Studies on Magnesium’s Mechanism of Action in Digitalis-induced Arrhythmias

By Michael J. Specter, M.D., Esther Schweizer, and Robert H. Goldman, M.D.

SUMMARY
The mechanism by which magnesium affects digitalis-induced arrhythmias was studied in dogs with and without beta-receptor blockade. Digoxin was infused at a rate of 2.5 μg/kg/min until ventricular tachycardia developed, then half the animals were given MgSO₄, the other half saline. In animals given MgSO₄, sinus rhythm was immediately re-established; in animals given saline, ventricular tachycardia persisted. In animals with beta-receptor blockade, MgSO₄ was as effective in abolishing ventricular tachycardia as in those without beta-receptor blockade.

We found no evidence that magnesium re-activated digoxin-inhibited (Na⁺, K⁺)-ATPase, altered myocardial or microsomal digoxin binding, or acted via the autonomic nervous system. Magnesium’s direct effect on calcium and potassium fluxes across the myocardial cell membrane may be the mechanism of its antiarrhythmic action in digitalis-toxic arrhythmias.

Magnesium has been shown to be effective in the treatment of cardiac arrhythmias brought on by digitalis in man and experimental animals. The mechanism by which magnesium affects digitalis-toxic arrhythmias has not been clearly demonstrated. Seller and coworkers suggested that magnesium reverses digitalis-induced inhibition of membrane-bound sodium, potassium, adenosine triphosphatase (Na⁺, K⁺)-ATPase, thought to be the “digitalis receptor” involved in both the arrhythmogenic and inotropic effects of digitalis. Magnesium-deficiency is associated with increased myocardial digoxin uptake; hypermagnesemia might, therefore, decrease digitalis uptake by the heart. Magnesium decreases potassium efflux and intracellular calcium and might thereby offset the toxicity of digitalis. In addition, by interfering with the effects of calcium, magnesium causes autonomic blockade which might influence digitalis-toxic arrhythmias.

The present investigation was, therefore, designed to determine magnesium’s mechanism of action in arrhythmias induced by digitalis toxicity. Our studies demonstrate no evidence of magnesium reactivation of digoxin-inhibited (Na⁺, K⁺)-ATPase, or displacement of digoxin from the heart, or of change in magnesium’s effectiveness as an antiarrhythmic agent in animals with beta-blockade.

Methods
Thirty-five adult mongrel dogs weighing 12.7-21.8 kg were anesthetized intravenously with sodium pentobarbital, 30 mg/kg, with small supplemental doses given as needed, then intubated with auffed endotracheal tube and ventilated with a Harvard respirator to maintain arterial P₀₂ above 80 mm Hg and pH between 7.35 and 7.45. A catheter was placed in the ascending aorta via the carotid artery and connected to a Statham P23Db pressure transducer for both pressure and blood gas determinations. A catheter was also placed in a jugular vein for blood sampling, and both femoral veins for drug administration. Lead II of the electrocardiogram was used to record heart rate and rhythm, which were also displayed continuously on an oscilloscope. Rectal temperature was maintained constant at 37 ± 2°C with a heating pad. All physiologic recordings were made on a Honeywell Electronics Medical System multichannel photographic recorder. Arterial blood gases and pH were determined with a Corning Model 165 blood gas analyzer.

In preliminary experiments, seven animals were given 2.5 μg/kg/min digoxin intravenously until ventricular tachycardia (four consecutive premature contractions) developed. Once ventricular tachycardia developed, the digoxin infusion was continued and the animals were then given 7 cc of 25% magnesium sulfate (MgSO₄) intravenously over 30 seconds with continuous recording of the electrocardiogram. Within 30 seconds after MgSO₄ administration, sinus rhythm returned and persisted for 2.5 to 14 min, average 6.1 ± 1.4 min (mean ± standard error of the mean). Subsequently, animals were instrumented and divided into five groups (table 1). Group I and II animals were given randomly-labeled H⁺-digoxin (kindly supplied by Dr. Stanley T. Bloomfield, Burroughs-Wellcome Laboratory, Inc., Research Triangle, Durham, North Carolina), diluted with cold digoxin to specific activity 15 μg/mg, via an infusion pump at a rate of 2.5 μg/kg/min until ventricular tachycardia developed. Group I animals were then given 7 cc of 25% MgSO₄ over 30 seconds, while group II animals...
received normal saline. One and one-half minutes after the beginning of the administration of MgSO₄ or saline, blood samples were obtained for serum digoxin and electrolyte concentrations and the animals were sacrificed. Group III and group IV animals were treated exactly like groups I and II, respectively, except that these animals received no digoxin. These animals served as controls for subsequent sodium, potassium adenosine triphosphatase, (Na⁺, K⁺)-ATPase, and magnesium adenosine triphosphatase, Mg⁺⁺-ATPase, determinations.

Animals in group V were instrumented as above. The vagus nerves were exposed in the neck and sectioned and the chronotropic response to isoproterenol, 0.2 μg/kg intravenously, was tested before and after the administration of sotalol, 1 mg/kg infused intravenously over ten minutes. This drug was used because it is a beta-adrenergic receptor blocking drug with no local anesthetic or intrinsic sympathomimetic activity. Sotalol decreased the chronotropic effect of 0.2 μg/kg isoproterenol 93.5 ± 3.5%. After the isoproterenol challenge, animals in group V were handled as in group I except that blood samples were obtained both at the onset of toxicity and after MgSO₄ administration when sinus rhythm was restored.

Biochemical Analysis

After the animals were sacrificed, their hearts were removed, blotted dry and frozen until analysis. Left ventricle digoxin concentration was assayed as described previously. Recovery of digoxin from left ventricular tissue averaged 85 ± 3%. No corrections were made for recovery. Serum digoxin was measured by placing 1.0 ml of serum in 14 ml aquasol (New England Nuclear Corporation, Boston, Mass.). Before counting, all samples were stored in the dark at 10°C until chumiluminescence had fully decayed. All samples and standards were counted three times in a Packard Tribar liquid scintillation counter to accumulate at least 4000 counts. The automatic external standards channel to ratio method was used to determine counting efficiency.

Serum sodium and potassium were measured with an Instrumentation Laboratory Model 143 flame photometer. Serum magnesium and calcium were determined by atomic absorption spectrophotometry using a Perkin-Elmer Model 290 atomic absorption spectrophotometer.

A modification of the method of Akera, Larsen, and Brody, as described previously, used to measure myocardial (Na⁺, K⁺)-ATPase and (Mg⁺⁺)-ATPase. Microsomal-bound digoxin was determined by extracting an aliquot of the microsomal pellet, obtained in the preparation of myocardial microsomal ATPase, with 10 ml of Bray’s solution. The microsomal fraction was shaken overnight at 24°C, then counted as described.

Enzyme was always prepared by the same individual from a single set of four hearts, one from each animal in groups I-IV. Likewise, assays were always performed simultaneously on a single set of four enzyme preparations.

Percent inhibition of (Na⁺, K⁺)-ATPase was calculated by subtracting (Na⁺, K⁺)-ATPase enzyme activity in group I and II animals from that in their controls, prepared at the same time, divided by the activity in their controls (groups III and IV, respectively).

Protein yield was determined by dividing the milligrams of protein in the microsomal fraction of each animal by the grams of left ventricle used to obtain the microsomal fraction.

Statistical Analysis

The experimental data were evaluated for statistical significance using a calculator programmed to calculate means and standard errors and to perform t-test analysis. Unpaired t-tests were used for intergroup comparisons; paired t-tests were used for intra-animal comparisons.

### Table 1

<table>
<thead>
<tr>
<th>Hemodynamic Parameters</th>
<th>Arterial BP (mm Hg)</th>
<th>Heart Rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>No.</td>
<td>Control</td>
</tr>
<tr>
<td>I (Dig, Mg)</td>
<td>6</td>
<td>163.0 ± 7.9</td>
</tr>
<tr>
<td>II (Dig, No Mg)</td>
<td>6</td>
<td>128.3 ± 6.0</td>
</tr>
<tr>
<td>III (No Dig, Mg)</td>
<td>6</td>
<td>160.3 ± 11.3</td>
</tr>
<tr>
<td>IV (No Dig, No Mg)</td>
<td>6</td>
<td>124.7 ± 7.4</td>
</tr>
<tr>
<td>V (Dig, Mg, Sotalol, vagotomy)</td>
<td>4</td>
<td>149.0 ± 10.9</td>
</tr>
</tbody>
</table>

Mean = standard error of mean.
No. refers to number of animals in each group.
Before Mg is at time of toxicity for groups I, II, and V, and at time of sacrifice for groups III and IV.
After Mg is at time of sacrifice for groups I, II, and V. One animal in group I developed asystole after MgSO₄; its heart rate prior to asystole was used to calculate the heart rate response to Mg.

*P < 0.05 compared with control.
†P < 0.025.
‡P < 0.01.
¶P < 0.10 compared with control.
††P < 0.05.
*P < 0.025.
‡‡P < 0.01.

Abbreviations: Dig = digoxin; BP = blood pressure.
Results

Hemodynamics

The infusion of digoxin increased systolic and diastolic pressure in all animals except one, while heart rate response was variable until toxicity. MgSO₄ decreased both arterial pressure and heart rate in all toxic and control animals (table I). All animals with toxicity reverted to sinus rhythm within 30 seconds after the end of MgSO₄ administration, followed in one animal by sinus bradycardia, then asystole. Group V animals with beta-blockade and bilateral vagotomy had a response to MgSO₄ similar to the group I animals. In animals not given digoxin, MgSO₄ administration slowed the sinus rate.

Time to toxicity was similar in groups I, II, and V: 56.0 ± 2.9, 55.8 ± 2.1, and 53.8 ± 4.2 min, respectively. At the time of sacrifice, all animals given MgSO₄, except the one which developed asystole, were in sinus rhythm, while all animals in group II who received digoxin but no MgSO₄ were in ventricular tachycardia.

Biochemical Analysis

Left ventricular digoxin concentrations were determined for group I animals given digoxin and MgSO₄, and group II animals given only digoxin. In animals given digoxin and MgSO₄ (group I), left ventricular concentration was 590.5 ± 35.0 ng/g, while in animals given only digoxin (group II), left ventricular digoxin averaged 570.5 ± 24.8 ng/g (P = NS). In group I animals, microsomal-bound digoxin was 39.9 ± 4.1 ng/mg protein while in group II animals, microsomal-bound digoxin was 32.9 ± 2.1 ng/mg (P = NS). In groups I and II, serum digoxin concentrations were similar at time of sacrifice, 201.5 ± 29.7 and 183.2 ± 17.3 ng/ml, respectively. In group V serum digoxin concentration was 193.8 ± 20.3 ng/ml at toxicity and 196.2 ± 20.9 ng/ml at time of sacrifice. Using a paired t-test, there was no significant difference in serum digoxin concentration between time of toxicity and time of sacrifice. Using an unpaired t-test, serum digoxin concentrations were similar at time of sacrifice in groups I, II, and V.

The (Na⁺, K⁺)-ATPase and (Mg++)-ATPase activity in group III animals given saline and group IV animals given MgSO₄ was measured. There was no significant difference in either (Na⁺, K⁺)-ATPase or (Mg++)-ATPase between groups III (given saline) and IV (given MgSO₄). In group III, (Na⁺, K⁺)-ATPase activity was 23.1 ± 1.7 μM Pi/mgP/hr; in group IV, it was 22.7 ± 1.8 μM Pi/mgP/hr. (Mg++)-ATPase was 3.7 ± 0.5 μM Pi/mgP/hr in group III, and 4.9 ± 0.3 μM Pi/mgP/hr in group IV. (Na⁺, K⁺)-ATPase was 9.6 ± 1.1 μM Pi/mgP/hr in group I and 10.7 ± 1.4 μM Pi/mgP/hr in group II animals. (Mg++)-ATPase was also similar in group I and II animals: 4.2 ± 0.7 and 4.9 ± 0.2 μM Pi/mgP/hr, respectively. The percent inhibition of (Na⁺, K⁺)-ATPase in group I and II animals given digoxin was compared to their simultaneously prepared and assayed controls in groups III and IV. (Na⁺, K⁺)-ATPase activity was 57.9 ± 4.0% less in group I than in group III animals, and 52.1 ± 6.1% less in group II than in group IV animals. The percent inhibition was similar in animals given MgSO₄ and those animals not given MgSO₄. Microsomal protein yield was 0.363 ± 0.015 mg protein/gram tissue in animals not given MgSO₄, and 0.373 ± 0.019 mg protein/gram tissue in animals given MgSO₄ (P = NS). Microsomal protein yield was also similar in animals given digoxin and MgSO₄ and those given only digoxin, 0.370 ± 0.018 and 0.383 ± 0.019 mg protein/gram tissue, respectively.

Serum potassium, magnesium, and calcium concentrations are given in table 2 for animals which received digoxin. Serum sodium concentration was similar in all groups. Serum potassium increased in all groups given digoxin, but this increase was not statistically significant. Serum potassium concentration was lower in group V animals than in group I and II animals. The reason for this is not clear. Control serum samples in group V dogs were, however, ob-

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Potassium (mEq/L)</th>
<th>Calcium (mg %)</th>
<th>Magnesium (mg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Mg</td>
<td>After Mg</td>
<td>Before Mg</td>
</tr>
<tr>
<td>Group I</td>
<td>4.17</td>
<td>4.37</td>
<td>12.23</td>
</tr>
<tr>
<td></td>
<td>±0.14</td>
<td>±0.10</td>
<td>±0.51</td>
</tr>
<tr>
<td>Group II</td>
<td>4.63</td>
<td>4.73</td>
<td>12.55</td>
</tr>
<tr>
<td></td>
<td>±0.12</td>
<td>±0.08</td>
<td>±0.60</td>
</tr>
<tr>
<td>Group V</td>
<td>3.80</td>
<td>3.97</td>
<td>11.13</td>
</tr>
<tr>
<td></td>
<td>±0.09</td>
<td>±0.24</td>
<td>±0.19</td>
</tr>
</tbody>
</table>

Values given are means ± standard error of the mean.
After Mg refers to time of sacrifice in groups I and V.
Before Mg refers to time of sacrifice in group II and to time of toxicity in group V.
*P < 0.01 compared to control time.

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tained after isoproterenol administration, a drug which increases blood glucose concentration and thereby stimulates insulin release which will, in turn, drive extracellular potassium into cells. Serum calcium concentration did not change significantly after MgSO₄ administration. Magnesium levels increased at least fivefold 1-1/2 minutes after the administration of MgSO₄. Since serial blood samples were not obtained after magnesium administration, this may not represent the peak serum magnesium concentration attained.

Discussion

The cardiac glycosides inhibit the activity of (Na⁺, K⁺)-ATPase, an enzyme essential for the maintenance of intracellular sodium and potassium concentration. The loss of myocardial potassium after inhibition of (Na⁺, K⁺)-ATPase may be causally related to digitalis-induced arrhythmias. Administration of magnesium has been shown to decrease coronary sinus-femoral artery potassium difference after digitalis administration. It has, therefore, been suggested that magnesium exerts its antiarrhythmic effects by decreasing digitalis-induced potassium loss from the myocardium directly or by reactivating (Na⁺, K⁺)-ATPase inhibited by digitalis. Our study offers no support for the hypothesis of Seller and coworkers, that magnesium reactivates digitalis-inhibited (Na⁺, K⁺)-ATPase, since (Na⁺, K⁺)-ATPase activity was similar in our group I and II animals. Percent inhibition of (Na⁺, K⁺)-ATPase was also similar in animals given MgSO₄ and those not given MgSO₄. Although this study does not rule out the possibility that a fraction of left ventricular (Na⁺, K⁺)-ATPase was reactivated by MgSO₄, and this effect was diluted out by the (Na⁺, K⁺)-ATPase not reactivated by MgSO₄, there is no evidence of the presence of such a fraction. Vassale et al., however, found that Purkinje fibers exposed to ouabain develop toxicity earlier than ventricular muscle fibers. This could be due to differences in (Na⁺, K⁺)-ATPase between Purkinje fibers and ventricular muscle fibers. Another reason for our finding of no reactivations of (Na⁺, K⁺)-ATPase by magnesium is the low Km, 0.8-1.25 mM, for magnesium activation of (Na⁺, K⁺)-ATPase. This low Km suggests that (Na⁺, K⁺)-ATPase may be fully activated at magnesium concentrations found in either the extracellular fluid or the intracellular compartment associated with membrane-bound (Na⁺, K⁺)-ATPase.

Our study also indicates that MgSO₄ administration does not displace digoxin from the left ventricle or a microsomal fraction of left ventricular tissue containing the (Na⁺, K⁺)-ATPase, since left ventricular and microsomal-bound digoxin were similar in group I, given digoxin and MgSO₄, and group II, given only digoxin.

A number of studies have suggested a synergistic or additive action between the sympathetic nervous system and digitalis in producing digitalis-toxic arrhythmias. Other studies do not support this conclusion. Since magnesium blocks sympathetic ganglia, we studied dogs with cervical vagotomy and beta-adrenergic receptor blockade (group V). Magnesium was as effective in these animals in abolishing digitalis-induced arrhythmias as in group I dogs without beta-adrenergic receptor blockade. Thus, the sympatholytic effects of magnesium are not responsible for its antiarrhythmic properties.

Wittenberg et al. showed that increasing heart rates increased ventricular automaticity during digitalization, while slowing the heart rate decreased ventricular automaticity. Since magnesium consistently decreased heart rate in our study, this is one possible mechanism for its antiarrhythmic actions. Using conscious dogs, however, Ghani and Smith found that magnesium chloride abolished digitalis-toxic arrhythmias even though heart rate increased. The reason for the different effects of magnesium on heart rate may be related to the use of conscious animals in that study while we used anesthetized animals.

In isolated perfused canine Purkinje fiber bundles, Rosen et al. found that ouabain-induced toxicity was associated with decreases in action potential amplitude, resting membrane potential, maximal slope of phase 0 depolarization, and action potential duration. These changes are the opposite of those produced by magnesium in isolated perfused rabbit hearts. Magnesium also prolongs the effective refractory period of ventricle fibers, and this may improve propagation of premature beats and prevent localized block and disorganization of excitation fronts.

Shine and Douglas noted that hypermagnesemia rapidly decreases calcium uptake and potassium efflux and exchange without affecting sodium exchange. Since loss of potassium from the heart attributed to (Na⁺, K⁺)-ATPase inhibition should be accompanied by changes in sodium exchange as well, Shine and Douglas' study also suggests that magnesium's antiarrhythmic effects are independent of an effect on (Na⁺, K⁺)-ATPase activity.

Conclusions

Magnesium is effective in abolishing digitalis-induced arrhythmias, but does not reanimate digitalis-inhibited (Na⁺, K⁺)-ATPase or displace digoxin from the heart or a microsomal fraction containing (Na⁺, K⁺)-ATPase. Magnesium does not appear to act as an antiarrhythmic agent because of its sympatholytic
effects. Direct effects on calcium and potassium fluxes across the myocardial cell may be the mechanism of its antiarrhythmic action in digitalis-toxic arrhythmias.

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