SHORT COMMUNICATION

Intra-erythrocyte sodium and (Na\(^+\),K\(^+\)-activated)-ATPase concentration and urinary aldosterone excretion in spontaneously hypertensive rats

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Summary

1. Intra-erythrocyte sodium, potassium, ATP and (Na\(^+\),K\(^+\)-activated)-ATPase concentrations and urinary aldosterone excretion were compared in 3-month-old spontaneously hypertensive rats (n = 11) and normotensive Wistar-Kyoto control rats (n = 11).

2. Spontaneously hypertensive rats exhibited significantly higher intra-erythrocyte sodium concentration (5.5 ± 1.3 vs 4.0 ± 1.1 mmol/l of erythrocytes, P < 0.01). No significant difference was found in intra-erythrocyte potassium, ATP or (Na\(^+\),K\(^+\)-activated)-ATPase concentration.

3. Mean urinary aldosterone excretion was significantly lower in spontaneously hypertensive rats (66.3 ± 6.5 pmol/24 h) than in Wistar-Kyoto rats (90.5 ± 10.6 pmol/24 h, P < 0.01). No significant relationship between urinary aldosterone and intra-erythrocyte sodium concentration was found in spontaneously hypertensive or Wistar-Kyoto rats or in the pooled group.

4. These results are thus consistent with previous findings of an increased intracellular sodium concentration in spontaneously hypertensive rats, but do not support the hypothesis that aldosterone is a dominant regulator of intracellular sodium concentration.

Key words: (Na\(^+\),K\(^+\)-activated)-ATPase, intra-erythrocyte sodium, spontaneously hypertensive rat, urinary aldosterone.

Introduction

Increased intracellular concentration of sodium and/or an increased erythrocyte and leucocyte permeability to sodium has been found in hypertensive man (Wessels, Junge-Hültsing & Losse, 1967; Edmondson, Thomas, Hilton, Patrick & Jones, 1975; Postnov, Orlov, Schvchenko & Adler, 1977; Garay & Meyer, 1979) and in both the Hebrew and Okamoto-Aoki types of spontaneously hypertensive rats (Ben-Ishay, Aviram & Viskoper, 1975; Postnov, Orlov, Gulak & Schvchenko, 1976; Yamori, Nara, Horie & Ohtaka, 1977). It has further been suggested (Friedman, Nakashima, McIndoe & Friedman, 1976; Friedman & Friedman, 1976) that intracellular sodium may play a central role in protein synthesis necessary for the structural hypertrophic changes of blood vessels characteristic of the hypertensive state.

An altered membrane permeability to sodium may thus be associated with an increased vascular reactivity and hence influence peripheral vascular resistance. Mineralocorticoids have been shown to increase blood pressure in man through an increase in peripheral resistance (Abboud, 1974; Distler & Philipp, 1979). Furthermore the increased sodium concentration in erythrocytes of spontaneously hypertensive rats has been reported to disappear after adrenalectomy, which indicates that mineralocorticoids might play a role even in enucleated cells (Postnov et al., 1976). We therefore wanted to reinvestigate intracellular sodium and (Na\(^+\),K\(^+\)-activated)-ATPase concentrations in spontaneously hypertensive rats and relate these findings to the excretion of aldosterone.

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Materials and methods

Animals

Experiments were performed on 3-month-old male spontaneously hypertensive rats of the Wistar strain and age-matched male normotensive Wistar–Kyoto control rats. Eleven animals of each strain were used. Body weight was the same (approx. 300 g) in both groups, and mean arterial blood pressure was higher in spontaneously hypertensive rats (135 ± 2 mmHg) than in Wistar–Kyoto rats (109 ± 2 mmHg). The rats were kept individually in metabolic cages for 3 weeks before the urine collection for aldosterone determination. The cages were kept at room temperature and a humidity of 63%. Standard pellet R3 (Anticimex) diet containing 7.8 mmol of sodium/100 g and tap water containing less than 0.5 mmol of sodium/l were given ad libitum.

Determinations

ATPase. Ouabain sensitive and non-sensitive ATPase was analysed by a modification of the method of Whaun & Oski (1969). Inorganic phosphate was determined in the supernatant as described by Fiske & Subbarow (1925). Protein concentration of the membrane suspension was analysed by the method of Lowry, Rosebrough, Farr & Randall (1951). The ATPase activities are expressed as nmol of inorganic phosphate generated h⁻¹ mg⁻¹ of protein.

ATP. Determinations were performed as a microassay by using an analysis kit from Boehringer-Mannheim (West Germany, Cat. no. 15979) on EDTA-treated blood; concentrations are expressed as mmol/l of erythrocytes.

Sodium and potassium. Concentrations in erythrocytes were determined as described by Bengtsson, Gennser & Nilsson (1970). No correction was made for trapped plasma or water.

Aldosterone. Excretion in the urine was measured with a radioimmunoassay as described by McKenzie & Clement (1974). The urine was hydrolysed with hydrochloric acid to pH 1 before radioimmunoassay. Cross-reactions with other steroids have been shown to be less than 0.015% for all naturally occurring steroids (Brown, Swander & McKenzie, 1976).

Results

Determinations

Intra-erythrocyte. The results of parallel analyses of erythrocytes in spontaneously hypertensive and Wistar–Kyoto rats are shown in Table 1. The total ATPase, (Na⁺,K⁺-activated)-ATPase and ATP levels were similar in both groups. The sodium concentration was, however, significantly higher (P < 0.01) in spontaneously hypertensive rats whereas the potassium concentration was similar in both groups.

Urinary aldosterone and its intra-erythrocyte relationships. Urinary aldosterone excretion was significantly lower in spontaneously hypertensive rats than in Wistar–Kyoto rats (66.3 ± 6.6 vs 90.5 ± 10.6 pmol/24 h, P < 0.01). Linear regression analyses between urinary aldosterone excretion and each of the intra-erythrocyte variables determined revealed no significant relationships, neither in the combined group of spontaneously hypertensive and Wistar–Kyoto rats nor in any of the two strains separately. Linear correlation analyses between urinary aldosterone excretion and intra-erythrocyte sodium concentration gave r = −0.31 in the combined group and r = −0.20 in spontaneously hypertensive rats, both not significant.

Discussion

We have performed these experiments on the assumption that the spontaneously hypertensive rat is a good model of the, perhaps, most common variant of human essential hypertension. There are, however, observations that

<table>
<thead>
<tr>
<th>Group</th>
<th>Sodium (mmol/l)</th>
<th>Potassium (mmol/l)</th>
<th>ATP (mmol/l)</th>
<th>Total ATPase (nmol P₁ generated h⁻¹ mg⁻¹ of protein)</th>
<th>(Na⁺/K⁺-activated) ATPase (nmol P₁ generated h⁻¹ mg⁻¹ of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneously hypertensive rats</td>
<td>5.5* ±</td>
<td>100.6 ±</td>
<td>0.58 ±</td>
<td>2995 ±</td>
<td>1515 ±</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>3.5</td>
<td>0.23</td>
<td>255</td>
<td>154</td>
</tr>
<tr>
<td>Wistar–Kyoto rats</td>
<td>4.0 ±</td>
<td>97.6 ±</td>
<td>0.57 ±</td>
<td>3029 ±</td>
<td>1521 ±</td>
</tr>
<tr>
<td></td>
<td>1.07</td>
<td>5.5</td>
<td>0.14</td>
<td>196</td>
<td>137</td>
</tr>
</tbody>
</table>
question the validity of that assumption. For example, spontaneously hypertensive rats metabolize ingested sodium differently from man, having a much lower urinary and much higher faecal excretion (Berglund, Lundin, Herlitz, Ricksten, Göthberg & Hallbäck, 1979). The present study also illustrates that spontaneously hypertensive rats exhibit higher intraerythrocyte (Na⁺,K⁺-activated)-ATPase and lower sodium concentrations than those in humans.

The smooth muscle cell of the resistance vessels should be the most interesting cell type to study with respect to intracellular sodium concentration, aldosterone effects and the relationship to peripheral resistance (Friedman & Friedman, 1976). Many of the effects of aldosterone rely on modification of nuclear control of protein synthesis. Extrapolation of results obtained from the enucleate erythrocyte to smooth muscle cells must be made with caution.

Plasma aldosterone is probably the best mirror of the aldosterone effect at the cell membrane. In hypertensive man the urinary excretion of the renally derived oxo-conjugate and plasma aldosterone has been shown to be increased in parallel owing to a decreased hepatic metabolism of aldosterone (Genest, Nowaczynski, Boucher & Kuchel, 1978), whereas the secretion rate was normal. The metabolism of aldosterone has not been studied in detail in rats but there is reason to believe that the urinary excretion of the oxo-conjugate, at least roughly, reflects the plasma concentration.

The present study has two main findings. Firstly, we observed a higher intra-erythrocyte sodium concentration in spontaneously hypertensive rats than in Wistar–Kyoto rats, which is consistent with previous findings by Ben-Ishay et al. (1975), Postnov et al. (1976) and Yamori et al. (1977). The increased intracellular sodium concentration could be due either to a less efficient use of the available (Na⁺,K⁺-activated)-ATPase molecules or to an increased passive permeability to sodium ions through the erythrocyte membrane. The results of Postnov et al. (1976) favour the latter explanation. The concentrations of (Na⁺,K⁺-activated)-ATPase obtained in the two strains indicate that the capacity for active sodium transport is similar in spontaneously hypertensive and Wistar–Kyoto rats and the equal concentrations of ATP further show that access to the energy necessary for the above enzyme activity is also the same in the two strains. Secondly, the aldosterone excretion was lower in spontaneously hypertensive rats than in Wistar–Kyoto rats. This has previously been demonstrated by other investigators (Dietz, Schömig, Mann, Rascher, Lüth, Grünherz & Gross, 1978).

Acknowledgments

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References


Postnov, Y.U., Omlov, S., Gulak, P. & Schwenk, A. (1976) Allowed permeability of the erythrocyte membrane for sodium and


