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

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Abstract. Total Mg\textsuperscript{2+} content in plasma and erythrocytes did not significantly differ between WKY and SHR. Mg\textsuperscript{2+} efflux via Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport was 10% lower in non Mg\textsuperscript{2+}-loaded erythrocytes of SHR than in WKY, and 16% lower in Mg\textsuperscript{2+}-loaded erythrocytes of SHR. The activation of Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport in erythrocytes by Cl-, as tested by substitution of Cl- with SCN-, and the regulation of Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport by protein kinases, as tested by PMA and staurosporine, showed no differences between WKY and SHR. The reduction of Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport was explained by a reduction in the number of Na\textsuperscript{+}/Mg\textsuperscript{2+} antiporter molecules in SHR erythrocytes. Mg\textsuperscript{2+} efflux in KCl medium by K+/Mg\textsuperscript{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\textsuperscript{2+} in serum, total Mg\textsuperscript{2+} and free Mg\textsuperscript{2+} in erythrocytes of SHR and patients with essential hypertension was proposed. The role of Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport and [Mg\textsuperscript{2+}], in the pathogenesis of experimental and clinical hypertension was discussed.

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

The role of Mg\textsuperscript{2+} in the pathogenesis of essential hypertension has been extensively studied because Mg\textsuperscript{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\textsuperscript{2+} in hypertension see [8]. In humans, serum and erythrocytes are easily available, so that their Mg\textsuperscript{2+} content has been often determined in studies on the role of Mg\textsuperscript{2+} in hypertension. In patients with essential hypertension, serum Mg\textsuperscript{2+} concentration was found unchanged [9-11] or slightly reduced [11]. Controversial results were reported for erythrocytes of hypertensives: total Mg\textsuperscript{2+} content was unchanged [9, 11], reduced [11] or increased [10, 11]. Also, [Mg\textsuperscript{2+}], of erythrocytes was insignificantly changed [5, 11], reduced [5, 11, 12] or increased [5, 11, 13]. [Mg\textsuperscript{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 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 intracellular [Mg$^{2+}$]$_i$, as measured by $^{31}$P-NMR [18]. For more literature see [8].

A mechanism for reduced total Mg$^{2+}$ and [Mg$^{2+}$]$_i$ 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$^+$]$_i$ and a decrease in [Mg$^{2+}$]$_i$ 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® (pentobarbital sodium) was obtained from Abbott (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 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® 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. 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%.

### Abbreviations

- ACTH: adrenocorticotropic hormone
- MAP kinase: mitogen-activated protein kinase
- MDCK: Madin-Darby canine kidney cells
- [Na$^+$]$_i$, [Mg$^{2+}$]$_i$: intracellular concentrations of Na$^+$ and free Mg$^{2+}$
- PKC: protein kinase C
- PMA: phorbol 12-myristate 13-acetate
- pMg: -log [Mg$^{2+}$]
- 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.

**Mg\(^{2+}\) loading**

A 10% (v/v) erythrocyte suspension of WKY and SHR was incubated at 37°C for 30 min in Mg\(^{2+}\) loading medium containing (in mmol/L) 140 KCl, 50 sucrose, 5 D-glucose and 10 Hepes-Tris, pH 7.4, 12 MgCl\(_2\) and 6 mmol/L A 23187 (dissolved in dimethyl sulfoxide).

After 30 min, the ionophore was removed by incubating the cells 4 times in ionophore-free Mg\(^{2+}\) loading medium plus 1% bovine serum albumin for 10 min at 37°C. Thereafter, the erythrocytes were washed 2 times in cold incubation medium.

**Mg\(^{2+}\) 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 Mg\(^{2+}\), the supernatant was diluted with TCA. The final concentration of TCA was 5% (w/v), containing 0.1% (w/v) La\(_2\)O\(_3\) and 0.16% (v/v) HCl. Mg\(^{2+}\) was measured in triplicates by means of atomic absorption spectrometry (Perkin Elmer, 2380). Mg\(^{2+}\) efflux was calculated from the increase in extracellular Mg\(^{2+}\) 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.

**Erythrocyte Mg\(^{2+}\) content**

Prior to incubation, Mg\(^{2+}\) 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 La\(_2\)O\(_3\)-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.

**Table 1. Mg\(^{2+}\) concentration in plasma (mmol/L) and erythrocytes (mmol/L cells) of WKY and SHR.**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>WKY</th>
<th>SHR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Mg(^{2+})</td>
<td>8</td>
<td>0.71 ± 0.02</td>
<td>0.73 ± 0.02</td>
<td>0.2084</td>
</tr>
<tr>
<td>Erythrocyte Mg(^{2+})</td>
<td>14</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.

**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].

**Mg\(^{2+}\) content in plasma and erythrocytes**

As shown in table 1, the concentration of plasma Mg\(^{2+}\) was the same in WKY and SHR, confirming some of the previous findings [15-17]. Also, in our study the concentration of total erythrocyte Mg\(^{2+}\) was the same in WKY and SHR, which is in accordance with other authors [17].

**Mg\(^{2+}\) efflux from erythrocytes**

Intracellular free Mg\(^{2+}\) is the active fraction of total Mg\(^{2+}\) and is in equilibrium with functionally active Mg\(^{2+}\) complexes. Since [Mg\(^{2+}\)], can be regulated by Mg\(^{2+}\) efflux, we investigated Mg\(^{2+}\) efflux from erythrocytes of WKY and SHR.

As shown in figure 1, Mg\(^{2+}\) efflux from Mg\(^{2+}\)-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, Mg\(^{2+}\) efflux in KCl medium and KSCN medium did not differ significantly.

In Na\(^+\)-containing media, Mg\(^{2+}\) efflux of Mg\(^{2+}\)-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, Mg\(^{2+}\) efflux in KCl medium and KSCN medium did not differ significantly.

In Na\(^+\)-containing media, Mg\(^{2+}\) efflux of Mg\(^{2+}\)-loaded erythrocytes is almost completely mediated by Na\(^+\)/Mg\(^{2+}\) antiport. Moreover, in Mg\(^{2+}\)-loaded erythrocytes the Na\(^+\)/Mg\(^{2+}\) antiport operates under nearly V\(_{\text{max}}\) conditions. Therefore, the reduction in Na\(^+\)/Mg\(^{2+}\) antiport may be caused by a reduction in the number of Na\(^+\)/Mg\(^{2+}\) antiporter molecules.

Since, in vivo, erythrocytes are not loaded with Mg\(^{2+}\), and their Na\(^+\)/Mg\(^{2+}\) antiport is operating under “non Mg\(^{2+}\)-loaded conditions”, we next investigated Mg\(^{2+}\) efflux in non Mg\(^{2+}\)-loaded erythrocytes of WKY and SHR. These experiments were possible because the Na\(^+\)/Mg\(^{2+}\) antiport in rat erythrocytes is 65 times
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.

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.

<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</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.
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

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**Table 3.** Effect of PMA and staurosporine on Mg2+ efflux (µmol/L cells·2 h) of non Mg2+-loaded erythrocytes of WKY and SHR in NaCl medium and KCl medium. The concentration of PMA was 1 µmol/L, of staurosporine 0.5 µmol/L. A SHR is the difference of Mg2+ 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>Staurosporine</td>
<td>158.4 ± 4.1</td>
<td>137.9 ± 10.6</td>
<td>-20.5*</td>
</tr>
<tr>
<td>KCl</td>
<td>Control</td>
<td>127.0 ± 5.7</td>
<td>125.6 ± 9.4</td>
<td>-1.4 n.s.</td>
</tr>
<tr>
<td></td>
<td>PMA</td>
<td>241.0 ± 6.0</td>
<td>236.3 ± 12.1</td>
<td>-4.7 n.s.</td>
</tr>
<tr>
<td></td>
<td>Staurosporine</td>
<td>95.7 ± 5.6</td>
<td>104.6 ± 11.2</td>
<td>8.9 n.s.</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 Staurosporine in WKY versus SHR was always p < 0.05. Values for A PMA and A 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 Mg²⁺ content between WKY and SHR. In SHR, the activity of Na⁺/Mg²⁺ antiport in erythrocytes under $V_{\text{max}}$ conditions was reduced by 16%, as found with Mg²⁺-loaded erythrocytes, and by 10% in non Mg²⁺-loaded erythrocytes.

**Controversial results on Mg²⁺ content in plasma and erythrocytes in hypertension**

Controversial results have been reported for the content of Mg²⁺ 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 Mg²⁺ 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 Mg²⁺ deficiency should be considered as a means for elucidating the role of Mg²⁺ in human hypertension. In acute experimental Mg²⁺ deficiency, blood pressure is reduced by an increase in NO production [31, 32] and by hyper-tension due to increased release of histamine and serotonin [33]. The release of these substances is caused by a reduced Mg²⁺/Ca²⁺ antagonism in hypomagnesemia.

Chronic Mg²⁺ 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 Mg²⁺ 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 cortisol and thyroxine, induced by ACTH and TSH [39, 40]. The increased release of catecholamines will induce Mg²⁺ efflux, e.g. from cardiomyocytes [41, 42], lymphocytes [43], liver [44, 45] and from bones [46]. Furthermore, the increased catecholamines will reduce glomerular filtration. A decrease in Mg²⁺ excretion can thereby be expected. Thus, plasma Mg²⁺ concentration may increase in catecholamine-induced hypertension. On the other hand, catechola-mines increase intracellular lipolysis in fat cells. The resulting free fatty acids will bind Mg²⁺, followed by a reduction of [Mg²⁺], in fat cells. This may induce an uptake of extracellular Mg²⁺ into fat cells [47]. In patients or rats having a high fat content, this mechanism may lead to a reduction of plasma Mg²⁺. When both these counteracting effects are differently expressed in individuals, an opposite effect on plasma and erythrocyte Mg²⁺ may occur in different hypertensives. Thus, serum Mg²⁺ can be independent of blood pressure. Erythrocyte Mg²⁺ may follow the alteration in plasma Mg²⁺. Moreover, an increase in extracellular Mg²⁺ can reduce the release of catecholamines through competition with extracellular Ca²⁺.

Alltogether, in patients with essential hypertension and in SHR, catecholamines and other hormones may play the major role in the pathogenesis of hypertension. Moreover, Mg²⁺ deficiency must exceed a certain degree in order to increase catecholamines [38] and induce toxic effects [48-50].

As seen from the various Mg²⁺ supplementation experiments with SHR, Mg²⁺ 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, Mg²⁺ supplementation had no significant effect on blood pressure [19]. As with DOCA-salt-induced hypertension, Mg²⁺ supplementation reduced blood pressure only by 10% [54], whereas in another study, oral Mg²⁺ supplementation completely prevented hypertension in DOCA-salt-treated rats [55]. In patients, Mg²⁺ supplementation had only a small or no significant effect [56, 57], probably depending on Mg²⁺ dosage or drug pre-treatment, e.g. with diuretics.

**Effect of extracellular Mg²⁺ on VSMC in vitro**

Evidence of a role played by Mg²⁺ in the pathogenesis of hypertension comes from experiments with isolated blood vessels and blood vessel preparations. In vitro, it was possible to induce contraction and relaxation of smooth muscle cells by drastically reducing or increasing the extracellular Mg²⁺ concentration [58]. Also, the effect of vasoconstrictors was increased at drastically reduced extracellular Mg²⁺ concentrations [11, 58, 59], particularly in SHR [60]. This modulation of vascular tone is affected by an altered Ca²⁺ influx due to a changed Mg²⁺/Ca²⁺ competition [1]. Unfortunately, in these experiments, in the controls an extracellular Mg²⁺ concentration of 1.2 mmol/L was used in a protein-free incubation medium, which had twice the physiological concentration of free Mg²⁺ in plasma. In these in vitro experiments with drastically reduced extracellular Mg²⁺ concentrations, vasoconstriction occurred immediately. In contrast, during in vivo experiments
with rats, when the reduction of extracellular Mg²⁺ was less pronounced, hypertension occurred only after 15 weeks and there was an additional effect of vasoactive substances (see above). Thus, in Mg²⁺-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 Mg²⁺ itself.

**Down regulation of Na⁺/Mg²⁺ antiport in erythrocytes of SHR**

Mg²⁺ efflux from non Mg²⁺-loaded rat erythrocytes in NaCl medium, via Na⁺/Mg²⁺ antiport, and Mg²⁺ efflux in KCl medium by the 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 [20]. However, we detected no difference between WKY and SHR in the regulation of Mg²⁺ efflux by PKA- and staurosporine-sensitive protein kinases. Also the regulation of Na⁺/Mg²⁺ antiport by intracellular Cl⁻, as recently described by us [30], was not different between WKY and SHR. Since the regulation of Na⁺/Mg²⁺ antiport via PKC and intracellular Cl⁻ was not changed in SHR, the reduction of the Na⁺/Mg²⁺ antiport activity may have been caused by a reduction in the number of Na⁺/Mg²⁺ antiporter molecules in erythrocytes of SHR. The mechanism may be a down regulation in the biosynthesis of Na⁺/Mg²⁺ antiporter molecules in erythrocytes of SHR. As discussed below, in VSMC of SHR, activity of Na⁺/Mg²⁺ antiport was increased. From the different behavior of Na⁺/Mg²⁺ antiport in erythrocytes and VSMC a mutation of the Na⁺/Mg²⁺ antiporter as an alternative mechanism leading to a reduced V_max of Na⁺/Mg²⁺ antiport in erythrocytes of SHR seems to be less probable.

**Role of the Na⁺/Mg²⁺ antiport in hypertension**

No consistent change has been described for Na⁺/Mg²⁺ antiport of erythrocytes in hypertension. In one study, half of the hypertensive patients showed an increase in Na⁺/Mg²⁺ antiport in Mg²⁺-loaded erythrocytes. Consequently, it was suggested that only a subgroup of patients with essential hypertension may be Mg²⁺-sensitive [21]. In another study, with hypertensive patients suffering from latent tetany, no significant alteration of the Na⁺/Mg²⁺ antiport in Mg²⁺-loaded erythrocytes was found [20]. Obviously, different pathogenic mechanisms not reflected by an alteration of Na⁺/Mg²⁺ antiport in erythrocytes may have induced hypertension in different types of patients.

From experiments with angiotensin II, participation of the Na⁺/Mg²⁺ 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 [Mg²⁺], in VSMC, in platelets and MDCK cells [61-63]. The increase in blood pressure and the alterations in [Na⁺], and [Mg²⁺], could be attenuated by imipramine and quinidine, which are unspecific inhibitors of the Na⁺/Mg²⁺ antiport [22, 63]. These results led to the conclusion that the Na⁺/Mg²⁺ 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 [Mg²⁺], [Na⁺], increased by about 20 mmol/L, whereas the decrease in [Mg²⁺], amounted only to 0.1-0.2 mmol/L [61-63]. However, the effect of angiotensin II on [Mg²⁺], seems to be complex and unsettled. Short-term exposure for 5-10 min of isolated, cultured VSMC to angiotensin II reduced [Mg²⁺], by stimulation of the Na⁺/Mg²⁺ antiport in vivo with rats. After chronic incubation of VSMC cultured for 24-30 h with angiotensin II, [Mg²⁺], was increased due to the stimulation of TRPM 7 channels [64].

A reduction of [Mg²⁺], 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 [Mg²⁺], was lower by 0.066 mmol/L [65]. In some tissues of SHR, [Mg²⁺], 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 Mg²⁺ 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 Mg²⁺ amounted to about 10 mmol/kg dry weight [67, 68]. Similarly, [Mg²⁺], in platelets of patients with essential hypertension was lower by 0.21 mmol/L [69], although other authors found an increase in [Mg²⁺], by 0.08 mmol/L [14].

Cumulative evidence of these findings does not support the idea that activation of Na⁺/Mg²⁺ antiport in vascular smooth muscle plays a major role in the pathogenesis of hypertension. The reasons are the following. In the Na⁺/Mg²⁺ antiport the stoichiometric ratio of transported Na⁺:Mg²⁺ is 1:1 or 2:1, which is not in accordance with the drastic increase of [Na⁺], compared with the rather small decrease of [Mg²⁺]. Furthermore, it is uncertain whether the small measured decreases in [Mg²⁺], from 0.1 to 0.2 mmol/L are by themselves sufficient to induce hypertension. Metabolic and functional effects of Mg²⁺-
rel} on the pMg, and on the pMg-dependency of Mg$^{2+}$-activated enzymes and proteins. Moreover, intracellular Mg$^{2+}$/Ca$^{2+}$ competition depends on the Ca$^{2+}$ and Mg$^{2+}$ affinity of the Ca$^{2+}$-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 [Mg$^{2+}$], and the increase of [Ca$^{2+}$], in VSMC [70] and consequently the altered Mg$^{2+}$/Ca$^{2+}$ competition in VSMC may play a major role.

In the intracellular ionic alterations in hypertension, besides an activation of Na$^+$/Mg$^{2+}$ antiport, a change of other transport systems such as the Na$^+$/H$^+$ antiport, the Na$^+$/Ca$^{2+}$ 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 [Mg$^{2+}$], in various cell types of hypertensive patients and SHR may well be caused by an increased Mg$^{2+}$ efflux via Na$^+$/Mg$^{2+}$ 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, growth factors, 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 Mg$^{2+}$ content. The reduction in Na$^+$/Mg$^{2+}$ antiport in SHR erythrocytes is not related to the increase in blood pressure. Erythrocytes are not a suitable source for studying the role of Mg$^{2+}$ in the pathogenesis of clinical hypertension and SHR. Acute and chronic experimental Mg$^{2+}$ deficiency reduces or increases systolic blood pressure in rats, although the extracellular Mg$^{2+}$ concentration may be similarly reduced, indicating that the superimposed endothelium situation determines blood pressure. Severe and chronic experimental Mg$^{2+}$ deficiency is a risk factor for hypertension, mainly due to the chronically increased release of catecholamines. In vitro, only extremely low extracellular Mg$^{2+}$ concentrations can increase the contraction of vascular smooth muscle cells and reinforce the effects of vasoconstrictors due to a changed Ca$^{2+}$/Mg$^{2+}$ competition. An alteration of the Na$^+$/Mg$^{2+}$ antiport and Mg$^{2+}$ metabolism in VSMC may participate in the pathogenesis of SHR, angiotensin II-induced and essential hypertension. With respect to the Na$^+$/Mg$^{2+}$ ratio of the Na$^+$/Mg$^{2+}$ 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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