Evidence That Prostacyclin Mediates the Vascular Action of Magnesium in Humans

JERRY L. NADLER, SCOTT GOODSON, AND ROBERT K. RUDE

SUMMARY Evidence in vitro and in humans suggests that Mg\(^{2+}\) can alter systemic and renal vascular tone. However, the mechanism of these effects is not known. The role of vasodilator prostaglandin release and Ca\(^{2+}\) flux in Mg\(^{2+}\)-induced changes in blood pressure and renal blood flow was studied in 10 normal subjects maintained on a fixed 80-mEq Na\(^{+}\) and K\(^{+}\) diet. Magnesium sulfate infused at 200 mg/hr for 3 hours reduced systolic and diastolic blood pressure within 1 hour (from 119 ± 2 [SEM] to 109 ± 4 mm Hg systolic; from 74 ± 3 to 64 ± 4 mm Hg diastolic; \(p<0.02\)). This hypotensive response was seen in all subjects and persisted for 3 hours. The pulse rate did not change, but renal blood flow (p-aminohippurate clearance) increased (from 902 ± 78 to 1108 ± 130 ml/min/1.73 m\(^2\); \(p<0.05\)). The Mg\(^{2+}\) infusion produced a significant increase in the excretion of the stable prostaglandin I\(_{2}\) (PGI\(_{2}\)) metabolite 6-keto-PGF\(_{1\alpha}\) (from 96 ± 12 to 154 ± 16 ng/g creatinine; \(p<0.01\)). In contrast, urinary PGE\(_{2}\) was not altered (328 ± 75 vs 399 ± 145 ng/g creatinine; \(p>0.6\)). To evaluate the functional role of PGI\(_{2}\) release, the cyclooxygenase inhibitors indomethacin (75 mg) or ibuprofen (600 mg) were given before the Mg\(^{2+}\) infusion. Both cyclooxygenase blockers, given in doses that inhibited immunoreactive 6-keto-PGF\(_{1\alpha}\) release, completely prevented the Mg\(^{2+}\)-induced decline in blood pressure and increased renal blood flow. In addition, pretreatment with the Ca\(^{2+}\) channel antagonist nifedipine (20 mg sublingual) blocked the Mg\(^{2+}\)-stimulated rise in PGI\(_{2}\) and fall in blood pressure. These results suggest that Ca\(^{2+}\) flux and PGI\(_{2}\) release play a role in mediating the vascular action of Mg\(^{2+}\) in humans. (Hypertension 9: 379-383, 1987)

KEY WORDS • prostacyclin • magnesium sulfate • blood pressure • renal blood flow

INCREASING evidence suggests that changes in the concentration of Mg\(^{2+}\) can alter vascular smooth muscle tone and reactivity. Reduction of extracellular Mg\(^{2+}\) concentration perfusing isolated canine and human vessels produces vasoconstriction and potentiates the pressor effects of angiotensin II and norepinephrine.\(^1,2\) In contrast, increasing the levels of Mg\(^{2+}\) relaxes vascular smooth muscle and reduces pressor responses.\(^3\) Mg\(^{2+}\) depletion in rats not only increases blood pressure but also reduces microcirculatory blood flow,\(^4\) while in humans Mg\(^{2+}\) supplementation augments the reduction in blood pressure induced by diuretics.\(^5\)

The mechanism of Mg\(^{2+}\) vascular action is not fully understood. However, recent evidence indicates that Mg\(^{2+}\) may alter Ca\(^{2+}\) flux and intracellular Ca\(^{2+}\) levels.\(^6,7\)

Prostacyclin (PGI\(_{2}\)) is a potent vasodilator prostaglandin that is produced in vessels and the kidney.\(^8\) In vitro evidence and our recent studies in humans indicate that Ca\(^{2+}\) flux is a key signal for PGI\(_{2}\) release.\(^9,10\) However, the role of Mg\(^{2+}\) in PGI\(_{2}\) synthesis is not known. The current study was designed to investigate the role of Ca\(^{2+}\) flux and PGI\(_{2}\) release \(\cdot\) the renal and systemic vascular actions of Mg\(^{2+}\). The results suggest that PGI\(_{2}\) release plays a key role in mediating the vasodilator effects of Mg\(^{2+}\) in humans.

Subjects and Methods

Ten normal volunteers (7 men, 3 women), aged 21 to 44 years, were studied in the Clinical Research Center under informed consent after 4 days of equilibration on a constant 80-mEq Na\(^{+}\), 80-mEq K\(^{+}\) diet. On 1 day, the subjects received an infusion of dextrose and water and a 3-hour urine sample was collected in parallel to serve as a control. On another day, the
subjects received a 3-hour infusion of MgSO₄ (200 mg/hr) using a constant infusion pump (IMED, San Diego, CA, USA). To evaluate the role of vasodilator prostaglandin, the Mg²⁺ infusion was repeated on another day during cyclooxygenase blockade with either indomethacin (Indocin, 75 mg; Merck Sharp & Dohme, Philadelphia, PA, USA) or ibuprofen (Motrin; Upjohn, Kalamazoo, MI, USA). Three doses of the respective cyclooxygenase inhibitor were given 8 hours apart before the Mg²⁺ infusion. On another day a repeat Mg²⁺ infusion was given during Ca²⁺ channel blockade with nifedipine (Procardia, 20 mg sublingual; Pfizer, NY, USA). The 3-hour urine samples were collected during all infusions, and an aliquot was immediately frozen at −20°C for later prostaglandin assay. During the Mg²⁺ infusions p-aminohippurate (PAH) was infused, using a constant infusion method, to evaluate renal blood flow. PAH was begun 2 hours before each Mg²⁺ infusion to ensure steady state basal levels.

Plasma for PAH was obtained in triplicate for basal renal blood flow and then hourly during the Mg²⁺ infusion. PAH was measured by standard spectrophotometric technique,¹¹ and renal blood flow was calculated as estimated renal plasma flow divided by 1 − hematocrit normalized to 1.73 m² body surface area; values are reported in units of ml/min/1.73 m². Serum Mg²⁺ was determined by atomic absorption spectrophotometry before and at the completion of each infusion.

PGl₂ production was estimated by radioimmunoassay of the stable PGI₂ metabolite 6-keto-PGF₁α in urine, as previously described.¹² In brief, an acidified urine sample with authentic [³H]6-keto-PGF₁α (New England Nuclear, Boston, MA, USA) added for recovery was extracted with ethyl acetate and then purified on high resolution Sephadex LH-20 columns (80 × 1 cm), which separates the major prostaglandin metabolites, including 2,3-dinor-6-keto-PGF₁α. The peak fraction, as determined by [³H]6-keto-PGF₁α, is then assayed using a sensitive and specific 6-keto-PGF₁α antisera,¹³ and a second antibody method is used to separate bound from free ligand.¹⁴ Recovery of added tracer averages 60 ± 6%. Interassay variation is 12%, and intraassay variation is 6%. Sensitivity is 10 pg/ml, and the assay blank averages 2 pg/ml. This assay has been validated with several techniques including negative-ion gas chromatography–mass spectrometry.¹⁰ In addition, urinary PGE₂ excretion was determined by a published radioimmunoassay method after several purification steps.¹⁴

Infusions were performed during the same time of day (1300–1600) and in nonsmoking subjects, since both time of day and smoke inhalation can alter 6-keto-PGF₁α excretion in humans.¹⁵,¹⁶

Values are reported as the mean ± SEM. Prostaglandin values are expressed in units of nanograms per gram of creatinine. Each subject was used as his or her own control and prostaglandin samples were run in the same assay. For statistical analysis the paired t test was used to compare control and experimental values using a CLINFO computer system.

### Results

#### Mg²⁺ Infusion Alone

The infusion produced a significant rise in serum Mg²⁺ concentration (Table 1). Systolic and diastolic blood pressure was reduced within 1 hour of Mg²⁺ administration (from 119 ± 2 to 109 ± 4 mm Hg systolic; from 74 ± 3 to 64 ± 4 mm Hg diastolic; p < 0.02). This hypotensive response persisted for 3 hours (Figures 1 and 2). The pulse rate did not change (68 ± 4 vs 72 ± 2 beats/min; p > 0.5), while renal blood flow was significantly increased (from 902 ± 78 to 1108 ± 130 ml/min/1.73 m²; p < 0.05; Figure 3).

The Mg²⁺ infusion produced a marked increase in the excretion of immunoreactive 6-keto-PGF₁α (from 96 ± 12 to 154 ± 16 ng/g creatinine; p < 0.01; Figure 4). However, urinary PGE₂ levels were not altered (328 ± 75 vs 399 ± 145 ng/g creatinine; p > 0.6).

#### Effect of Cyclooxygenase Inhibition

The Mg²⁺ infusion in subjects who were pretreated with indomethacin or ibuprofen produced changes in serum and urinary Mg²⁺ similar to those seen with the Mg²⁺ infusion alone (see Table 1). However, the use of cyclooxygenase blockers totally prevented the Mg²⁺-induced decrease in systolic and diastolic blood pressure (see Figures 1 and 2). Several subjects showed increases in blood pressure. The pulse rate did not change (71 ± 2 vs 68 ± 2; p > 0.4). Similarly renal blood flow did not increase, and values were similar to baseline (850 ± 125 vs 902 ± 78; p > 0.1; see Figure 3).

The cyclooxygenase blockers given alone did not alter basal blood pressure (114 ± 3 mm Hg systolic; 70 ± 2 mm Hg diastolic). The PGE₂ excretion was not altered (328 ± 75 vs 399 ± 145 ng/g creatinine; p > 0.6).

### Table 1. Effect of Mg²⁺ Infusion Alone and With Inhibitors on Serum Mg²⁺ Concentration and Urinary Mg²⁺ Excretion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mg²⁺ infusion</th>
<th>Mg²⁺ + I (n = 10)</th>
<th>Mg²⁺ + N (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal serum Mg²⁺ (mg/dl)</td>
<td>2.0 ± 0.2</td>
<td>1.94 ± 0.2</td>
<td>1.83 ± 0.1</td>
</tr>
<tr>
<td>Postinfusion serum Mg²⁺ (mg/dl)</td>
<td>4.23 ± 0.2*</td>
<td>4.31 ± 0.4*</td>
<td>4.19 ± 0.2*</td>
</tr>
<tr>
<td>Urinary Mg²⁺ (mg/mg creatinine)</td>
<td>0.658 ± 0.08</td>
<td>0.655 ± 0.06</td>
<td>0.892 ± 0.06²</td>
</tr>
</tbody>
</table>

Values are means ± SEM. I = indomethacin, 75 mg, or ibuprofen, 600 mg; N = nifedipine, 20 mg sublingual.

*tp < 0.01, compared with basal values.

†tp < 0.05, compared with Mg²⁺ + 1 values.
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74 ± 6 mm Hg diastolic) or renal blood flow (896 ± 80 ml/min/1.73 m²). Both indomethacin and ibuprofen completely prevented the Mg²⁺-induced rise of 6-keto-PGF₁₀ and produced levels similar to control (82 ± 22 vs 95 ± 12 ng/g creatinine; p > 0.4).

Effect of Ca²⁺ Channel Blockade
Pretreatment with nifedipine did not alter the increase in serum or urinary Mg²⁺ levels after Mg²⁺ administration (see Table 1). However, urinary Mg²⁺ excretion was slightly higher when compared with the Mg²⁺ infusion during cyclooxygenase inhibition (see Table 1).

Systolic and diastolic blood pressure remained unchanged during the Mg²⁺ infusion with nifedipine (116 ± 4 vs 114 ± 2 mm Hg systolic; 76 ± 4 vs 72 ± 3 mm Hg diastolic; p > 0.5). In addition, nifedipine completely blocked the Mg²⁺-stimulated rise of 6-keto-PGF₁₀ excretion (see Figure 4) and produced levels similar to control levels.

Discussion
The vasodilator effects of Mg²⁺ have been known for many years. In 1942, administration of Mg²⁺ to subjects with hypertension was shown to reduce blood pressure. More recent evidence indicates that Mg²⁺ supplementation can enhance the hypotensive effect of diuretic therapy. A deficiency of cellular Mg²⁺ content can markedly potentiate the sensitivity of blood vessels to pressor agents, and intracellular Mg²⁺ levels are reduced in untreated essential hypertensive subjects. Therefore, Mg²⁺ deficiency may play a role in the development and maintenance of essential hypertension.

The current study in normal subjects reveals that MgSO₄ infusion significantly reduces systolic and dia-
stolic blood pressure and increases renal blood flow. These results are similar to those in a previous study and indicate that Mg can alter systemic and renal vascular tone.

The mechanism of Mg-induced vasodilation is not completely known. Evidence in vitro indicates that Mg may compete with Ca for binding sites in vascular tissue, thereby preventing Ca-induced vasoconstriction. However, other results have not been consistent and show variable changes in Ca flux or intracellular Ca levels with increasing Mg concentration. A recent study using the calcium-sensitive dye quin 2 reported that Mg produced a rise in intracellular Ca concentration in dispersed bovine parathyroid cells. Other evidence indicates that Mg may displace Ca from intracellular sites and reduce Ca efflux, the net result being a transient rise in intracellular Ca levels.

Considerable evidence suggests that the vasodilator prostaglandins PGI and PGE participate as protective modulators of systemic and renal blood flow during states of increased pressor activity or ischemia. In addition, PGI has been shown to be a key mediator of the vasodilator actions of bradykinin. In the present study, the Mg-induced decrease in blood pressure and increase in renal blood flow were completely blocked by pretreatment with two structurally distinct cyclooxygenase inhibitors, suggesting that vasodilator prostaglandin release mediates the vascular action of Mg. The MgSO infusion selectively increased PGI, formation, as reflected by immunoreactive 6-keto-PGF in urine, indicating that PGI is the major vascular prostaglandin mediating these effects. Under basal conditions urinary 6-keto-PGF primarily reflects renal vascular PGI production, while another PGI metabolite, 2,3-dinor-6-keto-PGF, generally reflects extrarenal PGI production. However, systemic PGI formation can produce a rise in urinary 6-keto-PGF under stimulated conditions. Therefore, the increase in immunoreactive 6-keto-PGF in the Mg infusion may reflect both renal and extrarenal PGI release. This suggestion is supported by the results showing a complete blockade of the Mg-induced decrease in systemic and renal vascular tone with cyclooxygenase inhibition.

The direct effect of Mg on PGI release could not be fully evaluated in this study since we infused MgSO. However, evidence in cultured human endothelial cells indicates that the Mg ion and not changes in SO or osmolality are responsible for the stimulation in PGI formation. Similarly, other evidence indicates that Mg and not the associated anion is primarily responsible for its vascular effects.

Previous evidence in vitro and our results in humans suggest that Ca flux activates phospholipase and is an important signal for PGI synthesis. Since Mg may alter the transport and intracellular levels of Ca, we evaluated the effect of the Ca channel blocker nifedipine. The dose of nifedipine used has been shown previously to block only the Ca-mediated and not basal PGI, production. In the present study, nifedipine completely prevented the Mg-induced stimulation of PGI release. Similarly, the Ca antagonist blocked the Mg-induced decrease in blood pressure. This finding suggests that the Mg-induced vasodilation is linked to PGI release by changes in Ca flux. This mechanism of vasodilation is not unique to Mg since other studies show that bradykinin-stimulated release of PGI is also calcium-dependent.

The increased excretion of immunoreactive 6-keto-PGF does not appear to be secondary to changes in renal blood flow. Previous studies in humans indicate that vasodilators that can increase renal blood flow, such as isoproterenol, nifedipine, and prazosin, do not increase immunoreactive 6-keto-PGF, levels. In addition, vasopressor agonists, such as angiotensin II and norepinephrine, either do not change or increase 6-keto-PGF, levels.

The precise reason for the selective increase in PGI without changes in PGE is not totally clear from this study. One possible explanation is that PGE is less sensitive in vitro and in humans to changes in Ca flux. In addition, studies in renal medullary tissue indicate that increases in Mg do not stimulate PGE synthesis. Therefore, arachidonic acid released in response to changes in extracellular Mg in humans is converted primarily into PGI, resulting in unaltered levels of PGE.

In summary, these results suggest that PGI plays a key role in the systemic and renal vasodilator effects of Mg. These findings may provide a physiological basis for the use of Mg in disorders of altered vascular tone such as essential hypertension and preeclampsia.

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