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 \textit{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 \textit{via} the unspecific choline exchanger was not significantly reduced in SHR and was equally affected by PMA and staurosoporine 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\(^{2+}\)] 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\(^{2+}\)] of erythrocytes was insignificantly changed [5, 11], reduced [5, 11, 12] or increased [5, 11, 13]. [Mg\(^{2+}\)] 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 Mg²⁺ concentration in serum as well as in erythrocytes of SHR. While some authors found no change in serum Mg²⁺ [15-17] or erythrocyte Mg²⁺ [17] in SHR, others reported a reduction in the concentration of plasma Mg²⁺ [11, 18, 19], erythrocyte total Mg²⁺ [16, 19] or intraerythrocyte [Mg²⁺]ᵢ as measured by ³¹P-NMR [18]. For more literature see [8].

A mechanism for reduced total Mg²⁺ and [Mg²⁺]ᵢ in hypertension may be an increase in Mg²⁺ efflux via Na⁺/Mg²⁺ antiport. In Mg²⁺-loaded erythrocytes of hypertensive patients, the Na⁺/Mg²⁺ antiport did not significantly differ from normotensives [20], while in another study, 45% of patients with essential hypertension showed an increase in Na⁺/Mg²⁺ antiport [21].

In rats with experimentally induced hypertension caused by chronic infusion of angiotensin II, an increase in [Na⁺]ᵢ and a decrease in [Mg²⁺]ᵢ was found in platelets, which was normalized by imipramine and quinidine as inhibitors of Na⁺/Mg²⁺ antiport. From these results the authors concluded that an increase in Na⁺/Mg²⁺ antiport may play a role in the development of hypertension [22]. To date, the Na⁺/Mg²⁺ antiport has not been studied in erythrocytes of SHR. To clarify the role of the Na⁺/Mg²⁺ antiport in hypertension, we investigated Mg²⁺ efflux from Mg²⁺-loaded and non Mg²⁺-loaded erythrocytes of WKY and SHR.

### Materials and methods

#### Materials

Nembutal® (pentobarbital sodium) was obtained from Abott (North Chicago, IL, USA), PMA from SIGMA® (Taufkirchen, Germany), and staurosporine from CALBIOCHEM® (Bad Soden, Germany). All other chemicals were purchased at the highest purity available from Merck®, Darmstadt, Germany. Filtered, de-ionized and virtually Mg²⁺-free water with a resistance of 15-18 MΩ/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 Mg²⁺ 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® 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

### Abbreviations

ACTH: adrenocorticotropic hormone

MAP kinase: mitogen-activated protein kinase

MDCK: Madin-Darby canine kidney cells

Na⁺, [Mg²⁺]ᵢ: intracellular concentrations of Na⁺ and free Mg²⁺

PKC: protein kinase C

PMA: phorbol 12-myristate 13-acetate

pMg: -log [Mg²⁺]

TCA: trichloroacetic acid

TRPM7: transient receptor potential melastatin 7

TSH: thyroid stimulating hormone

VSMC: vascular smooth muscle cells
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 x 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 x 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 Vmax 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

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**Table 1. Mg²⁺ concentration in plasma (mmol/L) and erythrocytes (mmol/L cells) of WKY and SHR.**

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Mg²⁺</td>
<td>0.71 ± 0.02</td>
<td>0.73 ± 0.02</td>
<td>0.2084</td>
</tr>
<tr>
<td>Erythrocyte Mg²⁺</td>
<td>1.95 ± 0.03</td>
<td>1.93 ± 0.04</td>
<td>0.3596</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

**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 (ΔMg\(^{2+}\) efflux) between WKY and SHR is given as a percentage of Mg\(^{2+}\) efflux from erythrocytes of WKY.

<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>0.0443</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 ± 0.40</td>
<td>2.40 ± 0.35</td>
<td>n/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl/KSCN</td>
<td>1.23 ± 0.21</td>
<td>1.21 ± 0.20</td>
<td>n/A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean values ± S.E.; n = number of experiments; p = significance of difference between WKY and SHR.
Na+/Mg2+ antiport, and Mg2+ efflux in KCl medium by K+/Mg2+ 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 Mg2+ efflux from non Mg2+-loaded erythrocytes of WKY and SHR.

As can be seen from table 3, PMA (1 μmol/L) significantly stimulated Mg2+ efflux from erythrocytes of both rat strains in NaCl medium by about 50%, and in KCl medium by about 90%. Staurosporine (0.5 μmol/L) significantly reduced Mg2+ efflux in both strains in NaCl medium by about 20%. In KCl medium, staurosporine significantly inhibited Mg2+ efflux in NaCl medium and KCl medium by 25% and 17%, respectively. These results show that within experimental error, Na+/Mg2+ antiport and K+/Mg2+ antiport were equally affected by PMA and staurosporine in both rat strains via the choline exchanger. Remarkably, in both rat strains the K+/Mg2+ antiport was more sensitive to PMA than the Na+/Mg2+ antiport, as already found with Sprague-Dawley rats [29].

We previously reported that the Na+/Mg2+ 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 Mg2+ efflux differently. In both rat strains, Mg2+ efflux from Mg2+-loaded erythrocytes suspended in NaCl medium was, significantly, 17% higher than in NaSCN medium (figure 1). In KCl medium, Mg2+ efflux from Mg2+-loaded erythrocytes was only slightly higher than Mg2+ efflux in KSCN medium (figure 1).

In non Mg2+-loaded erythrocytes of WKY and SHR, Mg2+ efflux in NaCl medium was about 2.45 times (145%) significantly higher than in NaSCN medium (table 2). In KCl medium, Mg2+ efflux was only about 1.2 times (20%), but significantly, higher in WKY and SHR than in KSCN medium. The large difference between Mg2+ efflux in NaCl medium and in NaSCN medium is due to the activation of Na+/Mg2+ antiport by Cl−. The small difference between Mg2+ 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+/Mg2+ antiport in non Mg2+-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+/Mg2+ antiport between WKY and SHR. The Mg2+ content of plasma and erythrocytes was also assessed for the purpose of comparison with the

<table>
<thead>
<tr>
<th>Medium</th>
<th>Effector</th>
<th>WKY</th>
<th>SHR</th>
<th>ASHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></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>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></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>Staurosporine</td>
<td>114.0**</td>
<td>110.7**</td>
<td></td>
</tr>
</tbody>
</table>
|        | A Staurosporine | -145.3**       | -131.7**    
| NaCl   | PMA/Control   | 1.48         | 1.52         |
|        | Staurosporine | 0.82         | 0.80         |
| KCl    | PMA/Control   | 1.90         | 1.88         |
|        | Staurosporine | 0.75         | 0.83         |

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 Δ PMA and Δ Staurosporine in WKY versus SHR was always p < 0.05. Values for Δ PMA and Δ Staurosporine 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.

**Controversies on Mg$^{2+}$ content in plasma and erythrocytes in hypertension**

Controversial results have been reported for the content of Mg$^{2+}$ in plasma and erythrocytes in patients with essential hypertension and SHR. The fact that there are such controversial results has often been ignored. It is improbable that these were due to erroneous measurements. Usually, in the various studies, the difference in the mean values between controls and hypertensives was relatively small and the individual values of both groups overlapped. Uncontrolled conditions of Mg$^{2+}$ metabolism may interfere and may be responsible for some controversial results. In the following, we have tried to explain some of these controversial results.

The results of experimental Mg$^{2+}$ deficiency should be considered as a means for elucidating the role of Mg$^{2+}$ in human hypertension. In acute experimental Mg$^{2+}$ deficiency, blood pressure is reduced by an increase in NO production [31, 32] and by hyperemia due to increased release of histamine and serotonin [33]. The release of these substances is caused by a reduced Mg$^{2+}$/Ca$^{2+}$ antagonism in hypomagnesemia.

Chronic Mg$^{2+}$ deficiency in rats for more than 15 weeks induces hypertension [7, 34-37]. This may be caused by the chronically increased release of catecholamines, particularly when Mg$^{2+}$ deficiency is combined with stress [38]. Also, in SHR, more catecholamines are released by an increase in the activity of the sympathico-adrenal system [39, 40]. In addition, SHR had a permanently increased release of blood pressure is reduced by an increase in NO production [31, 32] and by hyperemia due to increased release of histamine and serotonin [33]. The release of these substances is caused by a reduced Mg$^{2+}$/Ca$^{2+}$ antagonism in hypomagnesemia.

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 *in vitro* experiments with drastically reduced 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 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$^{2+}$.

Altogether, in patients with essential hypertension and in SHR, catecholamines and other hormones may play the major role in the pathogenesis of hypertension. Moreover, Mg$^{2+}$ deficiency must exceed a certain degree in order to increase catecholamines [38] and induce toxic effects [48-50].

As seen from the various Mg$^{2+}$ supplementation experiments with SHR, Mg$^{2+}$ played only a minor role during early stages in the development of hypertension up to an age of about 10 weeks [19, 51-53]. Thereafter, when hypertension was established in SHR, Mg$^{2+}$ supplementation had no significant effect on blood pressure [19]. As with DOCA-salt-induced hypertension, Mg$^{2+}$ supplementation reduced blood pressure only by 10% [54], whereas in another study, oral Mg$^{2+}$ supplementation completely prevented hypertension in DOCA-salt-treated rats [55]. In patients, Mg$^{2+}$ supplementation had only a small or no significant effect [56, 57], probably depending on Mg$^{2+}$ dosage or drug pre-treatment, e.g. with diuretics.

**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 *in vitro*, it was possible to induce contraction and relaxation of smooth muscle cells by drastically reducing or increasing the extracellular Mg$^{2+}$ concentration [58]. Also, the effect of vasoconstrictors was increased at 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 staurosporine [20]. However, we detected no difference between W KY and SHR in the regulation of Mg$^{2+}$ efflux by PKA- 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 regulation 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 $V_{\text{max}}$ 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 hypertension may be Mg$^{2+}$-sensitive [21]. In another study with hypertensive patients suffering from latent tetany, no significant alteration of the Na$^+/Mg^{2+}$ antiport in Mg$^{2+}$-loaded erythrocytes was found [20]. Obviously, different pathogenic mechanisms not reflected by an alteration of Na$^+/Mg^{2+}$ antiport in erythrocytes may have induced hypertension in different types of patients.

From experiments with angiotensin II, participation of the Na$^+/Mg^{2+}$ antiport in the pathogenesis of hypertension was concluded. When hypertension was induced by chronic treatment of rats with angiotensin II, the increase in blood pressure was combined 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 reasonable 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 angiotensin II on [Mg$^{2+}$], seems to be complex and unsettled. Short-term exposure for 5-10 min of isolated, 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$^+$], 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 stoichiometric 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 hypertension. Metabolic and functional effects of Mg$^{2+}$...
rely 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 WYK 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 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 *vivo*, 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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