Effects of atropine, acetylsalicylic acid, FPL 55712, and phentolamine on increased histamine responsiveness of cat lung strips under conditions of airway inflammation

P. Bánovčin, P. Višňovský, J. Hanáček, L. Plank, J. Korpás

ABSTRACT: Our recent in vitro studies on airways smooth muscle of cats with turpentine oil inflammation showed an increase of isometric tension of the lung strips to histamine application. This communication describes the effect of atropine, acetylsalicylic acid, FPL 55712, and phentolamine on the histamine contractions of the lung strips derived from control and experimental groups of cats. Pretreatment of the lung strips with atropine and acetylsalicylic acid had no significant effect on histamine induced contraction. FPL 55712 significantly decreased the mean values of isometric contractions after the low doses of histamine in experimental groups of strips. The isometric contractions after higher doses of histamine were not affected by FPL 55712 in both groups of strips. The significant increase of histamine contractions of the lung strips induced by experimental inflammation was reduced by phentolamine. The role of alpha adrenergic receptors in the increased responsiveness of the inflamed lung tissues to histamine is discussed.

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One of the characteristic features of asthma is the increased responsiveness of the airways to non-specific stimuli including chemical, physical, pharmacological and infectious stimuli. The mechanisms involved in the modulation of the non-specific bronchial reactivity are not known but the role of airway inflammation has recently been stressed [1-3]. This hypothesis is based on the observation that exogenous factors increasing non specific bronchial reactivity can cause airway inflammation. Although the participation of cholinergic reflexes in the expression of airway hyperresponsiveness is well recognised, a solely neurological mechanism could not account for all its features [4].

In previous works we reported that experimentally induced airway inflammation increased the responsiveness of the cat lung strips to histamine in vitro [5, 6]. This communication describes the effects of atropine, acetylsalicylic acid, FPL 55712 and phentolamine on the histamine contractions of the lung strips derived from control groups of cats and cats suffering from experimentally induced inflammation.

Methods

The experiments were conducted with 21 male and female cats weighing from 2.0 to 3.5 kg.

Model of airway inflammation

To induce the experimental inflammation, a method described in the previous paper was used [7]. The cats were anaesthetized with thiopental sodium (30 mg·kg⁻¹, i.p.). The cervical part of trachea was exposed. A cannula was then introduced into the trachea, and through it 5 mg of turpentine oil aerosol was administered, for 2 minutes, to spontaneously breathing cats. The cannula was then removed and the incision was sutured. The lung strips were prepared 48 h after the administration of turpentine oil.

The experimental inflammatory process in the airways was evaluated in living animals on the basis of their behaviour and auscultation of the lungs. Part of the trachea and lung parenchyma of every cat was taken for histological assessment of the inflammation.

In vitro method

At the above interval after administration of the aerosol cats from the experimental group and healthy (control) animals were anaesthetized with thiopental sodium (30 mg·kg⁻¹, i.p.) and exsanguinated. Their thoracic cavities were opened and the lungs were removed. The lung parenchymal strip (20x3x3 mm) was cut out from

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the diaphragmatic lobe of the lung according to Lutcher et al. [8] and placed in an organ bath containing Krebs-Henseleit solution (NaCl 112.9, KCL 4.7, CaCl$_2$ 2.0, MgSO$_4$ 0.5, NaHCO$_3$ 24.9 and glucose 11.1 mol-'$^1$) at 37°C and pH 7.4, saturated with 95% O$_2$ and 5% CO$_2$. After 20 min initial tension of the preparations at 4 g and after an adaptational interval of 30 min at a working tension at 2 g, histamine was added cumulatively to the organ bath. The various histamine concentrations used ($10^{-9}$-$10^{-3}$ mol-l$^{-1}$; final bath concentrations) were always in the same volume (0.2 ml). Atropine, phenolamine and FPL 55712 were added to the organ bath 5 min, and acetylsalicylic acid 30 min, before the addition of histamine. In this study, comparisons of contraction responsive curves in paired strips from the same cats were used to test the effect of pretreatment. On each strip only one dose-response curve was determined. Mechanical responses were recorded isometrically, using a static-dynamic M1000 tensometric apparatus, and were registered on an MTA 175 Kutesz linear recorder.

The following substances were employed: Acetylsalicylic acid (Spofa), atropine (Spofa), FPL 55712 (Fisons), histamine (Spofa), phenolamine (Ciba) and thiopental sodium (Spofa).

**Data Analysis**

Histamine responses were plotted as a percent of maximal force at the highest concentration ($10^{-3}$ mol-l$^{-1}$) of the control group. Individual concentration-response curves were drawn by hand and ED$_{50}$ value was defined as the concentration of agent producing a half maximal response. The maximal isometric tension was elicited after the dose $10^{-3}$ mol-l$^{-1}$ of histamine. However, the dose $10^{-2}$ mol-l$^{-1}$ of histamine had to be used in some lung strips to elicit the maximal tension. ED$_{50}$ values were a measure of the sensitivity of the preparations and the potency of the agonist. Student’s t-test was used to test the probability that observed differences between means were due to chance. Differences that produced p values of less than 0.05 were accepted as significant. In the figures the mean values±s.e are presented.

Table 1. – ED$_{50}$ values for histamine in control and experimental groups of lung strips

<table>
<thead>
<tr>
<th>Treated by:</th>
<th>Control groups</th>
<th>Experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without</td>
<td>$1.7 \times 10^{-1.0.32}$</td>
<td>$1.8 \times 10^{-1.0.80}$</td>
</tr>
<tr>
<td>Atropine</td>
<td>$1.06 \times 10^{-1.0.20}$</td>
<td>$1.8 \times 10^{-1.0.56}$</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>$1.6 \times 10^{-1.0.72}$</td>
<td>$1.75 \times 10^{-1.0.65}$</td>
</tr>
<tr>
<td>FPL 55712</td>
<td>$1.2 \times 10^{-1.0.66}$</td>
<td>$2.0 \times 10^{-1.0.71}$</td>
</tr>
<tr>
<td>Phenolamine</td>
<td>$2.3 \times 10^{-1.0.63}$</td>
<td>$2.2 \times 10^{-1.0.90}$</td>
</tr>
</tbody>
</table>

**Results**

Histamine ($10^{-9}$-$10^{-3}$ mol-l$^{-1}$) produced a dose related contraction of the cat lung parenchymal strip. In the control group (n=11), the maximal isometric tension of the lung strips was 1.06±0.20 g. The ED$_{50}$ value for histamine was $1.7 \times 10^{-1.0.32}$ mol-l$^{-1}$. In the strips, a difference between control histamine response and those obtained from cats with experimentally induced airway inflammation (experimental group; n=10) was evident. The maximal isometric tension of the lung strips was 1.80±0.19 g, a value significantly different (p<0.01) from that of the control group. The ED$_{50}$ for histamine (1.8$x10^{-3}$ + 0.80 mol-l$^{-1}$) was not significantly different when compared with control group.

The possible participation of the cholinergic “M” receptors in the histamine-induced contraction of the lung strips was examined by pretreatment with atropine (final bath concentration $10^{-4}$ mol-l$^{-1}$). The application of the atropine to the organ bath did not change the initial tension of the control as well as experimental lung strips. No significant effect of atropine pretreatment in both groups of lung strips was observed. The isometric tension developed after $10^{-3}$ mol-l$^{-1}$ histamine was treated in the control group with atropine 1.06±0.18 g (n=11), and the ED$_{50}$ value for histamine was $1.8 \times 10^{-2.56}$ mol-l$^{-1}$. In the experimental group, the lung strips pretreated with atropine (n=10) the maximal isometric tension developed after the histaminewas 1.72±0.18 g. The ED$_{50}$ value for histamine was $1.6 \times 10^{-1.0.71}$ mol-l$^{-1}$.

The application of acetylsalicylic acid ($10^{-4}$ mol-l$^{-1}$) the inhibitor of prostaglandin synthesis - to the organ bath did not change the initial tone of the lung strips of the control group. In the experimental group of strips acetylsalicylic acid produced a slow loss of the baseline tension (0.9±0.03g) in four of the ten lung strips. The histamine induced contraction of the lung strips of both groups was not significantly influenced by acetylsalicylic acid. The maximal isometric tension in the control (n=11) and experimental (n=10) groups, both pretreated with acetylsalicylic acid was $0.91 \pm 0.16$ g and $1.73 \pm 0.20$ g, respectively. No significant changes in the ED$_{50}$ values for histamine were observed ($1.6 \times 10^{-0.72}$ mol-l$^{-1}$; $1.75 \times 10^{-1.0.65}$ mol-l$^{-1}$).

On the other hand, the antagonist of the leukotriene C$_4$ and leukotriene D$_4$ (LTC$_4$, LTD$_4$) FPL 55712 in dose $10^{-4}$ mol-l$^{-1}$ decreased the mean values of isometric contractions after the low doses of histamine in both groups of strips. However, this decrease was significant (p<0.05) in the experimental group only. The isometric contractions after higher doses of histamine were not affected by FPL 55712 in both groups of the lung strips. The maximal tensions were 1.19±0.16 g and 1.89±0.17 g, respectively. No significant differences in ED$_{50}$ values for histamine were observed in treated lung strips ($1.2 \times 10^{-1.0.66}$ mol-l$^{-1}$) control group, n=11; $2 \times 10^{-1.0.71}$ mol-l$^{-1}$) experimental group, (n=10) when compared with ED$_{50}$ values for histamine obtained in the paired lung strips without pretreatment. The FPL 55712 did not change the base-line tension of the lung strips in both groups.

Similarly, the application of the adrenergic alpha-receptor blocker phenolamine ($10^{-4}$ mol-l$^{-1}$) to the organ bath did not change the initial tension of the control as well as experimental lung strips. Figure 1
shows the effect of phentolamine pretreatment on histamine induced contractions of control (n=11) and experimental (n=10) groups of strips. The mean values of the isometric contractions were decreased in both groups, but significantly in the experimental group only. The ED₅₀ values for histamine were not significantly changed (treated control group: 2.3x10⁻³±0.63 mol·L⁻¹; treated experimental group: 2.2x10⁻³±0.90 mol·L⁻¹). The maximal isometric tension of the treated controls was 0.92±0.14 g, and the maximum tension developed in the treated experimental group was 1.35±0.15 g. The significant increase of the histamine contractions of the lung strips induced by experimental inflammation was reduced by phentolamine.

![Figure 1](image-url)

**Fig. 1.** - Effect of phentolamine on histamine response of the lung strips from control (C) and experimental (E) groups of cats. Statistical significance: *p<0.05; **p<0.01. (T=pretreatment of the lung strips by 10⁻⁴ mol·L⁻¹ phentolamine).**

**Discussion**

In our previous in vitro experiments we observed that the contractile responses of the lung strips to histamine were enhanced depending on the stage of the development of airway inflammation [3, 6]. One of the possible explanations is that airway smooth muscle contraction can be augmented by interaction between histamine and simultaneous cholinergic stimulation [9-11]. We tested this possible mechanism of enhanced histamine responsiveness of the lung strips under conditions of airway inflammation by pretreatment of the lung strips with atropine. Stephens et al [12] reported that atropine reduced the tension developed by tracheal smooth muscles in vitro in response to histamine. In our experiments atropine did not change the histamine contractions in control as well as in experimental groups of strips. This is in agreement with results reported by Skocor et al [13]. Our results suggest no participation of cholinergic mechanisms in histamine-induced contraction of cat lung strip.

The role of the prostaglandins in the inflammatory process is well documented [14, 15]. Their possible participation in the increased smooth muscle responsiveness was analysed by pretreatment with acetylsalicylic acid. The application of acetylsalicylic acid to the organ bath did not change the initial tone of the lung strips of the control group. In the experimental group of lung strips, acetylsalicylic acid produced a loss of the base-line tension in four out of ten lung strips. We suppose that under conditions of experimental inflammation of the airways the synthesis of bronchoconstricting cyclo-oxygenase products is increased. Another possible explanation is that the number or activity of receptors for endogenous bronchoconstrictive prostaglandins is increased [16]. In this study, the pretreatment of the control as well as of the experimental group of lung strips with acetylsalicylic acid does not significantly change the histamine responsiveness and the increased responsiveness of the lung strips of the experimental group persist. In experiments of Weichman et al. [17] the meclofenamic acid (a cyclo-oxigenase inhibitor) enhanced the histamine contraction of guinea pig trachea but histamine contraction of guinea pig lung strip remained unchanged. This is in agreement with our results.

Another group of metabolites of arachidonic acid, leukotrienes have potent myotrophic activity on airway smooth muscles [18]. However, their role in the inflammatory reactions is not fully understood. We studied the influence of FPL 55712 on histamine-induced contractions of the cat lung strips. Application of FPL 55712 to the organ bath did not change the initial tension of the lung strips of control and experimental groups. This indicates no participation of the leukotrienes which can be blocked by FPL 55712 in regulation of initial tension of the cat lung strip. Similarly, the histamine-induced contractions of the control lung strips were not significantly influenced by FPL 55712. This is in agreement with results obtained in canine tracheal smooth muscle [19] and guinea pig lung strip [18]. In the experimental group of lung strips the responses to lower concentrations of histamine were significantly decreased. However, the responses to higher concentrations of histamine remained unchanged. In experiments of Crease and BACH [20], 10⁻⁵ mol·L⁻¹ LTC₄ did not affect the concentration-response relationship for histamine-induced contraction. Higher LTC₄ and LTD₄ concentrations produced contractile responses. This increase in the base-line tension was associated with a decrease in the maximum response to histamine, but a combined response to LTC₄ or LTD₄ and histamine (3·10⁻⁴ mol·L⁻¹) was not significantly different from the maximum control response to histamine alone. These results
agree with those of Anderson and Goldberg [21]. We suppose that under inflammatory conditions low histamine concentrations elicit the production of leukotrienes and their additive effect on histamine contraction is blocked by FPL 55712.

Phentolamine, a non-selective antagonist of the alpha adrenergic receptors, was the only drug able to eliminate the significance of the difference in the responsiveness between the control and the inflamed lung strip groups. These results point out the role of alpha adrenergic receptors in the increased reactivity of the inflamed lung tissue to histamine. An antihistaminic effect of phentolamine can be excluded. Phentolamine did not show any effect in the uninfamed controls. In addition, pretreatment of the lung strips of control and experimental groups by clemastine (an antagonist of the histamine H₃ receptors) depresses the contractile response to histamine in both groups leaving the significant differences in their reactivity unchanged [22]. A role for alpha adrenergic enhancement of the histamine-induced contraction of the airways smooth muscle has been proposed by Anderson and Nilson. Douglas et al [23] demonstrated an inhibition of the histamine-induced bronchoconstriction in guinea pigs after the pretreatment with phentolamine suggesting direct interaction with histamine. At present, the mechanism remains unknown. In the observations of Kneussl and Richardson [24] bronchial smooth muscle taken at autopsy from persons with no evidence of lung disease contracted when exposed to norepinephrine after pretreatment by histamine, whereas the muscle from patients with the chronic obstructive lung disease or bronchopneumonia contracted without any pretreatment and the contraction could be blocked by phentolamine. Henderson et al [25] observed exaggerated alpha-adrenergic responses in the patients suffering from asthma in comparison to normal subjects. There is also evidence from receptor binding studies in guinea-pigs suggesting an increase in the number of alpha receptors and a decrease of beta receptors in an animal model of chronic asthma [26]. Further studies are required to investigate the role of alpha-adrenergic receptors in bronchial hyperreactivity.

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References

ANALYSIS OF INCREASED HISTAMINE RESPONSIVENESS OF LUNG STRIP


