Atrial natriuretic peptide, altitude and acute mountain sickness

J. S. MILLEDGE, J. M. BEELEY, S. McARTHUR AND A. H. MORICE
Clinical Research Centre, Northwick Park Hospital, Harrow, Middlesex, U.K.

(Received 27 July 1988/12 April 1989; accepted 4 May 1989)

SUMMARY

1. To investigate the mechanisms of acute mountain sickness, 22 subjects travelled to 3100 m by road and the following day walked to 4300 m on Mount Kenya. Control measurements were made over 2 days at 1300 m before ascent and for 2 days after arrival at 4300 m. These included body weight, 24 h urine volume, 24 h sodium and potassium excretion, blood haemoglobin, packed cell volume, and symptom score for acute mountain sickness. In 15 subjects blood samples were taken for assay of plasma aldosterone and atrial natriuretic peptide.

2. Altitude and the exercise in ascent resulted in a marked decrease in 24 h urine volume and sodium excretion. Aldosterone levels were elevated on the first day and atrial natriuretic peptide levels were higher on both altitude days compared with control.

3. Acute mountain sickness symptom scores showed a significant negative correlation with 24 h urinary sodium excretion on the first altitude day. Aldosterone levels tended to be lowest in subjects with low symptom scores and higher sodium excretion. No correlation was found between changes in haemoglobin concentration, packed cell volume, 24 h urine volume or body weight and acute mountain sickness symptom score.

4. Atrial natriuretic peptide levels at low altitude showed a significant inverse correlation with acute mountain sickness symptom scores on ascent.

Key words: fluid balance, hypoxia, sodium excretion.

Abbreviations: AMS, acute mountain sickness; ANP, atrial natriuretic peptide; PCV, packed cell volume.

INTRODUCTION

Altitude hypoxia has important effects on fluid balance [1] and there seems to be a crucial difference in response between subjects who go on to get acute mountain sickness (AMS) and those who remain free of symptoms. It is thought that AMS victims have an antidiuresis on ascent to altitude [1, 2], whereas the physiological response to acute hypoxia is one of diuresis [3]. However, human data are sparse and conflicting.

In the usual situation of mountain travel, ascent is accompanied by exercise, which has its own effect on fluid balance [4]. Of the hormones that influence fluid and sodium balance there has been considerable work on the effect of altitude on the renin-aldosterone system with and without exercise [5], but little in relation to AMS. Antidiuretic hormone release does not seem to be influenced by altitude [6, 7].

There has been no work reported as yet on altitude, AMS and atrial natriuretic peptide (ANP). ANP is released into the circulation after physiological manoeuvres which distend the cardiac atrium. Changes in plasma ANP within the physiological range result in a natriuresis [8–10]. ANP clearly plays an important part in the control of blood volume and is therefore likely to be of importance in the fluid balance changes of both the physiological and pathological responses to hypoxia (i.e. AMS).

The Royal Naval and Royal Marines Mountaineering Association Expedition to East Africa afforded an opportunity to study the effect of a rapid ascent to altitude (4300 m) on plasma ANP, plasma aldosterone and urinary excretion of sodium in a large number of subjects, some of whom would be expected to get AMS.

MATERIALS AND METHODS

Subjects

There were 22 expedition members who all acted as subjects for the study. Hormone measurements were limited to 15 subjects for technical reasons. Biographical details of the subjects are shown in Table 1 and all gave informed consent to the study.

Protocol

The altitude/time profile of the expedition is shown in Fig. 1, which shows the protocol for blood sampling and...
Table 1. Biographical details of subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>20M, 2F</td>
<td>34</td>
<td>21–56</td>
</tr>
<tr>
<td>Hormone</td>
<td>15</td>
<td>15M</td>
<td>36</td>
<td>21–56</td>
</tr>
<tr>
<td>studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Altitude/time profile of the expedition showing the timing of blood sampling (•) and 24 h urine collections (□).

Diet during control days was mainly from army catering. The sodium content, as reflected by the urinary excretion of 169 (sd 44) mmol, would seem to have been normal. The diet on the mountain was standard British Armed Forces composite rations from which subjects selected what they wished to eat. During the first 2 days at altitude this was invariably much less than the ration provided, the sodium content of which was 250 mmol. Subjects drank ad libitum; water bottles were used on the walk up to the base camp.

Control measurements included 24 h urine collection for 2 consecutive days (07.00 hours to 07.00 hours) and blood sampling at 04.00 hours and 09.00 hours for haemoglobin, packed cell volume (PCV), aldosterone and ANP estimation. The 04.00 hours sample was taken with subjects supine; the 09.00 hours sample was taken after subjects had been up and about for 1–2 h and was taken with subjects seated. Body weight was measured on rising in the morning (after emptying the bladder) using an electronic balance, which weighed to 0.1 kg.

Assays

Twenty-four hour urine collections were started at 07.00 hours on the morning of ascent from 3100 m and continued for the next 3 days. Twenty-four hour urine volume was measured, aliquots were taken, preserved in dry ice in a cryostat and sodium and potassium were analysed by flame photometry after return to London. Blood sampling was carried out at the same times and under the same conditions as at low altitude on the first and second mornings at base camp (4300 m).

PCV was measured in duplicate using a Compur M 1100 battery operated microcentrifuge. Haemoglobin estimation was by the cyanmethaemoglobin method using a battery operated Compur miniphotometer. Blood for ANP estimation was collected into chilled tubes containing ethylenediaminetetra-acetate and 0.2 ml of aprotinin, and for determination of aldosterone into heparinized tubes. Blood was separated within 15–20 min and plasma was stored in the cryostat with dry ice for transport back to the U.K. for analysis.

Aldosterone was estimated by radioimmunoassay using a commercial kit ['Coat-a-Count' TKAL2, Diagnostic Products, (UK) Ltd, Wallingford, Oxon., U.K.]. ANP was estimated by an established radioimmunoassay [10] modified by the use of 50 μl of sheep anti-ANP antibody. Preliminary extraction was on a Sep-Pak C18 cartridge using 60% acetonitrile. The 95% confidence detection limit of the assay was 2.5 fmol per tube (2.5 pmol/l of plasma). A number of samples had values below this and in the statistical analysis were given a value of 2 pmol/l.

AMS scoring

AMS symptoms were scored daily by each subject using a proforma, with symptoms assessed on a 0–3 scale, 0 representing no symptoms at all, 1, mild, 2, moderate and 3, severe symptoms. The symptoms scored were: headache, anorexia, nausea/vomiting, quality of sleep. Subjects were instructed to complete the proforma each morning relating to the previous 24 h. Compliance was checked by one of two observers. Symptom scoring was started on the morning of the day at 3100 m (when there were virtually no symptoms) and continued for 5 days at base camp (4300 m) by which time all AMS symptoms had resolved. The scores quoted in the Results section are the total for all 6 days for individual subjects.

Statistics

The effects of ascent to altitude were compared with control values by use of Student’s paired t-test. Correlations with AMS symptoms scores were assessed using Kendall’s rank test. P<0.05 was considered significant.
RESULTS

Effects of altitude on the whole group

Table 2 shows the effect of ascent to 4300 m (plus exercise) on the 24 h urine volume, 24 h sodium and potassium excretion, haemoglobin, PCV and body weight. The urine volume on the first 2 days at high altitude was reduced by 42% and the sodium excretion by 44% of low altitude values. Potassium excretion was reduced by 20% of control values. There was a reduction in body weight by a mean of 1 kg. Haemoglobin and PCV tended to rise on the first day at altitude and to fall back towards control levels by the second day. This change was significant for PCV on the first day.

Hormone assay results

The results of aldosterone and ANP measurement in 15 subjects are shown in Table 3. During the control period at low altitude there was a significantly higher value for both hormones at 09.00 hours than at 04.00 hours (P<0.0001, aldosterone; P<0.02, ANP). At altitude aldosterone levels were also higher at 09.00 hours than at 04.00 hours (P<0.02), but for ANP there was no difference.

On the first day at altitude aldosterone concentration rose, but fell back to control levels by the second day. ANP concentration also rose on going to altitude, although this rise was only significant for the 04.00 hours sample on the second day (P<0.025). On the first day the mean level was higher but the scatter was greater due mainly to one very high result (45 pmol/l). If this subject is excluded the rise in ANP for the remaining 14 subjects at 04.00 hours on the first altitude day is significant (P<0.02, paired t-test). There was a significant inverse correlation between aldosterone concentration and 24 h sodium excretion (r=0.50). There was no significant

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>AMS symptom score</th>
<th>Aldosterone (mmol/l)</th>
<th>ANP (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of day (hours)…</td>
<td>Low altitude</td>
<td>High altitude</td>
<td>Low altitude</td>
</tr>
<tr>
<td></td>
<td>04.00</td>
<td>09.00</td>
<td>04.00</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>37</td>
<td>119</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>35</td>
<td>105</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>77</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>38</td>
<td>68</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>76</td>
<td>204</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>80</td>
<td>238</td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>94</td>
<td>114</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>104</td>
<td>166</td>
</tr>
<tr>
<td>9</td>
<td>23</td>
<td>32</td>
<td>91</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>113</td>
<td>272</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>102</td>
<td>153</td>
</tr>
<tr>
<td>12</td>
<td>15</td>
<td>116</td>
<td>228</td>
</tr>
<tr>
<td>13</td>
<td>11</td>
<td>94</td>
<td>183</td>
</tr>
<tr>
<td>14</td>
<td>7</td>
<td>38</td>
<td>110</td>
</tr>
<tr>
<td>15</td>
<td>11</td>
<td>38</td>
<td>141</td>
</tr>
<tr>
<td>Mean</td>
<td>75</td>
<td>151*</td>
<td>130†</td>
</tr>
</tbody>
</table>

Table 2. Twenty-four hour urine volume, 24 h urinary sodium and potassium excretion, haemoglobin, PCV and body weight in 22 subjects

The control values are the mean of 2 days measurements. Haemoglobin and PCV were measured in samples taken at 09.00 hours. Results are mean (sd). Statistical significance (paired t-test): *P<0.002 compared with control.

Table 3. AMS symptom score, plasma aldosterone concentration and plasma ANP concentration

Samples below the detection limit of the ANP assay (2.5 pmol/l) are shown as 2.0 pmol/l. Low altitude values are the mean of samples on 2 control days. Statistical significance (paired t-test): *P<0.05 compared with 04.00 hours; †P<0.05 compared with low altitude.
correlation between aldosterone and ANP concentrations.

Results in relation to AMS symptoms

AMS symptom scores showed no significant correlation with body weight, haemoglobin concentration, PCV or 24 h urine volume, but there was a significant inverse correlation between 24 h sodium excretion on the first day at altitude and AMS scores (\(P<0.02\), Kendal's rank test)(Fig. 2).

Table 3 shows the AMS symptom scores for the 15 subjects in whom hormone measurements were made. The correlation coefficient (\(r\)) between aldosterone concentration and AMS symptom scores was 0.54 and 0.72 for 04.00 hour samples on day 1 (Fig. 3) and day 2 at high altitude, respectively. However, these were not significant (Kendal's rank test).

For ANP concentration the most significant correlation with AMS symptom scores was with the 04.00 hours samples at low altitude. Fig. 4 shows the mean of the 2 days at low altitude plotted against AMS scores. An inverse correlation is seen (\(r = -0.58\), \(P<0.03\), Kendal's rank test). At altitude the correlation coefficients for the relationship were \(r = -0.51\) and \(r = -0.52\) on the first and second days' 04.00 hours samples, respectively, but these were not significant.

DISCUSSION

In interpreting the results of this study the two stimuli of exercise and altitude hypoxia must be kept in mind, since the subjects were unacclimatized to both forms of stress. Exercise (of some hours duration) and chronic hypoxia have opposite effects on haemoglobin concentration and PCV. Exercise causes a decrease in PCV \([4]\) while hypoxia causes a rise in PCV, in both cases the changes over a few days being due to changes in plasma volume. The net result when both stimuli are experienced will depend upon the degree of each stimulus. In this study there was a small mean increase in both haemoglobin concentration and PCV, suggesting that hypoxia was the more important stimulus for control of plasma volume.

In the case of urine volume and aldosterone concentration the effect of exercise appeared to override the effect

Fig. 2. AMS symptom score totalled for the 6 days at altitude starting on the day of ascent from 3100 to 4300 m (day 0) plotted against the 24 h urinary sodium excretion for this day, ending at 07.00 hours on the first morning at 4300 m. \(r = -0.5\) (\(P=0.02\) by Kendal's rank test).

Fig. 3. AMS symptom score totalled for 6 days at altitude starting on the day of ascent from 3100 to 4300 m (day 0) plotted against plasma aldosterone concentration in the 04.00 hours samples at high altitude (4300 m) on the morning after arrival. \(r = 0.54\).

Fig. 4. AMS symptom score totalled for 6 days at altitude starting on the day of ascent from 3100 to 4300 m (day 0) plotted against plasma ANP concentration for the mean of two 04.00 hours samples at low altitude (1300 m). \(r = -0.58\) (\(P=0.03\) by Kendal's rank test).
of hypoxia in that there was a marked reduction in 24 h urine volume, in 24 h sodium excretion and a rise in aldosterone concentration, all typical of this type of exercise at low altitude [11]. Altitude hypoxia without exercise results in a fall in aldosterone secretion rates [12] and plasma concentration [13].

We chose to sample blood for ANP at 04.00 hours because of the report that ANP concentration peaked at that time [14], although a later study [15] showed no such peak. We found that levels were higher at 09.00 hours than at 04.00 hours at low altitude but there was no difference at high altitude. Although it is well known that ANP levels rise on lying down, they probably fall during the night [15] when no food or fluid is ingested and fluid is lost into the urine and by insensible means. The 09.00 hours sample was taken after morning tea and breakfast which would have corrected the mild night-time dehydration. The 04.00 hours sample had the advantage that subjects were in a basal state, rested and fasted. As a result there was less scatter in all measurements from this blood sample.

ANP studies at altitude in man have not previously been reported. Baertschi et al. [16] have shown that profound hypoxia causes release of ANP from isolated heart muscle strips, and Winter et al. [17] showed a rise in ANP plasma concentration in rats made chronically hypoxic in a chamber. The finding in the present study of an increase in ANP on going to altitude may either be due directly to hypoxia or to an increase in right atrial pressure secondary to hypoxic vasoconstriction of the pulmonary vasculature. Exercise causes a rise in ANP levels [18], but after short-term exercise concentrations fall to pre-exercise values in an hour. The effect of longer-term exercise may be of greater duration and might affect levels even some 12 h later; such studies have not been reported.

The main aim of this study was to correlate the various hormone changes with symptom scores for AMS. It has been reported that subjects who develop AMS have an anti-diuresis in contrast to those free of symptoms who have a diuresis on ascent to altitude [2]. Those who have gained weight (assumed to be due to fluid retention) have higher AMS scores than those who have lost weight on arrival at altitude [19]. We were not able to confirm these findings in that we failed to find significant correlations between 24 h urine volume or weight changes and AMS symptom scores. However, we did find a significant inverse correlation between 24 h sodium excretion on the day of ascent and AMS scores.

It is likely that the reduced sodium excretion seen in this study was due to exercise which induces activation of the renin-aldosterone system. This has been found previously in subjects on a controlled diet undertaking this form of exercise at both low altitude [11] and on ascent to high altitude [5]. The rise in ANP with altitude (or exercise) would have the opposite effect on sodium excretion, but under the circumstances of this study would appear to have less influence. There was some evidence of a correlation between aldosterone levels at altitude and AMS scores. There was rather better evidence for an inverse correlation between ANP concentration and AMS scores. Differences in levels of both these hormones between subjects with and without AMS could contribute to the differences in urinary sodium excretion.

The most unexpected finding was the negative correlation between the ANP levels (04.00 hours sample) before ascent to high altitude and subsequent AMS scores. If this finding is confirmed it would suggest that susceptibility to AMS depends upon a subject's normal control value for ANP. It has been shown that ANP not only has the opposite effect on sodium handling by the kidney as does aldosterone, but also inhibits aldosterone excretion from the adrenal gland in response to angiotensin II [20]. A rise in ANP on going to altitude therefore may account for the hitherto unexplained reduction in aldosterone concentration on exposure (without exercise) to altitude and the blunting by hypoxia of the aldosterone response to angiotensin II induced by exercise [21].

Resistance to AMS may occur as a result of the following events. On going to altitude ANP levels rise due to hypoxia and exercise (if undertaken), there is inhibition of aldosterone excretion and of sodium reabsorption by the kidneys. This results in a sodium-lead diuresis (or if exercise is taken, at least less sodium retention). This loss of sodium reduces any tendency to oedema formation in the brain or lungs consequent upon other mechanisms initiated by hypoxia.

REFERENCES