Characterization of the histamine receptors in the guinea-pig lung: evidence for relaxant histamine H₃ receptors in the trachea

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1 The histamine receptors were characterized on isolated circular segments of trachea and pulmonary arteries from the guinea-pig. The motor responses to histamine H₁-, H₂- and H₃-receptor agonists and antagonists were tested and the responses obtained were analysed in relation to the respiratory epithelium and the vascular endothelium.

2 Histamine induced a biphasic response in trachea and in pulmonary arteries. In low concentrations, histamine acted as a potent relaxant agent of precontracted segments and in moderate concentrations it constricted both precontracted and resting segments. When arterial segments from different parts of the pulmonary vascular tree were compared, only small interregional differences in the vasomotor response were seen.

3 Mepyramine caused a parallel shift to the right of the histamine-induced concentration-response curves for both the trachea and the pulmonary artery, indicating a contractile H₁-receptor. Cimetidine did not affect the histamine-induced contraction of the trachea, but a shift to the left was evident for low concentrations of histamine in the pulmonary artery. This is consistent with a dilator H₂-receptor in the pulmonary artery. The pA₂-value for mepyramine in the pulmonary artery, 8.75, was not affected by the presence of cimetidine. Thioperamide, a selective H₃ antagonist, shifted the concentration-response for the trachea to the left. Schild analysis for histamine and mepyramine yielded a line with a slope of 0.61, whereas the same analysis in the presence of thioperamide yielded a line with a slope of 1.05 and an approximated pA₂-value of 9.57. These results indicate the presence of a relaxant H₃ receptor in the trachea. In precontracted tracheal segments, application of mepyramine and cimetidine did not affect the low dose histamine relaxation. Thioperamide caused a parallel shift of the histamine concentration-response curve to the right, supporting the suggestion of a dilator H₃-receptor in the trachea. The pA₂-value for thioperamide, in the presence of mepyramine, was 7.79. In precontracted pulmonary arteries the histamine-induced dilatation was small. In the presence of mepyramine a rather strong histamine-induced dilatation became evident and this concentration-response curve could be shifted to the right by cimetidine, with a pA₂-value of 6.49. This is compatible with a dilator H₂-receptor.

4 The H₁-receptor agonists, thiazolyethylamine, 2-methylhistamine and pyridylethylamine and the rather unselective H₂-agonist, 4-methylhistamine, induced contraction of resting tracheal and pulmonary arterial segments. In precontracted segments of trachea, all H₁ and H₂ agonists studied induced a dilator response. The two rather unselective histamine receptor agonists 2-methylhistamine and 4-methylhistamine were about 100 times more potent than other H₁ and H₂ agonists tested. In the pulmonary artery, the H₂ agonists, impromidine, dimaprit and 4-methylhistamine induced a concentration-dependent relaxation. The relaxation of the pulmonary artery, elicited by the H₁ agonists, thiazolyethylamine and pyridylethylamine, was smaller, but more potent than the response induced by the H₂ agonists. This may reflect the presence of a separate dilator H₁-receptor.

5 R- α -methylhistamine induced a three phased response in precontracted tracheal segments. In low concentrations, a concentration-dependent dilator response appeared. At moderate concentrations, a stage with a plateau or a small contraction was seen, followed at high concentrations by a new concentration-dependent relaxation. The first dilator phase was similar to that obtained for histamine in the same preparation.

6 Removal of the epithelium or endothelium enhanced the contractile histamine response in both the trachea and the pulmonary artery as well as the dilator response in the trachea. These results support the hypothesis that the endothelial layer may serve as a barrier against the penetration of certain mediators. In the precontracted pulmonary artery, the small initial dilatation was abolished whereas the second dilatation seen in the presence of mepyramine was slightly reduced. This may reflect the influence of two separate histamine receptors, one of which is associated with the release of an endothelium-dependent dilator factor or factors.

7 In the guinea-pig trachea, histamine-induced contraction is mediated through H₁-receptors whereas dilatation probably involves an H₃-receptor on the smooth muscle. The guinea-pig pulmonary artery appears to be endowed with a contractile H₁ receptor on the smooth muscle cells and a dilator H₁ receptor located on the endothelium. A dilator H₂ receptor on the smooth muscle cells seems, at least *in vitro*, to be the most potent mediator of histamine-mediated pulmonary arterial dilatation.

Keywords: Histamine; histamine receptors; R- α -methylhistamine; H₃-receptors; pulmonary blood vessels; trachea

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Introduction

Histamine has long been recognized as an important chemical messenger (Hill, 1990; Schwartz *et al.*, 1990). It is distributed within mast cells in almost all mammalian tissues and has an established role as a mediator of inflammation and allergy (Bevan, 1982). Histamine can probably also function as a neurotransmitter or neuromodulator (Hill, 1990; Schwartz *et al.*, 1990; Ishikawa & Sperelakis, 1987). In the lung, histamine is one among several autacoids that possesses potent physiological properties. Immunological reactions can stimulate histamine release within the lung from mast cells and basophils (Riley & West, 1953; Lichtenstein, 1975), inducing bronchoconstriction, contraction of vascular smooth muscle and increased vascular permeability (Adams & Lichtenstein, 1979; Valentovic *et al.*, 1992).

Three classes of histamine receptors, H_1 , H_2 and H_3 , have been identified in vertebrates (Ash & Schild, 1966; Black *et al.*, 1972; Arrang *et al.*, 1983). H_1 receptors are coupled to inositol phosphate hydrolysis and mediate a variety of responses, including smooth muscle contraction, increased vascular permeability and hormone release (Hill, 1990). H_2 receptors are coupled to adenylate cyclase via a G_s -protein and the functional H_2 -responses include smooth muscle relaxation and inhibition of the immune system (Foreman *et al.*, 1985). H_3 -receptors were originally recognized in rat brain where they appeared to be involved in the feedback control of both histamine synthesis and release (Arrang *et al.*, 1987). Binding studies suggested that H_3 -receptors also existed in guinea-pig lung (Arrang *et al.*, 1987).

Histamine causes bronchoconstriction through stimulation of H_1 -receptors. Although H_2 - and H_3 -receptors have been demonstrated in lung parenchyma and airways, their role is unclear (Barnes & Ichinose, 1989). In isolated blood vessels, vasoconstriction is usually mediated through H_1 -receptors located on the vascular smooth muscle, whereas vasodilatation can involve the action of H_1 and/or H_2 -receptors (Edvinsson & Owman, 1975; Ottosson *et al.*, 1989; Krstic *et al.*, 1991). In certain smooth muscle preparations, one and the same concentration of exogenously applied histamine, may activate both constrictor and dilator mechanisms through different histamine receptors at separate locations. A histamine H_1 -induced contraction could therefore theoretically mask a dilator H_1 -, H_2 - or H_3 -receptor-mediated response (Mikkelsen *et al.*, 1984). These technical obstacles together with the lack of highly selective H_1 agonists (Hill, 1990) have probably contributed to the difficulties in obtaining a clearcut picture of the histamine receptor-mediated dilator responses.

H_3 -receptors seem to modulate cholinergic neurotransmission in guinea-pig trachea acting at both parasympathetic ganglia and at postganglionic nerve fibres (Ichinose *et al.*, 1989). Furthermore, these receptors also inhibit non-adrenergic, non-cholinergic bronchoconstriction (Ichinose & Barnes, 1989) and plasma extravasation (Ichinose *et al.*, 1990). In cerebral arteries, exogenous histamine has also been reported to activate H_3 -receptors causing relaxation (Ea Kim & Oudart, 1988; Ea Kim *et al.*, 1992).

The present study was designed to characterize the H_1 -, H_2 - and H_3 -receptors in guinea-pig trachea and pulmonary arteries and to study the involvement of the respiratory epithelium and vascular endothelium, respectively, in the motor responses elicited.

Methods

Young male guinea-pigs (200–300 g) were killed by a blow on the neck. The lungs, including the heart and trachea, were quickly removed and immersed in a cold ($+4^\circ\text{C}$) buffer solution (for composition, see below). An arterial branch system was isolated from either side of the lung. Circular segments were dissected at four different levels; main pul-

monary artery (MPA, located next to the heart), large lobar arteries (second to third branches), small lobar arteries (SLA, fourth to fifth branches) and intrasegmental arteries (ISA, ninth to tenth branches). In addition, the distal portion of the trachea was dissected free of surrounding tissue. The vessels and the trachea were used in the experiments either immediately or, occasionally, following overnight storage in a cold buffer solution. Circular segments were mounted on two L-shaped metal prongs. One prong was connected to a force displacement transducer attached to a computer for continuous registration of isometric tension and the other to a displacement device. The mounted segments were immersed in small (2.5 ml) temperature-controlled (37°C) tissue baths containing the buffer solution. The solution was equilibrated with 5% CO_2 in O_2 , giving a pH of 7.4.

Initially, a tension of 0.2–3 mN (depending on the dissecting level/diameter) was applied to the arterial segments and 2–3 mN was applied to the tracheal segments (Cardell *et al.*, 1990). The segments were subsequently allowed to stabilize at this level of tension for 90 min. The contractile ability of each segment was then examined by exposure to a potassium rich (60 mM) buffer solution (for composition, see below). Only when two reproducible contractions had been elicited was the individual segment used in further studies. The integrity of the vascular endothelium was assessed at the end of the experiments by obtaining a dilator response to acetylcholine (10^{-6} M) (Furchgott, 1984). The presence of tracheal epithelium was confirmed by staining with a 5% silver nitrate solution followed by light microscopy (Abrol *et al.*, 1984). There were no differences in the response to the histamine agonists when concentration-response curves obtained by cumulative application were compared to those obtained by a single dose procedure (Cardell *et al.*, 1990).

In a separate study, the vascular endothelium was removed by rubbing the intimal surface with a small wooden stick inserted via one cut end of the pulmonary artery. The tracheal epithelium was removed according to the same procedure (Goldie *et al.*, 1986). Verification of the absence of endothelium or epithelium was always checked by the use of silver nitrate and acetylcholine (see above). The responses to potassium and to the maximally effective concentration of histamine were completely reversible. Studies of responses in the absence and presence of antagonists could consequently be carried out by successive cycles of agonist exposures in each segment. The rubbing experiments were carried out in matched pairs of segments, with one segment in each series having an intact endothelium or epithelium. The log concentration-response relationship was approximated by linear regression analysis of the data within the 20% to 80% interval (Cardell *et al.*, 1993). The pD_2 value (i.e. the negative logarithm of the concentration eliciting half the maximum response, $\text{EC}_{50}/\text{IC}_{50}$) and $\text{E}_{\text{max}}\%/I_{\text{max}}\%$ (the maximal contraction/dilatation elicited by an agonist expressed as a percentage of the contraction induced by 60 mM K^+ /agonist precontraction) was calculated for each experiment. The concentration ratios (CR) were defined as the ratio of the EC_{50} value in the presence and absence of a given concentration of agonist (B). The pA_2 was calculated as described by Arunlakshana & Schild (1959) and modified by Tallarida *et al.* (1979); $\log (\text{CR}-1)/\text{B}$.

Solutions and drugs

The following solutions were used: (a) standard buffer solution (mM): NaCl 119, KCl 4.6, CaCl_2 1.5, MgCl_2 1.2, NaHCO_3 15, NaH_2PO_4 1.2 and glucose 11; (b) 60 mM K^+ buffer solution: as above, but substituting equimolar amounts of NaCl with KCl. Analytical-grade chemicals and twice-distilled water were used for preparing all solutions.

The following drugs were used: R- α -methylhistamine (kindly donated by Dr J.C. Schwarz, Unité de Neurobiologie

et Pharmacologie de l'INSERM, Centre Paul Broca, Paris, France), 2-pyridylethylamine, 2-methylhistamine, 2-thiazolylethylamine, dimaprit, 4-methylhistamine, impromidine (kind gifts from the Smith, Kline & French Laboratories, Welwyn Garden City, U.K.), acetylcholine chloride (Sigma, U.S.A.), atropine sulphate (Sigma, U.S.A.), cimetidine hydrochloride (S.K.F., U.K.), 5-hydroxytryptamine creatinine sulphate (Serva, Germany), ketanserine hydrochloride (Janssen, Belgium), mepyramine (ACO, Sweden), noradrenaline hydrochloride (Sigma, U.S.A.), prazosin hydrochloride (Pfizer, U.S.A.), propranolol (ICI, U.K.), prostaglandin F_{2α} (Astra, Sweden), spantide (Ferring AB, Sweden), thioperamide (Sigma, U.S.A.), vasoactive intestinal peptide (Peninsula, U.S.A.), yohimbine (Serva, Germany). All agents were dissolved in, and further diluted in, saline containing 1% bovine serum albumin (Behringwerke, Marburg, Germany) and used in the experiments within 30 min to avoid any possible degradation. The concentrations of the agents are expressed as the final molar concentration in the tissue bath.

Statistics

Statistical differences between means were tested by analysis of variance (ANOVA) followed by Scheffe's method (Wallenstein *et al.*, 1980). Statistical significance was assumed when $P < 0.05$.

Results

Histamine responses and regional differences

Histamine elicited a strong concentration-dependent contraction of tracheal segments ($E_{\max}$ %, 116 ± 31 ; pD_2 , 5.03 ± 0.44 ; $n = 24$) and of pulmonary arteries ($E_{\max}$ %, 116 ± 31 ; pD_2 , 5.31 ± 0.28 ; $n = 22$) (Figure 1a,b). The relaxant effects of histamine were studied after precontraction with either prostaglandin F_{2α}, 5-hydroxytryptamine or acetylcholine (trachea). Low concentrations of histamine induced a concentration-dependent dilatation of the precontracted trachea ($I_{\max}$ %, 27 ± 17 ; pD_2 , 7.20 ± 0.61 ; $n = 25$) followed by a contraction at higher concentrations (Figure 2). In the precontracted pulmonary artery only a small histamine-induced dilatation was seen ($I_{\max}$ %, 9 ± 3 ; $n = 6$). The presence of mepyramine (10^{-6} M), unmasked a strong concentration-dependent histamine-induced arterial dilatation ($I_{\max}$ %, 69 ± 13 ; pD_2 , 6.02 ± 0.16 ; $n = 5$) (Figure 3) and increased the tracheal dilatation ($I_{\max}$ %, 56 ± 11 ; pD_2 , 7.23 ± 0.25 ; $n = 6$). No differences in the histamine-induced dilator responses were seen between the different precontracting agents.

In order to investigate regional variations in the histamine responses of the pulmonary vascular tree, arteries from four different levels (MPA, LLA, SLA, ISA) were compared. Only

Figure 1 Concentration-response of histamine in guinea-pig trachea (a) and pulmonary artery (LLA) (b). Contractile effects of histamine are indicated by (●). Mepyramine 1×10^{-8} M (pD_2 ; LLA, 4.26 ± 0.29 ; ▽), 1×10^{-7} M (pD_2 ; trachea, 4.02 ± 0.23 ; LLA, 3.42 ± 0.22 ; △) and 1×10^{-6} M (pD_2 ; trachea, 3.78 ± 0.09 ; ○) induced a parallel shift to the right of the histamine-induced concentration-response curves for both the trachea and the pulmonary artery. Cimetidine 1×10^{-5} M (□) caused a slight shift to the left of the histamine curve in the LLA (pD_2 , 5.46 ± 0.37 ; $n = 11$). Thioperamide 1×10^{-6} M (◆) shifted the histamine curve for the trachea to the left and the rightward shift induced by mepyramine was more pronounced in the presence of thioperamide (mepyramine; 1×10^{-8} M (▼), 1×10^{-7} M (▲) and 1×10^{-6} M (■)). Responses are expressed as a percentage of potassium (60 mM)-induced contraction (K^+) and each point is the mean with s.d. Trachea, control; K^+ = 3.37 ± 2.89 mN; $n = 24$; antagonist experiments $n = 5-6$. Pulmonary artery, control; K^+ = 3.61 ± 2.22 mN; $n = 22$; antagonist experiments $n = 8-12$.

Figure 2 Biphasic response of histamine in tracheal segments precontracted with acetylcholine 1×10^{-6} M (●). In the presence of mepyramine 1×10^{-6} M the contraction seen at high histamine concentrations (3×10^{-6} M and 1×10^{-5} M) was abolished whereas the relaxation remained (○). The dilator concentration-response curve of histamine in the presence of mepyramine (1×10^{-6} M) was shifted to the right in parallel by the H_3 antagonist thioperamide 1×10^{-7} M (pD_2 , 6.54 ± 0.50 ; ▽) and 1×10^{-6} M (pD_2 , 5.50 ± 0.50 ; △). Thioperamide 1×10^{-5} M abolished the histamine-induced dilator response (□). Responses are expressed as a percentage of acetylcholine-induced contraction and each point is the mean with s.d. Control $n = 25$, antagonist experiments $n = 5-9$.

relatively small differences in sensitivity (pD_2) and maximally contractile response ($E_{max}\%$) were seen. The large arteries, MPA and LLA, exhibited slightly higher histamine sensitivity (pD_2 , 5.19 ± 0.39 ; $n = 16$ and 5.31 ± 0.28 ; $n = 22$, respectively) than the small arteries SLA and ISA (pD_2 , 4.88 ± 0.37 ; $n = 8$ and 4.80 ± 0.17 ; $n = 4$, respectively). In the smallest arteries, ISA (ninth to tenth branches), the relative maximal contraction was also smaller ($E_{max}\%$; 103 ± 11) than in the larger arteries ($E_{max}\%$: MPA, $127 \pm 33\%$; LLA, $116 \pm 31\%$; SLA, $121 \pm 37\%$) (Figure 4). Dilator experiments revealed no significant differences between these arterial regions (not illustrated).

Antagonist experiments

Mepyramine caused a parallel shift to the right of the histamine-induced concentration-response curves for both the trachea and the pulmonary arteries (Figure 1a,b). The dextral displacement of the concentration-response curves was used in a Schild analysis. The concentration-ratios for mepyramine yielded a line with the slope of 0.61 ($r = 0.98$, $y = 5.03 - 0.61x$) without and 1.05 ($r = 0.99$; $y = 10.05 - 1.05x$) with the presence of thioperamide. In the presence of thioperamide mepyramine caused a concentration-dependent shift of the

Figure 3 Response of histamine in pulmonary arterial segments precontracted with prostaglandin $F_{2\alpha}$ 3×10^{-6} M (●). In the presence of mepyramine 1×10^{-6} M a strong histamine-induced relaxation became evident (pD_2 , 6.02 ± 0.16 ; ○). Cimetidine 1×10^{-7} M (pD_2 , 5.93 ± 0.19 ; ▽) and 1×10^{-5} M (pD_2 , 4.58 ± 0.29 ; △) in the presence of mepyramine (1×10^{-6} M) shifted the concentration-response curve in parallel to the right. Responses are expressed as a percentage of prostaglandin $F_{2\alpha}$ -induced contraction and each point is the mean with s.d. Control, $n = 6$, antagonist experiments $n = 6-9$.

histamine responses, showing a pA_2 -value for mepyramine in the trachea of 9.57. In the pulmonary artery mepyramine yielded a line with a slope of 1.12 ($r = 0.99$, $y = 9.76 - 1.12x$), suggesting a simple competitive antagonism at one receptor site. The resulting pA_2 values were 8.25 in trachea and 8.75 in the pulmonary artery, respectively. Cimetidine did not affect the histamine-induced concentration-response curve for the trachea (e.g. cimetidine 10^{-7} M; $E_{max}\%$, 110 ± 23 ; pD_2 4.93 ± 0.29 ; $n = 5$; cimetidine 10^{-5} M; $E_{max}\%$, 103 ± 29 ; pD_2 5.41 ± 0.39 ; $n = 4$). A small shift to the left was seen in the pulmonary artery, but this leftward shift was only significant for low concentrations of histamine (10^{-7} M, 3×10^{-7} M and 10^{-6} M) (Figure 1b). Thioperamide did not affect the histamine-induced concentration-response curve for the pulmonary artery, but a leftward shift was seen in the trachea ($E_{max}\%$, 121 ± 31.7 ; pD_2 5.55 ± 0.27 ; $n = 7$). In the precontracted trachea, application of mepyramine (10^{-7} M or 10^{-6} M) abolished the contraction seen at high histamine concentrations (Figure 2) and consequently the maximal dilator response ($I_{max}\%$) increased, without significantly changing the pD_2 -values (mepyramine 10^{-6} M; $I_{max}\%$, 56 ± 11 ; pD_2 , 7.23 ± 0.25 ; $n = 6$). Addition of cimetidine (10^{-5} M) did not further change the dilator response ($I_{max}\%$, 54 ± 18 ; pD_2 , 7.17 ± 0.03 ; $n = 5$) nor did mepyramine in higher concentrations (10^{-5} M). The H_3 -antagonist, thioperamide, in the presence of mepyramine (10^{-6} M), caused a parallel shift to the right of the histamine-induced dilatation

Figure 4 Regional variations in the histamine-induced contractile response of the pulmonary vascular tree. Circular segments from four different levels were tested. Main pulmonary artery (MPA, located next to the heart, $\blacktriangle$), large lobar arteries (LLA, second to third branches, $\bullet$), small lobar arteries (SLA, fourth to fifth branches, $\blacksquare$) and intrasegmental arteries (ISA, ninth to tenth branches, $\blacklozenge$). Responses are expressed as a percentage of potassium (60 mM)-induced contraction (K^+) and each point is the mean with s.d. (MPA, $K^+ = 3.11 \pm 2.05$ mN, $n = 16$; LLA, $K^+ = 3.61 \pm 2.22$ mN, $n = 22$; SLA, $K^+ = 2.37 \pm 1.25$ mN, $n = 8$ and ISA, $K^+ = 1.03 \pm 0.49$ mN, $n = 4$).

(Figure 2). Schild analysis yielded a line with the slope of 0.77 ($y = 5.98 - 0.77x$) with a corresponding pA_2 -value of 7.79. When thioperamide was added in the absence of mepyramine, the histamine-induced dilatation became gradually inhibited and at a thioperamide concentration of 10^{-5} M the dilator response was totally abolished. The contractile response to thioperamide at higher concentration remained unaffected (Figure 2).

In the precontracted pulmonary artery, low concentrations of histamine induced only small dilator responses, roughly 10% of the precontraction level, followed by a strong contraction at high concentrations. In the presence of mepyramine, the contraction was abolished and a concentration-dependent dilatation was unmasked (Figure 3). A change of the mepyramine concentration from 10^{-7} M to 10^{-6} M did not affect the $I_{max}\%$ and pD_2 -values for the dilatation. However, if the experiments were performed with both cimetidine and mepyramine in the bath, cimetidine in rising concentrations caused a parallel shift of the concentration-response curve to the right, without any further change in $I_{max}\%$ (Figure 3). A Schild plot yielded a line with the slope of 1.10 ($y = 7.14 - 1.10x$) and a pA_2 -value of 6.49. Thioperamide did not affect the histamine-induced arterial dilator response.

In tracheal and pulmonary arterial segments precontracted by histamine (10^{-5} M), mepyramine induced a total relaxa-

tion with a pD_2 value of 6.42 ± 0.18 for the trachea ($n = 6$) and 7.61 ± 0.25 for the arteries ($n = 6$). No significant differences were seen in this mepyramine-induced dilatation when cimetidine (10^{-5} M) was present in the bath (pD_2 , 6.30 ± 0.20 in trachea and 7.75 ± 0.30 in arteries, $n = 4$). Furthermore, addition of cimetidine to histamine precontracted arteries did not induce any dilator response ($n = 6$).

H₁ and H₂ agonist experiments

H₁-receptor agonists (thiazolyethylamine, 2-methylhistamine, and pyridylethylamine) induced contractions of tracheal and pulmonary arterial segments at rest. The order of potency was the same for both types of tissues; 2-methylhistamine > pyridylethylamine > thiazolyethylamine. H₂-agonists (impromidine and dimaprit) induced no or very small contractions, whereas 4-methylhistamine induced a moderate contraction.

In precontracted segments of trachea the histamine agonists induced a dilator response, followed by a constriction for H₁-agonists at higher concentrations. The contractile response was abolished with mepyramine 10^{-6} M. Scrutinizing the dilator response, the two rather unselective H₁ and H₂ receptor antagonists, 2-methylhistamine and 4-methylhistamine, induced a potent concentration-dependent relaxation. These relaxations were not affected by the presence of mepyramine (10^{-6} M) and cimetidine (10^{-6} M). Other H₁ and H₂ agonists were less potent. In the pulmonary artery, H₂-receptor agonists (impromidine, dimaprit and 4-methylhistamine) induced a more uniform concentration-dependent relaxation with the following potency order; impromidine > 4-methylhistamine > dimaprit. These relaxations were abolished with cimetidine (10^{-5} M). H₁ agonists (thiazolyethylamine and pyridylethylamine) induced a small relaxation (about 5–10% of the arterial precontraction level) starting at 10^{-7} M. At 10^{-6} M, the small dilator responses reversed to strong contraction. Even though the dilator responses of the two tested H₁-receptor agonists were much smaller than the responses induced by the three H₂-receptor agonists, they induced their dilator responses in low concentrations. The potency and effectiveness of the histamine agonists, on resting and precontracted trachea and pulmonary artery segments are summarized in Table 1.

H₃ agonist experiments

R- α -methylhistamine, an H₃ receptor agonist, induced a three-phased motor response of precontracted tracheal segments. At low concentrations, a concentration-dependent dilator response was seen and at moderate concentrations an intermediate stage with a plateau or a small contraction appeared. This was followed at high concentrations by a new concentration-dependent relaxation (Figure 5). The first dilator effect of R- α -methylhistamine in the trachea was similar to that obtained for histamine. The maximal dilator response as well as the corresponding pD_2 -values were calculated separately for the two relaxing phases (First dilation: acetylcholine precontraction; $E_{max}\%$, 25 ± 11 ; pD_2 7.58 ± 0.47 ; $n = 10$. 5-Hydroxytryptamine precontraction; $E_{max}\%$, 26 ± 12 ; pD_2 7.47 ± 0.15 ; $n = 6$. Second dilation: 5-hydroxytryptamine precontraction; $E_{max}\%$, 111 ± 43 ; pD_2 4.44 ± 0.31 ; $n = 6$). The onset of R- α -methylhistamine-induced dilator responses in the trachea occurred less than 20 s after its addition to the bath. No difference was found between the relaxation induced by R- α -methylhistamine on tracheal segments precontracted by ACh, prostaglandin F_{2 α} (PGF_{2 α}) or 5-HT. However, in tracheal segments precontracted by histamine, only one dilator phase was seen ($E_{max}\%$, 124 ± 25 ; pD_2 4.38 ± 0.23 ; $n = 16$), corresponding to the second dilatation seen in the ACh, PGF_{2 α} or 5-HT precontracted segments (Figure 5). The presence of mepyramine (10^{-8} M, 10^{-5} M) and cimetidine (10^{-8} M, 10^{-5} M) in the bath did not affect the dilator effects of R- α -methylhistamine (nor did it affect the first and second dilatation of ACh, PGF_{2 α} or

Table 1 Motor responses of histamine H₁- and H₂-receptor agonists in guinea-pig trachea and pulmonary large lobar artery

Contraction	<i>n</i>	<i>E</i> _{max} %	Trachea		<i>n</i>	<i>E</i> _{max} %	Pulmonary artery	
			<i>pD</i> ₂				<i>pD</i> ₂	
Thiazolyethylamine	4	49 ± 18	4.29 ± 0.29		12	127 ± 29	5.11 ± 0.75	
Pyridylethylamine	8	83 ± 27	4.73 ± 0.23		9	130 ± 55	4.82 ± 0.41	
2-Methylhistamine	6	98 ± 18	5.14 ± 0.18		10	131 ± 62	5.47 ± 0.43	
4-Methylhistamine	4	32 ± 8	4.39 ± 0.38		6	62 ± 13	3.97 ± 0.13	
Impromidine	4	0	—		4	14 ± 8	3.90 ± 0.33	
Dimaprit	4	0	—		4	0	—	
Dilatation	<i>n</i>	<i>I</i> _{max} %			<i>n</i>	<i>I</i> _{max} %		
			<i>pD</i> ₂				<i>pD</i> ₂	
Thiozolyethylamine	7	76 ± 20	5.26 ± 0.63		5	15 ± 8	7.77 ± 0.32	
Pyridylethylamine	3	63 ± 9	6.11 ± 0.15		6	12 ± 7	7.50 ± 0.47	
2-Methylhistamine	5	58 ± 21	7.61 ± 0.26		NP			
4-Methylhistamine	5	46 ± 14	7.12 ± 0.25		5	35 ± 19	6.48 ± 0.56	
Impromidine	4	48 ± 13	5.46 ± 0.21		7	48 ± 16	7.54 ± 0.34	
Dimaprit	6	41 ± 25	5.50 ± 0.52		5	60 ± 4	5.73 ± 0.33	

Maximal contractile response (*E*_{max} %), expressed as a percentage of the contraction induced by 60 mM potassium; maximal dilator response (*I*_{max} %), expressed as a percentage of precontraction and sensitivity (*pD*₂) expressed as the negative logarithm of the concentration eliciting half the maximal response.

NP, not performed. The values represent the mean ± s.d.

Figure 5 The three phased motor response induced by the H₂-receptor agonist R-α-methylhistamine on guinea-pig tracheal segments precontracted with acetylcholine 1×10^{-6} M (●), prostaglandin F_{2α} 1×10^{-6} M (■) and simple one phased dilator response induced by the same H₂ agonist in tracheal segments precontracted by histamine 3×10^{-5} M (□). The symbols (□) indicate that the concentration-dependent dilator response of R-α-methylhistamine on segments precontracted by prostaglandin F_{2α} 1×10^{-6} M is not affected by the presence of mepyramine 1×10^{-6} M. Responses are expressed as a percentage of precontraction and each point is the mean with s.d. *n* = 5–9.

Table 2 R-α-methylhistamine induced motor response of guinea-pig pulmonary large lobar arterial segments

Pulmonary artery	<i>n</i>	R-α-methylhistamine contraction	
		<i>I</i> _{max} %	<i>pD</i> ₂
RαMeHA control	18	68 ± 36	3.82 ± 0.18
RαMeHA + Mep 10^{-9} M	6	70 ± 26	3.93 ± 0.22
RαMeHA + Mep 10^{-8} M	3	11 ± 6	3.54 ± 0.17 ^a
RαMeHA + Mep 10^{-7} M	3	0	—
RαMeHA + Cim 10^{-7} M	5	89 ± 28	4.11 ± 0.29
RαMeHA + Mep 10^{-5} M	5	74 ± 41	3.74 ± 0.15
Pulmonary artery	<i>n</i>	R-α-methylhistamine dilatation	
		<i>I</i> _{max} %	<i>pD</i> ₂
RαMeHA control ^b	6	0	—

Maximal contractile response (*E*_{max} %), expressed as a percentage of the contraction induced by 60 mM potassium; maximal dilator response (*I*_{max} %), expressed as a percentage of precontraction and sensitivity (*pD*₂) expressed as the negative logarithm of the concentration eliciting half the maximal response. RαMeHA, R-α-methylhistamine; Mep, mepyramine; Cim, cimetidine; ^acomplete concentration-response curve could not be obtained; ^bprostaglandin F_{2α} precontraction. The values represent the mean ± s.d.

5-HT precontracted segments nor the segments precontracted by histamine). This dilator response was not affected by the muscarinic receptor antagonist, atropine (10^{-6} M), the α₁-, α₂- and β-adrenoceptor antagonists prazosin (10^{-6} M), yohimbine (10^{-6} M) and propranolol (10^{-6} M), respectively, or the tachykinin antagonist, spantide (10^{-6} M). There were no contractile effects of R-α-methylhistamine (10^{-10} – 3×10^{-5} M) on the tracheal segments.

High concentrations of R-α-methylhistamine induced a concentration-dependent contraction of pulmonary arteries at rest. This contraction was competitively blocked by mepyramine whereas cimetidine was without effect (Table 2). In the precontracted artery dilator responses were not seen after application of R-α-methylhistamine (10^{-10} – 3×10^{-5} M). Addition of mepyramine (10^{-6} M) or cimetidine (10^{-6} M) did not reveal any masked dilator response.

Removal of the tracheal epithelium and vascular endothelium

Removal of the epithelium in the trachea shifted the concentration-response curve for the histamine-induced contraction to the left and increased the maximal contractile response (pD_2 5.74 ± 0.23 , $P < 0.01$; E_{max} %, 162 ± 29 ; $P < 0.01$; $n = 9$). The potency of the dilator response to histamine was also increased (pD_2 8.51 ± 0.36 , $P < 0.05$; I_{max} %, 25 ± 11 ; $n = 6$) (Figure 6a).

In the pulmonary arterial segments, removal of the endothelium affected the histamine-induced contractile response in the same way as the epithelium displacement in the trachea, and thus led to a slightly higher sensitivity and an enlargement of the maximum contraction (not significant) (pD_2 5.54 ± 0.15 ; E_{max} %, 133 ± 41 ; $n = 6$) (Figure 6b). In the precontracted pulmonary artery, the small initial dilatation was abolished (Figure 6c) whereas the larger dilatation seen in the presence of mepyramine was only slightly reduced (pD_2 5.91 ± 0.24 ; I_{max} %, 66 ± 23 ; $n = 6$). The contractile responses seen at higher histamine concentrations were potentiated (Figure 6c).

Discussion

Histamine in low concentrations acts as a relaxant agent of precontracted guinea-pig isolated pulmonary arterial and tracheal segments, whereas in high concentrations it induces contraction of both precontracted and resting segments. This

discussion will mainly focus on the histamine-induced dilator response, since the concept of histamine H₁ receptor-mediated contraction of isolated tracheal and pulmonary arteries is already well established (Ash & Schild, 1966; Timmerman, 1991).

The responses to histamine in isolated vascular preparations vary considerably depending upon the species (Toda, 1986), the type of vessels (Van de Voorde & Leusen, 1984) and the region within the vascular bed from which the tissue has been derived (Shirai *et al.*, 1987; Tsuru *et al.*, 1987). In the present study, arteries from various parts of the pulmonary vascular tree were examined, but only small intra-regional differences in the histamine-induced contractile response were seen. Histamine contraction was slightly more potent in large, proximal arteries than in small, more distally located arteries. When studying relaxations, no significant differences were seen.

The concept of H₁-receptor-mediated contraction of the trachea and the guinea-pig pulmonary artery is supported by the mepyramine-induced rightward shift of the concentration-response curves obtained. The slope of 0.61 in the trachea implicates involvement also of another site, possibly a H₃ receptor. The finding of a slope 1.05 in the presence of thioperamide supports this idea. The slope did not differ from unity for the pulmonary artery which suggests a simple competitive antagonism at one receptor site. Dissociation constant (pA_2) values of 8.0–9.3 for mepyramine and 6.1–7.0 for cimetidine have been reported in different test preparations (Ottoosson *et al.*, 1988). Thus, the pA_2 values found in the present study, 9.57 and 8.75 for mepyramine

Figure 6 Enhancement of the histamine response in tracheal segments. Filled symbols represent the control and open symbols represent segments where the epithelium and endothelium, respectively, have been removed by rubbing the luminal side of the specimens. (a) Histamine-induced contraction of resting (●, ○, $n = 9$) and acetylcholine precontracted (■, □, $n = 9$) tracheal segments. Responses are expressed as a percentage of potassium (60 mM)-induced contraction (●, ○) and acetylcholine-induced precontraction (■, □). Since in these experiments, the contraction induced by potassium (60 mM) corresponded to the contraction induced by acetylcholine 1×10^{-6} M both types of curves could be compared in the same diagram. (b) Histamine-induced contractions of control (●) and rubbed (○, $n = 6$) resting pulmonary arterial segments. Responses are expressed as a percentage of potassium (60 mM)-induced contraction. (c) The histamine-induced biphasic response of pulmonary arterial segments precontracted by prostaglandin $F_{2\alpha}$ 1×10^{-6} M (■) is exchanged for an enhanced contractile response by rubbing (□, $n = 7$) and an endothelium independent relaxation by adding of mepyramine 10^{-6} M (△ and ▲, $n = 6$). Responses are expressed as a percentage of acetylcholine 1×10^{-6} M-induced precontraction. Each point is the mean with s.d.

and 6.49 for cimetidine, are in accordance with those found in histamine receptor subtype characterizations carried out previously.

In some species, bronchodilatation is mediated via H_2 -receptors (Okpako *et al.*, 1978; Chand & De Roth, 1979). In contrast, Chand & Eyre (1978) and Vincic *et al.* (1984) have described a histamine-induced tracheal relaxation not affected by H_2 -antagonists and have suggested the existence of H_1 -receptor subtypes or a third histamine receptor in the trachea. In the present study, histamine (10^{-9} M– 10^{-6} M) induced a relaxation of precontracted tracheal segments followed by a contraction at higher concentrations. In the presence of mepyramine, the contractile component was abolished, and the maximal dilatation increased without any change in the pD_2 -value. Since there was no difference in the dilator responses between mepyramine at concentrations of 10^{-6} M and 10^{-5} M, the involvement of a 'traditional' dilator H_1 -receptor is less likely. Cimetidine did not affect the dilator response, which speaks against the involvement of H_2 receptors. Consequently, an H_3 receptor must be considered. The selective H_3 receptor antagonist, thioperamide (10^{-5} M), totally inhibited the dilator response of histamine in precontracted tracheal segments. The contractile responses seen at higher histamine concentrations were not affected. In the presence of mepyramine, thioperamide induced a parallel shift to the right of the dilator histamine response. The slope was 0.77 and the pA_2 -value 7.79, supporting the involvement of an H_3 receptor. Unexpectedly, all the tested H_1 and H_2 agonists induced a concentration-dependent relaxation of the precontracted tracheal segments. Two of them, the H_1 agonist, 2-methylhistamine, and the H_2 agonist, 4-methylhistamine, were about 100 times more potent than the rest, and their pD_2 -values corresponded with the value obtained for histamine during the same conditions. Both agonists are known to be potent but rather unselective. R - α -methylhistamine is about 15 times more potent than histamine as an H_3 agonist, but possesses only 1% of the activity of histamine on H_1 and H_2 receptors (Arrang *et al.*, 1987). This rather selective H_3 agonist induced a three phased response on precontracted tracheal segments, two dilator parts with a plateau or slight contraction in between. The slight contraction was eliminated by treatment with mepyramine, leaving the two dilator parts unaffected. Cimetidine did not affect this biphasic dilator response. The first dilatation, induced by low concentrations of R - α -methylhistamine, was slightly more potent than the dilatation induced by histamine in the same preparation. The second dilatation was elicited only at very high concentrations of R - α -methylhistamine. In tracheal segments precontracted by histamine, only the late second dilatation was seen. These data imply that the first 'low dose' dilatation induced by R - α -methylhistamine could be the result of an involvement of an H_3 -receptor, whereas the second 'high dose' dilatation may be the result of a more unspecific action.

The respiratory epithelium modifies the reactivity of the tracheal smooth muscle in response to several drugs, including histamine (Barnes *et al.*, 1985). In the present study, removal of the respiratory epithelium increased the potency of the histamine-induced contractions about 6 times and the relaxation about 10 times. The fact that removal of epithelium increases the sensitivity of tracheal segments not only to several contracting stimuli, but also to dilator agents (isoprenaline and adenosine), has also been reported by Holroyde (1986). These results suggest that the epithelium represents a permeability barrier the removal of which facilitates the access of exogenous substances (both contractile and relaxant) (Munakata *et al.*, 1989; Iriarte *et al.*, 1991). However, the possibility of a simultaneously existing mechanism for release of an epithelium-dependent relaxing factor(s) in response to histamine cannot be ruled out (Farmer *et al.*, 1986; Fedan *et al.*, 1988; VanHoutte, 1988). Recent reports have established that at least part of the inhibitory response in the trachea is mediated by nitric oxide

(Belvisi *et al.*, 1992), but the question as to whether nitric oxide is released directly from nerves in the airways, from airway epithelial cells and/or from endothelial cells of the bronchial vessels is open.

Histamine-induced relaxation can generally be mediated through H_2 -receptors located on the muscle cells (Toda, 1986; Stepanovic *et al.*, 1988) but in many isolated vascular preparations, relaxation elicited by histamine is dependent upon the presence of an intact endothelium (VanHoutte *et al.*, 1986; Chen & Suzuki, 1989). Further, depending on the species and the vessels, this endothelium-dependent relaxation occurs through activation of H_2 -receptors (Van de Voorde & Leusen, 1984; Sercombe *et al.*, 1986) and/or H_1 -receptors (Sato & Inui, 1984; Abacioglu *et al.*, 1987; Szarek *et al.*, 1992) located on the endothelial cells resulting in a subsequent release of inhibitory substances. In the present experiments, precontracted pulmonary arteries exhibited a biphasic response to histamine. In the presence of mepyramine, histamine caused a concentration-dependent relaxation at all concentrations tested. Addition of cimetidine caused a parallel shift of this curve to the right. Schild analysis revealed a pA_2 -value of 6.49 for cimetidine, indicating an H_2 -receptor. The presence of a dilator H_2 -receptor is further supported by the studies of contractile responses, where cimetidine enhanced the response to low concentrations of histamine. Hence, this could reflect the elimination of the influence from a dilator cimetidine-sensitive receptor (Boe *et al.*, 1980). When the endothelium was mechanically removed, the small histamine-induced dilatation seen prior to the following contraction disappeared and the remaining contraction was slightly enhanced. With mepyramine present in endothelium denuded preparations, histamine still induced a strong dilator response. The relaxation was slightly reduced from 69% in unrubbed to 51% in the rubbed segments without any significant change of the potency. These results show that histamine has a strong direct relaxant effect on precontracted pulmonary arteries. Furthermore, according to the presented results, the presence of an intact endothelium seems to contribute to the relaxant effect of histamine. The endothelium-dependent part of the relaxation could be mediated through a H_1 receptor triggered release of an endothelium derived relaxing factor (nitric oxide) and/or a cyclo-oxygenase product (Abacioglu *et al.*, 1987; Ortiz *et al.*, 1992). The importance of this endothelium-dependent component cannot be evaluated from the present results since the contractile H_1 receptor on the vasculature will tend to mask such a response. Investigations with the use of substances interfering with the formation of nitric oxide and cyclo-oxygenase products will be needed to solve this question.

The finding that the H_1 receptor agonists, 2-pyridylethylamine and 2-thiazolyethylamine induce dilatation (10–15%) of precontracted pulmonary arteries supports the notion of dilator H_1 receptors on the endothelium. The tested H_2 receptor agonists had the following order of potency: impromidine > 4-methylhistamine > dimaprit, and induced relaxations between 35–60%. According to Abacioglu *et al.* (1987), the dose-related relaxant effect of impromidine is independent of vascular endothelium which supports the assumption of a dilator H_2 -receptor on the vascular smooth muscle. R - α -methylhistamine did not induce any dilator responses in precontracted pulmonary arteries, but in high concentration this H_3 agonist induced a concentration-dependent contraction in segments at rest. The concentrations needed to induce this contraction were about 100 times higher than the concentration of histamine producing the same effect. The R - α -methylhistamine elicited contraction could be gradually blocked by mepyramine in rising concentrations, indicating that R - α -methylhistamine in high concentrations probably could also activate H_1 -receptors.

In conclusion, histamine induced a biphasic effect of guinea-pig trachea and pulmonary arteries. At low concentrations a relaxation was seen in precontracted segments

followed by contraction at higher concentrations. The contraction of either specimen was mediated through histamine H₁-receptors. In the pulmonary artery, the main part of the relaxant response seems to be mediated via H₂ receptors on the smooth muscle. However, the involvement of an endothelium-dependent relaxing factor or factors cannot be excluded. In the trachea, histamine-induced relaxation may involve H₃ receptors on the airway smooth muscle. Furthermore, the results support the suggestion that exogenous his-

tamine could activate H₃ receptors and consequently this third class of receptors might exist in tissues other than nerve terminals.

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References

- ABACIOGLU, N., ERCAN, Z.S., KANZIK, H., ZENGIL, H., DEMIRY-UREK, T. & TURKER, R.K. (1987). Endothelium-dependent relaxing effect of histamine on the isolated guinea-pig main pulmonary artery strips. *Agents Actions*, **22**, 30–35.
- ABROL, R.P., HUGHES, W.M., KRUEGER, G.A. & COOK, D.A. (1984). Detection of endothelium in cerebral blood vessels. *J. Pharmacol. Methods*, **12**, 213–219.
- ADAMS, G.K. & LICHTENSTEIN, L. (1979). *In vitro* studies of antigen-induced bronchospasm: effect of antihistamine and SRS-A antagonist on response of sensitized guinea pig and human airways to antigen. *J. Immunol.*, **122**, 555–562.
- ARRANG, J.-M., GARBARG, M., LANCELOT, J.-C., LECOMTE, J.-M., POLLARD, H., ROBBA, M., SCHUNACK, W. & SCHWARTZ, J.-C. (1987). Highly potent and selective ligands for histamine H₃-receptors. *Nature*, **327**, 117–123.
- ARRANG, J.-M., GARBARG, M. & SCHWARTZ, J.-C. (1983). Autoinhibition of brain histamine release mediated by a novel class (H₃) of histamine receptor. *Nature*, **302**, 832–837.
- ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.
- ASH, A.S.F. & SCHILD, H.O. (1966). Receptors mediating some actions of histamine. *Br. J. Pharmacol.*, **27**, 427–439.
- BARNES, P.J., CUSS, F.M. & PALMER, J.B. (1985). The effect of airway epithelium on smooth muscle contractility in bovine trachea. *Br. J. Pharmacol.*, **86**, 685–692.
- BARNES, P.J. & ICHINOSE, M. (1989). H₃ receptors in airways. *Trends Pharmacol. Sci.*, **10**, 264.
- BELVISI, M.G., STRETTON, C.D., YACOUB, M. & BARNES, P.J. (1992). Nitric oxide is the endogenous neurotransmitter of bronchodilator nerves in humans. *Eur. J. Pharmacol.*, **210**, 221–222.
- BEVAN, M.A. (1982). *Pharmacology of Histamine Receptors*. ed. Ganellin, G.R. & Parsons, M.E. pp. 103–145. Bristol: Wright.
- BLACK, J.W., DUNCAN, W.A.M., DURANT, C.J., GANELLIN, C.R. & PARSONS, E.M. (1972). Definition and antagonism of histamine H₂-receptors. *Nature*, **236**, 385–390.
- BOE, J., BOE, M.-A. & SIMONSSON, B.G. (1980). A dual action of histamine on isolated human pulmonary arteries. *Respiration*, **40**, 117–122.
- CARDELL, L.O., UDDMAN, R. & EDVINSSON, L. (1993). A novel ET_A-receptor antagonist, FR 139317, inhibits endothelium-induced contractions of guinea-pig pulmonary arteries, but not trachea. *Br. J. Pharmacol.*, **108**, 448–452.
- CARDELL, L.O., UDDMAN, R. & EDVINSSON, L. (1990). Analysis of endothelin 1-induced contractions of guinea-pig trachea, pulmonary veins and different types of pulmonary arteries. *Acta Physiol. Scand.*, **139**, 103–111.
- CHAND, N. & DE ROTH, L. (1979). Dual histamine receptor mechanism in guinea-pig lung. *Pharmacology*, **19**, 185–190.
- CHAND, N. & EYRE, P. (1978). Spasmolytic action of histamine in airway smooth muscle of horse. *Agents Actions*, **8**, 191–198.
- CHEN, G. & SUZUKI, H. (1989). Direct and indirect actions of acetylcholine and histamine on intrapulmonary artery and vein muscles of the rat. *Jpn. J. Physiol.*, **39**, 51–65.
- EA KIM, L., JAVELLAUD, J. & OUDART, N. (1992). Endothelium-dependent relaxation of rabbit middle cerebral artery to histamine H₃-agonist is reduced by inhibitors of nitric oxide and prostacyclin synthesis. *Br. J. Pharmacol.*, **105**, 103–106.
- EA KIM, L. & OUDART, N. (1988). A highly potent and selective H₃ agonist relaxes rabbit middle cerebral artery, *in vitro*. *Eur. J. Pharmacol.*, **150**, 393–396.
- EDVINSSON, L. & OWMAN, C. (1975). A pharmacologic comparison of histamine receptors in isolated extracranial and intracranial arteries *in vitro*. *Neurology*, **25**, 217–276.
- FARMER, S.G., FEDAN, J.S., HAY, D.W.P. & RAEBURN, D. (1986). The effects of epithelium removal on the sensitivity of guinea-pig isolated trachealis to bronchodilator drugs. *Br. J. Pharmacol.*, **89**, 407–414.
- FEDAN, J.S., HAY, D.W.P., FARMER, S.G. & RAEBURN, D. (1988). Epithelial cells: modulation of airway smooth muscle reactivity. In *Asthma: Basic Mechanisms and Clinical Management*. ed. Barnes, P.J., Rodger, I.W. & Thomson, N.C. pp. 143–162. London: Academic Press.
- FOREMAN, J.C., RISING, T.J. & WEBBER, S.E. (1985). A study of the histamine H₂-receptor mediating relaxation of the parenchymal lung strip preparation of the guinea-pig. *Br. J. Pharmacol.*, **86**, 465–473.
- FURCHGOTT, R.F. (1984). The role of endothelium in the responses of vascular smooth muscle to drugs. *Annu. Rev. Pharmacol. Toxicol.*, **24**, 175–197.
- GOLDIE, R.G., PAPADIMITRIOU, J.M., PATERSON, J.W., RIGBY, P.J., SELF, H.S. & SPINA, D. (1986). Influence of the epithelium on responsiveness of guinea-pig isolated trachea to contractile and relaxant agonists. *Br. J. Pharmacol.*, **87**, 5–14.
- HILL, S.J. (1990). Distribution, properties, and functional characteristics of three classes of histamine receptor. *Pharmacol. Rev.*, **42**, 45–83.
- HOLROYDE, M.C. (1986). The influence of epithelium on the responsiveness of guinea-pig isolated trachea. *Br. J. Pharmacol.*, **87**, 501–507.
- ICHINOSE, M. & BARNES, P.J. (1989). Inhibitory histamine H₃-receptors on cholinergic nerves in human airways. *Eur. J. Pharmacol.*, **163**, 383–386.
- ICHINOSE, M., BELVISI, M.G. & BARNES, P.J. (1990). Histamine H₃-receptors inhibit neurogenic microvascular leakage in airways. *J. Appl. Physiol.*, **68**, 21–25.
- ICHINOSE, M., STRETTON, C.D., SCHWARTZ, J.C. & BARNES, P.J. (1989). Histamine H₃-receptors inhibit cholinergic bronchoconstriction in guinea pig airways. *Br. J. Pharmacol.*, **97**, 13–15.
- IRIARTE, C.F., PASCUAL, R., VILLANUEVA, M.M., ROMAN, M., ORTIZ, J.L., CORTIJO, J. & MORCILLO, E. (1991). Epithelium modulates the reactivity of sensitized guinea pig trachea: influence of the surface of drug entry. *J. Pharm. Pharmacol.*, **43**, 392–395.
- ISHIKAWA, S. & SPERELAKIS, N. (1987). A novel class (H₃) of histamine receptors on perivascular nerve terminals. *Nature*, **327**, 158–160.
- KRSTIC, M.K., STEPANOVIC, R.M., VUCKOVIC, S.M. & KRSTIC, S.K. (1991). Endothelium-independent relaxation of the rat femoral artery caused by activation of histamine H₂-receptors. *Arch. Int. Pharmacodyn.*, **313**, 15–22.
- LICHTENSTEIN, L.M. (1975). The mechanism of basophil histamine release induced by antigen and calcium ionophore. *J. Immunol.*, **114**, 1692–1699.
- MIKKELSEN, E., SAKR, A.M. & JESPERSEN, L.T. (1984). Studies of the effect of histamine in isolated human pulmonary arteries and veins. *Acta Pharmacol. Toxicol.*, **54**, 86–93.
- MUNAKATA, M., HUANG, I., MITZNER, W. & MENKES, H. (1989). Protective role of epithelium in the guinea pig artery. *J. Appl. Physiol.*, **66**, 1547–1552.
- OKPAKO, D.T., CHAND, N. & EYRE, P. (1978). The presence of inhibitory histamine H₂-receptors in guinea-pig tracheobronchial muscle. *J. Pharm. Pharmacol.*, **30**, 181–186.
- ORTIZ, J.L., LABAT, C., NOREL, X., GORENNE, I., VERLEY, J. & BRINK, C. (1992). Histamine receptors on human isolated pulmonary arterial muscle preparations: effects of endothelial cell removal and nitric oxide inhibitors. *J. Pharmacol. Exp. Ther.*, **260**, 762–767.
- OTTOSSON, A., JANSEN, I. & EDVINSSON, L. (1988). Characterization of histamine receptors in isolated human cerebral arteries. *Br. J. Pharmacol.*, **94**, 901–907.
- OTTOSSON, A., JANSEN, I. & EDVINSSON, L. (1989). Pharmacological characterization of histamine receptors in the human temporal artery. *Br. J. Clin. Pharmacol.*, **27**, 139–145.
- RILEY, J.F. & WEST, G.B. (1953). The presence of histamine in tissue mast cells. *J. Physiol.*, **120**, 528–537.

- SATOH, H. & INUI, J. (1984). Endothelial cell-dependent relaxation and contraction induced by histamine in the isolated guinea-pig pulmonary artery. *Eur. J. Pharmacol.*, **97**, 321–324.
- SCHWARTZ, J.C., ARRANG, J.-M. & POLLARD, H. (1990). A third histamine receptor type. Characterization, localisation and functions of the H_3 -receptor. *Agents Actions*, **30**, 13–23.
- SERCOMBE, R., VERRECHIA, C., PHILIPSON, V., OUDART, N., DIMITRIADOU, V., BOUCHAUD, C. & SEYLAZ, J. (1986). Histamine-induced contractions and dilatation of rabbit middle cerebral arteries in vitro: role of endothelium. *Blood Vessels*, **23**, 137–153.
- SHIRAI, M., SADA, K. & NINOMIYA, I. (1987). Nonuniform effects of histamine on small pulmonary vessels in cats. *J. Appl. Physiol.*, **62**, 451–458.
- STEPANOVIC, R.M., KUTUSIC, Z.S., KRSTIC, S.K. & KRSTIC, M.K. (1988). Analysis of the response of the guinea-pig common carotid artery to histamine. *Period. Biol.*, **90**, 305–307.
- SZAREK, J.L., BAILLY, D.A., STEWART, N.L. & GRUETTER, C.A. (1992). Histamine H_1 -receptors mediate endothelium-dependent relaxation of rat isolated pulmonary arteries. *Pulm. Pharmacol.*, **5**, 67–74.
- TALLARIDA, R.J., COWAN, A. & ADLER, M.W. (1979). pA_2 and receptor differentiation: a statistical analysis of competitive antagonism. *Life Sci.*, **25**, 637–654.
- TIMMERMAN, H. (1991). Histamine agonists and antagonists. *Acta Otolaryngol.*, **479**, 5–11.
- TODA, N. (1986). Mechanism of histamine-induced relaxations in isolated monkey and dog coronary arteries. *J. Pharmacol. Exp. Ther.*, **239**, 529–535.
- TSURU, H., KOHNO, S., IWATA, M. & SHIGEI, T. (1987). Characterization of histamine receptors in isolated rabbit veins. *J. Pharmacol. Exp. Ther.*, **243**, 696–702.
- VALENTOVIC, M.A., BALL, J.G., MORENAS, M., SZAREK, J.L. & GRUETTER, C.A. (1992). Influence of nitrovasodilators on bovine pulmonary histamine release. *Pulm. Pharmacol.*, **5**, 97–102.
- VAN DE VOORDE, J. & LEUSEN, I. (1984). Effect of histamine on aorta preparations of different species. *Arch. Int. Pharmacodyn. Ther.*, **268**, 95–105.
- VANHOUTTE, P.M. (1988). Editorial. Epithelium derived relaxing factor: myth or reality? *Thorax*, **43**, 665–668.
- VANHOUTTE, P.M., RUBANYI, G.M., MILLER, V. & HOUSTON, D.S. (1986). Modulation of vascular smooth muscle contraction by the endothelium. *Annu. Rev. Physiol.*, **48**, 307–320.
- VINCEK, K., BLACK, J. & SHAW, J. (1984). Relaxation and contraction responses to histamine in the human lung parenchymal strip. *Eur. J. Pharmacol.*, **98**, 201–210.
- WALLENSTEIN, S., ZUCKER, C.L. & FLEISS, J. (1980). Some statistical methods useful in circulation research. *Circ. Res.*, **47**, 1–9.

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