Magnesium Deficiency Causes Transcriptional Downregulation of Kir2.1 and Kv4.2 Channels in Cardiomyocytes Resulting in QT Interval Prolongation

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Background: Mechanisms for QT interval prolongation and cardiac arrhythmogenesis in hypomagnesemia are poorly understood. This study investigated the potential molecular mechanism for QT prolongation caused by magnesium (Mg) deficiency in rats by using the patch clamp technique and molecular biology.

Methods and Results: Male Wistar rats were fed an Mg-free diet or a normal diet for up to 12 weeks. There was QT prolongation in the ECG of Mg-deficient rats, and cardiomyocytes from these rats showed prolongation of action potential duration. Electrophysiological studies showed that inward-rectifying K⁺ current (I_k1) and transient outward K⁺ current (I_to) were decreased in Mg-deficient cardiomyocytes, and these findings were consistent with the downregulation of mRNA, as well as protein levels of Kir2.1 and Kv4.2. In Mg-deficient cardiomyocytes, transcription factors, GATA4 and NFAT, were upregulated, whereas CREB was downregulated. In contrast to Mg deficiency, cellular Mg²⁺ overload in cultured cardiomyocytes resulted in the upregulation of Kir2.1 and Kv4.2, which was accompanied by the downregulation of GATA4 and NFATc4, and the upregulation of CREB. Activation of NFAT and inhibition of CREB reduced Kv4.2-I_to, whereas Kir2.1-I_k1 was reduced by CREB inhibition but not by NFAT activation.

Conclusions: Intracellular Mg deficiency downregulates I_k1 and I_to in cardiomyocytes, and this is mediated by the transcription factors, NFAT and CREB. These results provide a novel mechanism for the long-term QT interval prolongation in hypomagnesemia.

Key Words: Electrical remodeling; Hypomagnesemia; I_k1; I_to

Magnesium (Mg) is the second most abundant intracellular cation next to potassium (K) and the fourth most abundant cation in the body, playing an important role in many cellular functions. It is an essential element that regulates membrane stability and has neuromuscular, cardiovascular, immune and hormonal functions. Mg or magnesium ion (Mg²⁺) deficiency leads to a wide variety of metabolic abnormalities and clinical consequences. Mg deficiency has also been identified as a risk factor for cardiovascular diseases such as coronary artery disease. Epidemiological observations suggest that patients dying suddenly from ischemic heart disease have lower concentrations of myocardial tissue Mg and K than patients dying from other causes. Hypomagnesemia has also been shown to contribute to the onset and maintenance of various arrhythmias. Supraventricular arrhythmias in hypomagnesemia commonly include tachycardia and atrial premature contractions. Ventricular arrhythmias observed in patients with hypomagnesemia are usually associated with prolonged QT intervals. On the cellular level, hypomagnesemia is often associated with prolonged action potential duration (APD). Intracellular Mg²⁺ certainly has a role in the regulation of the inward rectifier potassium channel current (I_k1), and an increase in intracellular Mg concentration can block I_k1. The block of I_k1 by increased intracellular Mg²⁺ appears to prolong APD and QT interval. Based on this mechanism, a reduction of intracellular Mg²⁺ would be expected to cause QT shortening; however, hypomagnesemia is often accompanied by QT prolongation, and hypermagnesemia is often associated with QT shortening. Accordingly, the direct action of intracellular Mg²⁺ on I_k1 may not be correlated with changes in QT intervals on the surface electrocardiogram (ECG). Conventional understanding of QT changes with changes in extracellular Mg²⁺ is based on the notion that Mg²⁺ is a natural intrinsically blocker of the Ca²⁺ channel. Increased extracellular Mg blocks the inward Ca²⁺ current and shortens the QT interval, whereas decreased extracellular Mg²⁺ may increase the inward Ca²⁺ current and prolong the QT interval. Clinical findings that
Mg$^{2+}$ acts as an antiarrhythmic agent suppressing a wide spectrum of supraventricular and ventricular arrhythmias support the view that Mg modulates the inward Ca$^{2+}$ current. However, this understanding may seem at odds with the fact that atrioventricular conduction block is often observed in hypomagnesemic patients and in experimental animals with Mg deficiency. An important limitation of our current understanding of the effects of Mg$^{2+}$ on cardiac electrical properties is that most studies have evaluated only the immediate effects of changing intracellular and/or extracellular Mg concentrations on transmembrane ionic currents. Therefore, we planned to investigate the long-term effects of Mg deficiency on the phenotype of cardiac excitability to understand the transcriptional role of intracellular Mg$^{2+}$ in cardiomyocytes. The results of our study demonstrate that Mg deficiency causes downregulation of the Kir2.1-I,K1 and Kv4.2-I,G1 channels in cardiomyocytes.

**Methods**

For more details regarding the methods used in this study, please refer to Supplementary File.

**Animal Model**

All experimental protocols met the guidelines stipulated by the Physiological Society of Japan and the Ethical Committee of Oita University for Animal Experiments (Approved number F004003). Oita University granted an exemption from the requirement of ethics approval. Eight-week-old male Wistar rats weighing 250–280 g were used to develop a model of Mg deficiency. Rats were fed with an Mg-free diet (AIN-93M modified; Oriental Yeast Co., Ltd, Saga, Japan) or a normal diet (Oriental Yeast Co., Ltd. containing 0.26 g Mg/100 g) for up to 12 weeks.

**Surgical Procedures and ECG Recording**

A signal transmitter was implanted in each rat 3 days prior to the ECG recording.

**Measurement of Electrolyte Concentrations**

Serum concentrations of Mg, Ca, Na$^+$, K$^+$, and Cl$^-$ were measured in rats fed a normal diet or an Mg-free diet. Intracellular Mg concentration in cardiomyocytes and red blood cells were used as an index of the intracellular Mg concentration, as described previously.  

**Isolation of Cardiomyocytes**

Adult ventricular cardiomyocytes and neonatal cardiomyocytes were enzymatically prepared.

**Cellular Electrophysiological Recordings**

Whole-cell current clamp and voltage clamp experiments were performed.

**Real-Time Polymerase Chain Reaction (PCR) and Western Blotting**

Gene-specific primers for real-time PCR are listed in Supplementary Table 1. Western blotting was performed with the primary antibodies (Supplementary File).

**Adenoviral Vectors**

A recombinant adenovirus containing GATA4 was constructed, as described previously.

**Statistical Analysis**

Data are expressed as means±SE. Comparisons between groups and among groups were performed with either paired/unpaired t-tests or ANOVA followed by post-hoc tests using the Tukey procedure. Differences were considered significant at P<0.05. Numbers of cell/sample/patch/trace used in the study are shown in parentheses in each figure.

**Results**

**Animal Model of Mg Deficiency**

We created a model of Mg deficiency by feeding rats with an Mg-free diet for up to 12 weeks. Serum Mg, Na$^+$, Cl$^-$, K$^+$, Ca and intracellular Mg concentrations in control rats (normal diet) and Mg-deficient rats (Mg-free diet) are shown in Figure 1. Serum Mg (extracellular Mg) concentration was significantly decreased in Mg-deficient rats at week 2, and the reduction continued until the end of the

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**Figure 1.** Changes in extracellular and intracellular electrolyte concentrations in animals fed a normal diet or a magnesium (Mg)-deficient diet for up to 12 weeks. (A) Changes in extracellular (serum) and intracellular (erythrocyte) Mg concentrations. (B) Mg content in myocardium derived from control (veh) and hypomagnesemic rat at week 12. (C) Changes in serum concentrations of sodium (Na), chloride (Cl), potassium (K) and calcium (Ca). *P<0.05, †P<0.001, compared with week 0 (Con) or vehicle (Veh).
12-week observation period. Intracellular Mg concentration was significantly decreased at 3 weeks and this continued until the end of the 12-week observation period. Intracellular and extracellular Mg concentrations at week 4 were 6.85±0.23 mg/dL and 2.34±0.08 mg/dL in control rats, and 4.44±0.24 mg/dL and 0.68±0.05 mg/dL in Mg-deficient rats, respectively (P<0.05) in Figure 1A. Mg content in the myocardium was also significantly reduced at week 12 (Figure 1B) in Mg-deficient rats, indicating that the animal model of Mg deficiency represents intracellular Mg depletion. In addition, the Mg-free diet resulted in an increase of serum Ca concentrations at week 2 and later (Figure 1C). In contrast, serum Na⁺, K⁺ and Cl⁻ concentrations were unchanged throughout the 12-week observation period.

ECG Changes in Mg Deficiency
Mg-deficient rats exhibited ECG abnormalities including sinus tachycardia, QT prolongation, and premature ventricular contractions (PVSc) (Figure 2A). Heart rate gradually increased with the duration of the Mg-free diet. The QT interval and QT/RR interval ratio were significantly increased at week 2 and later. After 2 weeks of an Mg-free diet, the QT interval was 68.0±0.9 ms (control, 56.5±1.3 ms, P<0.05), and the QT/RR ratio was 0.43±0.01 (control, 0.30±0.01, P<0.05). In contrast, the PR interval and QRS duration were not changed by 12 weeks of an Mg-free diet (Figure 2B). PVCs were often recorded in Mg-deficient rats (Figure 2A-e); however, PVCs were not observed in the control rats.

Action Potentials in Mg-Deficient Cardiomyocytes
The APD in ventricular cardiomyocytes was recorded in control and Mg-deficient rats at week 6 by the current-clamp method (Figure 2D,E). The APD at 50% repolarization (APD50), APD at 75% repolarization (APD75) and APD at 90% repolarization (APD90) were significantly prolonged in Mg-deficient cardiomyocytes compared with those in control cardiomyocytes (Figure 2E).
Changes in K⁺ Channel Expression in Mg-Deficient Hearts
In order to clarify the long-term effect of Mg deficiency on APD prolongation, we performed real-time RT-PCR analyses to examine ion channel expression focusing on K⁺ channels in cardiomyocytes (Figure 3A,B). Kir2.1-mRNA expression was significantly downregulated by 38.5% in hypomagnesemic ventricular myocytes. Also, Kv4.2-mRNA expression was significantly downregulated by 19.1%. Reduction of Kir2.1- and Kv4.2-channel expression was confirmed by the measurement of protein levels (Figure 3C,D). However, other K⁺ channels, including KvLQT1 and rERG, were unchanged in Mg-deficient hearts.

I⁺K and Ito in Ventricular Mg-Deficient Cardiomyocytes
We recorded I⁺K and Ito from isolated ventricular cardiomyocytes of control and Mg-deficient rats. I⁺K was depressed with an increase of intracellular (pipette) Mg²⁺ concentration from 0.1 mmol/L to 5 mmol/L both in control and Mg-deficient cardiomyocytes. Importantly, I⁺K was significantly smaller in Mg-deficient cardiomyocytes than control cardiomyocytes over a wide range of intracellular Mg²⁺ concentrations. Furthermore, slope conductance at −80 mV was significantly decreased in Mg-deficient compared with control cardiomyocytes, regardless of the difference in recording conditions (Figure 4B,D,F). In comparison with cardiomyocytes from control rats, Mg-deficient cardiomyocytes had significantly smaller I⁺K: chord conductance at −80 mV (P<0.05) by 27.8–33.6%, as demonstrated in the I-V curves (Figure 4A,C,E and Supplementary Figure 1). A comparable electrophysiological finding was obtained, corroborating a reduction of 4-AP-sensitive Ito density in Mg-deficient cardiomyocytes (Figure 4G–J). Ito density at +60 mV was 65.3±10.6 pA/pF in control cardiomyocytes and 45.0±8.9 pA/pF in Mg-deficient cardiomyocytes. These electrophysiological features of Mg-deficient cardiomyocytes were highly consistent with the changes in Kir2.1 and Kv4.2 mRNA and protein levels (Figure 3). Thus, these results suggest that Mg deficiency modulates the functional expression of the Kir2.1 and Kv4.2 channels, which has a long-term effect on cardiac electrical properties.

[Mg²⁺]-Loaded Cardiomyocytes Exhibit an Increase of Kir2.1 and Kv4.2 Expression
In order to confirm the significance of intracellular Mg as a modulator of these ion channels, we examined whether intracellular Mg²⁺ loading for 24h affects Kir2.1 and Kv4.2 gene expression. We evaluated the effect of the addition of an Mg ionophore at various concentrations of MgCl₂ in the culture medium on the expression of Kir2.1 and Kv4.2 in neonatal cardiomyocytes (Figure 5). The expression of Kir2.1 and Kv4.2 in neonatal cardiomyocytes was significantly upregulated by 24h by an increase of extracellular Mg²⁺ only when the Mg²⁺ ionophore was added to the culture medium. The expression of Kv4.2 was significantly increased, in the same fashion, by an increase in extracellular Mg²⁺ in combination with the Mg²⁺ ionophore. Of note, the increase of Kir2.1-mRNA was much larger than that of Kv4.2-mRNA in response to the Mg ionophore in 1 mmol/L extracellular Mg²⁺. These findings corresponded with a larger reduction of Kir2.1-mRNA than Kv4.2-mRNA in Mg-deficient cardiomyocytes (Figure 4).

Mg²⁺-Dependent Changes in Transcription Factors and Signal Transduction
We performed quantitative analyses of mRNA levels for several cardiac transcription factors that could potentially affect cardiac excitation (GATA4, NFATc4, Csx/Nkx2.5, CREB) in adult ventricular myocytes and neonatal cardiomyocytes. This analysis was done to elucidate the transcriptional actions of intracellular Mg²⁺ (Figure 6). In the Mg-deficient heart, expression of NFATc4 was significantly upregulated by 56%, and GATA4 was upregulated by 34%. In contrast, the expression of CREB was significantly downregulated by 31%. To confirm the action of Mg²⁺, we evaluated changes in these transcription factors when intracellular Mg²⁺ was elevated by the use of 0–2 mmol/L extracellular MgCl₂ and the Mg²⁺ ionophore. Under these conditions, the expression of GATA4 and NFATc4 were significantly downregulated by 39% and 26%, respectively (Figure 6E,F), whereas expression of CREB was upregulated by 61% (Figure 6H) with 2 mmol/L
MgCl₂. On the contrary, expression of Csx/Nkx2.5 in cardiomyocytes was unchanged either in Mg-deficient hearts or when intracellular Mg²⁺ was increased (Figure 6C,G). Additional experiments were performed to determine the roles of NFATc4, GATA4 and CREB for the regulation of Kir2.1 and Kv4.2 expression without modifying the intracellular Mg²⁺ concentration. Because GSK3β inhibition is postulated to activate endogenous NFAT signaling in cardiomyocytes without elevating intracellular Ca²⁺ concentration or activating calcineurin, we applied a GSK3β inhibitor, 6-bromoindirubin-3'-oxime (BIO), to mimic the potential action of Mg deficiency on the Kv4.2 and Kir2.1 channels (Figure 7). Activation of NFAT signaling reduced expression of Kv4.2 without affecting Kir2.1. A non-specific GSK3β inhibitor, LiCl, also reduced the Kv4.2 but not the Kir2.1 channel in cardiomyocytes (Supplementary Figure 2). In contrast, inhibition of CREB signals by blocking protein kinase A reduced both Kir2.1 and Kv4.2 expression. And also in contrast, overexpression of GATA4 by a recombinant adenovirus containing GATA4 (Ad-GATA4) had no effect on the expression of these ion channels. Electrophysiological assessment of I_K1 and Ito firmly supported the significance of NFAT activation for the downregulation of Ito, and of CREB inhibition for the downregulation of I_K1 and Ito (Figure 7C–F), which is highly consistent with the transcription changes in these muscles.
Mg Deficiency Downregulates K Channel Expression

channels in Figure 7A and B. We also noted that a phosphodiesterase inhibitor, 3-isobutyl 1-methylxanthine (IBMX), by itself significantly increased I_K1 by 62% (Figure 7D) and I_to by 31% (Figure 7F), which also supports the notion that CREB is a positive regulator of Kir2.1 and Kv4.2 transcription.

Discussion

Major Findings

This study provided new evidence that chronic Mg deficiency depressed expression of Kir2.1 and Kv4.2 channels, leading to a decrease of I_K1 and I_to, which prolonged the APD in cardiomyocytes and the QT interval in the intact rat. Conversely, the application of an Mg^{2+} ionophore to increase the intracellular Mg^{2+} concentration resulted in upregulation of the Kir2.1 and Kv4.2 channels. These findings suggest that abnormal cardiac excitability and arrhythmias in Mg deficiency arises not only from an immediate effect of reduced serum Mg on cardiac electrical properties, but also from longer-term changes in Kir2.1- and Kv4.2-K^+ channels.

Figure 5. Increase of Kir2.1 and Kv4.2 channels in magnesium (Mg)^{2+}-overloaded cardiomyocytes. Relative mRNA expression of (A), Kir2.1 (Upper column for Kir2.1, Lower column for GAPDH) and (B) Kv4.2 isoforms (Upper column for Kv4.1, Lower column for GAPDH) in neonatal cardiomyocytes cultured for 24 h at different MgCl_2 concentrations (0–2 mmol/L) with or without an Mg ionophore (1 μmol/L). mRNA expression levels were determined by using real-time polymerase chain reaction (PCR). *P<0.05, compared with control [Mg ionophore (−) and 0 mmol/L MgCl_2].

Figure 6. Changes of GATA4, NFATc4 and CREB in magnesium (Mg)-deficient and Mg^{2+}-overloaded cardiomyocytes. Significant increase of GATA4 and NFATc4, and reduction of CREB in Mg-deficient cardiomyocytes. Significant reduction of GATA4 and NFATc4, and an increase of CREB in Mg^{2+}-overloaded cardiomyocytes at week 6. Relative mRNA expression of (A) GATA4; (B) NFATc4; (C) Csx/Nkx2.5; and (D) CREB, where mRNA was extracted from control and Mg-deficient rat cardiomyocytes at week 6. Relative mRNA expression of (E) GATA4; (F) NFATc4; (G) Csx/Nkx2.5; and (H) CREB, where mRNA was extracted from neonatal rat cardiomyocytes cultured for 24 h at different MgCl_2 concentrations (0–2 mmol/L) with or without an Mg ionophore (1 μmol/L) as indicated. Representative mRNA expression of GATA4, NFATc4, Csx/Nkx2.5 and CREB are shown in the upper columns (GAPDH in lower columns). *P<0.05, compared with control [Mg ionophore (−) and 0 mmol/L MgCl_2].
The prevalence of hypomagnesemia in hospitalized patients ranges from 8% to 30%. Hypomagnesemia is found in 19–44% of patients with chronic heart failure and 33% of patients with diabetes mellitus (DM). Conversely, DM is also associated with Mg deficiency, probably due to urinary Mg2+ loss. Ai et al demonstrated that the I to channel (Kv4.2) was downregulated in diabetic rat hearts. A major cardiac complication among these diseases includes a prolongation of the QT interval, although the molecular mechanisms have not yet been clarified. To address these issues, we created an animal model of Mg deficiency by feeding animals with an Mg-free diet. A traditional understanding of the mechanism for arrhythmias in Mg deficiency includes a direct effect of Mg2+ loss both in the extracellular and intracellular fluid. Mg 2+ is commonly known as a natural Ca2+ channel blocker. Therefore, an intravenous injection of MgSO4 causes a decrease of Ca2+ channel currents, especially the L-type Ca 2+ current, and this should shorten the QT interval. Consequently, Mg2+ loss should be accompanied by an increase of ICa.L, which may eventually cause QT prolongation. Because an increase of ICa.L should increase the intracellular Ca2+ concentration, activation of the Na+-Ca2+ exchanger current or transient inward current (ITI) would also be expected to prolong the QT interval. In fact, the frequency of PVCs is increased in patients with hypomagnesemia, and this finding was confirmed in our animal model (Figure 2A–e). Nevertheless, augmentation of ICa.L in the chronic phase of hypomagnesemia is not necessary for the development of abnormal cardiac excitability, because QT prolongation is often accompanied by prolongation of atrioventricular conduction. In contrast, intracellular Mg2+ concentrations below the physiological range may lead to QT prolongation, because transient Iκ1 becomes nearly zero, reflecting the absence of Mg2+ block. However, the loss of Mg2+ block may not sufficiently explain the prolongation of QT intervals in clinical and experimental Mg deficiency for the following reasons: (1) APDs are obviously prolonged in cardiomyocytes derived from Mg-deficient rats even when the intracellular Mg2+ is normal (Figure 2); (2) Iκ1 is...
consistently smaller in Mg-deficient cardiomyocytes by 28–32% under normal intracellular Mg\(^{2+}\) conditions in voltage-clamp experiments (Figure 4); and (3) intracellular Mg\(^{2+}\) concentrations <50% of normal may not be achievable in vivo (Figure 1A). In this context, we successfully demonstrated that during the chronic phase of Mg\(^{2+}\) deficiency, a moderate loss of intracellular Mg\(^{2+}\) (by ~35%) caused downregulation of the expression of Kir2.1 and Kv4.2 channels and a reduction of Ik\(_{\text{to}}\) and IK1. We also provided evidence that the expression of the Kir2.1 and Kv4.2 channels was upregulated by increasing intracellular Mg\(^{2+}\). These findings strongly indicate that intracellular Mg\(^{2+}\) is a pivotal regulator of ion channel expression in cardiomyocytes.

**Ik\(_{\text{to}}\) as a Substrate of Arrhythmogenicity**

Kir2.1 channel current or cardiac Ik\(_{\text{to}}\) current plays an important role in shaping normal cellular action potentials, stabilizing the cellular resting membrane potential. Several studies indicate that Ik\(_{\text{to}}\) plays a role in ventricular arrhythmias, as suggested in recent studies utilizing guinea pig heart models of ventricular fibrillation. And vice versa, depletion of Ik\(_{\text{to}}\) would likely increase the susceptibility of aberrant excitation of cardiomyocytes, consequently resulting in frequent ventricular premature contractions in this rat model that was fed an Mg-free diet (Figure 2A-e), indicating the importance of the Ik\(_{\text{to}}\) channel under specific disease conditions.

**Cellular Environment for Ion Channel Remodeling**

The Ca\(^{2+}\)/calmodulin system is an important Ca\(^{2+}\) sensor that responds to intracellular Ca\(^{2+}\) changes on both a beat-to-beat and tonic basis. Ca\(^{2+}\)/calmodulin binding activates a variety of downstream mediators, including calcineurin (Cn) and Ca\(^{2+}\)/calmodulin kinase II. In cardiac myocytes, activated Ca\(^{2+}\)/calmodulin increases CREB expression, which leads to upregulation of cardiac ion channels. Thus, NFAT is an important mediator of responses to changes in intracellular Ca\(^{2+}\) concentration. It is well established that Cn/NFAT signaling downregulates Ik\(_{\text{to}}\) leading to QT prolongation. Because hypomagnesemia is often observed in the setting of simultaneous hypocalcemia, concomitant metabolic derangements make it difficult to assign a specific mechanism for hypomagnesemia alone. For example, upregulation of NFATc4 and downregulation of CREB in this study may possibly be attributed to hypocalcemia. However, this is unlikely because hypercalcemia rather than hypocalcemia was observed in our animal model from week 2 to 12, presumably due to osteoclastic bone absorption in response to severe Mg depletion. Moreover, increasing intracellular Mg\(^{2+}\) concentration using an Mg\(^{2+}\) ionophore increased the expression of Kir2.1 and Kv4.2, and this confirms the importance of intracellular Mg\(^{2+}\) as a transcription modulator of ion channels that is independent of intracellular Ca\(^{2+}\) (Figure 8). These data suggest that intracellular Mg\(^{2+}\) might be a strong modulator of the transcription factors, CREB and NFAT.

**Intracellular Mg\(^{2+}\) and Pathophysiological Cellular Signals**

We were not able to determine the precise mechanism for the K\(^{+}\) channel reduction during chronic Mg deficiency. We demonstrated that a transcription factor, CREB, was downregulated in Mg deficiency and upregulated by high levels of intracellular Mg\(^{2+}\). Of note, Craig et al found that the CREB B-ZIP domain binds to the consensus CRE site >10-fold more in the presence of 10 mmol/L MgCl\(_2\). Moll et al also found that a certain Mg\(^{2+}\) concentration is needed to prevent non-specific electrostatic interactions between CREB and DNA. These findings strongly support the idea that Mg\(^{2+}\) is an important component of the cellular milieu that governs transcription activity in cardiomyocytes. Because the Kir2.1 gene has several putative CREB binding sites in its promoter region (−53b), transcriptional downregulation of Kir2.1 by CREB has been proposed in hypomagnesemia.

NFAT is a family of transcription factors involved in diverse cellular functions, and NFAT3 has been implicated in cardiac hypertrophy. In animal and clinical models of cardiac hypertrophy, Ik\(_{\text{to}}\) is reduced and this prolongs APD. Recent studies revealed that Cn dephosphorylates NFATc1-4 leading to a reduction of Ik\(_{\text{to}}\). This is an attractive mechanism because Cn/NFAT signals are necessary and sufficient to induce cardiac hypertrophy. In our study, the heart weight of rats at week 8 and later was significantly larger in Mg-deficient rats than control rats (Supplementary Table 2), and cell capacitance in the Mg-deficient cardiomyocytes was significantly larger than that in normal cardiomyocytes (Supplementary Figure 3). In addition, NFATc4 was upregulated in Mg deficiency and downregulated when the intracellular Mg\(^{2+}\) concentration was elevated, which indicates that intracellular Mg\(^{2+}\) may account for NFAT signaling in cardiac hypertrophy independently of intracellular Ca\(^{2+}\) overload. Rosow et al reported that the K\(_{\text{v}}\)4.2 gene has putative NFAT binding sites in its promoter region, and could be downregulated depending upon NFATc4 activity, which indirectly supports our conclusions. Based on the facts that GATA4 is a sufficient regulator of cardiomyocyte hypertrophy, that GATA4 expression was upregulated with cellular hypertrophy in our rat model of Mg deficiency, and that Ik\(_{\text{to}}\) is consistently downregulated in hypertrophied cardiomyocytes, we attempted to clarify the relation between GATA4 and Kv4.2 in Mg-deficient cardiomyocytes. However, despite the clinical evidence that left ventricular mass in patients with left ventricular hypertrophy was inversely correlated with serum Mg concentration, we were unable to observe altered expression of K\(_{\text{v}}\)4.2 by adenosine-mediated overexpression of GATA4 in cardiomyocytes (Figure 7B).

**Study Limitations**

Among more than 1,800 transcription factors in humans, we focused on 4 transcription factors, CREB, NFAT, GATA4, and Csx/Nkx2.5, to examine signaling mechanisms that might link the effects of Mg deficiency to changes in K\(^{+}\) channel transcription. We selected these 4 transcription factors, based on the results of previous studies. The activation of CREB was shown to be important for long-term cellular memory and cardiac electrical remodeling. In addition, the Cn/NFAT pathway was shown to be involved in the regulation of Ik\(_{\text{to}}\). Furthermore, GATA4 activation was shown to be involved in disease-associated downregulation of the K\(_{\text{v}}\)4.2 gene, and Nkx/Csx2.5 was shown to play an important role in cardiac electrical development. Although the present study results provide compelling evidence for a relationship between Mg deficiency and downregulation of the K\(_{\text{v}}\)4.2-Ik\(_{\text{to}}\) and Kir2.1-Ik\(_{\text{to}}\) channels mediated by the transcription factors, NFAT and CREB, we cannot exclude the possibility that other ion channels and/or tran-
scription factors may also contribute. To completely elucidate all possible factors, genome-wide analysis of factors affecting Mg-deficiency dependent electrical remodeling in cardiomyocytes would be needed. Moreover, post-translational control of ion channel transgenes would also be required. The strongest limitation of this study in extrapolating the results to clinical practice is that the isoforms underlying the I_{Ca} channel of rat and human ventricular myocytes are markedly different;^{39} \text{K}v_{4.2}\text{-I}_{\text{Ko}} is not required for repolarization of human cardiac action potentials. Consequently, caution must be used in extrapolating the results of this work to the human heart studies.

**Conclusions**

Our present study demonstrated a hitherto unknown function of intracellular Mg^{2+} as a long-term modulator of cardiac excitability. Furthermore, we provided a novel mechanism for APD and QT prolongation in Mg deficiency. Prolongation of APD in Mg-deficient cardiomyocytes is mainly attributed to a decrease of K^{+} channel density in the sarcolemma caused by the transcriptional downregulation of Kir2.1-I_{K1} and \text{K}v_{4.2}\text{-I}_{\text{Ko}}. Notably, upregulation of NFAT and downregulation of CREB was consistently associated with the reduction of the \text{K}v_{4.2} and Kir2.1 channels. These findings expand our understanding of how intracellular Mg^{2+} in cardiomyocytes modulates cellular protein expression, and represent an important step in understanding the molecular mechanisms that mediate the cardiac electrophysiological effects of Mg deficiency.

**Acknowledgment**

The authors wish to thank Y. Akiyoshi for her technical assistance.

**Funding Source**

This study was supported, in part, by Grants-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (KAKEN, Nos. 15590759, 17590775) to K.O.

**Disclosures**

None.

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Supplementary Files

Please find supplementary file(s); http://dx.doi.org/10.1253/circj.CJ-20-0310