

Plasma histamine in asthmatic and control subjects following exercise: influence of circulating basophils and different assay techniques

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ABSTRACT Arterial plasma histamine concentrations were measured after exercise in 10 subjects with extrinsic atopic asthma, 10 who were non-atopic and non-asthmatic and seven who were atopic but non-asthmatic, by a single isotope radioenzymatic assay. Significantly higher plasma histamine concentrations were found in the asthmatic subjects before exercise than in the non-atopic controls ($p < 0.05$). The mean histamine concentration rose after exercise in all groups but the increased levels were not significantly different from pre-exercise values. Similarly, mean circulating basophil counts increased in all groups after exercise, and a highly significant correlation was found between basophil counts and whole blood histamine concentrations ($p < 0.001$). *In vitro* studies showed that there was a significant correlation between the number of basophils added to plasma samples and the concentrations of histamine subsequently detected. Although the mean concentrations of plasma histamine and whole blood histamine and number of basophils in the atopic control group were intermediate between those found in the atopic asthmatic and non-atopic controls, none of the differences was significant. Venous plasma histamine concentrations after exercise were measured in a further five subjects with extrinsic atopic asthma and five non-atopic, non-asthmatic subjects before and after exercise with the more sensitive and specific double isotope radioenzymatic assay. Concentrations of plasma histamine measured by this assay were about one tenth of those measured by the single isotope radioenzymatic assay. Although a small rise in mean plasma histamine concentration occurred in both groups after exercise there was no significant difference in these levels either between or within the groups. We find no evidence from these studies on measurement of peripheral blood histamine to support the hypothesis that mast cell mediator release is implicated in the pathogenesis of exercise induced asthma.

Exercise induced asthma is a phenomenon which can be demonstrated in varying proportions of patients with both extrinsic atopic asthma and cryptogenic non-atopic asthma.¹ Several observations suggest that mast cells and their mediators may play a part in the pathogenesis. A short refractory period occurs after an episode of exercise induced asthma, during which exercise can no longer lead to airflow obstruction, suggesting the possibility of depletion of mediator stores from mast cells.² Sodium cromoglycate, a drug thought to act by stabilising

mast cells, reduces the airway response to exercise.³ Further, recent studies have suggested that significant increases in circulating plasma histamine can be shown in asthmatics developing airflow obstruction after exercise when compared with non-atopic controls.⁴

It has long been known that the inhalation of histamine can cause bronchoconstriction in man⁵ and its only origin, at least in human lung tissue, appears to be the mast cell.⁶ Plasma histamine has been shown by some investigators to increase during asthma induced by allergen bronchial provocation.^{7 8} Raised histamine concentrations have been found in circulating blood during spontaneously occurring asthmatic attacks⁹ and diurnal changes in peak expiratory flow rate have been shown to correlate

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with fluctuations in venous plasma histamine concentrations.¹⁰ These studies support the idea that histamine is important in the pathogenesis of asthma in man and suggest that mediator release from mast cells plays a part in the development of the disease. Studies of changes in concentrations of circulating histamine before and after exercise induced asthma have, however, given conflicting results. Both Hartley *et al*¹¹ and McFadden *et al*¹² failed to show significant increases in arterial plasma histamine during exercise induced asthma using a single isotope radioenzymatic assay. On the other hand, Barnes and Brown⁴ used a more sensitive and specific assay technique and reported a clear difference in the levels of plasma histamine between asthmatic subjects who developed exercise induced asthma and control subjects who were similarly tested.

Exercise produces a series of complex physiological changes and it has been known for some time that an increase in total circulating white blood cells is induced by this stimulus.¹³ Previous work^{14, 15} has shown a close association in both asthmatic and normal subjects between peripheral whole blood histamine concentrations and circulating basophil counts after exercise since most of the histamine in whole blood is derived from basophil polymorphonuclear leucocytes (basophils).¹⁶ The purpose of our study was to measure changes in plasma histamine before and after exercise in atopic asthmatic subjects. To assess whether atopy itself might influence histamine levels we included both atopic and non-atopic individuals in our control groups. Basophils were counted in all samples on which histamine assays were performed and histamine itself was measured by the two available sensitive assay techniques—namely, the single and double isotope radioenzymatic assays. *In vitro* experiments were performed to assess the influence of increasing numbers of basophils on measured levels of histamine in pooled plasma.

Methods

The *in vivo* study was performed in two parts. For the first part 27 male subjects with a mean age of 25.2 years gave informed consent to participate in the project, which had been approved by the hospital ethical committee. Ten had extrinsic asthma as shown by a greater than 15% change in forced expiratory volume in one second (FEV₁) after inhalation of a bronchodilator and an extrinsic cause for their asthma had been identified from the history and by bronchial provocation testing. At the time of study their FEV₁ was greater than 70% of that predicted for height and age, and their maintenance treatment was intermittent inhaled β_2 adrenergic

stimulants only. All were atopic—that is, they showed one or more positive responses to skinprick tests with a battery of 23 common inhalant and food allergens. Two groups of control subjects were studied—namely, 10 non-asthmatic subjects with negative responses to skinprick tests with common allergens (non-atopic controls) and seven non-asthmatic subjects who showed one or more positive responses to skinprick tests with common allergens (atopic controls). All were subjected to a standard exercise test under environmentally stable conditions, with a mean temperature of 19°C and relative humidity of 56%. The test consisted of running for six minutes on a treadmill set at a 5° gradient at a constant speed tolerated by the individual and resulting in a postexercise pulse rate of at least 160 beats per minute. All tests were performed in the morning. All the asthmatic subjects had abstained from all treatment for at least 24 hours before the study and no strenuous exertion had been performed in the previous 12 hours. Only one cup of tea or coffee was allowed for breakfast. Pulmonary function was monitored by measurement of peak expiratory flow rate with a Wright's peak expiratory flow meter (Clement Clarke International, Harlow) and FEV₁ with a Vitalograph (Buckingham) dry bellows spirometer. Readings were taken five minutes before and 5, 10, 15, 20, 30, and 60 minutes after exercise. Blood for histamine estimations and basophil counts was obtained from a forearm artery cannulated 30 minutes before exercise with a 20F Medicut cannula (Sherwood Medical Industries, St Louis, USA) inserted under 2% lignocaine local anaesthesia and intermittently perfused with heparinised saline solution. Samples were taken five minutes before and 0.5, 5, 10, 15, and 60 minutes after exercise. Ethylenediaminetetra-acetate (EDTA) 20 μ l was added to every 2 ml of blood taken for measurement of plasma histamine. The samples were centrifuged immediately at 4°C and plasma was separated and stored at -40°C.

In the second part of the *in vivo* study 10 male subjects with a mean age of 24.1 years were studied. Five had atopic asthma and five were non-atopic and non-asthmatic. The protocol for this study was identical to that of the first except that venous rather than arterial blood was taken and the plasma was stored in liquid nitrogen at a temperature of at least -70°C.

Two methods were used to assay plasma histamine. Histamine in the arterial samples in the first part of the study was measured by a modification of the technique described by Bruce and coworkers.¹⁷ In brief, this is a single isotope radioenzymatic assay in which histamine is extracted from plasma into acetic acid and converted into ¹⁴C-1-methyl histamine by

histamine-*N*-methyl transferase obtained from pig brain, in the presence of adenosyl-¹⁴C-1-methionine. The labelled histamine is then extracted and counted in a scintillation counter and the concentration of histamine calculated by reference to a standard curve. In our laboratory the intra-assay coefficient of variation for this technique is 8%, with a sensitivity of 1.8 nmol/l. Venous plasma histamine from the subjects in the second half of the project was measured by the double isotope radioenzymatic assay described by Brown *et al*¹⁸ as well as the single isotope assay. In brief, this technique is similar to the single isotope one but incorporates a second isotope to provide a control to correct for variations in methylation and losses in recovery and has an additional thin layer chromatography step, which is thought to increase the specificity of the assay. In this assay the histamine-*N*-methyl transferase is obtained from rat kidney. In our hands the intra-assay coefficient of variation is 7% and the limit of sensitivity 0.5 nmol/l.

Whole blood histamine was measured by the automated fluorometric assay described by Siraganian and Brodsky.¹⁹ The sensitivity of this assay is 3 nmol/l, which is adequate for measurement of levels of histamine in whole blood, which are considerably higher than those in plasma.

Basophil counts were made on anticoagulated blood samples taken at the same time as those for histamine assay. The blood was stained with toluidine blue, which preferentially stains basophils, and the red cells were lysed by saponin. Counts were made in a Fuchs-Rosenthal counting chamber.

For the *in vitro* study venous blood anticoagulated with 0.01 M EDTA was obtained from nine non-atopic, non-asthmatic subjects and eight patients with extrinsic atopic asthma. Each 20 ml sample of blood was mixed with 5 ml of a 6% dextran solution and allowed to sediment at room temperature for one hour. The sedimented red cells were discarded and the leucocyte rich supernatant was centrifuged at 90 *g* for 15 minutes at 4°C. The supernatant was discarded and the cell pellet resuspended in Tyrode's solution and kept on ice until required. Aliquots of the leucocyte suspension containing 10–50 × 10³ basophils/ml were layered on to either 1 ml of cooled (4°C) pooled plasma, previously prepared from blood taken from several non-atopic, non-asthmatic volunteers, or Tyrode's solution. The cells were gently mixed and the tubes were then centrifuged at 500 *g* for 10 minutes at 4°C. The purpose of this *in vitro* study was to simulate the conditions achieved when whole blood samples were obtained from the subjects in the *in vivo* study and allow measurement of any histamine release resulting simply from centrifugation of basophils through plasma. Hista-

mine concentrations in the plasma or Tyrode's supernatant solution and in the residual cell pellets were determined by the double isotope technique.

Statistical analysis of the results of measurement of histamine in whole blood and plasma was made with non-parametric tests, as there was no evidence that the variables measured were normally distributed and for this reason geometric means were calculated. The Wilcoxon matched pairs signed rank test was used for within group comparison, the Mann-Whitney U-test for between group comparison, and Kendall's rank correlation coefficient (the Tau test) for correlation between groups.²⁰ Statistical analysis of the results of the *in vitro* studies was performed with linear regression analysis and Student's *t* test.

Results

The results of the first part of the *in vivo* study are collectively displayed in graphic form in figure 1. The FEV₁ fell significantly in the asthmatic group after exercise, the maximum fall of 25.6% (±4.9%) occurring 10 minutes after the test. The maximum fall in FEV₁ was less than 2% (SEM 0.7%) in the non-atopic group and less than 3.5% (2.5%) in the atopic control group. Changes in PEF_r were similar to those in FEV₁.

Before exercise plasma histamine concentrations were significantly higher (*p* < 0.05) in the atopic asthmatic group (26.2 ± 14.2 nmol/l: geometric mean and standard errors) than in the non-atopic controls (9.3 ± 3.8 nmol/l), although there was no significant difference between the mean concentrations before exercise in the asthmatic group and in the atopic control subjects. The mean values of plasma histamine increased in all groups after exercise. This increase was greatest in the asthmatic patients and lowest in the non-atopic controls. There were, however, no significant differences between the changes in the concentrations of plasma histamine when the results in the different groups were compared or when pre-exercise levels of this mediator were compared with the levels at any particular time after exercise. Similarly, whole blood histamine increased in all groups after the exercise test. The greatest rise occurred in the asthmatic group and the lowest rise in the non-atopic controls. Once again the atopic control subjects had mean levels intermediate between those of the other two groups. Levels of whole blood histamine at any particular time after exercise did not, however, differ significantly from pre-exercise values and there were no significant differences between the groups.

Circulating basophil counts were similar in all three groups before exercise. The number of cells/ml was 17.9 ± 6.1, 17.3 ± 4.1, and 20.0 ± 4.6 × 10³ in the atopic

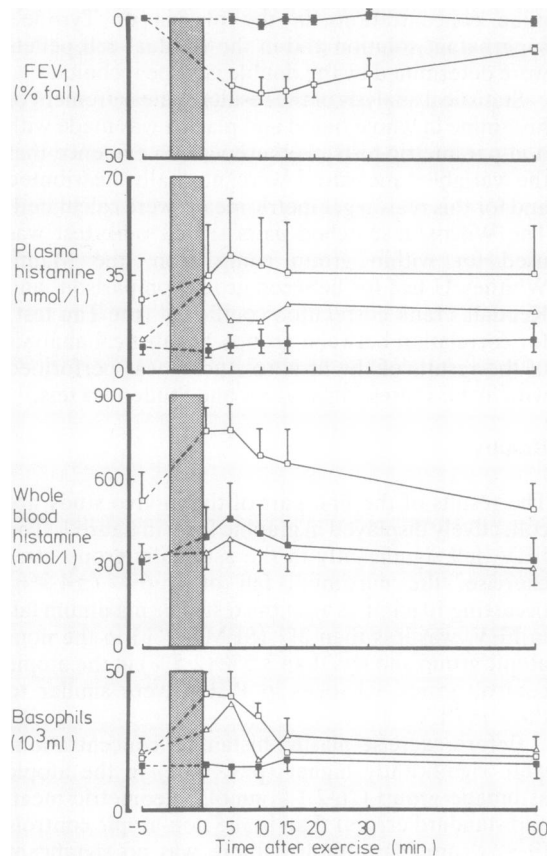


Fig 1 Changes in FEV₁, plasma histamine concentration as measured by the single isotope radioenzymatic assay, whole blood histamine, and numbers of basophils before and after exercise in asthmatic, non-atopic, and atopic subjects, expressed as the geometric means and standard errors. The pre-exercise measurements were taken five minutes before the start of exercise. □ asthmatic subjects; ■ non-atopic controls; △ atopic controls. The hatched area indicates the exercise stress test. There was no significant change in FEV₁ in either of the control groups and the data for these have been combined in the figure. The standard errors for the atopic group have been omitted as they overlie the values for the other groups and are of similar magnitude.

asthmatic, the non-atopic control, and the atopic control groups respectively (geometric means and standard errors). Significantly higher basophil counts were found in the atopic asthmatic group 30 seconds ($41.1 \pm 8.5 \times 10^3$ cells/ml) and five minutes ($40.4 \pm 8.8 \times 10^3$ cells/ml) after exercise than before exercise ($p < 0.05$). Although numbers of circulating basophils also increased after exercise in the atopic control group this change was not significant when counts before and after exercise were compared. There was no significant alteration in basophil counts

after exercise in the non-atopic control group. A highly significant correlation was found between the number of circulating basophils and whole blood histamine concentrations in the asthmatic patients ($p < 0.001$).

Plasma histamine concentrations measured before and after exercise in the second part of the study by both the double and the single isotope radioenzymatic assay are shown in figure 2. The concentrations measured by the double isotope technique are about

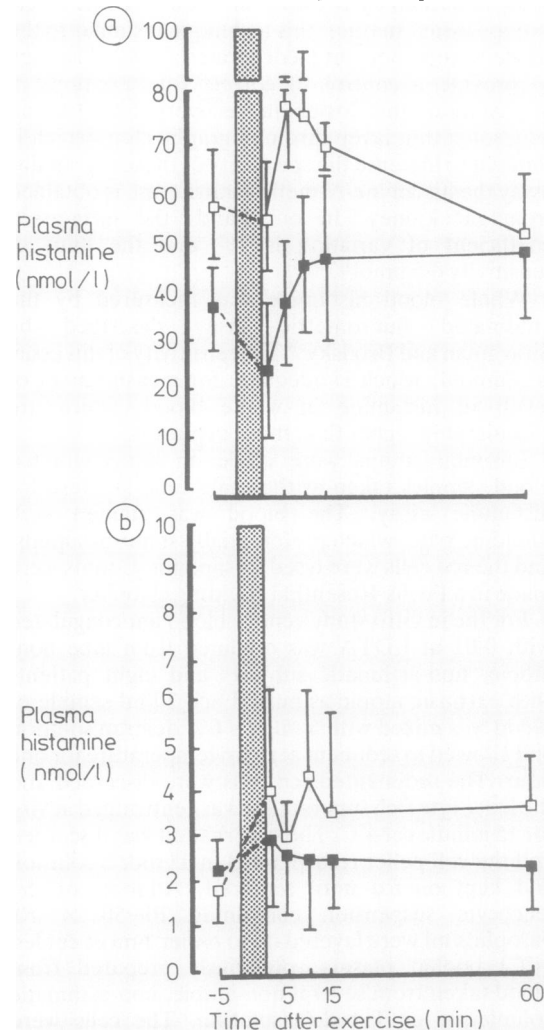


Fig 2 Venous plasma histamine concentration in asthmatic and non-atopic subjects after exercise testing, (a) as measured by single isotope radioenzymatic assay (upper graph) and (b) as measured by the double isotope radioenzymatic assay (lower graph). □ asthmatic subjects; ■ non-atopic controls. The hatched area indicates the exercise period.

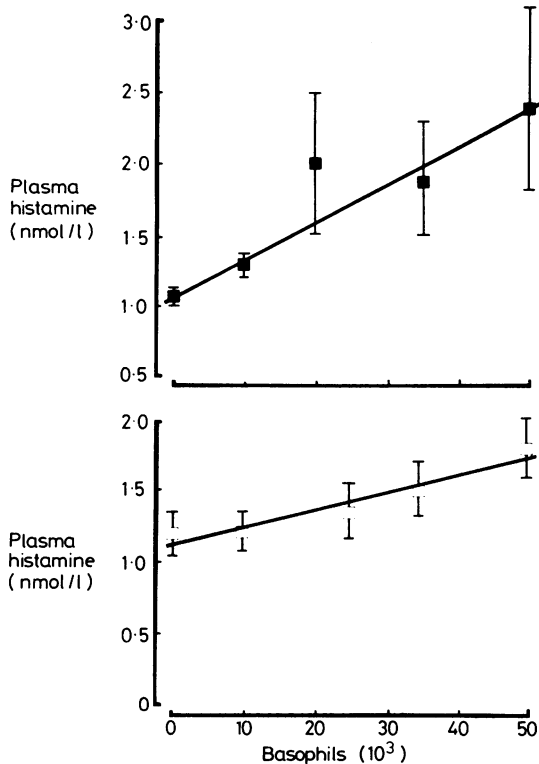


Fig 3 The *in vitro* relationship between numbers of basophils added and plasma histamine concentration as measured by the double isotope radioenzymatic assay after centrifugation of leucocytes through pooled plasma: basophils obtained from nine non-atopic controls (■) and eight asthmatic subjects (□).

one tenth those measured by the single isotope method. Before exercise the concentrations of histamine in venous blood measured by the double isotope assay was $1.8 \pm_{0.3}^{1.3}$ nmol/l in the group of asthmatics and in the non-atopic control group $2.0 \pm_{0.8}^{1.3}$ nmol/l, and these differences were not significant. A small rise in concentrations of plasma histamine occurred after exercise in both groups but at no time after exercise did the levels differ significantly from the values before exercise. Further, there were no significant differences between the histamine concentrations in venous plasma in the two groups as measured by either assay technique.

The results of the *in vitro* studies are shown in figure 3. There was a significant correlation between the concentrations of histamine detected in plasma after centrifugation and the numbers of basophils added from the blood both of patients with extrinsic atopic asthma ($r = 0.9$, $p < 0.05$) and of non-atopic, non-asthmatic subjects ($r = 0.9$, $p < 0.01$). There were no significant differences in the levels of

histamine measured in the pooled plasma after centrifugation of basophils from either of the two groups of subjects. Differences were, however, detected in the levels of histamine when basophils from these two groups of subjects were centrifuged through either pooled human plasma or Tyrode's solution. Centrifugation of basophils from non-atopic subjects through Tyrode's solution led to the release of 3.0% (SEM 1.4%) of total cellular histamine compared with 8.0% (1.9%) of total cellular histamine when these cells were centrifuged through pooled plasma. Similarly, centrifugation of basophils from patients with extrinsic atopic asthma through Tyrode's solution led to the release of 2.7% (0.6%) of total cellular histamine whereas centrifugation of these cells through pooled human plasma caused the release of 9.1% (2.2%) of the total cellular histamine. The differences between the amounts of histamine released when basophils were centrifuged through Tyrode's solution and through pooled plasma were highly significant— $p < 0.0025$ for basophils from non-atopic, non-asthmatic subjects and $p < 0.005$ for basophils from those with extrinsic atopic asthma.

Discussion

The results of the first part of the *in vivo* study, shown in figure 1, illustrate a temporal relationship between changes in mean concentrations of plasma histamine, whole blood histamine, basophil counts, and decreases in FEV₁ in the 10 patients with extrinsic atopic asthma. Nevertheless, few of these changes reached significance. Indeed, the only significant change in any of the groups between the values before and after exercise was the increase in basophils seen in the asthmatic patients. The only other significant difference was a higher concentration of plasma histamine measured by the single isotope assay before exercise in the group with extrinsic atopic asthma than in the non-atopic, non-asthmatic group. These results indicate that although there was a significant decrease in FEV₁ in the asthmatic patients this was not associated with a significant rise in plasma histamine. There was a substantial rise in whole blood histamine in the asthmatic group, which correlated significantly with changes in basophil counts. Comparison of the two available methods for measurement of plasma histamine showed that levels of this mediator were considerably lower when measured by the double isotope assay. This technique ought to be more specific for histamine than the single isotope assay by virtue of the additional steps in the assay; the single isotope technique may additionally be measuring other methylated compounds, including perhaps metabolites of histamine.

Our observations on changes in plasma histamine in asthma induced by exercise are at variance with those reported by Barnes and Brown.⁴ They reported a significant difference between plasma histamine levels in asthmatic and control subjects before exercise and a significant rise in plasma histamine after exercise only in the asthmatic patients who developed exercise induced asthma. They inferred that since increases in plasma histamine were seen only in the group developing an asthmatic reaction this reflected activation and release of mediators from lung mast cells. (In fact this group has repeated the work since the original report was published and has been unable to reproduce the finding of an increase in plasma histamine after exercise.²¹) They did not measure changes in numbers of circulating basophils and we, like others, have shown a highly significant correlation between changes in basophils and levels of whole blood histamine.¹⁴ Possibly changes in plasma histamine could result from release of this mediator from circulating basophils, either in vivo or, perhaps more likely, during the technical procedures required for the separation of plasma from whole blood in vitro.

We have shown that basophils can release up to 10% of their total histamine during a centrifugation procedure similar to that required for the separation of plasma from whole blood. The finding that basophils may "spontaneously" release histamine in vitro has been described by others,²² though there is as yet little evidence to suggest that histamine may also be released into circulating blood in vivo. Further, in our study there was a significant correlation between the amount of histamine released into plasma and the number of basophils added to samples of pooled human plasma. Our results also suggest that plasma itself might be having a "deleterious" effect on basophils. When equivalent numbers of basophils were centrifuged through an equivalent volume of plasma or Tyrode's solution, the amount of histamine released into plasma was significantly greater than that released into Tyrode's solution. There is evidence to suggest that membrane changes can occur during passage of cells through relatively dense media such as serum or ficol. Indeed, it has been shown that purification of rat mast cells through such density media can cause the loss or inactivation of IgE and other receptors^{23, 24} as well as causing the "spontaneous" release of substantial amounts of cellular histamine.²⁵ Our results suggest the possibility that the variations seen in the measurement of plasma histamine in different studies may be the result, at least in part, of differences in numbers of circulating basophils and release from them of histamine during the in vitro processes concerned in the measurement of this mediator. This underlines

the need both to count the number of circulating basophils and to take appropriate care in the collection of samples and subsequent separation of plasma from leucocytes.

Histamine itself has a half life of 60 seconds in peripheral blood²¹ and it would not be altogether surprising if any changes in the concentrations of this mediator were hard to detect. The absence of such changes in peripheral blood is not necessarily contrary to the hypothesis that mediator release from mast cells plays a part in the pathogenesis of exercise induced asthma. Nevertheless, we find no evidence from these studies on measurement of peripheral blood histamine to support the hypothesis that exercise induced asthma is the result of mediator release from lung mast cells. In our opinion, future studies on the possible pathogenic role of mast cells in exercise induced asthma should concentrate on measuring changes in mediators other than histamine, which ideally should have a long plasma half life and be specific to lung mast cells. Certainly any study measuring mediators which are also contained in circulating blood cells must consider and monitor these cells when using current assay techniques.

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