was routinely present in the medium in order to block H₁ receptors.

Calculations and statistics

The apparent pA₂ value of the test compounds against histamine (mouse brain cortex slices) or R-αMH (guinea-pig ileum strips) was calculated according to formula 4 of Furchgott (1972): pA₂ = log ([E']/[E] - 1) - log [B], where [E'] and [E] are the concentrations of the agonist producing the half-maximum effect in the presence and absence of the antagonist, respectively, and [B] is the concentration of the antagonist. In mouse brain cortex slices, the maximum inhibitory effect obtainable with histamine was consistently about 70%; thus, the concentration producing an inhibition of 35% was used for determination of the pEC₅₀ and apparent pA₂. In guinea-pig ileum strips, the maximum effect obtainable with agonists is highly variable ranging from less than 30% to nearly 100%; only tissues in which the maximum effect of the first curve was more than 30% were considered. For determination of the pEC₅₀ and apparent pA₂, the concentrations producing the half-maximum effect in the individual tissues were used.

Results are given as means ± s.e.mean of *n* experiments (functional experiments) or of *n* experiments in triplicate (binding experiments). In order to evaluate whether the displacement of [³H]-N^α-methylhistamine binding by the drugs under study is better fitted by a one- or two-site model, the *F*-test was used (partial *F*-test; de Lean *et al.*, 1982). The pK_i and the pA₂ values of the test compounds were compared by linear regression analysis; the Bonferroni correction (Holm, 1979) was used since the same data were subjected repeatedly to the regression analysis.

Drugs

The following were used: N^α-[methyl-³H]-methylhistamine dihydrochloride, (−)-[ring-2,5,6-³H]-noradrenaline base (NEN, Dreieich, Germany); atropine sulphate, histamine dihydrochloride (Merck, Darmstadt, Germany); desipramine hydrochloride (CIBA-Geigy, Wehr, Germany); 2,5-diphenyloxazole (PPO), 1,4-di-[2-(5-phenyloxazolyl)]-benzene (POPOP; Zinser, Frankfurt, Germany); imetit, mepyramine, R-(−)-α-methylhistamine dihydrogen maleate, N^α-methylhistamine dihydrochloride and the test compounds (for chemical structures, see Table 1) numbers 3, 4, 8 and 9 (free bases), number 1 (dihydrochloride) and numbers 2, 5, 6, 7 and 10 (maleates) were synthesized at the Institut für Pharmazie (Freie Universität, Berlin, Germany; the synthesis of the novel H₃ receptor antagonists will be described elsewhere); rauwolscine hydrochloride (Roth, Karlsruhe, Germany).

Results

Affinities of the test compounds for H₃ receptor binding sites

We have recently shown that [³H]-N^α-methylhistamine binds saturably to an apparently homogeneous class of receptors

Table 1 Chemical structures, affinities for H₃ binding sites and antagonistic potencies in two functional H₃ receptor models of ten newly synthesized compounds and of thioperamide

Compound	n	Chemical structure				Binding experiments	Functional experiments	
		R ¹	R ²	R ³	R ⁴		Mouse brain cortex slices ^b	Guinea-pig ileum strips ^c

1	2	H	NH	NH	NH-CH ₂ -	8.68	9.20	8.81
2	2	H	0	0	(CH ₂) ₂ -	8.52	8.28	7.23
3	2	H	NH	N-C-OC(CH ₃) ₃	NH-CH ₂ -	7.98	8.67	7.38
4	3	H	NH	N-C-OC(CH ₃) ₃	NH-	7.86	7.28	6.93
5	2	CH ₃	NH	0	(CH ₂) ₂ -	7.76	7.98	7.27
6	2	H	NH	0	(CH ₂) ₂ -	7.66	7.47	6.81
7	2	H	NH	0	(CH ₂) ₂ -	7.63	7.18	7.21
8	2	H	NH	0	NH-	7.58	7.07	7.26
9	2	H	NH	S	(CH ₂) ₂ -	7.57	7.40	7.51
10	2	H	NH	0	(CH ₂) ₂ -	7.56	7.27	6.64
Thioperamide						8.31, 7.19 ^d	8.67 ^e	8.79

^apK_i values were determined from the concentration-response curves (shown in Figure 1) of the novel H₃ receptor antagonists for their inhibitory effect on [³H]-N^α-methylhistamine binding to rat brain cortex membranes. Means of 4–8 experiments; the s.e.mean values (not shown) ranged from 0.02 to 0.10.

^bThe apparent pA₂ value of 1 was obtained from the concentration-response curves (shown in Figure 2) of histamine for its inhibitory effect on the electrically-evoked tritium overflow from superfused mouse brain cortex slices (preincubated with [³H]-noradrenaline). The apparent pA₂ values of the other newly synthesized compounds were obtained from corresponding concentration-response curves (not shown). Means of 4–11 experiments; the s.e.mean values (not shown) ranged from 0.06 to 0.26.

^cThe apparent pA₂ value of 1 was obtained from the concentration-response curves (shown in Figure 3) of R-(–)-α-methylhistamine for its inhibitory effect on the electrically induced contraction in guinea-pig ileum strips. The apparent pA₂ values of the other newly synthesized compounds and of thioperamide were obtained from corresponding concentration-response curves (not shown). Means of 8–20 experiments; the s.e.mean values (not shown) ranged from 0.04 to 0.16.

^dThe values, which represent the pK_i for H_{3A} and H_{3B} binding sites, respectively, are taken from West *et al.* (1990; almost identical binding assay as in the present study).

^eFrom Schlicker *et al.* (1992).

Figure 1 Inhibition of specific [³H]-N^α-methylhistamine binding to membranes from rat brain cortex by ten newly synthesized H₃ receptor antagonists. Means ± s.e.mean from 4–8 experiments (in triplicate) are shown (for some data points, s.e.mean is contained within the symbol): (□) 10; (■) 7; (Δ) 6; (▲) 2; (◇) 9; (◆) 5; (○) 1; (●) 3; (⊗) 4 and (⊗) 8. For details of compounds, see Table 1.

(n_H ~ 1) in rat brain cortex membranes, with a B_{max} of 98 ± 6 fmol mg⁻¹ protein and a K_D of 0.70 ± 0.03 nmol l⁻¹ (Kathmann *et al.*, 1993). The displacement of the specific binding of [³H]-N^α-methylhistamine 0.2 nmol l⁻¹ in this preparation by the ten newly synthesized compounds is shown in Figure 1. Analysis of the competition curves by computer programmes and subsequent statistical evaluation by the *F*-test showed that for 7 of the 10 compounds a one-site fit is better than a two-site fit in all individual experiments. With respect to 10, a two-site fit was better in 2 of 6 experiments, and with respect to 4 and 6 a two-site fit was better in one of 5 and 6 experiments, respectively. For the established H₃

receptor antagonist thioperamide, a two-site fit is consistently superior to a one-site fit (West *et al.*, 1990). The pK_i values are listed in Table 1.

Antagonistic potencies of the test compounds at H₃ receptors in the mouse brain cortex

Tritium overflow, evoked by the first period of electrical stimulation (S₁), was 6.89 ± 0.06% of tissue tritium in 33 controls; the S₂/S₁ value amounted to 1.09 ± 0.02. Histamine, which was present in the medium during S₂ only, inhibited the electrically evoked tritium overflow (S₂/S₁) in a concentration-dependent manner. The maximum inhibitory effect was about 70%; the concentration producing an inhibition of 35% (EC₅₀) was 0.13 μmol l⁻¹ (Figure 2). Compound 1, which was present in the medium throughout superfusion, shifted to the right the concentration-response curve of histamine for its inhibitory effect on the electrically evoked tritium overflow (Figure 2). Similar rightward shifts of the concentration-response curve of histamine were also obtained for the other test drugs (not shown). The apparent pA₂ values determined for the ten compounds are given in Table 1. The compounds by themselves did not affect the electrically evoked tritium overflow (S₁) (not shown).

Antagonistic potencies of the test compounds at H₃ receptors in the guinea-pig ileum

Electrical stimulation increased the tension in the guinea-pig ileum strips by 6.0 ± 0.09 mN (n = 125). The electrically induced contraction was abolished by atropine 1 μmol l⁻¹ (n = 8, not shown). R-αMH inhibited the electrically induced contraction in a concentration-dependent manner. The maximum effect was variable; the EC₅₀ (i.e. the concentration producing

the half-maximum inhibitory effect in the individual preparations) was 17 nmol l^{-1} (Figure 3). The effect of **R**- α MH was mimicked by imetit ($\text{EC}_{50} 7 \text{ nmol l}^{-1}$) and by N^{α} -methylhistamine ($\text{EC}_{50} 13 \text{ nmol l}^{-1}$; $n = 8$ each; results not shown).

The concentration-response curve of **R**- α MH was shifted to the right by **1** (Figure 3), by the other test compounds and by thioperamide (not shown). The apparent pA_2 values of the ten newly synthesized compounds and of thioperamide are given in Table 1. The novel H_3 receptor antagonists and thioperamide did not affect the electrically induced contraction by themselves (not shown).

Figure 2 Antagonism by **1** of the inhibitory effect of histamine on the electrically evoked tritium overflow from superfused mouse brain cortex slices preincubated with $[^3\text{H}]$ -noradrenaline. (○) Histamine; (●) plus compound **1** $0.01 \mu\text{mol l}^{-1}$. The medium contained desipramine $1 \mu\text{mol l}^{-1}$ plus rauwolfscine $1 \mu\text{mol l}^{-1}$ and, in some slices, **1** throughout superfusion and histamine from 62 min of superfusion onward. Tritium overflow was evoked twice after 40 and 90 min (S_1 , S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1). Tritium overflow is given as a percentage of the S_2/S_1 value in the corresponding controls (no histamine; not shown). Means $\pm$ s.e. mean of 4–9 experiments.

Figure 3 Antagonism by **1** of the inhibitory effect of **R**-(-)- α -methylhistamine (**R**- α MH) on the electrically induced contraction of guinea-pig ileum strips: (●) **R**- α MH; (○) plus compound **1** $0.01 \mu\text{mol l}^{-1}$. The preparations were exposed to medium containing mepyramine $1 \mu\text{mol l}^{-1}$ and were stimulated continuously. After construction of the first concentration-response curve to **R**- α MH, the strips were washed and then equilibrated with **1** for 20–30 min and a second concentration-response curve to **R**- α MH was generated. Values are expressed as percentages of the effect obtained with **R**- α MH $1 \mu\text{mol l}^{-1}$ (in the first concentration-response curve), which produces the maximum inhibitory effect ($75 \pm 8\%$). Means $\pm$ s.e. mean of 4 experiments. In each of the 4 tissues, a third concentration-response curve to **R**- α MH was constructed in the presence of **1** $0.1 \mu\text{mol l}^{-1}$ (not shown).

Correlations of the affinities and potencies of the test compounds and of thioperamide

The affinities (pK_i) of the newly synthesized H_3 receptor antagonists and of thioperamide for $[^3\text{H}]$ - N^{α} -methylhistamine binding and their potencies (apparent pA_2) in the two functional H_3 receptor models (mouse brain cortex; guinea-pig ileum) were compared by linear regression analysis (Figure 4 and 5). Thioperamide was included in the regression analysis since it is the reference H_3 receptor antagonist and, in addition, is capable of differentiating between H_{3A} and H_{3B} binding sites.

When the apparent pA_2 values of the eleven H_3 receptor antagonists in the mouse brain cortex model were compared to their pK_i values for H_3 binding sites and, in the case of thioperamide, to its pK_i for H_{3A} binding sites, a highly significant correlation was obtained. When, however, the pK_i of thioperamide for H_{3B} binding sites was used instead, the correlation was no longer significant (Figure 4a). Comparison of the apparent pA_2 values of the H_3 receptor antagonists in

Figure 4 Correlation of the affinities (pK_i) of ten newly synthesized compounds and of thioperamide (T), as measured by displacement of specific $[^3\text{H}]$ - N^{α} -methylhistamine binding, with their potencies (apparent pA_2), as determined by their abilities to antagonize (a) the effect of histamine on the electrically evoked tritium overflow from mouse brain cortex slices and (b) the effect of **R**-(-)- α -methylhistamine on the electrically induced contraction in guinea-pig ileum strips. S.e. means are shown; in some instances, s.e. mean is smaller than the symbol. The numbers of the compounds correspond to those given in Table 1. Data (taken from Table 1) were statistically analyzed by linear regression analysis. Two regression lines were calculated for the data points shown in either panel. Thus, the apparent pA_2 values of the 11 drugs were compared to their pK_i values for H_3 binding sites and to the pK_i value of thioperamide for H_{3A} sites (●) or its pK_i for H_{3B} sites (○). Only the regression lines for the first type of analysis are shown. In (a), the equations of the regression lines are: $y = 1.58x - 4.64$ (H_{3A}) and $y = 0.88x + 0.95$ (H_{3B}). In (b), the equations of the regression lines are: $y = 1.25x - 2.43$ (H_{3A}) and $y = 0.28x + 5.24$ (H_{3B}).

Figure 5 Correlation of the potencies (apparent pA_2) of ten newly synthesized compounds and of thioperamide (T), as determined by their ability to antagonize the effect of histamine on the electrically evoked tritium overflow from mouse brain cortex slices, with their potencies as determined by their ability to antagonize the effect of R-(α)-methylhistamine on the electrically induced contraction of guinea-pig ileum strips. Data (taken from Table 1) were statistically analyzed by linear regression analysis. S.e.means are shown; in some instances, s.e.mean is smaller than the symbol. The numbers of the compounds correspond to those given in Table 1. The equation of the regression line is: $y = 0.79x + 2.00$.

the guinea-pig ileum model with their pK_i for H₃ sites and the pK_i of thioperamide for H_{3A} sites also yielded a significant correlation. Again, replacement of the pK_i for H_{3A} sites by the pK_i for H_{3B} sites resulted in the loss of a significant correlation (Figure 4b). When the apparent pA_2 values in the two functional H₃ receptor models were compared, a significant correlation occurred (Figure 5).

Discussion

It was the aim of the present study, based on ten recently synthesized H₃ receptor ligands, to examine further the problem of H₃ receptor heterogeneity and to demonstrate structure-activity relationships for the novel compounds. We determined the affinities of the compounds for H₃ binding sites labelled by [³H]-N^m-methylhistamine in rat brain cortex membranes (according to West *et al.*, 1990, modified by Kathmann *et al.*, 1993). Furthermore, the potencies of the compounds for the H₃ receptor modulating noradrenaline release (an H_{3A} receptor according to the H₃ receptor subclassification postulated by West *et al.*, 1990) were determined in mouse brain cortex slices (according to Schlicker *et al.*, 1992). Finally, the potencies of the compounds were also determined in another functional H₃ receptor model, i.e. in the guinea-pig ileum (see Vollinga *et al.*, 1992, for references). Under the experimental conditions used in the present study (which were similar to those in the study of Vollinga *et al.*, 1992), the electrically induced contraction can be ascribed to the release of acetylcholine (since it was abolished by atropine). The inhibitory effect of R- α MH on the electrically induced contraction is mediated via H₃ receptors; thus, the H₃ receptor antagonist thioperamide (Arrang *et al.*, 1987) antagonized the effect of R- α MH with a pA_2 value of about 8.5. In addition, the recently described H₃ receptor agonist, imetit (Garbarg *et al.*, 1992; Howson *et al.*, 1992; van der Goot *et al.*, 1992; Kathmann *et al.*, 1993) potentially mimicked the effect of R- α MH.

Two approaches were chosen to obtain further evidence in favour of the heterogeneity of H₃ receptors. First, the effects of the novel H₃ receptor antagonists on the specific binding of [³H]-N^m-methylhistamine to rat brain cortex membranes were studied. The compounds, unlike thioperamide (West

et al., 1990) and burimamide (West *et al.*, 1990; Kathmann *et al.*, 1993), exhibited monophasic displacement curves although three compounds (4, 6, 10) produced a biphasic displacement in a minority of experiments. This might suggest that these compounds exhibit slightly different affinities for the H_{3A} and H_{3B} sites, which cannot be detected easily. The chemical structures of these compounds might represent a starting point for the synthesis of H₃ receptor antagonists differentiating more markedly between H_{3A} and H_{3B} sites.

Second, we determined the antagonistic potencies (expressed as apparent pA_2 values) of the novel compounds and of thioperamide in two functional H₃ receptor models which differ with respect to (i) the species (mouse vs. guinea-pig), (ii) the tissue (cerebral cortex vs. ileum) and (iii) the transmitter (noradrenaline vs. acetylcholine). It has been shown for other types of presynaptic receptors, including α_2 -adrenoceptors (see Trendelenburg *et al.*, 1993, for references) and 5-hydroxytryptamine receptors (see Hoyer & Middlemiss, 1989, for review), that their pharmacological properties strongly depend on these three parameters. However, the two functional H₃ receptors examined in the present study appear to be very similar, if not identical, as shown by the significant correlation between the antagonistic potencies of the eleven compounds. In addition, comparison of the apparent pA_2 values obtained in both models shows that the difference is lower than 0.9 log units for each compound if one takes into account that in the guinea-pig ileum the average level of pA_2 values was lower by 0.42 log units than in the mouse brain. Comparison of the potencies of the compounds with their affinities (expressed as pK_i) for H₃ binding sites yielded a significant correlation for both functional H₃ receptor models, if the pK_i value of thioperamide for H_{3A} sites was considered but not if its pK_i value for H_{3B} sites was used instead. Our data are compatible with the view that the H₃ receptor in the guinea-pig ileum, like that in the mouse brain cortex (Schlicker *et al.*, 1992), belongs to the H_{3A} subclass.

Considering the affinities and potencies of the compounds at the H₃ binding sites, the following structure-activity relationships may be deduced. (1) The amide 7, the corresponding thioamide 9 as well as the comparable urea derivative 8 do not differ in their affinity for H₃ receptors. (2) The guanidine 1 possesses the highest affinity of all of the test compounds, possibly due to the high basic capacity of this functional group and its ability to form hydrogen bonds. The guanidine moiety, which is protonated under physiological conditions, might interact with a carboxylate moiety of the H₃ receptor. An interaction between the $-NH_3^+$ of histamine and the carboxylate group of an aspartic acid residue has been postulated for H₁ and H₂ receptors (see Ganellin *et al.*, 1992, for references). Compared to 1, the affinity of the corresponding guanidine ester 3 is lower. The reason may be that the basic capacity of the guanidine moiety as well as its ability to form hydrogen bonds is attenuated by substitution with the *t*-butoxy-carbonyl residue, which exhibits an inductive effect (-I) although steric effects cannot be totally excluded. (3) Comparison of the different cyclic alkyl residues of compounds 6, 7 and 10 reveals almost the same affinities. Thus, steric effects do not appear to play a major role for the affinity for H₃ receptors. One might assume that, with respect to this part of the molecule, hydrophobic interactions are more important for the binding to the receptor than steric effects.

In conclusion, ten novel compounds, including amides, guanidine esters as well as a guanidine, an ester, a thioamide and an urea derivative, which have been synthesized according to the general construction pattern for H₃ receptor antagonists described by Lipp *et al.* (1992), exhibit intermediate to high affinities and potencies at H₃ receptors. The novel compounds, for which some structure-activity relationships could be derived, do not differentiate between H_{3A} and H_{3B} sites. The H₃ receptors in the two functional models, i.e. in the mouse brain cortex and in the guinea-pig ileum, closely resemble each other. The H₃ receptor in the guinea-pig ileum

(like that in the mouse brain cortex; Schlicker *et al.*, 1992) may belong to the H_{3A} subclass according to West *et al.* (1990).

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