Arterial plasma histamine levels at rest, and during and after exercise in patients with asthma: effects of terbutaline aerosol

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ABSTRACT Eight asthmatic patients and two normal subjects performed two identical exercise tests 140 minutes apart (first test preceded by inhalation of saline and the second by terbutaline sulphate). A ninth asthmatic patient exercised twice after placebo 40 minutes apart. Arterial plasma levels of histamine and cyclic AMP, expiratory flow rates and volumes were measured at rest and during and after exercise. After the first test the mean±SEM fall in PEFR was 45·2±2·6%. In five asthmatics there was an increase in plasma histamine (mean±SEM 14·8±3·3 pmol ml⁻¹) coinciding with exercise-induced asthma (EIA). Histamine levels returned to pre-exercise values within 30 minutes. After terbutaline these five patients had histamine levels greater than those observed before, during, or after the first test. This effect may have been the result of changes in pulmonary microcirculation. After the second test the levels decreased indicating no further release of histamine in response to exercise. No EIA occurred in these patients after terbutaline. The other patients and the two normal subjects had little or no change in histamine throughout the study. The one patient in whom exercise was repeated after placebo demonstrated less histamine release and less EIA after the second test.

As early as 1966 McNeill and his associates suggested that chemical mediators of bronchoconstriction such as histamine and slow reacting substance of anaphylaxis (SRS-A) may be released in response to exercise. The evidence for mediator release as a causal factor in exercise-induced asthma (EIA) has been derived from studies of repeated exercise challenge and the prevention of EIA by pharmacological inhibitors of varying specificity. The occurrence of a refractory period in some patients when exercise challenge is repeated within one hour is consistent with the hypothesis that mediators are depleted as a result of the initial exercise and take some time to be replenished. Furthermore, drugs which inhibit antigen-induced mediator release in vitro such as diethylcarbamazine, sodium cromoglycate, and the beta-adrenoceptor agonists prevent EIA in a significant proportion of asthmatic patients.

A recent study which measured histamine in the venous plasma of asthmatic patients before and after exercise failed to demonstrate an increase in levels of histamine in the post-exercise period. However histamine is inactivated in the systemic circulation so that small increases may not be reflected in venous plasma levels.

In a preliminary study of patients with asthma we found an increase in arterial plasma histamine coinciding with post-exercise bronchoconstriction and this increase was reduced by pre-treatment with terbutaline sulphate. Histamine levels were determined by fluorometric method. We have now extended these studies in a similar group of patients using a more sensitive and specific radio-enzymatic assay for histamine.

Methods

Nine male asthmatic patients who had moderate to severe EIA documented during a routine laboratory assessment volunteered for the study after a
full explanation of the experimental protocol as approved by the Hospital Ethics Review Committee. All patients were taking regular aerosol salbutamol. Seven required aerosol steroids or sodium cromoglycate or both for adequate control of their symptoms, and one patient was taking oral prednisone. Two normal non-asthmatic male subjects volunteered as controls.

Anthropometric data, atopic status (positive prick skin test with weal size greater than 2·0 mm to one or more of eight common allergens), medications, and values for peak expiratory flow rates (PEFR) and forced expiratory volume in one second (FEV₁) are given in table 1.

Table 1  Anthropometric data and values for peak expiratory flow rate (PEFR) and forced expiratory volume in one second (FEV₁) before exercise in the nine asthmatic patients (1-9) and the two control subjects (10, 11)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Atopic</th>
<th>Medication</th>
<th>Percentage predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*PEFR</td>
</tr>
<tr>
<td>1</td>
<td>32</td>
<td>176</td>
<td>75</td>
<td>+</td>
<td>S, B</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>172</td>
<td>73</td>
<td>+</td>
<td>S, B, C</td>
<td>72</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>169</td>
<td>75</td>
<td>—</td>
<td>S, B</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>168</td>
<td>66</td>
<td>+</td>
<td>S, P, C</td>
<td>53</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>161</td>
<td>67</td>
<td>+</td>
<td>S</td>
<td>98</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>172</td>
<td>78</td>
<td>—</td>
<td>S, B</td>
<td>97</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>181</td>
<td>86</td>
<td>—</td>
<td>S, C</td>
<td>74</td>
</tr>
<tr>
<td>8</td>
<td>42</td>
<td>184</td>
<td>55</td>
<td>+</td>
<td>S, B, T</td>
<td>65</td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>181</td>
<td>92</td>
<td>+</td>
<td>S</td>
<td>88</td>
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<td>10</td>
<td>25</td>
<td>181</td>
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<td>—</td>
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<td>109</td>
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<tr>
<td>11</td>
<td>21</td>
<td>182</td>
<td>78</td>
<td>—</td>
<td>—</td>
<td>108</td>
</tr>
</tbody>
</table>

S=salbutamol aerosol; P=prednisone; T=theophylline; C=disodium cromoglycate; B=beclomethasone dipropionate.
*Values measured before first exercise test.
†Values measured after terbutaline immediately before the second exercise test.

Patients were asked to refrain from taking any caffeine-containing beverages or asthma medication for at least six hours before the studies, which were performed in the morning. For the patient taking theophylline 12 hours had passed since the last tablet.

EXERCISE TESTS
Surface electrodes were placed on the chest and back for the continuous monitoring of heart rate (Devices Recorder, UK). A cannula was inserted into a radial or brachial artery under local anaesthesia. A three-way tap was used for blood collection and intermittent flushing of the cannula with heparinised saline. The subject then rested for at least 20 minutes.

Baseline measurements of FEV₁, forced vital capacity (FVC), and PEFR were made using an Avionics (California, USA) treadmill, set at a speed and slope sufficient to raise the heart rate above 165 beats per minute. After two minutes of running the patient was asked to walk at the same speed so that blood could be collected. Peak expiratory flow rates and FEV₁ were then measured immediately and the patient resumed running for the next five to seven minutes. On cessation of exercise a blood collection was made and then again 5, 10, 15, and 30 minutes later. When blood was taken the collection always preceded the measurements of PEFR which were made at 1, 3, 5, 7, 10, 15, 20, and 30 minutes after exercise. Forced expiratory volume in one second was measured five and 10 minutes after exercise.

In one patient the values for PEFR and FEV₁ samples were aspirated carefully leaving one ml of plasma above the sedimented red cells. The plasma for all determinations was immediately frozen and stored at −20°C until analysed.

The patient exercised for eight to 10 minutes on an Avionics (California, USA) treadmill, set at a speed and slope sufficient to raise the heart rate above 165 beats per minute. After two minutes of running the patient was asked to walk at the same speed so that blood could be collected. Peak expiratory flow rates and FEV₁ were then measured immediately and the patient resumed running for the next five to seven minutes. On cessation of exercise a blood collection was made and then again 5, 10, 15, and 30 minutes later. When blood was taken the collection always preceded the measurements of PEFR which were made at 1, 3, 5, 7, 10, 15, 20, and 30 minutes after exercise. Forced expiratory volume in one second was measured five and 10 minutes after exercise.

In one patient the values for PEFR and FEV₁ samples were aspirated carefully leaving one ml of plasma above the sedimented red cells. The plasma for all determinations was immediately frozen and stored at −20°C until analysed.
Plasma histamine in exercise-induced asthma

had returned to the pre-exercise level within 30 minutes and a second exercise test was performed 10 minutes later. Thus for this patient a period of 40 minutes separated two identical exercise studies.

The remaining eight patients were administered 2 ml of 0-9% saline containing 5 mg of terbutaline sulphate via a Hudson nebulizer in an identical fashion to placebo. Blood was collected at 20 and 60 minutes after terbutaline and in three patients an additional collection was made at 90 minutes. At this time a further 5 mg of terbutaline was administered to all patients in an identical manner to the initial dose. Twenty minutes later blood was collected and flow rates and volumes were measured. A second exercise test was then performed with the slope and speed of the treadmill and the duration of the exercise being identical to the first test. Heart rate was monitored throughout both exercise tests. A period of two hours and 20 minutes separated the two exercise tests. After the second test, blood was collected and measurements of flow rates and volumes were made at the same time intervals as those described for the first test.

Exercise was performed in an air conditioned room, the temperature and humidity were recorded on each test day, and care was taken to keep the room at a constant temperature and water content.

ASSAYS

The method for histamine assay was modified from the microassay procedure described by Taylor et al.12 Briefly, histamine methyltransferase (HMT) solution was freshly prepared each day from homogenised mouse brain. HMT-S-adenosyl-L-(methyl-3H) methionine (3H-SAMe) reactant solution was prepared by adding 10 μl 3H-SAMe to 1-0 ml HMT immediately before use in the assay. Then each of five plasma samples (400 μl) was incubated with 50 μl HMT-3H-SAMe reactant solution at 37°C for one hour. An internal standard of one ng histamine was added to two samples. The reaction was stopped by addition of 20 μl 5M NaOH containing methylhistamine (10 μg ml⁻¹). The 3H-methylhistamine formed was extracted into chloroform (3 ml) and the radioactivity in organic phase was determined by liquid scintillation spectrometry. With each assay, blanks and external standards of histamine were also determined. The sensitivity of the assay was 4-5 pmol ml⁻¹ and the recovery rate 71-2±4-5% SEM. The coefficient of variation for the plasma levels taken at rest ten minutes apart was 32%.

Cyclic AMP was assayed in triplicate using an Amersham Kit (Radio-Chemical Centre, Amersham Buckinghamshire, UK).

HISTAMINE INHALATION CHALLENGE

Subsequently eight of the nine subjects returned to the laboratory for an inhalation challenge test with histamine diphosphate as described by Chai et al.13 A Dosimeter (Rosenthal-French, Baltimore, Maryland, USA) connected to a cylinder of compressed air (20 psi and a setting of 0-6 sec) delivered nebulised histamine. Five inspiratory capacities of histamine at an initial concentration of 0-03 mg ml⁻¹ were given and measurements of PEFR and FEV₁ were made 90 seconds later. This procedure was immediately repeated with two-fold increases in concentration of histamine solution until the patient recorded a fall in PEFR and FEV₁ equivalent to that observed after the first exercise test.

The decrease in PEFR and FEV₁ in response to exercise and histamine challenge was quantified as follows:

\[
\text{Pre-challenge value} \quad \text{Lowest post-challenge value} \\
\text{Percentage fall= } \frac{\text{Pre-challenge value} - \text{Lowest post-challenge value}}{\text{Pre-challenge value}} \times 100
\]

The dose of inhaled histamine producing a fall in PEFR or FEV₁ of 20% (PD₂₀) and the dose equivalent to that observed on exercise (PDₑₓ) was assessed from the histamine dose-response curve and expressed in cumulative dose units (1 unit=1 inhalation of 1mg ml⁻¹ histamine solution).13

Statistical analysis was carried out using a t test for paired values in the same subject or an unpaired t test between groups of subjects. A two-tailed test was used to assess the level of statistical significance and a p value <0.05 has been taken as a statistically significant difference.

Since the sensitivity of the histamine assay was 4-5 pmol ml⁻¹ an increase in arterial plasma histamine after exercise was considered as significant when the highest post-exercise level of histamine was both (a) greater than 9 pmol ml⁻¹ and (b) greater than three standard deviations of the four measurements of the pre-exercise level of histamine.

Predicted normal values for PEFR were taken from the data of Cotes14 and for FEV₁, from those of Goldman and Becklake.15

RESULTS

The degree of airways obstruction present at rest before the first exercise test varied between the patients with only two having values for flow rates and volumes within normal limits (table 1).
Exercise-induced asthma occurred in all patients after the first exercise test and the fall in PEFR and FEV₁ was greater than 29% of the pre-exercise level. The two normal subjects had falls in PEFR less than 10%. Individual values for the maximum post-exercise percentage falls in PEFR and FEV₁ and arterial plasma histamine levels at rest, during and after exercise are given in table 2. At a time coinciding with the greatest fall in flow rates there was a significant increase in histamine levels in five of the nine patients. There was no significant increase in the histamine levels in response to exercise in the two normal subjects.

There was no correlation between the change in histamine level from rest to post-exercise and the percentage fall in PEFR (r=0.26 p<0.1, n=9) or percentage fall in FEV₁ (r=0.43, p<0.1, n=9).

For group 1, however, the highest levels of histamine coincided with the maximum fall in PEFR.

Values for histamine returned to the pre-exercise level within 30 minutes and there was no significant difference in levels of histamine between the two groups at this time.

Patient 1 performed a second exercise test 40 minutes after the start of the first exercise test. During the second test there was a rise in plasma histamine similar to that observed during the first test. However after exercise the plasma histamine level decreased and the values were lower compared with the first test (fig 2). The percentage fall in PEFR was also less after the second test (29-6% and 13% respectively).

The remaining eight patients performed a second exercise test after terbutaline sulphate. Compared with values after placebo, flow rates were significantly higher at rest (p<0.025) during (p<0.05) and after exercise (p<0.001). Those in group 1 (n=4) showed a marked increase in plasma histamine after terbutaline. By contrast there was little or no change in histamine levels in group 2 and the two normal subjects. Individual values for histamine and the maximum fall in PEFR, and PEFR after exercise were significantly different from their levels at rest.

On the basis of plasma histamine levels, the patients have been divided into two groups (table 2). Group 1 includes five patients who had a significant increase in the level of histamine after exercise, and group 2 contains the four patients whose values for histamine after exercise were not significantly different from pre-exercise levels.

The mean values ±1 SEM for PEFR (expressed as a percentage of the predicted normal value), histamine (pmol ml⁻¹), and cyclic AMP (pmol ml⁻¹) levels for the two groups are illustrated in fig 1. Values for PEFR and cyclic AMP were similar at rest, during and after exercise in each group but plasma histamine levels after exercise were statistically significantly higher in group 1 (p<0.05).

### Table 2 Plasma histamine levels at rest, during and after exercise, post-exercise falls in peak expiratory flow rate (PEFR) and one second forced expiratory volume (FEV₁), and the dose of histamine required to induce a fall in FEV₁ and PEFR of 20% (PD₂₀) and a fall equivalent to that observed after exercise (PDEX)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Plasma histamine pmol ml⁻¹</th>
<th>Exercise</th>
<th>Post-exercise</th>
<th>%Fall</th>
<th>Inhaled histamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
<td>Post-exercise</td>
<td>1-15 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.3±1.5</td>
<td>3.2±1.4</td>
<td>22.4±1.9</td>
<td>5.9±0.8</td>
<td>29.6</td>
</tr>
<tr>
<td>2</td>
<td>6.0±1.0</td>
<td>12.2±1.6</td>
<td>17.0±1.4</td>
<td>9.0±0.8</td>
<td>47.4</td>
</tr>
<tr>
<td>3</td>
<td>12.1±1.1</td>
<td>28.6±2.0</td>
<td>39.6±1.9</td>
<td>6.5±0.7</td>
<td>43.4</td>
</tr>
<tr>
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<td>3.8±0.5</td>
<td>14.8±1.1</td>
<td>0.0±0.0</td>
<td>50.1</td>
</tr>
<tr>
<td>5</td>
<td>5.8±0.9</td>
<td>5.2±0.8</td>
<td>10.6±0.7</td>
<td>6.9±0.6</td>
<td>50.8</td>
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<td>Mean</td>
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<td>10.6</td>
<td>20.9</td>
<td>5.7</td>
<td>44.3</td>
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<td>4.5</td>
<td>1.3</td>
<td>8.7</td>
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<td>2.6±0.7</td>
<td>0.8±0.2</td>
<td>0.0±0.0</td>
<td>47.2</td>
</tr>
<tr>
<td>8</td>
<td>1.2±0.3</td>
<td>3.9±0.5</td>
<td>5.0±0.5</td>
<td>3.5±0.4</td>
<td>52.3</td>
</tr>
<tr>
<td>9</td>
<td>10.2±1.3</td>
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<td>4.1</td>
<td>4.8</td>
<td>46.3</td>
</tr>
<tr>
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<td>1.9</td>
<td>2.1</td>
<td>2.3</td>
<td>9.0</td>
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<td>0.69</td>
<td>0.27</td>
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<td>0.29</td>
<td>0.88</td>
<td>0.37</td>
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</tr>
</tbody>
</table>

*PD=provocative dose of histamine expressed in units (1 unit = 1 inhalation of 1 mg ml⁻¹ histamine solution).
†Mean ± 1 SEM of four measurements.
### Plasma histamine in exercise-induced asthma

**Fig 1** Mean values ± SEM for peak expiratory flow rate expressed as a percentage of the predicted normal value, arterial plasma histamine and cyclic AMP levels at rest, during, and after exercise after the administration of nebulised saline and terbutaline sulphate. The solid line represents the data for the asthmatic patients in group 1 and the broken line patients in group 2.

**Table 3** Effect of terbutaline on plasma histamine before, during, and after exercise and post-exercise falls in peak expiratory flow rate (PEFR) and one second forced expiratory volume (FEV₁).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Plasma histamine pmol ml⁻¹</th>
<th>Exercise</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Post-drug*</td>
<td>Rest</td>
<td>Exercise</td>
<td>Post-exercise</td>
<td>1-15 min</td>
<td>30 min</td>
<td>%Fall</td>
</tr>
<tr>
<td>2</td>
<td>29.8 ± 0.7</td>
<td>21.0 ± 1.6</td>
<td>2.1 ± 0.7</td>
<td>6.9 ± 1.0</td>
<td>4.9 ± 0.7</td>
<td>0.0</td>
<td>4.2</td>
</tr>
<tr>
<td>3</td>
<td>23.8 ± 2.3</td>
<td>23.8 ± 2.3</td>
<td>4.0 ± 0.7</td>
<td>21.8 ± 1.7</td>
<td>2.3 ± 0.5</td>
<td>5.3</td>
<td>9.0</td>
</tr>
<tr>
<td>4</td>
<td>25.8 ± 1.7</td>
<td>6.5 ± 0.9</td>
<td>3.3 ± 0.8</td>
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</tr>
<tr>
<td>5</td>
<td>24.9</td>
<td>14.0</td>
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<tr>
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<tr>
<td>SEM</td>
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<td>4.8</td>
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**Group 2**

<table>
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<th></th>
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<tbody>
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<td>6</td>
<td>0.6 ± 0.2</td>
<td>0.6 ± 0.2</td>
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</tr>
<tr>
<td>8</td>
<td>8.5 ± 0.8</td>
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<td>3.1 ± 0.3</td>
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<td>14.9 ± 0.9</td>
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**Normal subjects**

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</thead>
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<td>11</td>
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<td>0.11</td>
<td>0.20</td>
<td>0.60</td>
<td>0.10</td>
<td>6.0</td>
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</table>

*Highest value obtained at rest after terbutaline

†Mean ± 1 SEM of four measurements.
FEV₁ are given in table 3. The mean values ±1 SEM for PEFR, histamine and cyclic AMP are illustrated in fig 1. In group 1, histamine levels immediately before exercise were higher than those before the first exercise test though the difference was not significant. During exercise three of the four patients demonstrated falls in plasma histamine to lower levels than before exercise and values for histamine were still lower after exercise compared with rest. In these three patients there were no significant post-exercise changes in plasma histamine, PEFR, or FEV₁. One patient (4) had a significant increase in plasma histamine after exercise associated with a fall in PEFR of 23·5% of the pre-exercise value. In group 2 there was no significant change in the levels of histamine after exercise in the presence of terbutaline; however one patient (9) had a small increase of 6·4 pmol ml⁻¹.

Plasma cyclic AMP levels after terbutaline were higher than after placebo at rest, during, and after exercise (p<0·01, p<0·025, p<0·001, respectively, n=8).

The mean value for heart rate measured during the last minute of exercise was 179±1·2 SEM and this was not significantly different to the value measured during the first test (mean 176±4 SEM).

Histamine inhalation tests were performed on eight patients. Individual values for doses of histamine producing 20% falls in PEFR and FEV₁ and doses producing equivalent falls to those observed after exercise are given in table 2. For group 1 patients less than seven dose units were required for falls in PEFR or FEV₁ of 20% from the pre-challenge values. Four of the patients in this group required less than 22 dose units to induce the same fall in PEFR and FEV₁ as that observed in response to the first exercise test. However one patient (5), who was sensitive to the lower doses of histamine, appeared to be less sensitive at the higher doses compared with the other four patients in the group. In group 2, two of the three patients appeared to be less sensitive to the effects of inhaled histamine compared with group 1. However the differences between the two groups were not statistically significant.

Discussion

This study demonstrates that in some patients with asthma a detectable increase in arterial plasma histamine will occur at a time when exercise has induced an increase in airways resistance.

Histamine release has previously been reported in...
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in response to non-immunological stimuli such as cold, vibration and heat.\textsuperscript{16-18} In our patients with demonstrable histamine release the time course after exercise challenge was similar to that reported for other physical stimuli—that is, release of histamine within one minute of challenge, a peak value within 15 minutes, and a return to the base level within 30 minutes.

In the previous study using a fluorometric assay for histamine we found similar quantitative changes to those reported here.\textsuperscript{11} However the fluorometric technique proved to be less sensitive and less reproducible compared with the single isotopic enzymatic assay due to lower recovery rates and possible interference of spermidine.\textsuperscript{19} The sensitivity and reproducibility of the measurements in this study suggested that a significant increase in plasma histamine had occurred in response to exercise when the values for histamine were greater than 9 pmol ml\textsuperscript{-1} and more than three standard deviations from the resting level. The resting levels of histamine in arterial plasma reported here are similar to those reported by others for venous plasma in non-asthmatic subjects.\textsuperscript{20}

The histamine released in response to exercise may have originated from tissue mast cells or circulating basophils which are considered the sole source of histamine in the blood.\textsuperscript{21} Mast cells are common in the submucosa\textsuperscript{22} and histamine-containing cells have been reported in the lumen of human bronchi.\textsuperscript{23} It is possible that mast cell degranulation occurs in the bronchial mucosa in response to heat and water loss from the respiratory tract during exercise.

In a recent study using hyperventilation with air at \textdegree{}11°C as a provoking stimulus for asthma, Deal \textit{et al}\textsuperscript{24} reported no consistent change in arterial histamine levels. However three of the five subjects investigated had changes in histamine levels greater than 18 pmol ml\textsuperscript{-1} after challenge and the levels of histamine were of a similar magnitude to those reported in this study for group 1 patients.

The role of histamine (and other putative mediators of bronchoconstriction) in the persistence of airways obstruction after plasma levels have returned to normal is not clear. The emergence of histamine in arterial blood after terbutaline inhalation suggests that the histamine was available locally in the lung for some considerable time after exercise. Thus it is possible that continuing airflow obstruction was related to local accumulation of histamine within the interstitium of the lung without prolonged elevation of plasma histamine in the systemic arterial circulation. Other data have suggested discrepancies between blood levels of histamine and concentration at receptor sites, since asthmatic patients have been given intravenous histamine in high dosage (0.2 mg kg\textsuperscript{-1} min\textsuperscript{-1}) with little or no change in lung function.\textsuperscript{25}

The relatively low levels of plasma histamine in this study compared with those detected in the venous drainage of limbs with cold-induced urticaria\textsuperscript{27} may be caused by several factors. In our studies it was not possible to sample blood as close to the site of presumed release. Moreover, if histamine release was occurring primarily in the bronchial mucosa and interstitium of the lung it may have been inactivated by diamine oxidase before entering the pulmonary microcirculation so that systemic arterial levels may have been an insensitive index of local pulmonary release. The increase in cardiac-output associated with exercise may "dilute" the plasma concentrations of histamine. This possibility is exemplified by the observed fall in plasma histamine during exercise from the high resting levels after terbutaline and a return towards pre-exercise levels in the 15 minutes after exercise.

The failure to detect a significant increase in histamine levels in some patients after exercise may have been the result of differences in rates of histamine deactivation, or in blood flow to areas of bronchoconstriction and in feedback inhibition of histamine release via H\textsubscript{2} receptors.\textsuperscript{26} It is also possible that the patients in group 2 were exquisitely sensitive to small undetectable releases of histamine in the lung but this seems most unlikely in view of the relatively high threshold to inhaled histamine in two of the patients. The fact that this group of patients also failed to have an increase in plasma histamine after terbutaline supports the suggestion that histamine was not released after the initial exercise challenge.

The only patient who exercised twice within 40 minutes showed less histamine release after the second test corresponding to substantially less EIA as measured by a fall in PEFR. The severity of EIA and lack of spontaneous recovery within 30 minutes precluded similar studies on the other eight patients. Further investigations are required to elucidate the role of histamine release in the post-exercise refractory state.\textsuperscript{2,3} In addition to inducing bronchodilatation in all patients, terbutaline appeared to cause a marked increase in the plasma level of histamine at rest which could not be attributed to a direct effect of the drug on the release of histamine from circulating basophils since we could not detect any increase in whole blood histamine levels after 30 minutes incubation.
with terbutaline. However, terbutaline may have a relaxant effect on venular endothelial cells and vascular smooth muscle, inducing a fall in pulmonary vascular resistance and increasing blood flow to areas previously constricted by hypoxia or histamine release. In this way terbutaline may have “flushed out” the previously released histamine from pockets of sequestration in poorly perfused areas of the pulmonary vasculature resulting in higher levels of plasma histamine after inhalation. Alternatively the emergence of histamine after terbutaline may have been unrelated to the drug and merely a characteristic of the normal time course for histamine release after exercise. In this situation a second attack of asthma might be expected after exercise challenge but there are only isolated reports of late reactions after EIA.

Sodium cromoglicate and diethylcarbamazine are both thought to prevent EIA by inhibiting mediator release from mast cells. The mechanism by which adrenoceptor agonists inhibit EIA is unclear. In group 1 exercise did not induce an increase in the levels of plasma histamine after terbutaline. This observation suggests that the drug may have had some inhibitory effect on the release of histamine from the mast cell. However the aerosol effectively inhibited EIA in group 2 who had little or no significant release of histamine.

Beta-adrenoceptor agonists have been shown to prevent mediator release from mast cells in vitro from human lung fragments and from rat peritoneal mast cells in vivo, but we are not aware of previous reports of this action in man.

The changes in cyclic AMP in response to exercise and the effect of terbutaline observed in this study are similar to those reported in a separate study from this laboratory. The effects of circulating cyclic AMP on mediator release are not clear but increased plasma levels may reflect increases in intracellular cyclic AMP, and this has been discussed by Kunitada et al. There appeared to be no relation between the change in cyclic AMP and the release of histamine in group 1 patients. However the values of cyclic AMP were highest after exercise in the presence of terbutaline, and it is possible that a critical threshold of cyclic AMP is required to prevent the release of mediators. In this study the changes in plasma levels of cyclic AMP appeared unrelated to the change in flow rates. Values for cyclic AMP after placebo were higher after exercise at a time when bronchoconstriction occurred. Hydrocortisone has been shown to stimulate adenyl cyclase in leucocytes and to restore diminished leucocyte adenyl cyclase responsiveness to isoprenaline in some asthmatic children. Our patients receiving corticosteroids (either aerosol beclomethasone or oral prednisone) were equally divided between groups 1 and 2 so it is unlikely that steroid therapy could account for differences in mediator release.

Mast cells release many mediators of bronchoconstriction of which histamine is but one major constituent. Histamine was chosen as a marker of mast cell degranulation with the reservation that it need not be the main or only mediator involved in the induction of exercise asthma. However, in view of the close relationship between the release of histamine and other mediators such as SRS-A it is likely that the measurement of histamine provides a representative profile of mediator release.

The results of this study suggest that chemical mediators are released in some patients with EIA but further studies are required to elucidate the role of mediator release in the aetiology of EIA.

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Thorax 1981 36: 259-267
doi: 10.1136/thx.36.4.259

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