

INCREASED HISTAMINE H₁ RECEPTOR BLOCKADE BY CHLORPHENIRAMINE IN TRACHEAL CHAINS OF ASTHMATIC GUINEA PIGS

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ABSTRACT

Receptor affinity and drug delivery to the receptor sites could be determinant factors for the increased bronchial responsiveness seen in asthma. Competitive antagonism blockade which is measured as dose ratio-1 (DR-1) depends only on these two factors. Therefore, in this study we have examined histamine H₁ blockade by chlorpheniramine on isolated tracheal chains of asthmatic compared to control guinea pigs.

An experimental model of asthma was induced in guinea pigs by injection and inhalation of ovalbumin (OA), and tracheal chains of asthmatic and control animals (for each group n=12) were prepared. The responses of tracheal chains to cumulative concentrations of histamine (H) in the absence and presence of 5 nM chlorpheniramine was measured, and the effective concentration of H causing 50% of maximum response (EC₅₀ H) was obtained. The chlorpheniramine blockade (DR-1) was calculated by (post chlorpheniramine EC₅₀H/EC₅₀ H)-1. The response of tracheal chains to 0.1% OA relative to the contraction obtained by 10 mM methacholine was also measured.

The tracheal response of asthmatic guinea pigs to OA was significantly higher than that of control animals (mean ± SEM, 57.03±4.99 vs. 3.92±1.14, *p*<0.001). Histamine H₁ receptor blockade by chlorpheniramine (DR-1) was also significantly higher in tracheas of asthmatic compared to control animals (17.34±3.89 vs. 4.11±1.08, *p*<0.01). There was a significant correlation between (DR-1) and tracheal responses to OA (*r*= 0.51, *p*<0.05).

This enhanced histamine H₁ receptor blockade in tracheal chains of asthmatic animals confirms our previous findings which was predicted to be due to increased drug delivery to the receptor. Drug delivery could also be a determinant factor for bronchial responsiveness to most stimulating agents in asthma.

Keywords: Histamine H₁ receptors, chlorpheniramine blockade, asthma, bronchial responsiveness

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INTRODUCTION

The most characteristic feature of asthma is bronchial hyperresponsiveness to a wide variety of inhaled physical, chemical, pharmacological and immunological stimuli. Responsiveness to many agents, including pharmacological agonists, can be accurately quantified; and the level of responsiveness has been shown to correlate loosely with the severity of asthma.¹ However, the mechanisms of the bronchial response to pharmacological agonists such as histamine and methacholine is highly complex and inadequately understood. The response to agonist substances involves many stages, including access to receptor sites and receptor occupancy. Receptor occupancy results in activation and the intracellular synthesis and release of second messengers leading to other intracellular reactions which finally cause airway narrowing. It is mainly due to this complexity that little is known about the underlying cause(s) of bronchial hyperresponsiveness in asthma.

To elucidate this complex causation we have chosen to compare competitive antagonists' blockade at the tracheobronchial tree of asthmatic and normal subjects. A pure competitive antagonist merely has to gain access to the receptor and bind to it. Receptor occupancy leads to a quantifiable rightward shift in the dose-response curve of the corresponding agonist. Based on *in vitro* steady-state experiments of Arunlakshana and Schild,² the degree of rightward shift measured as dose ratio (DR) is determined only by the concentration of antagonist at the receptor (II), which depends on dose and delivery and receptor affinity (ka).

In previous studies we demonstrated that competitive antagonism by atropine, chlorpheniramine, and propranolol at the bronchial muscarinic, histaminic (H₁), and adrenergic receptors was greater in asthmatic patients than in normal subjects.³⁻⁵ The highest level of blockade was seen in asthmatic patients who were most sensitive to inhaled methacholine, histamine, and isoprenaline and the lowest level in the least sensitive normal subjects.

However, in our *in vivo* study of competitive antagonism blockade in the tracheobronchial tree, a steady state could not be achieved due to several mechanisms, including: 1) local metabolism of agonist and antagonist substances, 2) removal of substances by local blood flow, and 3) background cholinergic, adrenergic and histaminergic effects. So applying the principles of Arunlakshana and Schild's study to non-steady state conditions *in vivo* is problematic. Therefore, in the present *in vitro* study, we have reexamined chlorpheniramine blockade at histamine H₁ receptors in tracheal chains of control and experimentally-induced asthmatic guinea pigs.

METHODS

Induction of guinea pig model of asthma

Guinea pig models of asthma were induced as previously described.^{6,7} Briefly, 24 male guinea pigs (500-800 g) were divided randomly into 2 groups of 12. Animals of group one were sensitized to OA (Sigma Chemical Co. Ltd., UK) by injecting 100 mg i.p. and 100 mg s.c. on day one and a further 10 mg i.p. on day 8. From day 14 sensitized animals were exposed to an aerosol of 4% OA for 18±1 days, 4 min daily. The aerosol was administered in a closed chamber, dimensions 30×20×20 cm. Group 2 served as controls and were treated identically, except that 0.9% saline vehicle alone was used throughout.

Tissue preparations

Guinea pigs were killed by a blow on the neck on day 30-32, and tracheas were removed. Each trachea was cut into 10 rings (each containing 2-3 cartilaginous rings). All the rings were then cut open opposite the trachealis muscle, and sutured together to form a tracheal chain.^{7,8}

Tissue was then suspended in a 10 mL organ bath (organ bath 61300, BioScience Palmer-Washington, Sheerness, Kent, UK) containing Krebs-Henseliet solution of the following composition (mM): NaCl 120, NaHCO₃ 25, MgSO₄ 0.5, KH₂PO₄ 1.2, KCl 4.72, CaCl₂ 2.5 and dextrose 11.

The Krebs solution was maintained at 37°C and gassed with 95% O₂ and 5% CO₂. Tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1h while it was washed with Krebs solution every 15 min.

Table I. Values of tracheal response to ovalbumin, histamine (EC₅₀ H), and histamine H₁ receptors' blockade by chlorpheniramine (DR-1) in control (n= 12) and sensitized guinea pigs (n= 12) and statistical differences between the two groups. Values are quoted as both arithmetic mean±SEM and geometric mean. In comparing values between the two groups, both the unpaired "t" test and the Mann Whitney "U" test were employed.

Tracheal response	Control	Asthmatic	p value "t" test	p value "U" test
Albumin response	3.92±1.14	57.03±4.99	p<0.001	
Geometric mean	2.89	54.63		p<0.001
EC ₅₀ H (µM)	26.25±5.34	26.92±3.49	p= 0.918	
Geometric mean	22.31	24.08		p<0.05
DR-1	4.11±1.08	17.34±3.89	p<0.01	
Geometric mean	2.57	11.76		p<0.005

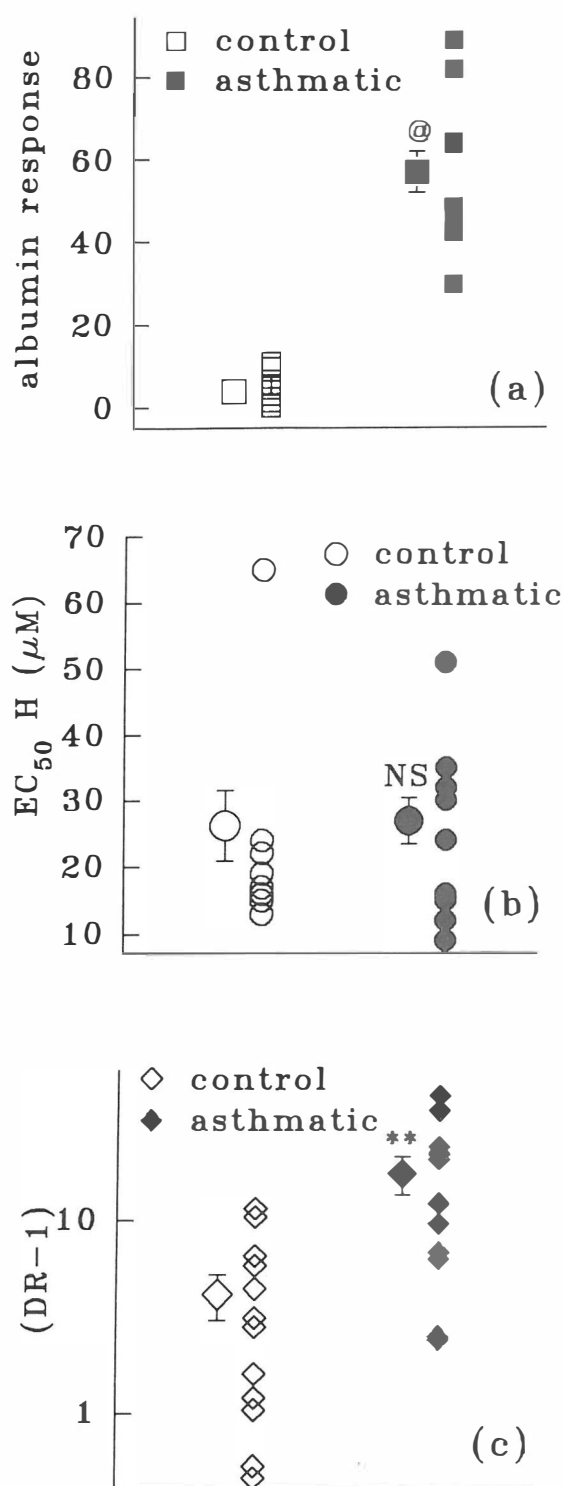


Fig. 1. Individual values and mean \pm SEM (big symbols with bars) of tracheal response to ovalbumin (a), histamine ($EC_{50} H$) (b), and histamine H_1 receptor blockade by chlorpheniramine ($DR-1$) (c), in control ($n=12$) and asthmatic guinea pigs ($n=12$) and statistical differences between the two groups.

NS: non-significant difference, ** $p < 0.01$, @: $p < 0.001$ compared to control group.

Measurement of tracheal response to ovalbumin

The tracheal response of all animals to 0.1% solution of OA was measured as follows:

1) 0.25 mL of 4% OA solution was added to the organ bath.

2) The degree of tracheal chain contraction was recorded after 15 minutes.

3) The degree of tracheal chain contraction was then expressed as a proportion (in percentage) of the contraction obtained by 10 mM methacholine hydrochloride (MW=196, Sigma Chemical Co. Ltd., UK).

Assessment of tracheal response to histamine and histamine H_1 receptor blockade

1) In each experiment two cumulative log concentration-response curves (LCRC) of histamine phosphate (MW=308, BDH Chemical Co. Ltd., UK) - induced contraction of tracheal chains were obtained, one 10 min after introducing a 5 nM concentration of chlorpheniramine maleate (MW=391, Sigma Chemical Co. Ltd., UK) in the organ bath (adding 0.05 mL of 1 μM chlorpheniramine solution to organ bath = post-chlorpheniramine histamine response curve), and the other 10 min after adding the same volume of saline (baseline histamine response curve).

2) Cumulative log concentration-response curve of the tracheal chain to increasing concentrations of histamine (0.1 μM to 10 mM) was obtained with addition of consecutive concentrations every 2 min. To obtain the curve, the percentage of contraction of the tracheal smooth muscle due to each concentration of histamine in proportion to the maximum contraction obtained by the final concentration of histamine (10 mM), in the baseline histamine response curve, was calculated and plotted against the log concentration of histamine.

3) The effective concentration of histamine causing 50% of the maximum response ($EC_{50} H$) of baseline and the post-chlorpheniramine histamine response curve in each experiment was measured (expressed as $EC_{50} H$ and post-chlorpheniramine $EC_{50} H$, respectively).

4) The tracheal response to histamine was considered as $EC_{50} H$.

5) The histamine H_1 receptor blockade by chlorpheniramine was assessed as dose ratio minus one ($DR-1$) which was calculated by: (post-chlorpheniramine $EC_{50} H/EC_{50} H$)-1.

The experiments for measuring the tracheal response to OA, post-chlorpheniramine histamine response curve, and baseline histamine response curves in each tracheal chain were performed randomly with 1h resting periods between each two experiments while washing the tissues every 10 min. Tracheal responses to histamine were tested on incubated tissues with 1.4 μM indomethacin 30 min prior and during obtaining LCRC in the presence of

Histamine H₁ Receptor Blockade in Asthma

Table II. Correlations between isolated tracheal response of control and asthmatic guinea pigs to ovalbumin(OA), histamine (EC₅₀H) and histamine H₁ receptor blockade by chlorpheniramine (DR-1).

Statistical test	OA-response vs. EC ₅₀ H	DR-1 vs. OA-response	DR-1 vs. EC ₅₀ H
Least squares regression (r)	-0.034	0.51	-0.312
p value	p= 0.873	p<0.05	p= 0.137
Coefficient of determination (r ²)	0.0012	0.26	0.097
Spearman rank correlation (r _s)	-0.157	0.506	-0.18
p value	p= 0.465	p<0.05	p= 0.399

both saline and chlorpheniramine. In all experiments responses were recorded on a kymograph (ET8 G-Boulitte, Paris) and measured after fixation.

Statistical analysis

The data of tracheal response to OA, tracheal response to histamine (EC₅₀H), and histamine H₁ receptor blockade (DR-1) were quoted as both arithmetic mean ± SEM and geometric mean because it has been shown previously that these values on *in vivo* studies are non-normally distributed.⁹ In comparing these values between asthmatic and control guinea pigs, both the unpaired "t" test and the Mann Whitney "U" test were employed. Tracheal responses to OA were related to tracheal responses to histamine (EC₅₀H), and both of these to (DR-1), using the least square regression and also using Spearman rank correlation to avoid any assumption of normal distribution of the data.

RESULTS

Tracheal response to ovalbumin

Tracheal response to OA in tracheal chains of asthmatic animals (57.03 ± 4.99, range 29.8-89) was significantly higher than in control animals (3.92 ± 1.14, range 0-11.1, p < 0.001), (Table I, Fig. 1a).

Tracheal response to histamine

The mean value of EC₅₀H in tracheal chains of asthmatic animals (26.92 ± 3.49 μM, range 9.0-51.0 μM) was not significantly different from that of control animals (26.25 ± 5.34 μM, range 13.0-65.0 μM), (Table I, Fig. 1b).

Chlorpheniramine blockade (DR-1)

The rightward shift of the post-chlorpheniramine

histamine response curve compared to the baseline histamine response curve in tracheal chains of asthmatic animals was greater than in control animals (Fig. 2). Mean DR-1 in tracheal chains of asthmatic animals (17.34 ± 3.89, range 2.4-44.8) was 4.1 times greater than in control animals (4.11 ± 1.08, range 0.46-11.46, p < 0.01) (Table I, Fig. 1c). The value of DR-1 in the most sensitive trachea of asthmatic animals was 97.4 times greater than the least sensitive trachea of the control animals.

Relationship between bronchial response to stimuli (ovalbumin and histamine) and chlorpheniramine blockade

There was no significant correlation between tracheal response to ovalbumin and EC₅₀H (r = -0.034, p = 0.873), or between DR-1 and EC₅₀H (r = -0.312, p = 0.137) (Table II). However, there was a significant correlation between DR-1 and the tracheal response to OA (r = 0.51, p < 0.05, r² = 0.26) (Table II, Fig. 3).

DISCUSSION

This study showed an increased tracheal response to ovalbumin of sensitized compared to control guinea pigs, which was very similar to the bronchial hyperresponsiveness of asthmatic patients. This increased tracheal response confirms the induction of asthma in sensitized guinea pigs. The results of this study also demonstrated that the histamine H₁ receptor blockade by chlorpheniramine is enhanced in tracheal chains of asthmatic guinea pigs. The results of this *in vitro* study confirm our previous *in vivo* findings with atropine,³ chlorpheniramine,⁴ and propranolol.⁵

The difference of chlorpheniramine blockade (DR-1) between asthmatic and control tracheal chains (4.1 fold)

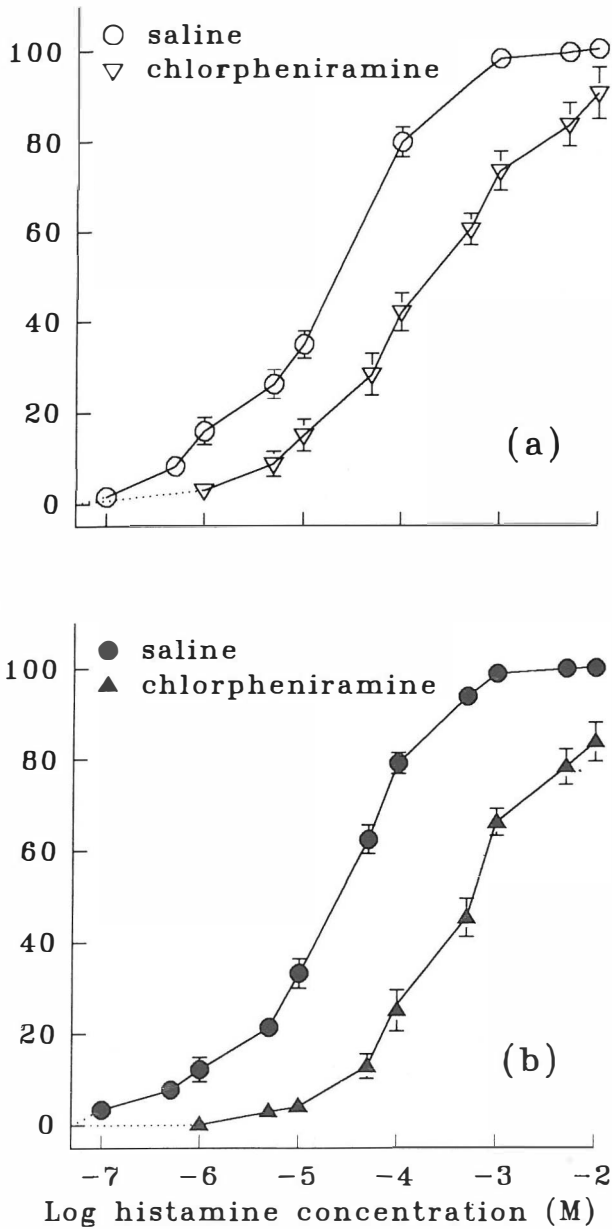


Fig. 2. Cumulative log concentration-response curves of histamine induced contraction of isolated trachea in the presence of saline and chlorpheniramine of control (a) and sensitized (asthmatic) guinea pigs (b) (for each group n= 12).

in the present study was fairly similar to our previous *in vivo* results for differences of antagonism blockade between asthmatic and normal subjects (the increased antagonism blockade in asthmatic patients compared to normal subjects of inhaled atropine, chlorpheniramine, and isoprenaline were 6.5, 6.8, and 11 fold, respectively). According to a classical pharmacological study,² the consistent antagonism blockade of the present *in vitro* study and our previous *in vivo* studies indicated that in asthma either receptor affinity (K_a) or drug delivery to

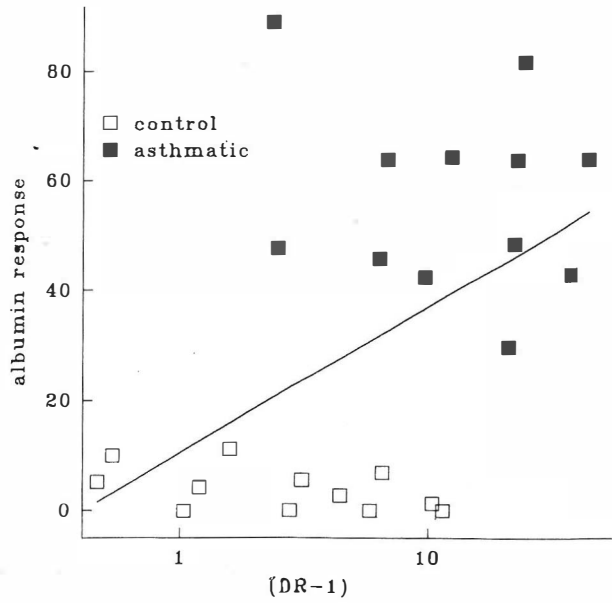


Fig. 3. Correlation between chlorpheniramine blockade (DR-1) and tracheal response to ovalbumin of control and asthmatic guinea pigs. $r= 0.51, p<0.05$.

the receptor (II) or both of these factors are increased. The fact that three receptor systems showed enhanced competitive antagonism blockade in asthma on *in vivo* studies and one receptor system on an *in vitro* study suggest that the abnormality lies with [I] rather than K_a . This conclusion is also supported by *in vitro* experiments which suggest that receptor affinity for a given antagonist shows little variation between species and tissues.¹⁰ We therefore suggest that this enhanced antagonism blockade may be caused by epithelial damage leading to increased epithelial permeability and accessibility of ligands to the receptor sites.

Epithelial damage is a well-recognized feature in asthma as inflammation^{13,14} and this appears to increase the permeability to small charged molecules.¹⁵ Airway inflammation in asthma has been known for a long time even in mild disease.¹⁶ One of the consequences of this inflammation is airway epithelial damage.¹⁷ In fact, several *in vitro* studies have shown that epithelial damage leads to increased bronchial responsiveness to different pharmacological agonists.^{8,18,19} Serosal vs. mucosal application of agonist ligands also leads to increased bronchial responsiveness,²⁰ but denudation of epithelium diminished this increased responsiveness.²¹ The cause of increased bronchial responsiveness in all of these studies was increasing permeability and easier access of ligands to the receptors. In addition, a significant correlation has been observed between epithelial damage and bronchial hyperresponsiveness in asthma.^{12,22} There is also a close

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association between airway inflammation, epithelial damage and bronchial hyperresponsiveness both in sensitized animals²³ and in asthmatic patients.²⁴ Thus airway inflammation can cause epithelial damage; and this, in turn, can result in better access of ligands to the active sites in the airways, causing the bronchial hyperresponsiveness observed in asthma.

However, our previous studies³⁻⁵ also showed enhanced blockade when pharmacological antagonists were administered by i.v. injection; and this cannot be due to increased epithelial permeability. Alternatively, if antagonist ligands were more slowly metabolized in asthmatic bronchi, this would increase [I] with both routes of administration. The strongest possibility is an increased tissue permeability due to airway inflammation. If the permeability of a physical barrier of some kind close to the receptor was increased in asthma, it could increase diffusion of antagonist ligands however administered and would explain the variation in DR-1 produced by both routes of administration. In addition, the results of the present *in vitro* study cannot be fully explained by increased epithelial permeability because the barrier role of epithelium against ligand diffusion²⁵ may be appreciated only when perfused tracheal or bronchial tubes are exposed to ligands from the mucosal sides but not in the model used in our study. A strip of tracheal smooth muscle is covered by epithelium only from one side and easily exposed to chemicals from the remaining three sides; this mostly excludes the barrier function of the epithelium. The increased chlorpheniramine blockade in tracheal chains of asthmatic guinea pigs shown in this study, as well as the increased atropine, chlorpheniramine, and propranolol blockade in our previous studies³⁻⁵ is perhaps due to the higher concentration of antagonists at the receptor sites achieved by increased epithelial and tissue permeability leading to an increase in [I].

The results of this study also showed significant correlations between histamine H₁ receptor blockade by chlorpheniramine DR-1 and the tracheal response to ovalbumin. The significant correlation between DR-1 and tracheal response to OA in the present study, as well as other antagonists' blockade and agonists' responsiveness in previous studies³⁻⁵ indicates that bronchial hyperresponsiveness to most stimuli in asthma, at least in part, is due to increased bronchial epithelial and tissue permeability which is perhaps due to airway inflammation in asthma.

Although our previous *in vivo* studies³⁻⁵ showed that differences in bronchial responsiveness to pharmacological agonists between asthmatic and normal subjects are higher than the differences in competitive antagonism blockade between the groups (asthmatics were 54.6, 22.5. and 15 times more sensitive to

methacholine, histamine, and isoprenaline, respectively, compared to normal subjects; but antagonist blockade for atropine, chlorpheniramine, and propranolol were 6.5, 6.8, and 11 times higher in asthmatic patients, respectively), the present *in vitro* study indicated increased chlorpheniramine blockade at histamine H₁ receptors but did not show enhanced tracheal response to histamine in asthmatic compared to control animals. It is difficult to explain why the tracheal response to histamine was not increased in asthmatic animals. Thompson et al.²⁶ and Sadeghi-Hashjin et al.²⁷ also showed that removal of epithelium from feline and bovine trachea did not influence the responsiveness of tracheal strips to histamine. This discrepancy could be explained by differences between *in vitro* and *in vivo* conditions. It might be thought that *in vivo* results could be affected by autonomic background effects, blood flow, and other similar factors. The non-significant relationship between *in vivo* airway respo

agents and *in vitro* sensitivity of isolated airway smooth muscle to the same agents²⁸⁻³⁰ supports this hypothesis. However, other studies showed an increased tracheal response to histamine in the absence of epithelial barrier.^{8,18,20,21} Masaki et al.³¹ have also shown a significant correlation between bronchial responsiveness of sensitized guinea pigs on *in vivo* and *in vitro* experiments. Therefore, another explanation for this discrepancy could be the differences between experimentally induced asthma and actual asthma disease. Obviously all aspects of asthma cannot be produced in the animal model of this disease; and this seems to be the strongest explanation for this discrepancy.

In conclusion, this study demonstrated enhanced histamine H₁ receptor blockade by chlorpheniramine in tracheal chains of asthmatic guinea pigs which confirms our previous *in vivo* results. The cause of this enhanced antagonism blockade at the tracheobronchial tree in asthma is perhaps increased epithelial and tissue permeability due to airway inflammation. The increased epithelial and tissue permeability of the airways is, at least in part, responsible for the bronchial hyperresponsiveness to most stimuli seen in asthma.

REFERENCES

1. Britton JR, Burney PG, Chinn S, Paracosta A, Tattersfield AE: The relationship between change in airway reactivity and change in respiratory symptoms and medication in a community study. *Am Rev Respir Dis* 138: 530-534, 1988.
2. Arunlakshana O, Schild HO: Some quantitative uses of drug antagonists. *Br J Pharmacol* 14: 48-58, 1959.
3. Boskabady MH, Snashall PD: Enhanced muscarinic

- receptor blockade with atropine in the asthmatic tracheobronchial tree: evidence for increased drug delivery. *Am Rev Respir Dis* 145: 756-761, 1992.
4. Boskabady MH, Snashall PD: Histamine (H₁) receptor affinity and bronchial responsiveness to histamine in man. *Eur Respir J* 3(Suppl 10): 131S, 1990.
 5. Boskabady MH, Snashall PD: Increased airway responsiveness to isoprenaline in asthma. *Eur Respir J* 4(suppl 14): 460s, 1991.
 6. McCaig D: Comparison of autonomic responses in the trachea isolated from normal and albumin-sensitive guinea-pig. *Br J Pharmacol* 92: 809-816, 1987.
 7. McCaig D, De Jonckheere S: Effect of two Ca²⁺ modulator in normal and albumin-sensitized guinea-pig trachea. *Eur J Pharmacol* 249: 53-63, 1993.
 8. Holroyde MC: The influence of epithelium on the responsiveness of guinea-pig isolated trachea. *Br J Pharmacol* 87: 501-507, 1986.
 9. Gillett MK, Briggs BA, Snashall PD: The influence of aerosol retention and pattern of deposition on bronchial responsiveness to atropine and methacholine in man. *Am Rev Respir Dis* 140: 1727-1733, 1989.
 10. Bowman WC, Rand MJ (eds.): *Textbook of Pharmacology*. 2nd ed., Oxford: Blackwell Scientific Publications, p. 39.25, 1980.
 11. Laitinen LA, Heino M, Laitinen A, Kava T, Haantela T: Damage of airway epithelium and bronchial reactivity in patients with asthma. *Am Rev Respir Dis* 131: 599-606, 1985.
 12. Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB: Bronchial biopsies in asthma: an ultrastructural quantitative study and correlation with hyperactivity. *Am Rev Respir Dis* 140: 1745-1753, 1989.
 13. Lundgren R, Soderberg M, Horstedt P, Stenling R: Morphological studies of bronchial mucosal biopsies from asthmatics before and after ten years of treatment with inhaled steroid. *Eur Respir J* 1: 883-889, 1988.
 14. Martin RJ, Cicutto LC, Smith HR, Ballord RD, Szeffler SJ: Airway inflammation in nocturnal asthma. *Am Rev Respir Dis* 143: 351-357, 1991.
 15. Ilowite JS, Bennett WD, Sheetz MS, Groth ML, Nierman DM: Permeability of bronchial mucosa to ^{99m}Tc-DTPA in asthma. *Am Rev Respir Dis* 139: 1139-1143, 1989.
 16. Busse W, Banks-Schlegel SP, Larson GL: Childhood versus adult-onset asthma. *Am J Respir Crit Care Med* 151: 1635-1639, 1995.
 17. Filley WV, Kephart GM, Holley KE, Gleich GJ: Identification by immunofluorescence of eosinophil granule MBP in lung tissue of patients with bronchial asthma. *Lancet* July 3: 11-15, 1982.
 18. Flavahan NA, Aarhus LL, Rimele TJ, Vanhoutte PM: Respiratory epithelium inhibits bronchial smooth muscle tone. *J Appl Physiol* 58: 834-838, 1985.
 19. Mapp CE, Boniotti A, Papi A, Chitano P, Saetta M, Di Stefano A, Ciaccia A, Fabbri LM: The effect of phosphoramidon and epithelium removal on toluene diisocyanate-induced contraction in guinea-pig bronchi. *Eur Respir J* 5: 331-333, 1992.
 20. Fedan JS, Nutt ME, Frazer DG: Reactivity of guinea-pig isolated trachea to methacholine, histamine and isoproterenol applied serosally versus mucosally. *Eur J Pharmacol* 190: 337-345, 1990.
 21. Munakata M, Huang I, Mitzner W, Menkes H: Protective role of epithelium in the guinea-pig airway. *J Appl Physiol* 66: 1547-1552, 1989.
 22. Beasley R, Roche WR, Roberts JA, Holgate ST: Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 139: 806-817, 1989.
 23. Padrid P, Snook S, Finucane T, Shiue P, Cozzi P, Solway J, Leff AR: Persistent airway hyperresponsiveness and histologic alteration after chronic antigen challenge in cats. *Am J Respir Crit Care* 151: 184-193, 1995.
 24. Booth H, Richmond I, Ward C, Gardiner PV, Harkawat R, Walters HE: Effect of high dose inhaled fluticasone propionate on airway inflammation in asthma. *Am J Respir Crit Care Med* 152: 45-52, 1995.
 25. Mitchell HW, Willet KF, Sparrow MP: Perfused bronchial segment and bronchial strip: narrowing vs. isometric force by mediators. *J Appl Physiol* 66: 2704-2709, 1989.
 26. Thompson DC, Wells JL, Altieri RJ, Diamond L: The effect of epithelium removal on non-adrenergic, non-cholinergic inhibitory response in isolated central airways of the cat and guinea pig. *Eur J Pharmacol* 145: 231-237, 1988.
 27. Sadeghi-Hashjin G, Henricks PAJ, Folkerts G, Verheyen AKCP, Van der Linde HJ, Nijkamp FP: Bovine tracheal responsiveness *in vitro*: role of the epithelium and nitric oxide. *Eur Respir J* 9: 2286-2293, 1996.
 28. Roberts JA, Rodger IW, Thomson NC: Airway responsiveness to histamine in man: effect of atropine on *in vivo* and *in vitro* comparison. *Thorax* 40: 261-267, 1985.
 29. Armour CL, Black JL, Berend N, Woolcock AJ: The relationship between bronchial hyperresponsiveness to methacholine and airway smooth muscle structure and reactivity. *Respir Physiol* 58: 223-233, 1984.
 30. Cerina J, Laduire M, Le R, Labat C, Raffestin B, Bayol A, Brink C: Comparison of human bronchial muscle responses to histamine *in vivo* with histamine and isoproterenol *in vitro*. *Am Rev Respir Dis* 134: 57-61, 1986.
 31. Masaki Y, Munakata M, Amishima M, Homma Y, Kawalami Y: *In vivo*, *in vitro* correlation of acetylcholine airway responsiveness in sensitized guinea-pigs. *Am J Respir Crit Care Med* 149: 1494-1498, 1994.

