Histamine-induced inhibition of neutrophil chemotaxis and T-lymphocyte proliferation in man

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Histamine inhibits *in vitro* human neutrophil chemotaxis and T-lymphocyte proliferation via H_2 receptors. The aim of this study was to verify these inhibitory effects of histamine in man *in vivo*. Healthy volunteers were challenged with histamine by intravenous (1 mg), subcutaneous (1 mg) and inhalatory (2.4 mg) routes. Venous blood was taken before and at different times after challenge. Neutrophil chemotaxis was studied by the Boyden assay and T-lymphocyte proliferation by counting H_3-thymidine incorporation in cultured mononuclear cells. Plasma histamine was measured by radioimmunoassay. Histamine infusion caused transient systemic symptoms as well as a significant decrease of neutrophil chemotaxis ($\bar{x} - 26\% \pm 6$) and of PHA-pulsed T-lymphocyte proliferation ($\bar{x} - 16\% \pm 6$) 4 h after histamine challenge. Subcutaneous injection of histamine caused only a significant decrease of neutrophil chemotaxis ($\bar{x} - 24\% \pm 15$) 4 h after injection. Histamine inhalation was well tolerated and caused a significant depression of neutrophil chemotaxis ($\bar{x} - 40\% \pm 15$) and of T-lymphocyte proliferation ($\bar{x} - 27\% \pm 6$) 2 and 4 h after the challenge. Histamine challenges were always accompanied by a rapid and transient rise in plasma histamine. Inhalation of an H_2 agonist (impromidine) but not of an H_1 agonist (betahistine) caused a decrease of neutrophil chemotaxis and of T-lymphocyte proliferation. Oral pretreatment with an H_2 antagonist (cimetidine) before histamine inhalation prevented histamine-induced decrease of neutrophil chemotaxis and T-lymphocyte proliferation, whereas astemizole, an H_1 antagonist, had no effect. In conclusion, during the few hours following administration, exogenous histamine in man causes a depression of neutrophil chemotaxis and T-lymphocyte proliferation via H_2 receptors. These observations are consistent with the concept that endogenous histamine may exert some modulatory effects on inflammatory and immune cells in man.

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Histamine has been recognized since the beginning of this century as a pro-inflammatory mediator playing an important role in allergic inflammation. During the last decade, a new concept has emerged which also presents histamine as a dampening modulator of inflammatory and immunologic events (3, 14). For example, histamine, via H_2 receptors, may depress *in vitro* antigen-mediated histamine release from basophil, eosinophil and neutrophil chemotaxis and a variety of both T- and B-cell functions (13, 21). In a previous paper, we reported a depression of neutrophil chemotaxis and of T-lymphocyte proliferation following histamine inhalation in man (6). The purpose of this study was to compare the effects of histamine following systemic (i.v. and s.c.) and inhalatory administrations and to further characterize the subtype of receptor involved by using specific H_1 and H_2 agonists.

Subjects and methods

At 0800 healthy volunteers received histamine by intravenous, subcutaneous or inhalatory routes and were followed for 24 h. Venous blood was taken before histamine administration and at different time intervals following the challenge to measure plasma histamine, neutrophil chemotaxis and T-lymphocyte proliferation. All the subjects gave their written informed consent and the protocol of this study was approved by the hospital Ethics Committee.

Subjects

The study comprised 12 healthy males (20–29 years old), taking no medication. They had normal pulmonary function and no allergic background on the basis of history, negative skin tests and RAST for a battery of allergens. Their body weight was between 60 and 75 kg and not exceeding 120% of the theoretical weight according to the tables of the Metropolitan Life Insurance Company (15). Some of these 12 subjects also took part in experiments with specific histamine agonists and antagonists.

Histamine intravenous and subcutaneous injections

Intravenous (i.v.) or subcutaneous (s.c.) administration of histamine took place at 0800 after an overnight fast. An indwelling intravenous catheter was placed in the left antecubital fossa to allow blood sampling before and after histamine injection. One milligram of histamine dihydrochloride (Sigma, St Louis, MO, USA) was diluted in 100 ml normal saline and infused intravenously in the right arm for 20 min with a syringe pump (Abott-Life Care, USA). Blood pressure measured by sphygmomanometry and pulse rate were monitored every 5 min until a stable base line was reached and every 5 min throughout the histamine infusion. In some experiments, histamine was injected subcutaneously (0.5 or 1 mg) in the right forearm of the volunteers.

Histamine inhalation

An aerosol of histamine or saline was generated by an ultrasonic De Vilbiss nebulizer (output 0.8 ml/min) loaded with 10 ml of solution. Histamine dihydrochloride was dissolved at 0.1% in saline. Inhalation challenges were performed at 0800 according to a technique well standardized in our laboratory. In a sitting position and wearing a nose clip, the subjects inhaled the aerosol for 3 min at tidal volume through a mouthpiece. The forced expiratory volume in 1 s (FEV_1) was measured with a water-sealed spirometer (Jaeger) before and 3 min after the histamine inhalation. Before starting the experiment, all the subjects had a $FEV_1 \geq 80\%$ of the predicted values (19). To study the subtype receptor involved, specific H_1- (betahistine, Duphar, UK) or H_2- (impromidine, SKF, UK) receptor agonists were used instead of histamine. In some experiments, subjects received astemizole (Janssen, Beerse, Belgium), an H_1-receptor antagonist, or cimetidine (SK, Rixensart, Belgium), a histamine H_2-receptor antagonist, at a therapeutic dose (20 and 800 mg/day^{-1} respectively) for 3 days, and 2 h before histamine inhalation.

Neutrophil 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 leukocytes and platelets was removed and pooled into a plastic tube. The concentration of leukocytes was determined by counting (haemocytometer) and adjusted with Hank's balanced salt solution to 2.10^6 cells/ml. Neutrophil chemotaxis was studied by a micropore filter technique using a modified Boyden chamber (5). Briefly, 2 ml of the leukocyte suspension (2.10^6 cells/ml) was 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 (10 mg/ml – Sigma). The chambers were incubated for 3 h at 37°C in humidified air. After incubation, the filters (Millipore, 5-µm porosity) were removed, stained with Diff Quick (Harleco). Chemotaxis was measured by counting neutrophils on both faces of the filter, at high power (×440), in 10 microscopic fields. The number of neutrophils present on the lower face of the filter was expressed as a percentage of the number of neutrophils remaining on the upper face of the filter (normal value $\bar{x}$:560% ± 120; range 450–680). All samples were performed in duplicate and the mean calculated. In this study, neutrophil chemotaxis was expressed as a percentage of the basal values, i.e. chemotaxis measured before histamine administration. Preliminary experiments have shown that oral intake of astemizole or cimetidine by human volunteers has no significant effect on neutrophil 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, UK) and resuspended to 1.10^6 cells/ml 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% CO_2) 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. Seventeen hours before completion of the culture, 20 µl of tritiated thymidine (methyl ^{3}H-thymidine; 50 µCi/ml, Amersham, UK) was added to the wells. The cells were harvested and the incorporation of the labelled

thymidine in the lymphocytes was measured in counts per minute (cpm) by a beta-counter (LKB). All the samples were run in triplicate and the mean cpm calculated. The results were expressed as a percentage of the basal values, i.e. spontaneous (in the absence of PHA) and PHA-pulsed lymphocyte proliferation was measured before histamine challenge. Normal values of spontaneous or PHA-pulsed lymphocyte proliferation in healthy volunteers ($n = 50$), were, respectively, 1000 cpm $\pm$ 280 and 95 000 cpm $\pm$ 15 000. 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, 10 and 15 min and 4 h after histamine administration. 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) (16). Its sensitivity was 0.1 ng/ml with an intra- or interassay variation coefficient of $<8\%$.

Statistical analysis

Student's paired t test was used for statistical comparison; $P<0.05$ was considered to be statistically significant. Results were presented as mean $\pm$ SD.

Results

Effect of i.v. or s.c. administration of histamine

Healthy volunteers received histamine or saline intravenously or subcutaneously and their cell functions were studied before and after challenge. Before injection, all the subjects had basal neutrophil chemotaxis ($\bar{x}$ 520% $\pm$ 90) and T-lymhocyte proliferation [$\bar{x}$ (spontaneous): 1040 cpm $\pm$ 260 – $\bar{x}$ (pulsed):99 300 cpm $\pm$ 12 000] within normal limits.

Intravenous histamine. The four subjects receiving intravenous histamine experienced a flush, an increased heart rate (mean + 30% $\pm$ 15 of basal value) and a decrease of diastolic blood pressure (mean – 25% $\pm$ 8 of basal value) during the infusion. They did not complain of headache, gastrointestinal or respiratory symptoms and their FEV_1 was unchanged 3 min after the end of the infusion.

Fig. 1 shows the effect of infusion of saline or histamine on neutrophil chemotaxis and T-lymphocyte proliferation in these four volunteers. Saline was without any effect. By contrast, histamine

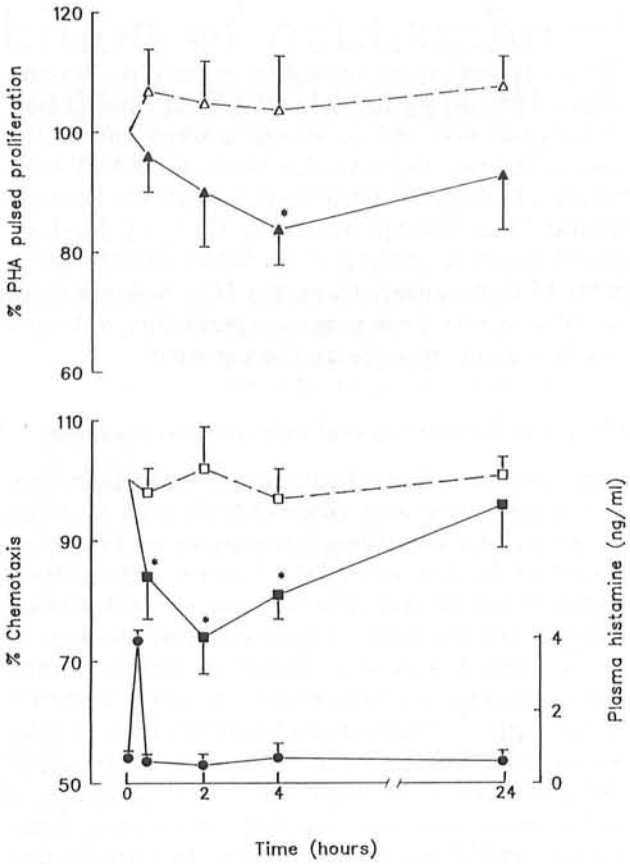

Fig. 1. Effect of intravenous infusion of histamine (black symbols) or saline (white symbols) on neutrophil chemotaxis and T-lymphocyte proliferation, measured *ex vivo*, in 4 men. Plasma histamine (●) after histamine infusion is also represented (* $P<0.05$).

infusion induced a rapid and significant decrease of neutrophil chemotaxis 30 min, 2 and 4 h after the end of the perfusion ($P<0.05$). This inhibitory effect was maximal at the 2nd h with a mean inhibition of 26% $\pm$ 6. Spontaneous and pulsed T-lymphocyte proliferation decreased slightly 30 min and 2 h after histamine infusion. Only the decrease observed at 4 h was significant ($P<0.05$). Twenty-four hours after the perfusion, basal values of chemotaxis and T-lymphocyte proliferation were again reached. As expected, histamine plasma level peaked during the perfusion (mean 3.9 ng/ml $\pm$ 0.3) and returned to basal values (mean 0.6 ng/ml $\pm$ 0.2) 5 min after the end of the perfusion (Fig. 1). Leukocytosis and white cell differential counts (coulter) were not modified 30 min, 2 h and 4 h after histamine injection (Table 1).

Subcutaneous histamine. Histamine 0.5 and 1 mg, or saline was injected subcutaneously in four normal volunteers. Saline and histamine 0.5 mg caused neither local nor systemic symptoms and were without any significant effect on neutrophil chemotaxis and T-lymphocyte proliferation. Subcutaneous injection

Table 1. Mean leucocyte (total and differential) count before and following histamine injection in 4 normal volunteers

	Before	After histamine injection		
		30 min	2h	4h
WBC·mm^{-3}	7800±1110	7750±1200	7920±1400	7830±1300
% Neutrophil	73±15	76±16	75±12	71±8
% Lymphocyte	23±7	21±6	20±6	24±4
% Basophil	1±1	1±2	2±1	1±1
% Eosinophil	1±1	1±1	1±2	1±1
% Monocyte	2±2	1±1	2±1	3±3

of 1 mg histamine caused a local erythema only at the site of the injection and a significantly increased heart rate (mean + 20% ± 7 of basal value). Four hours later, a significant decrease ($P<0.05$) of neutrophil chemotaxis (mean − 24% ± 15) and no effect on T-lymphocyte proliferation were observed in the four volunteers. Mean plasma histamine level increased to 3 ng/ml (± 0.4) 5 min following injection of 1 mg histamine and returned to normal limits 10 min later.

Effect of histamine inhalation. The effects of inhalation of histamine (aerosolized dose: 2.4 mg) or saline on neutrophil chemotaxis and T-lymphocyte proliferation are shown in Fig. 2. Inhalation of histamine induced a significant decrease of neutrophil chemotaxis (mean − 40% ± 15) 2 and 4 h after the challenge ($P<0.01$). Twenty-four hours later, a slight inhibition was still observed. Spontaneous and PHA-pulsed T-lymphocyte proliferation also significantly decreased 2 and 4 h following histamine inhalation ($P<0.05$). The mean inhibition of pulsed T-lymphocyte proliferation was about 27% (± 6) 2 and 4 h after histamine inhalation. Basal levels were again reached 24 h after the challenge. By contrast, saline inhalation had no effect on neutrophil chemotactic response and T-lymphocyte proliferation. Histamine inhalation was well tolerated by our volunteers who reported neither flush nor respiratory or systemic symptoms. No variation of FEV_1 was observed following the challenge. Plasma histamine concentration increased as a consequence of histamine inhalation (Fig. 2). Mean plasma histamine level, which was 0.4 ng/ml (± 0.2) before inhalation, rose to 2.4 ng/ml (± 0.5) 5 min after the end of inhalation and returned to normal limits 10 min later.

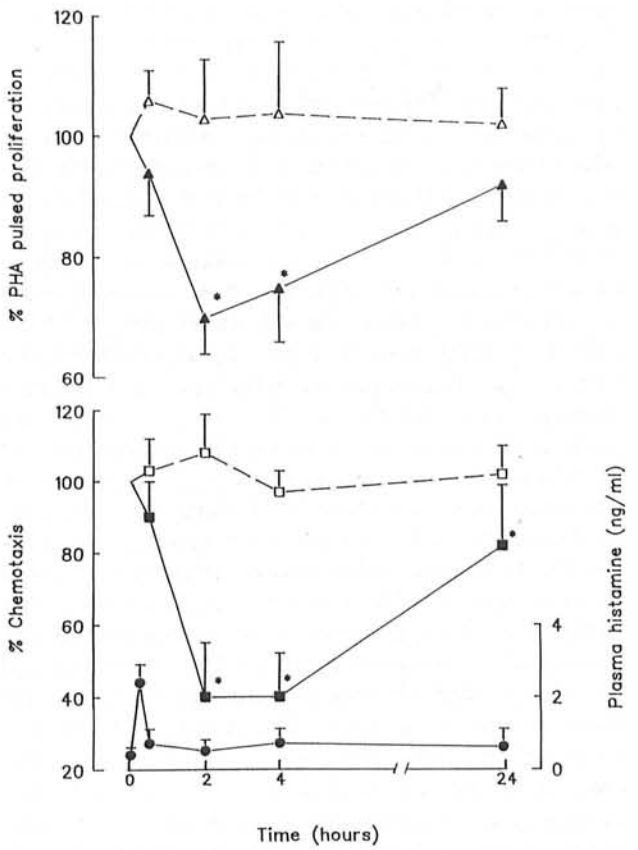

Fig. 2. Effect of histamine (black symbols) or saline (white symbols) inhalation on neutrophil chemotaxis and T-lymphocyte proliferation, measured *ex vivo*, in 4 men. Changes of plasma histamine (●) after histamine inhalation are also represented (* $P<0.05$).

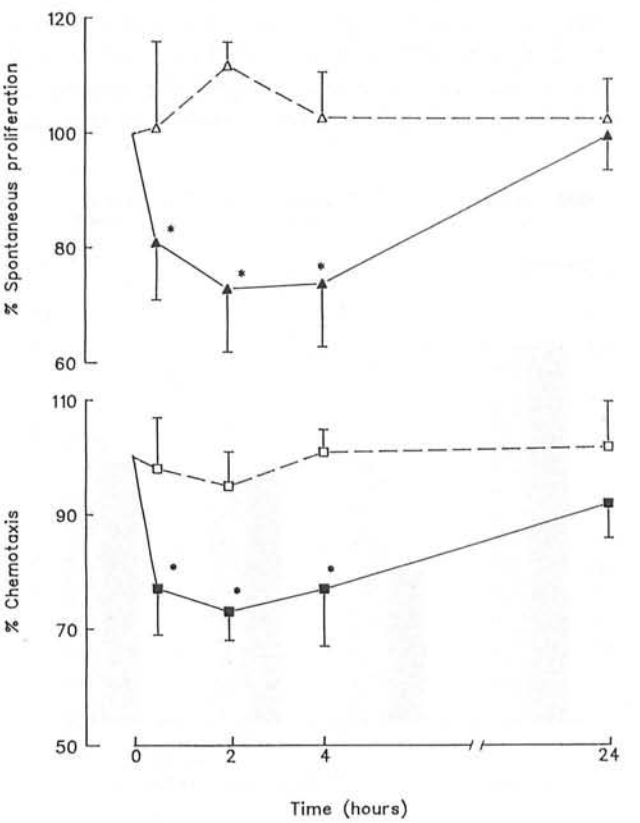

Fig. 3. Effect of H_1 (white symbols) or H_2 (black symbols) agonist inhalation on neutrophil chemotaxis and T-lymphocyte proliferation, in man. ($n=4$), (* $P<0.05$).

Effect of histamine H_1- and H_2-receptor agonists. As shown in Fig. 3, inhalation of betahistine (aerosolized dose: 2.4 mg), an H_1 agonist, had no effect on neutrophil chemotaxis and T-lymphocyte proliferation. In contrast, inhaled impromidine (aerosolized dose: 2.4 mg), an H_2 agonist, caused a significant decrease of neutrophil chemotaxis (mean inhibition: $27\% \pm 5$) and of spontaneous T-lymphocyte proliferation (mean inhibition: $27\% \pm 11$) 0.5–2 and 4 h after aerosol challenge. Pulsed T-lymphocyte proliferation tended to decrease although this was not statistically significant.

Effect of histamine H_1- and H_2-receptor antagonists. Four subjects were pretreated orally with astemizole (H_1 antagonist) or cimetidine (H_2 antagonist) for 3 days, and the effect of histamine inhalation on neutrophil chemotaxis and T-lymphocyte proliferation was compared to that obtained prior to H_1 or H_2 blockade. Inhibition of neutrophil chemotaxis and lymphocyte proliferation, which had been obvious 2 and 4 h after histamine challenge in the absence of premedication, was totally prevented by cimetidine ($P < 0.05$), whereas astemizole had no effect ($P > 0.1$) (Fig. 4).

Discussion

This study clearly shows that systemic (i.v. or s.c.) or inhalatory administration of histamine in man may cause a decrease of neutrophil chemotaxis and of T-lymphocyte proliferation (studied *ex vivo*) during the hours after challenge. Interestingly, histamine seems to be more active in inhibiting these leukocyte functions when administered by inhalation than by injection. Several possibilities could account for this difference. First, the aerosolized dose is more effective than the injected dose. Although it is difficult to extrapolate the amount of histamine reaching the lung in these circumstances. It may be greater after inhalation than after i.v. or s.c. injection. It is also possible that i.v. injected histamine is more quickly metabolized by methyltransferase and diaminoxydase, which are abundant in the pulmonary vascular endothelium (20). A further possibility is that inhaled histamine would react with specific receptor(s) on the respiratory epithelium or on some lymphocytes of the bronchial-associated lymphoid tissue (14, 15). Both cell types bear H_1 and H_2 receptors (7) and their activation could induce the secretion of cytokines suppressive of neutrophil chemotaxis and lymphocyte proliferation.

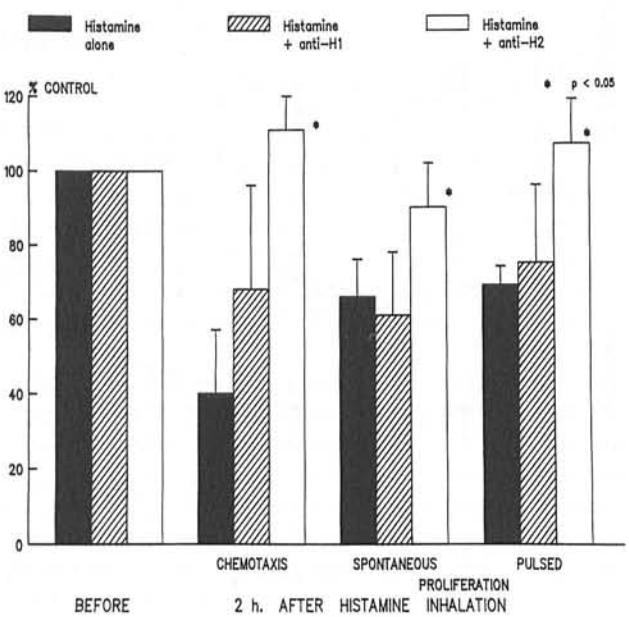

Fig. 4. Effect of oral pretreatment with astemizole (20 mg/day^{-1}, 3 days) or cimetidine (8000 mg/day^{-1}, 3 days) on the decrease of neutrophil chemotaxis or T-lymphocyte proliferation induced by inhaled histamine in man ($n = 4$).

Since inhalation of histamine was particularly well tolerated by volunteers, we favoured this route of administration for studying the subtype of histamine receptor involved. We found that inhaled impromidine, an H_2 agonist, but not betahistine, an H_1 agonist, inhibited neutrophil chemotaxis and T-lymphocyte proliferation. Moreover, oral pretreatment of volunteers with an H_2 antagonist completely prevented the inhibitory effects of inhaled histamine on these cells, whereas an H_1 antagonist had no effect, thus proving an effect on H_2 receptors. Human polymorphonuclears have indeed H_2 receptors on their surface and *in vitro* their activation causes an inhibitory action on several leukocytic functions (9, 13). Histamine H_2 type receptors are also present on different T-lymphocyte subsets ($CD8^+$, $CD4^+$) and their stimulation can be accompanied by the release of lymphokines which may suppress T-lymphocyte functions (21, 23). As expected, plasma histamine increased rapidly to approximate 10-fold basal values following i.v., s.c., or inhalatory administration of histamine. Fifteen minutes after challenge, basal level was again reached, whereas the effects on neutrophils and lymphocytes persisted for several hours. The same prolonged action of histamine can be seen when purified neutrophils are incubated *in vitro* with histamine (10^{-7} mol), washed and then tested 2 h later in a Boyden chamber (unpublished personal data). The reasons for the prolonged effect of histamine *in vivo* (and *in vitro*) are not clear at the present time. Little is known about the affinity of H_2 receptors for their agonists in inflammatory cells. Stimulation of H_2 receptors causes a rise in intracellular cAMP which persists for at least 1 h (17). The mechanisms by which changes in cAMP concentration are related to the observed effect of histamine remain poorly understood. Ulti-

mately, these events may involve contractile elements, such as microtubules and microfilaments which play an important role in cell locomotion and duplication. Several lines of evidence show that histamine modulates animal inflammatory cells *in vitro* and *in vivo* (1, 4). Histamine also exerts many negative effects on human leukocytes *in vitro* (3). In man, cimetidine enhances delayed skin reactions to tuberculin and to other recall antigens (2, 10). Our data, showing that systemic administration of histamine in man negatively influences neutrophil chemotaxis and T-lymphocyte proliferation, suggest that histamine may indeed exert physiologically a modulating role in inflammatory and immune responses in man.

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