Na\(^+\)/Mg\(^{2+}\) antiport in erythrocytes of spontaneously hypertensive rats: role of Mg\(^{2+}\) in the pathogenesis of hypertension

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Abstract. Total Mg\(^{2+}\) content in plasma and erythrocytes did not significantly differ between WKY and SHR. Mg\(^{2+}\) efflux via Na\(^+\)/Mg\(^{2+}\) antiport was 10% lower in non Mg\(^{2+}\)-loaded erythrocytes of SHR than in WKY, and 16% lower in Mg\(^{2+}\)-loaded erythrocytes of SHR. The activation of Na\(^+\)/Mg\(^{2+}\) antiport in erythrocytes by Cl\(^-\), as tested by substitution of Cl\(^-\) with SCN\(^-\), and the regulation of Na\(^+\)/Mg\(^{2+}\) antiport by protein kinases, as tested by PMA and staurosporine, showed no differences between WKY and SHR. The reduction of Na\(^+\)/Mg\(^{2+}\) antiport was explained by a reduction in the number of Na\(^+\)/Mg\(^{2+}\) antiporter molecules in SHR erythrocytes. Mg\(^{2+}\) efflux in KCl medium by K\(^+\)/Mg\(^{2+}\) antiport via the unspecific choline exchanger was not significantly reduced in SHR and was equally affected by PMA and staurosporine in WKY and SHR. An explanation for some controversial results, unchanged or reduced concentration of Mg\(^{2+}\) in serum, total Mg\(^{2+}\) and free Mg\(^{2+}\) in erythrocytes of SHR and patients with essential hypertension was proposed. The role of Na\(^+\)/Mg\(^{2+}\) antiport and [Mg\(^{2+}\)]\(^i\) in the pathogenesis of experimental and clinical hypertension was discussed.

Keywords: SHR, WKY, erythrocytes, Mg\(^{2+}\) content, Mg\(^{2+}\) efflux, Na\(^+\)/Mg\(^{2+}\) antiport, hypertension

The role of Mg\(^{2+}\) in the pathogenesis of essential hypertension has been extensively studied because Mg\(^{2+}\) modulates vascular smooth muscle tone and reactivity [1-6], and regulates both the cell growth as well as the cell differentiation involved in vascular remodeling in hypertension [7]. For a recent extensive review on the role of Mg\(^{2+}\) in hypertension see [8].

In humans, serum and erythrocytes are easily available, so that their Mg\(^{2+}\) content has been often determined in studies on the role of Mg\(^{2+}\) in hypertension. In patients with essential hypertension, serum Mg\(^{2+}\) concentration was found unchanged [9-11] or slightly reduced [11]. Controversial results were reported for erythrocytes of hypertensives: total Mg\(^{2+}\) content was unchanged [9, 11], reduced [11] or increased [10, 11]. Also, [Mg\(^{2+}\)]\(^i\) of erythrocytes was insignificantly changed [5, 11], reduced [5, 11, 12] or increased [5, 11, 13]. [Mg\(^{2+}\)]\(^i\), was increased in the platelets of patients with essential hypertension [14]. These discrepant results were often ignored or insufficiently explained.
An experimental model for studying the mechanisms of hypertension involves spontaneously hypertensive rats, e.g. SHR with the related Wistar rat strain WKY as a control. Controversial results also exist for the total Mg\(^{2+}\) concentration in serum as well as in erythrocytes of SHR. While some authors found no change in serum Mg\(^{2+}\) [15-17] or erythrocyte Mg\(^{2+}\) [17] in SHR, others reported a reduction in the concentration of plasma Mg\(^{2+}\) [11, 18, 19], erythrocyte total Mg\(^{2+}\) [16, 19] or intraerythrocyte [Mg\(^{2+}\)], as measured by \(^{31}\)P-NMR [18]. For more literature see [8].

A mechanism for reduced total Mg\(^{2+}\) and [Mg\(^{2+}\)]\(_{\text{i}}\) in hypertension may be an increase in Mg\(^{2+}\) efflux via Na\(^{+}/\)Mg\(^{2+}\) antiport. In Mg\(^{2+}\)-loaded erythrocytes of hypertensive patients, the Na\(^{+}/\)Mg\(^{2+}\) antiport did not significantly differ from normotensives [20], while in another study, 45% of patients with essential hypertension showed an increase in Na\(^{+}/\)Mg\(^{2+}\) antiport [21].

In rats with experimentally induced hypertension caused by chronic infusion of angiotensin II, an increase in [Na\(^{+}\)]\(_{\text{i}}\) and a decrease in [Mg\(^{2+}\)]\(_{\text{i}}\) was found in platelets, which was normalized by imipramine and quinidine as inhibitors of Na\(^{+}/\)Mg\(^{2+}\) antiport. From these results the authors concluded that an increase in Na\(^{+}/\)Mg\(^{2+}\) antiport may play a role in the development of hypertension [22].

To date, the Na\(^{+}/\)Mg\(^{2+}\) antiport has not been studied in erythrocytes of SHR. To clarify the role of the Na\(^{+}/\)Mg\(^{2+}\) antiport in hypertension, we investigated Mg\(^{2+}\) efflux from Mg\(^{2+}\)-loaded and non Mg\(^{2+}\)-loaded erythrocytes of WKY and SHR.

### Materials and methods

#### Materials

Nembutal\(^{\text{R}}\) (pentobarbital sodium) was obtained from Abott (North Chicago, IL, USA), PMA from SIGMA\(^{\text{R}}\) (Taufkirchen, Germany), and staurosporine from CALBIOCHEM\(^{\text{R}}\) (Bad Soden, Germany). All other chemicals were purchased at the highest purity available from Merck\(^{\text{R}}\), Darmstadt, Germany. Filtered, de-ionized and virtually Mg\(^{2+}\)-free water with a resistance of 15-18 M\(\Omega/cm\) was used for the solutions.

**WKY and SHR**

All experiments were paired, comparing male WKY as controls and their male hypertensive variant SHR, both groups aged 18-64 weeks and weighing 300-450 g. Within this range, the Mg\(^{2+}\) efflux in NaCl medium and KCl medium were independent of age for each animal group. WKY and SHR were obtained from DIMED Schönwalde GmbH, Schönwalde, Germany.

The blood pressure of prewarmed (38 °C) conscious rats was measured indirectly with a tail-cuff method equipped with an opto-electronic pulse sensor coupled to a computer (TSE blood pressure monitor, 2009002 series, TSE GmbH, Bad Homburg, Germany).

**Preparation and incubation of red blood cells**

On the same day, blood (6-8 mL) was obtained consistently from one anesthetized male WKY, and one male SHR (50 mg/kg Nembutal\(^{\text{R}}\) i.p.). The abdominal vein (vena cava inferior) was catheterized with a heparinized syringe. The blood was transferred to heparinized tubes, diluted 1:3–1:5 with NaCl medium consisting of (in mmol/L) 150 NaCl, 5 D-glucose and 10 Hepes-Tris, pH 7.4. The cell suspension was centrifuged at 1000 x g for 10 min at 24 °C. The plasma and buffy coat containing the white cells were aspirated and discarded. The sedimented red cells were washed twice at 24 °C in NaCl medium. Finally, the red cells were resuspended and incubated with gentle shaking as a 10% (v/v) suspension in one of the following media, each containing (in mmol/L) 5 D-glucose and 10 Hepes-Tris, pH 7.4: a) 150 NaCl (NaCl medium), b) 150 KCl (KCl medium), c) 150 NaSCN (NaSCN medium) and d) 150 KSCN (KSCN medium).

To minimize hemolysis, the cells were handled with utmost caution, temperature was kept at 24 °C, and centrifugation was carried out at 1000 x g. Usually, hemolysis ranged from 0.5% through 1.5%. The
studies were consistently performed as paired experiments in the different media.

**Mg²⁺ loading**

A 10% (v/v) erythrocyte suspension of WKY and SHR was incubated at 37 °C for 30 min in Mg²⁺ loading medium containing (in mmol/L) 140 KCl, 50 sucrose, 5 D-glucose and 10 Hepes-Tris, pH 7.4, 12 MgCl₂ and 6 μmol/L A 23187 (dissolved in dimethyl sulfoxide). After 30 min, the ionophore was removed by incubating the cells 4 times in ionophore-free Mg²⁺ loading medium plus 1% bovine serum albumin for 10 min at 37 °C. Thereafter, the erythrocytes were washed 2 times in cold incubation medium.

**Mg²⁺ efflux**

At the beginning and end of an experiment, 1 mL aliquots of the cell suspensions of one WKY and one SHR were centrifuged at 1000 × g for 10 min. To determine Mg²⁺, the supernatant was diluted with TCA. The final concentration of TCA was 5% (w/v), containing 0.1% (w/v) La₂O₃ and 0.16% (v/v) HCl. Mg²⁺ was measured in triplicates by means of atomic absorption spectrometry (Perkin Elmer, 2380). Mg²⁺ efflux was calculated from the increase in extracellular Mg²⁺ concentration during the time interval, and was related to the original cell volume measured by hematocrit. Hematocrit was determined by centrifugation at 1500 × g for 10 min. Mg²⁺ efflux was corrected for hemolysis. Erythrocyte Mg²⁺ content was measured for this purpose. Hemolysis was measured by determining hemoglobin at 557 nm.

**Erythrocyte Mg²⁺ content**

Prior to incubation, Mg²⁺ was extracted from an aliquot of sedimented erythrocytes with 5% (v/v) TCA and was measured after appropriate dilution of the extract with TCA and La₂O₃-HCl as described above.

**Statistical analysis**

Data were expressed as means ± S.E., and statistical significance of the difference between two values was determined by Student’s paired and two tailed t test. A p value < 0.05 was considered significant.

### Results

**Systolic blood pressure in WKY and SHR**

Systolic blood pressure was 139 ± 2 mmHg in WKY and 210 ± 6 mmHg in SHR (mean values ± S.E., n = 8). These values are in accordance with the results of other authors for WKY and SHR of about the same age [18, 23-25], but lower blood pressure values for WKY and SHR have also been reported [26].

**Mg²⁺ content in plasma and erythrocytes**

As shown in table 1, the concentration of plasma Mg²⁺ was the same in WKY and SHR, confirming some of the previous findings [15-17]. Also, in our study the concentration of total erythrocyte Mg²⁺ was the same in WKY and SHR, which is in accordance with other authors [17].

**Mg²⁺ efflux from erythrocytes**

Intracellular free Mg²⁺ is the active fraction of total Mg²⁺ and is in equilibrium with functionally active Mg²⁺ complexes. Since [Mg²⁺], can be regulated by Mg²⁺ efflux, we investigated Mg²⁺ efflux from erythrocytes of WKY and SHR.

As shown in figure 1, Mg²⁺ efflux from Mg²⁺-loaded erythrocytes was significantly lower in SHR than in WKY. The difference amounted to 16% in NaCl medium and NaSCN medium. In erythrocytes of WKY and SHR, Mg²⁺ efflux in KCl medium and KSCN medium did not differ significantly.

In Na⁺-containing media, Mg²⁺ efflux of Mg²⁺-loaded erythrocytes is almost completely mediated by Na⁺/Mg²⁺ antiport. Moreover, in Mg²⁺-loaded erythrocytes the Na⁺/Mg²⁺ antiport operates under nearly $V_{\text{max}}$ conditions. Therefore, the reduction in Na⁺/Mg²⁺ antiport may be caused by a reduction in the number of Na⁺/Mg²⁺ antiporter molecules.

Since, *in vivo*, erythrocytes are not loaded with Mg²⁺, and their Na⁺/Mg²⁺ antiport is operating under “non Mg²⁺-loaded conditions”, we next investigated Mg²⁺ efflux in non Mg²⁺-loaded erythrocytes of WKY and SHR. These experiments were possible because the Na⁺/Mg²⁺ antiport in rat erythrocytes is 65 times

<table>
<thead>
<tr>
<th>n</th>
<th>WKY</th>
<th>SHR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Mg²⁺</td>
<td>8</td>
<td>0.71 ± 0.02</td>
<td>0.73 ± 0.02</td>
</tr>
<tr>
<td>Erythrocyte Mg²⁺</td>
<td>14</td>
<td>1.95 ± 0.03</td>
<td>1.93 ± 0.04</td>
</tr>
</tbody>
</table>

Mean values ± S.E.; n = number of rats; p = significance of difference between WKY and SHR.
more active than in human erythrocytes [27].

Tables 2 and 3 show that erythrocytes of SHR suspended in NaCl medium had a significantly 10% lower Mg\(^{2+}\) efflux via Na\(^+/\)Mg\(^{2+}\) antiport than erythrocytes of WKY. A similar result was found by other authors quite recently [28]. The extent of reduction of Na\(^+/\)Mg\(^{2+}\) antiport of Mg\(^{2+}\) -loaded and non Mg\(^{2+}\) -loaded erythrocytes of SHR is very similar. Therefore, the reduction of Na\(^+/\)Mg\(^{2+}\) antiport in non Mg\(^{2+}\) -loaded erythrocytes of SHR can also be explained by a reduced number of Na\(^+/\)Mg\(^{2+}\) antiporter molecules and not by a changed regulation of Na\(^+/\)Mg\(^{2+}\) antiport in these cells (see below).

Mg\(^{2+}\) efflux in KCl medium by K\(^+\)/Mg\(^{2+}\) exchange via the unspecific choline exchanger was not significantly reduced in SHR, which agrees with our results for Mg\(^{2+}\)-loaded erythrocytes (see above this Section).

Regulation of Mg\(^{2+}\) efflux in WKY and SHR

We have previously shown that Mg\(^{2+}\) efflux from non Mg\(^{2+}\)-loaded rat erythrocytes in NaCl medium, via

![Figure 1. Mg\(^{2+}\) efflux from Mg\(^{2+}\)-loaded erythrocytes of WKY and SHR in different media. Erythrocytes were loaded with 12 mmol/L Mg\(^{2+}\). Mean values ± S.E.; n = 5; * p < 0.05, significance of difference between WKY and SHR.](image)

<table>
<thead>
<tr>
<th>Medium</th>
<th>WKY</th>
<th>SHR</th>
<th>Δ Mg(^{2+}) efflux (%)</th>
<th>n</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>211.6 ± 6.6</td>
<td>190.8 ± 6.2</td>
<td>-10</td>
<td>17</td>
<td>0.6443</td>
</tr>
<tr>
<td>NaSCN</td>
<td>84.4 ± 3.0</td>
<td>79.4 ± 2.2</td>
<td>-6</td>
<td>4</td>
<td>0.0988</td>
</tr>
<tr>
<td>KCl</td>
<td>146.8 ± 4.5</td>
<td>141.7 ± 5.0</td>
<td>-4</td>
<td>18</td>
<td>0.2015</td>
</tr>
<tr>
<td>KSCN</td>
<td>119.7 ± 10.9</td>
<td>116.8 ± 11.9</td>
<td>-2</td>
<td>5</td>
<td>0.4330</td>
</tr>
<tr>
<td>NaCl/NaSCN</td>
<td>2.51</td>
<td>2.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl/KSCN</td>
<td>1.23</td>
<td>1.21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean values ± S.E.; n = number of experiments; p = significance of difference between WKY and SHR.

Table 2. Mg\(^{2+}\) efflux (μmol/L cells·2 h) in different media from non Mg\(^{2+}\)-loaded erythrocytes of WKY and SHR. Effect of Cl is given by the ratios of Cl/SCN. The difference in Mg\(^{2+}\) efflux (Δ Mg\(^{2+}\) efflux) between WKY and SHR is given as a percentage of Mg\(^{2+}\) efflux from erythrocytes of WKY.
Na+/Mg\(^{2+}\) antiport, and Mg\(^{2+}\) efflux in KCl medium by K+/Mg\(^{2+}\) antiport, via the unspecific choline exchanger, were activated by the PKC activator PMA and inhibited by PKC inhibitors, as well as by the less specific PK inhibitor staurosporine [29]. In this study we have therefore investigated the effect of PMA and staurosporine on Mg\(^{2+}\) efflux from non Mg\(^{2+}\)-loaded erythrocytes of WKY and SHR.

As can be seen from Table 3, PMA (1 \(\mu\)mol/L) significantly stimulated Mg\(^{2+}\) efflux from erythrocytes of both rat strains in NaCl medium by about 50% and in KCl medium by about 90%. Staurosporine (0.5 \(\mu\)mol/L) significantly reduced Mg\(^{2+}\) efflux in both strains in NaCl medium by about 20%. In KCl medium, staurosporine significantly inhibited Mg\(^{2+}\) efflux in NaCl medium and KCl medium by 25% and 17%, respectively. These results show that within experimental error, Na+/Mg\(^{2+}\) antiport and K+/Mg\(^{2+}\) antiport were equally affected by PMA and staurosporine in both rat strains, via the choline exchanger. Remarkably, in both rat strains the K+/Mg\(^{2+}\) antiport was more sensitive to PMA than the Na+/Mg\(^{2+}\) antiport, as already found with Sprague-Dawley rats [29].

We previously reported that the Na+/Mg\(^{2+}\) antiport is activated by intracellular Cl\(^-\) [30]. Accordingly, in the present study we investigated whether in erythrocytes of WKY and SHR, substitution of Cl\(^-\) by SCN\(^-\) in the incubation media affects Mg\(^{2+}\) efflux differently. In both rat strains, Mg\(^{2+}\) efflux from Mg\(^{2+}\)-loaded erythrocytes suspended in NaCl medium was, significantly, 17% higher than in NaSCN medium (Figure 1). In KCl medium, Mg\(^{2+}\) efflux from Mg\(^{2+}\)-loaded erythrocytes was only slightly higher than Mg\(^{2+}\) efflux in KSCN medium (Figure 1).

In non Mg\(^{2+}\)-loaded erythrocytes of WKY and SHR, Mg\(^{2+}\) efflux in NaCl medium was about 2.45 times (145%) significantly higher than in NaSCN medium (Table 2). In KCl medium, Mg\(^{2+}\) efflux was only about 1.2 times (20%), but significantly, higher in WKY and SHR than in KSCN medium. The large difference between Mg\(^{2+}\) efflux in NaCl medium and in NaSCN medium is due to the activation of Na+/Mg\(^{2+}\) antiport by Cl\(^-\). The small difference between Mg\(^{2+}\) efflux in KCl medium and in KSCN medium can be explained by the lyotropic effect of anions on the choline exchanger. The higher Cl\(^-\) sensitivity of Na+/Mg\(^{2+}\) antiport in non Mg\(^{2+}\)-loaded erythrocytes is in agreement with our previous results with Sprague-Dawley rats [30].

**Discussion**

The aim of the present study was to determine whether there is a difference in erythrocyte Na+/Mg\(^{2+}\) antiport between WKY and SHR. The Mg\(^{2+}\) content of plasma and erythrocytes was also assessed for the purpose of comparison with the

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### Table 3. Effect of PMA and staurosporine on Mg\(^{2+}\) efflux (\(\mu\)mol/L cells·2 h) of non Mg\(^{2+}\)-loaded erythrocytes of WKY and SHR in NaCl medium and KCl medium. The concentration of PMA was 1 \(\mu\)mol/L, of staurosporine 0.5 \(\mu\)mol/L. A SHR is the difference of Mg\(^{2+}\) efflux between WKY and SHR.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Effector</th>
<th>WKY</th>
<th>SHR</th>
<th>A SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>Control</td>
<td>192.3 ± 4.9</td>
<td>173.2 ± 12.6</td>
<td>-19.1*</td>
</tr>
<tr>
<td></td>
<td>PMA</td>
<td>285.1 ± 4.9</td>
<td>264.0 ± 16.0</td>
<td>-21.1*</td>
</tr>
<tr>
<td></td>
<td>A PMA</td>
<td>92.8**</td>
<td>90.8**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Staurosporine</td>
<td>158.4 ± 4.1</td>
<td>137.9 ± 10.6</td>
<td>-20.5*</td>
</tr>
<tr>
<td></td>
<td>A Staurosporine</td>
<td>-33.9**</td>
<td>-35.3**</td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>Control</td>
<td>127.0 ± 5.7</td>
<td>125.6 ± 9.4</td>
<td>-1.4**</td>
</tr>
<tr>
<td></td>
<td>PMA</td>
<td>241.0 ± 6.0</td>
<td>236.3 ± 12.1</td>
<td>-4.7**</td>
</tr>
<tr>
<td></td>
<td>A PMA</td>
<td>114.0**</td>
<td>110.7**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Staurosporine</td>
<td>95.7 ± 5.6</td>
<td>104.6 ± 11.2</td>
<td>8.9**</td>
</tr>
<tr>
<td></td>
<td>A Staurosporine</td>
<td>-145.3**</td>
<td>-131.7**</td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>PMA/Control</td>
<td>1.48</td>
<td>1.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Staurosp/Control</td>
<td>0.82</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>PMA/Control</td>
<td>1.90</td>
<td>1.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Staurosp/Control</td>
<td>0.75</td>
<td>0.83</td>
<td></td>
</tr>
</tbody>
</table>

Mean values ± S.E.; n = 6; * significance of difference between control WKY and control SHR was always p < 0.05; n.s. = non significant difference between WKY and SHR; ** significance of A PMA and A Staurosporin in WKY versus SHR was always p < 0.05. Values for Δ PMA and Δ Staurosporin were not significantly different between WKY and SHR (not shown).
controversial results presented in the literature. We found that there was no difference in plasma and erythrocyte Mg\(^{2+}\) content between WKY and SHR. In SHR, the activity of Na\(^+\)/Mg\(^{2+}\) antiport in erythrocytes under \(V_{\text{max}}\) conditions was reduced by 16%, as found with Mg\(^{2+}\)-loaded erythrocytes, and by 10% in non Mg\(^{2+}\)-loaded erythrocytes.

**Controversial results**

Evidence of a role played by Mg\(^{2+}\) in the pathogenesis of hypertension comes from experiments with isolated blood vessels and blood vessel preparations. In vitro experiments with drastically reduced extracellular Mg\(^{2+}\) concentrations may increase in catecholamine-induced hypertension. On the other hand, catecholamines increase intracellular lipolysis in fat cells. The resulting free fatty acids will bind Mg\(^{2+}\), followed by a reduction of [Mg\(^{2+}\)]\(_{\text{i}}\) in fat cells. This may induce an uptake of extracellular Mg\(^{2+}\) into fat cells [47]. In patients or rats having a high fat content, this mechanism may lead to a reduction of plasma Mg\(^{2+}\). When both these counteracting effects are differently expressed in individuals, an opposite effect on plasma and erythrocyte Mg\(^{2+}\) may occur in different hypertensives. Thus, serum Mg\(^{2+}\) can be independent of blood pressure. Erythrocyte Mg\(^{2+}\) may follow the alteration in plasma Mg\(^{2+}\). Moreover, an increase in extracellular Mg\(^{2+}\) can reduce the release of catecholamines through competition with extracellular Ca\(^{2+}\).

**Effect of extracellular Mg\(^{2+}\) on VSMC in vitro**

Evidence of a role played by Mg\(^{2+}\) in the pathogenesis of hypertension comes from experiments with isolated blood vessels and blood vessel preparations. In vitro experiments with drastically reduced extracellular Mg\(^{2+}\) concentrations [11, 58, 59], particularly in SHR [60]. This modulation of vascular tone is affected by an altered Ca\(^{2+}\) influx due to a changed Mg\(^{2+}\)/Ca\(^{2+}\) competition [1]. Unfortunately, in these experiments, in the controls an extracellular Mg\(^{2+}\) concentration of 1.2 mmol/L was used in a protein-free incubation medium, which had twice the physiological concentration of free Mg\(^{2+}\) in plasma. In these in vitro experiments with drastically reduced extracellular Mg\(^{2+}\) concentrations, vasoconstriction occurred immediately. In contrast, during in vivo experiments
with rats, when the reduction of extracellular Mg\(^{2+}\)
was less pronounced, hypertension occurred only
after 15 weeks and there was an additional effect of
vasoactive substances (see above). Thus, in Mg\(^{2+}\)-
deficient rats, vasoconstriction and hypertension
may be produced to a major degree by the chronic
increase of catecholamines, and only to a minor
degree by a decrease in Mg\(^{2+}\) itself.

**Down regulation of Na\(^+/\)Mg\(^{2+}\) antiport**
in erythrocytes of SHR

Mg\(^{2+}\) efflux from non Mg\(^{2+}\)-loaded rat erythrocytes in
NaCl medium, *via* Na\(^+/\)Mg\(^{2+}\) antiport, and Mg\(^{2+}\)
efflux in KCl medium by the K\(^+/\)Mg\(^{2+}\) antiport, *via*
the unspecific choline exchanger, were activated by the
PKC activator PMA and inhibited by PKC inhibitors
as well as by the less specific PK inhibitor stauro-
sporine [29]. However, we detected no difference
between WKY and SHR in the regulation of Mg\(^{2+}\)
efflux by PMA- and staurosporine-sensitive protein
kinases. Also the regulation of Na\(^+/\)Mg\(^{2+}\) antiport by
intracellular Cl\(^-\), as recently described by us [30], was
not different between WKY and SHR. Since the regu-
lation of Na\(^+/\)Mg\(^{2+}\) antiport *via* PKC and intracellular
Cl\(^-\) was not changed in SHR, the reduction of the
Na\(^+/\)Mg\(^{2+}\) antiport activity may have been caused by
a reduction in the number of Na\(^+/\)Mg\(^{2+}\) antiporter
molecules in erythrocytes of SHR. The mechanism
may be a down regulation in the biosynthesis of
Na\(^+/\)Mg\(^{2+}\) antiporter molecules in erythrocytes of
SHR. As discussed below, in VSMC of SHR, activity of
Na\(^+/\)Mg\(^{2+}\) antiport was increased. From the different
behavior of Na\(^+/\)Mg\(^{2+}\) antiport in erythrocytes and
VSMC a mutation of the Na\(^+/\)Mg\(^{2+}\) antiporter as an
alternative mechanism leading to a reduced *V*\(_{max}\)
of Na\(^+/\)Mg\(^{2+}\) antiport in erythrocytes of SHR seems to
be less probable.

**Role of the Na\(^+/\)Mg\(^{2+}\) antiport in hypertension**

No consistent change has been described for
Na\(^+/\)Mg\(^{2+}\) antiport of erythrocytes in hypertension.
In one study, half of the hypertensive patients
showed an increase in Na\(^+/\)Mg\(^{2+}\) antiport in Mg\(^{2+}\)-
loaded erythrocytes. Consequently, it was suggested
that only a subgroup of patients with essential hyper-
tension may be Mg\(^{2+}\)-sensitive [21]. In another study
with hypertensive patients suffering from latent
tetany, no significant alteration of the Na\(^+/\)Mg\(^{2+}\) anti-
port in Mg\(^{2+}\)-loaded erythrocytes was found [20].
Obviously, different pathogenic mechanisms not
reflected by an alteration of Na\(^+/\)Mg\(^{2+}\) antiport in
erthrocytes may have induced hypertension in dif-
ferent types of patients.

From experiments with angiotensin II, participa-
tion of the Na\(^+/\)Mg\(^{2+}\) antiport in the pathogenesis of
hypertension was concluded. When hypertension
was induced by chronic treatment of rats with angio-
tensin II, the increase in blood pressure was com-
bined with an increase of [Na\(^+\)], and a decrease of
[Mg\(^{2+}\)], in VSMC, in platelets and MDCK cells [61-63].
The increase in blood pressure and the alterations in
[Na\(^+\)], and [Mg\(^{2+}\)], could be attenuated by imipramine
and quinidine, which are unspecific inhibitors of the
Na\(^+/\)Mg\(^{2+}\) antiport [22, 63]. These results led to the
conclusion that the Na\(^+/\)Mg\(^{2+}\) antiport may play a
role in the pathogenesis of hypertension [22]. But as
a matter of fact, in these cells there was no reason-
able stoichiometric ratio in the alterations of [Na\(^+\)],
and [Mg\(^{2+}\)]. [Na\(^+\)], increased by about 20 mmol/L,
whereas the decrease in [Mg\(^{2+}\)], amounted only to
0.1-0.2 mmol/L [61-63]. However, the effect of angio-
tensin II on [Mg\(^{2+}\)], seems to be complex and
unsettled. Short-term exposure for 5-10 min of iso-
lated, cultured VSMC to angiotensin II reduced
[Mg\(^{2+}\)], by stimulation of the Na\(^+/\)Mg\(^{2+}\) antiport in
*vivo* with rats. After chronic incubation of VSMC
cultured for 24-30 h with angiotensin II, [Mg\(^{2+}\)],
was increased due to the stimulation of TRPM 7 channels
[64].

A reduction of [Mg\(^{2+}\)], was also found with the
aorta of SHR compared with WKY. In the aorta of
SHR, [Na\(^+\)], was higher by 11.8 mmol/L and [Mg\(^{2+}\)],
was lower by 0.066 mmol/L [65]. In some tissues of
SHR, [Mg\(^{2+}\)], was lower when compared with WKY:
in VSMC by 0.2 mmol/L [66] or 0.12 mmol/L [63], and
in striated muscle by 0.13 mmol/L [66]. Again, there
was no reasonable stoichiometric ratio in [Na\(^+\)]
increase and decrease of total Mg\(^{2+}\) content in VSMC
of SHR with WKY as control. The difference in total
Na\(^+\) amounted to about 250 mmol/kg dry weight,
and the difference in total Mg\(^{2+}\) amounted to about 10
mmol/kg dry weight [67, 68]. Similarly, [Mg\(^{2+}\)], in
platelets of patients with essential hypertension was
lower by 0.21 mmol/L [69], although other authors
found an increase in [Mg\(^{2+}\)], by 0.08 mmol/L [14].

Cumulative evidence of these findings does not
support the idea that activation of Na\(^+/\)Mg\(^{2+}\) antiport
in vascular smooth muscle plays a major role in the
pathogenesis of hypertension. The reasons are the
following. In the Na\(^+/\)Mg\(^{2+}\) antiport the stoichiom-
metric ratio of transported Na\(^+/\)Mg\(^{2+}\) is 1:1 or 2:1, which
is not in accordance with the drastic increase of
[Na\(^+\)], compared with the rather small decrease of
[Mg\(^{2+}\)]. Furthermore, it is uncertain whether the
small measured decreases in [Mg\(^{2+}\)], from 0.1 to 0.2
mmol/L are by themselves sufficient to induce hyper-
tension. Metabolic and functional effects of Mg\(^{2+}\)-
relate on the pMg, and on the pMg-dependency of Mg²⁺-activated enzymes and proteins. Moreover, intracellular Mg²⁺/Ca²⁺ competition depends on the Ca²⁺ and Mg²⁺ affinity of the Ca²⁺-binding proteins. The difference in the intracellular pMgs between WKY and SHR, when calculated from the literature, was small and amounted only to 0.10 for erythrocytes [18], 0.13 for aorta [65], 0.08 [63] and 0.19 [66] for VSMC, 0.12 for striated muscle [66] and 0.19 for platelets [69]. The reduction of [Mg²⁺], and the increase of [Ca²⁺], in VSMC [70] and consequently the altered Mg²⁺/Ca²⁺ competition in VSMC may play a major role.

In the intracellular ionic alterations in hypertension, besides an activation of Na+/Mg²⁺ antiport, a change of other transport systems such as the Na⁺/H⁺ antiport, the Na⁺/Ca²⁺ antiport, the Na⁺,K⁺,Cl⁻ symport and the Na⁺,K⁺ pump may be involved. The Na⁺/H⁺ antiport and the Na⁺,K⁺,Cl⁻ symport are activated by angiotensin II [71]. Their individual contributions to hypertension are not defined. For further details see [35, 71-75]. The reduction in [Mg²⁺] in various cell types of hypertensive patients and SHR may well be caused by an increased Mg²⁺ efflux via Na⁺/Mg²⁺ antiport, induced by increased catecholamines, as shown for various cell types mentioned above.

In addition, further mechanisms, e.g. reactive oxygen species, various signal transducing enzymes as tyrosine kinases and MAP kinases, transcription factors and chemokines may play a role in the complex multifactorial mechanism of essential and experimental hypertension. These mechanisms have already been reviewed in detail [76].

### Conclusion

The genetic defect leading to hypertension in SHR is not reflected by the erythrocyte Mg²⁺ content. The reduction in Na⁺/Mg²⁺ antiport in SHR erythrocytes is not related to the increase in blood pressure. Erythrocytes are not a suitable source for studying the role of Mg²⁺ in the pathogenesis of clinical hypertension and SHR. Acute and chronic experimental Mg²⁺ deficiency reduces or increases systolic blood pressure in rats, although the extracellular Mg²⁺ concentration may be similarly reduced, indicating that the superimposed endocrine situation determines blood pressure. Severe and chronic experimental Mg²⁺ deficiency is a risk factor for hypertension, mainly due to the chronically increased release of catecholamines. In vitro, only extremely low extracellular Mg²⁺ concentrations can increase the contraction of vascular smooth muscle cells and reinforce the effects of vasoconstrictors due to a changed Ca²⁺/Mg²⁺ competition. An alteration of the Na⁺/Mg²⁺ antiport and Mg²⁺ metabolism in VSMC may participate in the pathogenesis of SHR, angiotensin II-induced and essential hypertension. With respect to the Na⁺/Mg²⁺ ratio of the Na⁺/Mg²⁺ antiport, the additional alteration of other transport systems and signal transducing mechanisms may play a major role in these types of hypertension.

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