

# Exercise induced asthma and endogenous opioids

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**ABSTRACT** Concentrations of endogenous opioid peptides in the plasma are increased during exercise and these substances have been implicated in the pathogenesis of asthma induced by chlorpropamide and alcohol in diabetic patients. This work was undertaken to determine whether exercise induced asthma might be mediated by endogenous opioids. Plasma  $\beta$  endorphin, met-enkephalin, and adrenocorticotrophic hormone (ACTH) concentrations were measured in five asthmatic patients and five normal volunteers breathing cold air during exercise. In four of the patients the effect of an infusion of naloxone on FEV<sub>1</sub> was also measured during exercise induced asthma. Exercise produced acute bronchoconstriction in all asthmatics, characterised by a fall in FEV<sub>1</sub>, whereas no change occurred in normal subjects. There was no difference in plasma met-enkephalin,  $\beta$  endorphin, and ACTH concentration between the two groups. Infusion of naloxone neither prevented nor worsened exercise induced asthma. These data suggest that endogenous opioids probably do not play a part in the development of exercise induced asthma.

Since their discovery<sup>1-2</sup> endogenous opioids have been shown to play a part in both physiological and pathophysiological conditions. Their role in the response to chronic pain,<sup>3</sup> in neuroendocrine regulation (for review see ref 4), and in narcotic dependence<sup>5</sup> is well established. Their possible role in respiratory regulation is still under investigation.<sup>6</sup>

It has recently been reported that endogenous peptides with opiate like activity, such as enkephalin, may mediate the asthma and facial flush observed in some diabetics treated with chlorpropamide when they drink alcohol.<sup>7</sup> Both sodium cromoglycate and the specific opiate antagonist were able to prevent the asthmatic reaction, which could be reproduced by an enkephalin analogue with opiate like activity.<sup>8</sup> It is also known that various types of exercise increase plasma concentrations of endogenous opioids such as  $\beta$  endorphin.<sup>9-11</sup> The present study was therefore undertaken to investigate whether exercise induced asthma might also be related either to an increase in circulating opioid peptide— $\beta$  endorphin or met-enkephalin or both—or to increased sensitivity to endogenous opiates, or to both. For this purpose plasma  $\beta$  endorphin and met-enkephalin immunoreactivities were measured in asthmatic patients

breathing cold, dry air during exercise. In four of these asthmatic subjects the effect of naloxone perfusion on the respiratory variables during exercise induced asthma was also measured.

## Methods

### SUBJECTS AND PROTOCOL

Five patients suffering from exercise induced asthma and five healthy subjects (three men and two women in both cases) were studied. The subjects of the two groups did not differ in age, weight, or body surface area. The mean ages of the asthmatic and control subjects respectively were 29.2 (SE 4.2) and 30.3 (2.8) years, and the mean weights 65 (4.7) and 67.2 (3.6) kg. The mean body surface area was 1.72 (0.1) m<sup>2</sup> for the asthmatic patients and 1.82 (0.6) m<sup>2</sup> for the control subjects. The patients were not receiving local or systemic corticosteroid treatment. The controls had not smoked or taken any medication or alcohol for at least 24 hours before the experiment.

The study protocol was approved by the ethical committee of the Department of Medicine, University Hospital of Geneva. Each subject (patients and normal volunteers) gave his written informed consent before starting the study.

Three days before the experiment a preliminary test determined the work load required to raise the heart rate to at least 80% of the predicted maximum.<sup>12</sup> The experiments were all performed between 8 and 10 am

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After insertion of a cubital intravenous catheter the subjects were allowed to relax for at least 45 minutes. Forced expiratory manoeuvres were performed 15 minutes and immediately before the exercise as well as 0, 5, 10, 15, 20, and 30 minutes afterwards. The forced expiratory volume for one second ( $FEV_1$ ) was measured with a Fleisch pneumotachograph, connected to an 8805 B amplifier, and a Hewlett-Packard integrator 88 and recorded on a recorder. The best  $FEV_1$  out of two or three forced expirations was selected. The challenge consisted of 10 minutes of exercise on a bicycle ergometer. During the first two to four minutes the work load was increased stepwise to reach a stable level for the last six minutes. Throughout the test the subjects breathed through a mouthpiece and received dry, cool air generated by an apparatus similar to that described by Strauss *et al.*<sup>12</sup>

Twenty five millilitre blood samples were collected in chilled heparinised tubes at various times before and after the exercise. The tubes were immediately spun down at 4°C and the plasma was flash frozen and stored at -20°C until assayed.

**NALOXONE INFUSION**

The effect of naloxone was studied in four of the asthmatics (two men and two women). The protocol was as described above, except that the subjects received, in random order and double blind fashion, two infusions, with a minimum interval of one week between them—namely naloxone (0.4 mg/min) and saline, infused by micropump through an indwelling venous cannula. The infusion was started five minutes before the beginning of the exercise test and continued for 20 minutes afterwards. Cardiorespiratory variables were measured as described above but no blood samples were taken. The protocol was performed twice on three of the patients and once on the fourth.

**RADIOIMMUNOASSAYS**

Plasma adrenocorticotrophic hormone (ACTH),  $\beta$  endorphin (COOH terminal  $\beta$  lipotrophin), and met-enkephalin were measured by radioimmunoassay after extraction.<sup>13-15</sup> The  $\beta$  endorphin assay used an antiserum showing equimolar cross reactivity with  $\beta$  lipotrophin. Met-enkephalin was assayed by oxidising the samples with  $H_2O_2$  after extraction and using an antiserum with high specificity for met-enkephalin sulphoxide.<sup>15</sup> The met-enkephalin antiserum had unique specificity with no cross reaction with leu-enkephalin, purified human  $\beta$  endorphin,  $\beta$  lipotrophin, or a large number of other hypothalamic, pituitary, and gastrointestinal peptides.<sup>15</sup> The limits of detection of these assays for plasma measurements were 8, 15, and 2.5 pg/ml for ACTH,  $\beta$  endorphin, and met-enkephalin respectively and the mean extraction rates for these peptides were 63%, 75%, and 29%.

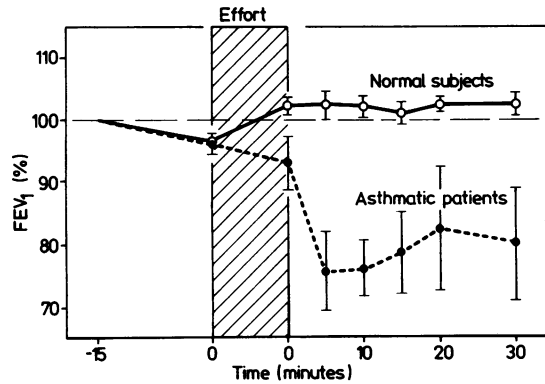


Fig 1 Percentage change (means and standard errors) in  $FEV_1$  in response to 10 minutes of exercise (shaded area) in normal subjects and asthmatic patients. (Baseline values taken as 100%).

The intra-assay coefficients of variation for the ACTH and  $\beta$  endorphin assays were less than 8%, and less than 9% for met-enkephalin. The inter-assay coefficients of variation were respectively 15% and 12%.

**STATISTICS**

The statistical methods used were analysis of variance for repeated measurements to analyse the change in opioid levels as a function of time, and the Mann-Whitney rank test to compare differences between two groups. All results are expressed as means with standard errors in parentheses.

**Results**

During the last six minutes of exercise the temperature of the inspired air and the ventilation volume per

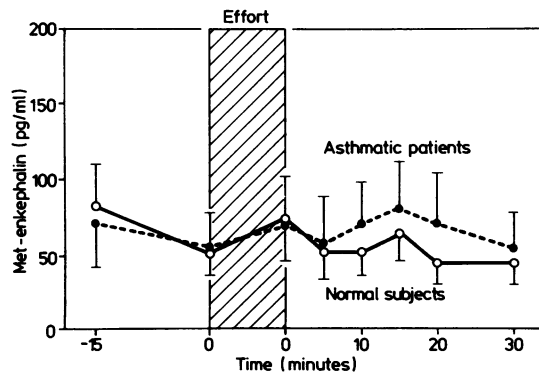


Fig 2 Plasma met-enkephalin concentrations (means and standard errors) in response to 10 minutes of exercise (shaded area) in normal subjects and asthmatic patients.

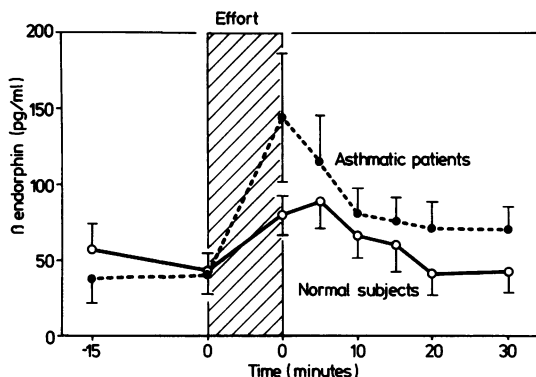


Fig 3 Plasma  $\beta$  endorphin concentrations (means and standard errors) in response to 10 minutes of exercise (shaded area) in normal subjects and asthmatic patients.

minute (in relation to body surface area) was similar in the asthmatic and the control subjects (unpaired *t* test). The values were respectively  $-11.2^{\circ}$  ( $1^{\circ}$ C) and  $39.1$  ( $2.8$ )  $\text{l min}^{-1} \text{min}^{-2}$  in the asthmatics and  $-9.9^{\circ}$  ( $0.9^{\circ}$ C) and  $38.2$  ( $4$ )  $\text{l min}^{-1} \text{min}^{-2}$  in the control subjects. Heart rates when expressed as percentages of predicted maximal heart rate were similar in the two groups (92 (3) in the asthmatic and 95 (2) in the control subjects).

In asthmatics  $\text{FEV}_1$  decreased within 10 minutes of the exercise to 32–82% of the basal value obtained 15 minutes before the exercise. In control subjects  $\text{FEV}_1$  did not change significantly, varying after exercise from 94% to 107% of the basal value (fig 1).

Plasma met-enkephalin concentration did not change significantly during the experiment and there was no difference between the normal subjects and the

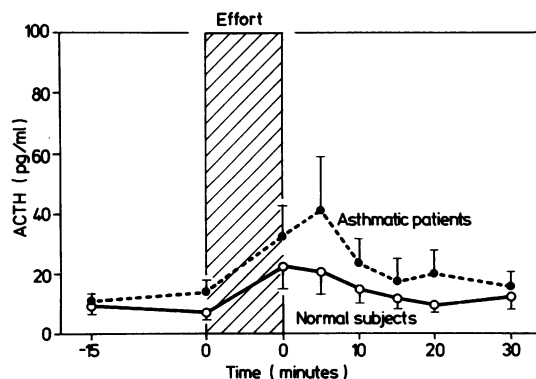


Fig 4 Plasma adrenocorticotrophic hormone (ACTH) concentrations (means and standard errors) in response to 10 minutes of exercise (shaded area) in normal subjects and asthmatic patients.

#### Postexercise percentage change in $\text{FEV}_1$ after administration of placebo and naloxone

Subject No	Test	Placebo	Naloxone
1	I	-15	-9
	II	-20	-13
2	I	-10	-17
	II	-11	-9
3	I	-59	-45
	II	-66	—
4	I	-6	-12

asthmatic patients (fig 2). Plasma  $\beta$  endorphin concentration on the other hand increased significantly at the end of the work test in both groups ( $p < 0.02$ ) (fig 3). The maximal percentage increment above basal values was 106% in the control group and 249% in the asthmatic patients group. This difference in increments was not significant ( $p = 0.1428$ , Mann-Whitney rank sum test). Plasma  $\beta$  endorphin concentrations returned to the basal values earlier in the controls than in the asthmatic subjects. Plasma ACTH concentrations in both groups showed a pattern similar to that of  $\beta$  endorphin (fig 4).

There was no correlation between the heart rate at the end of the exercise and the increase in  $\beta$  endorphin levels after the exercise.

#### NALOXONE INFUSION

In patients 1 and 2 the protocol consisting of naloxone and saline infusions in random order was repeated twice. Another asthmatic (patient 3) performed the test twice with saline and only once with the naloxone infusion. At the beginning of the second test with naloxone he felt unwell while exercising and we had to stop the experiment. The fourth patient had only one saline and one naloxone infusion, which was followed by generalized urticaria, starting 12 hours after the infusion, lasting for 36 hours, and requiring treatment. As shown in the table, there was no significant difference in the decrease in  $\text{FEV}_1$  between subjects receiving saline and those receiving naloxone.

#### Discussion

Exercise induced asthma is not a disease but an expression of bronchial hyperreactivity where the nature of the challenge is exercise. Since the occurrence of exercise induced asthma is clearly associated with hyperventilation in an environment of dry, cool air,<sup>16</sup> we used exercise or a cycle ergometer associated with a dry, cold air generator. This type of challenge has been shown to be the most potent and reproducible means of producing exercise induced asthma.<sup>17,18</sup> When exposed to this test the asthmatic

subjects, as expected, developed acute bronchoconstriction, characterised by a fall in the FEV<sub>1</sub>, whereas the normal subjects showed no change. Despite this striking difference in airway response, there was no significant difference in plasma met-enkephalin,  $\beta$  endorphin, or ACTH concentrations between the two groups.

The absence of significant differences in  $\beta$  endorphin and met-enkephalin plasma concentrations between normal subjects and asthmatic patients appears to rule out these circulating opioids as a cause of exercise induced asthma. The increase in  $\beta$  endorphin, which was sustained slightly longer in the asthmatic group, might be related to the more severe stress resulting from bronchoconstriction. The absence of a similar stress response in met-enkephalin concentrations is not surprising since this peptide is not affected by stresses such as surgery and insulin induced hypoglycaemia.<sup>19</sup> On the other hand, since plasma met-enkephalin has a short half life, possibly an increase in the plasma concentration of this peptide could be missed at the periphery. A rise in plasma met-enkephalin concentrations, however, is unlikely to have been missed through insensitivity of the radioimmunoassay. Indeed, in three recent studies using the same met-enkephalin assay with the same characteristics we and others were able to show increases in plasma met-enkephalin concentrations in chronic renal failure, exercise, and acupuncture.<sup>19-21</sup> The absence of changes in opioid concentrations in the peripheral circulation does not, of course, rule out their local release and a role in exercise induced asthma. Two main theories have been proposed to explain the bronchoconstriction associated with hyperventilation and airway cooling in asthmatic patients. One attributes a predominant role to the vagus nerve<sup>22-23</sup> whereas the other emphasises the part played by mediator substances released from mast cells in the airways.<sup>17,24</sup> Opioid peptides are known to be present in several neural pathways. They could function as neurotransmitters or neuromodulators or act locally as hormones. A role for these peptides might thus be compatible with either theory, for they could modulate either the effect of the vagus nerve or the release of mediators from the mast cells.

To test this hypothesis, we decided to block the possible opioid effects by infusing the specific opiate antagonist naloxone. The dose of naloxone chosen was similar to that previously reported to block the induction of asthma by chlorpropamide and by alcohol and to reverse the asthma induced by an enkephalin analogue.<sup>7</sup> We therefore postulated that this dose should suffice to block both the naloxone sensitive  $\mu$  and the naloxone insensitive  $\delta$  receptors. Even at this high dose, however, naloxone neither

blocked nor worsened exercise induced asthma. These results do not appear to support the hypothesis that such asthma is mediated by a local effect of opioid peptides, whether they act as neurotransmitters or as local hormones. We recognise that the number of subjects tested with naloxone was small and does not therefore preclude the existence of subgroups with different responses. The occurrence of undesirable side effects in two out of four patients prevented us from extending the study.

Although investigation of the effect of exercise on endogenous opioids was not the purpose of this study, we observed a significant rise in  $\beta$  endorphin and ACTH concentrations after the exercise test but only a variable and non-significant effect on met-enkephalin concentrations. The rise of the two peptides ACTH and  $\beta$  endorphin, derived from the common precursor pro-opiomelanocortin,<sup>25</sup> has already been reported by others<sup>9-11</sup> after physical exercise. On the other hand, the absence of significant changes in met-enkephalin concentration was surprising, since exercise is associated with adrenal catecholamine secretion and met-enkephalin is cosequestered with catecholamines in the chromaffin granules of the adrenal medulla. A recent study, however, reports a significant rise in plasma met-enkephalin concentrations after one hour of treadmill exercise, although the response also showed considerable heterogeneity.<sup>20</sup> Perhaps therefore our exercise test was either too short or not strenuous enough to produce increase in this peptide at the peripheral level.

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