Low [Mg$^{2+}$]$_e$ Enhances Arterial Spontaneous Tone via Phosphatidylinositol 3-Kinase in DOCA-Salt Hypertension

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Abstract—Phosphatidylinositol 3-kinase (PI3K) has been implicated in low extracellular Mg$^{2+}$ concentration ([Mg$^{2+}$]$_e$)-induced aortic contraction, and Mg$^{2+}$ deficiency has been associated with hypertension. Moreover, arterial PI3K activity is increased in hypertensive deoxycorticosterone (DOCA)-salt rats. We hypothesized that low [Mg$^{2+}$]$_e$ activates PI3K, eliciting enhanced vascular contraction, PI3K activity, and norepinephrine (NE)-induced contraction. Spontaneous tone was monitored in endothelium-denuded aortic strips from sham and DOCA-salt rats exposed to low Mg$^{2+}$ (0.15 mmol/L), high Mg$^{2+}$ (4.8 mmol/L), or normal (1.17 mmol/L) physiologic salt solution (PSS) in isolated tissue baths. LY294002 (20 μmol/L), a PI3K inhibitor, or vehicle was added (30 minutes), followed by NE (10$^{-9}$ to 3×10$^{-5}$ mol/L). Low [Mg$^{2+}$]$_e$, significantly enhanced tone in aortas from DOCA-salt and sham rats compared with normal PSS (DOCA-salt low [Mg$^{2+}$]$_e$, +51.5±7.0 vs DOCA-salt normal PSS, +7.1±1.4% of initial phenylephrine [PE] contraction), LY294002 and incubation with high Mg$^{2+}$ PSS decreased tone in aortas from DOCA-salt rats (low [Mg$^{2+}$]$_e$, LY294002, −87.5±8.8; normal PSS LY294002, −81.7±13.7; and high [Mg$^{2+}$]$_e$, −31.2±10.8% of initial PE contraction). Low [Mg$^{2+}$]$_e$, leftward-shifted NE-induced aortic contractions in sham and thus matched the shift observed with DOCA (−log EC$_{50}$ mol/L: sham PSS, −7.7±0.1; DOCA-salt PSS, −8.2±0.1; sham low [Mg$^{2+}$]$_e$, −8.2±0.1; and DOCA-salt low [Mg$^{2+}$]$_e$, −8.1±0.1). Moreover, this shift was inhibited by LY294002. In conclusion, low [Mg$^{2+}$]$_e$, might activate PI3K, leading to enhanced tone and agonist-induced contraction observed in aortas from DOCA-salt hypertensive rats. (Hypertension. 2004;43:125-130.)

Key Words: magnesium ■ kinase ■ deoxycorticosterone ■ hypertension, sodium-dependent ■ phosphorylation

Hypertension is associated with altered arterial responsiveness, spontaneous tone development (non–agonist-induced contraction), and vascular remodeling. Phosphatidylinositol 3-kinase (PI3K) plays crucial functional roles in spontaneous tone development and hyperreactivity in thoracic aortas from deoxycorticosterone acetate (DOCA)–salt and Nω-nitro-L-arginine hypertensive rats. PI3K activity and protein, specifically the p110δ class IA PI3K catalytic subunit, is upregulated in the aorta in experimental hypertension. PI3K catalytic subunits can directly associate with L-type Ca$^{2+}$ channels and increase Ca$^{2+}$ current to the cell. Moreover, Ca$^{2+}$-induced spontaneous tone in aortas from DOCA-salt rats is dependent on PI3K. However, it is unclear what stimulates the increase in PI3K activity in the condition of hypertension. Mg$^{2+}$ deficiency is found in hypertension and is also a mechanism suggested to activate a PI3K-dependent signaling pathway. Thus, altered Mg$^{2+}$ might be one explanation for the increase in PI3K observed in hypertension.

Mg$^{2+}$ is a mineral required by every cell of the body and is necessary for >300 biochemical reactions, including maintenance of normal muscle and nerve function, heart rhythm, energy metabolism, and protein synthesis. Intracellular Mg$^{2+}$ concentration ([Mg$^{2+}$]$_e$) regulates contractile proteins; modulates transmembrane transport of calcium, sodium, and potassium; acts as a essential cofactor in activation of ATPases; and influences DNA and protein synthesis. Mg$^{2+}$ concentrations are inversely proportional to blood pressure, with hypomagnesemia associated with hypertension. Mg$^{2+}$ deficiency induces cardiovascular alterations such as elevated blood pressure, enhanced agonist-mediated reactivity, attenuated responses to vasodilators, and increased vascular tone. Serum and erythrocyte Mg$^{2+}$ concentrations are lower in DOCA-salt spontaneously hypertensive rats (SHR) compared with SHR. Serum Mg$^{2+}$ is lower in vascular smooth muscle cells of SHR versus Wistar-Kyoto rats. In the experimental DOCA-salt rat model of hypertension, plasma total and free Mg$^{2+}$ concentrations are significantly lower compared with sham. This study is in contrast to a previous report of similar serum Mg$^{2+}$ concentrations in DOCA-salt and sham rats. The differences in measurements of Mg$^{2+}$ might be due to the technique by which Mg$^{2+}$ was measured, a debate that is also found in measurement of Mg$^{2+}$ levels in humans. Nonetheless, multiple investigators have found that dietary Mg$^{2+}$ supplementation attenuates hypertension in the DOCA-salt rat model of hypertension.
Mg$^{2+}$ utilization/activation of Ca$^{2+}$, Na$^+/K^+$-ATPase, tyrosine kinases, protein kinase C, and mitogen-activated protein kinase components have been implicated in altered vascular tone and/or cellular growth. If Mg$^{2+}$ is a modulator of PI3K, then low Mg$^{2+}$ might be one mechanism that amplifies PI3K activity. Thus, we hypothesized that a decrease in Mg$^{2+}$, a tool for activation of the PI3K signaling cascade, elicits enhanced vascular contraction represented by spontaneous tone development and enhanced norepinephrine (NE)-induced contraction in the aorta.

**Methods**

**DOCA-Salt Hypertension**

Male Sprague-Dawley rats (250 to 300 g; Charles River Laboratories Inc, Portage, Mich) received a DOCA- (200 mg/kg SC, Sigma) impregnated silicone elastomer (Silastic, Dow Corning) implant and uninephrectomy under isoflurane anesthesia, as described previously. Postoperatively, DOCA-salt rats were given a solution of 1% NaCl and 0.2% KCl for drinking. Sham rats also received a uninephrectomy but no implant and drank normal tap water. All animals were fed standard rat chow and had ad libitum access to food and water. After 4 weeks of treatment, systolic blood pressures were measured by standard tail-cuff procedures.

**Isolated Tissue Bath Protocol**

Thoracic aortas were removed from pentobarbital- (60 mg/kg IP) anesthetized rats, cut into helical strips, and denuded of endothelial cells with a moistened cotton swab. The strips were pair-mounted (sham/DOCA) in isolated tissue baths containing warmed (37°C), aerated (95% O$_2$/CO$_2$) physiologic salt solution (PSS: 103 mmol/L NaCl, 4.7 mmol/L KCl, 1.18 mmol/L KH$_2$PO$_4$, 1.17 mmol/L MgSO$_4$, 7H$_2$O, 1.6 mmol/L CaCl$_2$, 2H$_2$O, 14.9 mmol/L NaHCO$_3$, 5.5 mmol/L dextrose, and 0.03 mmol/L Ca$_2$EDTA) for measurements of isometric force. Tissues were challenged with the α-adrenergic agonist phenylephrine (PE, 10$^{-6}$ mol/L) to ensure arterial strip viability. Functional integrity of endothelial cells was evaluated by testing endothelium-dependent relaxation to acetylcholine (10$^{-6}$ mol/L) in half-maximal PE-contracted strips. Tissues were incubated in PSS buffer containing low extracellular Mg$^{2+}$ concentration ([Mg$^{2+}$]$_e$) (0.15 mmol/L), normal, or high ([Mg$^{2+}$]$_e$) (4.8 mmol/L) PSS for 30 minutes. The buffer was changed every 10 minutes to permit equilibration. After 30 minutes of incubation, changes in spontaneous tone were recorded in response to the respective changes in Mg$^{2+}$. LY294002 (20 μmol/L) or vehicle (0.1% dimethyl sulfoxide) was incubated in the baths for 30 minutes before the addition of increasing concentrations of NE (1×10$^{-9}$ to 3×10$^{-6}$ mol/L).

**PI3K Activity Assay**

Rat thoracic aortas were cleaned and placed in isolated tissue baths, incubated in low [Mg$^{2+}$], and normal PSS for 30 minutes, allowed to develop tone as stated earlier, and then frozen in LN$_2$. Aortas were pulverized in an LN$_2$-cooled mortar and solubilized in PI3K lysis buffer (20 mmol/L Tris, pH 7.6; 10% glycerol; 1% NP-40; 140 mmol/L NaCl, 2.5 mmol/L CaCl$_2$, 1 mmol/L MgCl$_2$; 1 mmol/L Na$_3$VO$_4$; 1 mmol/L dithiothreitol; and 1 mmol/L phenylmethylsulfonyl fluoride). Equal amounts of protein were immunoprecipitated for PI3K with the antibody for the regulatory PI3K subunit p85α (5 μL, Upstate Biotechnology) and protein A-agarose beads (70 μL, Invitrogen). Northcott et al determined that p85α immunoprecipitates the p110α, p110β, and p110δ catalytic subunits. The PI3K assay was performed as previously described. The radioactive product corresponding to PI3-monophosphate was spotted by thin-layer chromatography and visualized with a Bio-Rad personal molecular imager FX system.

**Data Analyses**

Data are presented as mean±SEM. Potency values (−log EC$_{50}$ in mol/L) were determined with GRAPHPAD Prism software. Spontaneous tone measurements are reported as percent initial PE (10$^{-5}$ mol/L) contraction. NE-induced contractions are displayed as percent maximum NE-induced contraction to compensate for alterations in spontaneous tone. PI3-monophosphate–radiolabeled areas were quantified with National Institutes of Health IMAGE software, version 1.61. When 2 groups were compared, a Student t test was used. For multiple comparisons, an ANOVA followed by least significant difference analysis and Student-Newman-Keuls post hoc tests was performed with SAS version 8.2 statistical software. In all cases, a value of P≤0.05 was considered statistically significant.

**Results**

**Spontaneous Tone**

The systolic blood pressures of DOCA-salt and sham rats were 168±5 mm Hg and 116±3 mm Hg, respectively. To more closely examine the changes that occur in vascular smooth muscle, the arteries were endothelium-denuded. Spontaneous tone developed in aortas isolated from DOCA-salt rats (Figure 1A, second and fourth tracing), with minimal tone development in aortas from sham rats (Figure 1A, first and third tracing). Low Mg$^{2+}$ PSS (0.15 mmol/L) induced a significant increase in spontaneous tone in aortas from both DOCA-salt and sham rats (Figures 1A and 1B), clearly visible in the aortas from DOCA-salt rats (compare Figure 1A second and fourth tracings). LY294002 (20 μmol/L), a PI3K inhibitor, significantly inhibited spontaneous tone in aortas from DOCA-salt rats incubated in both normal and low Mg$^{2+}$ PSS compared with their respective vehicle controls (Figures 2A and 2B). Converse to the increase in tone elicited by low Mg$^{2+}$, aortic strips incubated in high Mg$^{2+}$ PSS showed reduced spontaneous tone with respect to its vehicle control (Figure 2C), albeit not to the same extent as when aortic strips were incubated in LY294002. The effects of altered [Mg$^{2+}$]$_e$ and/or LY294002 were reversible, because on reequilibration with normal PSS, the tissues returned to normal reactivity.

**PI3K Activity Assays**

PI3K activity assays were performed to determine whether the increase in low [Mg$^{2+}$]$_e$–induced spontaneous tone that was LY294002- and thus, likely PI3K-dependent, was reflected biochemically. In aortic samples exposed to PSS in the tissue bath, there was a trend, but no significant difference, for the increase in PI3K activity in aortas from DOCA-salt and sham rats incubated in PSS (Figure 3A, compare shaded bars and Figure 3B, compare radiolabeled PI3-monophosphate in first and third lanes). When aortic strips were incubated in low Mg$^{2+}$, there was also a trend for an increase in PI3K activity, albeit neither were statistically significant (Figure 3A and 3B).

**NE-Induced Contraction**

We last examined the effects of low [Mg$^{2+}$]$_e$ on NE-induced contraction. Low [Mg$^{2+}$], significantly leftward-shifted NE-induced contraction in aortas from the sham rats. The NE-induced contraction in aortas from sham rats in low [Mg$^{2+}$], was similar to that of the DOCA-salt rats incubated in either PSS or low Mg$^{2+}$: No further shift occurred in aortas from DOCA-salt rats when exposed to low [Mg$^{2+}$], (Figure 4A).
LY294002 (20 μmol/L) shifted the NE-induced aortic contraction of both the sham and DOCA-salt rats in the presence of low Mg²⁺ compared with vehicle, resulting in similar potencies (Figure 4B). When the LY294002-mediated inhibition of NE-induced contraction in aortas from sham and DOCA-salt rats was compared, there was no significant difference between the aortic strips incubated in normal PSS compared with low Mg²⁺ PSS (Figure 4C). Thus, low [Mg²⁺]e appears to elicit enhanced NE-induced contraction via PI3K in aortas from sham rats but not from DOCA-salt rats.

**Discussion**

Arterial spontaneous tone and enhanced contractility are considered vascular hallmarks of experimental hypertension, and Mg²⁺ deficiency has been found in multiple experimental models of hypertension.¹¹,¹⁴,²⁸,²⁹ In humans, the occurrence of Mg²⁺ deficiency and the benefits of Mg²⁺ supplementation have been debated, largely by which end point and how to measure Mg²⁺.³⁰⁻³³ However, even small alterations in Mg²⁺ might induce vascular alterations, increasing the risk for cardiovascular-related conditions. Mg²⁺ deficiency leads to spontaneous tone and enhanced agonist-induced contraction in normotensive conditions.³⁰⁻³³ PI3K mediates spontaneous tone and enhances NE-induced contraction observed in aortas from DOCA-salt hypertensive rats.¹ The current studies examined whether these phenomena are linked and whether a decrease in [Mg²⁺]e activates the PI3K signaling cascade, eliciting the increase in spontaneous tone, and enhances vascular contraction observed in hypertension. Low [Mg²⁺]e was also used as a tool to examine a non-receptor-dependent mechanism to activate PI3K, ultimately resulting in enhanced aortic spontaneous tone in DOCA-salt hypertension.

[Mg²⁺]e deficiency, through Mg²⁺ removal, induces contraction of aortas from normotensive rats via the activation of mitogen-activated protein kinase, PI3K, and SH₂ domain-
containing proteins. We determined that low [Mg\(^{2+}\)]\(_{e}\), created experimentally, enhanced spontaneous tone in aortas from both sham and DOCA-salt rats. The PI3K inhibitor LY294002 abolished this spontaneous tone, linking low [Mg\(^{2+}\)]\(_{e}\) with PI3K and spontaneous tone. Mechanistically, PI3K can directly alter Ca\(^{2+}\) flux in rat portal vein myocytes and enhance spontaneous tone in aorta from DOCA-salt rats. Reduction in [Mg\(^{2+}\)]\(_{e}\) results in a rapid concentration-dependent increase in [Ca\(^{2+}\)]\(_{i}\) in cerebral vascular smooth muscle cells. Moreover, low [Mg\(^{2+}\)]\(_{e}\) leads to an increase in c-fos and c-jun as well as an induction of the p65 subunit of nuclear factor \(\kappa\)-B in cerebral vascular smooth muscle cells. PI3K activity is increased in aortas from hypertensive DOCA-salt rats compared with sham rats. If low [Mg\(^{2+}\)]\(_{e}\) activates PI3K in vascular smooth muscle, as LY294002-induced elimination of spontaneous tone suggests, PI3K activity should be increased in the smooth muscle of the aorta. When endothelium-denuded aortic strips from DOCA-salt and sham rats were placed in the tissue bath and

![Figure 3](http://hyper.ahajournals.org/)

**Figure 3.** A, p85\(\alpha\)-Associated PI3K activity in aortas from DOCA-salt and sham rats that were incubated in normal PSS or low Mg\(^{2+}\) PSS in isolated tissue baths. Bars represent mean arbitrary units \(\pm\) SEM (n=6 to 7). B, Representative results from PI3K activity. Spots represent radioactive product corresponding to PI3-monophosphate from aortic homogenates from sham and DOCA-salt rats incubated in normal PSS and low Mg\(^{2+}\) PSS in isolated tissue baths.

![Figure 4](http://hyper.ahajournals.org/)

**Figure 4.** A, The effect of low Mg\(^{2+}\) levels on NE-induced aortic contraction in endothelium-denuded aortas from sham and DOCA-salt rats. B, Effect of the PI3K inhibitor LY294002 (20 \(\mu\)mol/L) on NE-induced contraction in endothelium-denuded aortas from sham and DOCA-salt rats incubated in low Mg\(^{2+}\) PSS. C, Comparison of the effects of LY294002 (20 \(\mu\)mol/L) on NE-induced contraction in aortas incubated in low Mg\(^{2+}\) and normal PSS in isolated tissue baths. Points represent mean \(\pm\) SEM (n=4 to 7). Values are the log EC\(_{50}\) of the NE-induced contraction in the presence of normal PSS, low Mg\(^{2+}\) PSS, and/or LY294002. *P=0.05 vs sham PSS, #P=0.05 vs sham low Mg\(^{2+}\), \(\dagger\)P=0.05 vs DOCA low Mg\(^{2+}\).
incubated in normal PSS and low [Mg\(^{2+}\)]\(_{e}\), PSS, there was a trend for increases in PI3K activity caused by low Mg\(^{2+}\) stimulation of PI3K. These data do not quantitatively repeat earlier studies, in which there was statistically significantly higher PI3K activity in the DOCA-salt compared with sham rats.\(^1\) However, in the present experiment, several differences in protocol might account for our not observing a significant increase in PI3K activity, and these are all variables that might have masked and/or altered the magnitude of increase in PI3K activity: (1) tissues were incubated in altered salt conditions, (2) tension was pulled on the strips to achieve optimum length, (3) PI3K protein isolation buffer had Mg\(^{2+}\) present, and (4) more time elapsed until protein isolation. Nonetheless, there was a trend of increased activity in the arteries incubated in low Mg\(^{2+}\).

In summary, there was a small increase in spontaneous tone in aortas of the sham rats compared with a large increase observed in the aortas from DOCA-salt rats. Aortic strips incubated in low [Mg\(^{2+}\)]\(_{e}\) suggested a trend for increased activity in both sham and DOCA-salt rats. Taken together, these data suggest that although low Mg\(^{2+}\) levels can activate PI3K, PI3K is not the sole signal-transduction component that mediates spontaneous tone.

In DOCA-salt rats, Laurant et al\(^{16}\) determined that the blood pressure–lowering effect of Mg\(^{2+}\) supplementation in DOCA-salt hypertension was associated with lower in vivo cardiovascular reactivity to NE and angiotensin II. DiPette et al\(^{16}\) found no decrease in plasma Mg\(^{2+}\) concentration but did observe that Mg\(^{2+}\) supplementation reduced blood pressure, leading to the hypothesis of altered Mg\(^{2+}\) sensitivity in arteries. Other studies have shown that low [Mg\(^{2+}\)]\(_{e}\), potenti- ated NE-induced vasoconstriction in mesenteric arteries from SHR but not Wistar-Kyoto rats and altered vasopressin-induced vascular contraction; high [Mg\(^{2+}\)]\(_{e}\), attenuated both vasopressin- and NE-induced vasoconstriction.\(^{12}\) Our studies demonstrated hyperreactivity to NE-induced contraction in aortas from sham rats incubated in low Mg\(^{2+}\) PSS, resulting in similar potency of aortas from DOCA-salt rats. However, in aortas from DOCA-salt rats, there was no further hyperreactivity to NE-induced contraction. LY294002 normalized the NE-induced enhanced contraction in all aortic strips. These data further support the idea that PI3K is responsible for enhanced NE-induced contraction, similar to previous studies, and that PI3K mediates the enhanced NE-induced contraction in the presence of low [Mg\(^{2+}\)]\(_{e}\).\(^3\) The lack of a further leftward shift in NE-induced contraction in aortas from DOCA-salt rats by low [Mg\(^{2+}\)]\(_{e}\), indicates that more than PI3K must be stimulated to further shift the contraction in the aortas or that NE-induced activation of PI3K in the DOCA-salt rats is already maximal, and NE, unlike spontaneous tone, might depend on PI3K activity to a lesser degree.

**Perspectives**

The cellular mechanisms by which [Mg\(^{2+}\)]\(_{e}\) deficiency–induced enhanced hyperreactivity occurs has been extensively studied in multiple experimental models and clinical settings yet has not been fully elucidated. Increases in spontaneous tone and enhanced NE-induced contraction are 2 vascular dysfunctions observed in hypertension, as well as under conditions of low [Mg\(^{2+}\)]\(_{e}\). Previous studies in our laboratory have demonstrated that there is a functional increase in PI3K dependent–induced spontaneous tone and NE-induced reactivity in DOCA-salt and NO-nitro-l-arginine rat models of hypertension.\(^1\) The current studies demonstrate that low [Mg\(^{2+}\)]\(_{e}\) might be one non–receptor-dependent mechanism by which PI3K activity is increased in the condition of hypertension, leading to vascular dysfunction by altering arterial spontaneous tone and NE-induced contraction. Multiple studies have also examined the role of Mg\(^{2+}\) supplementation experimentally as well as clinically in correcting and/or treating high blood pressure but thus far, have not clearly determined whether Mg\(^{2+}\) supplementation has any beneficial effects. Further studies addressing whether Mg\(^{2+}\) supplementation will revert signaling pathways upregulated in hyper tension back to “normal” would be useful. Limitations of the present and other studies are that we have no knowledge of the real cause and effect of alterations in Mg\(^{2+}\) concentrations in terms of hypertension and the vasculature, nor do we define the exact mechanism by which [Mg\(^{2+}\)]\(_{e}\) alters PI3K-dependent tone or interacts with Ca\(^{2+}\) channels. However, these studies do further our knowledge, in that low [Mg\(^{2+}\)]\(_{e}\), can cause LY294002- and thus, PI3K-dependent alterations in arterial contraction.

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