



The Effect of Exposure of Guinea Pig to Cigarette Smoke and their Sensitization in Tracheal Responsiveness to Histamine and Histamine Receptor (H_1) Blockade by Chlorpheniramine

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Abstract

Airway responsiveness to histamine and histamine H_1 receptor blockade by chlorpheniramine (CR-1) on guinea pig trachea were examined. Chronic obstructive pulmonary disease (COPD) and asthma were induced in guinea pigs by exposing them to cigarette smoke for 3 months and by sensitization with injection and inhalation of ovalbumin (OA). The responses of tracheal chains of COPD ($n=8$), COPD + asthma ($n=6$) and control animals ($n=8$) to histamine (EC_{50} H) and (CR-1) were measured.

The in vitro histamine responses of COPD and COPD + asthmatic guinea pigs in tracheal chains were significantly higher than those of control animals ($p<0.001$ and $p<0.01$, respectively). The CR-1 blockade was also significantly greater in trachea of COPD and COPD + asthma compared to that of controls ($p<0.01$ and $p<0.05$, respectively). There were significant correlations between EC_{50} H and (CR-1) ($r=-0.542$, $p<0.01$). The hematocrit in COPD and COPD + asthma groups was also significantly higher than in controls ($p<0.001$ for both groups). The contractility of tracheal chains to histamine in COPD + asthma animals was significantly greater than those of control and COPD groups ($p<0.05$ for both cases). The differences in contractility between COPD and COPD + asthmatic groups, however, suggests different basic mechanisms for AHR in COPD and asthma.

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1. Introduction

Chronic obstructive pulmonary disease (COPD) is a global health problem, reaching almost epidemic proportions in the developing world [1]. The understanding of basic mechanisms of the disease and the development of new strategies to prevent the progression of this condition presents a major challenge.

It is believed that in cigarette smokers the airflow obstruction is caused by parenchyma disease (emphysema) and/or by smoke-induced distortion of the structure of the small airways [2–4]. A model of cigarette smoke-induced lung disease has been described in guinea pigs, which develop airflow obstruction and emphysematous lung destruction [5].

The most characteristic feature of asthma is bronchial hyperresponsiveness to a wide variety of inhaled physical, chemical, pharmacological and immunological stimuli. There are reports regarding airway hyperresponsiveness to different stimuli also in animals exposed to cigarette smoke [6–11]. However, the mechanisms of the bronchial response to pharmacological agonists such as methacholine and histamine are highly complex and inadequately understood. Therefore, little is known regarding the mechanism(s) of increased airway responsiveness (AHR).

The mechanism(s) of action of a competitive antagonist measured as the degree of rightward shift or dose ratio or concentration ratio (DR or CR) is far simpler than that of an agonist. It depends only to concentration of antagonist at the receptor sites ($[I]$) and receptor affinity (K_a) [12]. Receptor blockade by a competitive antagonist could help in revealing the mechanism(s) of airway responsiveness. In previous

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studies we have demonstrated enhanced blockade of different receptors by their antagonists both in asthmatic patients [13–15] and sensitized animals [16,17].

In the present study the tracheal responsiveness of guinea pigs exposed to cigarette smoke alone (an animal model of COPD), and exposed to cigarette smoke + sensitized to OA (an animal model of COPD and asthma) to histamine and histamine (H_1) receptor blocked by chlorpheniramine was studied.

2. Material and methods

2.1. Animals, cigarette smoke exposure and sensitization

Twenty-two adult Dunkin–Hartley guinea pigs (400–500 g) of both sexes were divided into three groups, two experimental groups including cigarette smoke exposure alone (an animal model of COPD, $n=8$), and exposed to cigarette smoke + sensitized to OA (an animal model of COPD + asthma, $n=6$) and one control group ($n=8$).

Experimental animals were exposed to cigarette smoke as previously described [18–20]. The animals were exposed to cigarette smoke in an awake, restrained state and spontaneously breathing in a smoking chamber which was a modification of that described by Simani et al. [18]. Animals were placed in a Plexiglas box with their heads secured in a compartment (15 cm $\times$ 12 cm $\times$ 7 cm). Twenty-milliliter puffs of cigarette smoke was drawn out of cigarettes with syringes and then exhausted at a rate of two puffs per minute into the animal's head chamber. Exposure of animals to each cigarette lasted for 8–9 min, with a 10 min resting period between cigarettes. The animals were initially exposed to one commercial non-filter cigarette per day, and this dose was increased to a maximum of 5 cigarettes per day over a 2-week period. In a pilot study it was observed that animals could not tolerate the exposure of cigarette smoke of more than 5 cigarettes per day. The exposure to the smoke of 5 cigarettes per day, 6 days per week, continued for 3 months. The guinea pigs exposed to cigarette smoke were called COPD animals. The control animals were not exposed to cigarette smoke, and they were kept in the animal house under normal conditions for the same period of time.

Sensitisation of animals to ovalbumin (OA) was performed using the method described by McCaig [21–23] during their exposure period to cigarette smoke. Briefly, 6 guinea pigs were sensitised to ovalbumin (Sigma Chemical Ltd., UK) by injecting 100 mg i.p. and 100 mg s.c. on day 1 and a further 10 mg i.p. on day 8. From day 14 sensitised 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 cm $\times$ 20 cm $\times$ 20 cm.

The study plan was approved by the Ethical Committee of the Mashhad University of Medical Sciences.

2.2. Tissue preparation

Guinea pigs were killed by a blow on the neck. Trachea was removed and 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 [21,22] and then suspended in a 10 ml organ bath (organ bath 61300, BioScience Palmer-Washington, Sheerness, Kent, UK) containing Krebs–Henseleit solution of the following composition (mmol/l): NaCl 120, NaHCO_3 25, MgSO_4 0.5, KH_2PO_4 1.2, KCl 4.72, CaCl_2 2.5 and dextrose 11 maintained at 37 °C and gassed with 95% O_2 and 5% CO_2 . Tissue was suspended under isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

2.3. Measurement of tracheal responses to histamine and histamine (H_1) receptor blockade

In each experiment two cumulative log concentration–response curves (LCRC) of histamine phosphate (BDH Chemical Co. Ltd., UK) induced contraction of tracheal chain were obtained, one 10 min after producing 10 nM concentration of chlorpheniramine maleate (Sigma Chemical Ltd., UK) in organ bath (adding 0.1 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).

Cumulative log concentration–response curve of tracheal chain to increasing concentrations of histamine (0.1 μM –10 mM) was obtained with additions 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, in baseline histamine response curve, was calculated and plotted against log concentration of histamine.

The effective concentration of histamine causing 50% of maximum response (EC_{50} H) of baseline and post-chlorpheniramine histamine response curve in each experiment was measured (expressed as EC_{50} H and post-chlorpheniramine EC_{50} H, respectively). The tracheal response to histamine was considered as EC_{50} H. The histamine H_1 receptor blockade by chlorpheniramine was assessed as concentration ratio minus one (CR-1) which was calculated by: (post-chlorpheniramine EC_{50} H/ EC_{50} H) – 1.

The experiments to measure post-chlorpheniramine histamine response curve and baseline histamine response curve in each tracheal chain were performed randomly with 1 h resting period between each of the 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 both saline and chlorpheniramine. In all experiments responses were recorded on a kymograph (ET8 G-Boulitt, Paris, France) and measured after fixation.

2.4. Pathological evaluation

The lungs of all COPD and COPD + asthmatic animals underwent normal pathological evaluation using hematoxylin-eosin staining.

2.5. Statistical analysis

The data of tracheal responses to histamine (EC_{50} H), histamine H_1 receptor blockade (CR-1) maximum response, muscle contractility and hematocrit have been expressed as mean $\pm$ S.E.M. In comparing all values between COPD, COPD + asthmatic and control guinea pigs ANOVA test were employed. Tracheal response to histamine (EC_{50} H), was related to (CR-1), using the least square regression. Significance was accepted at $p < 0.05$ level.

3. Results

3.1. Histology

The following pathological changes were observed in the lungs of both COPD and COPD + asthmatic animals (Fig. 1).

(1) Increased intra-alveolar septum in all specimens (due to infiltration of leukocytes and penetration of smoke particle). (2) Increased lymphatic tissue in the lung parenchyma of all specimens. (3) Destruction of alveolar wall and existence of emphysema in the lungs of most animals. (4) Intra-alveolar bleeding in most of animal's lungs. In addition the following pathological changes were observed in the lungs of COPD + asthmatic animals (Fig. 1): epithelial damage and paranchymal eosinophilia.

3.2. Tracheal response to histamine

The EC_{50} H in tracheal chains of COPD animals ($20.13 \pm 2.4 \mu\text{M}$, range 8–28 μM) was significantly lower than in control animals ($34.37 \pm 1.79 \mu\text{M}$, range 28–45 μM , $p < 0.001$) (Table 1, Fig. 2a).

The EC_{50} H in tracheal chains of COPD + asthmatic animals ($24.16 \pm 1.52 \mu\text{M}$, range 19–28 μM) was significantly lower than in control animals ($34.37 \pm 1.79 \mu\text{M}$, range 28–45 μM , $p < 0.01$) (Table 1, Fig. 2a). However, there was no significant difference in EC_{50} H between COPD and COPD + asthmatic animals.

Table 1

Values of tracheal response to histamine (EC_{50} H), histamine (H_1) receptor blockade by chlorpheniramine (CR-1), hematocrit, contractility response of guinea pig tracheal chains (contraction response to 50 μM concentration of histamine), and maximum contraction in tracheal chains of control, COPD (for each group, $n = 8$) and COPD + asthmatic ($n = 6$) animals and statistical differences between the three groups

Tracheal response	Controls	COPD	COPD + asthma
EC_{50} (μmol)	34.37 ± 1.79	$20.13 \pm 2.4^{***}$	$24.16 \pm 1.52^{**\text{ns}}$
CR-1	0.40 ± 0.07	$2.35 \pm 0.52^{**}$	$1.07 \pm 0.19^{*\text{ns}}$
Contractility	0.18 ± 0.03	$0.19 \pm 0.03\text{NS}$	$0.38 \pm 0.07^{*+}$
Hematocrit	21.80 ± 0.91	$34.75 \pm 1.84^{***}$	$37.16 \pm 2.02^{***\text{ns}}$

Values are means $\pm$ S.E.M. Statistical differences between values in COPD and COPD + asthmatic groups with those of control animals: NS; nonsignificant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical differences between values in COPD with COPD + asthmatic groups: ns; nonsignificant, + $p < 0.05$.

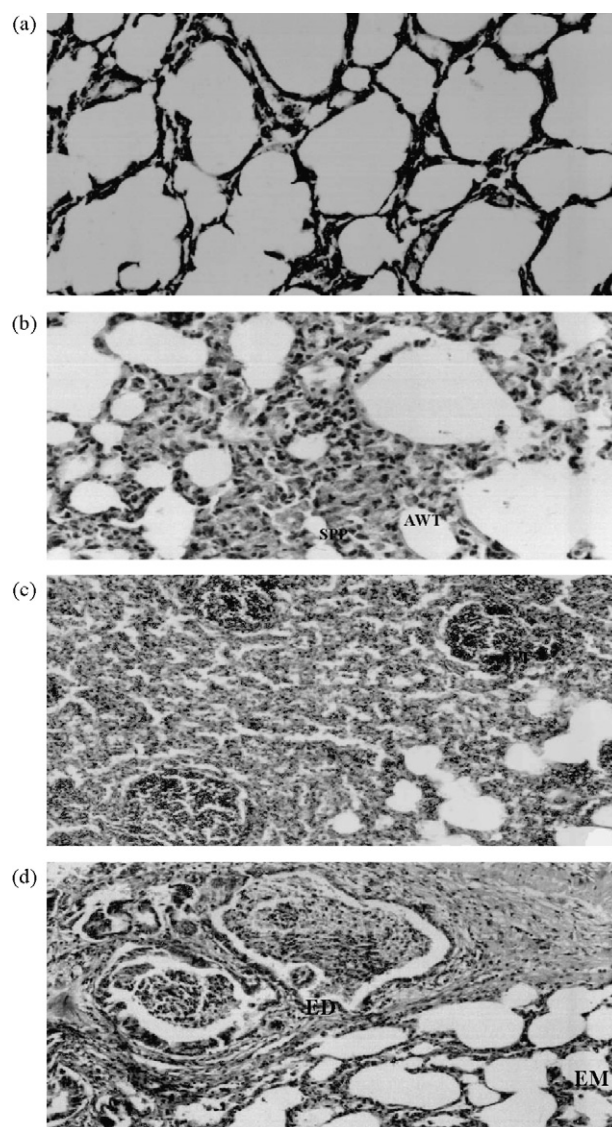


Fig. 1. Photograph of a lung specimen of control guinea pig (a), two lung specimens of COPD animals (b and c), and one lung specimens of COPD + asthmatic guinea pigs (d) with participation of smoke particles (SPP), alveolar wall thickening (AWT), increased lymphoid tissues (LT), emphysema (EM), and epithelial damage (ED) (magnification 40×10).

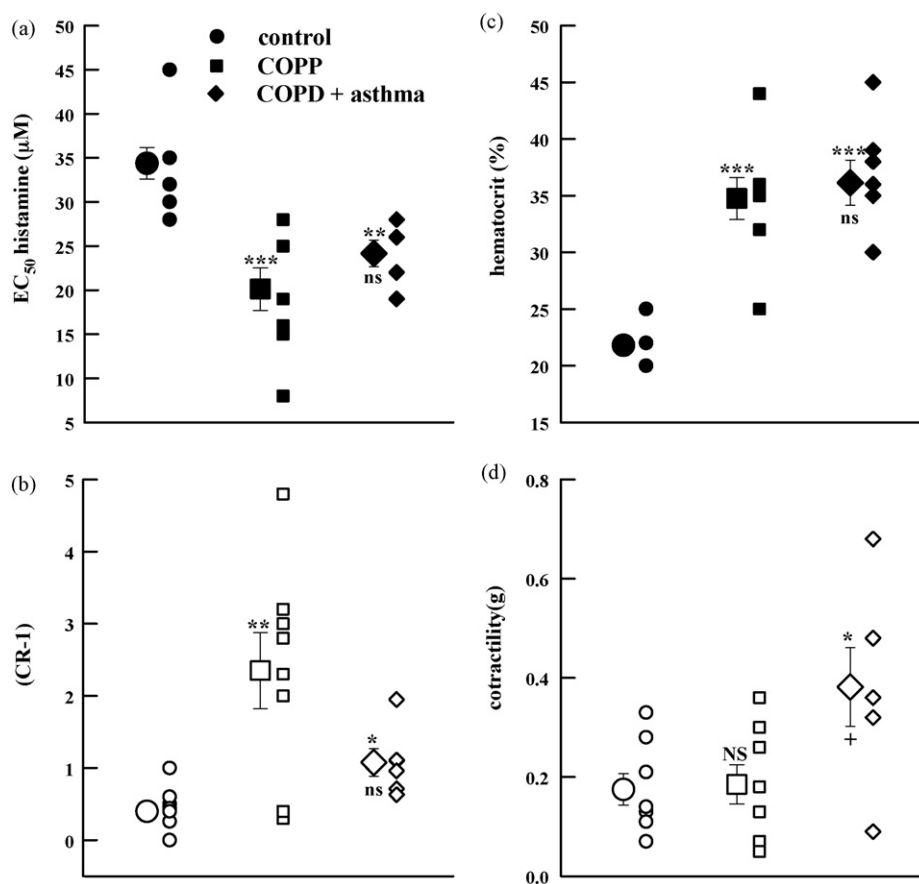


Fig. 2. Individual values and mean $\pm$ S.E.M. (big symbols with bars) of tracheal response to histamine (EC_{50} H) (a), histamine (H_1) receptor blockade by chlorpheniramine (CR-1) (b), hematocrit (c), and contractility response of tracheal chains (contraction response to 50 μ M concentration of histamine) (d) in tracheal chains of control (circle symbols), COPD (square symbols) (for each group, $n = 8$) and COPD + asthmatic animals (diamond symbols) ($n = 6$). Statistical differences between values in COPD and COPD + asthmatic groups with those of control animals: NS; nonsignificant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical differences between values in COPD with COPD + asthmatic groups: ns; nonsignificant, * $p < 0.05$.

3.3. Chlorpheniramine blockade (CR-1)

The rightward shift of the post-chlorpheniramine histamine response curve compared to the baseline histamine response curves in tracheal chains of both COPD and COPD + asthmatic animals was greater than that of control animals (Fig. 3). Mean CR-1 in tracheal chains of COPD animals (2.35 ± 0.52 , range 0.3–4.8) was 5.87 times greater than in control animals (0.40 ± 0.07 , range 0.1–0.7, $p < 0.01$) (Table 1, Fig. 2b).

The CR-1 value in tracheal chains of COPD + asthmatic animals (1.07 ± 0.19 , range 0.63–1.95) was 2.7 times greater than in control animals ($p < 0.05$) (Table 1, Fig. 2b). However, there were no significant differences in the values of CR-1 between COPD and COPD + asthmatic animals.

3.4. Relationship between bronchial response to histamine and chlorpheniramine blockade

There was a significant negative correlation between tracheal response to histamine (EC_{50} H) and histamine H_1

receptor blockade by chlorpheniramine ($r = -0.542$, $p < 0.01$; Fig. 4).

3.5. Contractility and maximum response

The post-chlorpheniramine maximum response in tracheal chains of control and COPD animals (99.13 ± 2.54 and 86.13 ± 10.11 , respectively) was not significantly lower than maximum response in the presence of saline ($100 \pm 0.00\%$ for both cases). However, the mean value of the post-chlorpheniramine maximum response in tracheal chains of COPD + asthmatic animals ($76.66 \pm 6.88\%$) was significantly lower than the maximum response in the presence of saline ($100 \pm 0.00\%$, $p < 0.05$).

The contractility in tracheal chains of COPD animals (0.185 ± 0.04 g, range 0.05–0.36) was not significantly higher than control animals (0.175 ± 0.03 g, range 0.07–0.33). However, the contractility in tracheal chains of COPD + asthmatic animals (0.38 ± 0.079 g, range 0.09–0.68) was significantly higher than those in both control and COPD animals ($p < 0.05$ for both cases).

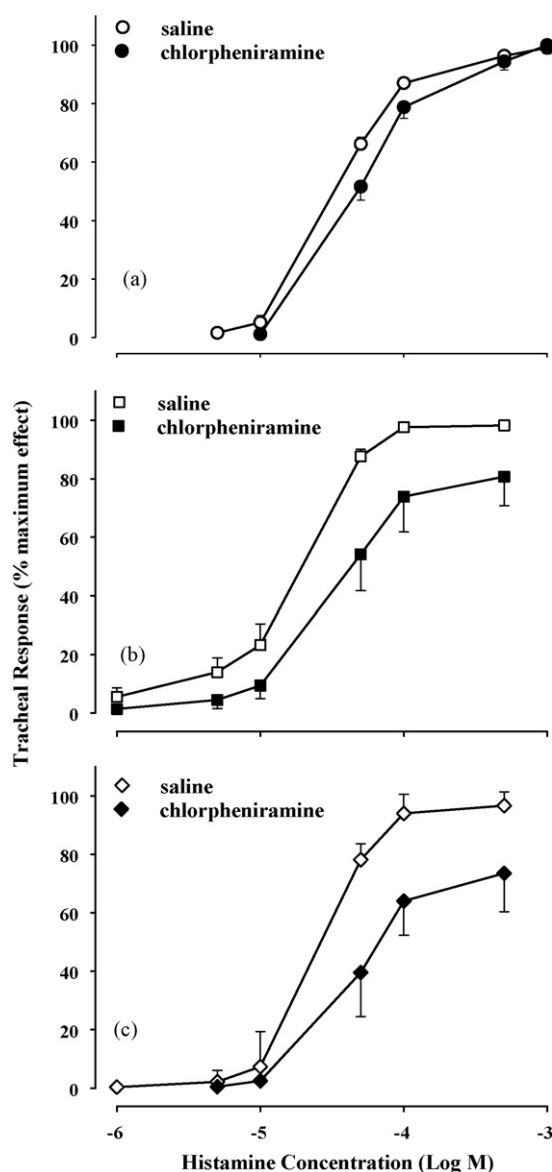


Fig. 3. Cumulative log concentration–response curves of histamine induced contraction of isolated trachea in the presence of saline and chlorpheniramine of control (a), COPD (b) (for each group, $n = 8$) and COPD + asthma guinea pigs (c) ($n = 6$).

3.6. Hematocrit

The hematocrit was significantly higher in COPD animals ($34.75 \pm 1.84\%$, range 25–44) than control animals ($21.8 \pm 0.91\%$, range 20–25, $p < 0.001$). The mean value of hematocrit in COPD + asthmatic animals ($37.16 \pm 2.02\%$, range 30–45), was also significantly higher than control animals ($21.8 \pm 0.91\%$, range 20–25, $p < 0.001$). However, the mean value of hematocrit in COPD + asthmatic animals was not significantly different than that of COPD animals.

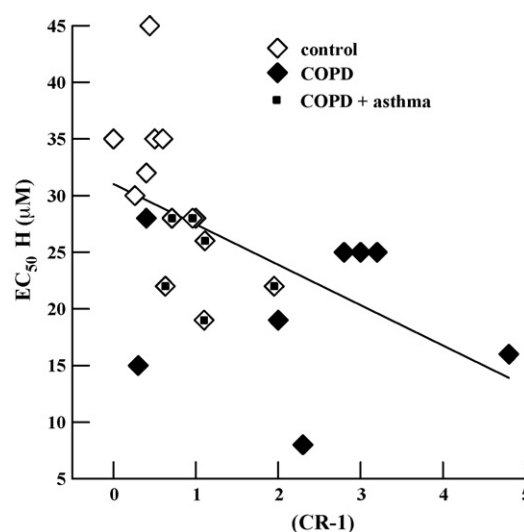


Fig. 4. Correlation between tracheal response to histamine ($EC_{50} H$) and histamine (H_1) receptor blockade by chlorpheniramine (CR-1) in all control, COPD, and COPD + asthma guinea pigs ($n = 22$): $r = -0.542$, $p < 0.01$.

4. Discussion

The present study showed increased in vitro tracheal response to histamine in guinea pigs exposed to cigarette smoke (COPD) and exposed to smoke + sensitized to ovalbumin (COPD + asthma) compared to control animals. The animals exposed to cigarette smoke showed increased intra-alveolar septum, infiltration of leukocytes, increased lymphatic tissue, destruction alveolar walls and intra-alveolar bleeding, i.e. the pathological changes in the lungs of these animals were similar to those of COPD patients [24–26]. The pathological changes in the lungs of animals exposed to cigarette smoke were also similar to those of previous studies [5,27,28]. The hematocrit in COPD and COPD + asthmatic animals were significantly higher than control group, too. The pathological changes in the lung of the guinea pigs exposed to cigarette smoke and increased hematocrit in these groups confirmed the induction of COPD in the experimental group of animals. The existence of eosinophils in lung parenchyma, epithelial damage in addition to the pathological changes of COPD animals, and increased tracheal response to histamine in COPD + asthmatic animals also confirmed the induction of experimental asthma in this group. Tracheal responsiveness to histamine seen in COPD and COPD + asthmatic guinea pigs was similar to the results of our previous study in asthmatic patients [14].

In addition several other studies have shown AHR in guinea pigs exposed to cigarette smoke to different stimuli [6–11]. The results of the present study, however, also demonstrated an increased histamine H_1 receptor blockade by chlorpheniramine (CR-1) in both COPD and COPD + asthmatic animals. The increased receptor blockade by a competitive antagonist was similar to the results of our previous in vitro results indicating increased muscarinic and histamine (H_1) receptors blockade by atropine [16] and chlor-

pheniramine [17] in sensitized guinea pigs, respectively. The consistent increase in antagonist blockade in the present study and our previous studies in sensitized animals [16,17] indicate that the cause of enhanced receptor blockade is increase of either receptor affinity (K_a) or drug delivery to the receptors ($[I]$) or both of these factors [12]. The fact that two receptor systems showed enhanced competitive antagonist blockade in sensitized animals [16,17] and one receptor system in cigarette exposed animals in *in vitro* studies suggests that the abnormality lies with $[I]$ rather than K_a . This conclusion is also supported by *in vitro* experiments, which predict that receptor affinity for a given antagonist shows little variation between species and tissues [29]. We therefore suggest that this enhanced antagonist blockade may be caused by epithelial damage leading to increased epithelial permeability and accessibility of ligands to the receptor sites.

In fact, the increased airway epithelial permeability to different agents has been demonstrated in asthmatic patients [30], animals exposed to cigarette smoke [18,31] and in smokers [32]. The existence of airway inflammation in asthmatic subjects [33], animals exposed to cigarette smoke [19,34,35], smokers [32,36], and COPD [37,38] is well documented. 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 increased receptor blockade by competitive antagonists. The association between airway inflammation, epithelial damage, and airway hyperresponsiveness has been reported both in sensitized animals [39] and in asthmatic patients [40].

Our previous studies [13–15] showed enhanced blockade, when the antagonists were administered by *i.v.* injection in asthmatic patients; and this cannot be due to increased epithelial permeability. The strongest possibility is the increased tissue permeability due to airway inflammation. If the permeability of a physical barrier of some kind close to the receptor was increased in COPD and asthma, it could increase diffusion of antagonist ligands administered by either way and would explain the variation in (CR-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 ligands diffusion [41] 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, using tracheal rings. A stripe of tracheal smooth muscle is covered by epithelium only from one side and easily exposed to chemicals from the remaining three sides, *i.e.* this mostly excludes the barrier function of the epithelium. The increased chlorpheniramine blockade in tracheal chains of COPD and COPD + asthmatic guinea pigs shown in the present study, as well as increased chlorpheniramine, atropine, and propranolol blockade in our previous *in vivo* studies in asthmatic patients [13–15] and enhanced atropine and chlorpheniramine blockade in tracheal chains of sensitized animal studies [16,17], is perhaps due to higher concentration of antagonists at the receptor sites achieved by

an increased epithelial and tissue permeability leading to an increase in $[I]$. In addition, destruction of lung parenchyma is also documented in exposed animals to cigarette smoke [19]. This can support an increased tissue permeability and better accessibility of ligands to the receptor sites.

The results of the present study showed significant correlations between histamine H_1 receptor blockade by chlorpheniramine (CR-1) and tracheal response to histamine *in vitro*. The significant correlation between (CR-1) and tracheal response to histamine in the present study, as well as blockade by other antagonists and agonist responsiveness in previous studies [13–17], indicates that bronchial hyperresponsiveness to different stimuli in COPD and asthma, at least in part, is due to increased bronchial epithelial and tissue permeability which is perhaps due to airway inflammation in these diseases. If this is true, the treatment of these diseases should be focused on preventing this increased permeability.

The present *in vitro* study indicated higher chlorpheniramine blockade at histamine H_1 receptors than tracheal response to histamine in tracheal chains of COPD guinea pigs compared to control animals (5.9 times for chlorpheniramine blockade and 1.7 times for histamine response). In COPD + asthmatic group while differences in EC_{50} H compared to control animals (1.4 times) was similar to COPD guinea pigs, the degree of chlorpheniramine block was smaller (CR-1 in COPD + asthmatic was 2.7 times that of control group). In fact, the results obtained in COPD + asthmatic group were closer to those of our *in vivo* study in asthmatic compared to normal subjects (6.8 times for chlorpheniramine blockade and 22.3 times for histamine response).

In addition, the contractility response of tracheal chains to histamine in COPD group was similar to the control animals, but contractility response in COPD + asthmatic animals was significantly greater than both COPD and control groups. These findings suggested that mainly the prejunctional mechanisms were responsible for increased tracheal responsiveness in COPD animals [42]. These mechanisms include: epithelial damage, neural control, inflammatory process and metabolism or absorption which affect antagonism blockade more than response to an agonist.

The results obtained in COPD + asthmatic groups suggested that in asthma both prejunctional and postjunctional mechanisms (including smooth muscle contractility, viscous and elastic load, swelling of the airway wall, and intraluminal exudate and secretions) are involved in causing AHR. In fact, the results of the present study were supported by the results of Verhoeven *et al.* study [43] indicating increased airway responsiveness to histamine and methacholine in both asthmatic and COPD patients. In addition, the results of the study of Verhoeven *et al.* also showed that the maximum response to methacholine in asthmatic subjects was higher than that in COPD patients.

The cause of small reduction in maximum contractile response of tracheal chain observed in the present of chlorpheniramine (Fig. 1) was perhaps due to a nonspecific effect of histamine which occurs when histamine H_1 receptors are

inhibited by chlorpheniramine. The other explanation for this finding is a nonspecific effect of chlorpheniramine. In fact the same findings are observed in our previous studies [44,45].

In conclusion, this study demonstrated enhanced histamine H₁ receptor blockades by chlorpheniramine in tracheal chains of COPD and COPD + asthmatic guinea pigs. The cause of this enhanced antagonist blockade at the tracheal chains is perhaps increased epithelial and tissue permeability due to airway inflammation. The increased epithelial and tissue permeability of the airways appears, at least in part, to be responsible for airway hyperresponsiveness seen in asthma and COPD. However, the results of the present study showed that the mechanisms of AHR in COPD are not identical to those of asthma disease.

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