Influence of Magnesium on Blood Pressure and the Effect of Nifedipine in Rats

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SUMMARY The influence of long-term alterations in dietary magnesium intake on blood pressure and on the antihypertensive effect of the calcium antagonist nifedipine was investigated in normotensive Wistar-Kyoto (WKY) and in spontaneously hypertensive rats (SHR). The rats were fed a diet either high (1%), normal (0.1%), or low (0.01%) in magnesium for 12 weeks (WKY) and 20 weeks (SHR), respectively. Nifedipine was added to the diet for 4 weeks in concentrations of 300 and 1000 ppm. Each dose was given for 2 weeks. Plasma and intraerythrocytic concentrations of sodium, potassium, and magnesium were measured before and at the end of nifedipine treatment. Blood was obtained by cardiac puncture. In the WKY and SHR, blood pressure was not influenced by magnesium intake. The blood pressure-lowering effect of nifedipine was most pronounced on normal dietary magnesium and was significantly suppressed in the magnesium-deficient rats. Plasma and intracellular total magnesium concentrations were consistently increased during high and reduced during low dietary intake of the ion. Intracellular sodium concentration increased during magnesium deficiency and was normalized by nifedipine. The marked and long-term alterations in plasma and intracellular concentrations of magnesium did not influence arterial blood pressure levels in either the normotensive WKY or the SHR. Therefore, dietary magnesium intake does not appear to play an important role in long-term regulation of blood pressure in rats. However, magnesium depletion attenuates the blood pressure-lowering effect of nifedipine. (Hypertension 9: 139-143, 1987)

KEY WORDS • magnesium • nifedipine • intracellular electrolytes • hypertension • spontaneously hypertensive rats

It has been suggested that the divalent cation magnesium plays an important role in the regulation of blood pressure. This suggestion is based on the observation that magnesium depletion enhances vascular reactivity to vasoconstrictor agents and that it may accelerate cardiovascular disease. In contrast, magnesium excess stabilizes vascular membranes and reduces vascular tone. An inverse relationship between intracellular free magnesium and systolic and diastolic blood pressure has been reported. Epidemiological data suggest that a diet rich in magnesium may protect against cardiovascular events. However, the available data on the long-term importance of magnesium for blood pressure regulation in human and experimental hypertension are limited and controversial.

In normotensive rats blood pressure has been reported to be reduced during magnesium deficiency. In spontaneously hypertensive rats (SHR), high dietary magnesium intake has been found to lower blood pressure. However, Günther et al. did not observe a relation between the development of hypertension and magnesium intake in SHR. In essential hypertension, a diet rich in magnesium appears to enhance the antihypertensive effect of thiazide diuretics.

Experimental evidence suggests that magnesium affects cell function and vascular tone through its effects on calcium handling. Magnesium deficiency may produce vasoconstriction by allowing excess entry and intracellular release of calcium. Therefore, changes in intracellular or extracellular concentrations of magnesium might also affect the therapeutic efficacy of calcium entry blockers.

In the present study, the influence of long-term alterations in dietary magnesium on blood pressure and
on the antihypertensive effect of nifedipine was investigated in normotensive Wistar-Kyoto rats (WKY) and in SHR. The results suggest that dietary magnesium intake is not important in long-term blood pressure regulation but that the vasodilator effects of calcium antagonists are partly dependent on intracellular and extracellular magnesium concentrations.

Materials and Methods

Ten-week-old SHR (Okamoto-Aoki strain) and normotensive WKY were obtained from Charles River Wega (Sulzfeld, West Germany). For 2 weeks before the experiment began, all rats received a diet with a normal magnesium content, during which the animals became accustomed to the blood pressure measurements. Distilled water was given ad libitum as drinking fluid throughout the experiment. All procedures followed were in accordance with the institutional guidelines.

The WKY and SHR were subdivided into three groups each. All experimental groups consisted of eight animals. The rats were fed a diet either high (1%), normal (0.1%), or low (0.01%) in magnesium (Ssniff; Soest, West Germany). Except for its concentration of magnesium, the composition of the three diets was identical. The different diets were prepared by adding monomagnesium-1-aspartate hydrochloride to a magnesium-deficient rat chow (0.01% Mg2+). The concentrations of magnesium in the diet were checked by atomic absorption spectrophotometry before feeding the chow to the rats. Mg-aspartate-HCl was chosen because it does not affect acid-base status, in contrast to such other magnesium salts as MgO, MgSO4, or MgCl2. The experimental period was 20 weeks for the SHR and 12 weeks for the WKY. From Weeks 9 to 12, the calcium antagonist nifedipine was added to the diet in concentrations of 300 and 1000 ppm. Each dose was given for 2 weeks. The daily food intake was randomly tested. The estimated intake did not differ between the experimental groups. Nifedipine was generously supplied by Bayer AG, Wuppertal, West Germany.

Body weight and systolic blood pressure were measured weekly. Systolic blood pressure was determined by a tail-cuff method in the conscious animal using a programmed electrosphygmomanometer (Infraton Tensiomet FIB 4/2; Boucke, Tübingen, West Germany) and a rat holder temperature control unit (Narco-Biosystems, Houston, TX, USA).

Blood for the measurements of plasma and intracellular total concentrations of sodium, potassium, and magnesium was obtained in a heparinized syringe by cardiac puncture with the rats under light ether anesthesia at the end of Weeks 8 and 12 of the experiment. From the obtained blood, aliquots were separated for the measurements of hematocrit and hemoglobin. The remainder was centrifuged at 4000 g at 0°C for 10 minutes, and plasma and buffy coat were removed. In plasma, concentrations of sodium and potassium were determined by flame photometry. The plasma concentrations of magnesium and the intracellular concentrations of sodium, potassium, and magnesium were measured by atomic absorption spectrophotometry (Model 2280; Perkin Elmer, Norwalk, CT, USA) as previously described by Duhm and Göbel.13 In brief, four aliquots of packed red blood cells (100 μl) were washed three times in 2 ml of a solution containing 150 mmol choline chloride, 10 mmol Tris morpholinepropanesulfonic acid, 5 mmol dextrose, and 1 mmol phosphoric acid, pH 7.4, at 37°C. The suspensions were centrifuged at 4000 g for 4 minutes. The packed red blood cells were hemolyzed with 1.6 ml of 6% 1-butanol and stored at 4°C until the measurements of intracellular concentrations of sodium, potassium, and magnesium were performed. Hemoglobin was determined in the hemolysates as cyanohemoglobin. The red blood cell cation contents were calculated from hemoglobin and cation concentrations in the lysates on the basis of the mean cellular hemoglobin content as determined in the fresh blood. Measurements were done in triplicate. Intracellular electrolyte concentrations are given as millimoles per liter of cells.

Statistical analysis was performed by analysis of variance and by Student's t test for paired and unpaired data. A p value below 0.05 was considered significant. All values are given as the mean ± SEM.

Results

In the normotensive WKY, systolic blood pressure was not influenced by either high or low magnesium intakes as compared with normal dietary magnesium (Figure 1). Nifedipine in a dietary concentration of 300 ppm lowered blood pressure significantly during normal magnesium intake. The higher dose of nifedipine (1000 ppm) had no additional effect. In the normotensive rats receiving a low or high magnesium intake, blood pressure was reduced only slightly by nifedipine (Figure 1). In the SHR, blood pressure was not influenced by the different dietary intakes of magnesium but fell significantly in all groups of rats when nifedipine, 300 ppm, was given (Figure 2). The effect of the calcium antagonist was slightly enhanced by increasing the dose to 1000 ppm. The response to nifedipine was most pronounced in the rats on normal magnesium intake. The blood pressure-lowering effect of nifedipine was significantly blunted during low dietary magnesium. After withdrawal of nifedipine, blood pressure rose to pretherapeutic levels in all groups.

The increase in body weight during the observation period was comparable in all groups and was not influenced by the concentration of magnesium in the diet (Table 1). Plasma concentrations of sodium and potassium were within the normal range in all dietary groups and were not altered by nifedipine (Table 2). As compared with normal magnesium intake (0.1%), the plasma concentrations of magnesium were significantly reduced during low and significantly increased during high magnesium intake in both the SHR and WKY (see Table 2).

Regardless of magnesium intake, intracellular sodi-
um and potassium concentrations were higher in the SHR than in the WKY, whereas intracellular total magnesium concentrations were similar in both groups of rats (Table 3). Intracellular sodium concentrations were elevated during magnesium depletion in both the SHR and WKY. Nifedipine lowered intracellular sodium in the rats receiving low or high dietary intakes of magnesium. Magnesium intake and treatment with nifedipine had no clear-cut influence on intracellular potassium concentrations. Intracellular magnesium concentrations were clearly reduced during magnesium depletion and slightly but significantly increased during high dietary magnesium. Nifedipine did not influence intracellular magnesium concentrations.

Discussion

In the present study, the influence of long-term alterations in dietary magnesium on blood pressure was investigated in normotensive WKY and SHR. Neither magnesium deficiency nor a diet high in magnesium appeared to influence blood pressure significantly.

As early as 1925 it was suggested that magnesium salts might be of value in the treatment of hypertension. Intra-arterial infusions of magnesium salts provoke an acute vasodilator response and attenuate the pressor effect of vasoconstrictor agents. The therapeutic efficacy of magnesium in eclamptic women is widely recognized and used. Magnesium appears to enhance the antihypertensive effect of diuretics, an effect that may be due to correction of diuretic-induced
hypomagnesemia. The rates of vascular disease seem to be lower in hard water areas than in soft water areas, an observation that has been partially ascribed to higher ingestion of magnesium. Whether magnesium intake is related to blood pressure in humans is controversial. No data are available on the influence of long-term dietary supplementation of magnesium in human hypertension. The results obtained in laboratory animals, mainly rats, are contradictory. In normotensive rats, blood pressure has been reported to be reduced during magnesium deficiency. In SHR, blood pressure has been found to be decreased with high magnesium intake and unaltered during magnesium deficiency. In the present study, blood pressure was not influenced despite clear-cut and long-term variations in magnesium intake and subsequent profound differences in plasma and intracellular concentrations of magnesium. Plasma concentrations of magnesium were similar to those obtained in previous studies.

Recently, Resnick et al. reported an inverse relationship between intracellular free magnesium in erythrocytes and blood pressure in essential hypertension. In the present study, no correlation could be observed between intracellular total magnesium concentration and systolic pressure in rats. Furthermore, intracellular total magnesium concentrations were not different between normotensive WKY and SHR. However, a relation between blood pressure and magnesium may have been missed in the present study, since intracellular free magnesium was not measured. When the data of Resnick et al. are compared with our values, it becomes clear that intracellular free magnesium may represent only 10 to 15% of intracellular total magnesium. Total and free magnesium may not necessarily be correlated. Therefore, an abnormal intracellular disposition of free magnesium in the SHR cannot be excluded.

In contrast to intracellular total magnesium, intracellular sodium and potassium concentrations both were significantly higher in the hypertensive animals. Dietary magnesium deficiency increased intracellular sodium in the SHR and WKY and may have been due to an inhibition of Na⁺,K⁺-adenosine triphosphatase during magnesium deficiency. It has been postulated that an increase in intracellular sodium may give rise to an increase in intracellular calcium concentration and thereby increase blood pressure. In the present study, however, blood pressure did not increase during magnesium deficiency despite a significant rise in intracellular sodium concentration. Administration of the calcium antagonist nifedipine lowered intracellular sodium in the magnesium-deficient and the magnesium-supplemented rats. Calcium antagonists are thought to lower peripheral vascular resistance by reducing the free calcium concentration in smooth muscle cells. Gray et al. observed that verapamil could reduce a defect in sodium transport in leukocytes in essential hypertension. They concluded that a reduced intracellular calcium content brought about by a calcium antagonist may increase the sodium efflux rate constant. This effect could explain the reduction in intracellular sodium concentration during treatment with nifedipine observed in the present study.

It has been proposed that the influence of magnesium on vascular smooth muscle is related to its influence on intracellular calcium metabolism. Magnesium is an antagonist of calcium entry into the cell. Furthermore, magnesium may antagonize calcium binding at intracellular sites, stimulate calcium influx into the sarcoplasmic reticulum, and retard calcium efflux into the extracellular space. Thus, the antihypertensive action of calcium antagonists may be altered by changes in dietary magnesium intake with subsequent changes in intracellular magnesium content. This hypothesis is supported by the findings of

### Table 3. Intracellular Electrolyte Concentrations After 8 Weeks of Different Magnesium Intakes and After an Additional 4 Weeks of Treatment with Nifedipine in SHR and Normotensive WKY

<table>
<thead>
<tr>
<th>Diet</th>
<th>Week</th>
<th>Sodium (mmol/L cells)</th>
<th>Potassium (mmol/L cells)</th>
<th>Magnesium (mmol/L cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01% Mg</td>
<td>8</td>
<td>5.86 ± 0.2*#</td>
<td>111.1 ± 2.7#</td>
<td>1.37 ± 0.04*</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>4.36 ± 0.15#</td>
<td>104.9 ± 2.4†+#</td>
<td>1.3 ± 0.05*</td>
</tr>
<tr>
<td>0.1% Mg</td>
<td>8</td>
<td>4.52 ± 0.11#</td>
<td>114.8 ± 1.9#</td>
<td>2.14 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>4.45 ± 0.07#</td>
<td>112 ± 1#</td>
<td>2.22 ± 0.02</td>
</tr>
<tr>
<td>1% Mg</td>
<td>8</td>
<td>4.56 ± 0.07#</td>
<td>115.4 ± 1.6#</td>
<td>2.3 ± 0.01†</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>4.04 ± 0.05†+§</td>
<td>107.9 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01% Mg</td>
<td>8</td>
<td>4.02 ± 0.08†</td>
<td>95.9 ± 2.2†</td>
<td>1.25 ± 0.04*</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3.49 ± 0.08†§</td>
<td>95.9 ± 1.5*</td>
<td>1.6 ± 0.3†</td>
</tr>
<tr>
<td>0.1% Mg</td>
<td>8</td>
<td>3.62 ± 0.1</td>
<td>102.5 ± 2.2</td>
<td>1.91 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3.89 ± 0.15</td>
<td>105.5 ± 1.1</td>
<td>2.07 ± 0.03</td>
</tr>
<tr>
<td>1% Mg</td>
<td>8</td>
<td>3.78 ± 0.05</td>
<td>94.7 ± 1.4†</td>
<td>2.21 ± 0.03†</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3.3 ± 0.05†</td>
<td>91.7 ± 1.4*</td>
<td>2.04 ± 0.13</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

* p < 0.001, †p < 0.01, ‡p < 0.05, compared with normal magnesium intake (0.1% Mg).

§p < 0.001, †p < 0.05, ‡p < 0.01, compared with Week 8 value.

#p < 0.001, compared with value for WKY.
Phillips and Robinson, 23 who observed an enhanced vasodilator response to verapamil in essential hypertension when magnesium was infused intra-arterially, whereas calcium infusions attenuated the effect of the calcium antagonist. In the present study, the blood pressure-lowering effect of nifedipine was significantly reduced during magnesium deficiency. The profound suppression of intracellular magnesium content during dietary magnesium depletion may have increased intracellular calcium concentration and altered calcium distribution within the cell. Therefore, the attenuated response to nifedipine during magnesium deficiency may be a consequence of changes in intracellular calcium activity. However, this explanation remains hypothetical, since intracellular and extracellular levels of total and ionized calcium were not determined in the present study.

In conclusion, even profound and long-term alterations in plasma and intracellular concentrations of total magnesium did not influence arterial blood pressure levels in either the normotensive WKY or the SHR. Therefore, dietary magnesium intake does not appear to play an important role in long-term regulation of blood pressure in rats. However, magnesium depletion attenuates the blood pressure-lowering effect of the calcium antagonist nifedipine.

References

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