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

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Abstract: The effects of various $[Mg^{2+}]_i$, particularly low $[Mg^{2+}]_i$, 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 $[Mg^{2+}]_i$ in the internal solutions with which the whole-cell recording was begun. With 0.03 to 1 mM $[Mg^{2+}]_i$, I_K was relatively stable after patch rupture, showing a minimal decay with time; with 3 mM $[Mg^{2+}]_i$, I_K rapidly declined; with $[Mg^{2+}]_i$ 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 magni-

tude in 10 min. The decline of I_K at low $[Mg^{2+}]_i$ 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 $[Ca^{2+}]_i$ and was also observed in ATP γ S-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 $[Mg^{2+}]_i$ higher than 0.01 mM is required to maintain I_K in guinea pig ventricular cells. The depression of I_K at low $[Mg^{2+}]_i$ appears to involve a phosphorylation-dephosphorylation-independent mechanism. [Japanese Journal of Physiology, 48, 81–89, 1998]

Key words: intracellular magnesium, delayed rectifier potassium current, ventricular myocyte, guinea pig.

Intracellular magnesium (Mg_i) is thought to play an important role in ion channel regulation in cardiac cells. An increase of Mg_i 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_i . 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_i . The delayed rectifier K^+ current (I_K) is also affected by Mg_i . 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_i effects remained unclear, though several possibilities have been considered [13].

The relationship between I_K and Mg_i in mammalian cardiac cells is not yet fully understood. We therefore examined the effects of varying $[Mg^{2+}]_i$ on I_K in guinea pig ventricular myocytes. It will be shown that, unlike I_K in frog atrium, I_K in these cells does not appreciably run up with $[Mg^{2+}]_i$ as low as 0.03 mM. Moreover, with $[Mg^{2+}]_i$ 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_i depletion, in contrast to the behavior of I_K .

Received on October 28, 1997; accepted on January 30, 1998

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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 Ω 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 μM nifedipine 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; $\text{K}_2\text{-ATP}$, 5; $\text{Li}_2\text{-GTP}$, 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 0.02 to 0.05 μM on an assumption that the solutions were contaminated with 10 to 30 μM 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; $\text{Na}_2\text{-ATP}$, 5; $\text{Li}_2\text{-GTP}$, 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 $36.0 \pm 0.5^\circ\text{C}$.

Statistics. The values are presented as mean $\pm$ 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 $[\text{Mg}^{2+}]_i$, 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 $29 \pm 12\%$ ($n=6$) with Mg^{2+} -deficient solution, $81 \pm 19\%$ ($n=6$) at 0.3 mM $[\text{Mg}^{2+}]_i$, and $35 \pm 12\%$ ($n=5$) at 3 mM $[\text{Mg}^{2+}]_i$.

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 $[\text{Mg}^{2+}]_i$, although with 0.03 mM $[\text{Mg}^{2+}]_i$ a transient increase in the tail cur-

rent like that seen with Mg²⁺-deficient solution was occasionally observed shortly after patch rupture. The magnitude of *I_K* tail current obtained 10 min after patch rupture was $80 \pm 21\%$ ($n=5$), $76 \pm 20\%$ ($n=6$), and $82 \pm 12\%$ ($n=6$) of the initial magnitude for 0.03, 0.1, and 1 mM [Mg²⁺]_i, respectively. These values were not significantly different from the value obtained with 0.3 mM [Mg²⁺]_i. At [Mg²⁺]_i lower than 0.01 mM (1 to 5 μM), however, the changes in *I_K* tail current were similar to those observed with Mg²⁺-deficient solution, and at 0.01 mM [Mg²⁺]_i the results were variable, *I_K* showing a rapid or slow decay de-

Fig. 1. Time course of changes in *I_K* tail current after patch rupture with pipette solutions with different [Mg²⁺]_i. 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 on repolarization to -40 mV after 1 s voltage step to $+30$ mV. Patch rupture was made at 0 time. The pipette solution was Mg²⁺-deficient (○), or contained 0.3 mM (△) or 3 mM Mg²⁺ (□). The mean of the data obtained from 5 to 6 cells is given for each pipette solution.

pending on the cell.

Thus at [Mg²⁺]_i 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 Mg²⁺ [12, 13]. A rapid decay of *I_K* magnitude during cell dialysis with high [Mg²⁺]_i was observed in frog atrial cells, and the underlying mechanism has been extensively studied [11–13]. Our results show that a presence of [Mg²⁺]_i 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 Mg²⁺-depleted cells.

Figure 2 shows typical changes in the *I_K* tail current and holding current observed when the cell was dialyzed with Mg²⁺-deficient internal solution. The holding current transiently shifted outwardly after patch rupture. This phenomenon was not observed if [Mg²⁺]_i 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 Mg²⁺ depletion without a shift of the holding current (not shown). Whatever the un-

Fig. 2. Time course of changes in *I_K* tail current and holding current after patch rupture with Mg²⁺-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-dependence

Fig. 3. Changes in I_K tail current during cell dialysis with Mg^{2+} -deficient internal solution. **A** and **B** show representative data obtained in a cell. The tail currents were elicited on repolarization to holding potential (-40 mV) after 1 s step to various test voltages. **A**: The magnitude of tail current is plotted against test voltages. The data were obtained at about 2 min ($\circ$), 4 min ($\triangle$), 6 min ($\square$), 8 min ($\bullet$), and 10 min ($\blacktriangle$) after patch rupture. **B**: The normalized magnitude of tail current is plotted against test voltages. The data in **A** were normalized relative to values at 80 mV and fitted to a Boltzmann relation, $y = 1/(1 + \exp[(V_h - V)/k])$, where V is test voltage, V_h the voltage at half-maximal activation (36.3 mV), and k the slope factor (16.3 mV). **C**: Summary of data obtained from 4 cells. The tail currents were obtained as in the experiment shown in **A** and were normalized. Only the data obtained at about 2 min ($\circ$) and 6 min ($\triangle$) after patch rupture are shown. The data were fitted to the above Boltzmann relation with $V_h = 30.4$ mV and $k = 14.9$ mV. The inset shows the original data that were normalized relative to the values at 80 mV obtained at about 2 min after patch rupture. The vertical lines show $\pm$ SD.

ent 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+} repletion in this cell. Similar observations were made in three other experiments. Figure 5 also shows that isoprenaline (1 μ M) applied after the Mg^{2+} repletion 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 Mg²⁺-deficient pipette solution. Data from the experiment shown in Fig. 2. Each graph shows a semi-logarithmic 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; τ_s and τ_f represent the slow and fast components, respectively.

taken to indicate that the Mg²⁺-containing solution effectively diffused into the cell after the pipette perfusion, since in similar experiments without Mg²⁺ repletion 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 Mg²⁺ depletion but resumed the sensitivity after Mg²⁺ repletion is unclear; one possible explanation is that some intracellular reaction steps following β -adrenergic stimulation required the presence of certain levels of [Mg²⁺]_i [21]. In the following, we investigated the nature of the *I_K* decline caused by intracellular Mg²⁺ depletion.

I_K in cardiac cells is thought to be sensitive to [Ca²⁺]_i. Increasing [Ca²⁺]_i 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 Ca²⁺ to the pipette solutions, which was expected to keep [Ca²⁺]_i at a very low level. However, the control of [Mg²⁺]_i with EDTA buffer also results in some changes in [Ca²⁺]_i. The [Ca²⁺]_i levels in the

Fig. 5. Effect of [Mg²⁺]_i on *I_K* tail current. 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 1 s voltage step to +30 mV. The initial patch rupture was made with Mg²⁺-deficient pipette solution at 0 time. Three minutes later (arrow), perfusion of the pipette with a solution containing 0.3 mM Mg²⁺ was begun. The effect of 1 mM isoprenaline (ISO) on the *I_K* tail current is also shown (bar).

pipette solutions with 0.3 mM Mg²⁺ and without added Mg²⁺ were calculated (see METHODS) to be 3.5×10⁻⁹ and 1.6×10⁻¹¹ M, respectively, assuming that the solutions were contaminated with 10 μM Ca²⁺, and these changes in [Ca²⁺]_i could have played a role in producing the observed decline of *I_K*. Therefore we examined this point by using an Mg²⁺-deficient pipette solution in which Ca²⁺ concentration was raised to 4×10⁻⁹ M by the addition of CaCl₂. With this pipette solution, however, the changes in *I_K* magnitude after patch rupture were very similar to those observed with the original Mg²⁺-deficient solution (three experiments, not shown). Thus we concluded that the observed *I_K* decline was not related to the decreased concentration of Ca²⁺ in the pipette solution.

The β -adrenergic regulation of cardiac *I_K* channels (for references, see [23]), like that of Ca²⁺ 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 Mg²⁺ 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 Mg²⁺ depletion. The cell was first dialyzed with a pipette solution containing 0.1 mM Mg²⁺ and 5 mM ATP γ S, a poorly hydrolyzable ATP analogue. ATP γ S was added to this solution in exchange for ATP. At 5 min after the patch rupture, 1 μM 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 $\text{ATP}\gamma\text{S}$. 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 $\text{ATP}\gamma\text{S}$ and 0.1 mM Mg^{2+} at 0 time. Then 1 μM 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 γ -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 $\text{ATP}\gamma\text{S}$ -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-dephos-

phorylation 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 μM 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 β -adrenergic stimulation during Mg^{2+} depletion. As shown in Fig. 7B, 1 μM 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 β -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).

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 $[Mg^{2+}]_i$ increased I_K with a half-maximal effect of about 20 nM 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 there-

after (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, $[Mg^{2+}]_i$ 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, $[Mg-ATP]_i$ was calculated to be 0.33 and 1.4 mM in the solutions with 0.005 and 0.03 mM $[Mg^{2+}]_i$, respectively. Therefore, there may be the question of whether I_K depended on free Mg^{2+} ions or 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 $[Mg^{2+}]_i$ solution, which contained a sub-millimolar level of $[Mg-ATP]_i$ (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 ATP γ S-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

$[\text{Mg}^{2+}]_i$ (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 $[\text{Mg}^{2+}]_i$ was low (0.1 and 0.3 mM), and the relationship between steady state magnitude of I_K and $[\text{Mg}^{2+}]_i$ (0.1 to 3 mM) was monotonic, with smaller I_K at higher $[\text{Mg}^{2+}]_i$ [13]. In our preparations, the run-up of I_K at low $[\text{Mg}^{2+}]_i$ was only transient, and I_K was relatively stable at 0.03 to 1 mM $[\text{Mg}^{2+}]_i$. Thus the dependence of I_K on $[\text{Mg}^{2+}]_i$ in guinea pig ventricular cells appears different from that in frog atrial cells. The monotonic decreases in I_K with increases in $[\text{Mg}^{2+}]_i$ 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 $[\text{Mg}^{2+}]_i$ and the initial transient increase in I_K at low $[\text{Mg}^{2+}]_i$ observed in this study (Fig. 1), but the rather stable behavior of I_K at 0.03 to 1 mM $[\text{Mg}^{2+}]_i$ 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+} depletion contrasted to the response of I_K . In most cells examined, I_{Ca} initially increased substantially, then declined only slowly during Mg^{2+} depletion (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+} depletion 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+} depletion was not due to any nonspecific effect of Mg^{2+} depletion on the ion channels. To explain the effects of Mg^{2+} depletion 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 $[\text{Mg}^{2+}]_i$ in mammalian cardiac cells is thought to be about 1 mM (for references, see [28, 29]), and it is unlikely that $[\text{Mg}^{2+}]_i$ falls to a level lower than 0.01 mM under physiological 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 experi-

mental 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 $[\text{Mg}^{2+}]_i$ 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]), $[\text{Mg}^{2+}]_i$ 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, $[\text{Mg}^{2+}]_i$ 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 $[\text{Mg}^{2+}]_i$, 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_{Kr}) 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_{Kr} 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_{Kr} is sensitive to Mg^{2+} depletion will be a subject of further investigation.

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan. One author (K.H.) extends thanks to Prof. K. Taki, Department of Emergency Medicine, Saga Medical School, for providing him an opportunity to do this work. Okadaic acid was a generous gift from Dr. Y. Tsukitani, Fujisawa Pharmaceutical Co., Tokyo, Japan.

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