Evidence That Prostacyclin Mediates the Vascular Action of Magnesium in Humans

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

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\(_{1a}\) (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\(_{1a}\) 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,\(^3\) 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 in 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-aminophippurate (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,\textsuperscript{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.

PGI2 production was estimated by radioimmunoassay of the stable PGI2 metabolite 6-keto-PGF1α in urine, as previously described.\textsuperscript{13} 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,\textsuperscript{15} and a second antibody method is used to separate bound from free ligand.\textsuperscript{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.\textsuperscript{10} In addition, urinary PGE2 excretion was determined by a published radioimmunoassay method after several purification steps.\textsuperscript{11}

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.\textsuperscript{12,16}

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; 71 ± 2 mm Hg diastolic).

### Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mg2+ infusion (n = 10)</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 ± 0.061</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.

\# \( p < 0.05 \), compared with Mg2+ + I values.
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-

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**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 F₁₀ (PGF₁₀) excretion. Values are means ± SEM. Asterisk indicates significant difference (p<0.01, paired Student's t test).
stolic blood pressure and increases renal blood flow. These results are similar to those in a previous study\textsuperscript{20} and indicate that Mg\textsuperscript{2+} can alter systemic and renal vascular tone.

The mechanism of Mg\textsuperscript{2+}-induced vasodilation is not completely known. Evidence in vitro indicates that Mg\textsuperscript{2+} may compete with Ca\textsuperscript{2+} for binding sites in vascular tissue, thereby preventing Ca\textsuperscript{2+}-induced vasoconstriction.\textsuperscript{21} However, other results have not been consistent and show variable changes in Ca\textsuperscript{2+} flux or intracellular Ca\textsuperscript{2+} levels with increasing Mg\textsuperscript{2+} concentration.\textsuperscript{6,7,22} A recent study using the calcium-sensitive dye quin 2 reported that Mg\textsuperscript{2+} produced a rise in intracellular Ca\textsuperscript{2+} concentration in dispersed bovine parathyroid cells.\textsuperscript{24} Other evidence indicates that Mg\textsuperscript{2+} may displace Ca\textsuperscript{2+} from intracellular sites and reduce Ca\textsuperscript{2+} efflux, the net result being a transient rise in intracellular Ca\textsuperscript{2+} levels.\textsuperscript{25}

Considerable evidence suggests that the vasodilator prostaglandins PGI\textsubscript{2} and PGE\textsubscript{2} participate as protective modulators of systemic and renal blood flow during states of increased pressor activity or ischemia.\textsuperscript{26-28} In addition, PGI\textsubscript{2} has been shown to be a key mediator of the vasodilator actions of bradykinin.\textsuperscript{29}

In the present study, the Mg\textsuperscript{2+}-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\textsuperscript{2+}. The MgSO\textsubscript{4} infusion selectively increased PGI\textsubscript{2} formation, as reflected by immunoreactive 6-keto-PGF\textsubscript{1a}, in urine, indicating that PGI\textsubscript{2} is the major vascular prostaglandin mediating these effects. Under basal conditions urinary 6-keto-PGF\textsubscript{1a} is 30% formed primarily into PGI\textsubscript{2}, resulting in unaltered levels of PGE\textsubscript{2}.\textsuperscript{30,31}

However, systemic PGI\textsubscript{2} formation can produce a rise in intracellular Ca\textsuperscript{2+} levels with increasing Mg\textsuperscript{2+} concentration,\textsuperscript{25} and indicates that Mg\textsuperscript{2+} can alter systemic and renal vascular tone such as essential hypertension and pre-eclampsia.

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

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