Effect of magnesium on vascular reactivity in NOS inhibition-induced hypertension

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ABSTRACT. This study investigated the effect of magnesium on the vascular reactivity of conduit and resistance arteries in a nitric oxide synthase inhibition-induced hypertension model. The aorta and third-order branches of the mesenteric artery were dissected from normotensive control and hypertensive rats, and their constriction and dilation responses in physiological saline solution containing normal (1.2 mM) or high (4.8 mM) magnesium concentrations were examined. The responses of the vessels were evaluated using potassium chloride (KCl) and phenylephrine (Phe), acetylcholine (ACh) and sodium nitroprusside. The Phe-induced constriction response of the aortic rings increased, whereas the ACh-induced dilation response decreased, in the hypertensive group compared to controls, in the presence of a normal magnesium concentration. High magnesium did not alter these responses in either group. Both the KCl- and Phe-induced constriction responses of the mesenteric arteries increased, and the ACh-induced dilation response decreased in the hypertensive group compared to controls, in the presence of a normal magnesium concentration. High magnesium significantly decreased the KCl and Phe-induced constriction and increased the ACh-induced dilation response of the mesenteric arteries in the hypertensive group, while it did not alter these responses in controls. This study suggests that high magnesium improves vascular reactivity of resistance-, but not conduit-type arteries in the nitric oxide synthase inhibition-induced hypertension model.

Key words: hypertension, magnesium, vascular reactivity

Hypertension is a complex and multifactorial disease and is associated with increased peripheral vascular resistance due to changes in vascular structure and function. The functional changes include increased contraction and/or decreased relaxation responses resulting in increased vascular tone [1, 2]. Magnesium is one of the important physiological regulators of blood vessel tone and it has been found that low magnesium levels are associated with hypertension. Low magnesium status has been suggested to contribute to increased peripheral vascular resistance and consequently, elevation of blood pressure in hypertension [3, 4]. Additionally, in vitro studies that investigated the effect of magnesium on vascular function have demonstrated that changes in extracellular magnesium concentrations alter vascular reactivity and tone. Low magnesium concentra-
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...tions increase the contractile response to various agonists such as endothelin-1 (ET-1) and phenylephrine (Phe), while high magnesium concentrations induce opposite effects [5, 6]. In addition, it has been suggested that low magnesium concentrations impair the acetylcholine (ACh)-induced dilation response due to inhibition of nitric oxide (NO) release in the coronary artery [7].

Several studies have reported an inverse relationship between intake of magnesium and hypertension: magnesium deficiency therefore is suggested to be an important risk factor for the development of hypertension [8]. Serum and intracellular magnesium concentrations were consistently lower in various experimental animal models of hypertension such as spontaneously hypertensive rats (SHR) and the deoxycorticosterone acetate (DOCA)-salt rat model. Additionally, magnesium concentrations were lower in tissue cultures of cardiomyocytes, striated muscle and arterial smooth muscle obtained from SHR than in those from normotensive control rats [9, 10].

In the model where hypertension is induced by chronic NO synthase (NOS) inhibition, and in which endothelial dysfunction predominates, low magnesium levels have been measured in many tissues such as erythrocytes, cerebral cortex, renal cortex and medulla, and renal artery and vein [11]. However, no studies have assessed the effect of magnesium on vascular reactivity in this model of hypertension. The goal of this study was to investigate the effect of high magnesium concentrations on vascular constriction and dilation responses in conduit and resistance arteries that play an important role in the modulation of peripheral vascular resistance and blood pressure.

Methods and materials

Animals

Twenty, male, Wistar rats (eight weeks old) were used in this study. The rats were housed in stainless steel cages, with a constant room temperature of 23 ± 2°C and a 12:12-h light-dark cycle. They had free access to standard rat diet (0.15% magnesium content) and drinking water. The experimental protocol was approved by the Animal Care and Usage Committee of Akdeniz University and it followed the guidelines for using animals in experimental research.

Groups

The animals were divided randomly into two groups: control (n = 10) and hypertensive (H; n = 10). Hypertension was induced by oral administration of the NOS inhibitor N-nitro-L-arginine methyl ester (L-NAME; 25 mg/kg/day), dissolved in drinking water, for four weeks in the H group [11-13]. The fluid and food consumption was monitored regularly.

Blood pressure measurement

The systolic blood pressure of the rats was measured using a non-invasive, tail-cuff method. The measurements were made at the start and end of the experiment. Data were obtained with a MAYBPHR 9610-PC unit and MP 150 data-acquisition system (BIOPAC Systems; Santa Barbara, CA, USA). The systolic blood pressure for each rat was calculated by taking the mean of at least four measurements.

Vascular ring preparation

At the end of the experiment, the rats were anesthetized with thiopental sodium (80 mg/kg, ip). Blood samples were drawn from the abdominal aorta, with heparin as the anticoagulant. The blood samples were centrifuged, and the plasma samples obtained were kept at -80°C until magnesium levels were measured. The thoracic aorta and mesenteric vascular bed were excised and transferred to dissecting dishes filled with ice-cold, physiological saline solution (PSS) containing (in) 110mM NaCl, 5mM KCl, 24mM NaHCO3, 1mM KH2PO4, 1mM MgSO4, 2.5mM CaCl2, 0.02mM EDTA, and 10mM glucose. Tissue samples were obtained from the aorta and mesenteric vascular bed for magnesium level measurement, and after immediate weighing; the tissues were kept at -80°C until measurements were performed. The plasma and tissue magnesium levels were measured using an atomic absorption spectrometer (Varian AA280FS, Mulgrave, Victoria, Australia).

Thoracic segments of the aorta and a third-order branch of the superior mesenteric artery were selected as conduit and resistance types of artery. Vessel samples were isolated, cleaned of connective tissue, and dissected into 2 mm-long strips, and then treated with 95% O2 and 5%
CO₂. They were then placed in an organ bath or myograph chamber containing PSS solution, 7.4 pH, at 37°C. Aortic rings were hung on steel wires and tied to an isometric transducer (FDT 1 A-10 MAY, Ankara, Turkey) by a tensed thread under a previously determined, 1g basal tension. They were washed for one hour at 15 minutes intervals. Mesenteric arteries were placed into a myograph set-up (Model 620M, Danish Myotech Technology, Aarhus N, Denmark). Basal wall tensions of vessel segments were calculated using a normalization program (LabChart Pro V7, Ad instruments, Bella Vista, Australia). Each vessel was allowed to rest at the predetermined basal tension for an hour at 37°C, and the PSS solution was changed every 15 minutes. The same experimental protocol was followed for all samples. After an hour-long resting period, the endothelium was examined following vitalization [20 mM KCl + 10⁻⁷ M phenylephrine (Phe, Sigma, St. Louis, USA)]. If the relaxation response to acetylcholine (ACh), (Sigma) was 60% or more, the endothelium was accepted as intact.

Experimental protocols for arteries

Rings were incubated for one more hour in PSS containing normal (1.2 mM) or high (4.8 mM) magnesium concentrations, before and during the applied protocols. During this period, the bath solution was changed every 15 minutes. In between all of the protocols applied, the vascular segments were left to rest for 30 minutes.

Assessment of vasoconstriction responses

The vasoconstriction responses of vessel rings were evaluated using KCl and Phe. The contraction of the vascular smooth muscle was examined by adding increasing doses of KCl (Merck, Darmstadt, Germany) to the bath fluid (20, 40, and 80 mM). The agonist-induced constriction response was evaluated by adding cumulative doses of Phe (10⁻⁶ to 3×10⁻⁵ M) to the bath solution.

Assessment of vasodilation responses

Before dose-response curves were initiated, all arterial rings were preconstricted with Phe (10⁻⁶ M). Endothelium-dependent relaxation was assessed using ACh (10⁻⁶ to 3×10⁻⁵ M). Endothelium-independent relaxation was assessed with sodium nitroprusside (SNP; 10⁻⁹ to 3×10⁻⁴ M, Merck). The Phe-induced steady state of contraction was considered to be 100%, and the relaxation responses were calculated as percentages of this contraction.

Analysis of results

All results are presented as means ± SE. The statistical significances of the dose-response curves were tested using repeated measures ANOVA, followed by the LSD post-hoc test. Differences in maximal effect of the drugs (Emax), systolic blood pressure levels, plasma and tissues magnesium content between the two groups were compared using the Mann-Whitney U test. Paired t tests were used to compare (Emax) and systolic blood pressure levels in the same groups. P value <0.05 was considered significant.

Results

Body weight and fluid and food consumption showed no differences between groups (data not shown).

Blood pressure

The initial systolic blood pressures were similar in both groups. Blood pressure was found to be increased at the end of the experiment in the H group compared to the control group and the basal levels of the H group animals (figure 1, p<0.001).
Table 1. Plasma (mmol/L) and vascular tissue magnesium concentrations (mg/g/wet weight) in control and hypertensive groups.

<table>
<thead>
<tr>
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<th>Control</th>
<th>Hypertensive</th>
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<tr>
<td>Plasma</td>
<td>1.37 ± 0.07</td>
<td>1.12 ± 0.1*</td>
</tr>
<tr>
<td>Aorta</td>
<td>0.071 ± 0.00</td>
<td>0.057 ± 0.01</td>
</tr>
<tr>
<td>Mesenteric vascular bed</td>
<td>0.090 ± 0.01</td>
<td>0.071 ± 0.01*</td>
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*P <0.05 Control versus Hypertensive group.

Plasma and tissue magnesium levels
In the H group of rats, the magnesium levels in plasma and in the mesenteric vascular beds were found to be significantly lower compared with those of the control group (p<0.05, table 1). Magnesium levels in the aortic tissues were found to be low; however, these differences were not significant when compared with those of the control group.

Responses of conduit arteries
All of the dose-response curves and the Emax values obtained for isolated aortic rings are presented in figure 2 and table 2, respectively.

The vasoconstriction responses
The vasoconstrictor dose-response curves and Emax values induced by Phe in the aortic rings were significantly increased in the H group rats compared with those of the control animals (p<0.01, p<0.001, respectively). High magnesium concentrations did not change the Phe-induced constriction responses either in the control or H groups. KCl-induced constriction responses (dose-response curves and Emax values) were similar in control and H rats, and a high magnesium concentration did not alter these responses.

The vasorelaxation responses
The vasorelaxant responses (dose-response curve and Emax values) to ACh were significantly lower in the aorta of the H rats compared with those of the control animals (p<0.001). In addition, high magnesium levels did not elicit significant changes in the responses of the aortas in either of the groups. SNP-induced relaxation responses (dose-response curves and Emax values) were similar in control and H rats, and high magnesium did not alter these responses.

Plasma and tissue magnesium levels
In the H group of rats, the magnesium levels in plasma and in the mesenteric vascular beds were found to be significantly lower compared with those of the control group (p<0.05, table 1). Magnesium levels in the aortic tissues were found to be low; however, these differences were not significant when compared with those of the control group.

Responses of resistance arteries
All of the dose-response curves and Emax values obtained for isolated mesenteric arteries are presented in figure 3 and table 3, respectively.

The vasoconstriction responses
The vasoconstrictor dose-response curves and Emax values for mesenteric arteries induced by Phe were significantly increased in H rats compared with those of the control animals (p<0.05, p<0.01, respectively). A high magnesium concentration significantly decreased the constriction responses to Phe in mesenteric arteries obtained from H animals (p<0.05). KCl-induced constriction responses (dose-response curves and Emax values) were significantly higher in H rats compared with those of the control rats (p<0.05). A high magnesium concentration significantly decreased Emax values, but did not change the dose-response curves to KCl in mesenteric arteries from H animals (p<0.01).

The vasorelaxation responses
The dose response curves to ACh were found to be similar in control and H animals, whereas Emax values were significantly decreased in mesenteric arteries obtained from H rats (p<0.01). A high magnesium concentration increased dilation responses (dose-response curves and Emax values) to ACh in mesenteric arteries from control animals (p<0.01), but only Emax values from H animals (p<0.05). SNP-induced relaxation responses (dose-response curves and Emax values) were similar in control and H rats, and a high magnesium level did not alter these responses.

Discussion
Impairment of vascular reactivity in hypertension is a well-known phenomenon. In this
Figure 2. Constriction and relaxation responses of aortas from control (C) and hypertensive (H) groups bathed in PSS containing 1.2 mM (C and H) or 4.8 mM magnesium. (C-Mg and H-Mg). Relaxation responses are expressed as a percentage of the pre-contraction levels with Phe (10^{-6} M). Cumulative dose-response curves to A) Phe, B) KCl, C) ACh and D) SNP in aortic rings. **P<0.01, ***P<0.001 control versus hypertensive group.

Table 2. Maximal constriction/dilation response values (Emax) to Phe, KCl, ACh and SNP in aortic rings from control and hypertensive groups under normal (1.2 mM) and high (4.8 mM) magnesium concentration incubations.

<table>
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<th>Control</th>
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<tr>
<td></td>
<td>Normal Mg (g)</td>
<td>High Mg (g)</td>
</tr>
<tr>
<td>Phe</td>
<td>2.30 ± 0.09</td>
<td>1.79 ± 0.26</td>
</tr>
<tr>
<td>KCl</td>
<td>3.17 ± 0.19</td>
<td>3.35 ± 0.21</td>
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<tr>
<td>ACh (%)</td>
<td>92.3 ± 3.8</td>
<td>91.7 ± 2.9</td>
</tr>
<tr>
<td>SNP (%)</td>
<td>99.9 ± 0.1</td>
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Phe; phenylephrine, KCl; potassium chloride, ACh; acetylcholine, SNP; sodium nitroprusside. ***P <0.001 control versus hypertensive group.
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**Figure 3.** Constriction and relaxation responses of third order branch of mesenteric artery from control (C) and hypertensive (H) groups bathed in PSS containing 1.2 mM (C and H) or 4.8 mM magnesium (C-Mg and H-Mg). Relaxation responses are expressed as a percentage of the pre-contraction levels with Phe (10⁻⁶ M). Cumulative dose-response curves to **A** Phe, **B** KCl, **C** ACh and **D** SNP in third order branch of mesenteric artery rings. *P < 0.05, **P < 0.01 control versus hypertensive group, #P < 0.05 normal versus high magnesium incubation within groups.

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<th>Control</th>
<th>Hypertensive</th>
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<tbody>
<tr>
<td></td>
<td>Normal Mg</td>
<td>High Mg</td>
</tr>
<tr>
<td>Phe (g)</td>
<td>2.12 ± 0.23</td>
<td>2.18 ± 0.20</td>
</tr>
<tr>
<td>KCl (g)</td>
<td>2.38 ± 0.17</td>
<td>2.86 ± 0.19</td>
</tr>
<tr>
<td>ACh (%)</td>
<td>89.0 ± 2.0</td>
<td>95.9 ± 1.5**</td>
</tr>
<tr>
<td>SNP (%)</td>
<td>94.9 ± 3.8</td>
<td>98.7 ± 0.5</td>
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</table>

Phe; phenylephrine, KCl; potassium chloride, ACh; acetylcholine, SNP; sodium nitroprusside.
*P < 0.05, **P < 0.01 control versus hypertensive group.
#P < 0.05, ##P < 0.01 normal versus high magnesium incubation within groups.
study we demonstrated, for the first time, the impairment of relaxant and constrictive vascular responses in conduit and resistance arterial segments in an NOS-inhibition-induced hypertensive model. Furthermore, high extracellular magnesium improved ACh-induced dilation, and decreased both KCl and Phe-induced constriction responses in resistance arteries.

Systemic hypertension is widely associated with altered arterial responsiveness, vascular remodeling and increased peripheral vascular resistance. Several epidemiological and clinical studies have strongly suggested that magnesium deficiency is associated with hypertension, and may participate in the pathogenesis of hypertension [1, 3, 14]. Magnesium, which is accepted as an important ionic modulator of vascular tone, attenuates agonist-induced vasoconstriction, and improves vascular relaxation responses, thus mitigating the increased peripheral vascular resistance in hypertension. Various experimental studies have also shown that changes in extracellular magnesium concentration affect the vascular reactivity and tone in some hypertension models [15-18].

Endothelium affects the vascular tonus by secreting many vasoactive agents, and contributes to the regulation of peripheral vascular resistance. NO is one of the most important agents that is produced by endothelial nitric oxide synthase (eNOS), and it plays a crucial role in regulation of vascular tone. Systemic hypertension induced by chronic NOS inhibition in rats was first defined as a new, experimental model of hypertension in 1992, allowing further investigation of the involvement of NO in cardiovascular physiology [12]. Several in vitro studies have reported that agonist-induced dilation responses are attenuated, whereas constriction responses are increased in various vascular beds in this hypertension model [12, 13, 19]. Previously, we demonstrated low magnesium levels in many tissues, such as erythrocytes, cerebral cortex, renal cortex and medulla, and renal vessels of NOS inhibition-induced hypertensive animals [11]. Low magnesium may cause an increase in intracellular calcium that leads to the increased myogenic tone seen in hypertension status [36]. There have been no investigations involving the effect of reduced magnesium levels on vascular reactivity in the NOS-inhibition hypertensive model, or the possible corrective effect on the vascular response of adding magnesium ions.

We asked whether high concentrations of magnesium could affect vascular reactivity of conduit (aorta) and resistance (third order branch of mesenteric artery) type arteries in the NOS inhibition-induced hypertension model. Since the results of these two different types of arteries are distinct, we discuss our results in two parts.

**Responses of aortic rings**

The contraction responses were evaluated using KCl as a hyperpolarizing factor and Phe as an agonist, in aortic rings. Our results demonstrated that KCl-induced constriction responses did not alter, whereas Phe-induced responses were greater in the aorta of H animals compared that those of the control group in the presence of PSS containing a normal magnesium concentration. Although some studies have demonstrated unchanged responses [20, 21], an increase in the adrenergic vascular contraction response in the NOS inhibition-induced hypertension model have been shown by many reports [22-25]. In the present study, the PSS that contained a high magnesium concentration did not cause any differences in the constriction responses of aortic rings obtained from both normotensive and hypertensive animals (C-Mg and H-Mg). Similarly, it has been previously demonstrated that high magnesium did not alter the constriction response of aortic rings to endothelin-1 in DOCA-salt hypertensive rats [17]. However, some investigations that used different hypertension models have demonstrated that vasoconstriction increases in magnesium deficiency, and that dietary magnesium supplementation causes a decrease in the contractile responses to various agonists [15, 26]. This discrepancy between these studies and our study may result from the diversity of experimental designs and/or hypertension models.

Relaxation responses were obtained using SNP, as an NO donor and ACh as an agonist in aortic rings. SNP-induced dilation responses did not alter, whereas ACh-induced responses significantly decreased in the aorta of H animals compared that those of the control group. These results are compatible with findings of previous studies [24, 27]. However, as with the constriction responses, in this study, high magnesium did not alter the dilation responses of aortic rings isolated from normotensive and hypertensive animals. Since there are no studies that examine
the impact of high extracellular magnesium on ACh- and/or SNP-induced relaxation responses of aortic rings in NOS inhibition-induced hypertension model, we cannot compare our results with others.

Conduit arteries, such as aorta, have not only a different vascular function from resistance arteries, they also exhibit different structural properties and receptor differences resulting in diverse vascular behaviors [28]. Firstly, the different effects of high magnesium on vascular reactivity of aorta from small mesenteric arteries may be due to the thickness of the wall. Secondly, incubation time might not be sufficient due to the much thicker wall of the aorta compared with mesenteric arteries. On the other hand, hypertensive animals compared to controls presented lower tissue magnesium levels in vessel segment. However, this was not statistically significant for the aorta. Diminution of magnesium levels might not contribute to deterioration in vascular responses in the aorta, so magnesium ions did not affect vascular responses.

Responses of mesenteric rings

Both KCl- and Phe-induced constriction responses were greater in mesenteric arterial segments of hypertensive compared with normotensive rats. The enhancement of the constriction response of resistance arteries in response to norepinephrine has been demonstrated in several studies using the NOS inhibition-induced hypertension model [23, 24]. However, in the present study, high magnesium attenuated the constriction responses of mesenteric arteries in hypertensive rats. Laurant et al. have shown attenuated vasopressin- and norepinephrine-induced vasoconstriction with high magnesium in SHR [6]. It has also been reported that a high extracellular magnesium concentration blunts vasoconstrictor actions [5, 29]: additionally, it is well-known that magnesium regulates intracellular free calcium levels through its negative modulatory effect on calcium channels [30-32]. Thus, the reducing effect of high magnesium on the constriction responses of mesenteric arteries as was demonstrated in this study, might be explained by the mechanism(s) proposed above. On the other hand, magnesium is known to have antioxidative properties and is suggested to preserve glutathione status [33]. Thus, the antiradical effects of magnesium might also contribute to improvement of vascular response.

ACh-induced responses significantly decreased, whereas SNP-induced dilation responses did not alter in mesenteric arteries from H animals, similar to the aortic ring relaxation responses. In our previous studies performed in similar animal hypertension models, we have shown that ACh-induced relaxation responses diminish in small arteries [34, 35]. In the present study, in contrast to the relaxation responses of aortic rings, high magnesium improved ACh-induced relaxation responses in mesenteric arteries, despite the fact that it did not alter SNP-induced vasorelaxation. These results suggest that high magnesium is capable of potentiating the vasorelaxant properties of ACh in mesenteric arteries. There are two hypotheses concerning how magnesium induces relaxation in arteries. One of them involves magnesium’s calcium ion antagonistic action in vascular smooth muscle, this being probably mediated via L-type Ca channels [36, 37]. A second proposes that magnesium also has modulatory effects on endothelium-derived relaxation responses [38]. The relaxatory effect of magnesium has been proposed to be dependent on endothelial function and endothelium-dependent magnesium-induced vasorelaxation has been consistently shown to be mediated by release of NO from endothelium [7, 39]. It should not be forgotten too that magnesium is a co-factor for adenylate cyclase, and it could be speculated to restore the vascular response, partly due to increasing the reduced magnesium levels by incubation with high magnesium ion concentrations [40].

Endothelial dysfunction is an important idea that is suggested to be involved in the pathogenesis of essential hypertension. This process manifest itself as an insufficiency in the production of endothelium-derived relaxing agents in response to ACh, as demonstrated in both humans and experimental animals [41]. Hypertension induced by chronic NOS inhibition is also thought to be a state related to endothelial dysfunction [34]. An ACh-response restored by incubation with magnesium ions suggests that magnesium supplementation may contribute to lowering blood pressure by improving vasoreactivity of resistance arteries that play an important role in the modulation of peripheral vascular resistance and blood pressure. There is mounting evidence showing that magnesium supplementation has a positive effect on hypertension in both animals.
and humans [42-44]. Accordingly, there was not a group that received magnesium-supplementation to the diet with NOS-inhibition hypertension may be the main limitation of this study.

In conclusion, the results of our study show that high extracellular magnesium has different effects on the vascular reactivity of conduit and resistance type arteries in the NOS inhibition-induced hypertension model. High magnesium attenuated constriction, but potentiated ACh-induced relaxation responses in mesenteric arteries, but not aortas. The reduced levels of magnesium in the NOS-induced hypertension model should be considered to be one of the contributory, pathophysiological factors that affect vascular tone.

Disclosure

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References


