Intracellular Mg$^{2+}$ Depletion Depresses the Delayed Rectifier K$^+$ Current in Guinea Pig Ventricular Myocytes

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Abstract: The effects of various [Mg$^{2+}$], particularly low [Mg$^{2+}$], on the delayed rectifier K$^+$ current ($I_K$) were studied in guinea pig ventricular myocytes with the patch clamp technique. The magnitude of $I_K$ was evaluated from the amplitude of its tail current elicited on repolarization following the depolarizing steps. The pipette-perfusion technique was also used. The initial variations of $I_K$ magnitude were dependent on [Mg$^{2+}$] in the internal solutions with which the whole-cell recording was begun. With 0.03 to 1 mM [Mg$^{2+}$], $I_K$ was relatively stable after patch rupture, showing a minimal decay with time; with 3 mM [Mg$^{2+}$], $I_K$ rapidly declined; with [Mg$^{2+}$] less than 0.01 mM, $I_K$ transiently increased after patch break, but declined progressively thereafter as the magnitude of $I_K$ decreased to about 30% of the initial magnitude in 10 min. The decline of $I_K$ at low [Mg$^{2+}$] showed the following features. The decline was accompanied little by changes in the voltage-activation relation or by changes in the kinetics of current deactivation. The decline was not related to changes in [Ca$^{2+}$], and was also observed in ATPγS-loaded, isoprenaline-stimulated cells, in which $I_K$ channels were presumed to be persistently phosphorylated. An application of okadaic acid did not prevent the decline of $I_K$ during Mg$^{2+}$ depletion. It is suggested that a presence of [Mg$^{2+}$] higher than 0.01 mM is required to maintain $I_K$ in guinea pig ventricular cells. The depression of $I_K$ at low [Mg$^{2+}$] appears to involve a phosphorylation-dephosphorylation–independent mechanism. [Japanese Journal of Physiology, 48, 81–89, 1998]

Key words: intracellular magnesium, delayed guinea pig.

Intracellular magnesium (Mg$^+$) is thought to play an important role in ion channel regulation in cardiac cells. An increase of Mg$^+$ decreases the inward calcium current ($I_{Ca}$) in frog [1, 2] and guinea pig [3] ventricular cells. Several types of K$^+$ channels in cardiac cells are modulated by Mg$^+$. Inward rectification of the ATP-sensitive K$^+$ channels [4, 5], the muscarinic K$^+$ channels [6], and the inwardly rectifying $I_{K1}$ channels [7–10] is due to a voltage-dependent block of the outward current by Mg$^+$. The delayed rectifier K$^+$ current ($I_K$) is also affected by Mg$^+$. In frog atrial cells, $I_K$ has been demonstrated to decrease with time (rundown) after initiation of the whole-cell configuration, when [Mg$^{2+}$] of the pipette solution was higher than 1 to 1.5 mM [11, 12]. When [Mg$^{2+}$] was lower than these values, $I_K$ increased with time (run-up). The mechanism underlying these Mg effects remained unclear, though several possibilities have been considered [13].

The relationship between $I_K$ and Mg$^+$ in mammalian cardiac cells is not yet fully understood. We therefore examined the effects of varying [Mg$^{2+}$] on $I_K$ in guinea pig ventricular myocytes. It will be shown that, unlike $I_{Ca}$ in frog atrium, $I_K$ in these cells does not appreciably run up with [Mg$^{2+}$], as low as 0.03 mM. Moreover, with [Mg$^{2+}$] less than 0.01 mM, $I_K$ rapidly declines with time after a transient run-up. The results also show that $I_{Ca}$ is not attenuated by such an Mg depletion, in contrast to the behavior of $I_K$. 

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METHODS

Preparation of single cells. Single ventricular cells were obtained from guinea pig hearts by using an enzymatic dissociation technique similar to one described elsewhere [14, 15]. Briefly, guinea pigs (250–330 g) were killed by a sodium pentobarbital overdose (70–90 mg/kg, I.P.). The chest was opened and the heart quickly excised. The heart was hung on a Langendorff-type perfusion system and perfused first with Tyrode solution, then with Ca\(^{2+}\)-free Tyrode solution. When the heartbeat ceased, the perfusate was changed to Ca\(^{2+}\)-free Tyrode solution containing collagenase (0.5 mg/ml) (Wako, Tokyo, Japan). After this enzyme treatment, the cells were dissociated in a high-K, low-Cl\(^-\) solution (“KB medium” [15]) and stored in this medium before use.

Patch clamp and recording technique. Whole-cell recordings were performed following the technique described by Hamill et al. [16]. The recording pipettes had a resistance of 1.5 to 2.5 M\(\Omega\) when filled with the pipette solution (internal solution). The cell was voltage-clamped at −40 mV, and rectangular depolarizing pulses (1 s duration) to +30 mV were applied every 10 s in most experiments to activate \(I_K\) and to create its tail current on return to the holding potential. In the experiments shown in Fig. 3, depolarizing pulses of different magnitude were applied every 5 s at various time points after the beginning of whole-cell recording to allow observance of the voltage-dependence of \(I_K\) quickly. In the experiments on \(I_{Ca}\) (Fig. 7), depolarizing pulses (250 ms duration) to 0 mV were applied from the holding potential of −40 mV. In some experiments, a pipette perfusion device [17] was used to change the solution perfusing the cell under the whole-cell configuration.

The whole-cell currents were recorded with a whole-cell clamp system (TM-1000, ACT ME, Tokyo, Japan), and data were stored on a digital audiotape by using a PCM data recorder (RD101T, TEAC, Tokyo, Japan) for later computer analysis (PC98 RL, NEC, Tokyo, Japan). The magnitude of the \(I_K\) tail current, which was considered to reflect the magnitude of the activated \(I_K\), was taken as the difference between the holding current and the outward current immediately after dissipation of the capacitive current transient on repolarization. In some experiments, a more detailed analysis with semilogarithmic plotting of the \(I_K\) tail against time was performed.

Solutions. The normal Tyrode solution contained (mM): NaCl, 140; KCl, 5.4; MgCl\(_2\), 0.5; CaCl\(_2\), 1.8; glucose, 10; HEPES, 5 (pH 7.4 with NaOH). The external solution used for recording \(I_K\) was made by adding 2 or 3 \(\mu\)M nicardipine to the normal Tyrode solution to block the Ca\(^{2+}\) current. The composition of the basic internal solution (without added Mg\(^{2+}\)) was (mM): K-aspartate, 80; KCl, 30; K\(_2\)-ATP, 5; Li\(_2\)-GTP, 0.1; EDTA, 2; HEPES, 5 (pH 7.2 with KOH). To obtain the internal solutions with various concentrations of free Mg\(^{2+}\) (0.001 to 3 mM), appropriate amounts of MgCl\(_2\) calculated according to Fabiato and Fabiato [18] and Tsien and Rink [19], were added to the above solution. For example, 0.65 and 9.9 mM MgCl\(_2\) were added to obtain the solutions containing 0.001 and 3 mM Mg\(^{2+}\), respectively. In this paper, the Mg\(^{2+}\)-deficient internal solution refers to the basic solution described above. It should be noted, however, that this solution probably contained free Mg\(^{2+}\) of about 0.2 to 0.05 \(\mu\)M on an assumption that the solutions were contaminated with 10 to 30 \(\mu\)M Mg\(^{2+}\). The external solution used to record \(I_{Ca}\) was made by omitting KCl in the normal Tyrode solution in exchange for equimolar CsCl. The Mg\(^{2+}\)-deficient internal solution for \(I_{Ca}\) contained (mM): Cs-aspartate, 90; CsCl, 30; Na\(_2\)-ATP, 5; Li\(_2\)-GTP, 0.1; EDTA, 2; HEPES, 5 (pH 7.2 with CsOH). A similar solution with free Mg\(^{2+}\) concentration of 0.1 mM was also prepared in a way similar to the above. All experiments were performed at 36.0±0.5°C.

Statistics. The values are presented as mean±SD. The statistical significance was evaluated by Student’s t-test, and \(p<0.05\) was considered significant.

RESULTS

Figure 1 shows the time course of changes in \(I_K\) tail current after establishment of the whole-cell configuration with three different concentrations of free Mg\(^{2+}\) in the pipette. With Mg\(^{2+}\)-deficient internal solution, the tail current transiently increased after patch rupture, but progressively decreased thereafter. With 3 mM [Mg\(^{2+}\)]\(_i\), the tail current monotonically decreased after patch rupture. In contrast, the tail current declined only slowly when the internal solution contained 0.3 mM Mg\(^{2+}\). Thus after 10 min of the cell dialysis, the magnitude of \(I_K\) tail current, relative to the initial magnitude observed within 20 s after patch rupture, was 29±12% (n=6) with Mg\(^{2+}\)-deficient solution, 81±19% (n=6) at 0.3 mM [Mg\(^{2+}\)]\(_i\), and 35±12% (n=5) at 3 mM [Mg\(^{2+}\)]\(_i\).

The internal solutions with other concentrations of Mg\(^{2+}\) were also examined. The changes in \(I_K\) tail current during cell dialysis with the solutions containing 0.03, 0.1, and 1 mM Mg\(^{2+}\) were essentially similar to those observed with 0.3 mM [Mg\(^{2+}\)]\(_i\), although with 0.03 mM [Mg\(^{2+}\)], a transient increase in the tail cur-
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rent like that seen with Mg$^{2+}$-deficient solution was occasionally observed shortly after patch rupture. The magnitude of $I_K$ tail current obtained 10 min after patch rupture was 80 ± 21% ($n=5$), 76 ± 20% ($n=6$), and 82 ± 12% ($n=6$) of the initial magnitude for 0.03, 0.1, and 1 mM [Mg$^{2+}$], respectively. These values were not significantly different from the value obtained with 0.3 mM [Mg$^{2+}$]. At [Mg$^{2+}$] lower than 0.01 mM (1 to 5 μM), however, the changes in $I_K$ tail current were similar to those observed with Mg$^{2+}$-deficient solution, and at 0.01 mM [Mg$^{2+}$], the results were variable, $I_K$ showing a rapid or slow decay depending on the cell.

Thus at [Mg$^{2+}$] of 0.03 to 1 mM, the magnitude of $I_K$ was relatively stable, showing a minimal decay during the whole-cell recording, under our experimental conditions. The observed slow decay could be due to a spontaneous rundown of the current. These results are different from those obtained in frog atrial cells, in which a clear run-up of $I_K$ was seen when the cells were dialyzed with internal solutions containing 0.1 to 0.3 mM Mg$^{2+}$ [12, 13]. A rapid decay of $I_K$ magnitude during cell dialysis with high [Mg$^{2+}$] was observed in frog atrial cells, and the underlying mechanism has been extensively studied [11–13]. Our results show that a presence of [Mg$^{2+}$], higher than 0.01 mM was required to maintain $I_K$ in guinea pig ventricular cells. In the following, we attempted to further characterize the rapid decline of $I_K$ observed in Mg$^{2+}$-depleted cells.

Figure 2 shows typical changes in the $I_K$ tail current and holding current observed when the cell was dialyzed with Mg$^{2+}$-deficient internal solution. The holding current transiently shifted outwardly after patch rupture. This phenomenon was not observed if [Mg$^{2+}$] was higher than 0.01 mM. Although we did not investigate this phenomenon in detail, changes in the conductance of inwardly rectifying $I_{K1}$ current were likely involved in this current shift, since such a shift of holding current was not observed when the external solution was a K$^+$-free Tyrode solution (not shown), and since it is well known that $I_{K1}$ current is suppressed by depletion of external K$^+$. Furthermore, when atrial cells, in which the density of $I_{K1}$ channels is scarce compared with that in ventricular tissue [20], were used in similar experiments, only changes in $I_K$ were observed during Mg$^{2+}$ depletion without a shift of the holding current (not shown). Whatever the un-

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**Fig. 1.** Time course of changes in $I_K$ tail current after patch rupture with pipette solutions with different [Mg$^{2+}$]. The magnitude of $I_K$ tail current, normalized with reference to initial magnitude (that is, within 20 s after patch rupture), is plotted against the time after patch rupture. $I_K$ tail currents were elicited against repolarization to −40 mV after 1 s voltage step to +30 mV. Patch rupture was made at 0 time. The pipette solution was Mg$^{2+}$-deficient (□), or contained 0.3 mM (△) or 3 mM Mg$^{2+}$ (●). The mean of the data obtained from 5 to 6 cells is given for each pipette solution.

**Fig. 2.** Time course of changes in $I_K$ tail current and holding current after patch rupture with Mg$^{2+}$-deficient pipette solution. Representative record. The magnitudes of $I_K$ tail current and of holding current are plotted against the time after patch rupture. The $I_K$ tail current was elicited on repolarization to holding potential (−40 mV) after 1 s voltage step to +30 mV. The inset shows the current traces (a, b, c, and d) obtained at time points indicated by the corresponding letters in the upper graph.
derlying mechanism, we considered that the shift of holding current and the changes in $I_K$ had no causal relationship. This view may also be supported by the observation that the $I_K$ tail current continued to decline even after the holding current became steady following the transient change (Fig. 2).

To investigate whether the decline of $I_K$ caused by intracellular Mg$^{2+}$ depletion had any voltage-depen-
dent features, the relationship between activation voltage and magnitude of $I_K$ tail current was determined periodically (about every 2 min) during the cell dialysis with Mg$^{2+}$-deficient solution. Figure 3, A and B, shows the results of these experiments. The $I_K$ tail currents following the various test voltages appeared to decrease with a similar time course during Mg$^{2+}$ depletion independent of the magnitude of test voltages (Fig. 3A), and the normalized magnitude of $I_K$ tail currents obtained at different time points showed a similar voltage-dependence (Fig. 3B). The results obtained in four similar experiments are summarized in Fig. 3C. In this figure, only the data obtained at two time points (about 2 and 6 min after patch rupture) during Mg$^{2+}$ depletion are shown for clarity, and it is apparent that the $I_K$ tail currents obtained at these time points had almost the same voltage dependence. These results suggest that Mg$^{2+}$ depletion caused a decline of $I_K$ without changing its voltage dependence.

In accordance with the above, the decline of $I_K$ during Mg$^{2+}$ depletion was accompanied little by changes in the kinetic property of $I_K$. Figure 4 shows a result of the analyses of the current deactivation. The deactivation time course of $I_K$ was fitted with the sum of two exponentials. The time constants of the fast and slow components of $I_K$ deactivation were 131 and 652 ms for $I_K$ recorded immediately after patch rupture (Fig. 4a), 128 and 598 ms for $I_K$ recorded when it was maximal (Fig. 4b, about 1.2 min after patch rupture), and 125 and 605 ms for $I_K$ recorded when it substantially decreased after the peak (Fig. 4c, about 4 min after patch rupture). These results show that the time constants of $I_K$ deactivation were nearly constant during Mg$^{2+}$ depletion. Similar observations were made in two other cells. Thus the kinetic property of $I_K$ did not appear to change appreciably during the course of experiment, although the deactivation kinetics could not accurately be analyzed for $I_K$ at a later stage (Fig. 4d) because of the smallness of the current amplitude.

The decline of $I_K$ caused by intracellular Mg$^{2+}$ depletion was irreversible. In the experiment shown in Fig. 5, the cell was first dialyzed with Mg$^{2+}$-deficient pipette solution, which resulted in a decline of $I_K$ tail current, as usual. An internal solution containing Mg$^{2+}$ (0.3 mM) was then introduced to the cell by using a pipette perfusion device. However, no recovery of the tail current occurred over a period of several min after the Mg$^{2+}$ depletion in this cell. Similar observations were made in three other experiments. Figure 5 also shows that isoproterenol (1 μM) applied after the Mg$^{2+}$ depletion clearly enhanced $I_K$. This was
Fig. 4. Analysis of deactivation time course of the $I_K$ tail currents obtained at different time points during cell dialysis with Mg$^{2+}$-deficient pipette solution. Data from the experiment shown in Fig. 2. Each graph shows a semilogarithmic plot of $I_K$ tail current. In a, b, c, and d, the tail currents denoted by the corresponding letters in the inset of Fig. 2 were analyzed. In a, b, and c, the decay of the $I_K$ tail current was fitted with two exponentials with the time constants indicated in each panel; $\tau_s$ and $\tau_f$ represent the slow and fast components, respectively.

Taken to indicate that the Mg$^{2+}$-containing solution effectively diffused into the cell after the pipette perfusion, since in similar experiments without Mg$^{2+}$ repulsion we observed that the depressed $I_K$ showed little sensitivity to isoprenaline (five experiments, not shown). The reason why the depressed $I_K$ was insensitive to isoprenaline during Mg$^{2+}$ depletion but resumed the sensitivity after Mg$^{2+}$ repulsion is unclear; one possible explanation is that some intracellular reaction steps following beta-adrenergic stimulation required the presence of certain levels of [Mg$^{2+}$], [21].

In the following, we investigated the nature of the $I_K$ decline caused by intracellular Mg$^{2+}$ depletion.

$I_K$ in cardiac cells is thought to be sensitive to [Ca$^{2+}$]. Increasing [Ca$^{2+}$], to $10^{-7}$ M, from $10^{-10}$ M, has been shown to cause an increase in $I_K$ in guinea pig ventricular cells [22]. In the present study, we did not add Ca$^{2+}$ to the pipette solutions, which was expected to keep [Ca$^{2+}$] at a very low level. However, the control of [Mg$^{2+}$], with EDTA buffer also results in some changes in [Ca$^{2+}$]. The [Ca$^{2+}$] levels in the pipette solutions with 0.3 mM Mg$^{2+}$ and without added Mg$^{2+}$ were calculated (see METHODS) to be $3.5 \times 10^{-9}$ and $1.6 \times 10^{-11}$ M, respectively, assuming that the solutions were contaminated with 10 $\mu$M Ca$^{2+}$, and these changes in [Ca$^{2+}$] could have played a role in producing the observed decline of $I_K$. Therefore we examined this point by using an Mg$^{2+}$-deficient pipette solution in which Ca$^{2+}$ concentration was raised to $4 \times 10^{-9}$ M by the addition of CaCl$_2$. With this pipette solution, however, the changes in $I_K$ magnitude after patch rupture were very similar to those observed with the original Mg$^{2+}$-deficient solution (three experiments, not shown). Thus we concluded that the observed $I_K$ decline was not related to the decreased concentration of Ca$^{2+}$ in the pipette solution.

The beta-adrenergic regulation of cardiac $I_K$ channels (for references, see [23]), like that of Ca$^{2+}$ channels (for references, see [24]), is mediated by a mechanism involving phosphorylation and dephosphorylation of the channel protein or related structure. We investigated whether the decline of $I_K$ observed during intracellular Mg$^{2+}$ depletion was related to such a phosphorylation-dephosphorylation-dependent mechanism. The experiment shown in Fig. 6 was designed to see whether phosphorylated $I_K$ channels were sensitive to Mg$^{2+}$ depletion. The cell was first dialyzed with a pipette solution containing 0.1 mM Mg$^{2+}$ and 5 mM ATPyS, a poorly hydrolyzable ATP analogue. ATPyS was added to this solution in exchange for ATP. At 5 min after the patch rupture, 1 $\mu$M isoprenaline was applied to the cell, and this resulted in a marked increase in the $I_K$ tail current. However, the
Fig. 6. Effect of Mg\(^{2+}\) depletion on the \(I_K\) enhanced persistently by isoprenaline in the presence of internal ATP\(_\gamma\)S. The magnitude of \(I_k\) tail current is plotted against time after patch rupture. The \(I_k\) tail currents were elicited on repolarization to -40 mV after a 1 s voltage step to +30 mV. A patch rupture was made with a pipette solution containing 5 mM ATP\(_\gamma\)S and 0.1 mM Mg\(^{2+}\) at 0 time. Then 1 \(\mu\)M isoprenaline (ISO) was added to the bath (bar). About 5 min after the isoprenaline was washed off, replacement of pipette solution with an Mg\(^{2+}\)-deficient one was begun (arrow). Note that the current did not decline after removal of the agonist, but it began to after introduction of the Mg\(^{2+}\)-deficient internal solution.

current did not decrease, and in this case it even tended to increase, after removal of the agonist. The persistent activation of \(I_k\) then observed can be attributed to persistent phosphorylation of the channel brought about by phosphorylation with hydrolysis-resistant \(\gamma\)-phosphate. Under this condition, switching the pipette solution to an Mg\(^{2+}\)-deficient one led to a decline of \(I_k\) tail current. The magnitude of tail current obtained at 10 min after the introduction of Mg\(^{2+}\)-deficient solution was about 50% of its maximum magnitude seen before the solution change (Fig. 6). Similar observations were made in two other ATP\(_\gamma\)S-loaded, isoprenaline-stimulated cells, and in these cells the magnitude of \(I_k\) tail current after 10 min perfusion with Mg\(^{2+}\)-deficient solution was 36 and 44% of its maximum magnitude.

Thus the activity of the persistently phosphorylated \(I_k\) channels appeared to decline in response to Mg\(^{2+}\) depletion, the rate and degree being comparable to those observed in ATP-loaded, nonstimulated cells (Fig. 1). The simplest explanation of these findings may be to assume that Mg\(^{2+}\)-depletion affected \(I_k\) channels through a phosphorylation-independent mechanism, regardless of whether they were phosphorylated. The relationship between the \(I_k\) decline and phosphorylation was further examined in the following.

\(I_k\) might be regulated by a phosphorylation-dephosphorylation cycle even under the basal conditions, that is, in the absence of stimulating agonists, in guinea pig ventricular cells [25], and Mg\(^{2+}\) depletion might depress \(I_k\) by affecting such a process. To test this possibility, we examined whether okadaic acid, an inhibitor of protein phosphatases, could modulate the behavior of \(I_k\) during Mg\(^{2+}\) depletion. The cells were dialyzed with Mg\(^{2+}\)-deficient solution containing 10 \(\mu\)M okadaic acid. Under this condition, the changes in \(I_k\) magnitude after patch rupture were similar to those observed in the absence of okadaic acid (four experiments, not shown), suggesting that the decline of \(I_k\) occurred independently of, if any, an okadaic acid-sensitive phosphorylation-dephosphorylation process. However, the initial transient increase in \(I_k\) tail current after patch rupture appeared to be more pronounced with okadaic acid; the tail current transiently increased 30 to 40% compared with the initial magnitude, whereas its increase was about 20% in control (Fig. 1). The nature of this difference is unknown.

The question remains as to whether Mg\(^{2+}\) depletion exerts deleterious effects specifically or nonspecifically on \(I_k\) channels. To obtain information on this point, we examined the effect of Mg\(^{2+}\) depletion on \(I_{Ca}\). When the whole cell recording was begun with Mg\(^{2+}\)-deficient internal solution, the magnitude of \(I_{Ca}\) initially increased to some extent (run-up), then slowly declined during Mg\(^{2+}\) depletion (Fig. 7A). However, the rate of \(I_{Ca}\) decline was much slower in comparison with the rate of \(I_k\) decline observed during Mg\(^{2+}\) depletion (Fig. 1); \(I_{Ca}\) was relatively well maintained after the initiation of Mg\(^{2+}\) depletion, its magnitude after 15 min being more than 80% of the maximum magnitude in most cells. In some cells, \(I_{Ca}\) declined little during Mg\(^{2+}\) depletion. Thus the behavior of \(I_{Ca}\) during Mg\(^{2+}\) depletion was quite different from that of \(I_k\), \(I_{Ca}\) showing only little or no decline. The slow decline of \(I_{Ca}\) observed in most cells may represent a spontaneous rundown of \(I_{Ca}\) channels.

However, \(I_{Ca}\), like \(I_k\), was insensitive to \(\beta\)-adrenergic stimulation during Mg\(^{2+}\) depletion. As shown in Fig. 7B, 1 \(\mu\)M isoprenaline had no effect on \(I_{Ca}\) in the cell that had been dialyzed with Mg\(^{2+}\)-deficient internal solution for 5 min. Similar results were obtained in five other cells. This finding suggests that as considered for the ineffectiveness of isoprenaline on \(I_k\) during Mg\(^{2+}\) depletion, completion of the \(\beta\)-adrenergic modulation of Ca\(^{2+}\) channel required the presence of intracellular Mg\(^{2+}\). When internal solutions containing 0.1 mM Mg\(^{2+}\) were used, \(I_{Ca}\) changed in a manner similar to the above after patch rupture, but it clearly increased in response to isoprenaline (not shown).
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Fig. 7. A: Time course of changes in $I_{Ca}$ during intracellular Mg$^{2+}$ depletion. The magnitude of $I_{Ca}$ is plotted against time after patch rupture. The $I_{Ca}$ was elicited by applying a 250 ms depolarizing pulse to 0 mV from a holding potential of −40 mV. The magnitude of $I_{Ca}$ was taken as the difference between the peak inward current at the beginning of the pulse and the minimum inward current at the end of the pulse. A patch rupture was made at 0 time. The inset shows an example of $I_{Ca}$ record with zero current level (dotted line). B: Effect of isoprenaline on $I_{Ca}$ in a cell perfused with Mg$^{2+}$-deficient solution. One micromolar isoprenaline was added to the bath 5 min after the patch rupture. The cell was exposed to the drug for 5 min. Records a, b, and c were obtained immediately before, during (3 min after the drug), and after the application of isoprenaline. The dotted line indicates zero current level.

DISCUSSION

The intracellular Mg$^{2+}$ depletion depressed $I_K$ in guinea pig ventricular cells. The findings that this depression was accompanied little by changes in the voltage-activation relation of $I_K$ (Fig. 3) or by changes in its deactivation kinetics (Fig. 4) suggested that Mg$^{2+}$ depletion reduced $I_K$ without affecting its voltage dependence. On the other hand, Williams and Beatch [26] recently reported a different result. They showed that decreases in [Mg$^{2+}$], increased $I_K$ with a half-maximal effect of about 20 mM in guinea pig ventricular cells. However, it was not clear in their study how the magnitude of $I_K$ changed with time after patch rupture, or whether $I_K$ kept a steady magnitude during prolonged Mg$^{2+}$ depletion. We did observe an increase in $I_K$ when the whole-cell recording was begun with Mg$^{2+}$-deficient internal solution, but it was only transient; $I_K$ progressively declined thereafter (Figs. 1 and 2). It seems difficult to simply compare the present results with the report by Williams and Beatch.

The presence of intracellular Mg$^{2+}$ appears to be essential for maintaining the functional $I_K$ channels in ventricular cells. In the present study, [Mg$^{2+}$], higher than 0.01 mM was required to prevent the rapid decline of $I_K$ during cell dialysis. Under our experimental conditions, however, decreasing the concentration of free Mg$^{2+}$ in the pipette solutions resulted in a concomitant decrease in the concentration of Mg-ATP complex. For example, [Mg-ATP], was calculated to be 0.33 and 1.4 mM in the solutions with 0.005 and 0.03 mM [Mg$^{2+}$], respectively. Therefore, there may be the question of whether $I_K$ depended on free Mg$^{2+}$ ions on Mg-ATP in the present study. It seems unlikely, however, that the low Mg-ATP level caused the decline of $I_K$ because of the following reasons. First, the rapid $I_K$ decline was observed even with the 0.005 mM [Mg$^{2+}$], solution, which contained a submillimolar level of [Mg-ATP], (0.33 mM). Second, when the cell was dialyzed with an ATP-free, Mg$^{2+}$-containing (0.3 mM) solution in which Mg-ATP was absent, $I_K$ declined little or only slowly for at least 20 min (data not shown). This is consistent with the observation of others; $I_K$ could be recorded stably in guinea pig ventricular cells dialyzed with ATP-free Mg$^{2+}$-containing solution [27] and in frog atrial cells dialyzed with a similar solution [11]. Thus we consider that free Mg$^{2+}$ ions play a key role in maintaining the $I_K$ channels.

As with the mechanism underlying the decline of $I_K$ during intracellular Mg$^{2+}$ depletion, our results suggested that the decline was not related to changes in the intracellular Ca$^{2+}$ concentration. Furthermore, the decline was considered to involve a phosphorylation-dephosphorylation-independent mechanism, since $I_K$ declined in response to Mg$^{2+}$ depletion in ATPγS-loaded, isoprenaline-stimulated cells in which the $I_K$ channels were presumed to be persistently phosphorylated (Fig. 6), and since an application of okadaic acid, a phosphatase inhibitor, did not prevent the decline of $I_K$ during Mg$^{2+}$ depletion. However, because okadaic acid has a high specificity to type 1- and type 2A-phosphatases [25], our results may not rule out the possibility that other types of phosphatases were involved. Therefore, our view that the $I_K$ decline during Mg$^{2+}$ depletion was mediated by a phosphorylation-dephosphorylation-independent mechanism should be examined in further experiments.

$I_K$ declined not only during intracellular Mg$^{2+}$-depletion, but also during cell dialysis with Mg$^{2+}$-rich (3 mM) solution (Fig. 1). A similar $I_K$ decline at high
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[Mg^{2+}], (1.5 to 10 mM) has also been observed in frog atrial cells [11–13], and this Mg^{2+} effect was reversible [12, 13]. In the frog atrial cells, however, $I_k$ increased with time (run-up) after patch break when [Mg^{2+}] was low (0.1 and 0.3 mM), and the relationship between steady state magnitude of $I_k$ and [Mg^{2+}], (0.1 to 3 mM) was monotonic, with smaller $I_k$ at higher [Mg^{2+}], [13]. In our preparations, the run-up of $I_k$ at low [Mg^{2+}] was only transient, and $I_k$ was relatively stable at 0.03 to 1 mM [Mg^{2+}]. Thus the dependence of $I_k$ on [Mg^{2+}], in guinea pig ventricular cells appears different from that in frog atrial cells. The monotonic decreases in $I_k$ with increases in [Mg^{2+}], in frog atrial cells were suggested to be due to allosteric binding of Mg^{2+} to the channels, which may modify their gating properties, and/or a result of Mg^{2+}-dependent modulation of intermediate enzymes, which may modify the channel properties [13]. These mechanisms might also explain the decline of $I_k$ at 3 mM [Mg^{2+}], and the initial transient increase in $I_k$ at low [Mg^{2+}], observed in this study (Fig. 1), but the rather stable behavior of $I_k$ at 0.03 to 1 mM [Mg^{2+}], may indicate that the mechanisms proposed for frog atrial cells operate only weakly in guinea pig ventricular cells.

The response of $I_{Ca}$ to Mg^{2+} depletions contrasted to the response of $I_k$. In most cells examined, $I_{Ca}$ initially increased substantially, then declined only slowly during Mg^{2+} depletions (Fig. 7). If the slow decline is attributed to a spontaneous rundown of $I_{Ca}$ channels, the finding indicates that $I_{Ca}$ increases in response to Mg^{2+} depletions in guinea pig ventricular cells. This is in agreement with the observations of others [1–3]. The finding also suggests that the rapid decline of $I_k$ during Mg^{2+} depletions was not due to any nonspecific effect of Mg^{2+} depletion on the ion channels. To explain the effects of Mg^{2+} depletions on $I_k$, we speculate that the $I_k$ channel protein or related structure has some specific sites, of which association with Mg^{2+} is essential to maintain the channel function. Alternatively, some Mg^{2+}-dependent intermediate enzymes or substances may control the channel activity. Further studies will be necessary to test these possibilities and to clarify the nature of the Mg^{2+}-dependence of cardiac $I_k$ channels.

The normal [Mg^{2+}], in mammalian cardiac cells is thought to be about 1 mM (for references, see [28, 29]), and it is unlikely that [Mg^{2+}], falls to a level lower than 0.01 mM under physiologic and pathological conditions. Therefore the present findings may have no direct relevance to the electrophysiological functions of cardiac cells. On the other hand, our results may provide a noteworthy matter for the experimental study. In the whole-cell studies performed on cardiac cells, the internal solution is usually buffered with EGTA and contains MgCl$_2$ and ATP, which are the major determinants of [Mg^{2+}], level. In a combination of 1 mM MgCl$_2$ and 5 mM ATP, which may be one of the popular combinations (for example, [22, 23]), [Mg^{2+}], is calculated to be about 0.02 mM. This value is not only far from the normal level, but also near the critical level for $I_k$ shown in the present study. With 0.5 mM MgCl$_2$ and 5 mM ATP, [Mg^{2+}], will take a value of less than 0.01 mM, at which $I_k$ may be depressed. Our results suggest that in the preparation of internal solutions with a combination of inorganic magnesium salts and Mg^{2+}-binding compounds, their concentrations should be selected carefully to obtain appropriate levels of [Mg^{2+}], especially in the studies on cardiac $I_k$.

Finally, the cardiac $I_k$ has been shown to have two distinct components; a rapidly activating one ($I_{kL}$) that shows a prominent inward rectification, and a slowly activating one ($I_{kS}$) that shows a minimal rectification [30, 31]. In the present study we analyzed $I_k$ without considering these components, the measured $I_k$ representing their total. Since fully activated $I_{kS}$ is about 10-fold larger than fully activated $I_{kL}$ in guinea pig ventricle [30], and since $I_{kS}$ should be the dominant component of $I_k$ activated by 1 s depolarizing pulses because of its slow kinetics [30, 31], it is expected that the analyzed $I_k$ mainly represented $I_{kS}$. We therefore consider that at least $I_{kS}$ was depressed by Mg^{2+} depletion. Whether $I_{kL}$ is sensitive to Mg^{2+} depletion will be a subject of further investigation.

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