Effects of Magnesium on the Tone of Isolated Human Coronary Arteries

Comparison With Diltiazem and Nitroglycerin

Tadashi Kimura, MD, Hirofumi Yasue, MD, Naritsugu Sakaino, MD,
Manabu Rokutanda, MD, Michihisa Jougasaki, MD, and Haruo Araki, MD

To study the effects of magnesium (Mg$^{2+}$) on human coronary arteries and to compare those effects with those of diltiazem and nitroglycerin, we measured the tension of ring segments from isolated human coronary arteries obtained at autopsy within 5 hours after death. Precontracted segments with 3×10^{-4} M prostaglandin F$_2$α were studied after adding cumulative concentrations of these agents (1.0–8.0 mM, 10^{-2}–10^{-3} M, and 10^{-10}–10^{-11} M, respectively). Mg$^{2+}$ significantly inhibited the tonic contraction compared with the time-matched controls at 1.0 and 2.0 mM (48.7±5.6% vs. 88.6±2.2%, p<0.01, 36.2±6.1% vs. 78.9±3.0%, p<0.01, respectively). 1.0 and 2.0 mM Mg$^{2+}$ did not suppress, but actually increased, the amplitude of periodic contraction, but 8.0 mM Mg$^{2+}$ reduced the amplitude compared with the controls (6.6±5.2% vs. 73.3±10.7%, p<0.01). Diltiazem at a concentration of 10^{-5} M moderately inhibited the tonic contraction, and reduced the amplitude of periodic contraction almost completely. Nitroglycerin reduced the tonic contraction almost completely at a concentration of 10^{-6} M but did not reduce the amplitude of periodic contraction at any concentration. We conclude that 1.0 and 2.0 mM Mg$^{2+}$ inhibits the tonic contraction and that 8.0 mM Mg$^{2+}$ inhibits the periodic as well as the tonic contraction of isolated human coronary arteries. Diltiazem inhibits the periodic contraction, whereas nitroglycerin suppresses tonic contraction without affecting the periodic contraction. (Circulation 1989;79:1118–1124)

Coronary artery spasm has an established and important role in the pathogenesis of not only variant angina but also of ischemic heart disease in general, including resting angina, exertional angina, acute myocardial infarction, and sudden cardiac death.1–4 However, the mechanism(s) by which coronary spasm occurs remains to be elucidated.

Epidemiologic studies have shown that the mortality from ischemic heart disease has an inverse correlation with the intake of magnesium (Mg$^{2+}$).5–7 Also, reduced Mg$^{2+}$ levels have been found in the serum of patients with ischemic heart disease.8–10 and an important role for Mg$^{2+}$ in ischemic heart disease has been postulated. In experimental studies in vitro, Mg$^{2+}$ deficiency has constricted coronary arteries in dogs, suggesting that Mg$^{2+}$ deficiency may cause coronary spasm and subsequent sudden death in humans.11 The responsiveness of coronary arteries to vasoactive agents, however, varies considerably among animal species.12 Moreover, isolated human coronary arteries are known to develop periodic contraction that is not usually observed in coronary arteries from dogs or pigs.12–17 Examination of human coronary arteries is necessary to clarify the role of Mg$^{2+}$ in the pathogenesis of coronary spasm in humans.

The purpose of this study was to examine the effects of Mg$^{2+}$ on contractile tension of isolated human coronary arteries and to compare them with those of diltiazem and nitroglycerin, which are the drugs widely used for the treatment of coronary spasm.18

Methods

Coronary arteries were obtained from 27 patients at autopsy (20 men and seven women; their ages ranged from 29 to 82 with a mean age of 63 years) 2–5 hours after death (mean, 2.6 hours). None had a history of ischemic heart disease. The dissected coronary arteries were immediately immersed in physiologic saline solution at 4°C. They were

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From the Division of Cardiology and Department of Pharmacology, Kumamoto University Medical School, 1-1-1, Honjo, Kumamoto City 860, Japan.

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Address for correspondence: Hirofumi Yasue, MD, Division of Cardiology, Kumamoto University Medical School, 1-1-1, Honjo, Kumamoto City 860, Japan.
cleaned free of connective tissue and adhesion fat and cut into 5-mm ring segments. Each segment was attached to two stainless steel hooks and incubated with Krebs solution in an organ bath of 10 ml. The resting tension was set to 2 g. The solution was oxygenated with 95% O₂-5% CO₂ and maintained at 37°C and pH 7.4. The Krebs solution consisted of (in mM) 120.0 NaCl, 5.9 KCl, 1.0 MgSO₄, 2.4 CaCl₂, 10.0 dextrose, 20.0 NaHCO₃, and 1.0 Na₂HPO₄. Mg²⁺-free solution was made by removing MgSO₄ from this Krebs solution. The developed tension was measured isometrically with a force transducer UL-20GR (Minebea, Tokyo, Japan). Before the experiments, the coronary segments were allowed to equilibrate in the bathing media for 60–90 minutes, during which the solutions were replaced every 10–15 minutes. The following drugs were used. Prostaglandin (PG) F₂α (Ono Pharmaceutical, Tokyo, Japan), diltiazem HCl (Tanabe Pharmaceutical, Osaka, Japan), and nitroglycerin (Nippon-kayaku, Tokyo, Japan). Mg²⁺ was used in the form of MgCl₂.

Because tone of coronary segments may change over the time course of the experiment, the effects of time alone on the tone of coronary segments were examined as the matched-time control study. Five experiments were done simultaneously for each heart. Two experiments were done in Mg²⁺-free Krebs solution: one to examine the effects of Mg²⁺ and the other to examine the effects of time alone. Three experiments were done in 1.0 mM Mg²⁺ Krebs solution: one to examine the effects of diltiazem, one to examine the effects of nitroglycerin, and one to examine the effects of time alone. Each coronary segment was assigned randomly to one of the five experiments because our preliminary experiment showed that even coronary segments from the same artery and tested simultaneously under identical conditions did not necessarily exhibit the same response of developed tension. In three hearts, coronary segments were examined only for the effects of Mg²⁺.

After 60–90 minutes of incubation, coronary segments were precontracted with 3 × 10⁻⁶ PGF₂α. Coronary segments that did not develop a tension of more than 1 g after exposure to PGF₂α were discarded from the study because these segments were assumed to be not viable. Coronary segments whose tension decreased to less than 70% of the first developed tension induced by PGF₂α within 60 minutes were also discarded from the study because effects of drugs might not be reflected enough in their tensions. Coronary segments that developed irregular periodic contraction or unstable tension during the incubation time after adding PGF₂α were also excluded from the study because of the difficulty in examining the effects of drugs on these segments.

After 80 minutes of incubation after PGF₂α administration when the tension stabilized, cumulative concentrations of Mg²⁺, diltiazem, or nitroglycerin (1.0–8.0 mM, 10⁻⁹–10⁻⁵ M, and 10⁻¹⁰–10⁻⁶ M, respectively) were added to the organ bath every 40 minutes. All drugs were given in 0.1-ml increments.

After the end of the pharmacologic experiments, coronary segments were fixed in 15% formalin. The fixed tissue was embedded in paraffin, and histologic sections were stained with hematoxylin and eosin and Verhoeff-van Gieson stain to identify atherosclerotic changes and degree of luminal narrowing. The area bounded by the internal elastic membrane (IEM) and the area of the coronary arterial lumen remaining patent were identified and traced under a light microscope with a drawing tube. The degree of luminal narrowing in cross-sectional area was measured with an electronic digitizing tablet with a resolution of 0.1 mm (Wacom, Saitama, Japan) and a microcomputer PC-8801 (CPU Z80, Nippon electric, Tokyo, Japan). The percent luminal narrowing in cross sectional area was calculated according to the formula: [(area bounded by IEM) – (area of patent and calculated with lumen area)]/(area bounded by IEM).

Statistical Analysis

Tension after addition of each drug was expressed as the percentage of the amount of preconstriction produced by PGF₂α. Significance of data was calculated with analysis of variance followed by Bonferroni’s correction for paired t test and Student’s t test for unpaired data. Data are expressed as mean±SEM. A p value less than 0.05 was considered statistically significant.

Results

A total of 237 coronary segments were examined, of which 16 segments did not develop tension of more than 1 g after exposure to PGF₂α. 60 segments had tension decreased to less than 70% of the initial tension induced by PGF₂α within 60 minutes, and 41 segments exhibited unstable or irregular tension after exposure to PGF₂α. Thus, 120 segments were finally examined for the effects of Mg²⁺, diltiazem, and nitroglycerin. Spontaneous regular periodic contraction developed in 12 (10.0%) of these segments before adding PGF₂α. Of these 12 segments, eight were in the normal Krebs solution, and the remaining four were in the Mg²⁺-free solution.

Effects of Mg²⁺

Tonic contraction developed in 45 (75.0%) of the 60 segments after exposure to PGF₂α in the Mg²⁺-free Krebs solution. Mg²⁺ was administered in 24 of the 45 segments, and the remaining 21 segments were used as time-matched controls. Figure 1 shows representative tracings of the tonic contraction induced by PGF₂α and its inhibition by Mg²⁺. The tonic contraction was sustained unless Mg²⁺ was given (Figure 1A). The administration of 1.0 mM Mg²⁺ reduced the tonic tension substantially, and an increase in Mg²⁺ concentration up to 8.0 mM caused further reduction in tonic tension (Figure 1B). In
nine (37.5%) of the 24 coronary segments, periodic contraction was provoked by 1.0 mM Mg²⁺ after reduction of tonic tension (Figure 1C). The provoked periodic contraction was then suppressed by Mg²⁺ at the higher concentrations of 4.0 and 8.0 mM in all of the nine segments.

Figure 2 summarizes the effects of Mg²⁺ on the tonic contraction induced by PGF₂α in 15 coronary segments that did not develop periodic contraction in response to 1.0 mM Mg²⁺. The tonic tension decreased significantly at 1.0 mM Mg²⁺ compared with the time-matched controls (48.7±5.6% vs. 88.6±2.2%, p<0.01). Higher concentrations of Mg²⁺ further reduced the tension, and almost full relaxation was observed at 8.0 mM (4.0±7.3% compared with 67.0±3.1% for the time-matched controls, p<0.01).

Periodic contraction was provoked by PGF₂α in 15 (25.0%) of the 60 coronary segments in the Mg²⁺-free Krebs solution. The effects of Mg²⁺ were examined in seven, and the remaining eight segments were used as time-matched controls. Figure 3 shows representative tracings. The administration of 1.0 mM Mg²⁺ reduced the tension of relaxation and increased amplitude of periodic contraction (Figure 3B). However, at 4.0 mM Mg²⁺, the periodic contraction was reduced, and at 8.0 mM, it was almost abolished. An irregularity of periodic contraction often developed after administration of Mg²⁺ (Figure 3). The periodic contraction of the control segments was sustained for 60 minutes (Figure 3A).

Figure 4 summarizes the effect of Mg²⁺ on the periodic contraction examined in seven coronary segments. Mg²⁺ reduced the peak tension dose-dependently, and a significant difference from the time-matched controls was observed at 4.0 and 8.0 mM (27.5±8.5% vs. 86.6±5.1%, p<0.01, and −0.1±4.6% vs. 80.7±6.0%, p<0.01, respectively). The amplitude of periodic contraction tended to increase at 1.0 and 2.0 mM but diminished at 4.0 mM and was completely abolished at 8.0 mM in five of the seven coronary segments (6.6±5.2% vs. 73.3±10.7%; p<0.01, compared with the time-matched controls).

**Effects of Diltiazem and Nitroglycerin**

Tonic contraction was induced by PGF₂α in 31 (51.7%) of the 60 coronary segments in 1.0 mM Mg²⁺ Krebs solution. Of the 31 segments, 10 were examined for the effects of diltiazem, 10 for those of nitroglycerin, and 11 were used as time-matched controls. Figure 5 shows the representative tracings. The tension of the control segment remained stable over the time of the experiment (Figure 5A). Diltiazem reduced the tonic contraction at the high concentrations of 10⁻⁷ and 10⁻⁵ M (Figure 5B).
Nitroglycerin decreased the tonic contraction dose dependently, and a significant difference from the time-matched control was observed at concentrations of $10^{-6}$ M or more (Figure 5C).

Figure 6 summarizes the effects of diltiazem and nitroglycerin on the tonic contraction. Diltiazem suppressed the tonic contraction only moderately at $10^{-6}$ and $10^{-5}$ M compared with the time-matched controls (53.0±6.9% vs. 75.9±6.5%, p<0.05, and 32.8±8.5% vs. 69.5±8.1%, p<0.01, respectively). Nitroglycerin decreased the tonic contraction dose dependently and significantly at concentrations of $10^{-8}$, $10^{-7}$, and $10^{-6}$ M compared with the time-matched controls (49.6±7.1% vs. 81.7±4.1%, $p<0.01$, 3.4±8.2% vs. 75.9±6.5%, p<0.01, and −5.1±6.6% vs. 69.5±8.1%, $p<0.01$, respectively).

Periodic contraction was induced by PGF$_{2\alpha}$ in 29 (48.3%) of the 60 coronary segments in 1.0 mM Mg$^{2+}$ Krebs solution. Of the 29 coronary segments, 10 were examined for the effect of diltiazem, nine for the effect of nitroglycerin, and 10 were used as time-matched controls. Figure 7 shows the representative tracings. Diltiazem reduced the amplitude of periodic contraction significantly and dose dependently at concentrations from $10^{-7}$ to $10^{-5}$ M compared with the time-matched controls (Figure 7B). Nitroglycerin, however, did not significantly decrease the amplitude of periodic contraction even at the high concentration of $10^{-6}$M (Figure 7C).

**Figure 4.** Plots of effects of cumulative concentrations of Mg$^{2+}$ on the periodic contraction induced by $3 \times 10^{-6}$ M PGE$_{2\alpha}$ in Mg$^{2+}$-free solution. Upper panel: Changes in the peak tension of the periodic contraction. Lower panel: Changes in the amplitude of oscillation caused by Mg$^{2+}$. Tension immediately before the administration of 1.0 mM Mg$^{2+}$ was defined as 100%. , time-matched control; ○, Mg$^{2+}$. Data were mean±SEM. *p<0.05, **p<0.01 vs. control. †p<0.01 vs. the first developed tension (100%).

**Figure 5.** Representative tracings of the effects of diltiazem and nitroglycerin on the tonic contraction induced by $3 \times 10^{-6}$ M prostaglandin (PG) F$_{2\alpha}$ in 1.0 mM Mg$^{2+}$ solution. Panel A: Time course of tonic contraction. Panel B: Effects of cumulative concentrations of diltiazem on the tonic contraction. Panel C: Effects of cumulative concentrations of nitroglycerin on the tonic contraction.

**Figure 6.** Plot of effects of cumulative concentrations of diltiazem and nitroglycerin on the tonic contraction induced by $3 \times 10^{-6}$ M PGE$_{2\alpha}$ in 1.0 mM Mg$^{2+}$ solution. Tension immediately before the administration of diltiazem or nitroglycerin was defined as 100%. , time-matched control. Data were mean±SEM. *p<0.05, **p<0.01 vs. control. †p<0.01 vs. the first developed tension (100%).
78.0±2.6%, p<0.01, respectively). On the other hand, nitroglycerin reduced the peak tension significantly compared with the time-matched controls only at the high concentration of 10⁻⁶ M (62.0±2.9% vs. 78.0±2.6%, p<0.01). Diltiazem reduced the amplitude of periodic contraction significantly and dose dependently at concentrations of 10⁻⁷, 10⁻⁶, and 10⁻⁵ M compared with the time-matched controls (61.1±14.7% vs. 111.0±8.6%, p<0.01, 22.5±9.7% vs. 96.4±9.0%, p<0.01, and 0% vs. 97.1±9.8%, p<0.01, respectively). In contrast, nitroglycerin did not reduce the amplitude of periodic contraction even at the high concentration of 10⁻⁶ M.

**Histologic Study**

Histologic examination was performed in all coronary segments. The percent luminal narrowing in cross-sectional area ranged from 9.4 to 62.1% (mean, 31.8%). No difference occurred in the degree of luminal narrowing among five study groups of coronary segments. Also, no significant correlation occurred between the degree of luminal narrowing and the degree of relaxation of tonic contraction in any of the Mg²⁺, diltiazem, and nitroglycerin studies; correlation coefficient (r) was -0.12 for the Mg²⁺ study (n=15), -0.27 for the diltiazem study (n=10), and 0.30 for the nitroglycerin study (n=10). No difference was found in the degree of luminal narrowing between the group of coronary segments

**Discussion**

Responsiveness of the human coronary arteries to various vasoactive agents has been studied in vitro with coronary segments obtained at autopsy or from the heart of recipients for cardiac transplantation. In the case of coronary segments obtained at autopsy, some inevitable time delay exists in starting experiments, and this may influence the responsiveness to some vasoactive stimuli. In this study, we studied human coronary segments obtained at autopsy within 5 hours after death. Although no significant changes in responsiveness of coronary arteries have been reported within this time period, the present study shows that nearly half of the coronary segments obtained within this time period either had unstable basal tone or did not develop enough tension in response to the agonist and, thus, were unsuitable for examining the effects of vasoactive agents. Moreover,
even coronary segments from the same artery and tested simultaneously under identical conditions did not necessarily show the same response. Furthermore, time alone may change the tone of coronary segments. Accordingly, we used only the coronary segments that developed enough tension in response to PGF$_{2\alpha}$ and that sustained stable tension over the time course of the experiment, and we randomized them to five types of experiment, including the time-matched control study.

Previous studies indicate that isolated human coronary arteries develop periodic contraction either spontaneously or in response to vasoactive agents such as PGF$_{2\alpha}$. This phenomenon has not been regularly observed in isolated coronary arteries from dogs and pigs, which are the most commonly used animal models. In the present study, periodic contraction occurred spontaneously in 10% and was induced in 48.3% of the coronary segments by PGF$_{2\alpha}$ in the normal physiologic solution. Mg$^{2+}$ suppressed PGF$_{2\alpha}$-induced periodic contraction only at the higher concentrations of 4.0 and 8.0 mM. At the lower concentrations of 1.0 and 2.0 mM, Mg$^{2+}$ provoked irregular periodic contraction after a substantial decrease in the tonic tension.

Previous studies have shown that extracellular calcium is essential for the development of periodic contraction in isolated human coronary arteries and that calcium antagonists suppress periodic contraction. In the present study, diltiazem, a calcium antagonist, suppressed periodic contraction markedly, which is in agreement with the previous reports. Thus, Mg$^{2+}$ seems to have a calcium antagonist-like action at the higher concentrations. Indeed, previous reports indicate that Mg$^{2+}$ inhibits the influx of calcium across the cell membrane.

Periodic contraction has been speculated to be dependent upon fluctuating membrane potentials triggered by certain groups of cells (pacemakers) that rhythmically depolarize and repolarize. Mg$^{2+}$ inhibits the intercellular propagation of stimulus, and because smooth propagation of stimulus is essential for arteries to contract rhythmically or to work as a single unit, Mg$^{2+}$ may suppress periodic contraction by interfering with the smooth propagation of stimulus. An irregularity of periodic contraction was often observed after adding low concentrations of Mg$^{2+}$ to the Mg$^{2+}$-free solution in the present study. This may have been due to the inhibition of propagation of stimulus from the leading pacemaker and the subsequent activation of multiple pacemakers resulting in irregular contraction of vascular smooth muscle.

Relaxation of PGF$_{2\alpha}$-induced tonic contraction occurred even at a physiologic concentration of 1.0 mM Mg$^{2+}$. The extent of relaxation by these low concentrations of Mg$^{2+}$ was substantial. These results are compatible with those of Turlapaty and Altura in isolated dog coronary arteries, who showed that the addition of a low concentration (1.2 mM) of Mg$^{2+}$ to the Mg$^{2+}$-free solution caused a reduction of both the resting tension and the responsiveness to various vasoactive agents. Altura and his coworkers speculated that low Mg$^{2+}$ concentrations or lack of Mg$^{2+}$ has a role in the genesis of vasospasm. Thus, the present study extends, as well as confirms, their previous reports on dog coronary arteries to human coronary arteries and supports the hypothesis that Mg$^{2+}$ deficiency may have a role in the pathogenesis of some coronary spasm in humans.

The mechanism(s) by which Mg$^{2+}$ reduces the tonic contraction is not clear. The calcium antagonist diltiazem reduced the tonic contraction moderately only at the high concentrations, although the drug suppressed the periodic contraction markedly or completely at the same concentrations. Mg$^{2+}$, on the other hand, reduced the tonic contraction substantially at the low concentrations of 1.0 and 2.0 mM, at which it did not inhibit but rather provoked the periodic contraction. This suggests that the inhibition of tonic contraction by Mg$^{2+}$ at the low concentrations is not mainly caused by the inhibition of calcium influx through cell membrane, which is sensitive to the calcium antagonist diltiazem. However, further study may be necessary to confirm this interpretation because we did not examine the effects of other calcium antagonists such as nifedipine and verapamil in this study.

Mg$^{2+}$ may reduce intracellular calcium concentration or compete with calcium for cellular binding sites. Mg$^{2+}$ deficiency is known to increase membrane permeability to calcium, resulting in increased intracellular calcium. A decrease in cyclic AMP in the absence of enough Mg$^{2+}$ has been speculated to result in an increased concentration of calcium ions within the cytoplasm because there would be less cAMP-mediated calcium sequestration. Also, Mg$^{2+}$ has been postulated to be an activator of calcium-dependent ATPase at the membrane that extrudes calcium from cells. Thus, in the Mg$^{2+}$-free solution, increased contractile tension developed probably because intracellular calcium of the coronary segments was increased. Restoration of Mg$^{2+}$ concentration in the organ bath to the physiologic level, that is, 1.0 mM, probably resulted in a decreased calcium concentration in the cytoplasm that, in turn, led to a reduction of the tension.

Nitroglycerin, which reduces intracellular calcium concentration without significant effects on calcium influx, reduced the tonic contraction markedly without affecting the periodic contraction. Thus, nitroglycerin has effects similar to those of low concentrations of Mg$^{2+}$ on the isolated human coronary arteries. This fact also supports the interpretation that relaxation of tonic contraction by the low concentrations of Mg$^{2+}$ resulted from reduction in intracellular calcium concentration without substantially blocking calcium entry into the cells.

Periodic contractions were observed more frequently in the physiologic Mg$^{2+}$ Krebs solution than in the Mg$^{2+}$-free solution and were frequently provoked by adding Mg$^{2+}$ to the Mg$^{2+}$-free solution to physi-
logic levels (1.0-2.0 mM). In all these cases, periodic contractions were provoked after the decrease of tonic contraction and thus did not indicate the elevation of tonic. The mechanism is unknown.

Coronary spasm has an established and important role in the pathogenesis of not only variant angina but also ischemic heart disease in general.1-4 and several reports indicate that a Mg2+ deficiency occurs in patients with ischemic heart disease.8-10 The present study shows that Mg2+ suppresses tonic contraction of isolated human coronary arteries in low concentrations and periodic contraction as well in high concentrations. However, because we used only coronary segments that developed a tension greater than 1 g after exposure to PGF2α and that were stable, these results apply only to the ring segments that fulfill that criteria. With this limitation, the results of the present study suggest that the decrease of Mg2+ in the body induced by various causes may increase coronary tone and lead to coronary spasm in some patients. Mg2+ sulfate has been widely used for the treatment of preeclampsia-eclampsia to inhibit vasoconstriction as well as contraction of the uterus, which is a smooth muscle organ.31 Thus, there is a possibility that Mg2+ may also be useful for the treatment and prevention of coronary spasm.

References

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