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ORIGINAL ARTICLE

Tracheal responsiveness to both isoprenaline and beta$_2$-adrenoreceptor blockade by propranolol in cigarette smoke exposed and sensitized guinea pigs

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Objective: Airway hyperresponsiveness is the main feature of asthma and also exists in cigarette smokers. In previous studies we have shown increased airway responsiveness to isoprenaline in asthmatic patients and smokers. In this study, tracheal responsiveness to isoprenaline and β-adrenergic receptor blockade was investigated in animals exposed to cigarette smoke (AECS) with or without sensitization by ovalbumin (OA).
Methodology: Guinea pigs were exposed to cigarette smoke over a 3-month period with or without sensitization by injection and inhalation of OA. Tracheal responses in AECS, AECS + sensitized and control animals ($n = 7$ for each group) to isoprenaline in the absence and presence of 20 nmol/L propranolol were measured and EC_{50} was established. The propranolol blockade (concentration ratio minus one (CR-1)) was calculated (post-propranolol $EC_{50}/EC_{50}) - 1$.
Results: The tracheal response of AECS and AECS + sensitized guinea pigs to isoprenaline was significantly higher than that of control animals (EC_{50}: 4.24 ± 0.54, 3.66 ± 0.53 and $7.71 \pm 0.68.79$ μmol for AECS, AECS + sensitized and control animals, respectively) ($P < 0.001$). There was no significant difference in EC_{50} between AECS and AECS + sensitized. CR-1 was also significantly higher in the trachea of AECS and AECS + sensitized compared with controls (13.39 ± 2.22 and 15.35 ± 2.95 vs. 3.10 ± 0.6, $P < 0.05$ in both cases). There was no significant difference in CR-1 between AECS and AECS + sensitized. There was a significant correlation between the tracheal response to isoprenaline (EC_{50}) and CR-1 ($r = -0.731$, $P < 0.001$). There was no significant difference in tracheal maximum response to isoprenaline between the three groups of animals.
Conclusions: The results of this study indicate an increased tracheal response to a β_2-adrenergic stimulating drug and enhanced β_2-adrenergic blockade by propranolol in both AECS and AECS + sensitized. These results suggest similar increase in airway responsiveness to β_2-adrenergic agonists and β_2-receptor blockade in AECS and AECS + sensitized guinea pigs.

Key words: beta$_2$-adrenoreceptor blockade, COPD, guinea pig, tracheal responsiveness.

INTRODUCTION

COPD is a global health problem, reaching almost epidemic proportions in the developing world.[1] Understanding the basic mechanisms of this disease and the development of new treatment(s) to prevent the progression presents a major challenge.

In cigarette smokers airflow obstruction is caused by parenchymal disease (emphysema) and/or by smoke-induced distortion of the structure of the small airways.[2–4] Investigation of the mechanisms of cigarette smoke-induced disease can be facilitated by using an animal model in which guinea pigs develop airflow obstruction and emphysematous lung destruction.[5]

One of the most characteristic features of asthma is bronchial hyperresponsiveness to a wide variety of inhaled physical, chemical, pharmacological and immunological stimuli. However, airway

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hyperresponsiveness (AHR) to different stimuli is also observed in animals exposed to cigarette smoke (AECS).[6–10]

Controversy exists regarding bronchial responsiveness to β_2-adrenergic agonists in asthma. Previous studies have shown very similar dose–response relationships to β-agonists in normal and asthmatic subjects.[11–14] Our previous studies showed an increased bronchial responsiveness to isoprenaline in asthmatics compared with normal subjects,[15] in sensitized compared with control animals[16] and in epithelium denuded trachea compared with intact epithelium trachea.[17] In addition, in recent studies, increased airway responsiveness to salbutamol in smokers compared with non-smoking subjects and asthmatics has been demonstrated.[18,19]

In the present study the effect of cigarette smoke on airway responsiveness to the β-agonist isoprenaline and to β_2-adrenergic blockade by propanolol was studied by examining tracheal responsiveness of guinea pigs exposed to cigarette smoke alone, and exposed to cigarette smoke and sensitized to ovalbumin (OA).

MATERIALS AND METHODS

Animals, cigarette smoke exposure and sensitization

Twenty-one adult Dunkin-Hartley guinea pigs of both genders and weighing 400–500 g were divided into three groups: one group was exposed to cigarette smoke only (AECS), one exposed to cigarette smoke and sensitized to OA (AECS + sensitized) and one control group ($n = 7$ for each group).

Experimental animals were exposed to cigarette smoke as previously described.[20,21] 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 and coworkers.[20] Animals were placed in a Plexiglas box with their heads secured in a compartment (15 cm × 12 cm × 7 cm). A syringe delivered the cigarette smoke. Twenty millilitre puffs of cigarette smoke were drawn out of the cigarettes with syringes and then exhausted at a rate of two puffs per minute into the animals' chamber. Exposure of animals to each cigarette lasted for 8–9 min, with a 10-min resting period between cigarettes. The animals were exposed initially to one commercial non-filter cigarette per day gradually increasing to a maximum of five cigarettes per day over a 2-week period. In a pilot study it was observed that animals could not tolerate the exposure to cigarette smoke of more than five cigarettes per day. The exposure to the smoke of five cigarettes per day, 6 days per week, continued for 3 months. The control animals were not exposed to cigarette smoke and kept in an animal house in normal conditions for the same period of time.

Sensitization of animals to OA was performed using the method described by McCaig.[22,23] Briefly, seven guinea pigs were sensitized to OA (Sigma Chemical Ltd, Poole, Dorset, 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 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 cm × 20 cm × 20 cm.

Tissue preparations

Guinea pigs were killed by a blow to the neck and the trachea removed. Each trachea was cut into 10 rings (each containing two to three cartilaginous rings). All the rings were then cut open opposite the trachealis muscle, and sutured together to form a tracheal chain.[23,24] The tracheal chain was then suspended in a 10-mL organ bath (organ bath 61 300, BioScience Palmer-Washington, Sheerness, Kent, UK) containing a 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.

The Krebs-Henseleit solution was maintained at 37°C and gassed with 95% O_2 and 5% CO_2. The tracheal chain was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

Measurement of tracheal response to isoprenaline and β_2-receptor blockade

In each experiment two cumulative log concentration–response curves of isoprenaline sulphate (Sigma Chemical Ltd) induced relaxation of precontracted tracheal chains were obtained. One of the curves was performed 10 min after producing a 20 nmol/L concentration of propranolol hydrochloride (Sigma Chemical Ltd) in the organ bath (post-propranolol response curve), and the other 10 min after adding the same volume of saline to the bath (baseline isoprenaline response curve). Isoprenaline concentrations were increased every 2 min (range from 10 nmol/L to 100 μmol/L), and the percentage of relaxation due to each value of isoprenaline concentration in proportion to the maximum contraction obtained with 10 μmol/L methacholine (Sigma Chemical Ltd) alone was plotted against the log concentration of isoprenaline.

The effective concentration of isoprenaline causing 50% of maximum response (EC_{50}) alone or post propranolol was measured and expressed as EC_{50} and post-propranolol EC_{50}, respectively. The isoprenaline EC_{50} for tracheal responses in the three animal groups were calculated and compared. The β_2-adrenergic receptor blockade by propranolol was assessed as the concentration ratio minus one (CR-1) which was calculated by: (post-propranolol EC_{50}/baseline EC_{50}) − 1.

Pathological evaluation

The lungs of some AECS and AECS + sensitized were randomly selected for normal pathological evaluation using haematoxin-eosin dye.

Statistical analysis

The data for the tracheal response to isoprenaline (EC_{50}), β_2-adrenergic blockade (CR-1) maximum response and haematocrit were quoted as mean ± SEM. In comparing all values between AECS, AECS + sensitized and control guinea pigs ANOVA with Tukey-Kramer post test was used. Tracheal response to isoprenaline (EC_{50}) was related to (CR-1) using the least square regression. Significance was accepted at $P < 0.05$.

RESULTS

Histology

The following pathological changes were observed in the lungs of AECS (Fig. 1): increased interalveolar septum in all specimens, increased lymphatic tissue in the lung parenchyma of all specimens, the destruction of alveolar wall and existence of emphysema in the lung of most animals and intra-alveolar bleeding in most of the animal lungs.

In addition, the following pathological changes were observed in the lungs of AECS + sensitized (Fig. 1): epithelial damage and parenchymal eosinophilia.

Tracheal response to isoprenaline

The mean value of isoprenaline EC_{50} in tracheal chains of AECS (4.24 ± 0.54 μM, range 2.8–6.0) was significantly lower than in control animals (7.71 ± 0.68 μM, range 5–10, $P < 0.001$) (Table 1, Fig. 2a). The most responsive trachea of AECS was 3.6 times more sensitive to isoprenaline than the least responsive trachea from control animals.

The mean value of EC_{50} in tracheal chains of AECS + sensitized (3.66 ± 0. 53 μM, range 2.0–6.0) was significantly lower than in control animals ($P < 0.01$) (Table 1, Fig. 2a). The most responsive trachea of AECS + sensitized was five times more sensitive to isoprenaline than the least responsive trachea from control animals. However, there was no significant difference in EC_{50} between AECS and AECS + sensitized.

Beta-adrenergic blockade (CR-1)

The rightward shift of the post-propranolol isoprenaline response curve compared with the baseline isoprenaline response curve in tracheal chains of AECS was greater than that of control animals (Fig. 3). Mean CR-1 in tracheal chains of AECS (13.39 ± 2.22, range 7.5–25.0) was 4.32 times greater than in control animals (3.10 ± 0.63, range 1.20–5.42, $P < 0.05$) (Table 1, Fig. 2b). The value of CR-1 of the most sensitive trachea of AECS animals was 20.1 times greater than that for the least sensitive trachea from control animals.

The rightward shift of the post-propranolol isoprenaline response curve compared with the baseline isoprenaline response curve in tracheal chains of AECS + sensitized was greater than that of control animals (Fig. 3). Mean CR-1 in tracheal chains of AECS + sensitized (15.35 ± 2.95, range 6.00–24.00) was 4.95 times greater than in control animals ($P < 0.05$) (Table 1, Fig. 2b). The value of CR-1 of the most sensitive trachea of AECS was 20.0 times greater than that of the least sensitive trachea from control animals. However, there was no significant difference in the value of CR-1 between AECS and AECS + sensitized.

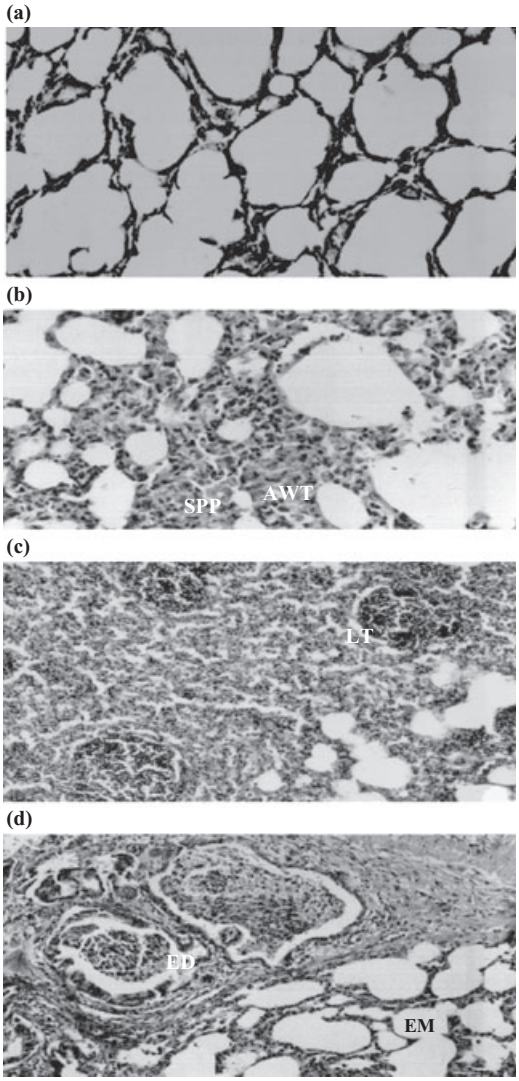

Figure 1 Photomicrograph of a lung specimen from control guinea pig (a) and from COPD animals (b,c), and a lung specimen of animals exposed to cigarette smoke and sensitized to ovalbumin (AECS + sensitized; d) showing smoke particles (SPP), alveolar wall thickening (AWT), increased lymphoid tissues (LT), emphysema (EM) and epithelial damage (ED).

Relationship between bronchial response to isoprenaline and propranolol blockade

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

Table 1 Tracheal response to isoprenaline (EC_{50}), β-receptor blockade by propranolol (CR-1), haematocrit and maximum relaxation response to isoprenaline in tracheal chains of control, cigarette exposed (AECS) and cigarette exposed + sensitized (AECS + sensitized) animals and statistical differences between the three groups ($n = 7$ for each group)

Tracheal response	Control	AECS	AECS + sensitized
EC_{50} (μmol)	7.71 ± 0.68	4.24 ± 0.54***	3.66 ± 0. 53*** ns
(CR-1)	3.10 ± 0.63	13.39 ± 2.22*	15.35 ± 2.95* ns
Maximum response	92.17 ± 6.05	85.86 ± 3.31 NS	89.57 ± 5.51 NS ns
Haematocrit	21.80 ± 0.91	34.57 ± 2.13***	37.16 ± 2.02*** ns

Values are quoted as mean ± SEM.

Statistical differences between values in AECS and AECS + sensitized groups with those of control animals: NS; non-significant, $*P < 0.05$, $***P < 0.001$; Statistical differences between values in AECS with AECS + sensitized groups: ns; non-significant.

β-adrenergic blockade by propranolol ($r = -0.731$, $P < 0.01$; Fig. 4).

Maximum response to isoprenaline

There was no significant difference between the mean values of maximum response to isoprenaline in tracheal chains of AECS (85.86 ± 3.31), AECS + sensitized (89.57 ± 5.51) and control animals (92.17 ± 6.05) (Table 1, Fig. 3).

DISCUSSION

This study showed increased tracheal response to isoprenaline in guinea pigs exposed to cigarette smoke and exposed to smoke + sensitized to OA compared with control animals. The histological findings in AECS showed increased interalveolar septum and lymphatic tissue, destruction of alveolar walls and intra-alveolar bleeding as was to that observed in previous studies.[5,25,26] The increased tracheal response to isoprenaline in AECS + sensitized confirmed the induction of experimental asthma and the tracheal responsiveness to a β-agonist drug was similar to our previous studies.[15–18]

Several other studies have shown AHR to different stimuli in guinea pig exposed to cigarette smoke.[6–10] However, the present study demonstrated an increased tracheal responsiveness to isoprenaline and β-adrenergic receptor blockade by propranolol (CR-1) in both AECS and AECS + sensitized. The increased receptor blockade by a competitive antagonist was similar to previous studies indicating increased muscarinic, histaminic (H_1) and β-adrenergic blockade by atropine,[27] chlorpheniramine[28] and propranolol[16] in sensitized guinea pigs, respectively. The consistent increase in antagonist blockade in the present study and in our previous studies in sensitized animals,[16,27,28] indicates that the cause of enhanced receptor blockade is due to an increase of either receptor affinity (Ka) and/or drug delivery to the receptors ([I]).[29] The fact that three receptor systems showed enhanced competitive antagonist blockade in sensitized animals[16,27,28] and one receptor system in cigarette exposed animals in *in vitro* studies suggests that the abnormality lies with [I] rather than Ka. This conclusion is also supported by *in vitro* experiments, that demonstrate that receptor affinity for a given antagonist shows little variation between species and tissues.[30] We therefore suggest that this enhanced antagonist blockade is caused by epithelial damage leading to increased epithelial permeability and accessibility of ligands to the receptor sites.

Increased airway inflammation and epithelial permeability to different agents has been demonstrated in asthmatic patients, AECS and in smokers.[20,21,31–39] The pathological findings in the present study showed lung tissue inflammation in both AECS and AECS + sensitized groups. The association between airway inflammation, epithelial damage and AHR has been reported both in sensitized animals[40] and in asthmatic patients.[41]

However, our previous studies[15,42,43] showed enhanced blockade when pharmacological antagonists were administered by i.v. injection in asthmatic patients; and this cannot be due to increased airway epithelial permeability possibly this occurred due to increased tissue permeability secondary to airway inflammation and would explain the variation in (CR-1) produced by both routes of administration. Furthermore, the results of the present *in vitro* study cannot be fully explained by increased airway epithelial permeability as the barrier role of epithelium against ligand diffusion[44] will only occur if the tracheal or bronchial tubes are exposed to ligands from the mucosal side. In our model, suspended tracheal rings were used and thus non-epithelial surfaces were exposed. Pathological examination did not show obvious airway epithelial damage in AECS animals although there was epithelial damage in the airways of AECS + sensitized guinea pigs.

The increased propranolol blockade in tracheal chains of AECS and AECS + sensitized guinea pigs, as well as the increased chlorpheniramine, atropine, and propranolol blockade in our previous *in vivo* studies in asthmatic patients[15,42,44] and enhanced atropine, chlorpheniramine and propranolol blockade in tracheal chains of sensitized animal studies,[16,27,28] could be due to higher concentration of antagonists at the receptor sites achieved by an increased epithelial and tissue permeability leading to an increase in [I]. Destruction of lung parenchyma has been documented in AECS[21] and thus, increased tissue

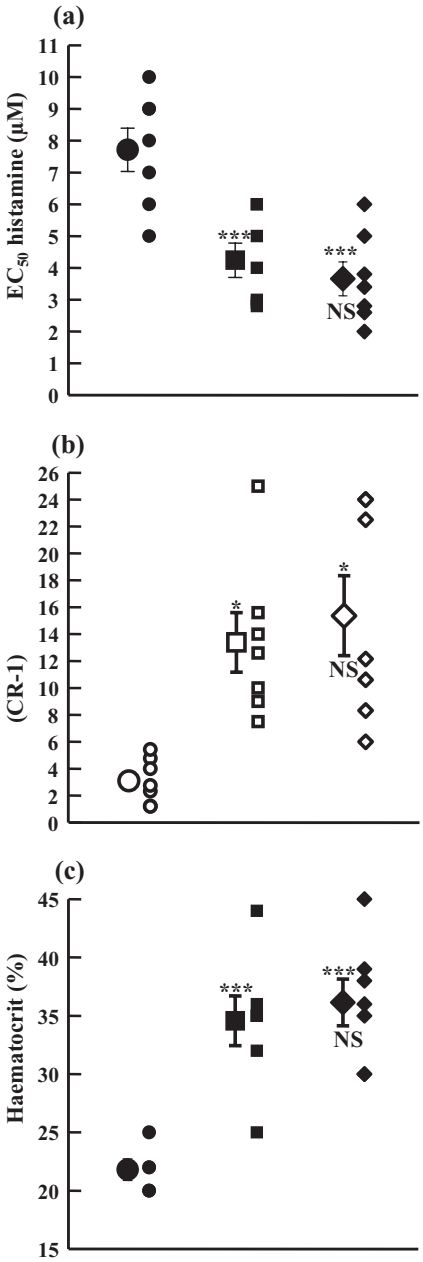

Figure 2 Individual values (○, ◇, □) and mean ± SEM (larger symbols with error bars) of tracheal response to isoprenaline (EC_{50}) (a), β-receptor blockade by propranolol (CR-1) (b) and haematocrit (c) in (○, ●) tracheal chains of control, (□, ■) animals exposed to cigarette smoke (AECS), and (◇, ◆) AECS + sensitized animals ($n = 7$ for each group). Statistical differences between values in AECS and AECS + sensitized groups with those of control animals: $^{*}P < 0.05$, $^{***}P < 0.001$. Statistical differences between values in AECS with AECS + sensitized groups: NS, non-significant.

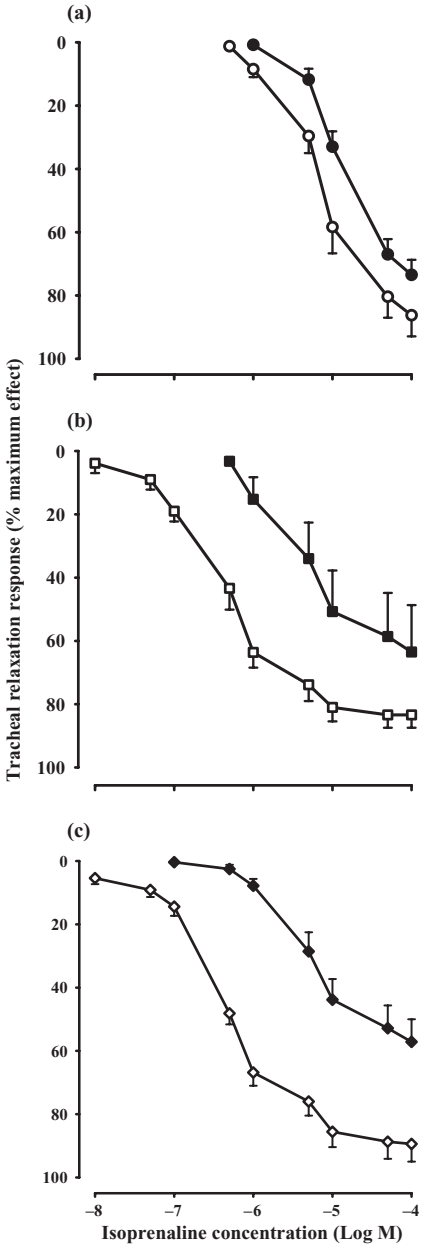

Figure 3 Cumulative log concentration–response curves of isoprenaline induced relaxation of isolated trachea in the presence of (○, ◇, □) saline and (●, ■, ◆) propranolol of (a, ○, ●) control, (b, □, ■) cigarette exposed (AECS) guinea pigs, and (c, ◇, ◆) AECS and ovalbumin-sensitized (AECS + sensitized) guinea pigs (for each group, $n = 7$).

permeability and better accessibility of ligands to the receptor sites is possible.

There was also a significant correlation between β-adrenergic receptor blockade by propranolol (CR-1) and tracheal response to isoprenaline and histamine. These data as well as that from our previous studies[15,42,43] suggest that bronchial hyperresponsiveness to different stimuli in AECS and asthma is due at least in part to increased bronchial tissue permeability. If substantiated, the treatment should be focused on preventing increased permeability.

Using a similar method to the present study but with exposure periods of 2 h/day for 2 weeks, enhanced bronchial smooth muscle contraction

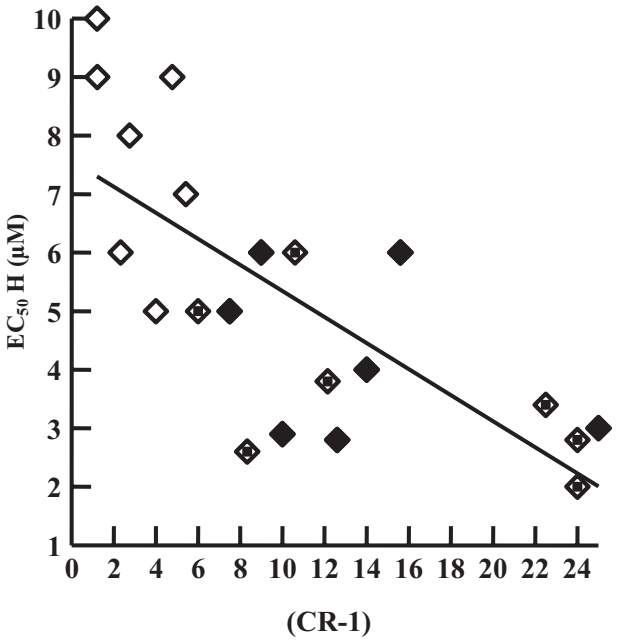

Figure 4 Correlation between tracheal response to isoprenaline (EC_{50}) and β-receptor blockade by propranolol (CR-1) in (◇) control, (◆) cigarette exposed (AECS), and (◈) AECS + ovalbumin-sensitized guinea pigs ($n = 21$): $r = -0.731$, $P < 0.001$.

induced by cigarette smoke exposure was shown.[45] Our previous study also showed an increased tracheal responsiveness to methacholine in cigarette smoke exposed guinea pigs.[46] Although, there are reports regarding the effect of smoking on enhanced airway responsiveness both in atopic humans[47] and sensitized animals,[48] the novel findings of the present *in vitro* study that there was a similar tracheal responsiveness to isoprenaline and β-adrenergic blockade (CR-1) in AECS and AECS + sensitized guinea pigs. These results suggest that airway responsiveness to β-agonists in early phase of AECS is similar to that of asthma and induction of asthma does not add further increase in this phenomenon. In addition, there was no significant difference in the maximum relaxant effect to isoprenaline between the three groups of animals (control, AECS and AECS + sensitized). These findings may indicate that maximum relaxation response to β-agonist drugs is more likely to occur in early phase of AECS.

The other possible explanation for the findings of the present study is an upregulation and increased expression of the receptors due to both sensitization and cigarette smoke However, upregulation and increased expression of the receptors would result to increased tracheal responsiveness to isoprenaline without affecting β-blockade and therefore this seems unlikely.

This study demonstrated similar increases in tracheal response to isoprenaline and enhanced adrenergic receptor blockade by propranolol in AECS and AECS + sensitized guinea pigs. These results suggest similar increases in airway responsiveness to β-adrenergic agonists and β-receptor blockade in AECS and AECS + sensitized guinea pigs and that maximum relaxation response to β-agonist drugs is obtainable only in early phase of AECS.

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REFERENCES

1 Rennard SI, Farmer SG. COPD in 2001. *Chest* 2002; **121** (Suppl. 5): 113s–115s.
2 Wright JL, Lawson LM, Pare PD, Kennedy S, Wiggs B, Hogg JC. The detection of small airways disease. *Am. Rev. Respir. Dis.* 1984; **129**: 898–994.
3 Matsuba K, Wright JL, Wiggs BR, Pare PD, Hogg JC. The change in airways structure associated with reduced forced expiratory Volume in one second (FEV_1). *Eur. Respir. J.* 1989; **2**: 834–9.
4 Bosken CH, Wiggs BR, Pare PD, Hogg JC. Small airway dimensions in smokers with obstruction to air flow. *Am. Rev. Respir. Dis.* 1990; **42**: 563–70.
5 Wright JL, Churg A. Cigarette smoke cause physiological and morphological changes of emphysema in the guinea pig. *Am. Rev. Respir. Dis.* 1990; **142**: 1422–6.
6 James AL, Dirks P, Ohtaka H. Airway responsiveness to intravenous and inhaled acetylcholine in the guinea pig after cigarette smoke exposure. *Am. Rev. Respir. Dis.* 1987; **136**: 158–162.
7 Dusser DJ, Djokic TD, Borson DB. Cigarette smoke induces bronchoconstrictor hyperresponsiveness to substance P and inactivates airways neutral endopeptidase in the guinea pig. *J. Clin. Invest.* 1989; **84**: 900–6.
8 Han-pin K, Ling-chung L. Sensory neuropeptides modulate cigarette smoke-induced decrease in neutral endopeptidase activity in guinea pig airways. *Life Sci.* 1995; **57**: 2187–96.
9 Lee LY, Lou YP, Hong JL, Lundberg JM. Cigarette smoke-induced bronchoconstriction and release of tachykinins in guinea pig lungs. *Respir. Physiol.* 1995; **99**: 173–81.
10 Hulbert WM, Mclean T, Hogg JC. The effect of acute airway inflammation on bronchial reactivity in guinea pig. *Am. Rev. Respir. Dis.* 1985; **132**: 7–11.
11 Harvey JE, Tattersfield AE. Airway response to salbutamol: effect of regular salbutamol inhalations in normal, atopic and asthmatic subjects. *Thorax* 1982; **37**: 280–7.
12 Holgate ST, Stubbs WA, Wood PJ, McCaughey ES, Alberti KGMM, Tattersfield AE. Airway and metabolic resistance to intravenous salbutamol: a study in normal man. *Clin. Sci.* 1980; **59**: 155–61.
13 Harvey JE, Baldwin CJ, Wood PJ, Alberti KGMM, Tattersfield AE. Airway and metabolic responsiveness to intravenous salbutamol in asthma: effect of regular inhaled salbutamol. *Clin. Sci.* 1981; **60**: 579–85.
14 Barnes PJ, Pride NB. Dose–response curves to inhaled beta-adrenoceptor agonist in normal and asthmatic subjects. *Br. J. Clin. Pharmacol.* 1983; **15**: 677–82.

15 Boskabady MH, Snashall PD. Bronchial responsiveness to beta-adrenergic stimulation and enhanced beta-blockade in asthma. *Respirology* 2000; **5**: 111–18.
16 Boskabady MH, Zarei A. Increased tracheal responsiveness to β-adrenergic agonist and antagonist in ovalbumin sensitized guinea-pigs. *Pharmacol.* 2004; **71**: 73–9.
17 Boskabady MH, Teymoory S. The influence of epithelium on the responsiveness of guinea pig trachea to β-adrenergic agonist and antagonist. *Med. Sci. Monit.* 2003; **9**: BR336–42.
18 Boskabady MH, Ferhadi H. Correlation between Airway Responsiveness to Salbutamol and Methacholine in Smokers. *Med. Sci. Monit.* 2005; **11**: CR344–50.
19 Boskabady MH, Saadatinejad M. Airwa responsiveness to a beta-adrenergic agonist (salbutamol) in asthma. *J. Asthma* 2003; **40**: 917–25.
20 Simani AS, Inoue S, Hogg JC. Penetration of the respiratory epithelium of guinea pigs following exposure to cigarette smoke. *Lab. Invest.* 1974; **31**: 75–81.
21 Sansores RH, Abboud RT, Becerril C *et al.* Effect of exposure of guinea pigs to cigarette smoke on elastolytic activity of pulmonary macrophages. *Chest* 1997; **112**: 214–19.
22 McCaig D, De Jonckheere S. Effect of two Ca^{2+} modulator in normal and albumin-sensitized guinea-pig trachea. *Eur. J. Pharmacol.* 1993; **249**: 53–63.
23 McCaig D. Comparison of autonomic responses in the trachea isolated from normal and albumin-sensitive guinea pig. *Br. J. Phamacol.* 1987; **92**: 809–16.
24 Holroyde MC. The influence of epithelium on the responsiveness of guinea-pig isolated trachea. *Br. J. Pharmacol.* 1986; **87**: 501–7.
25 Selman M, Montano M, Ramos B. Tobacco smoke-induced lung emphysema in guinea pigs is associated with increased interstitial collagenase. *Am. J. Physiol.* 1996; **271**: L734–L743.
26 Wright JL, Churg A. smoke-induced lung emphysema in guinea pig is associated with morphometric evidence of collagen breakdown and repair. *Am. J. Physiol.* 1995; **268**: L17–L20.
27 Boskabady MH, Adel-kardan S. Increased muscarinic receptor blockade by atropine in tracheal chains of ovalbomine-sensitized guinea pigs. *Pharmacol.* 1999; **58**: 300–8.
28 Boskabady MH, Harati M, Adel Kardan S. Enhanced chlorpheniramine blockade in isolated tracheal chains of asthmatic guinea-pigs. *Med. J. Islam. Rep. Iran* 1998; **12**: 265–71.
29 Arunlakshana O, Schild HO. Some quantitative uses of drug antagonists. *Br. J. Pharmacol.* 1959; **14**: 48–58.
30 Bowman WC, Rand MJ. *Textbook of Pharmacology*, 2nd edn. Blackwell Scientific Publications, Oxford, 1980; 25–39.
31 Ilowite JS, Bennett WD, Sheetz MS, Groth ML, Nierman DM. Permeability of bronchial mucosa to ^{99m}Tc-DTPA in asthma. *Am. Rev. Respir. Dis.* 1989; **139**: 1139–43.
32 Burns AR, Hosford SP, Dunn LA. Respiratory epithelial permeability after cigarette smoke exposure in guinea pigs. *J. Appl. Physiol.* 1989; **66**: 2109–16.
33 Taylor RG, Agnew JE, Francis RA, Pavia D, Clarke SW. Respiratory epithelial permeability is unrelated to bronchial reactivity and small airway function in young smokers and nonsmokers. *Eur. Respir. J.* 1988; **1**: 319–23.
34 Martin RJ, Cicutto LC, Smith HR, Ballord RD, Szefler SJ. Airway inflammation in nocturnal asthma. *Am. Rev. Respir. Dis.* 1991; **143**: 351–7.
35 Matsumoto K, Aizawa H, Iniue H. Eosinophilic airway inflammation induced by repeated exposure to cigarette smoke. *Eur. Respir. J.* 1998; **12**: 387–94.
36 Nisikawa M, Kakemisu N, Ito T. Superoxide mediates cigarette smoke-induced infiltration of neutrophils into the airways through nuclear factor-B activation and IL-8 mRNA expression in guinea pigs *in vivo*. *Am. J. Respir. Cell. Mol. Biol.* 1999; **20**: 189–98.
37 Finkelsteine R, Fraser RS, Ghezzo H. Alveolar inflammation and its relation to emphysema in smokers. *Am. J. Respir. Crit. Care Med.* 1995; **152**: 1666–72.
38 Pettersen CA, Adler KB. Airways inflammation and COPD. *Chest* 2002; **121**: 142s–150s.
39 Cosio MG, Majo J, Cosio MG. Inflammation of the airways and lung paranchyma in COPD: role of T cells. *Chest* 2002; **121**: 160s–165s.
40 Padrid P, Snook S, Finucane T *et al.* Persistent airway hyperresponsiveness and histologic alteration after chronic antigen challenge in cats. *Am. J. Respir. Crit. Care Med.* 1995; **151**: 184–93.
41 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.* 1995; **152**: 45–52.
42 Boskabady MH, Snashall PD. Enhanced muscarinic receptor blockade with atropine in the asthmatic tracheobronchial tree: evidence for increased drug delivery. *Am. Rev. Respir. Dis.* 1992; **145**: 756–61.
43 Boskabady MH, Snashall PD. Enhanced histamine H_1 receptor blockade with chlorpheniramine in the asthmatic tracheobronchial tree: further evidence for increased drug delivery in asthma. *Med. J. Islam. Rep. Iran* 1997; **11**: 115–22.
44 Mitchell HW, Willet KF, Sparrow MP. Perfused bronchial segment and bronchial strip: narrowing *vs.* isometric force by mediators. *J. Appl. Physiol.* 1989; **66**: 2704–9.
45 Chiba Y, Murata M, Ushikubo H *et al.* Effect of cigarette smoke exposure *in vivo* on bronchial smooth muscle contractility *in vitro* in rats. *Am. J. Respir. Cell. Mol. Biol.* 2005; **33**: 574–81.
46 Boskabady MH, Aslani MR, Tabatabaei A. The influence of epithelium and isoprenaline incubation on responsiveness of guinea-pig trachea to methacholine. *Pharmacology* 2006; **76**: 1–7.
47 Prieto L, Gutierrez V, Uixera S, Berto JM. Effect of cigarette smoking on airway responsiveness to adenosine 5′-monophosphate in subjects with allergic rhinitis. *Chest* 2003; **123**: 971–3.
48 Moerloose KB, Pauwels RA, Joos GF. Short-term cigarette smoke exposure enhances allergic airway inflammation in mice. *Am. J. Respir. Crit. Care Med.* 2005; **172**: 168–72.