Arterial plasma histamine after exercise in normal individuals and in patients with exercise-induced asthma


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Summary

1. Arterial plasma histamine concentrations, forced expiratory volume in 1.0 s (FEV$_{1.0}$) and peak expiratory flow rate were determined in nine patients with exercise-induced asthma and in five control subjects before and after 8 min of cycle-ergometer exercise.

2. In the controls neither FEV$_{1.0}$ nor peak expiratory flow rate fell by more than 5% in any individual during the 30 min postexercise period. The asthmatic patients all experienced a fall in FEV$_{1.0}$ or peak expiratory flow rate, or both, of 15% or more in the period 5–20 min after completion of the exercise.

3. There was no difference between the control subjects and the asthmatic patients in the plasma histamine response to exercise. In both groups there was an insignificant rise of about 40% during exercise, although the initial levels were higher in the asthmatic patients.

4. The mean plasma histamine peak of the asthmatic patients preceded the mean maximal fall of FEV$_{1.0}$ and peak expiratory flow rate by approximately 15 min. However, no positive correlation was found between rise in, or peak, plasma histamine levels and decrease in lung function.

5. Three non-atopic asthmatic patients had a significantly higher mean plasma histamine concentration during exercise than had the atopic subjects.

6. A strong positive correlation in asthmatic patients, and asthmatic and control subjects together was found between age and mean postexercise plasma histamine concentrations.

7. The results do not support a direct role for histamine in the production of exercise-induced asthma.

Key words: age, asthma, atopy, exercise, exercise-induced asthma, histamine.

Introduction

It has been known for centuries that exercise can induce an attack in asthmatic individuals, but this phenomenon was not investigated until relatively recently [1–3]. Exercise-induced asthma is non-specific and may occur in 70–90% of asthmatic patients [4]. For this reason it can be useful in diagnosis and its investigation may provide important information concerning the pathogenesis of asthma.

The mechanism of exercise-induced asthma is largely unknown. The initial stimulus comes perhaps from hyperventilation and airway cooling [5]. The onset of airways narrowing is typically delayed until 2–3 min after exercise is completed and there is evidence of a short refractory period after one attack of exercise-induced asthma, when another cannot be induced so easily [6]. This has led to the suggestion that mediator release from mast cells might be involved and that the refractory period might be
owing to the time taken to replenish mediator stores [7, 8]. Further indirect evidence for the involvement of mediators in exercise-induced asthma is the fact that sodium cromoglycate, a drug which is not a bronchodilator, but which is thought to stabilize mast cells, can prevent it in a high proportion of subjects [9, 10].

There is evidence that one such mediator, histamine, may at least be a useful peripheral marker of bronchial events. It has been shown that allergen inhalation can produce a significant rise in venous plasma histamine levels in susceptible asthmatic patients [11]. Bruce, Weatherstone, Seaton & Taylor [12] also found raised plasma histamine levels in patients admitted to hospital with severe asthma and that these levels fell to normal after therapy. This finding has been confirmed in a subsequent study [13].

Several workers have studied the possible role of histamine in exercise-induced asthma, without conclusive results [14–16].

In the present study the possibility that histamine may have a role as a mediator in exercise-induced asthma has been further examined by following arterial plasma concentrations in nine patients with asthma and five normal individuals before and after a period of cycle-ergometer exercise.

Materials and methods

All patients investigated were male and had proven exercise-induced asthma (>15% fall in peak expiratory flow rate or forced expiratory volume in 1·0 s (FEV1·0) after an exercise test). Their ages ranged from 19 to 49 years (mean 32·9 years). Six patients had multiple positive skin-prick tests to common allergens. Bronchodilator drugs were not taken within 12 h of the test. Sodium cromoglycate (one patient) was withheld for 24 h, but corticosteroid aerosols (four patients) were permitted. The control group comprised five healthy non-atopic males aged 28–39 years (mean 31·6 years) who were not taking any medication. All subjects gave their informed consent and the project was approved by the Ethical Committee of the hospital.

Each subject attended the laboratory for about 90 min on one occasion. Thirty minutes before exercise a small Venflon cannula (1·2 mm, 18G) was inserted percutaneously into the radial or brachial artery under local anaesthetic [2% (w/v) lignocaine]. After 25 min, arterial blood samples were collected and ventilatory function measured before exercise on a cycle ergometer. Further blood samples were taken during the last minute of exercise and 5, 15 and 30 min after completion of the exercise. Blood samples were analysed for lactate content and plasma histamine concentration. Those for lactate estimation were placed immediately in an equal volume of cold 8% (v/v) perchloric acid before enzymatic analysis (Sigma method no. 826-UV). Blood samples for histamine were kept in ice and centrifuged at 1500 g and 4°C for 10 min. Plasma was then carefully removed with a Pasteur pipette, leaving the buffy coat undisturbed, and stored at −20°C until analysis.

The exercise challenge consisted of 8 min of submaximal steady-state exercise on an electromagnetically-braked cycle ergometer (Lode). The load was selected to produce a heart rate of about 150 beats/min during the last 2 min of exercise. Oxygen uptake was measured continuously as described by Miller, Davies, Cole & Seaton [17]. The exercise tests were performed in a laboratory whose temperature ranged from 20 to 24°C. The humidity of the inspired air was not controlled.

Ventilatory function was assessed by means of the FEV1·0 (Vitalograph) and peak expiratory flow rate (Wright peak-flow meter). The best of three technically acceptable readings was recorded each time. Measurements were made 5 min and immediately before exercise and the means of these values used as the baseline. They were repeated immediately after exercise and 5, 10, 15, 20 and 30 min thereafter.

Plasma histamine was determined by a radioenzymatic method [18] based on those of Snyder, Baldessarini & Axelrod [19], Beaven, Jacobsen & Horakova [20] and Bruce [21]. Histamine methyltransferase (EC 2·1.1.8) was purified from pig brain [21] by a method based on those of Brown, Tomchick & Axelrod [22] and Lorenz, Reimann, Barth, Kusche, Meyer, Doenicke & Hutzel [23] for guinea-pig brain. Six fresh brains were homogenized in ice-cold sucrose solution (0·25 mol/l) and centrifuged at 105 000 g and 4°C for 60 min. Ammonium sulphate was added to the supernatant to give first 45%, then 70% saturation, and the precipitates were collected by centrifugation at 12 000 g. The second precipitate was dissolved in phosphate buffer (0·25 mol/l, pH 7·4) and ammonium sulphate removed by column chromatography with Sephadex G-200. The fractions containing histamine methyltransferase activity were pooled and kept in portions at −20°C; activity was monitored by the procedure described below with histamine as substrate.

Each standard test was assayed in duplicate. A volume of 500 μl of standard (100 nmol of histamine/l) or plasma was added to 500 μl of dilute acetic acid (50 mmol/l), mixed and heated
at 100°C for 5 min. After centrifugation 100 μl of supernatant was added to 200 μl of a solution prepared immediately before use by mixing 100 μl of S-[3H]adenosylmethionine (200–500 mCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.), 15 ml of phosphate buffer (0.1 mmol; The Radiochemical Centre, Amersham, Bucks., U.K.), 15 ml of phosphate buffer (0.1 mol/l, pH 7.4) and 5 ml of histamine methyltransferase preparation. The solution was mixed and incubated at 37°C for 5 min. After centrifugation 100 μl of perchloric acid (0.7 mol/l) to stop the reaction.

[3H]Methylhistamine was extracted into 5 ml of chloroform, after the addition of 200 μl of sodium hydroxide solution (10 mol/l), by inversion for 5 min. After centrifugation at 1000 g for 5 min the upper aqueous layer was removed and the chloroform washed with 1 ml of sodium hydroxide solution (3.3 mol/l); 4 ml of the chloroform extract was then dried in a scintillation vial and 3H radioactivity measured in a liquid-scintillation counter.

The exercise tests, blood sampling and measurements of pulmonary function were carried out in Cardiff, Wales, U.K.; the plasma samples were later taken, frozen in solid carbon dioxide, to Liverpool, U.K., where they were assayed for histamine. To eliminate any possibility of analytical bias, identification of the samples and the results of the pulmonary function tests were exchanged simultaneously with the results of the histamine analyses. Statistical analyses were performed by conventional Gaussian techniques as described by Armitage [24].

Results

The mean exercise performance of each group of subjects is shown in Table 1. There was no significant difference between the asthmatic patients and normal subjects in any of the measurements made.

In each of the asthmatic patients there was a decrease of at least 15% in either FEV1.0 or peak expiratory flow rate after the period of exercise. The mean maximal fall in FEV1.0 was 24.2% and in peak expiratory flow rate was 20.0%. For both measurements mean minima were seen at about 15 min after completion of the exercise. In individual patients the minimum values were found between 5 and 20 min after exercise. In the normal individuals no single measurement of FEV1.0 or peak expiratory flow rate fell by more than 5% compared with the pre-exercise level and indeed the mean values showed a slight rise in both FEV1.0 and peak expiratory flow rate after exercise of 0.06 and 0.36% respectively.

The initial mean plasma histamine level of the asthmatic patients was higher than that of the control subjects (Table 2, Fig. 1; P < 0.05). During the last minute of exercise the mean plasma histamine level of the control subjects rose by 42% compared with the initial level, but this rise was not significant. Subsequent values returned towards the initial level.

In the asthmatic patients the mean plasma histamine level rose by 48% during exercise, but neither during nor after exercise was the change in mean histamine level significant. At no time after exercise was there a significant difference between the mean plasma histamine levels in the two groups of subjects.

From mean values, the lowest FEV1.0 and peak expiratory flow rate followed the mean plasma histamine peak by 15 min, but in two asthmatic patients and two control subjects there was no postexercise increase in plasma histamine. The non-atopic patients had a significantly higher mean plasma histamine concentration during exercise than the atopic patients (P < 0.05). There were no significant differences between those patients who used inhaled corticosteroids and those who did not.

There was no evidence of a positive correlation between the absolute or percentage fall in FEV1.0 or peak expiratory flow rate and the maximum rise in histamine after exercise. Rather, there appeared to be a negative correlation. The correlation coefficients between the percentage falls in FEV1.0 and peak expiratory flow rate and the peak postexercise plasma histamine were -0.79 (P = < 0.02) and -0.73 (P = < 0.05) respectively.

There was a strong correlation between the mean postexercise plasma histamine concentration and age (Fig. 2). The mean postexercise plasma histamine concentration for each subject is derived from the average of the concentrations during and 5, 15 and 30 min after exercise. This was significant in the case of the

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Asthmatic patient</th>
<th>Normal subject</th>
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</thead>
<tbody>
<tr>
<td>Cardiac frequency in last 2 min of exercise (beats/min)</td>
<td>148 ± 2.0</td>
<td>146 ± 5.4</td>
</tr>
<tr>
<td>Total ventilation (l BTPS)</td>
<td>330 ± 10</td>
<td>354 ± 39</td>
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<tr>
<td>Total oxygen uptake (mmol)</td>
<td>590 ± 16</td>
<td>627 ± 55</td>
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<tr>
<td>Max. arterial lactate concn. (mmol/l)</td>
<td>8.9 ± 2.2</td>
<td>6.2 ± 1.2</td>
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The initial mean plasma histamine level of the asthmatic patients was higher than that of the control subjects (Table 2, Fig. 1; P = 0.052). During the last minute of exercise the mean plasma histamine level of the control subjects rose by 42% compared with the initial level, but this rise was not significant. Subsequent values returned towards the initial level.

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Table 2. Plasma histamine concentrations of nine patients with exercise-induced asthma and of five control subjects before and after exercise on a cycle ergometer for 8 min

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Age (years)</th>
<th>Histamine concn. (nmol/l)</th>
<th>After Exercice (min)</th>
<th>Pre</th>
<th>During</th>
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<td>A1*</td>
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<td>17</td>
<td>13</td>
<td>10</td>
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<tr>
<td>A2†</td>
<td>45</td>
<td>15</td>
<td>23</td>
<td>15</td>
<td>28</td>
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<td>A3</td>
<td>23</td>
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<td>A4</td>
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<td>A5†</td>
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* Patients having maintenance beclomethasone by inhalation
† Non-atopic patients.

Fig. 1. Plasma histamine concentrations (means ± 1 SEM) in the nine asthmatic patients (●) and five control subjects (○) measured immediately before and at intervals during and after 8 min of cycle-ergometer exercise.

Fig. 2. Comparison between the mean postexercise plasma histamine concentration and the age of the subject in the patients with asthma (▲) and the control subjects (●). The correlation coefficient for the patients alone is +0.75 (P < 0.02) and for all the subjects is +0.71 (P < 0.01).

Asthmatic patients (P < 0.02) and, although only five normal subjects were examined, this relationship might also hold for the normal group. Certainly the oldest normal individual had the highest mean plasma histamine concentration and the youngest had the lowest. When the data for control subjects and asthmatic patients were combined, the correlation between the post-exercise plasma histamine concentration and age remained highly significant (P < 0.01). The age of the patient was not significantly correlated with the pre-exercise plasma histamine concentration.
Discussion

The importance of histamine in allergic reactions has been demonstrated in a number of systems in vitro, including human skin, leucocyte and lung preparations [25–29]. However, histamine has a very short half-life in the circulation [30, 31] and is normally present in very low concentrations which are barely detectable by previous methods of assay. This may have contributed to the controversy regarding the role of histamine in human asthma. However, with a sensitive enzymatic assay, Bhat et al. [11] demonstrated raised venous plasma histamine concentrations during provoked bronchospasm in asthmatic patients and Bruce et al. [12] detected significantly raised plasma concentrations in severe asthma. Simon, Stevenson, Arroyave & Tan [32] were also able to correlate venous plasma histamine levels with the severity of asthma in a group of asthmatic outpatients. Such studies support the idea that histamine may have a role in bronchial asthma or at least act as a marker of mast-cell degranulation if it is itself not the direct cause of airway narrowing.

Mean plasma histamine levels were higher throughout the study period in the asthmatic group, but only the initial difference came close to statistical significance. This may simply reflect the small numbers in each group, and the variation within the asthmatic group. We found that mean plasma histamine concentrations rose during exercise in both normal individuals and patients with asthma, but this rise above pre-exercise levels did not achieve statistical significance. The highest postexercise histamine value recorded in the control subjects was 17 nmol/l. This value was equalled or exceeded in all but two of the asthmatic patients. We cannot exclude the possibility that the amount of histamine in the tissues is one factor in the development of asthma after exercise in some individuals. Duner & Pernow [33], using a biological assay, found significant elevations of whole-blood and plasma histamine in a group of normal subjects during heavy cycle-ergometer exercise of 18 to 24 min duration. This was accompanied by a leucocytosis. A shorter period of stair-climbing exercise did not produce an increase in plasma histamine levels in six normal subjects or nine asthmatic patients [34], although marked leucocytosis was again demonstrated. The asthmatic patients in that study did not all have exercise-induced asthma. The first study of histamine specifically in exercise-induced asthma was by Granerus et al. [14], who collected urine samples before and after exercise and were unable to show any increase in histamine release in three patients. McFadden & Soter [15], using a protocol and assay method similar to our own, were unable to demonstrate any rise in arterial plasma histamine in ten subjects with exercise-induced asthma but, in contrast, Ferris et al. [16], using a fluorimetric assay, found a significant mean rise of 105% in six subjects with exercise-induced asthma after treadmill running. Data from their control subjects was not given in their report and, as we found in the present study, normal subjects may also show apparently large rises in plasma histamine when they are expressed in percentage terms.

We were unable to correlate changes in lung function with individual plasma histamine levels. Subjects with more severe exercise-induced asthma did not necessarily have greater changes in plasma histamine. On the contrary, there was some evidence of a negative correlation. This agrees with the findings of Bhat et al. [11], who were also unable to demonstrate greater rises in plasma histamine in patients with more severe airways narrowing during allergen-induced bronchospasm. Apart from the variability of histamine measurements another factor that may be important in this context is the sensitivity of the individual asthmatic subject to histamine. We have not made any attempt to assess this in these patients by performing, for example, histamine-inhalation challenge tests. Similarly, there was only evidence of a negative correlation between atopy and plasma histamine values, in that the three non-atopic individuals had the highest histamine levels during exercise.

Although McFadden & Soter [15] were unable to demonstrate any rise in plasma histamine in subjects with exercise-induced asthma, they found that those individuals who benefited from premedication with sodium cromoglycate tended to have higher mean histamine levels. This implies that mast-cell degranulation may be particularly important in certain individuals. Ferris et al. [16] found that terbutaline, presumably acting as a mast-cell stabilizing drug, reduced the rise in plasma histamine in their group of subjects.

We exercised five of these asthmatic patients again, several months after the study we have described, to assess the effect of sodium cromoglycate and found no correlation between the plasma histamine levels found in the present study and the subjects who later derived benefit from the drug. Two of the subjects (nos. A1 and A8, Table 2) had partial prevention of their exercise-induced asthma, whereas three did not (nos. A3, A6 and A9).

Interestingly, a positive correlation was found
between the age of the subject and the plasma histamine level after exercise (Fig. 2). The reason for this relationship with age is not at all apparent and perhaps demands further study.

The source of histamine in blood is obviously of great importance. Most of the histamine that is present in whole blood is present in the leucocytes, particularly basophils [35]. The increase in whole-blood histamine which Duner & Pernow [33] found in their normal subjects was almost certainly due to the leucocytosis accompanying exercise. We did not perform basophil counts in the present study and it could be argued that raised plasma histamine levels during exercise reflect release of histamine from increased numbers of basophils during sampling, rather than formation at a distant site during exercise. We have shown no difference in the response of arterial plasma histamine to exercise between patients with exercise-induced asthma and normal control subjects. Thus our findings do not support a role for histamine in exercise-induced asthma, but it may be that small amounts of histamine released in the bronchi during exercise could cause pronounced local effects, either directly or by initiating reflex vagal bronchoconstriction, without altering arterial plasma histamine levels. One approach to this problem is to measure frequent transpulmonary histamine levels by simultaneously sampling pulmonary and systemic arterial blood, but no information is yet available in man by this invasive approach. An alternative method is to study the effects of specific histamine H₁-receptor antagonists on exercise-induced asthma. Anti-histamines given orally or by injection in conventional doses do not prevent exercise-induced asthma [7], although larger doses may do so [36]. We have more recently found that the H₁-receptor antagonist clemastine can significantly reduce exercise-induced asthma when given by inhalation [37] and further studies of the role of histamine in this condition are required.

References

Histamine and exercise-induced asthma


