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Hypomagnesemia: Characterization of a Model of Sudden Cardiac Death

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Objectives. **We sought to compare the incidence of sudden death in rats treated with magnesium-deficient and control diets and to address the electrophysiologic characteristics associated with these end points.**

Background. **Although magnesium deficiency is associated with an increased incidence of sudden cardiac death in patients, there has been no clear cause and effect relation because of a number of covariables, including diuretic use, hypokalemia, digitalis use and left ventricular dysfunction.**

Methods. **Hypomagnesemic rats and their paired control rats underwent in vivo electrophysiologic studies and measurements of the total calcium and magnesium content of their cardiac ventricles.**

Results. **Serum magnesium levels were 0.5 ± 0.3 mEq/liter (mean ± SD) in hypomagnesemic animals and 1.2 ± 0.9 mEq/liter in control animals. A modest but significant prolongation of the repolarization time was seen at the apical epicardial site (83 ± 8 ms in hypomagnesemic rats vs. 68 ± 13 ms in control rats, $p < 0.05$), but not at the other sites studied. Bradyarrhythmias and tachyarrhythmias were observed in 82% of the hypomagnesemic rats during the in vivo electrophysiologic studies, compared with 0% in the control group. During these studies, sudden, unexpected asystolic deaths were observed in 4 of 11 hypomagnesemic rats and 0 of 8 control rats. Polymorphic nonsustained ventricular tachycardia was provoked by rapid pacing in 5 of 11 hypomagnesemic rats and 0 of 8 control rats. Three of six hypomagnesemic rats exposed to auditory stimuli developed seizures, followed immediately by sudden deaths—two due to asystole and one due to ventricular fibrillation—although no end points occurred in the control animals.**

Conclusions. **In this model, magnesium deficiency results in sudden cardiac death. The presence of startle induction of sudden death preceded by seizures suggests that sudden cardiac death results from a neurologic trigger.**

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An association between magnesium deficiency and sudden cardiac death has been reported in humans (1–7). However, a cause and effect relation was not defined in humans owing to a number of covariables, including diuretic use, hypokalemia, digitalis use and left ventricular dysfunction. Not only does controversy exist as to whether chronic in vivo magnesium depletion causes sudden cardiac death, but little is known about whether magnesium depletion results in tachyarrhythmias, bradyarrhythmias and other electrophysiologic effects or whether these electrophysiologic effects are related to changes in myocardial calcium or magnesium. Accordingly, the purposes of this study were 1) to define the incidence of sudden cardiac death and of bradyarrhythmias and tachyarrhythmias; 2) to identify abnormalities of repolarization; and 3) to determine the myocardial content of calcium and magnesium in a rat model of chronic magnesium depletion.

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Methods

Animals. Pairs of male weanling Sprague-Dawley rats were randomly allocated to either a semisynthetic magnesium-free fodder (containing <0.002% magnesium) or the standard rodent chow diet containing 0.21% magnesium. Deionized water containing no detectable magnesium was fed ad libitum. Between days 14 and 21 hypomagnesemic rats (mean [±SD] weight 167 ± 16 g) and their paired control rats (mean weight 244 ± 44 g) underwent a number of investigations: 1) in vivo electrophysiologic studies; 2) in vivo electrocardiographic measurements obtained during provocative auditory stimulation; 3) measurements of serum magnesium and potassium concentrations; and 4) measurements of the total calcium and magnesium content in the cardiac ventricles. A larger number of rats were studied in the hypomagnesemic group (Table 1) because the animals that died before experimentation were replaced. These experiments conform to the position of the American Heart Association on research animal use.

In vivo electrophysiologic studies. Rats were premedicated with 7.5 mg/kg body weight of diazepam intraperitoneally and

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Table 1. Distribution of Hypomagnesemic and Control Rats According to Procedure

Procedure	Hypomagnesemic Rats (n = 66)	Control Rats (n = 44)
In vivo study	11	8
In vivo provocative study	6	6
Unwitnessed death	8	0
Witnessed death	6	0
Other death (instrumentation)	11	6
Blood samples	10	9
Histologic studies	8	9
Measurement of total calcium and magnesium	6	6

then anesthetized with alpha-chloralose (55 mg/kg) and morphine (66 mg/kg) intraperitoneally. A tracheotomy tube was inserted and the rats were ventilated with 50% nitrous oxide and oxygen using a Harvard respirator. Body core temperature was maintained at 37°C by a heat lamp and heating pad. A sternotomy was performed and the heart was exposed. Two pairs of epicardial and two pairs of endocardial bipolar electrodes with an interelectrode distance of ~5 mm were attached to the apical and basal aspects of the heart surfaces, respectively. Epicardial leads were constructed by baring a loop of AS634 Kooner Teflon-coated multifilament stainless-steel wire and sutured to the epicardium with 7-0 silk suture. The endocardial leads were placed in the ventricle by threading a Teflon-coated monofilament stainless-steel wire (0.003-in. bare, 0.0045-in. Teflon-coated diameter) through a 27-gauge needle and forming a 1-mm hook with a bared end. The needle and wire were inserted through the myocardium into the left ventricle. The needle was then removed from the ventricle and slid back on the wire to a position outside the chest wall. The bared hook was then pulled back against the endocardium.

Epicardial and endocardial electrograms were recorded at a ventricular pacing length of 150 ms using an Electronics for Medicine amplifier with a bandpass of 0.03 to 5,000 Hz. Programmed electrical stimulation involved the introduction of rapid pacing trains of 8 beats at a cycle length of 110 ms and a single extrastimulus at twice the diastolic threshold and a pulse width of 2.5 ms using a constant current Bloom stimulator to induce ventricular tachycardia/fibrillation. The end point of the programmed electrical stimulation was the induction of sustained ventricular tachycardia. Inducible ventricular tachycardia was defined as consecutive ventricular depolarizations at a cycle length <100 ms.

In vivo provocative study. Rats randomized to the hypomagnesemic diet commonly died of inadvertent startle reactions. These startle reactions occurred while personnel were handling either the cages or the rats. To define the mechanisms of the inadvertent startle-induced deaths, we recorded electrocardiograms during maneuvers designed to startle the animals. Six conscious hypomagnesemic rats and their paired controls were exposed to 100 dB of white noise. Electrodes were implanted into the animals the day before the experiment to allow the animal to recover from the operation. The electrocardiograms (ECGs) were monitored continuously before, during and immediately after the auditory stress testing.

Measurement of total ventricular calcium and magnesium content. Total ventricular calcium and magnesium content was determined in six hypomagnesemic and six paired control rats. To wash out the blood from the coronary bed, the hearts were perfused retrogradely in a Langendorff perfusion apparatus with deionized water containing no detectable magnesium or calcium. The cardiac ventricles were weighed and dried at 150°C until a constant dry weight was reached. The dry samples were then digested with a mixture of hydrochloric acid and nitric acid (3:1) at 95°C, followed by a hydrogen peroxide reaction. Calcium and magnesium were then measured by ICP (atomic emission with argon plasma). Measurements of calcium and magnesium were performed by Chemex Labs Alberta, Inc. The results were expressed as micrograms of calcium or magnesium per gram of dry weight of the cardiac ventricles. Using this procedure, the average efficiency for calcium and magnesium was 100.2% calcium and 99.4% magnesium.

Pathologic studies. Pathologic specimens were fixed in formalin, dehydrated in graded alcohols, cleared with xylene and embedded in paraffin wax (Surgiplast). Sections were stained with hematoxylin and eosin and with Gomori trichrome.

Statistical analysis. Results are expressed as mean value ± SD. One-way analysis of variance was used to examine the differences among the multiple groups. When a significant difference was noted, Dunnett's multiple range test was used to define intergroup comparisons. The Student *t* test was used to determine the differences between paired and unpaired observations. The null hypothesis was rejected when the two-tailed p value was <0.05.

Results

Animals. One hundred ten animals were randomized to the two dietary treatments: 66 to the hypomagnesemic diet and 44 to the control diet (Table 1). For all the experiments, the rats were studied at a mean of 18 ± 4 days on the dietary treatment. The serum magnesium level of rats on the magnesium-deficient diet was significantly reduced compared with that of the control animals. Serum magnesium concentrations were 0.5 ± 0.3 mEq/liter in hypomagnesemic animals and 1.2 ± 0.9 mEq/liter in control animals (14 ± 2 days on diet) ($p < 0.05$). Serum potassium concentrations were in the normal range and were not statistically different between the two groups (hypomagnesemic 5.2 ± 1 mEq/liter vs. control 5.2 ± 0.8 mEq/liter). After 7 to 11 days the animals on the hypomagnesemic diet began to exhibit signs of hyperactivity, vasodilation, irritability, skin rash and seizures. These signs were in keeping with the severe reduction in the serum magnesium levels measured in these rats. The hypomagnesemic rats developed the following characteristics: ectodermal lesions (94%), unexpected and unwitnessed deaths (12%)

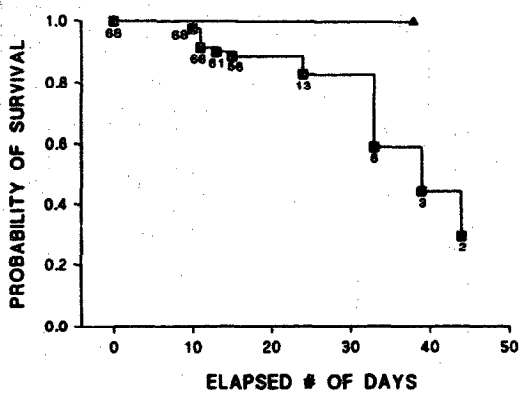


Figure 1. Life-table analysis of unexpected deaths in hypomagnesemic versus control rats ($p < 0.05$).

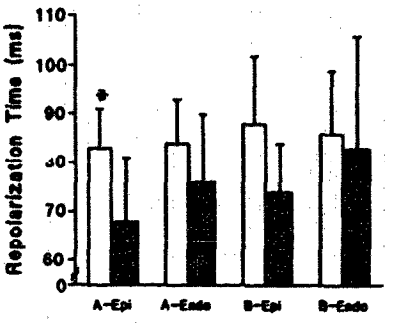


Figure 3. Mean ventricular repolarization times measured at different recording sites in the in vivo invasive study. Recordings were obtained at a basic cycle length of 150 ms. **Open bars** = hypomagnesemic rats; **solid bars** = control rats; A-Epi = apical epicardial site; A-Endo = apical endocardial site; B-Epi = basal epicardial site; B-Endo = basal endocardial site. *$p < 0.05$.

and inadvertent startle-induced seizures preceding sudden deaths (9%). None of these characteristics were observed in the rats receiving the control diet. The time course of their unexpected deaths is shown in Figure 1. The deaths that occurred during the in vivo electrophysiologi· studies and during the in vivo provocative studies were not included in Figure 1.

In vivo electrophysiologic characteristics. Nineteen consecutive animals underwent in vivo electrophysiologic testing. Ventricular tachycardia was induced by the extrastimulus technique in 5 of the 11 (45%) hypomagnesemic rats compared with 0 of the 8 control rats ($p < 0.05$). Figure 2 shows an example of the polymorphic ventricular tachycardia induced during rapid pacing in hypomagnesemic animals. In four of five animals the tachyarrhythmia was polymorphic and self-terminated. In one animal the tachyarrhythmia app monomorphic. Of the 11 hypomagnesemic rats, ((36%) developed sudden unexpected asystole, compared with 0 of 8 control rats ($p < 0.05$).

Figure 3 summarizes in vivo repolarization times measured at different electrocardiographic recording sites. Modest but significant prolongation in repolarization time was noted in the hypomagnesemic rats when recording at the apical site at the epicardium (hypomagnesemic 83 ± 8 ms vs. control 68 ± 13 ms, $p < 0.05$). Figure 4 compares typical recordings of the repolarization time measured at the apical site of the epicardium for a hypomagnesemic rat and a control rat. Similar trends were noted at the base of the epicardium, but the difference was not statistically significant. No differences were noted in repolarization times recorded at the apical or basal endocardial sites.

Figure 2. Example of ventricular tachycardia induced by programmed stimulation in a hypomagnesemic rat (3.5 s/display). APEX EPI = apical epicardial site; APEX ENDO = apical endocardial site; BASE EPI = basal epicardial site; ECG = electrocardiogram.

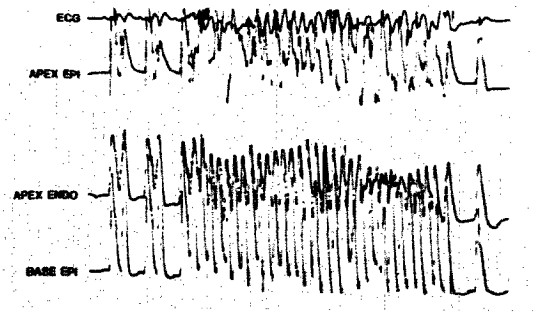
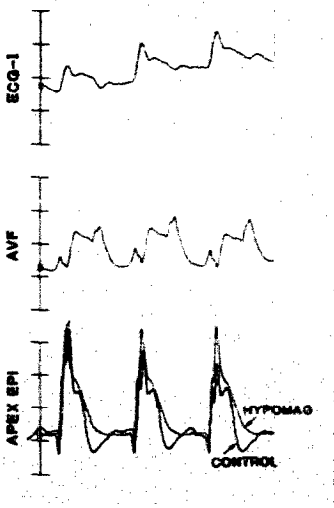


Figure 4. Example of surface electrocardiographic (ECG) recordings (leads I and aVF) measured in a hypomagnesemic (HYPOMAG) rat and epicardial recordings measured at the apex of the epicardium (APEX EPI) (0.4 s/display). CONTROL = control rat.



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In vivo provocation of arrhythmias. All six hypomagnesemic rats exposed to the auditory stimuli developed seizures. Three of the six animals died after the auditory stimulation: two due to asystole and one due to ventricular fibrillation. Figure 5 shows examples of ECG traces recorded from paired control (upper panel) and hypomagnesemic (lower panel) rats immediately after the auditory stimulation. The ECG of the control rats was not modified by the white noise, although the paired hypomagnesemic rats developed bradyarrhythmias or tachyarrhythmias within 2 min of the onset of the seizures. Of the six animals studied, four developed ventricular arrhythmias, including fatal ventricular fibrillation in one rat. Two rats developed a polymorphic ventricular tachycardia with beat to beat oscillation of the QRS axis and a long short initiation sequence (Fig. 5B), characteristics similar to torsade de pointes ventricular tachycardia. The remaining rat had nonsustained polymorphic ventricular tachycardia. None of the six paired control rats exposed to auditory stimulation developed seizures, ventricular tachycardia or fibrillation or asystole.

Total ventricular calcium and magnesium. The total calcium and magnesium content of the cardiac ventricles measured in six hypomagnesemic rats and six paired control rats was not significantly different. In the hypomagnesemic animals, the ventricular calcium content was similar to the values for the paired control animals (602 ± 100 μg/g and 534 ± 130 μg/g of dry tissue, respectively, p = NS). The myocardial magnesium content was similar for the hypomagnesemic and control animals (1,020 ± 45 μg/g and 983 ± 37 μg/g of dry tissue, respectively; p = NS).

Pathologic findings. In this study, the hypomagnesemic rats manifested some of the general and specific cardiovascular effects commonly observed during chronic hypomagnesemia. The general effects observed in our study included growth retardation, ulceration of the skin, irritability, hyperactivity and seizures. The cardiovascular effects included focal myocardial calcification and fibrosis. In fact, in our study, 50% of the hypomagnesemic rats showed patchy and focal fibrosis. The extent of fibrosis was similar in animals with or without sudden death.

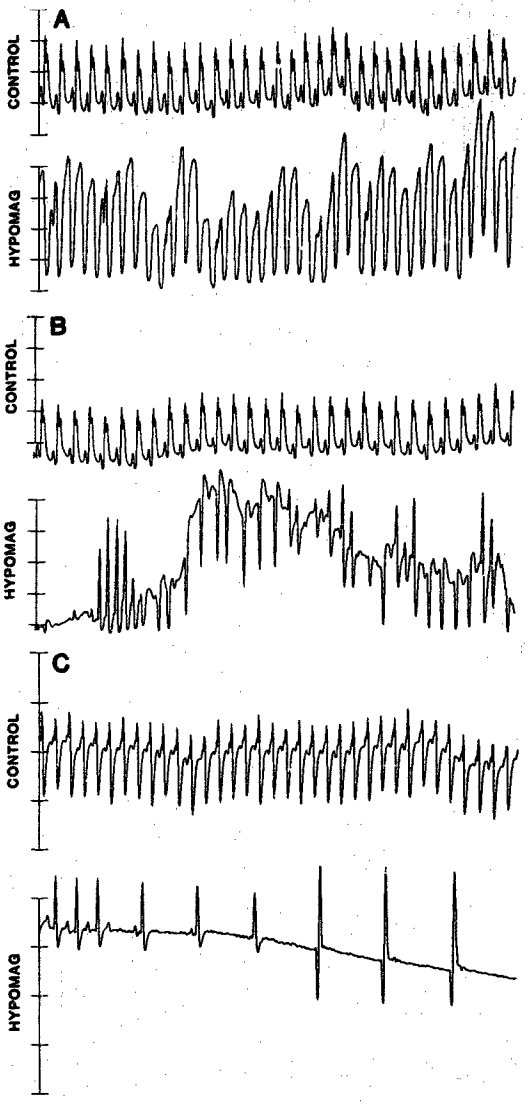

Figure 5. Examples of electrocardiographic recordings from control and hypomagnesemic rats (HYPOMAG) immediately after auditory-induced seizures (10 s/display).

Discussion

Sudden deaths occurred frequently in hypomagnesemic rats. All episodes of spontaneous and witnessed sudden death occurred after inadvertent startle, and seizures consistently preceded death. The in vivo provocation with white noise stress similarly caused seizures, and the subsequent deaths were preceded by either ventricular tachycardias or asystole. Some episodes of ventricular tachycardia induced by the white noise frequently had characteristics similar to torsades de pointes, consisting of a short–long short initiation sequence and beat to beat oscillation in the QRS axis (Fig. 5B). Similar episodes of polymorphic ventricular tachycardias were also induced by programmed electrical stimulation in these hypomagnesemic rats. The electrophysiologic features recorded in the hypomagnesemic rats was prolongation of repolarization, which was spatially heterogeneous, suggesting that this electrophysiologic mechanism may contribute to arrhythmogenesis in these animals. However, the startle induction of sudden death preceded by seizure implies a neurologic contribution to arrhythmogenesis. These results, taken together, suggest the following pathophysiologic hypothesis: sudden cardiac death in this model results from an interaction of neurologic triggers and an

underlying cardiac abnormality consisting of heterogeneous prolongation of ventricular repolarization.

Electrophysiologic manifestations of hypomagnesemia. It has been reported that chronic magnesium depletion has little effect on in vitro action potential configuration in the absence of other electrolyte disturbances (4,6,8–10). In humans, severe hypomagnesemia can result in repolarization alternans and prominent U waves, but only minimal prolongation of the QT interval in the absence of hypocalcemia or hypokalemia (8–12). Our in vivo data are in keeping with the results of these previous human studies in that hypomagnesemia produced only modest prolongation of repolarization and this prolongation was spatially heterogeneous.

Arrhythmias associated with hypomagnesemia. Hypomagnesemia in humans is generally associated with congestive heart failure, digitalis use, chronic diuretic use, hypokalemia and hypocalcemia. The presence of these covariables complicates the assessment of a cause and effect relation between hypomagnesemia and sudden cardiac death. The hypomagnesemic model of sudden death described in the present rat study has the advantages that serum potassium was not reduced, digitalis was not used and congestive heart failure was not evident. Thus, the contribution of these potential covariables was eliminated. In keeping with the data from our rat model, some cases of ventricular tachycardia have been reported in the setting of hypomagnesemia in the absence of structural heart disease and digitalis use in humans (7,13–14).

We have also shown that the association between hypomagnesemia and auditory stress can precipitate polymorphic ventricular tachycardias with features similar to those of torsades de pointes. This is in keeping with data from previous reports in both humans and animals, which support the association between stress-induced malignant arrhythmias and sudden death (15,16). Torsades de pointes induced by emotional stress in the presence of hypomagnesemia has been reported in humans (17). In our study, sudden cardiac death occurred in some hypomagnesemic animals after bradyarrhythmia and asystole. This is in keeping with human data previously published, suggesting an association between hypomagnesemia and the occurrence of torsades de pointes preceded by bradyarrhythmia (18–20).

Effects of hypomagnesemia on the calcium and magnesium content of the heart. Controversy exists as to whether hypomagnesemia increases intracellular calcium content (1,21). One study has shown that chronic magnesium depletion may produce an increase in the total calcium content in the heart (24), whereas other studies have reported that magnesium depletion did not change free intracellular calcium in either cultured or freshly isolated cardiac myocytes (22,23). Our results also show that the total cardiac content of calcium was similar in both groups.

Chronic treatment with a magnesium-deficient diet reduced the serum magnesium concentration of the treated rats by 58% compared with the control animals. However, the total magnesium content in the heart remains unchanged. Other groups have also reported similar findings (23–26). It is possible that a longer hypomagnesemic treatment period is required to decrease the cardiac magnesium content.

Pathologic studies. In this study, the histologic findings observed in the chronic hypomagnesemic animals are in keeping with the results of previous studies (10,26–30). The prevalence of the cardiac manifestations associated with hypomagnesemia was not a determinant of sudden cardiac death, because the extent of fibrosis was similar in the presence and absence of sudden death.

Conclusions. To our knowledge, no previous study has reported spontaneous sudden death during hypomagnesemia in the absence of superimposed experimental infarction, digitalis treatment, diuretic use or hypokalemia. Our data suggest that sudden cardiac death associated with hypomagnesemia in humans may be caused by the hypomagnesemia, per se, and its associated spatially heterogeneous prolongation of repolarization. Results of this study are in keeping with the hypothesis that sudden cardiac death in hypomagnesemic rats results from the interaction of a neurologic trigger and dispersion of ventricular repolarization.

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