Plasma ionized magnesium during acute hyperventilation in humans

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(Received 10 January/30 April 1996; accepted 24 May 1996)

INTRODUCTION

Acute overbreathing has been associated with, among other things, hypoxaemia, pulmonary disease, drugs, pain, infections and anxiety [1]. The clinical syndrome of acute overbreathing tetany includes abnormal sensations of the extremities and circumpalmar areas, tonic rigidity in the muscles of the hands and feet, sensations of lightheadedness and altered consciousness [1]. In addition, respiratory alkalosis, the acid–base disorder that results from overbreathing, precipitates dangerous cardiac arrhythmias and predisposes to coronary vasoconstriction [2, 3]. The disturbances linked with acute respiratory alkalosis have traditionally been attributed to hypocalcaemia [1, 4, 5] and hypokalaemia [1, 6, 7]. However, mild to moderate acute respiratory alkalosis results in a mild increase in the circulating potassium concentration [8] but with inconsistent change in ionized calcium [9, 10]. Magnesium plays a critical role in the maintenance of normal membrane function, and magnesium depletion is often associated with cardiac arrhythmias or vasoconstriction [11–13]. Data on circulating magnesium in acute hyperventilation are scanty. Circulating magnesium exists in the ionized state and in the undissociated form, either bound to proteins, primarily albumin, or complexed to anions such as bicarbonate, citrate and phosphate [11–13]. Technology for detecting circulating ionized magnesium, the most interesting form with respect to physiological and biological properties, is now available in the form of new magnesium-selective electrodes [14–16]. The opportunity has therefore been seized to investigate the effect of acute hyperventilation on circulating magnesium.

METHODS

Experimental design

Eight healthy men volunteered to participate in the study. The median age of the group was 26 (range 23–46) years, median body weight 75.2 (62.4–83.8) kg and median height 1.825 (1.652–1.875) m. The subjects attended the laboratory after an overnight fast, ate a standard breakfast (caloric content 2950 kJ, sodium 19 mmol, potassium 10 mmol, phosphate 8 mmol, calcium 7 mmol and magnesium 1.8 mmol) and remained seated throughout the experiment. After placing a cannula into a cubital vein, the subjects rested for 30 min. Hyperventilation was induced, synchronizing the respiratory rate at 20/min with a metronome and instructing the subjects to breathe as deeply as possible over a period of
Blood pressure and heart rate were measured, and blood samples drawn anaerobically without stasis into heparinized (heparin 10 units/ml) tubes before starting hyperventilation ('before hyperventilation'), before concluding the hyperventilation assay ('during hyperventilation') and 30 min after hyperventilation ('after hyperventilation'). Haematocrit, pH, carbon dioxide pressure, ionized calcium and magnesium, and sodium, potassium and chloride were measured immediately (less than 5 min) after blood collection.

The remaining samples were stored for determination of total calcium and magnesium, inorganic phosphorus, protein and albumin.

The study had been approved by the local ethics committee and informed written consent was obtained.

**Analytical procedures**

All measurements were performed in triplicate. Plasma ionized magnesium was determined at the prevailing pH with a magnesium-specific ion electrode apparatus (AVL 988-4/Mg Analyzer) containing the neutral carrier-based membrane ETH 7025. This apparatus has recently been characterized [14-16].

Ion-selective electrodes were used for the measurement of plasma ionized calcium, potassium, sodium and chloride, blood pH and carbon dioxide pressure. Plasma bicarbonate concentration was calculated using the Henderson-Hasselbalch equation. Total protein (biuret method), albumin (Bromcresol Purple method), inorganic phosphorus (ammonium molybdate method), total calcium (creolphthalein complexone method) and magnesium (Xylice Blue I method) were measured colorimetrically on an autoanalyzer. Haematocrit was assessed by means of a microhaematocrit centrifuge.

The circulating free magnesium or calcium fraction was calculated by dividing the ionized by the corresponding total concentration.

Under conditions of a constant erythrocyte volume, an increase in haematocrit is associated with a decrease in plasma volume. It has been shown on the basis of mathematical and experimental considerations that proportionally the change in haematocrit is not numerically equal to a change in plasma volume [18, 19]. Hence, the relative plasma volume during hyperventilation was determined from haematocrit measured before (HK_b) and during (HK_d) hyperventilation using the equation [18]:

\[
\frac{C_d}{C_b} = \frac{1}{1 - HK_b} \times \left(1 + \frac{1}{HK_b - HK_d}\right)
\]

The relative intravascular magnesium or calcium mass during hyperventilation was subsequently calculated from the total plasma concentration before (C_b) and during (C_d) hyperventilation as well as from the relative plasma volume during hyperventilation using the equation [17]:

\[
\frac{C_d}{C_b} \times \frac{1}{1 - HK_b} \times \left(1 + \frac{1}{HK_b - HK_d}\right)
\]

All values are expressed as median and ranges and sometimes as individual values. Statistical evaluations were made by means of the Friedman test (non-parametric analysis of variance for repeated measurements), using the Bonferroni adjustment. A P-value of less than 0.05 was accepted to indicate statistical significance.

**RESULTS**

The results of the study are shown in Table 1. Voluntary hyperventilation was associated with an increase in heart rate of 13 (2-41) beats/min and with tingling and numbness in the hands (n=8) and feet (n=4). Five volunteers had carpopedal spasm and two sensations of lightheadedness. Blood pressure was not modified by voluntary overbreathing.

Voluntary overbreathing resulted in a decrease in carbon dioxide pressure of 12.9 (9.1-24.0) mmHg, a decrease in plasma bicarbonate concentration of 1.5 (0.1-4.6) mmol/l and an increase in blood pH of 0.13 (0.08-0.26). Plasma sodium and chloride were not modified by voluntary overbreathing. Overbreathing was associated with an increase in the plasma potassium concentration of 0.56 (0.19-0.41) mmol/l. Upon cessation of hyperventilation, plasma potassium concentration fell by 0.21 (0.21-0.45) mmol/l. Plasma ionized calcium concentration was not influenced by hyperventilation. On the contrary, the total plasma calcium concentration increased significantly. Overbreathing was associated with a persistent tendency towards hypophosphataemia, which decreased by 0.42 (0.29-0.55) mmol/l.

The total plasma protein and albumin concentration increased. The haematocrit increased by 0.02 (0.01-0.03), indicating a relative plasma volume of 0.92 (0.89-0.96).

The effects of voluntary overbreathing on circulating magnesium are shown in both Table 1 and Fig. 1. The total plasma magnesium concentration was not modified during and after hyperventilation. On the contrary, hyperventilation was associated with a reduction in the ionized magnesium concentration of 0.05 (002-0.15) mmol/l and in the free magnesium fraction of 0.06 (0.01-0.19). The tendency towards ionized hypomagnesaemia and low free magnesium fraction tended to persist 30 min after hyperventilation.

The effects of hyperventilation on the relative circulating mass of calcium and magnesium are shown in Fig. 2. During hyperventilation the rela-
Table 1. Clinical and laboratory findings in eight healthy subjects before, during and after voluntary overbreathing. Results are given as median and ranges. Statistical significance: *P < 0.05 and **P < 0.01 compared with before and after hyperventilation; †P < 0.05 compared with before ventilation; §P < 0.05 compared with before and during hyperventilation. §The factor by which values in mmHg should be multiplied in order to convert them into kPa is 0.1333.

<table>
<thead>
<tr>
<th></th>
<th>Before hyperventilation</th>
<th>During hyperventilation</th>
<th>After hyperventilation</th>
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<tbody>
<tr>
<td>Blood pH</td>
<td>7.39 (7.34–7.43)</td>
<td>7.51** (7.44–7.65)</td>
<td>7.42† (7.37–7.44)</td>
</tr>
<tr>
<td>Carbon dioxide pressure (mmHg)§</td>
<td>43.9 (35.8–52.7)</td>
<td>30.8** (19.7–41.4)</td>
<td>38.6† (35.9–44.9)</td>
</tr>
<tr>
<td>Plasma bicarbonate (mmol/l)</td>
<td>25.6 (23.0–28.9)</td>
<td>23.7* (21.0–31.3)</td>
<td>24.2 (23.4–28.3)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>75 (58–82)</td>
<td>88† (72–119)</td>
<td>76 (60–86)</td>
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<tr>
<td>Blood pressure (mmHg)</td>
<td>118 (111–130)/71 (61–80)</td>
<td>129 (110–130)/68 (60–78)</td>
<td>118 (112–128)/76 (58–80)</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>0.43 (0.40–0.44)</td>
<td>0.45* (0.41–0.46)</td>
<td>0.43 (0.39–0.45)</td>
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<tr>
<td><strong>Plasma</strong></td>
<td></td>
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<tr>
<td>Sodium (mmol/l)</td>
<td>138.5 (136.6–140.7)</td>
<td>138.8 (137.0–140.7)</td>
<td>138.4 (137.1–141.4)</td>
</tr>
<tr>
<td>Chloride (mmol/l)</td>
<td>104.8 (103.0–107.2)</td>
<td>105.0 (102.6–106.8)</td>
<td>104.5 (103.3–106.5)</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>3.93 (3.73–4.24)</td>
<td>4.12* (3.55–4.33)</td>
<td>3.81† (3.61–4.26)</td>
</tr>
<tr>
<td>Phosphate (mmol/l)</td>
<td>1.29 (0.99–1.76)</td>
<td>0.86** (0.64–1.94)</td>
<td>0.97† (0.67–1.42)</td>
</tr>
<tr>
<td>Protein (g/l)</td>
<td>73.0 (70.1–85.2)</td>
<td>77.3* (69.6–94.3)</td>
<td>75.3 (69.3–80.3)</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>42.1 (39.2–45.1)</td>
<td>43.4* (37.9–46.6)</td>
<td>42.8 (37.4–45.1)</td>
</tr>
<tr>
<td><strong>Plasma calcium</strong></td>
<td></td>
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</tr>
<tr>
<td>Total (mmol/l)</td>
<td>2.39 (2.27–2.45)</td>
<td>2.57* (2.34–2.62)</td>
<td>2.40 (2.35–2.48)</td>
</tr>
<tr>
<td>Ionized (mmol/l)</td>
<td>1.6 (1.24–1.29)</td>
<td>1.26 (1.20–1.30)</td>
<td>1.26 (1.23–1.29)</td>
</tr>
<tr>
<td>Free fraction</td>
<td>0.53 (0.51–0.55)</td>
<td>0.52 (0.47–0.55)</td>
<td>0.50 (0.47–0.54)</td>
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<tr>
<td><strong>Plasma magnesium</strong></td>
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</tr>
<tr>
<td>Total (mmol/l)</td>
<td>0.81 (0.73–0.88)</td>
<td>0.82 (0.71–0.91)</td>
<td>0.80 (0.73–0.85)</td>
</tr>
<tr>
<td>Ionized (mmol/l)</td>
<td>0.54 (0.47–0.72)</td>
<td>0.50** (0.45–0.57)</td>
<td>0.52† (0.48–0.62)</td>
</tr>
<tr>
<td>Free fraction</td>
<td>0.68 (0.63–0.82)</td>
<td>0.61** (0.59–0.66)</td>
<td>0.66 (0.61–0.76)</td>
</tr>
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</table>

Fig. 1. Influence of voluntary overbreathing at a respiratory rate of 20/min on total plasma magnesium, plasma ionized magnesium and free magnesium fraction in eight healthy volunteers. The filled symbols (left and right) denote the values measured before (left) and after (right) overbreathing; the open symbols (middle) denote the values measured during overbreathing.
Fig. 2. Relative intravascular mass of calcium (filled symbols) and magnesium (open symbols) during hyperventilation in eight healthy subjects. The relative intravascular magnesium mass was significantly decreased to 0.94 (0.89–0.99). In contrast, the relative intravascular calcium mass was unmodified at 1.00 (0.92–1.04). The horizontal line (value of 1.0) denotes the relative intravascular mass of calcium or magnesium before hyperventilation.

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DISCUSSION

The most important new findings in this study are that acute overbreathing reduces the circulating ionized magnesium concentration and the intravascular magnesium mass. Extracellular magnesium deficiency therefore represents a conceivable cause for the tetanic disturbances and the cardiac complications that are precipitated by hyperventilation [1–3]. Furthermore, the study provides a possible explanation for the effect of magnesium on anginal attacks induced by hyperventilation in patients with variant angina [20].

The traditional theory [1, 4, 5] that overbreathing accompanies an increased circulating calcium fraction bound to anionic proteins has not been supported by experimental proof [9, 10]. In the present study, voluntary overbreathing reduced circulating ionized magnesium but without any influence on total magnesium and ionized calcium concentrations. The decreased free magnesium fraction noted during overbreathing indicates that magnesium complexes to anions or binds to anionic proteins. The tendency towards a low free magnesium fraction may not result from increased complexation to anions such as phosphate and bicarbonate, whose concentration is decreased by acute hyperventilation, as shown in this and other studies [21]. Changes in the concentration of plasma proteins are associated with a stable fraction of ionized magnesium [11–13]. It is therefore speculated that an increased affinity of anionic plasma proteins for magnesium accounts for ionized hypomagnesaemia during acute hyperventilation in the present investigation. The higher affinity of anionic plasma proteins for magnesium than for calcium that we noted during hyperventilation is probably related to the fact that the ionic radius of the former, 65 pm, is smaller than that of the latter, 94 pm [11–13]. Preliminary observations in vitro support this notion [15].

In our subjects overbreathing was associated with a decreased relative intravascular magnesium mass. This parameter was calculated from the total magnesium and haematocrit concentrations before and during hyperventilation using an equation based on both mathematical and experimental considerations [17–19]. Renal magnesium wasting is unlikely to explain the decreased intravascular magnesium mass, as alkalosis strongly reduces the urinary magnesium excretion [12]. Increased perspiration of magnesium is also unlikely to be the cause because of the low sweat concentration of this ion [12]. Increased sympathoadrenergic activity, as indicated by increased heart rate and catecholamine plasma levels, is an anticipated effect of acute overbreathing [17] that stimulates cellular magnesium uptake [12, 22]. It is therefore tempting to assume that intracellular shifts of magnesium underlie the decreased intravascular magnesium mass noted during hyperventilation.

Studies on circulating magnesium during hyperventilation have hitherto been hindered by the use of total magnesium as a reflection of extracellular homeostasis of this ion. The use of ion-selective electrodes to measure free magnesium [14–16] has allowed us to demonstrate ionized hypomagnesaemia in the clinical setting of acute hyperventilation. This fact provides a possible explanation for the clinical syndrome of tetany, for some complications, such as cardiac arrhythmias and coronary vasoconstriction, and for the ability of magnesium to relieve anginal attacks in patients with variant angina [20]. However, the rather small changes in circulating ionized magnesium noted in our subjects during hyperventilation suggest that factors other than ionized hypomagnesaemia might also account for the disturbances associated with respiratory alkalosis. The use of magnesium to prevent the symptoms induced by overbreathing deserves evaluation.

REFERENCES

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Circulating magnesium during hyperventilation

351