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Histamine H_1 receptor antagonism by cetirizine in isolated guinea pig tissues: influence of receptor reserve and dissociation kinetics

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Abstract

We characterised histamine H_1 receptor antagonism by cetirizine and its enantiomers on isolated guinea pig ileum and trachea. Competitive or mixed (competitive and apparent noncompetitive) antagonism profiles were observed. The order of potency was: chlorpheniramine ≥ mepyramine>levocetirizine>cetirizine ≥ terfenadine>loratadine>dextrocetirizine. The inhibitory effects of cetirizine, levocetirizine, terfenadine and loratadine were slowly reversible compared to those of dextrocetirizine or mepyramine. Cetirizine and its enantiomers were inactive on L-type Ca^{2+} channels. Reduction of the histamine H_1 receptor reserve by dibenamine in the ileum (100-fold higher than in the trachea) showed a gradual change from the competitive profile of dextrocetirizine and mepyramine to a mixed profile. The present results show that cetirizine and levocetirizine are selective competitive but slowly reversible histamine H_1 receptor antagonists. Their mixed antagonism profile observed in the trachea can be explained by the small receptor reserve present in this tissue compared to the ileum and their very slow dissociation rate from the histamine H_1 receptor.

Keywords: Cetirizine; Histamine H_1 receptor antagonism; Receptor reserve; Dissociation kinetic; Ileum, guinea pig; Trachea, guinea pig

1. Introduction

Cetirizine has previously been described as a competitive histamine H_1 receptor antagonist on the guinea pig isolated ileum (Abe et al., 1994), but as a noncompetitive histamine H_1 receptor antagonist on the guinea pig isolated trachea (Kahler and Du Plooy, 1994) and bronchus (Advenier et al., 1991). The aim of the present study was to determine whether a difference in receptor reserve or dissociation rate from the receptor might explain the disparity observed between ileum and trachea responses to the histamine H_1 receptor antagonist effect of cetirizine. Because levocetirizine has recently been launched in Europe as an antihistamine under the commercial name of Xyzal™, the study was extended to the enantiomers of cetirizine in order to try to explain differences previously reported between the enantiomers (Moguilevsky et al., 1995). Reference histamine H_1 receptor antagonists used in these studies were chlorpheniramine, loratadine, mepyramine and terfenadine.

2. Materials and methods

2.1. Tissue preparation and measurement of mechanical activity

Rings of trachea or segments of ileum were prepared from male Dunkin–Hartley guinea pigs (250–450 g). Rings of aorta were prepared from male Wistar rats (300–500 g). Before dissection, the animals were sacrificed by stunning and exsanguination under guidelines approved by the UCB Pharma Ethical Committee. Tracheal preparations were mounted under a resting tension of 1 g in 20 ml jacketed organ baths containing modified Krebs' solution (112 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 1.0 mM KH_2PO_4, 1.4 mM $MgSO_4$, 2.5 mM $CaCl_2$, 11 mM glucose). Indomethacin (0.003 mM) and atropine (0.01 mM) were added in order to inhibit the development of spontaneous tone and the effect of epithelium derived relaxing factor, respectively.

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Ileum preparations were mounted under a resting tension of 1 g in 20 ml jacketed organ baths containing Tyrode solution (136.9 mM NaCl, 2.7 mM KCl, 11.9 mM $NaHCO_3$, 0.4 mM NaH_2PO_4, 1.1 mM $MgCl_2$, 1.8 mM $CaCl_2$ and 5.6 mM glucose). Aortae were mounted under a resting tension of 2 g in 20 ml jacketed organ baths containing Krebs' solution without indomethacin or atropine. Tissues were allowed to equilibrate for a period of 60 min. The bathing solution was maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. Isometric contractions were measured by a force-displacement transducer (HSE K30, Hugo Sachs Elektronik, Germany or IT1-25, EMKA Technologies, France) coupled to a computer system (IOX, EMKA Technologies) capable of automatic data acquisition and bath washout through electrovalves at defined times. Drugs were manually diluted and injected into the bath.

2.2. Determination of histamine H_1 receptor antagonism

At the end of the 60-min period of equilibration, the ileum was contracted three times with 1 μM histamine at 4-min intervals. After a 60-min period of incubation with the test drug, a noncumulative concentration–response curve to histamine was constructed in the absence or presence of the test drug. At the end of the 60-min period of equilibration, the trachea was contracted twice with 1 μM histamine at 30-min intervals. After a 60-min period of stabilisation, two cumulative concentration–response curves to histamine (10 nM to 1 mM) were successively constructed 90 min apart. The test drug or the solvent was incubated during 60 min prior to the second concentration–response to histamine. Some experiments with trachea were made in the presence of 1 μM thioperamide included in the Krebs' solution. Results were obtained from at least four individual experiments. Only one concentration of antagonist was tested on each tissue. Control tissues were treated with solvent only.

2.3. Determination of histamine H_1 receptor reserve

In order to determine the receptor reserve, the same protocol was used in the presence of dibenamine (3 μM) to irreversibly block the histamine H_1 receptors. The incubation time was adapted to reduce the maximal response by 25% as required by the method described by Furchgott (1966). This incubation was made before the concentration–response curve in experiments with the ileum or between the two concentration–response curves in experiments with tracheal tissue.

2.4. Determination of histamine H_1 receptor antagonism in the presence of a decreased receptor reserve

At the end of the 60-min period of equilibration, the ileum was contracted three times with 1 μM histamine at 4-min intervals. The ileum was then incubated with dibenamine (1 or 3 μM) for 15 min. After a 45-min washout period, the ileum was incubated for a 60-min period with the test drug and a noncumulative concentration–response curve to histamine was constructed in the absence or presence of the test drug. Results were obtained from at least four individual experiments. Only one concentration of antagonist was tested on each tissue. Control tissues were treated with solvent only.

2.5. Determination of the duration and the reversibility of the action

In order to estimate the duration of action and the reversibility of the action of the test drug, the ileum was contracted, after a 60-min period of stabilisation, six times with 1 μM histamine at 5-min intervals. The test drug was incubated for a period of 60 min. The ileum was then stimulated once with 1 μM histamine in the presence of the test drug after which the ileum was repetitively stimulated with 1 μM histamine every 5 min during a period of 175 min in the absence of the compound. After each stimulation, the ileum was washed with Tyrode solution. Results were obtained from at least four individual experiments.

2.6. Determination of Ca^{2+} channel antagonist properties

Rat aortic preparations were exposed to a solution containing a high concentration of potassium (KCl–Krebs' solution in which NaCl and KCl concentrations were 14 and 100 mM, respectively). Each preparation was allowed to contract to a steady-state response, after which the bathing solution was replaced by normal Krebs' solution. Each preparation was exposed three times to KCl–Krebs' solution at 30-min intervals. When the third contraction had stabilised, the test compound was added for a 120-min period. Only preparations in which the contractions were matched were used. Only one concentration of antagonist was tested on each tissue. Control tissues were treated with solvent only. The change in tension due to the test compound was expressed as a percentage of the induced relaxation of the initial contraction corrected by the relaxation observed with solvent in order to normalise the data.

2.7. Analysis of the results

Results are expressed as mean ± S.D. in the tables and mean ± S.E.M. in the graphs. Comparison between two means was made using Student's paired or unpaired *t*-test (Lambert, 1973). Comparison between several means was made using an *F*-test (variance analysis with one classification parameter) (Lambert, 1973). The concentration of histamine inducing half maximal response and the maximal response of each concentration–response curve was calculated by an iterative computer program (XLfit, ID Business Solutions, United Kingdom or Prism, GraphPad software, San Diego, USA) fitting the experimental data to a four

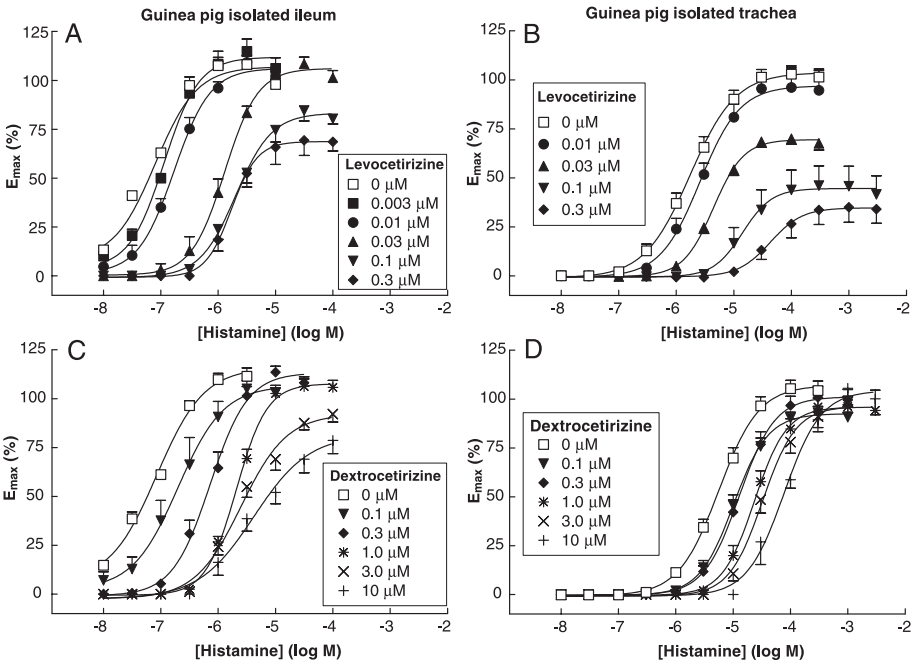

Fig. 1. Concentration–response curves to histamine elicited on the guinea pig isolated ileum (left panels A and C) or trachea (right panels B and D) in the absence or presence of various concentrations of levocetirizine (upper panels A and B) or dextrocetirizine (lower panels C and D). Symbol: control (□), 0.003 (■), 0.01 (●), 0.03 (▲), 0.1 (▼), 0.3 (♦), 1 (*), 3 (×) and 10 (+) μM. The ordinate is the maximal amplitude (E_{max}) of the contraction induced by histamine expressed as percentage of the maximal amplitude observed before the incubation with the compounds. The abscissa is the log molar concentration of histamine. Points represent mean values ($n = 5–10$). Vertical bars represent S.E.M. When S.E.M. bars are absent, the S.E.M. was smaller than the symbol. Solid lines represent the fitted curves (see Section 2).

parameter logistic equation: $Y = A+((B-A)/(1+(((10^{C})/(10^{X}))^{D})))$, where A = minimum Y, B = maximum Y, C = logarithm of the molar concentration of the agonist inducing 50% of B, D = slope factor, X = logarithm of the molar concentration of agonist and Y = observed effect. Other pharmacological parameters (pD_2, pD_2' and pA_2) were calculated as described by Van Rossum et al. (1963) or Arunlakshana and Schild (1959). pD_2 is defined as the negative logarithm of the molar concentration of agonist inducing 50% of the maximal effect induced by this agonist. pD_2' is defined as the negative logarithm of the molar concentration of antagonist that would produce a 50%

Table 1
pK_b, pA_2 and pD_2' values for various antagonists of histamine-induced contractions in guinea pig ileum or trachea

Tissue	Antagonist	pK_b (slope, n)	pA_2 (n)	pD_2' (n)
Ileum	Cetirizine	8.10 (1.05 ± 0.08, 24)	7.96 ± 0.38 (30)	5.52 ± 0.35 (12)
	Levocetirizine	8.40 (1.18 ± 0.12, 24)	8.29 ± 0.46 (30)	6.28 ± 0.24 (12)
	Dextrocetirizine	7.40 (0.93 ± 0.11, 24)	7.11 ± 0.45 (30)	4.44 ± 0.43 (11)
	Chlorpheniramine	– (0.83 ± 0.06, 30)[a]	9.38 ± 0.26 (30)	6.96 ± 0.24 (12)
	Loratadine	7.53 (1.13 ± 0.17, 28)	7.63 ± 0.48 (28)	5.84 ± 0.26 (11)
	Mepyramine	9.20 (0.92 ± 0.06, 20)	9.06 ± 0.19 (20)	–
	Terfenadine	8.13 (0.96 ± 0.09, 20)	8.09 ± 0.19 (20)	6.44 ± 0.45 (18)
Trachea	Cetirizine	7.31 (0.90 ± 0.14, 26)	7.25 ± 0.38 (26)	6.65 ± 0.32 (27)
	Levocetirizine	7.70 (1.25 ± 0.13, 17)	7.87 ± 0.23 (17)	7.03 ± 0.37 (17)
	Dextrocetirizine	– (0.76 ± 0.06, 28)[a]	6.39 ± 0.28 (28)	–
	Chlorpheniramine	– (0.77 ± 0.05, 18)[a]	8.75 ± 0.17 (18)	–
	Loratadine	7.29 (0.97 ± 0.09, 15)	7.28 ± 0.18 (15)	6.01 ± 0.52 (15)
	Mepyramine	– (0.85 ± 0.04, 24)[a]	8.54 ± 0.14 (24)	6.31 ± 0.60 (14)
	Terfenadine	– (0.72 ± 0.11, 16)[a]	7.22 ± 0.29 (16)	6.36 ± 0.48 (16)
Trachea[b]	Dextrocetirizine	– (0.78 ± 0.06, 18)[a]	6.71 ± 0.22 (18)	–
	Mepyramine	8.82 (0.89 ± 0.11, 24)	8.71 ± 0.30 (24)	6.75 ± 0.77 (17)

pK_b values are calculated according to Arunlakshana and Schild, and pA_2 or pD_2' values are calculated according to Van Rossum as described in Section 2. The results are given as means ± S.D. of n determinations.

[a] Schild slope different from 1.

[b] In the presence of 1 μM thioperamide.

reduction of the maximal effect induced by an agonist. pA_2 is defined as the negative logarithm of the molar concentration of antagonist that would produce a twofold shift to the right in the concentration–response curve for an agonist. The receptor reserve was determined as described by Furchgott (1966).

2.8. Drugs and chemicals

The following drugs were used: cetirizine (Zyrtec™, UCB Group, Brussels, Belgium), levocetirizine (Xyzal™, UCB Group) and dextrocetirizine were all synthesised at UCB Pharma Sector (Braine l'Alleud, Belgium). Chlorpheniramine, loratadine, mepyramine, terfenadine, histamine and dibenamine were purchased from Sigma-Aldrich (Bornem, Belgium). Stock solutions were made up either in distilled water or dimethylsulfoxide and subsequently diluted in distilled water. The solvent, even at the highest concentration present in the bath (0.1%), had no effect on muscle contraction.

3. Results

3.1. Histamine H_1 receptor antagonism in the guinea pig ileum and trachea

Histamine (Fig. 1) induced a concentration-dependent contraction of isolated guinea pig ileum and trachea with a pD_2 of 6.98 ± 0.33 (n = 34) and 5.71 ± 0.28 (n = 63), and a maximal effect of 4.44 ± 1.15 g (n = 34) and 3.65 ± 0.78 g (n = 63) for ileum and trachea, respectively. The effect of cetirizine and its two enantiomers on the contraction induced by histamine on isolated guinea pig ileum and trachea were tested in comparison with the effect induced by four reference compounds.

On the guinea pig ileum, mepyramine (3–300 nM), induced a shift to the right of the concentration–response curve to histamine without any decrease of the maximal amplitude. All other compounds induced a shift to the right followed at higher concentrations by a decrease of the maximal amplitude. The maximal decrease was about

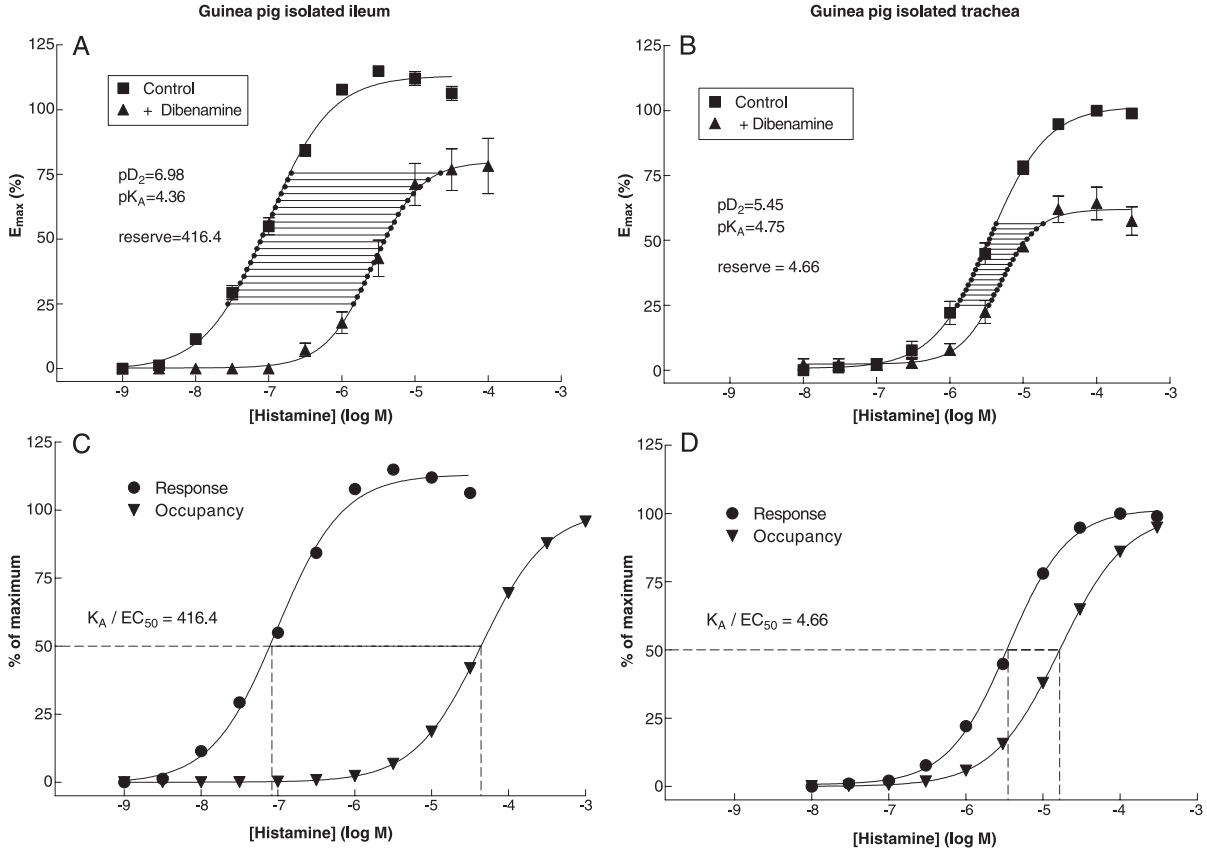

Fig. 2. Upper panels: concentration–response curves to histamine elicited on the guinea pig isolated ileum (panel A) or trachea (panel B) in the absence (■) or presence (▲) of 3 μM dibenamine (incubation period of 60 or 180 min for trachea or ileum, respectively, in order to obtain about 25% of inhibition). The ordinate is the maximal amplitude (E_{max}) of the contraction induced by histamine expressed as percentage of the maximal amplitude observed before the incubation with the compounds. The abscissa is the log molar concentration of histamine. Points represent mean values (n = 5–10). Vertical bars represent S.E.M. When S.E.M. bars are absent, the S.E.M. was smaller than the symbol. Solid lines represent the fitted curves (see Section 2). Lower panels: comparison in the ileum (panel C) and the trachea (panel D) of the response induced by histamine (●) related to receptor occupation (▼). The ordinate is the maximum expressed as percentage. The abscissa is the log molar concentration of histamine. Values are means of 5–10 experiments. Solid lines represent the fitted curves (see Section 2).

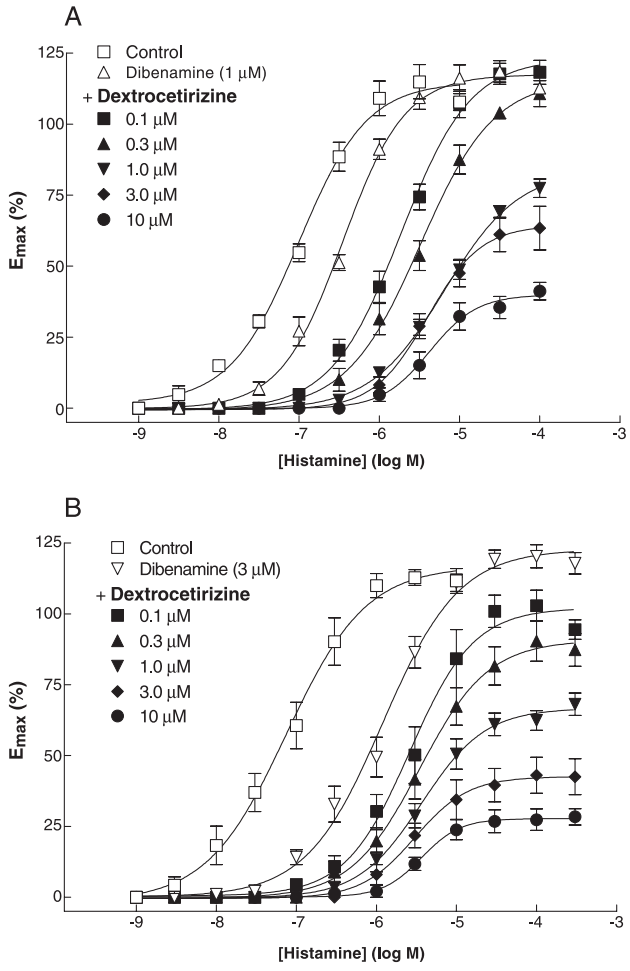

Fig. 3. Effect of dextrocetirizine [0.1 (■), 0.3 (▲), 1.0 (▼), 3.0 (♦) and 10 (●) μM] on guinea pig isolated ileum in the presence of a gradual decrease of the histamine H_1 receptor reserve due to the presence of 1 μM (△, panel A) or 3 μM (▽, panel B) dibenamine. Vehicle (DMSO 0.1%, □) was tested as control. The ordinate is the maximal amplitude (E_{max}) of the contraction induced by histamine expressed as percentage of the maximal amplitude observed before the incubation with the compounds. The abscissa is the log molar concentration of histamine. Points represent mean values ($n = 5–10$). Vertical bars represent S.E.M. When S.E.M. bars are absent, the S.E.M. was smaller than the symbol. Solid lines represent the fitted curves (see Section 2).

20% with 1 μM cetirizine, 0.3 μM levocetirizine, 100 μM dextrocetirizine and 0.1 μM chlorpheniramine, about 50% with 1 μM loratadine and about 80% with 1 μM terfenadine. The slope of the Schild analysis (taking into account only the concentrations inducing a shift without any decrease of the maximum) was not different from unity (Table 1), indicating the competitive nature (at least in part) existing between these compounds and histamine on the histamine H_1 receptors in the guinea pig ileum. pA_2 and/or pD_2' values calculated according to Van Rossum method are presented in Table 1. The rank order of potency of the compounds was: chlorpheniramine ≥ mepyramine>levocetirizine>cetirizine ≥ terfenadine> loratadine>dextrocetirizine.

On the guinea pig trachea, chlorpheniramine (up to 1–30 nM) and dextrocetirizine (0.1–10 μM) induced a shift to the right of the concentration–response curve to histamine without any decrease of the maximal amplitude. Cetirizine (0.03–1 μM), levocetirizine (0.01–1 μM), loratadine (0.03–1 μM), mepyramine (3–100 nM) and terfenadine (0.03–1 μM) induced both a shift to the right and a decrease of the maximal amplitude. The maximal amplitude of this decrease was about 20% with 0.1 μM mepyramine, about 40% with 1 μM loratadine and terfenadine and finally 70% with 1 μM cetirizine and 0.3 μM levocetirizine. The slope of the Schild analysis was less than unity except with cetirizine, levocetirizine and loratadine (Table 1). pA_2 and/or pD_2' values calculated according to the Van Rossum method are presented in Table 1. The rank order of potency was: chlorpheniramine ≥ mepyramine>levocetirizine>terfenadine ≥ cetirizine ≥ loratadine>dextrocetirizine. The slopes of the Schild plots (Table 1) were not changed when the experiments were done in the presence of 1 μM thioperamide.

The two enantiomers of cetirizine (Fig. 1) showed a similar antagonism profile on the guinea pig ileum but not in the trachea, where dextrocetirizine, unlike cetirizine and levocetirizine, did not decrease the maximal amplitude of the concentration–response curve to histamine. In terms of potency, levocetirizine was about 10–30-fold more potent than dextrocetirizine depending on the tissue investigated. All the tested compounds were more potent on the guinea pig ileum than on the guinea pig trachea.

3.2. Histamine H_1 receptor reserve determination in the guinea pig ileum and trachea

A 180-min period of incubation with 3 μM dibenamine (Fig. 2) induced both a shift to the right and a 25–30% inhibition of the maximal effect of the concentration–response induced by histamine on the isolated guinea pig ileum. The same effect (but with a smaller shift) was observed with an incubation period of 60 min on the isolated guinea pig trachea. Based on these curves, the histamine H_1 receptor reserve was calculated to be 100-fold

Table 2
pA_2 and pD_2' values for various antagonists of histamine-induced contractions in guinea pig ileum in the presence of dibenamine

Antagonist	Dibenamine concentration (μM)	pA_2 (n)	pD_2' (n)
Dextrocetirizine	1	6.97 ± 0.62 (24)	5.41 ± 0.31 (18)
Mepyramine	1	8.86 ± 0.34 (29)	6.59 ± 0.50 (18)
Cetirizine	3	6.93 ± 0.85 (29)	6.75 ± 0.28 (25)
Levocetirizine	3	7.43 ± 0.61 (28)	7.05 ± 0.34 (25)
Dextrocetirizine	3	6.18 ± 0.78 (23)	5.87 ± 0.41 (23)
Mepyramine	3	8.10 ± 0.77 (30)	7.30 ± 0.54 (30)

pA_2 or pD_2' values are calculated according to Van Rossum as described in Section 2. The results are given as means ± S.D. of n determinations.

greater for the ileum than the trachea (416.5 vs. 4.7). A full histamine response can be obtained with an occupation of only 10% of the receptors on the ileum, whereas 90–100% of the receptors must be occupied for the same response on the trachea.

3.3. Histamine H_1 receptor antagonism on the guinea pig ileum in the presence of dibenamine

When the receptor reserve was progressively decreased in the guinea pig ileum by the presence of increasing concentrations of dibenamine, the insurmountable part of the antagonism observed with dextrocetirizine (Fig. 3) was progressively increased. The same behaviour was observed with mepyramine, cetirizine and levocetirizine. pA_2 and pD_2' values calculated according to Van Rossum are reported in Table 2. The ranking of the compounds according to potency (Table 2) did not change.

3.4. Duration of action of histamine H_1 receptor antagonism on the guinea pig ileum

Repetitive stimulation of the guinea pig ileum with 1 μM histamine every 5 min induced reproducible contractions for a period of at least 170 min. An inhibition of 75% of this contraction was obtained after 60 min of incubation with 0.3 μM levocetirizine. This inhibition (Fig. 4) was very slowly reversed (after 170 min, the inhibition was still 60%). The same effect was observed with cetirizine and terfenadine while the inhibition induced by dextrocetirizine, chlorpheniramine, mepyramine and loratadine was quickly reversed (half recovery time about 11 min).

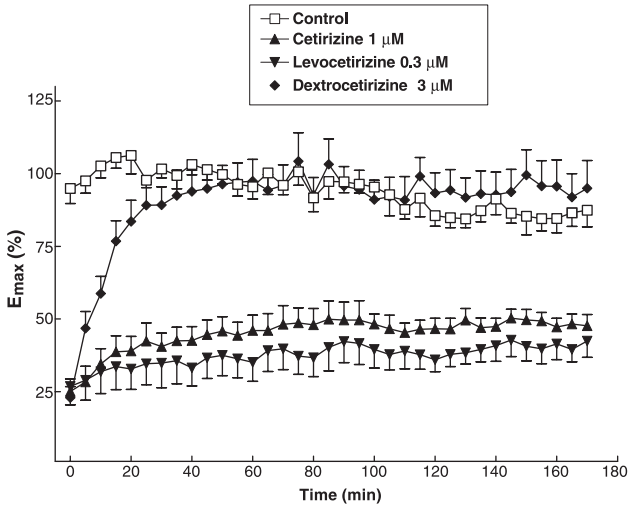

Fig. 4. Reversibility of the inhibitory effects induced after 60 min of incubation with cetirizine (1 μM, ▲), levocetirizine (0.3 μM, ▼) or dextrocetirizine (3 μM, ♦) on repetitive histamine-induced contractions of guinea pig isolated ileum. The ileum was contracted each 5 min by 1 μM histamine with intermediate wash after each contraction. The contraction was induced in the presence (first contraction at time 0 min) or in the absence (all the other contractions) of the test compound in the bath. Vehicle (DMSO 0.1%, □) was tested as control. The ordinate is the maximal amplitude (E_{max}) of the contraction induced by 1 μM histamine expressed as percentage of the maximal amplitude observed before the incubation with the compounds. The abscissa is the time expressed as min. Points represent mean values ($n=8$). Vertical bars represent S.E.M.

3.5. Determination of Ca^{2+} channel antagonism

The contraction induced by depolarisation of the rat aorta was not inhibited by cetirizine at concentrations as high as 10 μM. The same effect was observed with levocetirizine, dextrocetirizine or chlorpheniramine. Loratadine and specially terfenadine induced a consistent inhibition of the contraction induced by depolarisation of the rat aorta [apparent pD_2' of 4.43 ± 0.27 ($n=6$) and 5.74 ± 0.39 ($n=11$) for loratadine and terfenadine, respectively]. Nevertheless, terfenadine is 2.5–10-fold less potent than diltiazem or verapamil [apparent pD_2' of 6.14 ± 0.24 ($n=4$) and 6.80 ± 0.59 ($n=9$), respectively].

4. Discussion

This work demonstrates that histamine induces a maximal response of the guinea pig isolated trachea only when almost all the H_1 receptors are occupied. In contrast, histamine induced a maximal response of the guinea pig isolated ileum when only a small number of H_1 receptors occupied. As the maximal amplitude of a concentration–response curve depends on changes in the receptor reserve and in the efficiency of the coupling (Kenakin, 1999), this difference in the histamine H_1 receptor reserve between the two tissues should be taken into account in the interpretation of the effects induced by various H_1 receptor antagonists.

This work also demonstrates that cetirizine and levocetirizine antagonise histamine-induced contractions of the isolated guinea pig ileum and trachea. They induced a shift to the right of the concentration–response curve to histamine and at high concentrations provoked a decrease of the maximal amplitude (greater in the trachea), suggesting a mixed antagonistic profile (surmountable and insurmountable). In contrast, no decrease of the maximum response to histamine was observed with the distomer dextrocetirizine, mepyramine and chlorpheniramine in the isolated guinea pig trachea. The decrease of the maximum response to histamine was higher on the trachea than on the ileum with cetirizine and levocetirizine and lower with loratadine and terfenadine. As observed with all other histamine H_1 receptor antagonists tested, cetirizine and its enantiomers were more potent on the ileum than on the trachea. The enantiomeres displayed stereoselectivity on both tissues with levocetirizine being 30-fold more potent than dextrocetirizine.

Typical reversible competitive antagonism implies no reduction of the maximal amplitude of the concentration–response curve and an experimental Schild regression slope of unity. On the isolated trachea, cetirizine and levocetirizine decreased the maximal response to histamine by up to

70%, and the experimental Schild regression slope of dextrocetirizine was less than unity. A decrease of the maximal amplitude of the concentration–response curve or noncompliance of the experimental Schild regression slope to unity has several explanations (Kenakin, 1997): (i) the antagonism is not competitive, (ii) a drug-disposition mechanism or other nonequilibrium steady state obscures the competitive nature of the antagonism, (iii) the competitive antagonism of a heterogeneous receptor population subserving the same response is observed or (iv) multiple drug properties are expressed over the concentration range used to make the measurements. The first alternative is usually considered only after elimination of the other three because they can obscure true competitive antagonism to the point that it resembles true noncompetitive antagonism.

Explanation one: a drug-disposition mechanism or other nonequilibrium steady state obscures the competitive nature of the antagonism. In binding experiments, an incubation time of 60 min at 37 °C is sufficient to reach 85% of the equilibrium binding with the lowest concentrations of levocetirizine (10 nM). At higher concentrations, full equilibrium binding occurs within 60 min (Gillard et al., 2002). It can, thus, be assumed that under these experimental conditions, the compounds were tested close to equilibrium. Antagonists with slow dissociation kinetics (relative to agonist dissociation kinetics) can cause a limited shift of agonist concentration–response curves accompanied by reductions in maximal responses (Kenakin, 1984, 1997). Our results showed a slower histamine response recovery rate for cetirizine and levocetirizine compared to dextrocetirizine. This is in line with the kinetic binding data reported by Gillard et al. (2002) showing that levocetirizine has a much slower dissociation rate than dextrocetirizine ($t_{1/2}$ of 142 and 6 min, respectively). This might explain why a decrease of the maximal response was observed in the presence of cetirizine or levocetirizine but not in the presence of dextrocetirizine, mepyramine or chlorpheniramine (Dobashi et al., 1996, and the present observations). A decrease of the maximal response was also observed in the presence of loratadine or terfenadine which are characterised (Gillard et al., 2002) by dissociation kinetic similar or slightly higher to that of dextrocetirizine ($t_{1/2}$ of 5 and 37 min, respectively). In addition, high concentrations of an antagonist characterised by slow dissociation constant kinetics would be expected to reduce the number of spare receptors available to histamine. The fact that guinea pig trachea has a receptor reserve 100 times smaller than guinea pig ileum might therefore explain the greater decrease in the maximal amplitude observed in the trachea compared to ileum. The importance of the receptor reserve was also demonstrated by the fact that when the receptor reserve is reduced by dibenamine, the decrease of the maximal effect of the histamine response in the isolated guinea pig ileum observed in the presence of an antagonist such as dextrocetirizine is observed at lower concentrations.

Explanation two: the competitive antagonism of a heterogeneous receptor population subserving the same response is observed. Histamine may bind to various subtypes of histamine receptor inducing tracheal smooth muscle contraction or relaxation through various mechanisms. Besides activating histamine H_1 receptors, histamine also binds to and activates histamine H_2 or H_3 receptors (see Chand and Sofia, 1995 for review). Although histamine H_2 receptors have been demonstrated in lung parenchyma and airways, their role is unclear and cimetidine does not affect the histamine-induced contraction of the trachea (Cardell and Edvinsson, 1994). The histamine H_1 and H_3 receptors located on the epithelium mediate via the release of epithelium derived relaxing factor or epithelium derived contracting factor, relaxation or contraction of the tracheal smooth muscle. Finally, the histamine H_1 and H_3 receptors located on the vagal nerve terminals and/or on sensory c-fibres can contract or relax tracheal smooth muscle by increasing or decreasing release of acetylcholine or substance P. In binding studies, the affinity of cetirizine is at least 500 times in favour of histamine H_1 receptors vs. histamine H_2 or H_3 receptors (Gillard et al., 2002). Cardell and Edvinsson (1994) reported that mepyramine competitively antagonised histamine-induced contractions of isolated guinea pig trachea and that the Schild regression slope changed from 0.61 to 1.05 in the absence or the presence of thioperamide (histamine H_3 receptor antagonist), respectively. Such a change of slope was not observed with dextrocetirizine under our experimental conditions (the slope changes from 0.76 to 0.79 in the absence or the presence of thioperamide). This could be related to the difference in the slower dissociation rate of dextrocetirizine ($t_{1/2}$ of 6 min) compared to that of mepyramine ($t_{1/2}$ of 0.7 min) at the histamine H_1 receptor observed in binding studies (Gillard et al., 2002).

Explanation three: multiple drug properties are expressed in the concentrations used to make the measurement. This study failed to show any relaxation of isolated rat aorta contracted by 100 mM KCl in the presence of cetirizine or its enantiomers (10 μM), suggesting that these compounds do not interact with L-type Ca^{2+} channels unlike other antihistamines tested such as loratadine or terfenadine. Histamine contraction of the ileum is more sensitive to Ca^{2+}-antagonists such as verapamil than that of trachea (Ali et al.,1988; Valcheva and Belcheva, 1994). This observation might explain why loratadine and terfenadine induced a decrease of the histamine maximal response similar or higher in the guinea pig ileum than in the trachea. In contrast to loratadine or desloratadine (Cardelus et al., 1999; Gillard et al., 2003), no muscarinic antagonist effect (Gillard et al., 2003) was observed with cetirizine or its enantiomers on the guinea pig trachea stimulated by carbachol (muscarinic M_3 receptor assay) and the paced isolated guinea pig left atrium (muscarinic M_2 receptor assay). No effect (data not shown) was observed with cetirizine or its enantiomers on the rat myenteric plexus stimulated by [Des-

Arg[9]]-bradykinin (bradykinin B_1 receptor assay) or the guinea pig ileum stimulated by bradykinin (bradykinin B_2 receptor assay). Moreover, cetirizine and its enantiomers showed at least 500 times higher binding affinity for histamine H_1 receptors compared to a variety of other G-protein coupled receptors including histamine H_2 and H_3 subtypes, α_1-, α_2- and β-adrenoceptors, muscarinic receptors, dopamine D_2 receptors and 5-HT_{1A} and 5-HT_2 serotoninergic receptors (Gillard et al., 2002). The antagonist profile of cetirizine and its enantiomers is, therefore, very selective for histamine H_1 receptors.

Our data demonstrate that the influence of the degree of the histamine H_1 receptor reserve present in guinea pig ileum or trachea, and the effect of dissociation kinetics of cetirizine and its enantiomers from the histamine H_1 receptor can fully explain the distinct families of concentration–response curves observed with cetirizine and its enantiomers in these two tissues. These data are further evidence that cetirizine and levocetirizine are potent histamine H_1 receptor competitive antagonists with slow dissociation rates from the histamine H_1 receptor. Although possible, postulating a noncompetitive or an allosteric binding of cetirizine or levocetirizine to a site different from the histamine H_1 receptor is not required in order to explain the different pattern of antagonistic behaviour observed in various tissues (ileum, trachea) with these histamine H_1 receptor antagonists.

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