Effect of high salt intake on sodium, potassium-dependent adenosine triphosphatase activity in the erythrocytes of normotensive men

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SUMMARY

1. We measured ouabain-insensitive adenosine triphosphatase (ATPase), sodium, potassium-dependent adenosine triphosphatase (Na⁺,K⁺-ATPase) and intracellular Na⁺ and K⁺ in the erythrocytes of 19 healthy volunteers, before and after supplementation of their normal diet with 6.0-8.9 g of salt (102-137 mmol of NaCl) per day, for 5 days.

2. The subjects had a small but significant gain in weight. Mean plasma renin activity decreased from 1.57 to 0.73 pmol of angiotensin 1 h⁻¹ ml⁻¹ and plasma aldosterone from 0.46 to 0.24 nmol/l.

3. Total ATPase activity fell from 197.9 nmol of inorganic phosphate h⁻¹ mg⁻¹ during the control period to 173.5 during the high-salt period (P<0.0125). Na⁺,K⁺-ATPase activity fell from 162.2 to 141.4 nmol of inorganic phosphate h⁻¹ mg⁻¹ (P<0.05). Intracellular Na⁺ and intracellular K⁺ did not change.

4. These results are consistent with the hypothesis that salt-induced volume expansion causes the release of a factor inhibitory to the Na⁺ pump.

Key words: erythrocyte, salt, sodium, potassium-dependent adenosine triphosphatase.

Abbreviations: ATPase, adenosine triphosphatase; BP, blood pressure; Na⁺,K⁺-ATPase, sodium, potassium-dependent adenosine triphosphatase; Pᵢ, inorganic phosphate; PRA, plasma renin activity.

INTRODUCTION

It has been proposed that extra cellular fluid volume expansion may result in formation of a substance which inhibits the Na⁺,K⁺-pump [1–5]; it is further suggested that this inhibition of the Na⁺-pump results in vasoconstriction of arteriolar smooth muscle and so leads to systemic hypertension [1–3,6]. These concepts, if proven, may help to explain the relationship between salt, extracellular volume and blood pressure (BP). In support of these ideas, several laboratories have described inhibition of sodium, potassium-dependent adenosine triphosphatase (Na⁺,K⁺-ATPase) in various forms of experimental hypertension characterized by volume expansion [1–6]. Unfortunately, most of those models employed a variety of manoeuvres in addition to salt intake and it is not clear that the observed inhibition of Na⁺,K⁺-ATPase was the result of volume expansion alone.

Information on the effect of salt intake on the activity of the Na⁺,K⁺-pump in humans is very limited and often contradictory. This uncertainty led us to examine the effect of a moderately high salt intake, without other manipulations, on the ouabain-sensitive Na⁺,K⁺-ATPase in erythrocytes of a homogeneous population of normal young men. The question is important because it may shed light on the relationship between salt and BP, since inhibition of the Na⁺,K⁺-pump has been linked to increased peripheral resistance [1–9].

METHODS

Nineteen volunteers participated in this institutionally approved study. All were white males, 23–38 years old (mean age 27.1 years), normotensive and free of known disease. Four of the subjects had a family history of essential hypertension. The subjects were studied twice, before (control) and after (experimental) 5 days on increased salt intake. Salt intake during the control period was estimated by measuring 24 h urinary Na⁺ excretion in nine subjects. Sodium excretion was measured on the last day of the high-salt period in all subjects. During the experimental period each subject took their customary
diet supplemented with 6.0–8.0 g of NaCl in tablets (102–137 mmol) daily for 5 days. The studies were conducted just before and at the end of the high-salt period. Body weight, pulse rate and BP were measured. BP was always measured between 9.00 and 10.00 hours with a mercury sphygmomanometer by the same physicians. The average of three measurements, after 15 min supine and after 2 min standing, was recorded. Mean BP was calculated as diastolic BP plus one-third of the pulse pressure.

Techniques

Twenty millilitres of blood were obtained in heparinized tubes, placed on ice, and the erythrocytes separated by centrifugation at 6750 g. Plasma Na⁺ and K⁺ were measured by flame photometry. Total and ouabain-sensitive (Na⁺,K⁺) adenosine triphosphatase (ATPase) activity was measured in duplicate in the erythrocytes by the method of Cole et al. [10], slightly modified by us and described in detail elsewhere [11]. The activity of the enzyme was expressed as nmol of inorganic phosphate (Pi) released per hour of incubation per mg of membrane protein. To test the reproducibility of the method, ATPase activity was measured in a different group of nine subjects, on 2 different days, and the results were compared. Plasma renin activity (PRA) and plasma aldosterone were measured by standard techniques. Intracellular Na⁺ and K⁺ contents were measured in duplicate by a method previously described [11].

Table 1. Variables sensitive to salt intake during normal and experimental diet

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>High-salt diet</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>81.1 ± 8.3</td>
<td>81.7 ± 8.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plasma K⁺ (mmol/l)</td>
<td>4.37 ± 0.35</td>
<td>4.20 ± 0.21</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Supine BP (mmHg) (kPa)</td>
<td>90.1 ± 5.7</td>
<td>90.7 ± 7.0</td>
<td>NS</td>
</tr>
<tr>
<td>Standing BP (mmHg) (kPa)</td>
<td>92.1 ± 7.0</td>
<td>94.1 ± 8.1</td>
<td>NS</td>
</tr>
<tr>
<td>PRA (pmol of ANG I h⁻¹ ml⁻¹)</td>
<td>1.57 ± 0.76</td>
<td>0.73 ± 0.54</td>
<td>0.0001</td>
</tr>
<tr>
<td>Aldosterone (nmol/l)</td>
<td>0.46 ± 0.20</td>
<td>0.24 ± 0.08</td>
<td>0.0025</td>
</tr>
</tbody>
</table>

Table 2. Effect of high-salt intake on erythrocyte ATPase activity and intracellular Na⁺

<table>
<thead>
<tr>
<th>ATPase activity</th>
<th>Control</th>
<th>High-salt diet</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ATPase activity (nmol of P, h⁻¹ mg⁻¹ of membrane protein)</td>
<td>197.7 ± 52.4</td>
<td>173.5 ± 42.7</td>
<td>&lt;0.0125</td>
</tr>
<tr>
<td>Ouabain-insensitive ATPase activity (nmol of P, h⁻¹ mg⁻¹ of membrane protein)</td>
<td>34.7 ± 17.4</td>
<td>32.1 ± 17.0</td>
<td>NS</td>
</tr>
<tr>
<td>Na⁺,K⁺-ATPase activity (nmol of P, h⁻¹ mg⁻¹ of membrane protein)</td>
<td>162.2 ± 45.2</td>
<td>141.3 ± 29.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Intracellular Na⁺ (mmol/litre of cells)</td>
<td>7.61 ± 1.2</td>
<td>7.81 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Intracellular K⁺ (mmol/litre of cells)</td>
<td>89.7 ± 9.8</td>
<td>88.6 ± 7.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Corrected with the Bonferroni correction for five tests.

Statistical analysis

All results are means ± sd. Student's t-test for paired data was used to assess the differences. The P values were corrected for multiple comparisons of the data by the Bonferroni correction.

RESULTS

Mean Na⁺ intake during the control period was estimated by measuring Na⁺ excretion, in nine subjects, as an average of 147 mmol/day. Urinary Na⁺ excretion during the last 24 h of the high-salt experimental period, measured in 18 subjects, was 254 ± 72 mmol/24 h (mean ± sd). Table 1 presents data on variables likely to be affected by salt intake. Body weight rose slightly in most subjects and the difference for the group was significant. Mean supine and standing BP did not change. Mean PRA and plasma aldosterone fell significantly. Plasma K⁺ concentration also fell slightly but significantly.

Table 2 presents data on erythrocyte ATPase activity and cation content. Total ATPase and Na⁺,K⁺-ATPase activities fell significantly, whereas ouabain-insensitive ATPase activity did not change. Intracellular Na⁺ rose and intracellular K⁺ fell, but neither change was significant.

In the group of nine normal subjects, whose erythrocyte ATPase activity was measured twice, on different days, mean total ATPase activity was 246 ± 68 and 234 ± 73 nmol of P, h⁻¹ mg⁻¹ (P=NS), and Na⁺,K⁺-
Salt intake and adenosine triphosphatase activity

ATPase activity was 183 ± 71 and 185 ± 63 nmol of P, h⁻¹ mg⁻¹ (P = NS).

DISCUSSION

Since the early experiments of De Wardener, many studies have attempted to demonstrate the existence of humoral substances induced by volume expansion. Haddy and co-workers [3, 6], based on their own observations and on studies culled from the literature, suggested that volume expansion may induce the release of a factor which inhibits the Na⁺,K⁺-pump in all muscular components of the vascular system [6, 7]. This hypothesis is supported by reports from the laboratories of De Wardener & MacGregor [1, 8], and Blaustein and co-workers [2, 9], Gonick et al. [4], Gruber et al. [5], and others, in a variety of animal preparations. However, not all agree, and De Mendonca et al. [12] could not detect an effect of high salt intake on ouabain-sensitive Na⁺ efflux in rat erythrocytes. Observations in humans also suggest that volume expansion may inhibit the Na⁺,K⁺-pump. Poston et al. [13] showed inhibition of Na⁺ extrusion in normal leucocytes by a chromatographic fraction of urine from normal subjects undergoing saline expansion, and inhibition of ouabain-sensitive Na⁺ transport in leucocytes of normal subjects in mineralocorticoid escape [13]. Other investigators have described ion transport inhibitory properties in the urine of saline-expanded subjects [14–16], and in the plasma of salt-loaded subjects [17], Weissberg et al. [18], however, was not able to show a clear effect of plasma from salt-fed subjects on either Na⁺ transport or ouabain binding, and at least another investigator [19] found increased ²²Na efflux with increased salt intake. Unfortunately, in most experimental models hypertension induced by extracellular fluid volume expansion, other manoeuvres, such as partial nephrectomy, administration of deoxycorticosterone acetate and kidney wrapping, with or without salt administration, were performed, and it is not clear whether the reported observations were the result of volume expansion alone. Differences in age, race, sex or drug therapy, as well as differences in techniques, may explain these inconsistencies.

In contrast, a heightened salt intake was the sole experimental manoeuvre in our study, the activity of the enzyme was measured directly in the erythrocyte membrane, and we studied a homogeneous population of healthy, young, white, normotensive men.

Body weight, PRA and plasma aldosterone were measured as indicators of compliance with the ingestion of salt. The significant fall in PRA and plasma aldosterone, the small but significant increase in body weight, and the increased Na⁺ excretion, reflect the higher salt intake. The small decline in plasma K⁺ is also probably due to high salt intake. After 5 days on a high-salt diet there was a significant reduction of total and ouabain-sensitive Na⁺,K⁺-ATPase activity in the erythrocyte membrane, whereas ouabain-insensitive ATPase activity remained unchanged.

To test the reproducibility of the method we measured total ATPase and Na⁺,K⁺-ATPase activities twice, on two different days, in a separate group of subjects on normal salt intake and found no significant difference between the measurements.

Our finding is consistent with the hypothesis that volume expansion somehow results in inhibition of the Na⁺ pump. It is also consistent with the finding by Weissberg et al. [20] of diminished ouabain-sensitive ²²Na efflux rate by salt ingestion, and with the results of Boero et al. [21] after acute saline infusion.

It is likely, although unproved, that inhibition of the Na⁺ pump in the erythrocyte may reflect a similar inhibition in other tissues such as cardiac muscle and arteriolar smooth muscle. It is well known that under certain circumstances salt intake may increase BP, but the mechanism of this effect remains elusive. In our short-term study, BP did not rise. Whether prolonged salt intake and suppression of the Na⁺ pump for a longer period would have resulted in a higher BP in these normotensive subjects can not be determined from this study.

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REFERENCES


