Regulation of the Voltage-Gated Ca$^{2+}$ Current by Intracellular Free Mg$^{2+}$ Studied by Internal Perfusion of Single Cardiac Myocytes

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Introduction

Despite the fact that Mg$^{2+}$ is the major divalent cation in the cell, relatively little is known about the regulation of its intracellular concentration or the effects of changes in this on cellular function. Recently, it has become evident that a variety of different kinds of ion channels are affected by intracellular Mg$^{2+}$. For example, inward rectification of several kinds of K$^+$ channels including the inward rectifier, the ATP-dependent K$^+$ channel, and the acetylcholine (ACh)-activated K$^+$ channel has been shown to be caused by a time- and voltage-dependent blockade of the K$^+$ channel by intracellular Mg$^{2+}$ (Matsuda et al. 1987; Matsuda 1988; Horie and Irisawa 1987, 1989; Horie et al. 1987; Vandenbergh 1987). There are reasons to believe, however, that the effects of Mg$^{2+}$ on ionic channels are not limited to simple obstruction of the permeation pathway. For example, the delayed rectifier current (I_K) is reduced in amplitude by increasing intracellular Mg$^{2+}$, but the mechanism of this effect is rather insensitive to voltage, suggesting that Mg$^{2+}$ blocks this channel at a site that is remote from the pore itself (Duchatelle-Gourdon et al. 1989). Recently, Pusch et al. (1989) have shown that outward current through tetrodotoxin (TTX)-sensitive Na$^+$ channels is blocked in a voltage- and time-dependent fashion by intracellular Mg$^{2+}$ in a manner consistent with Mg$^{2+}$ blockage of ion permeation. However, in addition, internal Mg$^{2+}$ also affects inward current through these channels, implying another site of Mg$^{2+}$ action.

In this article, we will summarize work in my lab (White and Hartzell 1988, 1989; Hartzell and White 1989) on the modulation of voltage-gated Ca$^{2+}$ currents by intracellular Mg$^{2+}$. Voltage-gated Ca$^{2+}$ channels are present in most, if not all, excitable cells and play a key role in the initiation and maintenance of contraction of cardiac cells (Hartzell 1988). Thus, understanding the mechanisms by which these channels are affected by intracellular constituents such as Mg$^{2+}$ is an important step in understanding how these channels are regulated physiologically and how their malfunction may occur pathologically.
Complex effects of Mg$^{2+}$ have been found on $I_{Ca}$ amplitude and inactivation that can be partly explained by supposing that Mg$^{2+}$ binds to site in the permeation pathway, thus obstructing the passage of permeant ions and also altering the mode of channel inactivation. Furthermore, the binding of Mg$^{2+}$ to this site is modulated by channel phosphorylation.

Methods

Single myocytes were isolated from frog ventricle using enzymatic and mechanical dissociation procedure that have been described in detail elsewhere (Fischmeister and Hartzell 1987; White and Hartzell 1988; Hartzell and White 1989). $I_{Ca}$ was measured using the whole-cell configuration of the patch clamp technique (Hamill et al. 1981). $I_{na}$ was blocked with TTX. K$^+$ currents were blocked by both internal and external Cs$^+$. Sometimes internal tetraethylammonium (TEA) was also used. Currents were elicited by 200- or 400-ms duration voltage clamp pulses. They were leak-subtracted using the following procedure. Leak was determined by averaging several current traces evoked by voltage pulses from $-80$ mV to $-70$ mV. The mean holding current and leak current were calculated from the averaged trace and used to construct an idealized noise-free leak trace. The trace was then multiplied by the appropriate scaling factor, assuming a linear current–voltage (I–V) relationship for the leak, and subtracted from the $I_{Ca}$, $I_{na}$, or $I_{CaNa}$ traces. The voltage protocols for determining $I_{Ca}$ inactivation are based on the two-pulse paradigm of Hodgkin and Huxley (1952) and have already been published (Fischmeister and Hartzell 1986; Argibay et al. 1988). Half-inactivation was defined as the prepulse potential producing 50% of the inactivation produced by the same duration prepulse to 0 mV.

External solutions contained 115 mM NaCl, 20 mM CsCl, 0.3–3.0 mM MgCl$_2$, 1.8 mM CaCl$_2$ or 2 mM BaCl$_2$, 5 mM sodium pyruvate, 5 mM glucose, 0.001 mM TTX, and 10 mM HEPES (N-2-hydroxyethyl-piperazine-N’-2-ethane sulfonic acid), at pH 7.4. External Mg$^{2+}$ concentration was varied between 0.3 mM and 3 mM without compensation for changes in osmotic strength. When Ba$^{2+}$ was used as charge carrier, Ca$^{2+}$ was absent. When Na$^+$ was the charge carrier, the external solution contained 25 mM NaCl, 110 mM CsCl, 0.1 mM EGTA, and 10 mM HEPES at pH 8.5. The internal solution varied in composition and could be changed during recording by a system permitting continuous perfusion of the patch electrode (Hartzell and Fischmeister 1986; Fischmeister and Hartzell 1987).

The goal of these experiments was to examine the effects of changing free intracellular Mg$^{2+}$ concentration while maintaining MgATP and other ion concentrations as constant as possible. Composition of the solutions was calculated by a computer program developed by Godt and Lindley (1982), which was kindly provided by Dr. Robert Godt (Medical College of Georgia, Augusta). The stability constants were the same as those published by Godt and Lindley (1982). In these calculations, I assumed that Cs$^+$ was equivalent
to K⁺. Solution composition is given in more detail in Hartzell and White (1989).

Currents were fitted to sums of exponentials by a Marquart least squares procedure (FIT program, W. Goolsby, Emory University). Leak-subtracted currents were used for the least squares fitting routine, and the current between the peak inward current and the end of the pulse was subjected to the curve-fitting routine.

**Results**

**Effects of Mg²⁺ on Basal Ica and Ina Amplitude**

The effect of internal Mg²⁺ on Ica was examined by eliciting Ica with 400-ms voltage pulses from −80 mV holding potential to various potentials at 8-s intervals, [Mg²⁺], was altered by changing the solution in the recording pipette by means of a perfusion system. There were significant differences in the effect of [Mg²⁺] on Ica. At the peak of the I–V relationship, the effects of [Mg²⁺] on Ica amplitude were minimal, whereas the effects of Ba²⁺ (Ina) or Na⁺ (IcaNa) current through Ca²⁺ channels were greater. Specifically, in 15 cells that were perfused with both 0.3 mM Mg²⁺ and 3.0 mM Mg²⁺, Ica at 0 mV was reduced 11% ± 8.5% (mean ± SE) upon switching to the higher Mg²⁺ concentration. The largest reduction observed was 34%. In comparing two partially overlapping populations of cells that were perfused with either 0.3 mM or 3.0 mM Mg²⁺ or both concentrations, Ica in the presence of 3 mM Mg²⁺ was 20% less than it was in the presence of 0.3 mM Mg²⁺ (n = 97 for 0.3 mM and n = 45 for 3 mM Mg²⁺). The effects of internal Mg²⁺ on the amplitude of Ina were somewhat greater; Ina in the presence of 3.0 mM Mg²⁺ was reduced 36% compared with Ina in the presence of 0.3 mM Mg²⁺ in 25 cells studied. In approximately 10 cells in which we used Na⁺ as the charge carrier in the absence of any external divalent cations, increasing [Mg²⁺] caused a very significant decrease in IcaNa at −20 mV it was reduced by 50% – 80%.

In addition to producing these changes in maximal current amplitude, increasing [Mg²⁺] also shifted the I–V relationships to more negative potentials (Fig. 1). The magnitude of this effect was dependent upon the charge carrier. The I–V relationship for Ica, was shifted an average of 7 mV to the left, that for Ina, 10 mV, and that for IcaNa, 20 mV. Thus, some of the decrease in the currents observed at 0 mV is due to the shift in the I–V relationship. Despite this shift, however, the maximum current in the presence of 3 mM Mg²⁺ was smaller than in the presence of 0.3 mM Mg²⁺ when Ba²⁺ or Na⁺ was the charge carrier.

We can suggest two possible mechanisms by which the shift in the I–V relationship might be produced by [Mg²⁺]. [Mg²⁺] could shift the I–V relationship by screening a negative internal surface charge. Such a mechanism would be nonspecific and would not involve any direct effect on Ca²⁺ chan-
Fig. 1. Current-voltage relationships of $I_{\text{Ca}}$, $I_{\text{Ba}}$, and $I_{\text{Na}}$ with different [Mg$^{2+}$]. Current-voltage relationships were obtained by 400-ms pulses from $$-80 \text{ mV}$$ holding potential. Currents were leak subtracted by subtracting a linearly scaled current produced in response to a 10-mV depolarization. Solid squares, internal solution of 0.3 mM Mg$^{2+}$. Solid circles, internal solution of 3 mM Mg$^{2+}$. Permeant ion species were: 1.8 mM Ca, 3 mM Ba, 25 mM Na. Solutions are described in detail in Methods. Lower portion shows the percentage inhibition of inward current as a function of membrane potential produced by switching from 0.3 mM Mg$^{2+}$ to 3 mM Mg$^{2+}$.

nals. Alternatively, [Mg$^{2+}$] could compete for permeant ion binding to permeation sites in the channel in a voltage-dependent manner. These possibilities are explored in more detail below.

Effects of Mg$^{2+}$ on $I_{\text{Ca}}$ and $I_{\text{Ba}}$ Inactivation Under Basal Conditions

$Ca^{2+}$ Channel Inactivation

$I_{\text{Ca}}$ inactivation occurs by two mechanisms: one is voltage dependent and the other is Ca$^{2+}$ dependent. In general (Eckert and Chad 1984), these two mechanisms can be distinguished experimentally by using Ba$^{2+}$ or Na$^{+}$ as the charge carrier, because these ions do not activate the Ca$^{2+}$-dependent component of inactivation (Lee et al. 1985), or by buffering internal Ca$^{2+}$ to low levels with EGTA or BAPTA (Bechem and Pott 1985). In our studies, however, using Ba$^{2+}$ as the charge carrier slows inactivation much more than does Ca$^{2+}$ buffering (Argibay et al. 1988). Even with 25 mM BAPTA internally, $I_{\text{Ca}}$ inactivates more rapidly than does $I_{\text{Ba}}$ (H.C. Hartzell, unpublished data). This suggests that either it is impossible to buffer Ca$^{2+}$ at the site responsible for Ca$^{2+}$-dependent inactivation, perhaps because this site is in the permeation pathway, or that BA$^{2+}$ affects channel gating independently of Ca$^{2+}$-dep
dent inactivation. This possibility is strengthened by the observation that the inactivation kinetics of $I_{\text{Na}}$ depend upon the Ba$^{2+}$ concentration, even in the nominal absence of Ca$^{2+}$. Ignoring these possible complications, the effects of [Mg$^{2+}$] on $I_{\text{Ca}}$ and $I_{\text{Na}}$ inactivation were examined in order to gain insights into the mechanism of the Mg$^{2+}$ effect.

Before looking at the effects of [Mg$^{2+}$] on inactivation, the inactivation of $I_{\text{Ca}}$ and $I_{\text{Na}}$ was characterized. The rates of Ca$^{2+}$-dependent and voltage-dependent inactivation were estimated by a standard gapped two-pulse protocol consisting of a prepulse of varying duration and amplitude followed after a 3-ms interval by a 200-ms test pulse from $-80$ mV to 0 mV. The amplitude of the current elicited by the test pulse was expressed as a percentage of the current in the absence of a prepulse. Ca$^{2+}$ buffering in these experiments was moderate: The internal solution contained only 5 mM EGTA.

The $I_{\text{Ca}}$ inactivation curve (Fig. 2A) had a "U"-shape as described previously by others (Tillotson 1979; Eckert and Tillotson 1981; Mentrard et al. 1984; Hadley and Hume 1987; Argibay et al. 1988). Increasing inactivation of $I_{\text{Ca}}$ occurred with prepulses between $-60$ mV and 0 mV, with maximum inactivation occurring as prepulses neared 0 mV. With prepulses of short duration, inactivation decreased as prepulses became positive to 0 mV. This "relief from inactivation" (Lee et al. 1985) is due to the fact that the inactivation curve with Ca$^{2+}$ as the charge carrier is the sum of voltage-dependent inactivation, which is incomplete especially with short duration prepulses, and Ca$^{2+}$-dependent inactivation, which is greatest in magnitude at the peak of the I-V relationship ($\approx 0$ mV). The inactivation curves obtained in the same cell using Ba$^{2+}$ as the charge carrier reflect the voltage-dependent component of inactivation (Fig. 2B). Under these conditions, the inactivation curve was much less U-shaped, and relief from inactivation was usually negligible. The curve was sigmoidal with a plateau between 0 and +100 mV.

The time course of inactivation was evaluated by examining the effect of different duration prepulses to 0 mV on the response to the standard test pulse (Fig. 3A). The time course of voltage-dependent inactivation with Ba$^{2+}$ as the charge carrier was slower than inactivation produced by Ca$^{2+}$-dependent and voltage-dependent processes together with Ca$^{2+}$ as the charge carrier. With Ba$^{2+}$ as the charge carrier, 50% inactivation was produced by 60-ms prepulses, and steady-state inactivation was achieved with 700-ms prepulses. With Ca$^{2+}$ as the charge carrier, inactivation was more than twice as fast and was complete within 200 ms. The amount of Ca$^{2+}$-dependent inactivation relative to the voltage-dependent component was calculated as the ratio of the difference between the two curves and the Ba$^{2+}$ curve (Fig. 3B). Ca$^{2+}$-dependent inactivation contributed most to inactivation with short prepulses and declined as the amount of voltage-dependent inactivation increased.

Effects of [Mg$^{2+}$] on Inactivation Kinetics

[Mg$^{2+}$] had only a small effect on the kinetics of inactivation of $I_{\text{Ca}}$ at any potential (data for 0 mV shown in Fig. 4). $I_{\text{Ca}}$ decayed almost completely
within 200 ms with both 0.3 mM and 3.0 mM Mg\textsuperscript{2+}. Although a quantitative analysis of Ca\textsuperscript{2+} current kinetics in different \( [\text{Mg}\textsuperscript{2+}]_i \) remains to be accomplished, in many cells increasing \( [\text{Mg}\textsuperscript{2+}]_i \) caused a slight slowing of \( I_{ca} \) (Fig. 4). This is more evident in the lower set of traces in Fig. 4, which were obtained in the presence of a low concentration of cAMP, but was also true in the absence of cAMP (upper set of traces).
In contrast to the small effect of \([\text{Mg}^{2+}]\), on \(I_{\text{Ca}}\) kinetics, increasing \([\text{Mg}^{2+}]\) altered the inactivation of \(I_{\text{Na}}\) and \(I_{\text{Ca}^{\text{(No)}}}\) drastically (Fig. 5). With 0.3 mM \(\text{Mg}^{2+}\), \(I_{\text{Na}}\) at 0 mV decayed less than 50% in 200 ms and was still incompletely inactivated after 800 ms. Changing \([\text{Mg}^{2+}]\), to 3.0 mM produced a marked increase in \(I_{\text{Na}}\) decay such that the current was almost completely inactivated within 200 ms. The kinetics of inactivation of \(I_{\text{Na}}\) were altered at all potentials.
Fig. 4. Effect of $[\text{Mg}^{2+}]_i$ on $I_{\text{Ca}}$ kinetics. Results from two different cells are shown to show the range of effect. Upper panel, two current traces are superimposed. That indicated by triangles was obtained in 3 mM $[\text{Mg}^{2+}]_i$, and the unmarked trace was obtained in 0.3 mM $[\text{Mg}^{2+}]_i$. Lower panel, three traces are superimposed, which were obtained from a cell internally perfused with 1 mM cAMP. Smallest trace was obtained in 3 mM $\text{Mg}^{2+}$. It was then normalized to the same amplitude as a trace obtained in 0.3 mM $\text{Mg}^{2+}$ (unmarked trace). In both panels, to different extents, $I_{\text{Ca}}$ obtained in 3 mM $\text{Mg}^{2+}$ decayed more slowly than that in 0.3 mM $\text{Mg}^{2+}$.

Although the effect of $[\text{Mg}^{2+}]_i$ on $I_{\text{Ca}(\text{Na})}$ has not been analyzed as extensively, the results appear qualitatively the same as those obtained with $I_{\text{Ba}}$. $I_{\text{Ca}(\text{Na})}$ obtained at all potentials inactivated more rapidly with 3 mM $\text{Mg}^{2+}$ than with 0.3 mM $\text{Mg}^{2+}$.

A quantitative analysis of the kinetics of inactivation of $I_{\text{Ba}}$ is shown in Fig. 6. $\text{Ba}^{2+}$ currents were leak subtracted and fitted to the sum of two exponentials plus an offset ($I_{\text{Ba}} = A \exp(-t/\tau_1) + B \exp(-t/\tau_2) + C$). Each panel in Fig. 6 is a 3-dimensional plot showing the amplitudes (A, B, and C; Z-axis) and the time constants ($\tau_1$ and $\tau_2$; Y-axis) of each component as a function of test potential. Arbitrarily, the fast component was defined as having a time constant of less than 100 ms. The most noticeable difference

Fig. 5. Effect of $[\text{Mg}^{2+}]_i$ on the kinetics of $I_{\text{Ba}}$ and $I_{\text{Ca}(\text{Na})}$. Current traces from experiments similar to those described in Fig. 1 are shown. Left traces were obtained with 0.3 mM $\text{Mg}^{2+}$ and right traces with 3 mM $\text{Mg}^{2+}$. $I_{\text{Ba}}$ and $I_{\text{Ca}(\text{Na})}$ experiments were from different cells. $I_{\text{Ba}}$ was evoked by 800-msec duration pulses from $-80$ mV to the potential indicated. Traces were leak subtracted. External $\text{Mg}^{2+}$ concentration was 0 mM. $I_{\text{Ca}(\text{Na})}$ was obtained with 400-msec duration pulses from $-80$ mV to the potential indicated. Traces were leak-subtracted.
between the currents obtained in 0.3 mM and 3.0 mM Mg\textsuperscript{2+} was the relative magnitude of the offset. The offset was several-fold larger at almost every potential in 0.3 mM than it was in 3.0 mM Mg\textsuperscript{2+}. The rapidly inactivating component was the major component with 3.0 mM Mg\textsuperscript{2+} at all voltages, but with 0.3 mM Mg\textsuperscript{2+} the rapidly inactivating component was a minor component between +5 mV and -10 mV and was negligible at all other potentials. The fast time constant was similar with both 0.3 mM and 3.0 mM Mg\textsuperscript{2+} (between 30 and 60 ms), but the slow time constant was considerably slower in 0.3 mM Mg\textsuperscript{2+} than in 3.0 mM Mg\textsuperscript{2+}. Thus, these data show that increasing [Mg\textsuperscript{2+}] increased the relative magnitude of the noninactivating component of I\textsubscript{Na} and slowed the time constant and increased the amplitude of the slow component at all potentials. Thus, it appears that Mg\textsuperscript{2+} causes an increase in the voltage-dependent component of inactivation.

The effect of high [Mg\textsuperscript{2+}], on the amplitude and decay of I\textsubscript{Na} was at least partially reversible except after prolonged (>30 min) internal perfusion. However, it was seldom that we were able to reverse completely the effects of high [Mg\textsuperscript{2+}]. This irreversibility was associated with a gradual contracture of the cell with repeated stimulation. We supposed that the contracture was related to an accumulation of intracellular Ba\textsuperscript{2+}, because Ba\textsuperscript{2+} can activate the contractile machinery but is not chelated well by EGTA and is not pumped by
most Ca\(^{2+}\)-ATPases, including the sarcolemmal Ca\(^{2+}\)-ATPase and the sarco
cytoplasmic reticulum Ca\(^{2+}\)-pump. Accumulation of intracellular Ba\(^{2+}\), how
ever, does not explain the changes in inactivation kinetics, because increases in the
inactivation rate of I\(_{\text{Na}}\) were also observed when the cells did not undergo
contracture. Several experiments were performed with a very low stimulation
frequency (1 min\(^{-1}\) or less) to reduce Ba\(^{2+}\) influx. These cells did not visibly
contract, but I\(_{\text{Na}}\) inactivation was nevertheless increased by increasing [Mg\(^{2+}\)].
Furthermore, as shown above, similar changes in inactivation kinetics were
observed when Na\(^{+}\) was used as the charge carrier. The possibility should be
recognized, however, that the effects of [Mg\(^{2+}\)]\(_{i}\) on inactivation could be due to
complex alterations in cellular ionic homeostasis. For example, Mg\(^{2+}\),
Ba\(^{2+}\), Ca\(^{2+}\), and Na\(^{+}\) likely compete for binding to various sites in the cell.
Maintenance of homeostasis of divalent cations also involves various co-
transport mechanisms involving monovalent cations. Thus, altering one ion could
result in complex changes in ionic composition in the cell. Although specific,
testable hypotheses may not immediately come to mind, these problems are
worthy of causing the loss of a considerable amount of sleep.

Effects of [Mg\(^{2+}\)]\(_{i}\) on Quasi-Steady-State Inactivation

[Mg\(^{2+}\)]\(_{i}\) had characteristic effects on inactivation as measured by the gapped
two-pulse protocol with 200-ms duration prepulses (Fig. 7). With Ca\(^{2+}\) as the
charge carrier, increasing [Mg\(^{2+}\)]\(_{i}\) shifted half-inactivation about 5 mV in the
negative direction and depressed relief from inactivation considerably. Relief
from inactivation with prepulses to +100 mV decreased from 85% to 30% as
[Mg\(^{2+}\)]\(_{i}\) was increased from 0.3 mM. On average, with Ca\(^{2+}\) as the charge
carrier, increasing [Mg\(^{2+}\)]\(_{i}\) from 0.3 mM to 3.0 mM produced a 56% depres-
sion in relief from inactivation. With Ba\(^{2+}\) as the charge carrier, half-inacti-
vation was shifted 8 mV in a negative direction, and the plateau of inactivation
between 0 and +100 mV was diminished by more than 50% when [Mg\(^{2+}\)]\(_{i}\) was
increased. These results are consistent with the hypothesis that internal Mg\(^{2+}\)
increases the amount of voltage-dependent inactivation.

Since the inactivation protocol with 200-ms prepulses did not produce
steady-state inactivation of I\(_{\text{Na}}\), the difference in the inactivation curves with
different [Mg\(^{2+}\)]\(_{i}\) could have been caused by changes in the rate of inactivation
as well as in the level of steady-state inactivation. The results above show
clearly that the rate of inactivation is increased by increasing [Mg\(^{2+}\)]. In
addition, analysis of the rate of steady-state inactivation measured by the
standard two-pulse protocol suggests that steady-state inactivation occurs at
least twice as rapidly with 0.3 mM Mg\(^{2+}\) as with 3.0 mM Mg\(^{2+}\).

In addition to its effect on the rate of inactivation [Mg\(^{2+}\)], also increases the
steady-state level of inactivation. In the cell shown in Fig. 8, I\(_{\text{Na}}\) inactivation
was measured with the two-pulse protocol using 3-s prepulses. When the cell
was perfused with 3.0 mM Mg\(^{2+}\), half-inactivation was shifted in the negative
direction, and the steady-state level of inactivation was considerably less.
These results suggest that voltage-dependent inactivation is increased by [Mg$^{2+}$]. In order to test whether voltage-dependent inactivation was due entirely to a Mg$^{2+}$-dependent mechanism, we examined the kinetics of $I_{\text{Na}}$ in cells exposed both internally and externally to Mg$^{2+}$-free solutions (data not shown, see Hartzell and White 1989). The internal solution was similar to the 0.3 mM Mg$^{2+}$ solution except that MgCl$_2$ and ATP were omitted and EDTA was added. Under these conditions $I_{\text{Na}}$ was slower than that observed in 0.3 mM Mg$^{2+}$, but the current still exhibited both an inactivating component.
and a non-inactivating component. Thus, either voltage-dependent inactivation is caused entirely by a \( \text{Mg}^{2+} \)-dependent mechanism, or it is not possible to buffer intracellular \( \text{Mg}^{2+} \) completely by this method.

The effects of \([\text{Mg}^{2+}]_i\) were specific to the side of the membrane on which the \([\text{Mg}^{2+}]_i\) was manipulated. Changing \([\text{Mg}^{2+}]_i\) altered \(I_{\text{Ba}}\) amplitude, as has been reported previously, but had little or no effect on \(I_{\text{Ba}}\) or \(I_{\text{Cl}}\) inactivation. Increasing the external \(\text{Mg}^{2+}\) concentration had effects on the kinetics of inactivation that were qualitatively the opposite to those of increasing \([\text{Mg}^{2+}]_i\).
except that these effects were relatively small. Specifically, increasing \([\text{Mg}^{2+}]_o\) increased the time constant and amplitude of the slow component of inactivation and may have increased the offset slightly. Furthermore, the inactivation curves measured with 200-ms prepulses with \(\text{Ca}^{2+}\) as the charge carrier have the same U-shape with different external \(\text{Mg}^{2+}\) concentrations (data not shown, but see Hartzell and White 1989).

**Frog Ventricular Cells Exhibit Only L-type \(\text{Ca}^{2+}\) Channels**

The ability of internal \(\text{Mg}^{2+}\) to convert a slowly inactivating current to a rapidly inactivating one suggested the possibility that internal \(\text{Mg}^{2+}\) was converting or altering the expression of T- and L-type \(\text{Ca}^{2+}\) channels (Bean 1985): High \([\text{Mg}^{2+}]_i\) might favor the expression of T-type channels. However, we were unable to find any evidence for T-type channels in this preparation using different holding potentials or drugs including dihydropyridine \(\text{Ca}^{2+}\) channel antagonists, nickel, or tetramethylen. With 0.3 mM \(\text{Mg}^{2+}\) (Fig. 9A) the I–V relationships of \(I_{\text{Na}}\) from holding potentials of −100 mV and −40 mV and the difference between these curves were virtually identical in shape, and all had peaks near 0 mV. \(I_{\text{Na}}\) was completely blocked by 0.1–1.0 \(\mu M\) nifedipine at holding potentials positive to −50 mV and was blocked 80% at a holding potential of −100 mV. This incomplete block was most likely due to the voltage-dependent nature of the nifedipine block (Kass and Krafft 1987). With 3.0 mM \(\text{Mg}^{2+}\) (Fig. 9B), \(I_{\text{Na}}\) was almost completely inactivated at a holding potential of −40 mV, as expected from the effects of \(\text{Mg}^{2+}\) on the steady-state inactivation curve. The I–V relationship from a holding potential of −50 mV, however, had the same shape as that obtained from a −100 mV holding potential. \(I_{\text{Na}}\) was completely blocked by 1 \(\mu M\) nifedipine even from a holding potential of −100 mV under these conditions. Thus, there was no evidence for T-type \(\text{Ca}^{2+}\) channels in this preparation.

**Effects of \([\text{Mg}^{2+}]_i\) on \(I_{\text{Ca}}\) and \(I_{\text{Na}}\) Elevated by cAMP-Dependent Mechanisms**

In contrast to the small effect of \([\text{Mg}^{2+}]_i\), on the amplitude of basal \(I_{\text{Ca}}\) at 0 mV, \([\text{Mg}^{2+}]_i\), had a much larger effect on the amplitude of \(I_{\text{Ca}}\) elevated by cAMP-dependent mechanisms. This was true when cAMP levels were raised by β-adrenergic agonists, by forskolin, or by internal perfusion of the cell with cAMP. In Fig. 10, the top panel shows the amplitude of \(I_{\text{Ca}}\) measured near the peak of the I–V relationship (0 mV) as a function of time upon changing from 0.3 mM \(\text{Mg}^{2+}\) to 3 mM \(\text{Mg}^{2+}\). On the average, \(I_{\text{Ca}}\) at 0 mV decreased 65% in response to the increase in \([\text{Mg}^{2+}]_i\). However, as was observed for basal \(I_{\text{Ca}}\), the effect of \([\text{Mg}^{2+}]_i\) is voltage dependent (Fig. 10, middle panel), and \(I_{\text{Ca}}\) is reduced more at more positive potentials (Fig. 10, bottom panel).
Mechanisms of the Effect of Mg$^{2+}$ on cAMP-Elevated $I_{Ca}$

The effect of [Mg$^{2+}$], on the amplitude of $I_{Ca}$ elevated by cAMP-dependent mechanisms could be due to effects of [Mg$^{2+}$], on cAMP production, cAMP degradation, or phosphorylation of the Ca$^{2+}$ channel. Since $I_{Ca}$ was reduced by increasing [Mg$^{2+}$], even when the cell was perfused internally with cAMP (Fig. 10), this suggested that [Mg$^{2+}$], was not interfering with cAMP synthesis. In order to test whether the effects of cAMP observed were related to these mechanisms, we examined the effect of [Mg$^{2+}$], on $I_{Ca}$ elevated by various agents that bypass various steps in the cAMP-dependent phosphorylation
Fig. 10. Effects of changing [Mg$^{2+}$], on $I_{Ca}$ elevated by internal perfusion with 5 nM cAMP. 
Top panel, cell was initially perfused with 0.3 mM Mg$^{2+}$, and 5 nM cAMP was added to the 
perfusion where indicated ($t=2$ min). At $t=20$ min, internal solution was changed to 3 mM 
Mg$^{2+}$ and $I_{Ca}$ declined. Middle panel, Current–voltage relationship for $I_{Ca}$ obtained in the 
presence of 5 nM cAMP and either 0.3 mM or 3 mM Mg$^{2+}$. Bottom panel, percentage 
inhibition of $I_{Ca}$ by increasing [Mg$^{2+}$] from 0.3 mM to 3 mM was calculated as a function 
of membrane potential.
pathway. These experiments demonstrate that the effects of $[\text{Mg}^{2+}]_i$ are not on cAMP degradation or on the rate of channel phosphorylation.

Several experiments demonstrated that the effect of $[\text{Mg}^{2+}]_i$ was unlikely to be mediated by cAMP degradation. First, the effect of $[\text{Mg}^{2+}]_i$ on the cAMP concentration-I$_{\text{Ca}}$ response curve was to decrease the maximum I$_{\text{Ca}}$ that could be elicited, rather than to shift the concentration-response curve along the concentration axis, as would be expected if $[\text{Mg}^{2+}]_i$ increased the rate of cAMP degradation (Hartzell and Fischmeister 1986). This is shown in Fig. 11. I$_{\text{Ca}}$ was measured at 0 mV, and the effect of different concentrations of cAMP was measured with different $[\text{Mg}^{2+}]_i$. In the presence of 0.3 mM Mg$^{2+}$, a maximal I$_{\text{Ca}}$ of 2.3 nA was measured with concentrations of cAMP between 3 and 10 $\mu$M. In contrast, with 3 mM Mg$^{2+}$, even with 100 $\mu$M cAMP, the maximal I$_{\text{Ca}}$ that could be elicited was 1.4 nA. The average effect of $[\text{Mg}^{2+}]_i$ on the cAMP concentration-response curve is shown in Fig. 12. The effect of increasing $[\text{Mg}^{2+}]_i$ is to decrease the maximal I$_{\text{Ca}}$ with little change in the $EC_{50}$ for cAMP. These results are inconsistent with a mechanism involving stimulation of phosphodiesterase activity by internal Mg$^{2+}$. Additional support for this conclusion is given by the finding that the effect of $[\text{Mg}^{2+}]_i$ on I$_{\text{Ca}}$ elevated by
Fig. 12. cAMP concentration-\(I_{\text{Ca}}\) response curves obtained as shown in Fig. 11. Circle, 0.3 mM Mg\(^{2+}\); triangle, 1 mM Mg\(^{2+}\); square, 3 mM Mg\(^{2+}\). \(I_{\text{Ca}}\) density was calculated as the net Ca\(^{2+}\) current divided by cell capacity. Each point is the mean of 3–48 cells, error bars are SEM. Reproduced from *Science* 239:778–780 (1988), copyright 1988 AAAS (White and Hartzell 1988)
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Fig. 13. Effect of changing [Mg$^{2+}$] on $I_{Ca}$ elevated by internal perfusion with 8-bromo-cAMP. At $t = 7$ min, 10 µM 8-bromo-cAMP was added to the internal perfusion. Increasing [Mg$^{2+}$] from 0.3 mM to 3 mM during the period indicated produced a reversible decrease in $I_{Ca}$.

Fig. 14. Effect of changing [Mg$^{2+}$], on $I_{Ca}$ elevated by internal perfusion with catalytic subunit of cAMP-dependent protein kinase (provided by Dr. David Glass, Emory University). Top panel, [Mg$^{2+}$] was increased from 0.3 mM to 3 mM after perfusion with cAMP-dependent protein kinase (75 µg/ml) and $I_{Ca}$ increased. Lower panel, [Mg$^{2+}$] was decreased and $I_{Ca}$ decreased.
internal perfusion with the stable analogue of cAMP, 8-bromo-cAMP, was virtually the same as that observed when \( I_{Ca} \) was elevated by native cAMP (Fig. 13). Increasing \([\text{Mg}^{2+}]_i\), from 0.3 mM to 3.0 mM decreased cAMP-elevated \( I_{Ca} \) 63\% (\( n = 8 \)) and 8-bromo-cAMP-elevated \( I_{Ca} \) 60\% (\( n = 3 \)). Furthermore, \([\text{Mg}^{2+}]_i\) had a very similar effect on \( I_{Ca} \) that was elevated by internal perfusion with the catalytic subunit of cAMP-dependent protein kinase (Fig. 14). Decreasing \([\text{Mg}^{2+}]_i\) from 3 mM to 0.3 mM (top panel) increased \( I_{Ca} \) elevated by the catalytic subunit of cAMP-dependent protein kinase. Increasing \([\text{Mg}^{2+}]_i\) from 0.3 mM to 3.0 mM (bottom panel) had the opposite effect. The effect of \([\text{Mg}^{2+}]_i\) on the amplitude of \( I_{Ca} \) elevated by cAMP-dependent mechanisms was easily reversible and occurred equally regardless of whether the change was from low to high \( \text{Mg}^{2+} \) or from high to low \( \text{Mg}^{2+} \). In this regard, the effect of \( \text{Mg}^{2+} \) on the amplitude of \( I_{Ca} \) elevated by cAMP-dependent mechanisms differed from the effect of \( \text{Mg}^{2+} \) on the inactivation of \( I_{Na} \). As noted above, the effect of high \([\text{Mg}^{2+}]_i\) on \( I_{Na} \), inactivation was usually not completely reversible.

Finally, the effect of \([\text{Mg}^{2+}]_i\) on \( I_{Ca} \) amplitude is not due to changes in protein phosphatase activity, because changing \([\text{Mg}^{2+}]_i\), produces identical results in the presence of okadaic acid, a potent protein phosphatase inhibitor (Hartzell, in preparation). In addition, decreasing the amplitude of cAMP-elevated \( I_{Ca} \), \([\text{Mg}^{2+}]_i\), also altered the 200-ms inactivation curve for \( I_{Ca} \) (Fig. 15). The effect of \([\text{Mg}^{2+}]_i\) on the cAMP-elevated \( I_{Ca} \) inactivation curve was very similar to its effect on the basal \( I_{Ca} \) inactivation curve. The voltage at

![Fig. 15. Effect of \([\text{Mg}^{2+}]_i\) on 200-ms inactivation curve for \( I_{Ca} \) in the presence of cAMP. Inactivation was measured by a gapped two-pulse protocol as described in Fig. 2. Cell was perfused internally with 20 \( \mu \text{M} \) cAMP and \( \text{Ca}^{2+} \) was the charge carrier.](image-url)
which 50% inactivation was observed was shifted slightly to negative potentials, and the relief from inactivation was strongly depressed with increasing [Mg$$^{2+}$$].

**Discussion**

$I_{\text{Ca}}$ is partly responsible for the plateau of the cardiac action potential, may contribute to the pacemaker depolarization in the sinoatrial node, and is responsible for the influx of Ca$$^{2+}$$ ions that initiates excitation-contraction coupling in cardiac muscle (Hartzell 1989). $I_{\text{Ca}}$ is modulated by cAMP-dependent protein phosphorylation by neurotransmitters and drugs that influence $\beta$-adrenergic and cholinergic receptors on cardiac cells (Tsien 1977; Reuter 1983; Tsien et al. 1986; Trautwein and Pelzer 1985; Fischmeister and Hartzell 1986; Hartzell and Fischmeister 1987; Hartzell 1988).

The several effects of $[\text{Mg}^{2+}]_i$ on current through Ca$$^{2+}$$ channels differ in some respects but many of the mechanisms. First, $[\text{Mg}^{2+}]$, reduces the amplitude of $I_{\text{Ca}}$, $I_{\text{Na}}$, and $I_{\text{Ca(NA)}}$ in a voltage-dependent manner. The inward current is decreased more strongly with more positive depolarizations, as would be expected if Mg$$^{2+}$$ blocked the Ca$$^{2+}$$ channel at a point within the voltage gradient of the membrane. The observation that the ability of internal Mg$$^{2+}$$ to block inward current through Ca$$^{2+}$$ channels is related to the charge carrier supports this mechanism. If Mg$$^{2+}$$ competes for permant ion binding to permeation sites in the channel in a voltage-dependent manner, one would predict that the effect of internal Mg$$^{2+}$$ on current amplitude would be related to the relative affinities of permant ions for permeation sites, which is Ca$$^{2+}$$ > Ba$$^{2+}$$ > Na$$^{+}$$ (Hille 1984). Permamnt ions entering the cell would tend to compete for the Mg$$^{2+}$$ ions at the inner mouth of the channel. The kinetics of this block is rather fast, because the effect is seen within 5 ms, which is the time-to-peak of the Ca$$^{2+}$$ current. One expects a flickering blockade of single Ca$$^{2+}$$ channels to be produced by internal Mg$$^{2+}$$.$^1$ The finding that internal Mg$$^{2+}$$ is much more effective at blocking $I_{\text{Ca}}$ in the presence of cAMP than it is under basal conditions suggests that phosphorylation of the channel increases the affinity of the binding site for Mg$$^{2+}$$.$^1$ Consistent with this suggestion is the observation that recovery from inactivation is decreased by cAMP in a manner similar to the decrease produced by increasing Mg$$^{2+}$$.$^1$ One wonders whether the added negative charge on the channel introduced by phosphorylation might directly impose an additional attractive force on Mg$$^{2+}$$ or whether the effect of phosphorylation is indirect via a conformational change. In any event, an increase in the affinity of the channel for Mg$$^{2+}$$ is also indicated by preliminary experiments showing that when Na$$^{+}$$ was the charge carrier in the absence of cAMP, Mg$$^{2+}$$ decreased inward current amplitude drastically. For example, in the presence of 3 mM Mg$$^{2+}$, cAMP often produced only negligible increases in $I_{\text{Ca(NA)}}$, in contrast to significant increases with 0.3 mM [Mg$$^{2+}$$]$_i$. 
These effects of $[\text{Mg}^{2+}]_i$ on cAMP-elevated $I_{\text{Ca}}$ amplitude were not due to effects of $\text{Mg}^{2+}$ on channel phosphorylation, because they also occur when $I_{\text{Ca}}$ is elevated by 8-bromo-cAMP or the catalytic subunit of cAMP-dependent protein kinase. These results rule out an effect of internal $\text{Mg}^{2+}$ on phosphodiesterase, adenylate cyclase, or protein kinase activities.

The second documented effect of internal $\text{Mg}^{2+}$ is an alteration of the inactivation kinetics and the steady-state inactivation of $I_{\text{Ca}}$ and $I_{\text{Na}}$. Increasing $[\text{Mg}^{2+}]_i$ causes a dramatic increase in the voltage-dependent inactivation of $I_{\text{Na}}$. $I_{\text{Ca}}$ inactivation in heart appears to be mediated by two mechanisms, one voltage-dependent and the other $\text{Ca}^{2+}$-dependent (Kass and Sanguinetti 1984; Eckert and Chad 1984; Lee et al. 1985; Bechem and Pott 1985; Kass et al. 1986; Chad and Eckert 1986). Some laboratories, however, have proposed that $I_{\text{Ca}}$ inactivation is due entirely to a $\text{Ca}^{2+}$-dependent mechanism at least in snail neurons and GH$_3$ cells (Tillotson 1979; Eckert and Tillotson 1981; Chad and Eckert 1986), but other laboratories find only voltage-dependent inactivation (for example, Cohen and Lederer 1987). Both mechanisms clearly exist, but their proportion may depend upon the experimental conditions and the tissue studied. The existence of a $\text{Ca}^{2+}$-dependent mechanism is supported by the observations that inactivation increases with increasing intracellular or extracellular $\text{Ca}^{2+}$ concentration, is greatly slowed when ions other than $\text{Ca}^{2+}$ (such as $\text{Ba}^{2+}$) are used as the charge carrier, is reduced when intracellular $\text{Ca}^{2+}$ is chelated, and is slowed when the driving force for $\text{Ca}^{2+}$ entry is reduced (Eckert and Chad, 1984; Kass et al. 1986). The existence of “pure” voltage-dependent inactivation has been demonstrated by a two-pulse protocol in which the prepulse is given in the absence of $\text{Ca}^{2+}$ (Akaike et al. 1988) or by using $\text{Na}^{+}$ as the charge carrier (Hadley and Hume 1987). The kinetics of $I_{\text{Ca}}$ inactivation suggests that the rapid phase of inactivation is due to the $\text{Ca}^{2+}$-dependent component, whereas the slow phase is voltage dependent.

The U-shape of the inactivation curve (Fig. 2) can be viewed as the sum of voltage-dependent inactivation, which can be described by a Boltzmann relation with incomplete inactivation, and $\text{Ca}^{2+}$-dependent inactivation, which has the same shape as the $I_{\text{Ca}}$–$V$ relationship. For short duration prepulses $\text{Ca}^{2+}$-dependent inactivation predominates, whereas for longer duration prepulses voltage-dependent inactivation predominates. It may be for this reason that some investigators (for example, Cohen and Lederer, 1987) fail to find clear evidence of $\text{Ca}^{2+}$-dependent inactivation under conditions of steady-state inactivation.

With either $\text{Ca}^{2+}$ or $\text{Ba}^{2+}$ as the charge carrier, increasing $[\text{Mg}^{2+}]_i$ shifts the inactivation curve to the left and decreases relief from inactivation. The shift in the inactivation curve could be caused by the ability of $\text{Mg}^{2+}$ to screen negative surface charges on the internal surface of the membrane (Frankenhaeuser and Hodgkin 1957; Kass and Krafte 1987). A change in external divalent ion concentrations produces a shift in the $I_{\text{Ca}}$–$V$ relationship and inactivation curves that reflects divalent cations binding to and screening negatively charged groups on the cell surface (Frankenhaeuser and Hodgkin 1957; Kass and Krafte 1987). In heart cells, a 10-fold change in $[\text{Ca}^{2+}]_i$ shifts the
inactivation curve approximately 30 mV, whereas a 10-fold change in \([\text{Mg}^{2+}]_\text{i}\) shifts the curve only about 10 mV because of the lower affinity of \text{Mg}^{2+} for the surface charges (Kass and Krafte 1987). The shift in the inactivation curve is similar in magnitude but opposite in direction for the same changes in \([\text{Mg}^{2+}]_\text{e}\) and \([\text{Mg}^{2+}]_\text{i}\). That is, a 10-fold increase in \([\text{Mg}^{2+}]_\text{e}\) shifts the I–V relationship and the inactivation curves about 10 mV to the left, whereas a 10-fold increases in \([\text{Mg}^{2+}]_\text{i}\) shifts them 10 mV to the right. If this is true, then the internal surface charge is similar in magnitude to external surface charge. This might be expected considering the asymmetrical distribution of phospholipids in the plasma membrane: negatively charged phosphatidyl serines are often concentrated in the internal leaflet of the lipid bilayer. In addition, a negative internal surface charge may be partly due to the phosphate groups on phosphorylation sites on cytoplasmic domains of membrane proteins.

Although surface charge effects might explain the shifts in the inactivation and I–V curves, they are unlikely to explain the changes in relief from inactivation. Relief from inactivation observed with \text{Ca}^{2+} as the charge carrier is largely an expression of the fact that voltage-dependent inactivation is incomplete at voltages positive to 0 mV with low \([\text{Mg}^{2+}]_\text{e}\). The incomplete nature of voltage-dependent inactivation is demonstrated by the plateau of the steady-state inactivation curves with \text{Ba}^{2+} as the charge carrier and by the incomplete inactivation of \text{I}_\text{Na} at most potentials. The incomplete inactivation of \text{I}_\text{Na} with low \([\text{Mg}^{2+}]_\text{i}\) is not simply due to a shift in the inactivation parameters as a function of voltage, as would be expected from changes in surface charge. Rather, \([\text{Mg}^{2+}]_\text{e}\) seems to affect the amount of voltage-dependent inactivation at all voltages. Relief from inactivation is decreased by \text{cAMP} (Argibay et al. 1988) in a manner similar to that produced by increasing \([\text{Mg}^{2+}]_\text{e}\). It is worth considering that this decrease in relief from inactivation produced by \text{cAMP} might be related to accumulation of internal \text{Mg}^{2+} due to rapid hydrolysis of \text{ATP} for protein phosphorylation.

Although the kinetics of inactivation of \text{I}_\text{Na} is significantly accelerated by increasing \([\text{Mg}^{2+}]_\text{e}\), \text{I}_\text{Ca} inactivation is not as dramatically affected. Indeed, if increasing \([\text{Mg}^{2+}]_\text{e}\) has any effect on \text{I}_\text{Ca} kinetics, it is to slow inactivation. This may seem puzzling, considering the fact that internal \text{Mg}^{2+} accelerates \text{I}_\text{Na} inactivation. To explain these results, the following hypothesis is proposed. A single \text{Ca}^{2+} channel can be inactivated in either a voltage-dependent or \text{Ca}^{2+}-dependent manner, but not both. The rate of \text{Ca}^{2+}-dependent inactivation is determined by permeant \text{Ca}^{2+} ions, whereas the mode of inactivation is determined by the \text{Mg}^{2+} concentration at the cytoplasmic face of the channel. When \text{Mg}^{2+} is bound, the channel has a higher probability of inactivating by a voltage-dependent mechanism, whereas when \text{Mg}^{2+} is absent, the \text{Ca}^{2+}-dependent mechanism is favored. In the absence of \text{Ca}^{2+} as the charge carrier, \text{Ca}^{2+}-dependent inactivation is greatly slowed. The rate of inactivation of the macroscopic current is then determined by the fraction of the channels exhibiting voltage-dependent inactivation, which is set by the cytosolic \text{Mg}^{2+} concentration. In support of this hypothesis, it can be seen that \text{I}_\text{Ca} inactivation (at 0 mV) is often fitted by the sum of two exponentials with time constants of
results in the 13-ms component of inactivation slowing to 240 ms (Argibay et al. 1988). We believe that the 30-ms component represents voltage-dependent inactivation and the 13-ms component, Ca\(^{2+}\)-dependent inactivation. Increasing \([\text{Mg}^{2+}]_i\) causes a slowing of \(I_{\text{Ca}}\) inactivation due to an increase in the fraction of channels that exhibit voltage-dependent inactivation (the 30-ms component) rather than the Ca\(^{2+}\)-dependent component. In contrast, \(I_{\text{Na}}\) inactivation is speeded by increasing \([\text{Mg}^{2+}]_i\), because more channels shift to the voltage-dependent inactivation mode (30-ms component) from the Ca\(^{2+}\)-dependent mode (240-ms component due to the absence of Ca\(^{2+}\) as the charge carrier).

Since the relative contribution of the 30-ms voltage-dependent component increases with increasing \([\text{Mg}^{2+}]_i\), one could conclude that voltage-dependent inactivation is dependent upon \([\text{Mg}^{2+}]_i\). An obvious prediction of this hypothesis is that voltage-dependent inactivation should be eliminated when \([\text{Mg}^{2+}]_i\) is zero. Under these conditions, \(I_{\text{Na}}\) continued to exhibit a 30-ms inactivating component. It may be difficult to buffer \([\text{Mg}^{2+}]_i\) near the membrane to these low concentrations, and other ions may be able to substitute partially for Mg\(^{2+}\). It is postulated that voltage-dependent inactivation is stimulated by \([\text{Mg}^{2+}]_i\), and that a portion of voltage-dependent inactivation is Mg\(^{2+}\)-dependent. There are several studies that apparently argue against this hypothesis. For example, cardiac muscle L-type channels incorporated into bilayers exhibit voltage-dependent inactivation in the absence of internal and external Mg\(^{2+}\) (Rosenberg et al. 1988). Further experimentation will be required to resolve these questions.

It remains unclear whether the decrease in \(I_{\text{Na}}\) and \(I_{\text{Ca}(\text{Na})}\) amplitude produced by increasing \([\text{Mg}^{2+}]_i\) under basal conditions could be related to the more rapid inactivation under these conditions or whether different mechanisms mediate the effects of internal Mg\(^{2+}\) on inactivation and amplitude. The decrease in \(I_{\text{Ca}}\) amplitude by \([\text{Mg}^{2+}]_i\) in the presence of cAMP is unlikely to be related to the inactivation per se. It is possible that the binding of Mg\(^{2+}\) to the site in the permeation pathway that is responsible for the decrease in current amplitude results in a conformational change in the inactivation gate such that the voltage-dependent mode of channel inactivation is favored.

It has been pointed out by Lee et al. (1985) that voltage-dependent inactivation of Ca\(^{2+}\) channels may be an important mechanism of Ca\(^{2+}\)-channel inactivation during an action potential, because the Ca\(^{2+}\) transient lags considerably behind the Ca\(^{2+}\) current, and consequently, Ca\(^{2+}\)-dependent inactivation might be small under these conditions. If this is true, changes in \([\text{Mg}^{2+}]_i\) that occur physiologically might have significant effects on Ca\(^{2+}\) currents during the action potential. Although \([\text{Mg}^{2+}]_i\) alters action potential shape (Watanabe and Dreyfus 1972; Chesnais et al. 1975), it is not yet known what effects \([\text{Mg}^{2+}]_i\) has on the cardiac action potential. Additional studies are required to determine whether \([\text{Mg}^{2+}]_i\) is physiologically regulated in cardiac cells, and if so, what effects this has on cardiac physiology (White and Hartzell 1989).
References


Chesnais JM, Coraboeuf E, Sauviant MP, Vassas JM (1975) Sensitivity to H, Li, and Mg ions of the slow inward sodium current in frog atrial fibres. J Mol Cell Cardiol 7:627–642


Frankenhaeuser B, Hodgkin AL (1957) The action of calcium on the electrical properties of squid axons. J Physiol (Lond) 137:218–244


Hartzell HC (1989) Regulation of cardiac ion channels by catecholamines, acetylcholine, and second messenger systems. Prog Biophys Mol Biol 52:165–247


Tillotson D (1979) Inactivation of Ca conductance dependent on entry of Ca ions in molluscan neurons. Proc Natl Acad Sci USA 76:1497–1500


Vandenberg CA (1987) Inward rectification of a potassium channel in cardiac ventricular cells depends on internal magnesium ions. Proc Natl Acad Sci (USA) 84:2560–2564

