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 Mg²⁺ can alter systemic and renal vascular tone. However, the mechanism of these effects is not known. The role of vasodilator prostaglandin release and Ca²⁺ flux in Mg²⁺-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 m²; p<0.05). The Mg²⁺ infusion produced a significant increase in the excretion of the stable prostaglandin I₂ (PGI₂) metabolite 6-keto-PGF₁α (from 96 ± 12 to 154 ± 16 ng/g creatinine; p<0.01). In contrast, urinary PGE₁ was not altered (328 ± 75 vs 399 ± 145 ng/g creatinine; p>0.6). To evaluate the functional role of PGI₂ release, the cyclooxygenase inhibitors indomethacin (75 mg) or ibuprofen (600 mg) were given before the Mg²⁺ infusion. Both cyclooxygenase blockers, given in doses that inhibited immunoreactive 6-keto-PGF₁α release, completely prevented the Mg²⁺-induced decline in blood pressure and increased renal blood flow. In addition, pretreatment with the Ca²⁺ channel antagonist nifedipine (20 mg sublingual) blocked the Mg²⁺-stimulated rise in PGI₂ and fall in blood pressure. These results suggest that Ca²⁺ flux and PGI₂ release play a role in mediating the vascular action of Mg²⁺ 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 Mg²⁺ can alter vascular smooth muscle tone and reactivity. Reduction of extracellular Mg²⁺ 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 Mg²⁺ relaxes vascular smooth muscle and reduces pressor responses.³ Mg²⁺ depletion in rats not only increases blood pressure but also reduces microcirculatory blood flow, while in humans Mg²⁺ supplementation augments the reduction in blood pressure induced by diuretics.⁵

The mechanism of Mg²⁺ vascular action is not fully understood. However, recent evidence indicates that Mg²⁺ may alter Ca²⁺ flux and intracellular Ca²⁺ levels.⁶ ⁷ Prostacyclin (PGI₂) is a potent vasodilator prostaglandin that is produced in vessels and the kidney.⁹ In vitro evidence and our recent studies in humans indicate that Ca²⁺ flux is a key signal for PGI₂ release.⁹ ¹⁰ However, the role of Mg²⁺ in PGI₂ synthesis is not known. The current study was designed to investigate the role of Ca²⁺ flux and PGI₂ release in the renal and systemic vascular actions of Mg²⁺. These results suggest that PGI₂ release plays a key role in mediating the vasodilator effects of Mg²⁺ 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 MgSO<sub>4</sub> (200 mg/hr) using a constant infusion pump (IMED, San Diego, CA, USA). To evaluate the role of vasodilator prostaglandin, the Mg<sup>2+</sup> 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 Mg<sup>2+</sup> infusion. On another day a repeat Mg<sup>2+</sup> infusion was given during Ca<sup>2+</sup> 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 Mg<sup>2+</sup> infusions p-aminophippurate (PAH) was infused, using a constant infusion method, to evaluate renal blood flow. PAH was begun 2 hours before each Mg<sup>2+</sup> infusion to ensure steady state basal levels.

Plasma for PAH was obtained in triplicate for basal renal blood flow and then hourly during the Mg<sup>2+</sup> infusion. PAH was measured by standard spectrophotometric technique, 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 Mg<sup>2+</sup> was determined by atomic absorption spectrophotometry before and at the completion of each infusion.

PGI<sub>2</sub> production was estimated by radioimmunoassay of the stable PGF<sub>1β</sub> metabolite 6-keto-PGF<sub>1α</sub> in urine, as previously described. In brief, an acidified urine sample with authentic [3H]6-keto-PGF<sub>1α</sub> (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-PGF<sub>1α</sub>. The peak fraction, as determined by [3H]6-keto-PGF<sub>1α</sub>, is then assayed using a sensitive and specific 6-keto-PGF<sub>1α</sub> antisera, and a second antibody method is used to separate bound from free ligand. 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. In addition, urinary PGE<sub>2</sub> excretion was determined by a published radioimmunoassay method after several purification steps.

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-PGF<sub>1α</sub> excretion in humans. 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

#### Mg<sup>2+</sup> Infusion Alone

The infusion produced a significant rise in serum Mg<sup>2+</sup> concentration (Table 1). Systolic and diastolic blood pressure was reduced within 1 hour of Mg<sup>2+</sup> 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 Mg<sup>2+</sup> infusion produced a marked increase in the excretion of immunoreactive 6-keto-PGF<sub>1α</sub> (from 96 ± 12 to 154 ± 16 ng/g creatinine; \( p < 0.01 \); Figure 4). However, urinary PGE<sub>2</sub> levels were not altered (328 ± 75 vs 399 ± 145 ng/g creatinine; \( p > 0.6 \)).

#### Effect of Cyclooxygenase Inhibition

The Mg<sup>2+</sup> infusion in subjects who were pretreated with indomethacin or ibuprofen produced changes in serum and urinary Mg<sup>2+</sup> similar to those seen with the Mg<sup>2+</sup> infusion alone (see Table 1). However, the use of cyclooxygenase blockers totally prevented the Mg<sup>2+</sup>-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; 74 ± 3 mm Hg diastolic).

### Table 1. Effect of Mg<sup>2+</sup> Infusion Alone and With Inhibitors on Serum Mg<sup>2+</sup> Concentration and Urinary Mg<sup>2+</sup> Excretion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mg&lt;sup&gt;2+&lt;/sup&gt; infusion (n = 10)</th>
<th>Mg&lt;sup&gt;2+&lt;/sup&gt; + I (n = 10)</th>
<th>Mg&lt;sup&gt;2+&lt;/sup&gt; + N (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal serum Mg&lt;sup&gt;2+&lt;/sup&gt; (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 Mg&lt;sup&gt;2+&lt;/sup&gt; (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 Mg&lt;sup&gt;2+&lt;/sup&gt; (mg/g creatinine)</td>
<td>0.658 ± 0.08</td>
<td>0.655 ± 0.06</td>
<td>0.892 ± 0.06*</td>
</tr>
</tbody>
</table>

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

*\( p < 0.01 \), compared with basal values.

\( \text{tp} < 0.05 \), compared with Mg<sup>2+</sup> + I values.
Figure 1. Effect of MgSO₄ infusion alone (●) or with cyclooxygenase inhibition (○) on systolic blood pressure (BP). The circles represent individual subjects, while the lines show the mean values before and after 3 hours of infusion. I = indomethacin or ibuprofen. Asterisk indicates significant difference (p < 0.01, paired Student's t test).

Figure 2. Effect of MgSO₄ infusion alone (●) or with cyclooxygenase inhibition (○) on diastolic blood pressure (BP). Asterisk indicates significant difference (p < 0.01, paired Student's t test).

Figure 3. Effect of MgSO₄ infusion alone (Mg²⁺) or with cyclooxygenase inhibition (Mg²⁺ + I) on renal blood flow. Values are means ± SEM of nine subjects. Asterisk indicates significant difference (p < 0.05).

Figure 4. Effect of MgSO₄ infusion alone or with nifedipine (N) on immunoreactive 6-keto-prostaglandin F₁α (PGF₁α) excretion. Values are means ± SEM. Asterisk indicates significant difference (p < 0.01, paired Student's t test).

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-PGF₁α 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-PGF₁α 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^{2+} can alter systemic and renal vascular tone.

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

Considerable evidence suggests that the vasodilator prostaglandins PGI_{2} and PGE_{2} participate as protective modulators of systemic and renal blood flow during states of increased pressor activity or ischemia. Evidence in vitro indicates that the MgSO_{4} infusion selectively increased PGI_{2} formation, as indicated by immuno-reactive 6-keto-PGF_{1α} in urine, indicating that PGI_{2} is the major vascular prostaglandin mediating these effects. Under basal conditions urinary 6-keto-PGF_{1α} primarily reflects renal vascular PGI_{2} production, while another PGI_{2} metabolite, 2,3-dinor-6-keto-PGF_{1α}, generally reflects extrarenal PGI_{2} production. However, systemic PGI_{2} formation can produce a rise in urinary 6-keto-PGF_{1α} under stimulated conditions. Therefore, the increase in immuno-reactive 6-keto-PGF_{1α} during the Mg^{2+} infusion may reflect both renal and extrarenal PGI_{2} release. This suggestion is supported by the results showing a complete blockade of the Mg^{2+}-induced decrease in systemic and renal vascular tone by cyclooxygenase inhibition.

The direct effect of Mg^{2+} on PGI_{2} release could not be fully evaluated in this study since we infused MgSO_{4}. However, evidence in cultured human endothelial cells indicates that the Mg^{2+} ion and not changes in SO_{4} or osmolality are responsible for the stimulation in PGI_{2} formation. Similarly, other evidence indicates that Mg^{2+} and not the associated anion is primarily responsible for its vascular effects.

Previous evidence in vitro and our results in humans suggest that Ca^{2+} flux activates phospholipase and is an important signal for PGI_{2} synthesis. Since Mg^{2+} may alter the transport and intracellular levels of Ca^{2+}, we evaluated the effect of the Ca^{2+} channel blocker nifedipine. The dose of nifedipine used has been shown previously to block only the Ca^{2+}-mediated and not basal PGI_{2} production. In the present study, nifedipine completely prevented the Mg^{2+}-induced stimulation of PGI_{2} release. Similarly, the Ca^{2+} antagonist (nifedipine) blocked the Mg^{2+}-induced decrease in blood pressure. This finding suggests that the Mg^{2+}-induced vasodilation is linked to PGI_{2} release by changes in Ca^{2+} flux. This mechanism of vasodilation is not unique to Mg^{2+} since other studies show that bradykinin-stimulated release of PGI_{2} is also calcium-dependent.

The increased excretion of immuno-reactive 6-keto-PGF_{1α} 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 immuno-reactive 6-keto-PGF_{1α} levels. In addition, vasopressor agonists, such as angiotensin II and norepinephrine, either do not change or increase 6-keto-PGF_{1α} levels.

The precise reason for the selective increase in PGI_{2} without changes in PGE_{2} is not totally clear from this study. One possible explanation is that PGE_{2} is less sensitive in vitro and in humans to changes in Ca^{2+} flux. In addition, studies in renal medullary tissue indicate that increases in Mg^{2+} do not stimulate PGE_{2} synthesis. Therefore, arachidonic acid released in response to changes in extracellular Mg^{2+} in humans is converted primarily into PGI_{2}, resulting in unaltered levels of PGE_{2}.

In summary, these results suggest that PGI_{2} release plays a key role in the systemic and renal vasodilator effects of Mg^{2+}. These findings may provide a physiological basis for the use of Mg^{2+} in disorders of altered vascular tone such as essential hypertension and pre-eclampsia.

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References

8. Moncada SR, Gryglewski RJ, Bunting S, Vane JR. An enzyme isolated from arteries transforms prostaglandin endoperoxides...
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11. Harvey R, Brothers A. Renal extraction of para-aminomuconate and creatinine measured by continuous in vivo sampling of arterial and renal vein blood. Ann NY Acad Sci 1962;102:46-54


32. Watson KV, Moldow CF, Oghurn PL, Jacob HS. Magnesium sulfate: rationale for its use in preeclampsia. Proc Natl Acad Sci USA 1986;83:1075-1078


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