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The effect of L-arginine on guinea-pig and rabbit airway smooth muscle function *in vitro*

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Abstract

We have investigated the effects of L-arginine, D-arginine and L-lysine on airway smooth muscle responsiveness to spasmogens *in vitro*. Both L-arginine and D-arginine (100 mM) significantly reduced the contractile potency and maximal contractile response to histamine but not to methacholine or potassium chloride in guinea-pig epithelium-denuded isolated trachea. Similarly, the contractile response to histamine was significantly reduced by L-arginine (100 mM) in rabbit epithelium-denuded isolated bronchus. The amino acid L-lysine (100 mM) failed to significantly alter the contractile potency of histamine in guinea-pig isolated trachea ($P>0.05$). In guinea-pig isolated trachea precontracted with histamine, both L-arginine and D-arginine produced a concentration-dependent relaxation which was not significantly altered by epithelium removal or by the presence of the nitric oxide synthase inhibitor, N^G-nitro L-arginine methyl ester (L-NAME; 50 µM). Thus, at very high concentrations, arginine exhibit a non-competitive antagonism of histamine-induced contraction of isolated airway preparations that was independent of the generation of nitric oxide and was not dependent on charge. These observations confirm previous studies of cutaneous permeability responses and of contractile responses of guinea-pig isolated ileal smooth muscle. Taken together, the data suggest that high concentrations of arginine can exert an anti-histamine effect.

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Introduction

We have previously demonstrated the ability of both L-arginine and D-arginine, but not L-lysine (all 10 µmol/site) to significantly reduce histamine- but not bradykinin-induced plasma protein extravasation in guinea-pig skin (1). Furthermore, L-arginine and D-arginine but not L-lysine (all 100 mM) attenuated histamine- but not bradykinin-induced contraction of guinea-pig isolated ileum (1). These data suggested the ability of very high concentrations of both L-arginine and D-arginine to act as functional antagonists against H_1 receptor-mediated contraction (1). This is consistent with an earlier study demonstrating that arginine inhibits histamine- and, to a lesser extent, kallidin-induced responses in guinea-pig ileum and uterus (2). However, L-arginine at the same

high concentration had no significant effect on histamine-induced plasma protein extravasation in rabbit skin (3). *In vivo* and *in vitro* studies of guinea-pig airway responsiveness to histamine have shown enhancement of responses following treatment with nitric oxide synthase inhibitors and abrogation of this enhancement by concomitant treatment with L-arginine (4). In the present study, we have investigated the ability of high concentrations of arginine to alter the biological action of histamine on airway smooth muscle preparations *in vitro*.

Material and Methods

Tissue preparation

Albino guinea-pigs (300-500 g) were killed by cervical dislocation and the trachea was removed and placed in cold (4°C) Krebs-Henseleit solution aerated with 95% O_2 and 5% CO_2 and containing the cyclooxygenase inhibitor indomethacin (5 µM). Rabbits (NZW, 2-3 kg) were killed by an overdose of pentobarbitone (60 mg/ml) and the main bronchus was removed and placed in cold oxygenated Krebs-Henseleit solution. The epithelium can influence guinea-pig tracheal (5) and rabbit bronchial (6) smooth muscle responsiveness to spasmogens. Thus, the epithelium was removed from all preparations unless otherwise specified. Epithelium was removed with a cotton wool probe and its absence and presence were assessed histologically as described previously (5).

Guinea-pig tracheal and rabbit bronchial rings were suspended in 10-ml organ baths under an optimal tension of 1 g in Krebs-Henseleit solution containing indomethacin (5 µM) at 37°C and aerated with 95% O_2 and 5% CO_2. Changes in tension were measured isometrically using an FTO3 transducer connected to an Electromed amplifier and chart recorder (Grass Instruments Co., Beds, UK). Tissues were equilibrated for 30 min and the Krebs-Henseleit solution changed every 10 min.

Methacholine (10 µM) was added to the bath to induce maximal contraction. After the contractile response had reached a plateau, the tissues were washed 5 times over a 15-min period and allowed to equilibrate for a further 30 min.

Functional studies

Guinea-pig trachea. Cumulative concentration-effect curves for histamine (0.01 µM-1 mM), methacholine (0.01-100 µM) and potassium chloride (10-160 mM) were constructed before and following 20-min incubation in the presence of L-arginine, D-arginine or L-lysine (10-100 mM). Tissues were then washed 5 times over a 15-min period to remove the amino acids and tissues were allowed to equilibrate for a period of 30 min before concentration-effect curves for these spasmogens were constructed. In control tissue, concentration-response curves to the spasmogens were performed in the absence of the amino acids.

The effect of L-arginine (100 mM) and L-lysine (100 mM) on the pH of Krebs-Henseleit solution at room temperature and aerated with 95% O_2 and 5% CO_2 was analysed using an ABL 30 blood gas analyser.

In other studies, epithelium-intact and denuded tracheal preparations were contracted with histamine (EC_{50}) in the absence or presence of the nitric oxide synthase inhibitor N^G-nitro L-arginine methyl ester (L-NAME; 50 µM). Cumulative concentrations of L-arginine, D-arginine and L-lysine (1-100 mM) were superimposed on this histamine contractile response.

Rabbit bronchus. Cumulative concentration-effect curves for histamine (0.1 µM-1 mM) were performed in the absence and following 20-min incubation in the presence of L-arginine (100 mM).

Contractile responses were expressed as a percentage of the maximum response observed in the control tissue. Thus, EC_{50} and EC_{30} refer to the concentration of agonist

which gives 50% and 30%, respectively, of the maximum response observed in the control tissue.

Data analysis

Contractile responses to methacholine, histamine and potassium chloride were expressed as a percent of the maximal response (Emax) to the spasmogens obtained prior to incubation of airway preparations with the amino acids. The concentration of agonist that contracted guinea-pig tracheal preparations to 30% Emax (EC_{30}) was used as a measure of contractile agonist potency. The concentration of L- and D-arginine that reversed histamine-contracted tissue by 50% (EC_{50}) was used as a measure of relaxant agonist potency. Contractile and relaxant potency was expressed as the geometric mean together with 95% confidence limits. Tension values are represented as the arithmetic mean and standard error of the mean. The Student paired and non-paired *t*-test was performed to test significance between means. Data were considered statistically significant if $P<0.05$.

Drugs

Indomethacin, L-NAME, acetyl-ß-methylcholine chloride (methacholine chloride), histamine dihydrochloride, L-arginine, D-arginine, L-lysine hydrochloride were all from Sigma (St. Louis, MO). All drugs were dissolved in Krebs-Henseleit solution of the following composition: 117.6 mM NaCl, 5.4 mM KCl, 0.57 mM $MgSO_4.7H_2O$, 1.03 mM $KH_2.PO_4$, 25.0 mM $NaHCO_3$, 11.1 mM glucose and 2.5 mM $CaCl_2.2H_2O$.

Results

Guinea-pig trachea: contractile responses

Histamine. The contractile potency of histamine was not significantly altered in the absence or presence of L-arginine (10 and 30 mM; $P>0.05$) (data not shown). In contrast, L-arginine (100 mM) significantly reduced the contractile potency in response to histamine (EC_{30}, 95% confidence limits; control 0.7 µM (0.6-1.2), N = 6 *vs* L-arginine 100 µM (40-250), N = 6, $P<0.05$, Figure 1A). The maximum contractile response (Emax) to histamine was also significantly reduced in the presence of L-arginine (control 500 ± 150 mg *vs* L-arginine 200 ± 50 mg, N = 6, $P<0.05$). The contractile potency ($P>0.05$) and Emax ($P>0.05$) of histamine were not significantly different from controls after washing tissues to remove L-arginine.

D-arginine (10 and 30 mM; $P>0.05$) failed to significantly alter the contractile potency of histamine (data not shown). In contrast,

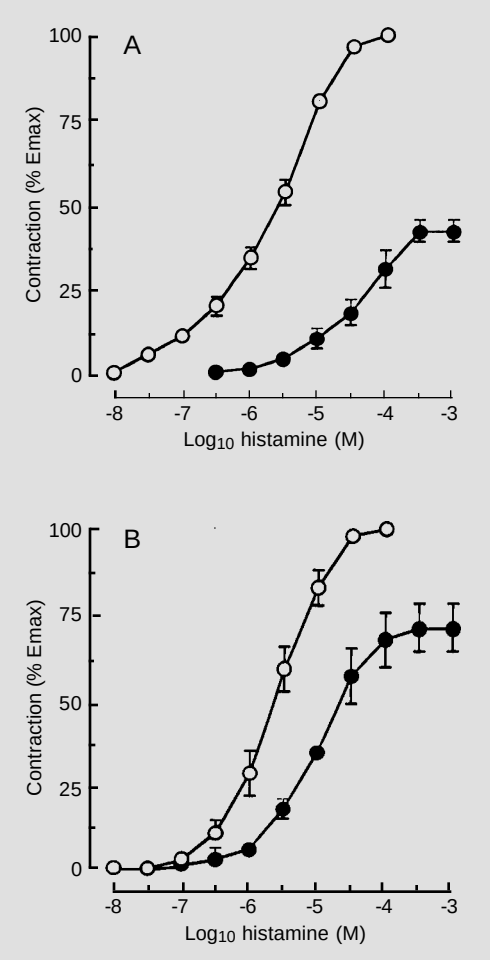

Figure 1 - Concentration-effect curves for histamine in the absence (open circles) or presence (closed circles) of L-arginine (100 mM) (A) or D-arginine (100 mM) (B) in guinea-pig isolated trachea. Each point represents the mean of 4-6 observations and vertical bars represent the SEM. Indomethacin (5 µM) is present throughout.

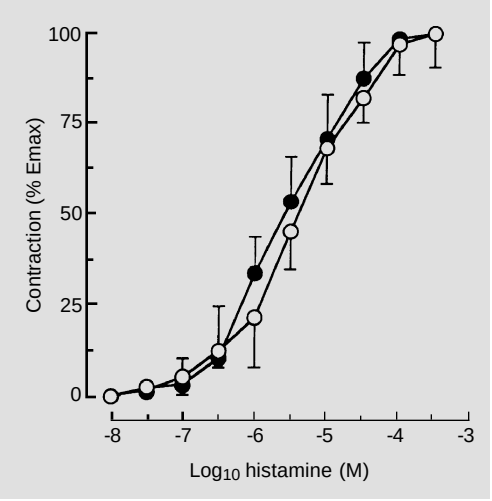

Figure 2 - Concentration-effect curves for histamine in the absence (open circles) or presence (closed circles) of L-lysine (100 mM) in guinea-pig isolated trachea. Each point represents the mean of 4-6 observations and vertical bars represent the SEM. Indomethacin (5 µM) is present throughout.

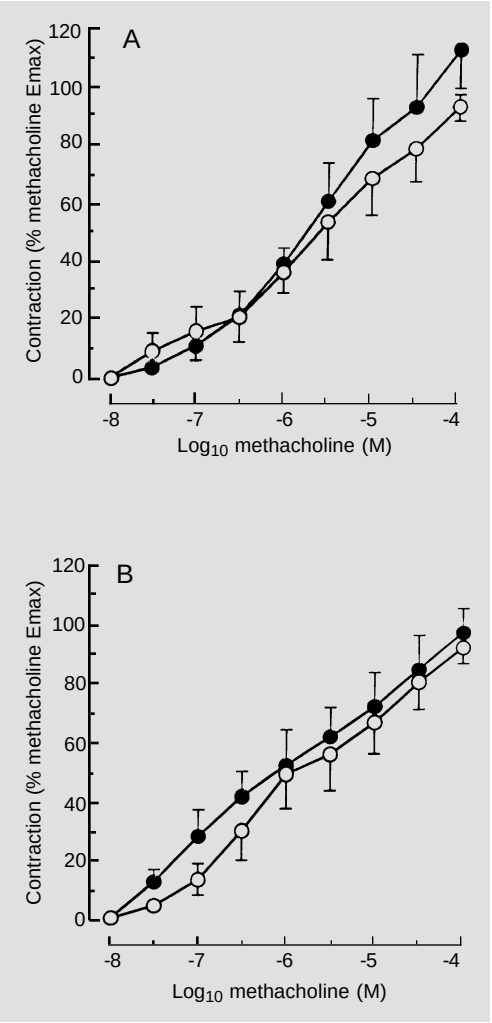

Figure 3 - Concentration-effect curves for methacholine in the absence (open circles) or presence (closed circles) of L-arginine (100 mM) (A) or D-arginine (100 mM) (B) in guinea-pig isolated trachea. Each point represents the mean of 4 observations and vertical bars represent the SEM. Indomethacin (5 µM) is present throughout.

D-arginine (100 mM) significantly reduced the contractile potency (EC_{30}, 95% confidence limits; control 1.0 µM (0.4-2.5), N = 4 *vs* D-arginine 8 µM (5-12), N = 4, P<0.05; Figure 1B) and Emax (control 650 ± 190 mg *vs* D-arginine 475 ± 140 mg, N = 4, P<0.05) of histamine. Following removal of D-arginine from the organ bath, the contractile potency (P>0.05) and Emax (P>0.05) of histamine were not significantly different from controls.

In contrast, L-lysine (100 mM) failed to significantly alter the contractile potency (EC_{30}, 95% confidence limits; control 1.2 µM (0.6-2.5), N = 4*vs* L-lysine 1.2 µM (0.8-2), N = 4, P>0.05; Figure 2) and Emax (control 650 ± 90 mg *vs* L-lysine 650 ± 130 mg, N = 4, P>0.05) of histamine.

Both L-arginine (100 mM) and L-lysine (100 mM) significantly reduced the pH of Krebs-Henseleit solution (control, pH: 7.218 ± 0.014, N = 3; L-arginine, pH: 7.033 ± 0.007, N = 3; L-lysine, pH: 6.943 ± 0.008, N = 3, P<0.05).

Methacholine. L-arginine (100 mM) failed to significantly reduce the contractile potency (EC_{30}, 95% confidence limits; control 0.47 µM (0.03-6.5), N = 4 *vs* L-arginine 0.56 µM (0.14-2.2), N = 4, P>0.05) and Emax (control 1.6 ± 0.4 g *vs* L-arginine 2 ± 0.6 g, N = 4, P>0.05) of methacholine (Figure 3A). Similarly, D-arginine (100 mM) also failed to significantly reduce the contractile potency (EC_{30}, 95% confidence limits; control 0.44 µM (0.06-3.2), N = 4 *vs* L-arginine 0.16 µM (0.04-0.69), N = 4, P>0.05) and Emax (control 1.6 ± 0.11 g *vs* L-arginine 1.6 ± 0.05 g, N = 4, P>0.05) of methacholine (Figure 3B).

Potassium chloride. L-arginine (100 mM) failed to significantly alter the contractile potency (EC_{30}, 95% confidence limits; control 3.2 mM (1.7-5.8), N = 6 *vs* L-arginine, 2.0 mM (0.8-5), N = 6, P>0.05) and Emax (control 700 ± 70 mg *vs* L-arginine 600 ± 60 mg, N = 6, P>0.05) of potassium chloride.

Guinea-pig trachea: relaxant responses

In histamine-contracted guinea-pig tracheal preparations, L-arginine (10-100 mM) induced a concentration-dependent relaxation in epithelium-intact and epithelium-denuded preparations (Figure 4). In the absence of L-NAME, epithelium removal failed to significantly alter the airway smooth muscle potency of L-arginine (EC_{50} 95% confidence limits; epithelium-intact, 26.9 mM (22.4-32.4), N = 6 *vs* epithelium-denuded, 16 mM (13.2-19.1), N = 6, P>0.05; Figure 4A). Similarly, epithelium removal failed to significantly alter the relaxant potency of L-arginine in the presence of L-NAME (EC_{50} 95% confidence limits; epithelium-intact, 26.3 mM (22.9-30.2), N = 6 *vs* epithelium-denuded, 20 mM (16.2-24.5), N = 6, P>0.05; Figure 4B). Furthermore, L-NAME failed to significantly attenuate the relaxant potency of L-arginine in epithelium-intact (P>0.05) or epithelium-denuded (P>0.05) preparations compared with preparations in the absence of L-NAME.

D-arginine (10-100 mM) also induced a concentration-dependent relaxation in epithelium-intact and -denuded preparations (Figure 5). In the absence of L-NAME, epithelium removal failed to significantly alter the airway smooth muscle potency of D-arginine (EC_{50} 95% confidence limits; epithelium-intact, 36.3 mM (28.3-46.5), N = 6 *vs* epithelium-denuded, 15.1 mM (12.3-18.6), N = 7, P>0.05; Figure 5A). Similarly, epithelium removal failed to significantly alter the relaxant potency of D-arginine in the presence of L-NAME (EC_{50} 95% confidence limits, epithelium-intact, 18.6 mM (14.8-23.4), N = 5 *vs* epithelium-denuded, 16.2 mM (13.2-19.5), N = 6, P>0.05; Figure 5B). Furthermore, L-NAME failed to significantly attenuate the relaxant potency of D-arginine in epithelium-intact (P>0.05) or epithelium-denuded preparations (P>0.05) compared with preparations in the absence of L-NAME.

In contrast to L-arginine, L-lysine (1-100 mM) failed to reverse histamine-induced contractile responses in guinea-pig epithelium-intact (N = 3) and epithelium-denuded (N = 3) tracheal preparations (data not shown).

Rabbit bronchus: contractile responses

L-arginine (100 mM) significantly reduced the contractile potency (EC_{30}, 95% confidence limits; control 10 µM (5.5-18), N = 8 *vs* L-arginine, 160 µM (91-275), N = 8, P<0.05) and Emax (control, 900 ± 160 mg *vs* L-arginine 300 ± 60 mg, N = 8, P<0.05) of

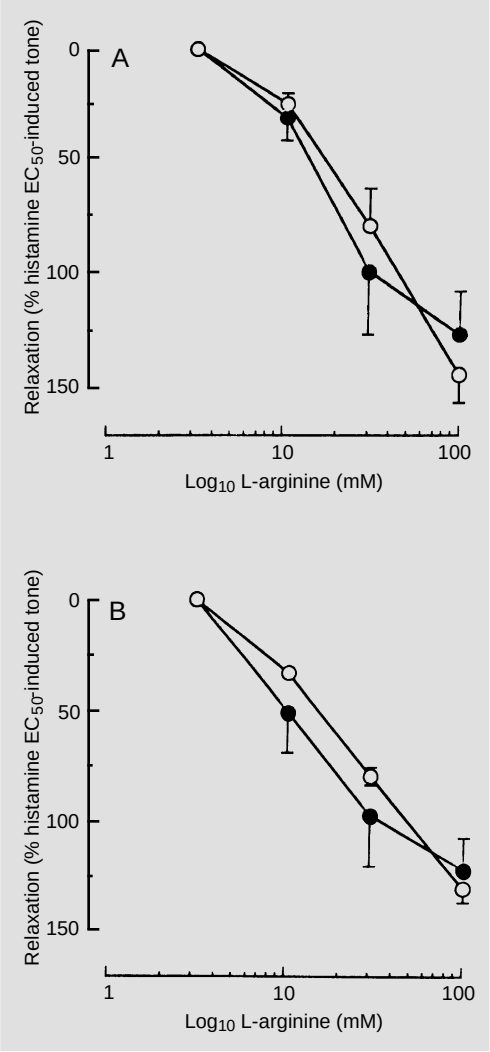

Figure 4 - Relaxation concentration-effect curves for L-arginine in absence (A) and presence (B) of L-NAME (50 mM) in epithelium-intact (open circles) and epithelium-denuded (closed circles) guinea-pig isolated trachea precontracted with histamine (EC_{50}). Each point represents the mean of 5-6 observations and vertical bars represent the SEM. Indomethacin (5 µM) is present throughout.

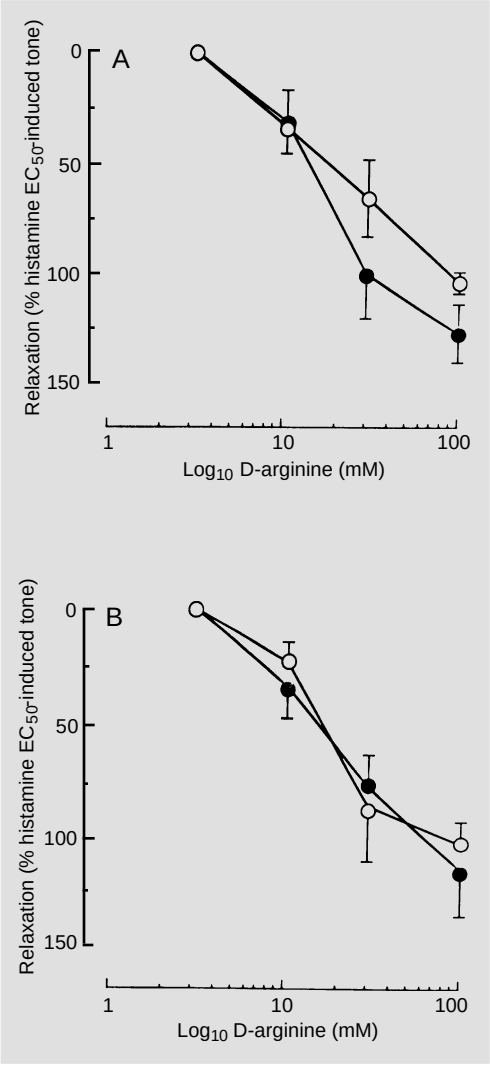

Figure 5 - Relaxant response curves for D-arginine in the absence (A) and presence (B) of L-NAME (50 µM) in epithelium-intact (open circles) and epithelium-denuded (closed circles) guinea-pig isolated trachea precontracted with histamine (EC_{50}). Each point represents the mean of 5-6 observations and vertical bars represent the SEM. Indomethacin (5 µM) is present throughout.

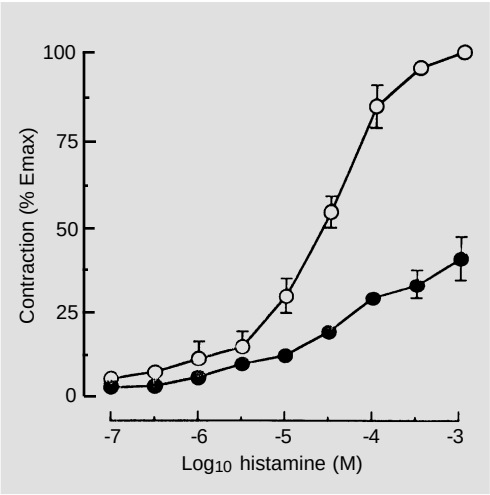

Figure 6 - Concentration-effect curves for histamine in the absence (open circles) or presence (closed circles) of L-arginine (100 mM) in rabbit isolated bronchus. Each point represents the mean of 8 observations and vertical bars represent the SEM. Indomethacin (5 µM) is present throughout.

histamine (Figure 6). Following removal of L-arginine from the organ bath, the contractile potency of and the contractile response to histamine were not significantly different from control ($P>0.05$).

Discussion

We have demonstrated that high concentrations (100 mM) of L- and D-arginine, but not L-lysine, reduced the Emax of histamine in guinea-pig isolated tracheal ring preparations, consistent with non-competitive antagonism of the contractile response to histamine. The same high concentration of L-arginine was also shown to reduce histamine responsiveness in rabbit bronchial preparations. Furthermore, in guinea-pig tracheal preparations the contractile response to histamine was reversed by L- and D-arginine in a concentration-dependent manner that was epithelium-independent and not mediated by L-NAME-sensitive pathways. In contrast, L-lysine did not relax tissues precontracted with histamine. Thus, it appears that L- and D-arginine can specifically interact with H_1-receptors and/or the microenvironment to selectively attenuate histamine-induced airway contractile responses.

A number of studies have demonstrated the ability of L- but not D-arginine to reverse the effect of nitric oxide synthase inhibitors on vascular smooth muscle relaxation (7) and on cholinergic nerve-induced contraction (8). Recently we have shown that L-NAME (0.1 µmol/site) inhibited bradykinin and histamine-induced plasma protein extravasation in guinea-pig skin (1). L-arginine (10 µmol/site) reduced the inhibitory effect of L-NAME on the bradykinin but not on the histamine response (1). In the absence of a nitric oxide synthetase inhibitor, high concentrations of L- and D-arginine also directly inhibited histamine-induced plasma protein extravasation in the skin and histamine-induced contraction of guinea-pig isolated ileum (1). This was most likely a conse-

quence of a non-competitive effect of L-arginine on H_1-receptors. Indeed, the lack of involvement of the nitric oxide synthetase pathway in this response is demonstrated by the lack of stereoselectivity observed between the effect of L- and D-arginine on histamine-induced contraction. In contrast, the nitric oxide synthase inhibitors L-NAME and N^G-monomethyl-L-arginine (L-NMMA) augmented histamine-induced bronchoconstriction in guinea-pigs that was reversed by aerosolised L- but not D-arginine, respectively (4). However, the effects of L- and D-arginine on histamine responsiveness in the absence of a nitric oxide synthetase inhibitor was not reported (4).

The ability of both L- and D-arginine to antagonise the histamine-, but not methacholine- or potassium chloride-induced contraction of epithelium-denuded guinea-pig trachea reported here is consistent with the ability of high concentrations of L- and D-arginine to inhibit the histamine- but not bradykinin-induced contraction of guinea-pig isolated ileum (1). Furthermore, the inhibitory response to these amino acids was non-competitive in nature and not dependent on charge as judged by the inability of L-lysine to inhibit the airway contractile response to histamine which we have also documented in the ileum (1). The inhibitory effect of L-arginine on the histamine-induced contraction could not be attributed to a fall in the pH of the Krebs-Henseleit solution, since L-lysine, which produced a similar effect on pH, failed to alter the contractile response to histamine in guinea-pig isolated trachea (this study) or guinea-pig ileum (1). Furthermore, the inability of L- and D-arginine to alter the contractile response to methacholine and potassium ions indicates that the action of these amino acids is not attributable to a nonspecific effect on airway smooth muscle function *per se*. The interaction between L-arginine and the H_1-receptor in airways is also not species dependent since a similar result was observed in rabbit isolated bronchus, although it should be noted that L-arginine does not inhibit histamine-induced plasma exudation in rabbit skin (3). This latter observation implies that the effect of L-arginine is not merely due to a chemical interaction with histamine.

Both L- and D-arginine reversed the histamine-induced contractile response by a mechanism that was epithelium-independent and not L-NAME-sensitive. L-lysine failed to relax histamine-contracted tissues, ruling out the possibility that transient changes in bath pH may have influenced smooth muscle tone. These data are also consistent with an interaction between high concentrations of L- and D-arginine and the H_1-receptor but do not confirm the observation that L- but not D-arginine (360 µM) relaxed histamine-induced contraction of guinea-pig perfused trachea denuded of epithelium and in the presence of L-NAME (4). The use of ring preparations, as opposed to tracheal segments, might account for the different findings since epithelium-derived nitric oxide might alter the permeability of the airway epithelium to various spasmogens that would not be a factor with ring preparations. The contractile response to methacholine or carbachol but not serotonin, leukotriene D_4 or arecoline was increased in the presence of L-NAME in guinea-pig perfused tracheal preparations (4). Whether differences in the lipophilicity of these agents (9,10) or their ability to simulate the release of nitric oxide (4) can account for these differential effects is not clear.

It was of interest that L-arginine was able to relax histamine-contracted tissues at concentrations that did not have any significant effect in antagonising the concentration-effect curve for histamine. This differential ability of L-arginine to reverse histamine-induced contractile responses compared with its effect on the histamine concentration-effect curve is analogous to the inability of the $beta_2$-adrenoceptor agonist, salbutamol, to shift the concentration-effect curves to

acetylcholine in human bronchus at concentrations which reversed acetylcholine-induced contractile responses (10) and of the phosphodiesterase type IV inhibitor, Ro 20-1724, to shift the concentration-effect curve to capsaicin in guinea-pig isolated trachea at concentrations which reversed capsaicin-induced contractile responses (11). These findings can be explained in terms of functional antagonism (12,13). Thus, if the receptor-subeffect response relationship to L-arginine has a much lower reserve than for histamine then one may expect only minimal antagonism of the concentration-effect curve for histamine. However, despite the lower reserve for L-arginine compared with histamine, L-arginine possesses a sufficient stimulus to reverse the contractile response to histamine.

In conclusion, we have demonstrated the ability of high concentrations of L- and D-arginine to interact with H_1-receptors in a non-competitive, reversible manner that is unrelated to changes in bath pH, chemical antagonism, charge or non-specific smooth muscle effects in airway tissue.

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