

Prevention of bronchoconstriction in sensitized guinea pigs: efficacy of common prophylactic drugs

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Accepted 23 April 2004

Abstract

Efficacy of β_2 -agonists (B2), anticholinergics, corticosteroids, anti-inflammatory drugs or antihistamines against methacholine, histamine, or allergen-induced lung constriction was tested in ovalbumin sensitized guinea pigs. Airway resistance (R_{aw}) and parenchymal damping (G) and elastance (H) were determined from low-frequency forced oscillatory input impedance (ZL). ZL was measured under control conditions, during iv infusions of methacholine, and following iv boluses of histamine or ovalbumin. In decreasing potency, ipratropium, salmeterol, fluticasone or cromoglycate reduced the methacholine-induced increases in R_{aw} and G . Only antihistamines had any effects on the histamine-induced increases in R_{aw} , G , and H . The ovalbumin-induced increases in R_{aw} , G and H in the control animals ($120 \pm 90\%$, $201 \pm 126\%$, $86 \pm 71\%$) were markedly reduced by pretreatments with antihistamines ($18 \pm 11\%$, $13 \pm 9\%$, $3 \pm 3\%$) and cromoglycate ($29 \pm 13\%$, $35 \pm 22\%$, $18 \pm 10\%$). Bronchoconstriction induced by muscarinic receptor stimulation is inhibited by anticholinergic, anti-inflammatory or β_2 -agonist pretreatment; antihistaminic or anti-inflammatory premedication is beneficial if the release of histamine via an exposure to allergen is responsible for the fall in lung function.

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Keywords: Mammals; Guinea pig; Mechanics of breathing; Airway resistance; Modulators; Histamine; Pharmacological agents; Anticholinergic; Antihistamine; β_2 -Agonist; Resistance; Airways; Upper airways; Bronchial constriction

1. Introduction

Patients with bronchial hyperreactivity (BHR) are at an increased risk of exacerbation of their symptoms following stimulation of irritant receptors in the airways, via cholinergic pathways, and/or the release

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of endogenous mediators. Management of patients diagnosed with BHR requires the use of prophylactic drugs to minimize the risk of induced bronchospasm, which enhances the morbidity and may also be potentially life-threatening (Caplan et al., 1990).

β_2 -Agonists (B2) (Nair et al., 1984; Cheung et al., 1992; Derom et al., 1992; Booth et al., 1993; Cockcroft et al., 1995; Rosenthal et al., 1999; Sont et al., 1999; Calhoun et al., 2001; Larj and Bleeker, 2002), anticholinergics (Nair et al., 1984; Larsson, 1987; Ulrik et al., 1992), inhaled corticosteroids (Juniper et al., 1990; Barnes and Pedersen, 1993; van Rensen et al., 1999; Sont et al., 1999; Corsico et al., 2000; Hofstra et al., 2000), anti-inflammatory drugs (Andersson and Bergstrand, 1981; Patel, 1984), and antihistamines (Nathan et al., 1979; Mauser et al., 1990) are commonly used in asthmatic and in allergic patients as routine treatment in the presence of BHR. β_2 -Agonists are routinely administered to patients with BHR so as to prevent or to treat the bronchospasm induced by different reflex mechanisms (Zachary and Evans, 1996). However, other forms of prophylactic treatment may also exert beneficial effects, particularly when the bronchospasm is induced by other triggering mechanisms via neural and/or humoral pathways. Although each of these medications has been investigated separately, their potential to prevent bronchospasm induced via different pathways has not been compared within a study. Furthermore, changes in the airway function in these previous studies have been estimated by spirometry (Cheung et al., 1992; Derom et al., 1992; Booth et al., 1993; Cockcroft et al., 1995; Rosenthal et al., 1999; Sont et al., 1999; Calhoun et al., 2001; Larsson, 1987; Ulrik et al., 1992; Andersson and Bergstrand, 1981; Patel, 1984; Juniper et al., 1990; Corsico et al., 2000; Hofstra et al., 2000; van Rensen et al., 1999; Larsson et al., 2001; Nathan et al., 1979) or by whole-body plethysmography (Nathan et al., 1979), thus, distinct changes in the airway dimensions and the parenchymal viscoelasticity have not been characterized separately.

Accordingly, the aims of the present investigations were to compare the ability of common forms of preventive treatment used in the presence of BHR to prevent an induced constriction via stimulation of the (i) muscarinic receptors, (ii) the histamine (HIS) receptors, or (iii) following an allergic reaction. Experiments were performed in ovalbumin

(OVA)-sensitized guinea pigs with consequent BHR. Since bronchoactive agents alter the airway mechanics and the lung tissue viscoelasticity (Hantos et al., 1992; Lutchen et al., 1994, 1996; Petak et al., 1997; Hall et al., 1999; Adamicza et al., 1999; Habre et al., 2001, 2002), the mechanical properties of these compartments were characterized separately by using the forced oscillation technique at low frequencies (Hantos et al., 1992; Lutchen et al., 1994, 1996; Petak et al., 1997, 1999; Hall et al., 1999; Adamicza et al., 1999; Habre et al., 2001, 2002).

2. Methods

2.1. Sensitization and study groups

After approval from the Institutional Ethics Committee, 50 young male, pathogen-free Hartley guinea pigs weighing 350–750 g were studied. To induce BHR, 44 animals were actively sensitized by daily exposure to aerosolized OVA (10 mg/ml in 0.9% sterile sodium chloride solution for 5 min) from Days 0 to 7 (Featherstone et al., 1988; Wu et al., 2000). Experiments were performed on Day 10. In order to avoid the development of tolerance to OVA, the animals were fed with food free of egg proteins and were kept under environmental conditions that were as stable as possible. Food and water were available *ad libitum*.

Animals were randomly assigned to one or the other of the following seven protocol groups. No sensitization was performed in control group C, while the sensitized animals in the other six groups either underwent no pretreatment (group CS), or were treated with long-acting β_2 -agonist (group B2), anti-inflammatory drug (group AI), inhalation steroid (group IS), anticholinergic agent (group AC), or antihistamine agents (group AH). The characteristics of the protocol groups are presented in Table 1. All prophylactic drugs were administered 1 h prior to OVA exposure.

2.2. Animal preparation

The guinea pigs were anesthetized by ip injection of urethane in a dose that produced deep anesthesia for 8–10 h. Tracheostomy was next performed, and a snugly fitting cannula (14-gauge, Braun, Melsungen,

Table 1
Summary of the protocol groups

Group	Number	Sensitization	Pretreatment	Mode of delivery	Duration of pretreatment (day)
C	6	No	None		
CS	9	Yes	None		
B2	8	Yes	Salmeterol 100 µg	Aerosol	5–10
AI	7	Yes	Cromolyn sodium 2 mg	Aerosol	5–10
IS	8	Yes	Fluticasone 100 µg	Aerosol	0–10
AC	5	Yes	Ipratopium bromide, 0.25 mg	Aerosol	5–10
AH	7	Yes	Clemastine 250 µg ranitidine 2 mg	Intraperitoneal	5–10

Germany) was inserted into the trachea. The guinea pigs were then mechanically ventilated (Model 683, Harvard Apparatus, South Natick, MA, USA) with a constant tidal volume of 1 ml/100 g and a frequency of 50–60 min⁻¹ at an FiO₂ of 0.3 in air. A 28-gauge silastic catheter (Portex, Hythe, GB) was subsequently introduced into the femoral artery. This catheter was connected to a calibrated pressure transducer (model 156 PC 06-GW2, Honeywell, Zürich, Switzerland) for the continuous monitoring of hemodynamic parameters such as blood pressure and heart rate. The jugular vein was cannulated with a similar silastic catheter for drug delivery. To ensure adequate analgesia, fentanyl was infused iv at a rate of 2 µg/kg/h. Midline sternotomy was performed and the chest was largely retracted. To avoid lung collapse with the chest open, a positive end-expiratory pressure of 2.5 cm H₂O was applied.

During the mechanical ventilation, the airway pressure was monitored (Validyne DP 45, Northridge, CA, USA). A heating pad (Miostar, Zürich, Switzerland) was applied to maintain the body temperature at 37 ± 0.5 °C, which was monitored via a rectal temperature sensor (Thermalert, model TH-8, Physitemp, Clifton, NJ, USA). Airway and arterial pressures, heart rate and rectal temperature were sampled at 50 Hz by an analogue/digital interface converter (Biopac, Santa Barbara, CA, USA), these signals were displayed and stored on a microcomputer. At approximately 30 min intervals, arterial blood samples were taken and analyzed radiometrically (model 505, Acid Base Laboratory, Copenhagen, Denmark).

2.3. Measurement of ZL

The forced oscillatory input impedance of the pulmonary system (ZL) was measured in the guinea pigs

to estimate the respective contributions of the airway and tissue mechanical properties to the total lung resistance, as described in detail previously (Petak et al., 1997; Hall et al., 1999; Habre et al., 2001). Briefly, a three-way tap was used to connect the tracheal cannula from the respirator to a loudspeaker-in-box system at end-expiration. During this short 6 s apneic period, small-amplitude pseudorandom pressure excitations generated by the loudspeaker were introduced into the trachea via a polyethylene wave-tube with known geometry. The forcing pressure signal contained 23 components in the frequency range of 0.5–21 Hz. The lateral pressures at the loudspeaker (P_1) and at the tracheal end (P_2) of the wave-tube were measured by two identical pressure transducers (model 33NA002D, ICSensors, Malpitas, CA, USA). The signals P_1 and P_2 were low-pass filtered (5th-order Butterworth, 25-Hz corner frequency) and sampled with an analogue-digital board of another microcomputer at a rate of 256 Hz. Fast Fourier transformation with 4 s time windows and 95% overlapping was used to calculate the pressure transfer functions (P_1/P_2) from the 6 s recordings. ZL was calculated as the load impedance of the wave-tube (Petak et al., 1997; Hall et al., 1999; Habre et al., 2001).

$$ZL = \frac{Z_0 \sin h(\gamma L)}{(P_1/P_2) - \cos h(\gamma L)}$$

where L is the length, Z_0 is the characteristic impedance, and γ is the complex propagation wave number of the wave-tube. The latter two parameters are determined by the geometrical data and the material constants of the tube wall and the air (Franken et al., 1981).

2.4. Separation airway and tissue mechanics

At low oscillation frequencies, the airways and the parenchyma exhibit distinctly different types of frequency-dependent behavior (Hantos et al., 1992; Lutchen et al., 1994, 1996; Petak et al., 1997, 1999; Hall et al., 1999; Adamicza et al., 1999; Habre et al., 2001, 2002). The airway properties have been shown to be manifested as a frequency-independent flow-resistive component in the real part and a linearly increasing component in the imaginary part of the ZL spectra. Conversely, both the parenchymal resistance in the real and the reactance in the imaginary part of the ZL spectra have been shown to decrease roughly inversely with increasing frequency. These distinctly different frequency dependences were utilized to separate the mechanical properties of the two compartments (Hantos et al., 1992; Lutchen et al., 1994, 1996; Petak et al., 1997, 1999; Hall et al., 1999; Adamicza et al., 1999; Habre et al., 2001, 2002). A model containing a frequency-independent airway resistance (R_{aw}) and inertance (I_{aw}) in series with a constant-phase tissue model (Hantos et al., 1992) including parenchymal damping (G) and elastance (H) was fitted to the ZL spectra by minimizing the relative differences between the measured and modeled impedance values:

$$ZL = R_{aw} + j\omega I_{aw} + \frac{(G - jH)}{\omega^\alpha}$$

where j is the imaginary unit, ω is the angular frequency ($2\pi f$) and $\alpha = 2/\pi \arctan(H/G)$. The R_{aw} and I_{aw} values were corrected for the resistance and inertance, respectively, of the measurement set-up, including the tracheal cannula.

2.5. Experimental protocol

After surgery, a 10–15 min period was allowed for the animal to reach a steady-state condition. Four ZL recordings were then made to establish the baseline. MCh or HIS was next administered in a random sequence to induce lung constriction. A 20 min period was allowed between the constrictor agents for the lung function to normalize; at the end of which another set of baseline ZL recordings was collected. MCh was infused iv at increasing doses of 1, 2, and 4 $\mu\text{g/kg/min}$. During each dose of MCh, ZL was mea-

sured at 1 min intervals for approximately 7–10 min, until four successive measurements revealed the same lung mechanical condition. HIS was administered iv in increasing boluses of 0.2, 0.4, and 0.8 $\mu\text{g/kg}$. ZL was recorded at 15 s, 1 min, and 2 min after the HIS bolus. Following the constrictor challenges, another period of 20 min was allowed for the animals to recover, and a third set of baseline ZL data was measured. Finally, an iv bolus of OVA (0.2 mg) was injected iv and the changes in ZL were followed at 1 min intervals for 10 min.

The ZL data collected under baseline conditions and those measured during steady-state constriction were averaged and used for the model fitting. The individual ZL data were fitted with the model after the administration of HIS and following the OVA challenges. In these cases, the peak response in R_{aw} in each animal was used for further analyses. The changes in the mechanical parameters were expressed as percentage changes from the baseline prior to the challenge.

2.6. Statistical analysis

Scatters in the parameters were expressed in SE values. The Kolmogorov–Smirnov test was used to test data for normality. Two-way repeated measures analysis of variances (ANOVA) with the constrictor agonist (MCh, HIS, or OVA) as the first and the prophylactic agent as the second variable was used to estimate the effects of pretreatment on the mechanical responses of the lungs to constrictor stimuli. The changes in the lung mechanical parameters in response to OVA in naive and in sensitized untreated animals were compared by one-way repeated measures ANOVA. Pairwise comparisons were performed by using the Student–Newman–Keuls multiple comparison procedures. Statistical tests were carried out with the significance level set at $P < 0.05$.

3. Results

Fig. 1 illustrates the real (resistance) and imaginary parts (reactance) of ZL and the corresponding model fits obtained in a representative non-treated sensitized guinea under control conditions, and during steady-state constrictions induced by increas-

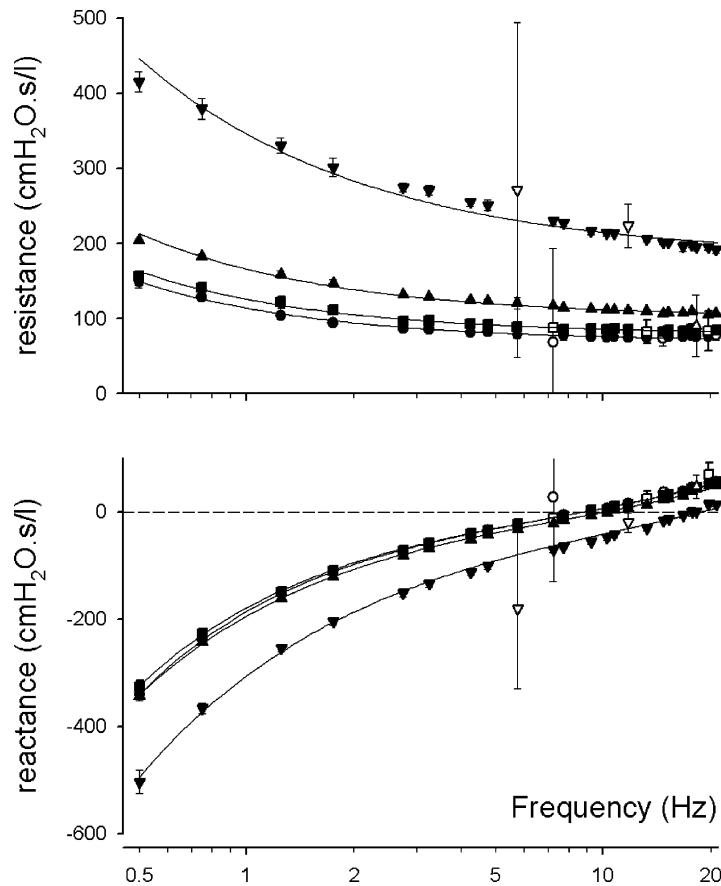


Fig. 1. Real (resistance) and imaginary parts (reactance) of pulmonary input impedance in a representative rat from the CS group under baseline conditions (●), and during iv methacholine infusions at a rate of 1 (■), 2 (▲), and 4 $\mu\text{g/kg/min}$ (▼). Symbols with bars represent mean $\pm$ S.D. values from 4 to 6 consecutive measurements. Lines indicate the corresponding model fits. Hollow symbols: data points that were excluded from model fits, because of corruption by cardiac artifacts.

ing doses of MCh infusion. Resistance values fall with increasing frequency in consequence of the decreasing contribution of the tissue resistance and approach plateaus corresponding to the flow resistances of the airways. The quasi-hyperbolic increases in the reactance curves reflect the elastic behavior of the parenchyma, while the linearly increasing inertive components dominate at high frequencies. The MCh-induced parallel increases in the resistance curves indicate dose-dependent elevations in Raw, while the enhanced frequency dependences suggest elevations in the parenchymal resistance. The changes in reactance curves were generally smaller, increases in the frequency dependence can only be seen at the highest dose of MCh. The

model fitted the impedance spectra well under each experimental condition with no systematic fitting error.

3.1. BHR induced by OVA sensitization

Sensitization to OVA induced marked and statistically significant increases in responsiveness to both MCh ($275 \pm 40\%$ versus $48 \pm 7.3\%$ increase in Raw in groups CS and C, respectively, at a MCh dose of 4 $\mu\text{g/kg/min}$, $P < 0.002$) and HIS ($95.6 \pm 10.4\%$ versus $25.9 \pm 13\%$ increase in Raw at a dose of 0.8 $\mu\text{g/kg}$, $P < 0.001$). Furthermore, all the sensitized guinea pigs responded to 0.2 mg OVA ($73 \pm 18\%$ peak increase in Raw), whereas the mechanical parameters

remained at the baseline level in the animals in group C ($-3.6 \pm 1.0\%$).

3.2. Effects of pretreatment on constrictor responses

Under baseline conditions, none of the mechanical parameters exhibited significant differences between the protocol groups. Figs. 2 and 3 depicts the changes in the airway and parenchymal mechanical parameters during MCh, HIS, and OVA challenges in the sensitized animals.

3.2.1. Methacholine challenges

MCh generally induced dose-dependent increases in the parameters Raw and G, and decreases in Iaw,

whereas the changes in H were much smaller. In group CS, MCh induced statistically significant increases in Raw at doses of 2 and 4 $\mu\text{g/kg/min}$, a significant decrease in Iaw at the highest dose, and statistically significant increases in the parenchymal mechanical parameters, with the changes being much greater in G. The animals in groups B2 and AC exhibited significantly smaller responses in Raw to the highest dose of MCh. Pretreatment with antihistamines in the animals in group AH did not lead to significant inhibitory effects on the MCh-induced changes in the lung mechanics. In contrast, pretreatment with ipratropium in group AC completely prevented the MCh-induced changes in the airway and tissue mechanical parameters, with no statistically significant increase in any lung

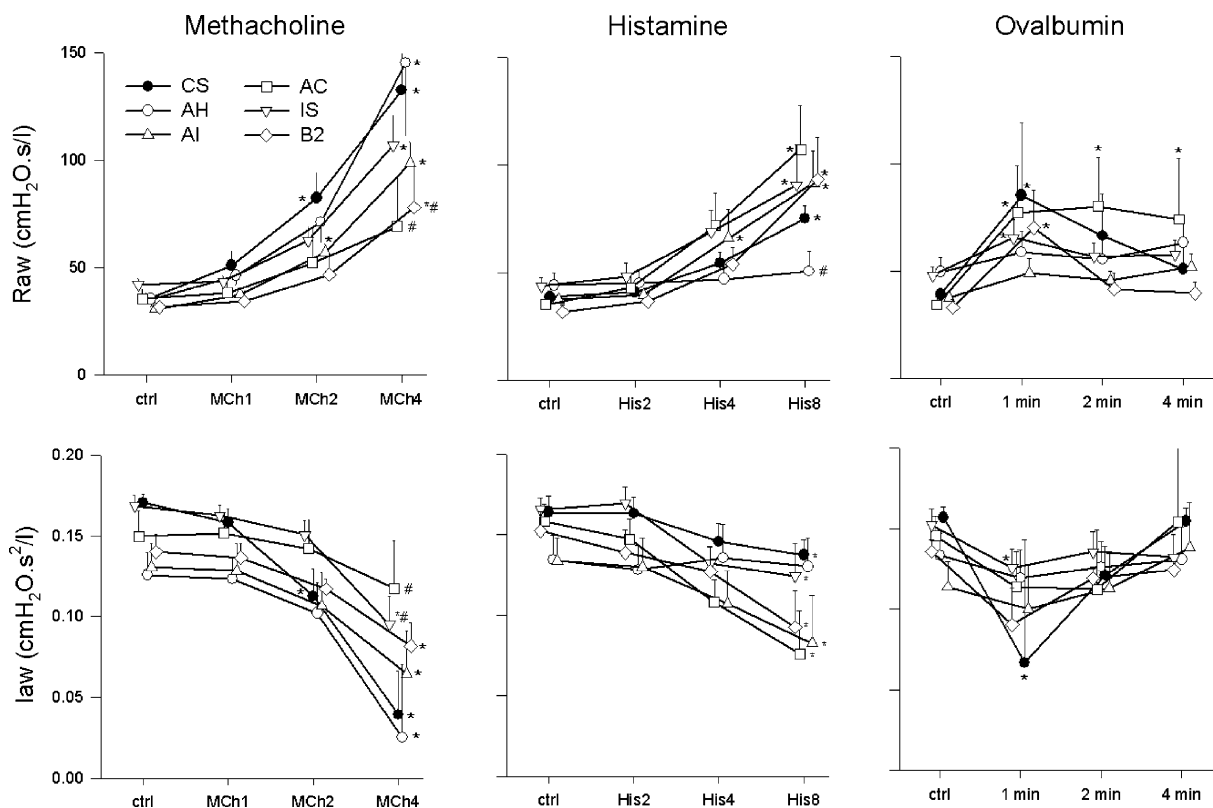


Fig. 2. Changes in airway resistance (Raw) and inertia (Iaw) during iv methacholine infusions (MCh 1, 2, and 4 correspond to an iv infusion rate of 1, 2 and 4 $\mu\text{g/kg/min}$, respectively), following iv histamine boluses (HIS2, HIS4 and HIS8 correspond to doses of 0.2, 0.4, and 0.8 $\mu\text{g/kg}$, respectively), and after ovalbumin administrations in sensitized control guinea pigs (CS) and in sensitized animals pretreated with antihistamine (AH), anti-inflammatory (AI), anticholinergic (AC), inhaled steroid (IS) or long-acting β_2 -agonist (B2) drugs. (*) $P < 0.05$ vs. the parameter value obtained under control conditions within the same group. (#) $P < 0.05$ vs. the parameter value obtained in group CS under the same conditions.

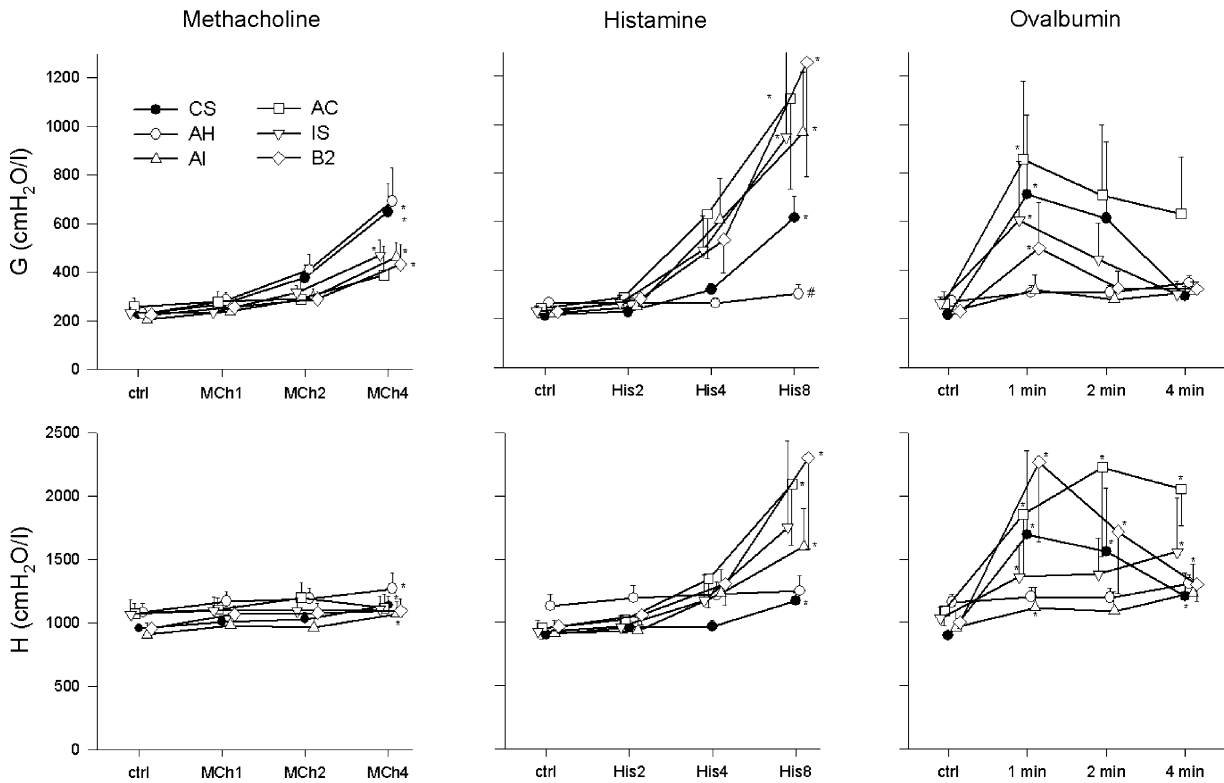


Fig. 3. Changes in parenchymal damping (G) and elastance (H) during iv methacholine infusions (MCh 1, 2 and 4 correspond to an iv infusion rate of 1, 2 and 4 $\mu\text{g/kg/min}$, respectively), following iv histamine boluses (His2, His4 and His8 correspond to doses of 0.2, 0.4, and 0.8 $\mu\text{g/kg}$, respectively), and after ovalbumin administrations in sensitized control guinea pigs (CS) and in sensitized animals pretreated with antihistamine (AH), anti-inflammatory (AI), anticholinergic (AC), inhaled steroid (IS) or long-acting β_2 -agonist (B2) drugs. (*) $P < 0.05$ vs. the parameter value obtained under control conditions within the same group. (#) $P < 0.05$ vs. the parameter value obtained in group CS under the same conditions.

mechanical parameter as compared with their baseline values.

3.2.2. Histamine challenges

HIS at 0.8 μg induced significant increases in R_{aw} and G in all groups except the guinea pigs pretreated with antihistamines; the animals in this group did not exhibit a significant change in any of the mechanical parameters measured. The decreases in I_{aw} were statistically significant for groups B2, AI, and AC, while increases in H were observed only in groups B2 and AC.

3.2.3. Allergic reactions

The iv administration of OVA resulted in significant increases in R_{aw} , G , and H , and decreases

in I_{aw} in the animals in groups CS, AC, IS, and B2 at 1 min. The peak responses in the mechanical parameters generally occurred 1 min following OVA administrations; prolonged responses were only observed in the animals of the AC group (Figs. 2 and 3).

Fig. 4 demonstrates the relative changes in the airway and parenchymal mechanical parameters at 1 min following the allergen exposures. No statistically significant inhibitory effects were observed in the groups AC, IS, and B2 with animals exhibiting substantial individual variability. Anti-inflammatory and antihistamine pretreatments resulted in significantly smaller changes in R_{aw} , I_{aw} , and G , whereas only antihistamine pretreatments prevented the OVA-induced increases in H significantly.

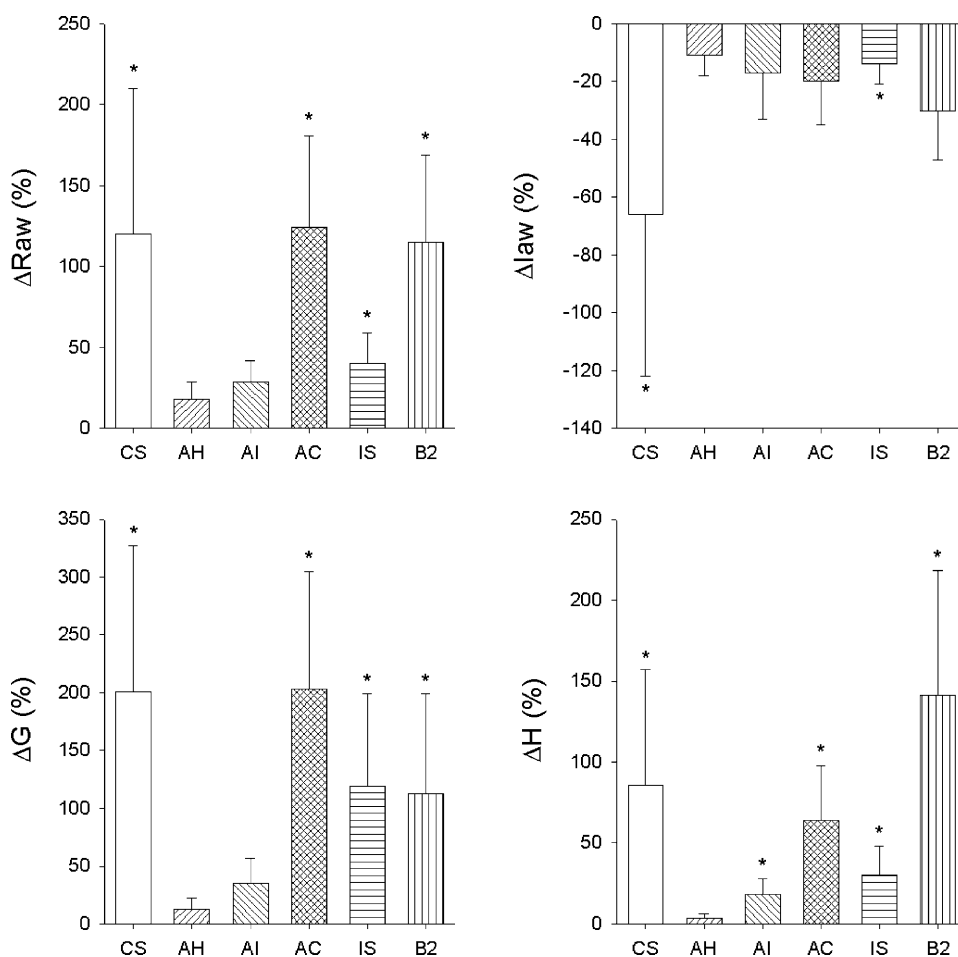


Fig. 4. Relative changes in airway resistance (Raw), inertance (law), and parenchymal damping (G) and elastance (H) 1 min following iv ovalbumin administrations in sensitized control guinea pigs (CS) and in sensitized animals pretreated with antihistamine (AH), anti-inflammatory (AI), anticholinergic (AC), inhaled steroid (IS) or long-acting β_2 -agonist (B2) drugs. (*) $P < 0.05$ vs. the parameter value obtained under control conditions within the same group.

4. Discussion

Bronchospasm of hyperreactive airways is one of the most frequent adverse events in allergic patients. Airway constriction may result from direct stimulation of the muscarinic receptors or indirectly via reflex pathways. Allergic reactions and the subsequent liberation of HIS or other constrictor mediators by mast cell degranulation may also compromise the lung function. In the present study, we have found that the pretreatment of animals with allergen-induced BHR with a long-acting β_2 -agonist or an anticholinergic is effective in protecting against lung constriction induced by

direct stimulation of the muscarinic receptors by MCh. Furthermore, pretreatment with a combination of H_1 and H_2 -receptor blockers abolished the HIS-induced bronchoconstriction, whereas the other prophylactic drugs had no beneficial effect. Finally, when the lung constriction resulted from an antigen challenge, pretreatments with antihistamine proved to be the most beneficial among the agents studied; administration of an anti-inflammatory drug inhibited the lung response of allergic origin significantly, while the other common prophylactic drugs had no significant beneficial protective effects against OVA-induced lung constriction in the sensitized animals.

4.1. Methodological considerations

Repeated sensitization with OVA has been shown to induce bronchial inflammation with subsequent BHR in guinea pigs. The results of the present study confirm the presence of BHR to exogenous constrictor stimuli and the development of an antigen-antibody reaction with severe lung constriction following this procedure (Featherstone et al., 1988; Wu et al., 2000). The choice of constrictor agonists covered the situations where cholinergic or HIS receptors are stimulated, and where the lung constriction results from an antigen-antibody reaction. MCh was infused, whereas OVA and HIS were administered as an iv bolus since the tachyphylaxis observed with the latter drug does not allow the maintenance of a steady-state constriction (Lutchen et al., 1994).

A description of the protective effects of prophylactic treatment on the airway and parenchymal compartments separately may be of importance, since different dilator drugs have been shown to exert distinctly different effects on the airway smooth muscle and on the parenchymal contractile elements (Petak et al., 1999). A long-acting β_2 -receptor agonist was demonstrated to act solely on the airways, whereas phosphodiesterase type 4 inhibition may also relax the contractile response of the parenchyma (Petak et al., 1999). Accordingly, forced oscillatory input impedance data were collected in the present study at frequencies encompassing the rate of spontaneous breathing. This measurement technique has been found to give an adequate description of the airway and parenchymal mechanics separately in small rodents under control conditions and following various constrictor challenges (Petak et al., 1997; Hall et al., 1999; Adamicza et al., 1999; Habre et al., 2001). The current ZL data exhibit a frequency dependence similar to those measured in various mammals (Hantos et al., 1992; Lutchen et al., 1994, 1996; Petak et al., 1997, 1999; Hall et al., 1999; Adamicza et al., 1999; Habre et al., 2001, 2002), and are in good agreement with those obtained previously in guinea pigs (Adamicza et al., 1999).

4.2. Effects of pretreatment on MCh-induced lung constriction

In agreement with previous findings in other rodents, the changes in lung mechanics during the iv

infusion of MCh were mainly due to the effects of airway narrowing (Petak et al., 1997, 1999; Habre et al., 2001). The changes in G together with the decreases in I_{aw} reflect the enhanced ventilation inhomogeneities (Lutchen et al., 1996; Petak et al., 1997). With the exception of the antihistamine, all of the prophylactic pretreatment agents applied in the present study inhibited this bronchoconstriction significantly. Moreover, the prevention of bronchoconstriction led to more uniform ventilation, which was manifested in smaller increases in G .

The prophylactic drugs applied in the present study exert their effects via different mechanisms. The current findings suggest that the most effective mechanism against MCh-induced bronchoconstriction of the hyperreactive airways is blockade of the muscarinic receptors by an anticholinergic drug, ipratropium. Application of this drug prevented significant changes in airway tone, suggesting that its routine use would be highly beneficial in situations where the cholinergic neural pathway is involved in the event of bronchospasm. With regard to the uniform findings reported previously (Nair et al., 1984; Larsson, 1987; Ulrik et al., 1992; Wu et al., 1992) and the functional antagonism, the blocking effect of ipratropium against a cholinergic bronchospasm was predictable, even during severe contractile stimuli. However, salmeterol and fluticasone exerted a similar degree of bronchoprotection up to the highest MCh dose (Fig. 2, MCh2), at which their effectiveness lagged behind the anticholinergic efficacy. Although, salmeterol and fluticasone did not completely block severe MCh-induced constriction, their beneficial effect is still noteworthy, while non-steroid anti-inflammatory treatment was less effective throughout the whole dose-response curve. These findings are in agreement with those demonstrating the effectiveness of both salmeterol (Derom et al., 1992; Booth et al., 1993; Rosenthal et al., 1999; Calhoun et al., 2001; Larj and Bleecker, 2002) and inhaled steroids (Sont et al., 1999; Juniper et al., 1990; Barnes and Pedersen, 1993; Corsico et al., 2000; Hofstra et al., 2000; van Rensen et al., 1999) in protecting against constriction induced via the cholinergic pathway, with a minimal effect of cromoglycate (Andersson and Bergstrand, 1981; Patel, 1984; Larsson et al., 2001). Since, inhaled steroids decrease inflammation and thus BHR only following long-term administration (Juniper et al., 1990; Barnes and Peder-

sen, 1993; van Rensen et al., 1999), while salmeterol exerts its beneficial effect after administration of only a single dose, the latter may also be the drug of choice to prevent the respiratory adverse effects observed during cholinergic stimulation of allergic sensitized airways.

4.3. Effects of pretreatment on histamine-induced lung constriction

The changes in the lung mechanical parameters after the administration of HIS agree with those observed previously in rabbits (Habre et al., 2002). The marked increases in Raw indicate the development of airway constriction. The increases in the tissue parameters are likely to be consequences of combined effects of intrinsic changes in the parenchymal viscoelasticity and a highly heterogeneous airway narrowing, developing especially in the lung periphery (Sekizawa et al., 1988) with a subsequent loss in lung volume (Lutchen et al., 1996).

In agreement with previous findings, by blocking the H₁ and H₂ receptors, antihistamine pretreatment exerted a highly significant protective effect against the lung constriction induced by HIS (Nathan et al., 1979; Greenberger et al., 1979; Tomioka and Yamada, 1982; Mauser et al., 1990; Habre et al., 2002) by totally abolishing the increases in the airway and parenchymal mechanical parameters. None of the other pretreatment agents blocked the lung responses to HIS in the sensitized guinea pigs. In accordance with previous results, the current findings confirm that short-term pretreatment with ipratropium or cromoglycate is ineffective in protecting against HIS-induced bronchoconstriction (Nair et al., 1984; Patel, 1984). In a majority of the previous studies, which demonstrated a bronchoprotective effect of inhaled corticosteroids, this prophylactic agent was administered for a prolonged period (4 weeks at least) (Juniper et al., 1990; Barnes and Pedersen, 1993; van Rensen et al., 1999; Hofstra et al., 2000). The short-term administration of inhaled corticosteroids is therefore unsuitable to protect against the adverse effects of HIS release in the lungs.

4.4. Effects of pretreatment on lung constriction induced by allergen exposures

Changes in the airway and parenchymal parameters following iv administration of the allergen in the sen-

sitized guinea pigs agree with those observed previously in sensitized rats (Hall et al., 1999) and followed similar pattern of change obtained following HIS challenges in the present study. Indeed, the increases in Raw following the HIS and OVA challenges correlated significantly ($r = 0.47$, $P = 0.01$), whereas changes in Raw following administrations of MCh and OVA were fairly independent ($r = -0.02$, $P = 0.9$). This finding indicates the dominance of endogenous release of HIS in the allergic reaction, while the cholinergic pathways played a minor role.

The results obtained in the different protocol groups suggest that prophylactic treatments with antihistamine and an anti-inflammatory drug are able to prevent both the airway and the parenchymal constriction induced by an allergic reaction in sensitized guinea pigs. Accordingly, the prophylactic use of these drugs may have beneficial effects in situations where there is a chance for the development of an antigen-antibody reaction.

The beneficial effect of antihistamine pretreatment in the presence of BHR is in accordance with previous results (Nathan et al., 1979; Mauser et al., 1990). Since, anti-inflammatory drugs reduce the capacity of the lung tissues to release histamine following antigen challenge (Andersson and Bergstrand, 1981), the effectiveness of cromolyn sodium against an allergen-induced lung response may be related to a smaller amount of endogenous histamine released following OVA administrations. The ineffectiveness of anticholinergics is not surprising in the view of the dominance of the endogenous HIS release after OVA challenge, while the relatively short-term administration may be responsible for the minor effects of the inhaled steroid. Conflicting results have been reported on the effects of a β_2 -agonist on hyperreactive airways. Salmeterol has been shown to have a beneficial effect on the airway tone in asthmatic patient (Rosenthal et al., 1999), whereas the regular use of salmeterol together with allergen exposure has been reported even to enhance the bronchial responsiveness to the allergen (Cockcroft et al., 1995; Larj and Bleeker, 2002). The current results confirm the findings of Calhoun et al. (2001) demonstrating that salmeterol alone does not have a significant effect on the lung constriction induced by an allergic reaction in sensitized guinea pigs.

4.5. Summary and conclusions

The results of the present study demonstrate that the agents commonly used against BHR do not provide equal protection against cholinergic, histamine and allergen-induced lung constriction. When cholinergic stimulation, as would be seen from stimulation of the irritant receptors in the airways, is expected to be the major cause of lung constriction, the prophylactic administration of ipratropium and/or salmeterol seems most appropriate since these agents are effective without requiring long-term administration. In the presence of allergy or atopy, however, when the release of HIS is responsible for the deteriorating lung function, antihistamine pretreatment affords significant bronchoprotection. In the presence of BHR, a combination of different prophylactic drugs may be mandatory to minimize the risk of lung constriction involving various pathways.

Acknowledgements

This work was supported by Swiss National Science Foundation Grant 3200-064899.01/1 (Bern, Switzerland) and Hungarian Scientific Research Grant OTKA F38340 (Budapest, Hungary). The authors thank Fabienne Fontao, technician at the Division of Anesthesiological Investigations, University of Geneva, Switzerland, for her excellent technical assistance.

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