Electrophysiological Effects of Magnesium on Cells in the Canine Sinus Node and False Tendon

W. Thomas Woods, Richard E. Katholi, Ferdinand Urthaler, and Thomas N. James

SUMMARY Sinus node cells in the isolated perfused canine right atrium and cells of the right ventricular false tendons were used to assess the effects of extracellular Mg²⁺ concentration ([Mg²⁺]₀) on cardiac electrical activity. Removal of Mg²⁺ from the perfusate into the sinus node led to an increase of 36% in sinus rate that was sustained for as long as Mg²⁺ was absent; doubling [Mg²⁺]₀ to 2 mmol/liter caused the sinus rate to decrease by 19%. During Mg²⁺-free perfusion, the accelerated sinus rate could be depressed by the addition of certain substances; verapamil and Mn²⁺ produced the same percent depression regardless of [Mg²⁺]₀, but tetrodotoxin and reduced [Na⁺] in the solution each brought about significantly greater depression of sinus rate when Mg²⁺ was absent. No change in maximum diastolic potential was observed in the sinus node cells when Mg²⁺ was withheld. In false tendon cells, on the other hand, removal of Mg²⁺ was accompanied by depolarization of the transmembrane potential to a stable level of approximately −40 mV, at which potential action potentials could not be elicited. Increasing [Mg²⁺]₀ to 4 mmol/liter caused slight hyperpolarization of false tendon cells, but maximum upstroke velocity of the action potential and overshoot were reduced in spite of the more negative resting transmembrane potential. In both types of tissue, sinus node and false tendon, all changes clearly began to reverse within 30 minutes after restoration of normal [Mg²⁺]₀, and recovery was complete by 60 minutes. Since neither atropine, 5 μg/ml, nor propranolol, 15 μg/ml, modified the responses to Mg²⁺ alteration, these results indicate that Mg²⁺ has a direct effect on transmembrane electrical processes. The functional response to changes in concentration of this cation depends upon the specific type of cell acted upon.

DEPLETION of magnesium ion (Mg²⁺) predisposes to certain tachycardias (Beller et al., 1974). Increased extracellular Mg²⁺ concentration ([Mg²⁺]₀) has been shown to slow the heart rate (Schmidt et al., 1965; Hashimoto et al., 1974; Seifen, 1968). A major cardiac effect of Mg²⁺ may be mediated by modifying the intracellular pool of Mg²⁺ available to mitochondrial and other enzymes (Burch and Giles, 1977). Another possibility is that the membrane enzyme system [(Na⁺-K⁺)-ATPase] responsible for maintenance of transmembrane gra-
dients of Na⁺ and K⁺ is depressed during Mg²⁺ depletion, since it requires Mg²⁺ for its activity (Neff et al., 1972; Langer and Brady, 1974). Still others have held, based on experimental evidence from isolated cardiac tissue, that Mg²⁺ exerts a more direct effect on the cardiac cell by modifying the transmembrane ionic current (Spector et al., 1975; Shine and Douglas, 1974). This is consistent with the observation that rapidly administered Mg²⁺ suppresses erratic electrical behavior in the heart (Zwillinger, 1935; Ghani and Rabah, 1977; Seller et al., 1970).

Both ventricular and atrial cells respond to hypomagnesemia similarly, with prolongation of the action potential being the major effect (Hoffman and Suckling, 1956). Removal of divalent cations from the superfusate has been shown to enhance slow inward Na⁺ current during the plateau of the action potential (Garnier et al., 1969), and addition of Mg²⁺ has been shown to diminish the same
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right ventricular false tendons that were quite variable in length and branching pattern. False tendons were obtained from the canine hearts (14 tendons in all) following pentobarbital anesthesia (30 mg/kg, iv) and were treated in every respect in the same way as were the atria, with the single exception that direct arterial perfusion was not carried out. When false tendons were affixed to the wax floor of the perfusion chamber, the cells of this tissue were readily accessible to impalement with the same microelectrode arrangement used for impalement of atrial cells. Cells within this tissue were stimulated with a Grass S4 instrument in combination with an SIU6 isolation unit and a CCU1A constant current unit. Rectangular stimulus pulses (2-msec duration) from two silver wires 2 mm apart were adjusted so as to be as close to threshold as possible.

Action potentials were recorded by the microelectrode technique previously reported (Woods et al., 1976), except that microelectrodes were made from glass 1 mm in outside diameter and were suspended from 30-gauge chlorided silver wire. When recording from cells with action potentials having a rapid upstroke (only the cells of the false tendon in this study), maximum upstroke velocity was monitored via the electronically differentiated signal and displayed simultaneously with the action potential trace. Upstroke velocity of sinus node action potentials was measured directly from the tracings.

Statistical analysis was performed using paired differences and the Student’s t-test; significant differences are expressed as probabilities [P < 0.01, P < 0.05, or P = NS (no significant difference)]. All grouped observations are expressed as mean ± one standard deviation.

Results

Effect of Mg$^{2+}$-Free Solution on Sinus Node Cells

When the arterial perfusion solution was replaced with one containing no MgSO$_4$, sinus rate slowly accelerated following a time course that most likely corresponds to washout of extracellular Mg$^{2+}$ (Page and Polimeni, 1972), so that within 45 minutes the maximum sinus rate was attained. The final rate was approximately 35% higher than the control rate (Table 1), and it remained stable for at least 4 hours of Mg$^{2+}$-free perfusion. Since removal of SO$_4^{2-}$ alone had no apparent effect, we attribute the changes to the removal of Mg$^{2+}$.

Neither β-adrenergic blockade with propranolol nor cholinergic blockade with atropine (Woods et al., 1978) altered this response. As soon as the control [Mg$^{2+}$], was restored in the sinus node perfusates, the rate began to fall and within 30 minutes had
TABLE 1  Change in Sinus Rate Produced by Changing [Mg2+]

<table>
<thead>
<tr>
<th>[Mg2+] (mmol/liter)</th>
<th>No.</th>
<th>Sinus rate (impulses/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20</td>
<td>148* ± 37</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>109 ± 27</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>88* ± 12</td>
</tr>
</tbody>
</table>

* Significantly different (P < 0.05) from control ([Mg2+] = 1 mmol/liter).

To investigate the mechanism of the hypomagnesemic sinus rate increase, six atria were subjected to several agents that have well-established chronotropic effects on sinus node cells, and that reduce inward current in relatively specific ways. Low Na+, TTX, VPML, Mn2+, and Ca2+-free solutions were perfused through the sinus node artery for appropriate periods of time, and the resultant decreases in sinus rate, both in the presence and absence of Mg2+, are summarized in Figure 1.

**Effect of Low [Na+]**

During perfusion with normal [Mg2+]o, a 60-second exposure to one-fourth the control [Na+] produced a consistent negative chronotropic effect that depressed the sinus rate from 124 ± 42 to 115 ± 34 impulses/min (P < 0.05) throughout the low-Na+ perfusion period. However, during perfusion with the Mg2+-free solution, the same low [Na+] given for 60 seconds brought about a significantly greater depression of sinus rate (from 133 ± 40 to 108 ± 22, P < 0.05). Regardless of whether Mg2+ was present in the test solutions, the sinus rates returned to control (124 ± 42 and 133 ± 40, respectively) within 1 minute after normal [Na+]o was restored in the sinus node perfusate.

**Effect of TTX**

TTX had no significant direct chronotropic effect under normal [Mg2+]o, 1 mmol/liter conditions; sinus rate was 116 ± 38 before and 114 ± 38 (P = NS) during a 15-second perfusion of TTX in all six atria tested. When Mg2+ was eliminated from the perfusate, the sinus rate first increased from 113 ± 37 to 147 ± 43 impulses/min (P < 0.01). When TTX was then administered, there was a profound negative chronotropic effect, with sinus rate falling from 147 ± 43 to 118 ± 32 impulses/min (P < 0.01), representing a complete return to the sinus rates observed before Mg2+ removal. This negative chronotropic action of TTX persisted even though Mg2+-free perfusion without TTX continued for at least 15 minutes (post-TTX rate was 117 ± 34 during this period). Restoration of [Mg2+]o led to no change in sinus rate but, afterward, all subsequent removals of [Mg2+]o produced the characteristic sinus rate increase.

**Effect of VPML and Mn2+**

VPML and Mn2+ were perfused into the sinus node artery separately and in random sequence, with complete recovery of sinus rate in between so that the independent effect of each could be adequately assessed. The chronotropic depression observed after a 15-second perfusion was similar for each, with VPML reducing sinus rate from 133 ± 50 to 112 ± 35 (P < 0.05) and Mn2+ reducing it from 132 ± 44 to 120 ± 32 (P < 0.05). Mn2+ depression of sinus rate was relatively brief, lasting from 30 to 60 seconds in each test. However, the bradycardia induced by VPML was of much longer duration, although return to control sinus rate was steadily progressive and complete within 152 ± 42 seconds. Perfusion without Mg2+ produced no significant change in the chronotropic response to VPML or Mn2+ (see Fig. 1) when considered as a percent change in control sinus rate.

**Effect of Low [Ca2+]o**

When Ca2+ was removed from the perfusate, the sinus nodes stopped firing altogether within 15 minutes; this effect was completely reversed within 15 minutes after Ca2+ was restored. The responses of sinus rate to Ca2+ removal were therefore assessed and tabulated after only 10 minutes of Ca2+-free perfusion (that is, before sinus arrest), to study the
response to Ca\(^{2+}\) depletion in the presence and absence of Mg\(^{2+}\). With Mg\(^{2+}\), 1 mmol/liter, the sinus rate fell by 15 \pm 12\% (P < 0.05) at the end of the 10-minute Ca\(^{2+}\)-free period. However, when Mg\(^{2+}\) was omitted, the response to the same Ca\(^{2+}\)-depletion was an increase in rate by 28 \pm 15\% (P < 0.05), an effect not unlike that due to Mg\(^{2+}\)-depletion. Longer exposure to the Ca\(^{2+}\)-free solution resulted in sinus node cell quiescence in each experiment.

Action potentials of sinus node cells underwent distinct changes during Mg\(^{2+}\)-free perfusion; these changes are characterized in Figure 2. The first remarkable change came after 20 minutes, at which time hypomagnesemic tachycardia had commenced; the typical action potential of sinus node cells had a more abrupt upstroke (Fig. 2, upper panels). The action potentials rose in general from a more negative takeoff potential because of the increased sinus rate, which had a priori a shorter cycle length. As sinus rate continued to accelerate, prominent prepotentials emerged from the leading edge of the pacemaker cell action potential upstroke (Fig. 2, lower panels), and these were present throughout the period of hypomagnesemic tachycardia. Despite searching, we have been unable to locate other atrial cells that were firing prior to these sinus node cells, and that might have generated early electrical signals giving origin to the prepotentials. We further noted that, when sinus rate was transiently slowed by each of the inward-current reducing manipulations (low Na\(^{+}\), TTX, VPML, or Mn\(^{2+}\)), the prepotentials disappeared; however, they persisted during the Ca\(^{2+}\)-free perfusion period. Although they are apparently rate-related, these prepotentials are not a general characteristic of rapidly firing sinus node cells. Pacing with external stimuli at rates encountered in this study produces no such prepotentials, only more rapid upstroke velocities arising from more negative takeoff potentials.

We believe it is particularly important that hypomagnesemia produced no change in maximum diastolic potential (—56 \pm 7 mV before and —60 \pm 9 during hypomagnesemia, P = NS). The increased rate of impulse formation in the sinus node thus cannot be ascribed to a general depolarization of the pacemaker cells.

Effect of Mg\(^{2+}\)-Rich Solution on Sinus Node Cells

Doubling [Mg\(^{2+}\)]\(_o\) consistently slowed the sinus rate by 19\% (P < 0.05) within 30 minutes in the seven atria tested (see Table 1); the new rate was maintained for at least 2 hours. This negative chronotropic effect was terminated by removal of the extra Mg\(^{2+}\). Sinus rate returned to control as soon as [Mg\(^{2+}\)]\(_o\) was returned to normal. No changes in sinus node action potential were observed during this treatment.

Effect of Mg\(^{2+}\)-Free Solution on False Tendon Cells

In contrast to the effect of low [Mg\(^{2+}\)]\(_o\), on the sinus node cells, its effect on cells of the false tendon may be primarily attributed to depolarization of the resting transmembrane potential. The sequence of events regularly observed in all 14 such cells was: gradual depolarization, followed by a period of spontaneous activity, and then further depolarization to a stable level. Since these cells were quiescent when normal [Mg\(^{2+}\)]\(_o\) was present, external stimuli were applied to study changes in the action potentials. The changes observed as Mg\(^{2+}\) in the superfusate was altered are given in Table 2. At 20 minutes, just prior to the period of spontaneous activity, the resting potential had decreased by 31\% (P < 0.01),

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**FIGURE 2**  *Sinus node action potentials during Mg\(^{2+}\) depletion*. These recordings illustrate the major changes that occurred in the shape of the sinus node action potential when Mg\(^{2+}\) was omitted from the arterial perfusate. The upper panels show that the maximum upstroke velocity (arrows) of the characteristic sinus node action potential increased after 20 minutes of exposure to the Mg\(^{2+}\)-free solution; maximum diastolic potential was unchanged. The lower panels show that, after 45 minutes of Mg\(^{2+}\)-free perfusion, sinus rate had increased, and that the sinus node action potentials were preceded by brief, low amplitude prepotentials. Vertical calibrations are in mV and horizontal calibrations are given in msec in each panel.
TABLE 2  Action Potential Characteristics of False Tendon Cells during Mg$^{2+}$-Free Superfusion

<table>
<thead>
<tr>
<th>Mg$^{2+}$ concentration (mmol/liter)</th>
<th>No.</th>
<th>RMP (mV)</th>
<th>Overshoot (mV)</th>
<th>dV/dt$_{\text{max}}$ (V/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (at 20 minutes)</td>
<td>14</td>
<td>-59* ± 14</td>
<td>17* ± 10</td>
<td>138* ± 58</td>
</tr>
<tr>
<td>0 (at 60 minutes)</td>
<td>14</td>
<td>-41* ± 18</td>
<td>No action potential</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>-85 ± 4</td>
<td>28 ± 3</td>
<td>231 ± 26</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>-91* ± 5</td>
<td>12* ± 10</td>
<td>156* ± 70</td>
</tr>
</tbody>
</table>

RMP = resting membrane potential; dV/dt$_{\text{max}}$ = maximum rate of voltage change during action potential upstroke.

* Significantly different ($P < 0.01$) from control (Mg$^{2+}$, 1 mmol/liter).

with the overshoot and maximum upstroke velocity declining by closer to 40% ($P < 0.01$ for each). By 60 minutes, spontaneous activity had subsided and the transmembrane potential was approximately half its original value. This level of transmembrane potential was maintained for at least another hour, and few or no action potentials could be elicited by external stimulation. Figure 3 shows the sequence of events during decreased [Mg$^{2+}$], in a single false tendon cell.

Recovery from these effects by restoration of Mg$^{2+}$ was essentially complete within 1 hour (resting transmembrane potential returned to $-85 \pm 4$ mV), although both overshoot ($24 \pm 2$ mV) and maximum upstroke velocity ($189 \pm 42$) reflected a slight residual effect. These responses to Mg$^{2+}$-free superfusion were readily repeated at least three times in each false tendon cell.

Effect of Mg$^{2+}$-Rich Solution of False Tendon Cells

Elevation of Mg$^{2+}$ to 2 mmol/liter had no significant effects on false tendon cells. However, Mg$^{2+}$, 4 mmol/liter, changed the resting transmembrane potential to a more negative level (see Table 2). Even though a more negative transmembrane potential was regularly observed (zero potentials were always checked), both overshoot and maximum upstroke velocity of action potentials declined ($P < 0.01$ for each) in Mg$^{2+}$, 4 mmol/liter (Fig. 4 and Table 2). All effects of elevated [Mg$^{2+}$], subsided within 1 hour after superfusion of the Mg$^{2+}$-rich solution was discontinued.

Discussion

Magnesium Ion and the Sinus Node

While others have shown that [Mg$^{2+}$], alters neural influences on cells of the sinus node (Toda and West, 1967; Somjen and Baskerville, 1968), our experiments dealt with its direct chronotropic effect. The responses to ionic current blockers shed some light on the mechanism of this direct effect. It is known that Mg$^{2+}$ normally interferes with inward current through the slow channel during the action potential (Gamier et al., 1969; Chesnais et al., 1971, 1975), and also that there is a blocking effect on the inward current which underlies diastolic depolarization (Hashimoto et al., 1974; Seifen, 1968). The functional consequence of Mg$^{2+}$ in the environment of sinus node pacemaker cells is a lower rate of firing. We observed that VPML and Mn$^{2+}$, both of which block slow inward current carried by Na$^+$ and Ca$^{2+}$ (Wit and Cranefield, 1974; Zipes and Fischer, 1974), were equally effective at the tested concentrations whether Mg$^{2+}$ was in the perfusate or not. It appears, therefore, that the cause of sinus rate increase by low [Mg$^{2+}$], may be
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The orderly propagation of impulses through the conduction system of the heart can be disrupted only in part an enhancement of inward current through the slow channel. This is supported by the observation that simultaneous removal of Ca²⁺ from the perfusate transiently potentiated this tachycardia, even though Ca²⁺ removal would be expected to diminish slow channel current (Seifen et al., 1964).

Although sinus rate is known to be relatively insensitive to TTX (Yamagishi and Sano, 1966; Tomlinson and James, 1968) or to small reductions in [Na⁺] (Toda, 1968; Noma and Irisawa, 1975), the sinus rate proved to be sensitive to manipulations of [Na⁺] during Mg²⁺-free perfusion. This could involve passage of inward Na⁺ current through the slow channel (reduced by lowering [Na⁺]) or passage of Na⁺ through the rapid Na⁺ channel (reduced either by lowering [Na⁺] or by TTX). The rapid Na⁺ channel for which TTX is thought to be relatively specific probably exists and can be activated in both sinus node cells (Kreitner, 1975; Noma et al., 1977) and atrioventricular node cells (Ruiz-Ceretti and Ponce Zumino, 1976), although it is apparently inactivated (by the less negative takeoff potential) in the former. When Mg²⁺ is removed, these rapid channels may become less inactivated (takeoff potential became more negative), and this may promote earlier generation of the impulse. Thus, when either TTX or low [Na⁺] reduced the flow of Na⁺ through these channels, impulse generation became delayed and actually returned to its original point in the sinus cycle. During Mg²⁺-free perfusion, the increased upstroke velocity of the action potential as well as the subsequent appearance of prepotentials in recordings from sinus node cells suggest that the primary pacemaking site may shift, as has been described during changes in sinus rate (Lu, 1970). With these characteristics, upstrokes of the impaled sinus node cells had action potentials resembling those seen during stimulation from an ectopic site. They were also similar to normal cells of the atroventricular node, which have many structural features similar to the sinus node cells (James and Sherf, 1974). On the other hand, with our epicardial exploring microelectrode we could not find cells which could be considered as new, distant driving pacemakers. Since all other atrial myocytes essentially retained their normal action potential characteristics, we believe that the primary pacemaker cells, even if their site shifted, remained within the sinus node during this acute depletion of Mg²⁺.

Magnesium Ion and False Tendon Cells

The function of Mg²⁺ in electrical activity of cells in the false tendon appears to depend on its effect on resting transmembrane potential, other changes being secondary to this. The only exception is that excess Mg²⁺ had an additional effect on these cells similar to that of a rapid inward current blocker, since there was decreased maximum action potential upstroke velocity and overshoot, although the resting potential was actually more negative. Thus, increased [Mg²⁺] would oppose the enhanced conduction velocity associated with a more negative resting transmembrane potential (Hoffman and Cranefield, 1960). Although the very low level of Mg²⁺ attained in this study probably never occurs in the human heart, we observed, over a relatively broad range of biological variation of this ion (0–2 mmol/liter), a significant change in the electrical behavior of these cells; thus, Mg²⁺ can facilitate depolarization or hyperpolarization, depending upon its concentration.

The consistent depolarization produced by rapid depletion of Mg²⁺ in cells of the false tendon suggests that Mg²⁺ is required for maintenance of normal membrane permeability to other electrophysiologically important ions. There is a minimum [Mg²⁺], below which the cell cannot maintain its ion selectivity, so that during Mg²⁺-free superfusion, a generalized permeability change occurs. If permeability to other ions increased and K⁺ permeability decreased, depolarization would ensue. Furthermore, the enzyme system, (Na⁺-K⁺)-ATPase, which maintains the asymmetric transmembrane gradients of Na⁺ and K⁺, requires Mg²⁺ for its activity. Whatever fraction of total transmembrane potential is contributed by the electrogenic activity of this ion pumping system (Isenberg and Trautwein, 1975) might be eliminated, thus enhancing depolarization. However, sinus node cells that also have this electrogenic mechanism (Noma and Irisawa, 1974) did not depolarize.

The orderly propagation of impulses through the conduction system of the heart can be disrupted
when the transmembrane potentials of its constituent cells fall to less negative levels; abnormal "slow response" activity can then be evoked and contribute to electrical instability (Cranefield, 1975). Judging from these experiments, depolarizing effects of diminished [$\text{Mg}^{2+}$], are entirely reversed by the replacement of Mg$^{2+}$, and the addition of excess Mg$^{2+}$ may further help restore a more negative transmembrane potential. An interesting contrast was provided by the observation that a decrease in [$\text{Mg}^{2+}$], had no effect on maximum diastolic potential in pacemaker cells of the sinus node. This suggests that the cell membrane permeability change caused by Mg$^{2+}$ depletion in false tendon cells may be already well developed in sinus node cells. This would be a fundamental difference between these two types of cardiac cells.

[$\text{Mg}^{2+}$], is held at a relatively fixed level in intact biological systems, but it is well known that hypomagnesemia is considered arrhythmogenic when associated with hypokalemia or digitalis toxicity (Beller et al., 1974; Seller et al., 1970; Iseri et al., 1975). If one could extrapolate from our results, we would expect that lowered [$\text{Mg}^{2+}$], would favor the development of supraventricular tachycardia (enhancement of normal automaticity activity), or of either reentrant or abnormally automatic ventricular tachycardia (due to progressive depolarization of ventricular cells). Increased [$\text{Mg}^{2+}$], on the other hand, appears to stabilize electrical activity in the heart (Ghani and Rabah, 1977) and has been administered therapeutically for this very purpose (Zwilling, 1935).

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