

Depression of polymorphonuclear chemotaxis and T-lymphocyte proliferation following histamine inhalation in man

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ABSTRACT: Histamine, *in vitro*, via H₂-receptor activation, exerts an inhibitory effect on polymorphonuclear (PMN) chemotaxis and T-lymphocyte proliferation. The aim of this study was to verify these histamine inhibitory effects in man. Healthy and asymptomatic asthmatic volunteers inhaled a histamine (0.1%), methacholine (0.1%) or saline aerosol for 3 min. Asthmatics were selected on the basis of low bronchial sensitivity to pharmacological agents. Blood was taken before and at different times following aerosol challenge. PMN chemotaxis was studied *in vitro* by the Boyden assay. T-lymphocyte proliferation was measured by counting H₃-thymidine incorporation in cultured mononuclear cells. Plasma histamine was measured by a specific and sensitive radioimmunoassay. Inhalation of histamine or methacholine caused a 22-43% decrease in forced expiratory volume in one second (FEV₁) in asthmatics only. In both groups, there was a transient increase of plasma histamine immediately following histamine inhalation, and 2 and 4 h later, a significant decrease of PMN chemotaxis and T-lymphocyte proliferation. Inhalation of methacholine or saline had no effect on leukocytes. Oral administration of an H₂-receptor antagonist, cimetidine, before histamine inhalation, prevented the decrease of PMN chemotaxis and T-lymphocyte proliferation, whereas astemizole, an H₁-antagonist, had no effect. In conclusion, histamine, at a dose commonly used for bronchial provocation tests, causes, in man, 2 and 4 h after inhalation, a depression of PMN chemotaxis (tested *in vitro*) and T-lymphocyte proliferation through activation of H₂-receptors.

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Histamine has long been considered a mediator of certain types of acute inflammation. Recently, however, a new concept has developed which presents histamine as a modulator of the physiological processes which regulate both immune and inflammatory events [1, 2]. It is well known that histamine exerts, *in vitro*, an inhibitory effect on various types of inflammatory cells via H₂-receptors. For example, histamine inhibits the release of basophilic mediators [3], chemotactic response of polymorphonuclear neutrophils (PMNs) [4], cytotoxicity and proliferation of T-lymphocytes [5] and NK activity [6]. To the best of our knowledge, these inhibitory effects of histamine have never been shown in man. Since inhalation of histamine is widely used in respiratory medicine to study bronchial responsiveness, we thought it interesting to investigate the effect of inhaled histamine on PMN chemotaxis and T-lymphocyte proliferation in normal volunteers and asthmatic patients. Asthmatics were included because it is known that PMNs from atopic asthmatics are, *in vitro*, more sensitive chemotactically than normal cells to the inhibitory effects of histamine [7]. Our results show clearly that, in

man, inhaled histamine causes a significant depression of these leucocyte functions. This conclusion is of theoretical interest because it provides evidence that histamine can modulate human inflammatory cells *in vivo*.

Subjects and methods

Inhalation challenges were performed on normal and asthmatic volunteers according to a technique well standardized in our laboratory. Blood samples were taken before and at different times after inhalation to study PMN chemotaxis, T-lymphocyte proliferation and plasma histamine levels. The protocol of this study was approved by the Ethics Committee of the University of Liège.

Subjects

Ten healthy individuals (8 males and 2 females; 21-28 yrs old) were studied. They had normal pulmonary function and no allergic background on the basis of history,

negative skin tests and radio allergosorbent test (RAST) for a battery of allergens. Six allergic asthmatics (4 males, 2 females; 19–25 yrs old), selected on the basis of low bronchial sensitivity to inhaled histamine (forced expiratory volume in one second (FEV_1) drop of about 30% only after 3 min of inhalation) were also studied. They were sensitive to house dust, dermatophagoïdes and other allergens on the basis of history, skin tests and RAST. They were, however, asymptomatic and received no treatment.

Inhalation challenges

Histamine or methacholine inhalation tests were performed at 8 am. Before starting the experiment, all subjects had an $FEV_1 \geq 80\%$ of the predicted value [8]. FEV_1 was measured with a Godart spirometer before and 3 min after the inhalation of saline, histamine or methacholine solution. The aerosol was generated by an ultrasonic De Vilbiss nebulizer (output 0.8 ml·min⁻¹) containing 10 ml of solution. Histamine dihydrochloride (Sigma, St-Louis, Mo, USA) and methacholine (Sigma) solutions 0.1% in saline were prepared extemporaneously.

The subject, in a sitting position, inhaled the aerosol for 3 min at tidal volume, through a mouthpiece, with a noseclip. In some experiments, the subjects received orally, astemizole (Janssen, Beerse, Belgium), a histamine H_1 -receptor antagonist, or cimetidine, a histamine H_2 -receptor antagonist (Smith Kline, Rixensart, Belgium), at a therapeutic dose (20 and 800 mg·day⁻¹, respectively) during 3 days and 2 h before histamine challenge.

PMN chemotaxis

Venous blood (20–40 ml) was drawn into 10 ml vacutainer tubes, each containing 143 USP units of lithium heparin, before and 0.5, 2, 4 and 24 h after the challenge. The blood was mixed and allowed to stand for 45 min at 37°C. The upper plasma layer rich in leucocytes and platelets was removed and pooled into a plastic tube. The concentration of leucocytes was determined by counting (haemocytometer) and adjusted with Hank's balanced salt solution to $2 \cdot 10^6$ cells·ml⁻¹. PMN chemotaxis was studied by a micropore filter technique using a modified Boyden chamber [6, 7]. Briefly, 2 ml of the leucocyte suspension ($2 \cdot 10^6$ cells·ml⁻¹) were placed in the upper compartment of the chamber and the same volume of chemoattractant in the lower compartment. The chemoattractant was autologous serum activated with zymosan (Sigma). The chambers were incubated for 3 h at 37°C in humidified air. After incubation, the filters (Millipore, 5 µ porosity) were removed, stained with Diff-Quick (Harleco). Chemotaxis was measured by counting PMN on both faces of the filter, at high power ($\times 440$), in 10 microscopic fields. The number of PMN present on the lower face of the filter was expressed as a percentage of the number of PMN remaining on the upper face of

the filter (normal value between 450–685%). All samples were performed in duplicate and the mean calculated. In this study, PMN chemotaxis was expressed as a percentage of the basal values, *i.e.* chemotaxis measured before inhalation. Preliminary experiments have shown that oral intake of astemizole or cimetidine by human volunteers has no significant effect on PMN chemotaxis.

T-lymphocyte proliferation

Heparinized venous blood (20 ml) was taken before and 0.5, 2, 4 and 24 h after the challenge. Mononuclear cells were obtained by density gradient centrifugation of blood on lymphoprep (Nyegaard, Oslo, Norway). Cells were washed three times in Hank's without Ca⁺⁺ and Mg⁺⁺ (Gibco, Scotland) and resuspended to $1 \cdot 10^6$ cells·ml⁻¹ in RPMI 1640 containing 5% heat inactivated fetal calf serum (Gibco). Triplicates (200 µl) of washed cells were incubated for 72 h (37°C, 5% CO₂) in plastic microwells (Nunc, Denmark), in the presence or absence of purified phytohaemagglutinin (PHA, Wellcome, Dartford, UK) to a final concentration of 1 µg·ml⁻¹. Seventeen hours before completion of the culture, 20 µl of tritiated thymidine (methyl 3H thymidine: 50 µCi·ml⁻¹; Amersham, UK) was added to the wells. The cells were harvested (Skatron) and the incorporation of the labelled thymidine in the lymphocytes was measured using a beta-counter (LKB). All the samples were run in triplicate and the mean calculated. The results were expressed as a percentage of the basal values, *i.e.* lymphocyte proliferation measured before inhalation challenge. The mean stimulation index was the ratio of counts per minute (CPM) of PHA stimulated lymphocytes to CPM of unstimulated lymphocytes (normal value 62 ± 12). Preliminary experiments have shown that oral intake of astemizole or cimetidine by human volunteers has no significant effect on T-lymphocyte proliferation.

Plasma histamine determinations

Venous blood (5 ml) was collected in a cold tube containing sodium ethylene diaminetetra-acetic acid (EDTA) before and 5 min, 10 min and 4 h after histamine challenge. Blood was immediately centrifuged at 4°C and the upper part of the plasma supernatant carefully collected. Histamine was measured by a specific and sensitive radioimmunoassay (Immunotech, Marseille, France) [10, 11]. Its sensitivity was 0.1 ng·ml⁻¹ with an intra or interassay variation coefficient of <8%.

Statistical analysis

Student's paired t-test was used to assess the significance of the difference between two means. When $p < 0.05$, the difference is statistically significant.

Results

Clinical tolerance

Normal subjects had no respiratory or systemic symptoms following inhalation of histamine or methacholine at a dose of 2.4 mg or lower. Four healthy volunteers, inhaling 15 mg of histamine, experienced some flushing, headache, tachycardia, and two had a slight decrease of systolic arterial pressure (-2.6 kPa). Asthmatics received a maximal dose of 2.4 mg of histamine or methacholine which caused an FEV₁ drop in all of them, ranging from 22-43% of basal values. No systemic reaction was observed.

Chemotaxis

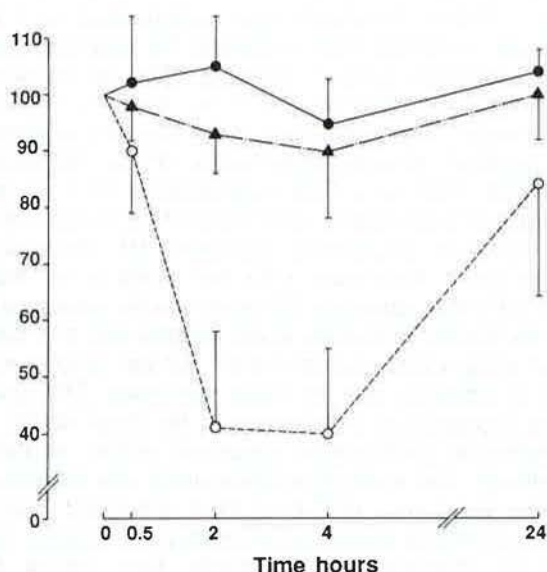


Fig. 1. - Effect of histamine 2.4 mg (O), methacholine 2.4 mg (▲) or saline (●) inhalation on PMN chemotaxis (% of basal values) in normal volunteers (n=4). PMN: polymorphonuclear neutrophils.

PMN chemotaxis and T-lymphocyte proliferation

PMN chemotaxis and T-lymphocyte proliferation were studied before and after histamine (2.4 mg), methacholine (2.4 mg) and saline aerosol challenges.

Before inhalation, all of the volunteers had basal PMN chemotaxis (range 450-685%) and spontaneous or PHA-induced T-lymphocyte proliferation (mean stimulation index 62±12) within normal limits.

As shown in figure 1, inhalation of histamine by normal volunteers caused a significant drop of PMN chemotaxis, 2 and 4 h after the challenge. The mean inhibition as a percentage of basal value was 60% (range 42-74%) for the same time values (p<0.05). Twenty four hours later, basal values were again reached (p=0.07). Saline or methacholine inhalation had no significant effect on PMN chemotactic response. Leucocyte and white cell differential counts (Coulter) were not modified following histamine inhalation (table 1). Similar results were observed in asthmatics, with a mean PMN chemotaxis inhibition of 55 and 52%, respectively, 2 and 4 h after the inhalation.

Figure 2 shows mean spontaneous and PHA pulsed T-lymphocyte proliferation (% of basal values) 0.5, 2, 4 and 24 h following histamine, methacholine and saline inhalation in normal subjects. Saline and methacholine had no significant effect on T-lymphocyte proliferation. In contrast, histamine caused a significant decrease of pulsed T-lymphocyte proliferation 2 and 4 h after inhalation (p<0.05). Spontaneous proliferation was only significantly decreased 2 h after inhalation (p<0.05). Twenty four hours later, basal levels were again reached. In the four asthmatics studied, the same results were observed.

Dose-effect relationship

The relationship between aerosolized histamine doses and the effect on PMN chemotaxis or PHA stimulated lymphocyte proliferation was studied in four normal volunteers. Results obtained 2 h following inhalation are shown in figure 3. Inhalation of 0.4 mg of histamine had no effect on PMN but caused a weak inhibition of T-lymphocyte proliferation (p=0.06). Histamine 15 mg inhibited PMN chemotaxis and T-lymphocyte proliferation to the same extent as that observed with a 2.4 mg dose.

Table 1. - Mean leucocytosis and white blood cell (WBC) differential count before and following histamine inhalation in four normal volunteers

	Before	After histamine inhalation		
		2 h	4 h	24 h
WBC·mm ⁻³	7,400±1,020	7,210±1,230	7,580±2,010	7,320±900
%				
Neutrophil	78±15	74±17	80±12	75±7
Lymphocyte	15±4	17±6	15±3	20±6
Basophil	3±2	3±1	2±3	1±4
Eosinophil	3±4	3±3	1±4	3±2
Monocyte	1±2	3±3	2±4	1±3

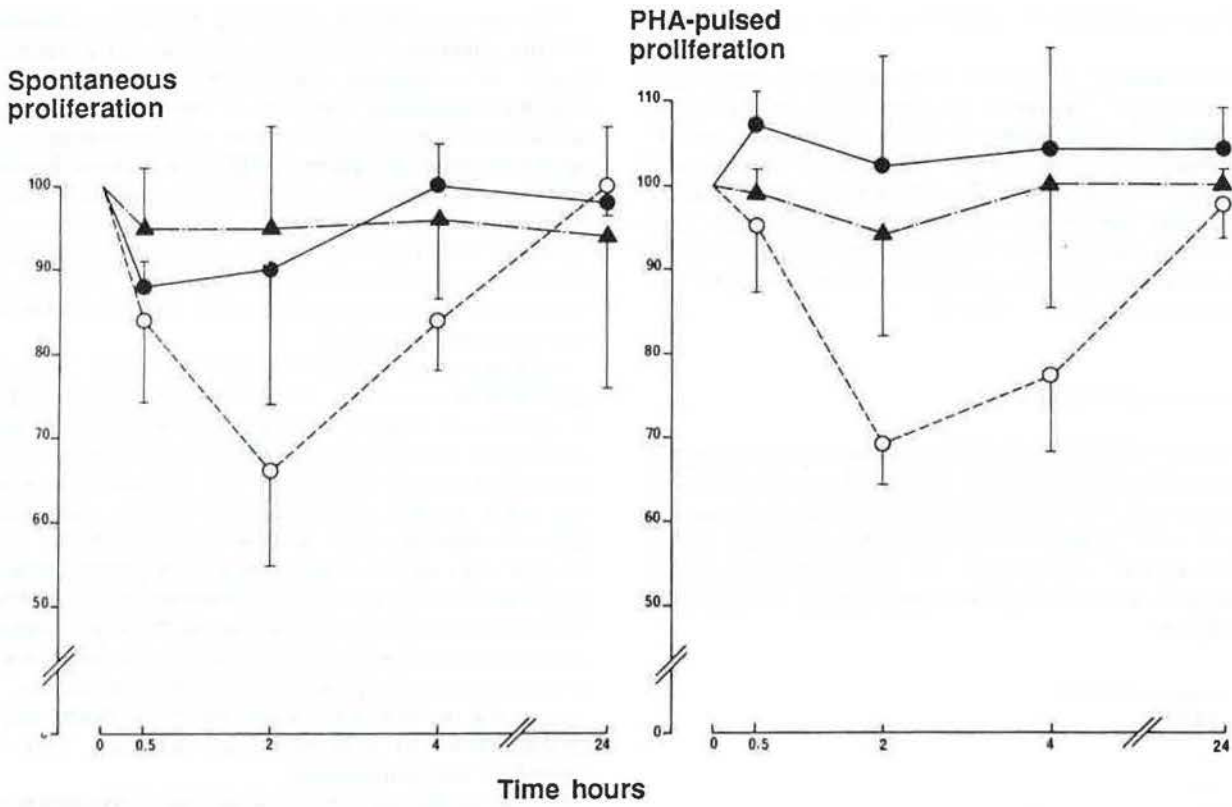


Fig. 2. - Effect of histamine 2.4 mg (○), methacholine 2.4 mg (▲) or saline (●) inhalation on spontaneous and PHA-pulsed T-lymphocyte proliferation (% of basal values) in normal volunteers (n=4); PHA: phytohaemagglutinin.

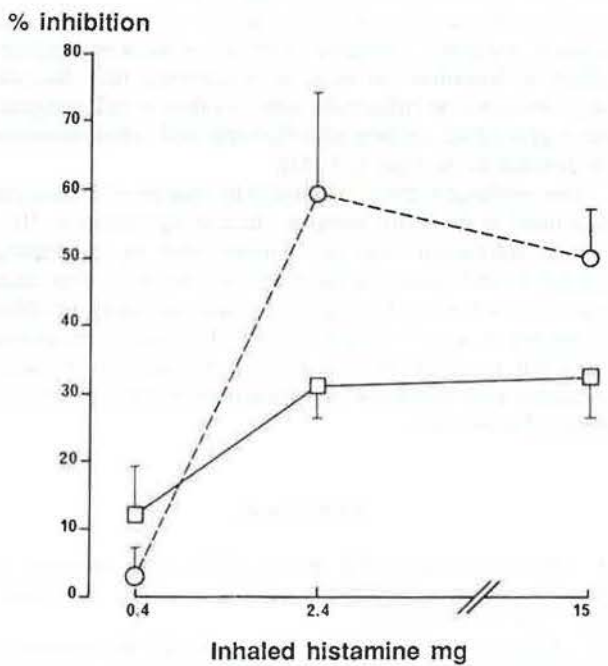


Fig. 3. - Dose-effect relationship between inhaled histamine and mean inhibition of PMN chemotaxis (○) or of PHA-pulsed T-lymphocyte proliferation (□) in man (n=4). PMN: polymorphonuclear neutrophils; PHA: phytohaemagglutinin.

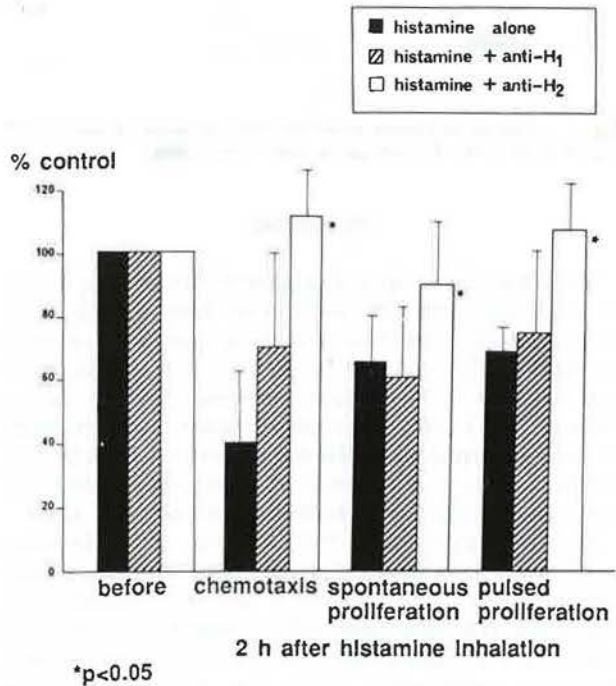


Fig. 4. - Effect of oral pretreatment with astemizole (20 mg·day⁻¹, 3 days) or cimetidine (800 mg·day⁻¹, 3 days) on the decrease of PMN chemotaxis or T-lymphocyte proliferation induced by inhaled histamine in man (n=4). PMN: polymorphonuclear neutrophils.

Effect of histamine H_1 - and H_2 -receptor antagonists

Four healthy volunteers were pretreated orally with astemizole or cimetidine for three days and the effect of histamine inhalation on PMN chemotaxis and T-lymphocyte proliferation was compared to that obtained prior to H_1 or H_2 blockade. Inhibition of PMN chemotaxis and lymphocyte proliferation which had been obvious 2 and 4 h after challenge was totally prevented by cimetidine ($p < 0.05$), whereas astemizole had no significant effect ($p > 0.1$) (fig. 4).

Histamine plasma levels

Figure 5 shows mean plasma histamine concentrations before and after histamine inhalation (2.4 mg) in four normal men. The mean plasma histamine level was $0.4 \text{ ng}\cdot\text{ml}^{-1}$ (± 0.2 ; range 0.3–0.7) before the challenge. It rose to $2.4 \text{ ng}\cdot\text{ml}^{-1}$ (± 0.5 ; range 2–3) 5 min after the end of inhalation and returned to normal limits 10 min after challenge.

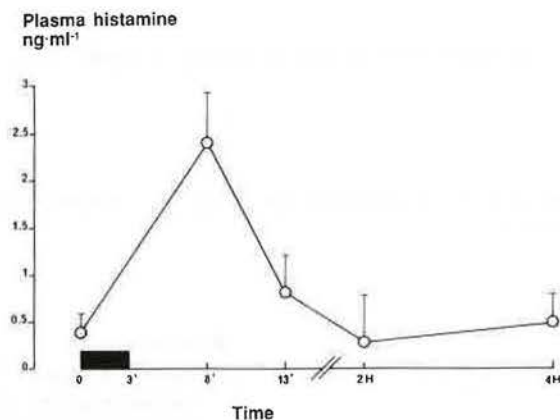


Fig. 5. – Change of plasma histamine after histamine inhalation (2.4 mg) in man ($n=4$). Aerosolization time 3 min (■).

Discussion

This study shows that inhalation of histamine (2.4 mg), in conditions commonly used in our laboratory to study bronchial responsiveness, causes a rapid and transient increase of plasma histamine in man. This is followed 2 and 4 h later by a significant decrease of PMN chemotaxis and of T-lymphocyte proliferation, both measured *in vitro* in normal and asthmatic volunteers. As expected, similar results were obtained following subcutaneous or intravenous injection of histamine (unpublished data).

It is well documented that histamine exerts *in vitro*, via H_2 receptor activation, an inhibitory effect on inflammatory cells [3–6]. We think that the inhibition of leucocytic functions observed is a direct effect of histamine on PMN and lymphocytes. Our experiments with histamine antagonists show that pretreatment with cimetidine completely blocks the inhibitory effect of inhaled histamine, and are thus in keeping with this conclusion.

We did not observe, following histamine inhalation 2.4 mg, changes in leucocyte or white cell differential counts or symptoms (tachycardia, arterial pressure changes) suggesting a release of endogenous catecholamines. Epinephrine which causes a rise in cellular cyclic adenosine monophosphate (AMP) is known to decrease PMN migration [12]. In four subjects, plasma levels of catecholamines (epinephrine, norepinephrine) were measured by a high performance liquid chromatography (HPLC) with electrochemical detection [13], and no significant variation was observed 10 and 30 min following histamine inhalation.

Endothelial cells bear histamine-receptors which are predominantly of the H_1 type. They can be activated by a number of factors including pharmacological agent (histamine, acetylcholine, bradykinin, etc.) and "stress" exerted on the cell membrane [14]. Stimulated endothelial cells release, amongst other things, endothelial derived relaxing factor and prostacyclin (PGI_2) [15]. Prostacyclin causes vasodilatation and inhibits platelet aggregation by the intracellular formation of cyclic AMP. The inhibitory effect of histamine on PMN and lymphocytes observed in man, does not seem to be secondary to a stimulation of the pulmonary endothelium because: 1) it is totally prevented by histamine H_2 blockade; and 2) methacholine, an endothelial cell activator, does not reproduce the phenomenon.

In vivo studies carried out on animals have shown that exogenous histamine can depress T-lymphocyte proliferation [16, 17]. In addition, cimetidine reduces the growth of lung metastases and prolongs survival of tumour bearing mice by inactivating suppressor cells [18, 19]. Histamine could suppress host defence mechanisms by H_2 -receptor activation of suppressor T-cells. In this way, the use of anti- H_2 drugs can have a protective effect against tumours in laboratory animals. Only sparse and indirect evidence supports such an immunomodulator effect of histamine in man. It is reported that delayed skin reactions to tuberculin and to other recall antigens are significantly enhanced following oral administration of cimetidine in man [20, 21].

The inhibitory effect of inhaled histamine on leucocyte functions is probably without clinical significance. Histamine inhalation tests are widely used in respiratory medicine and have a good systemic tolerance. Our data showing that inhaled histamine in man can dampen PMN chemotaxis, and T-lymphocyte proliferation (measured *in vitro*) is of theoretical interest because it provides evidence that histamine can modulate human inflammatory cells *in vivo*.

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Dépression du chimiotactisme des polymorphonucléaires et de la prolifération des lymphocytes T après inhalation d'Histamine chez l'homme. T.B. Bury et M.F. Radermecker.

RÉSUMÉ: Il est connu que l'histamine, *in vitro*, inhibe une stimulation des récepteurs H₂, le chimiotactisme des PMNs et la prolifération lymphocytaire. Le but de cette étude était de vérifier les effets inhibiteurs de l'histamine chez l'homme *in vivo*. Des volontaires sains et asthmatiques ont inhalé durant 3 min une solution d'histamine (0.1%), de méthacholine (0.1%) ou de liquide physiologique. Les asthmatiques avaient été sélectionnés sur base d'une réactivité bronchique basse vis à vis des agents pharmacologiques. Des prélèvements sanguins ont été réalisés avant et différents temps après le test afin d'étudier le chimiotactisme des PMNs, la prolifération lymphocytaire et le taux plasmatique d'histamine. L'inhalation d'histamine ou de méthacholine a entraîné, une chute du VEMS de 22-43% uniquement chez les patients asthmatiques. Dans les deux groupes il y avait une augmentation transitoire de l'histamine plasmatique juste après l'inhalation d'histamine et, 2 et 4 h plus tard, une diminution significative du chimiotactisme des PMNs et de la prolifération lymphocytaire. L'inhalation de méthacholine ou de liquide physiologique n'induisait aucune modification des fonctions leucocytaires. Un prétraitement réalisé à l'aide d'un antagoniste des récepteurs H₂ (cimetidine) prévenait la diminution du chimiotactisme des PMNs et de la prolifération lymphocytaire alors que le prétraitement réalisé à l'aide d'un anti-H₁ (astémisole) était sans effet. En conclusion, l'histamine, à une dose habituellement utilisée lors des tests de provocation bronchique, cause chez l'homme, 2 et 4 h après son inhalation, une diminution du chimiotactisme des PMNs et de la prolifération lymphocytaire par une stimulation des récepteurs H₂.

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