Histamine induced bronchoconstriction and end tidal inspiratory activity in man

N E L Meessen, C P M van der Grinten, S C M Luijendijk, H Th M Folgering

Abstract

Background – End tidal inspiratory activity (ETIA) in diaphragm and parasternal intercostal muscles can be evoked in man and in animals by administration of histamine. Exacerbations of asthma and administration of histamine are often accompanied by hyperinflation. The aims of the study were to determine (1) the magnitude of ETIA in response to histamine in man, (2) the relative contributions of chemical and mechanical stimulation of airway receptors to ETIA, and (3) the importance of ETIA to hyperinflation.

Methods – The effects of inhalation of histamine on the electrical activities of the diaphragm and parasternal intercostal muscles measured with surface electrodes were studied in 21 subjects. The experiments were repeated after inhalation of 600 mg of salbutamol to prevent histamine induced bronchoconstriction and concomitant mechanical stimulation of airway receptors. Subjects were connected to a closed breathing circuit to measure the changes in functional residual capacity (FRC) for the different experiments.

Results – The mean values of histamine induced ETIA were 60.6% and 46.9% of peak inspiratory activities during control conditions for the diaphragm and intercostal muscles, respectively. After salbutamol histamine induced ETIA was reduced to about one quarter of pre-salbutamol values. FRC increased 427 ml as a result of inhalation of histamine, but after salbutamol this increase was only 53 ml. The data for ETIA and FRC were interpreted as indicating that the contributions of airflow limitation and ETIA to histamine induced hyperinflation are comparable.

Conclusions – Histamine is a forceful stimulus for inducing ETIA. Both chemical and mechanical stimulation of airway receptors contribute to evoke ETIA, of which the contribution of mechanical stimulation is the more important. ETIA contributes substantially to histamine induced hyperinflation.

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Keywords: end tidal inspiratory activity, histamine, EMG, lung receptors, β2 agonist, bronchoconstriction.

EMG activity of inspiratory muscles at the end of expiration has been observed in response to administration of histamine both in animals and man. We have shown in cats that this so called end tidal inspiratory activity (ETIA) is a vagal reflex activity which is due to stimulation of rapidly adapting pulmonary receptors. Rapidly adapting pulmonary receptors can be stimulated by histamine in cats, dogs, and rabbits. It is not known whether they are stimulated by histamine by direct chemical effects or by indirect mechanical effects due to bronchoconstriction, or by a combination of both.

Exacerbations of asthma and administration of histamine are often accompanied by hyperinflation. Although both bronchoconstriction and ETIA are accepted as causes of hyperinflation in bronchial asthma, it has not yet been shown which is more important. The aims of the present study were therefore to determine (1) the magnitude of ETIA in response to histamine in man, (2) the relative contributions of chemical and mechanical stimulation of airway receptors to ETIA, and (3) the importance of ETIA to hyperinflation. To this end, experiments were performed on human subjects who were challenged with histamine both before and after administration of the β2 agonist salbutamol. Salbutamol provides protection against bronchoconstriction caused by different stimuli such as histamine, so in the latter experiment histamine induced bronchoconstriction was largely prevented.

Methods

SUBJECTS

Twenty one subjects (nine men) of age range 14–62 years participated in the study. Characteristics of the subjects are presented in table 1. Informed consent was obtained from all subjects who were naive with respect to the aims of the study which was approved by the ethical committee of the hospital.

MEASUREMENT OF RESPIRATORY MUSCLE

FUNCTION

Subjects were connected to a closed breathing circuit (fig 1) by a mouthpiece and oxygen was added to the inhaled gas. The transdiaphragmatic pressure was recorded by means of a catheter placed in the abdominal aorta. The transdiaphragmatic pressure was measured 3 cm above the diaphragm level. The mean intratracheal pressure was measured at the end of the expiratory phase.

Table 1 Mean (SE) characteristics of the subjects (n = 21)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SE)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>32.5 (2.5)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.9 (1.5)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.7 (2.6)</td>
</tr>
<tr>
<td>FEV1 (%)</td>
<td>3.6 (0.19)</td>
</tr>
<tr>
<td>FEV1 (%pred)</td>
<td>49.4 (3.2)</td>
</tr>
<tr>
<td>FVC (l)</td>
<td>78.9 (1.93)</td>
</tr>
<tr>
<td>Rrs (hPa.l.1-5)</td>
<td>3.1 (0.08)</td>
</tr>
<tr>
<td>Rrs (hPa.l.1-5)</td>
<td>3.5 (0.36)</td>
</tr>
</tbody>
</table>

FEV1 = forced expiratory volume in one second; FVC = forced expiratory vital capacity; Rrs = airway resistance determined at 6 Hz by forced oscillation technique.
Histamine and inspiratory muscle activity

The provocative concentration of histamine (PC_{20}) at which the forced expiratory volume in one second (FEV_{1}) fell by 20% of the pre-challenge value was determined on day 1 with a histamine challenge test using the method of Hargrave et al. On day 2 the effects of inhaling one single concentration of histamine on EMGs of the diaphragm and intercostal muscles and on the FRC were studied. This concentration was the one at which on day 1 FEV_{1}, had fallen by 20% or more or, in case of a non-responsive subject, the highest concentration used on day 1 (≥ 8 mg/ml). After the subject had been connected to the breathing circuit the recording was started. The recording included at least five stable control breaths and continued for the two minutes of histamine inhalation and a further 1.5 minutes thereafter. Subsequently, 600 μg of salbutamol (Ventolin) was administered by a standard metered dose inhaler. Fifteen minutes later the single dose histamine test was repeated. In order to evaluate the effects of histamine and salbutamol on airway mechanics, respiratory resistance and FEV_{1} were determined on four occasions: before histamine (control), after inhalation of histamine, 15 minutes after inhalation of salbutamol, and after a subsequent inhalation of histamine.

**PULMONARY FUNCTION MEASUREMENTS**

Changes in FRC (ΔFRC) in response to histamine were calculated from the spirogram. Forced vital capacity (FVC) and FEV_{1} were obtained by standard spirometric measurements (Fukuda Sangyo). All values were related to the reference values of the European Community for Coal and Steel.

Respiratory resistance was determined by means of the forced oscillation technique which has been described in detail elsewhere. It has been reported that respiratory resistance measured at low frequencies correlates well with airway resistance. In the present study the resistance at 6 Hz was used (R_{ns}).

**DATA ANALYSIS**

Mean values of end tidal EMG activity and of peak EMG activity were calculated from five consecutive breaths recorded about 60 seconds after the inhalation of histamine had been stopped. These mean values will be referred to as EMGet_{BST} and EMGpk_{BST}, respectively. In a similar way we determined EMGpk_{CTRL} from the recordings of five consecutive control breaths of the same experiment taken from the last part of the control period. ETIA is represented by EMGet. In order to minimise the effects of instrumental, intersubject, and intrasubject scatter in the measured EMG activity ETIA was expressed as a percentage of EMGpk_{CTRL} and, for the same reason, EMGpk_{CTRL} was also expressed as a percentage of EMGpk_{CTRL}.

Changes in FRC were determined from the difference in FRC between the same groups of breaths, five control breaths, and five breaths supplied to maintain the oxygen concentration in the circuit constant (21%). Gas was sampled continuously from the circuit for the measurement of the oxygen concentration (Taylor Servomex) and subsequently fed back into the system (fig 1). A pump maintained a constant bias flow in the circuit of at least 120 l/min which prevented rebreathing of CO_{2}. Exhaled CO_{2} was absorbed by soda lime. An aerosol of saline or histamine could be delivered by a jet nebuliser ( DeVilbiss 646) which was connected to the circuit near the mouthpiece (fig 1). The calibrated output of the nebuliser was 0.13 ml/min.

The electrical activities of the diaphragm and intercostal muscles were obtained from two pairs of electrodes, each pair consisting of two silver discs of 7 mm diameter which were glued to the skin, 2 cm apart. The electromyogram (EMG) of the intercostal muscles was obtained from electrodes placed parasternally in the left second intercostal space. The EMG of the diaphragm was obtained from another pair of electrodes placed between the mid-clavicular and mid-axillary line in the seventh or eighth right intercostal space. The raw EMG activities were amplified, filtered (200–1200 Hz), rectified, and fed into a leaky integrator with a time constant of 50 ms (Neurolog, Digitimer). The integrated activities of the diaphragm and intercostal muscles, the volume signal of the wedge spirometer, and the oxygen concentration of the gas in the breathing circuit were recorded with a multichannel X-t recorder (Kipp, The Netherlands). The responses of the diaphragm, intercostal muscles, and functional residual capacity (FRC) to inhalation of histamine were studied from these recordings. EMG activities were also monitored by an audio monitor.

All subjects were tested in the sitting position. They were seated in a relaxed posture and were asked to remain in that position until the end of the recording. Thus, with good cooperation from our subjects we were able to minimise the effect of the postural muscles on recorded EMG activities. To divert their attention from breathing they were asked to concentrate on a poster placed in front of them.

**EXPERIMENTAL PROTOCOL**

The provocative concentration of histamine (PC_{20}) at which the forced expiratory volume in one second (FEV_{1}) fell by 20% of the pre-challenge value was determined on day 1 with a histamine challenge test using the method of Hargrave et al. On day 2 the effects of inhaling one single concentration of histamine on EMGs of the diaphragm and intercostal muscles and on the FRC were studied. This concentration was the one at which on day 1 FEV_{1}, had fallen by 20% or more or, in case of a non-responsive subject, the highest concentration used on day 1 (≥ 8 mg/ml). After the subject had been connected to the breathing circuit the recording was started. The recording included at least five stable control breaths and continued for the two minutes of histamine inhalation and a further 1.5 minutes thereafter. Subsequently, 600 μg of salbutamol (Ventolin) was administered by a standard metered dose inhaler. Fifteen minutes later the single dose histamine test was repeated. In order to evaluate the effects of histamine and salbutamol on airway mechanics, respiratory resistance and FEV_{1} were determined on four occasions: before histamine (control), after inhalation of histamine, 15 minutes after inhalation of salbutamol, and after a subsequent inhalation of histamine.
60 seconds after the inhalation of histamine had been stopped. A mismatch between oxygen supply and oxygen uptake will cause the baseline of the spirometer signal to drift one way or the other which would affect the results for AFRC. The shift in the baseline of the spirometer signal is proportional to the deviation of the oxygen concentration from 21%.

The relationship between these two factors was determined experimentally by adding a known amount of pure oxygen to the system and by reading the corresponding change in oxygen concentration. With the help of this relationship we have corrected the raw data for AFRC for the aforementioned oxygen concentration related shift of the spirometer signal. The inaccuracy of the correction was ±6 ml. This error adds to the overall random error in the determination of AFRC.

All values reported are means (SE). Unless otherwise indicated, differences were evaluated for statistical significance using the Wilcoxon’s test for paired observations. A p value of <0.05 was considered to be significant. Statistical analysis was performed using the statistical software package SPSS/PC+ (SPSS Inc, Chicago, Illinois).

**Results**

**EFFECTS OF HISTAMINE ON EMG AND FRC**

Figure 2 shows the results for one subject’s recordings of integrated electrical activities of the diaphragm and intercostal muscles and of the corresponding changes in lung volume. The recordings show that, before inhalation of salbutamol, ETIA is evoked in the diaphragm and in the intercostal muscles in response to histamine as end tidal EMGs do not return to baseline levels (fig 2A). At the same time FRC is increased. After administration of salbutamol inhalation of histamine evokes hardly any ETIA and FRC did not change at all (fig 2B).

Before inhalation of salbutamol mean (SE) values of histamine induced ETIA obtained from 21 subjects were 60.6 (8.5)% and 46.9 (7.0)% of EMGpkCTRL for the diaphragm and intercostal muscles, respectively (fig 3). The mean increase in FRC (AFRC) was 427 (67) ml or 13.7 (2.1)% of FRC predicted (table 2, fig 4). In response to histamine, peak inspiratory EMG activity increased to 375 (46)% and 364 (49)% of EMGpkCTRL for the diaphragm and intercostal muscles, respectively (fig 5).

After inhalation of salbutamol histamine induced ETIA was 16.6 (4.1)% and 10.9 (2.7)% for the diaphragm and intercostal muscles, respectively (fig 3). Histamine induced ETIA was significantly lower after inhalation of salbutamol compared with pre-salbutamol values.

**Table 2** Mean (SE) responses of FRC, FEV₁, and Rsₜₑₑ before and after salbutamol (n = 21)

<table>
<thead>
<tr>
<th></th>
<th>Before salbutamol</th>
<th>After salbutamol</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Histamine</td>
</tr>
<tr>
<td>∆FRC (ml)</td>
<td></td>
<td>427 (67)</td>
</tr>
<tr>
<td>FEV₁ (l)</td>
<td>3.6 (0.19)</td>
<td>2.8 (0.21)***</td>
</tr>
<tr>
<td>Rsₜₑₑ (hPa l/s)</td>
<td>3.5 (0.36)</td>
<td>5.9 (0.48)***</td>
</tr>
</tbody>
</table>

∆FRC = change in functional residual capacity in response to histamine; FEV₁ = forced expiratory volume in one second; Rsₜₑₑ = airway resistance determined at 6 Hz. *p < 0.05; **p < 0.01; ***p < 0.001 control versus histamine.
Histamine was ETIA, subjects with functional (%) ETIA was 77.6 <0.05; AFRC in inhalation 3 Meani (p control) <0.01 higher vs ETIA, FRC, in subjects. Salbutamol. Changes with values Significantly (p <0.001) before salbutamol (Wilcoxon test). ETIA was abolished. Data obtained from 21 subjects. Error bars indicate 1 SE. ***p <0.001 compared to histamine before and after inhalation of 600 µg salbutamol. Before inhalation of salbutamol Rs, increased and FEV decreased significantly in response to histamine compared with control. After inhalation of salbutamol these responses to histamine were much diminished. Data represent means obtained from 21 subjects. Error bars indicate 1 SE. ***p <0.001; **p <0.01 (Wilcoxon’s test for paired observations). Note inverse scale for FEV.

*p <0.05; **p <0.01 before versus after salbutamol (Wilcoxon’s test). ETIA in the diaphragm was significantly (p <0.05) higher than in the intercostal muscles for both conditions.

**Effects of Histamine and Salbutamol on Respiratory Resistance and FEV**

Changes in airway diameter will influence respiratory resistance at 6 Hz (Rrs). Effects of histamine on Rs, and FEV, obtained from 21 subjects before and after inhalation of salbutamol are shown in table 2 and fig 6. Rs, increased significantly (p <0.001) in response to histamine compared with control. After salbutamol only a small increase in Rs, was found in response to histamine compared with control values after salbutamol (fig 6). FEV, decreased significantly compared with control values (p <0.001) in response to histamine (table 2, fig 6). After salbutamol a small but still significant (p <0.01) fraction of that response remained (table 2, fig 6).

For further interpretation of the results a subgroup was defined including only those subjects whose FEV, decreased by less than 2% in response to histamine after salbutamol. The mean values of ETIA, ΔFRC, and FEV, for this subgroup are presented in table 3.

**Discussion**

Inhalation of histamine evoked ETIA in the diaphragm and intercostal muscles in all of our subjects. After administration of salbutamol the magnitude of histamine induced ETIA was considerably diminished. Similarly, FRC increased significantly in response to histamine, while this increase was minimal after sal-
butamol. FEV₁ decreased and Rrs increased in response to histamine. After salbutamol, inhalation of histamine only slightly affected these indices of bronchoconstriction.

EMG AND SURFACE ELECTRODES
It has been reported that the electrical activity of the diaphragm detected by surface electrodes is comparable with that detected by oesophageal electrodes²⁻⁵ and it is concluded that there is only a minimal contamination from the activity of other chest muscles. In man electrical expiratory activity was found in the transversus abdominis muscle but not in the external oblique or the rectus abdominis muscles during expiration.⁶ It is not likely, therefore, that the EMG of the diaphragm detected by surface electrodes is contaminated by electrical activity of abdominal muscles during expiration. Furthermore, the relationship between surface and oesophageal EMGs has not been found to be modified by changes in lung volume.⁵⁻⁷ Contamination of inspiratory EMGs by expiratory activity is unlikely since our results for the EMGs of the diaphragm and intercostal muscles (figs 3 and 5) were comparable and were qualitatively the same as those found in our experiments in animals in which we used intramuscular electrodes. The use of surface electrodes to record EMGs of the diaphragm and intercostal muscles during the experimental conditions used in this study therefore appears valid.

INDICES OF BRONCHOCONSTRICTION
Several pulmonary function variables can quantify the patency of the Airways. FEV₁ decreases with increasing bronchoconstriction. It has been shown that Rrs is increased in patients with chronic obstructive pulmonary disease (COPD) and with asthma.²² The changes in FEV₁ and Rrs in response to histamine and after salbutamol were as expected (table 2, fig 6). After salbutamol these indices reached values which were not different from pre-salbutamol control values or even “better”. FEV₁ decreased significantly in response to histamine even after inhalation of salbutamol (table 2, fig 6). Thus, histamine induced bronchoconstriction was not fully prevented by salbutamol although mean values of FEV₁, after histamine (94.8% predicted) were comparable with pre-salbutamol control values (94.7% predicted). Furthermore, changes in FEV₁ and Rrs were very similar with respect to administration of histamine and salbutamol (fig 6).

PEAK INSPIRATORY EMG ACTIVITY
Peak EMG activity was increased significantly in response to histamine (about 3.5 times control values). This may have been due to a delayed off switch of inspiration because of increased airway resistance as well as to enhanced stimulation of inspiratory activity by airway receptors. This is supported by the observation that the increased peak EMG activity was no longer observed after bronchoconstriction had been prevented by salbutamol. Furthermore, mean minute ventilation after administration of histamine (8.7 (0.5) l/min) was not significantly different from control values (8.1 (0.5) l/min). Hence, changes in peak EMG activity cannot be attributed to changes in arterial carbon dioxide tension.

END TIDAL INSPIRATORY ACTIVITY
It has been shown that histamine can induce ETIA in rabbits,¹ cats,² and man.¹¹ In a previous study in cats we have shown that stimulation of rapidly adapting pulmonary receptors elicits ETIA, whereas ETIA is inhibited by stimulation of slowly adapting receptors.²⁵ Histamine induced bronchoconstriction is caused both by stimulation of H₁ receptors in bronchial smooth muscles and by stimulation of rapidly adapting pulmonary receptors via a vagal reflex.²⁸⁻²⁹ In animals the activity of rapidly adapting pulmonary receptors has been found to increase in response to inhalation of irritant gases³⁰ and by intravenous or intratracheal application of histamine.⁸⁹ It has been shown in dogs that when histamine is administered at intervals of longer than 15 minutes the responses of rapidly adapting pulmonary receptors are reproducible.³¹ In the further discussion we start from the assumption that the underlying mechanisms of ETIA in man are similar to those found in experimental animals summarised above.

The present study shows that inhalation of histamine is a powerful stimulus for inducing ETIA, both in the diaphragm and in intercostal muscles in man. After inhalation of salbutamol histamine induced ETIA was considerably reduced, indicating that bronchoconstriction was involved in causing ETIA and, by extrapolation of the abovementioned findings obtained in experimental animals, this would mean that the enhanced ETIA after administration of histamine is mainly due to mechanical stimulation of rapidly adapting pulmonary receptors by bronchoconstriction. This will be discussed in more detail below. Slowly adapting receptors are stimulated by increased lung volume, so if stimulation of slowly adapting receptors also inhibits ETIA in man, histamine induced hyperinflation may diminish the magnitude of histamine induced ETIA through rapidly adapting receptors.

After administration of salbutamol + histamine, FEV₁ and Rrs reached levels which did not differ from their control values before salbutamol (p = 0.71 and 0.69, respectively). This suggests that the remaining ETIA after salbutamol + histamine is due to direct chemical stimulation of rapidly adapting receptors. After salbutamol, however, a small but significant decrease in FEV₁ and increase in Rrs compared with control were still observed in response to histamine (fig 6). Thus, it cannot be ruled out that after salbutamol some mechanical stimulation of rapidly adapting receptors by histamine induced bronchoconstriction was still present. In the results a subgroup of nine subjects was defined. The FEV₁ in these sub-
Histamine and muscle was no salbutamol. To more be attributed of ETIA after salbutamol (CNAP) causes muscles somewhat (mean 95% confidence interval), therefore, ETIA is inferred from our previous findings that continuous negative airway pressure (CNAP) - which is a strong mechanical stimulus for rapidly adapting receptors - induces ETIA in cats and in man. The contribution of chemical stimulation to the total stimulation of rapidly adapting receptors may depend on the concentration of histamine used. In subjects with bronchial hyperresponsiveness a low dose of histamine causes a firm bronchoconstriction with corresponding mechanical stimulation of rapidly adapting receptors but, because of the low histamine concentration, chemical stimulation of the receptors may be small. The P0.5 of eight of our nine subjects in the subgroup discussed above was ≥ 8 mg/ml histamine, thus the concentration of histamine administered to these subjects was rather high (mean 12.5 (1.9) mg/ml). In the remaining 12 subjects the concentration was lower (mean 9.68 (2.03) mg/ml). It is likely, therefore, that the conclusion derived from the subgroup of the nine non-responsive subjects will also apply to the responsive subjects - namely, that mechanical stimulation of rapidly adapting receptors is the more important stimulus in histamine induced ETIA. The properties of rapidly adapting receptors suggest a positive feedback mechanism as stimulation of the receptors induces reflex bronchoconstriction which in turn stimulates the receptors. However, the concomitant increases in ETIA and flow limitation result in an increase in FRC which is beneficial by increasing the diameter of the airway.

ETIA and Hyperinflation
In asymptomatic asthmatic subjects the end expiratory pleural pressure has been shown to be more negative during histamine induced hyperinflation than can be accounted for by the chest wall relaxation pressure, indicating the presence of ETIA. Therefore, in addition to airflow limitation ETIA may be one of the causes of hyperinflation. As mentioned before, after administration of salbutamol + histamine the FEV1 and Rrs reached levels which were comparable to their pre-salbutamol control values. It is likely, therefore, that the small histamine induced increase in FRC after salbutamol (table 2, fig 4) is solely due to ETIA which is also small in that condition (fig 3). Siafakas et al. showed that there is a close proportional relationship between phrenic activity and the driving pressure of the respiratory system during inspiration. In static respiratory conditions this pressure is linearly related to lung volume in the middle of the range. In quiet breathing, as occurred in our experiments, static conditions are approximated at the turning points of inspiration and expiration. The findings of Siafakas et al suggest that, in control conditions with quiet breathing restricted to the mid-range of lung volumes, tidal volume (Vt) is proportional to peak EMG activity and, therefore, that ETIA alone may account for an increase in FRC equal to ETIA times Vt/Cap (AFRC = ETIA . Vt/Cap). Note that ETIA is expressed as a percentage of EMGpVt/Cap. Computation of this product for the total group of 21 subjects using the mean values of ETIA for the diaphragm and intercostal muscles resulted in the following estimated values for AFRC for ETIA alone: 328 (55) ml for the experiments before salbutamol and 90 (24) ml for the experiments after salbutamol. A comparison of these data with those measured for AFRC (table 2) suggests that the contribution of ETIA to histamine induced hyperinflation in our experiments is larger than that of flow limitation before salbutamol and that ETIA is the sole determinant of hyperinflation after salbutamol, which is consistent with the absence of flow limitation in that condition.

This study was supported by the Netherlands Asthma Foundation (NAF-88.26).

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