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# Loss of Cardiac Magnesium in Experimental Heart Failure Prolongs and Destabilizes Repolarization in Dogs

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***Objectives.*** **We sought to determine whether heart failure results in loss of cardiac magnesium sufficient to alter cellular electrophysiology.**

***Background.*** **Free magnesium has numerous intracellular roles affecting metabolism, excitability and RNA synthesis. Total cardiac magnesium content is reduced in heart failure, but it is unclear whether magnesium loss is primary or iatrogenic. Furthermore, it is unknown whether free magnesium levels are affected or whether a change in free magnesium would alter cellular electrophysiology.**

***Methods.*** **Eight mongrel dogs underwent demand ventricular pacing (VVI) at 250 beats/min for 3 weeks to induce heart failure. Sublingual epithelial magnesium was measured before pacing and at death. Left ventricular myocytes were isolated and loaded with Mag-Indo-1 to measure free magnesium ($[Mg^{2+}]_i$); myocytes from eight normal dogs served as controls. To test whether changes in $[Mg^{2+}]_i$ in this range could alter cellular repolarization, current-clamped myocytes were dialyzed with 0.5 or 1.0 mmol/liter $MgCl_2$.**

***Results.*** **Mean sublingual epithelial magnesium fell significantly in the paced animals, from 36.9 ± 0.5 to 33.9 ± 0.7 mEq/liter ($p < 0.01$). Mean cardiac $[Mg^{2+}]_i$ was significantly lower in the dogs with heart failure—0.49 ± 0.06 versus 1.06 ± 0.15 mmol/liter ($p < 0.003$). Time to 90% repolarization was significantly shorter in cells dialyzed with 1.0 mmol/liter compared with 0.5 mmol/liter $MgCl_2$ in myocytes from normal dogs or dogs with heart failure (596 ± 34 vs. 760 ± 58 ms in normal dogs and 586 ± 29 vs. 838 ± 98 ms in dogs with heart failure; $p < 0.05$ for each).**

***Conclusions.*** **Experimental heart failure results in both tissue and cardiac magnesium loss in the absence of drug therapy. Free cardiac magnesium is significantly reduced, possibly contributing to abnormal repolarization in heart failure.**

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Patients with congestive heart failure manifest a high incidence of sudden cardiac death (1). The role of electrolyte abnormalities in provoking sudden cardiac death in patients with heart failure has received increased attention (2). Patients with symptomatic or terminal heart failure manifest a reduction in cardiac magnesium (3,4), but it is not known whether this magnesium loss is due to diuretic or other drug use, concomitant disease or heart failure per se.

The significance of a reduction in cardiac magnesium is unknown. Magnesium is present in relatively large quantities in both myocytes and soft tissue. Although the free fraction of magnesium ($[Mg^{2+}]_i$) represents only ~5% of the total cellular content (5), the ionized species ($Mg^{2+}$) may significantly modulate cellular metabolism and excitability. Every adenosine triphospatase requires $Mg^{2+}$ as a cofactor (6), and many sarcolemmal ion channels are directly or indirectly affected by changes in $Mg^{2+}$. The L-type calcium and delayed-rectifier potassium currents ($I_{Ca}$ and $I_K$) are both antagonized by $[Mg^{2+}]_i$ (7), and changes in $[Mg^{2+}]_i$ within the physiologic range significantly alter the open probability and rate of adaptation of the ryanodine receptor (8).

Repolarization is prolonged and temporally variable in heart failure (9,10). A reduction in $[Mg^{2+}]_i$ could alter repolarization through effects on depolarizing and repolarizing currents (11). In this study we measured myocyte $[Mg^{2+}]_i$ to see whether experimental heart failure significantly affects the concentration of this important cation. We then manipulated cytosolic $Mg^{2+}$ to evaluate its effect on cellular repolarization.

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**Abbreviations and Acronyms**

AP = action potential
APD = action potential duration
$APD_{50}$ = time to 50% repolarization
$APD_{90}$ = time to 90% repolarization
DMSO = dimethyl sulfoxide
$I_{Ca}$ = L-type calcium current
$I_K$ = delayed rectifier potassium current
$Mg^{2+}$ = ionized magnesium
$[Mg^{2+}]_i$ = intracellular, ionized magnesium

## Methods

**Protocol.** Eight mongrel dogs underwent induction of heart failure through VVI pacing at 250 beats/min for 3 weeks in an approved protocol (12). To confirm that tissue magnesium was altered by heart failure, sublingual epithelial magnesium was measured before thoracotomy and at death (n = 6); ventricular myocytes were harvested for measurement of cardiac $[Mg^{2+}]_i$ and electrophysiology. In three animals, a Millar catheter was used to measure left ventricular performance, a practice then discontinued to expedite the harvest of the myocytes. Tissue magnesium and $[Mg^{2+}]_i$ were compared with those of eight control animals.

**Measurement of tissue magnesium.** Tissue magnesium concentration in sublingual epithelial cells was measured using energy dispersive X-ray analysis (EXA, Intracellular Diagnostics). This method assesses *total* cellular magnesium and cannot differentiate free from bound species. Previously we reported a strong correlation between the total cellular magnesium measured in the sublingual epithelium and that in atrial muscle taken at cardiopulmonary bypass in 18 patients undergoing cardiac surgery (13).

**Measurement of cardiac $[Mg^{2+}]_i$.** The heart was harvested <2 h after discontinuation of pacing. The hearts were arrested using St. Thomas cardioplegic solution, and the myocytes were enzymatically dispersed. Calcium-free Tyrode's solution, followed by collagenase, was infused into the left anterior descending coronary artery and myocytes were mechanically dispersed into HEPES-based buffer (pH 7.4, 25°C) containing 140 mmol/liter NaCl, 4.5 mmol/liter KCl, 10 mmol/liter HEPES, 1.2 mmol/liter $MgCl_2$ and 2 mmol/liter $CaCl_2$. Myocytes were incubated with the fluorescent indicator Mag-Indo-1 (AM-ester, Molecular Probes), using pleuronic and dimethyl sulfoxide (DMSO) at a final concentration of 10 μmol/liter for 10 min. Cells were studied on an inverted microscope (fluorescence excitation at 350 nm and fluorescence collected at 410 nm and 490 nm, 20°C) (14). A standard in vivo calibration of the probe was performed using 2 μmol/liter FCCP and 0.2 mmol/liter iodoacetic acid to de-energize the cells. To collapse concentration gradients, 2 μmol/liter nigericin, 10 ng/ml valinomycin and 20 μmol/liter 4-Br-A-23187 were used (12). The cells were superfused with varying amounts of $Mg^{2+}$ (from 0 to 50 mmol/liter) in buffer containing 123 mmol/liter KCl, 10 mmol/liter HEPES and 1 mmol/liter EGTA. One millimolar EDTA was added to the buffer containing 0 mmol/liter $Mg^{2+}$. Then $[Mg^{2+}]_i$ was fit to the relation

$$[Mg^{2+}] = K_{de}\frac{R - R_{min}}{R_{max} - R},$$

where R = the ratio of fluorescence measured at 410 nm over 490 nm. The observed $K_{de} = 5.9$, $R_{max} = 2.3$ and $R_{min} = 0.454$.

**Action potential recordings.** To test whether a 50% change in free magnesium could alter the time course and variability of action potential (AP) repolarization, myocytes from both a normal dog and a dog with heart failure were subjected to whole-cell current-clamp recordings before and after dialysis with either 0.5 mmol/liter $MgCl_2$ or 1.0 mmol/liter $MgCl_2$ using low resistance pipettes (1 to 3 MΩ; other contents were 0 mmol/liter adenosine triphosphate, 120 mmol/liter KCl, 6 mmol/liter NaCl, 20 mmol/liter HEPES and 5 μmol/liter EGTA). After establishing a giga-ohm seal in the voltage-clamp mode, minimal negative pressure was applied until a high resistance access was achieved; 200 APs were then recorded. To allow for dialysis of the cytosol, positive pressure was applied. Injection of pipette contents was confirmed by a sudden reduction in the size of the stimulus artifact and visualization of mild cell dilation. To confirm that the action potential duration (APD) was not altered by inadvertent dialysis during the initial recording, the electrophysiologic variables from normal and failing cells were also studied using the perforated-patch technique (which prevents movement of divalent cations from the pipette into the cell). These pipettes contained 1.0 mmol/liter $MgCl_2$ with a saturating concentration of amphotericin in DMSO (as well as 1.0 mmol/liter $CaCl_2$, 120 mmol/liter KCl, NaCl 6 mmol/liter, HEPES 20 mmol/liter and EGTA 5 μmol/liter). The cells were stimulated at 0.5 to 1.0 Hz (20°C) using an Axopatch 1-C amplifier. The mean rest membrane potential and time to 50% and 90% repolarization ($APD_{50}$ and $APD_{90}$) of 200 consecutive APs (for each cell) were recorded using Pclamp 6.0 software (Axon Instruments). The variability of the APD was assessed by taking the square root of APD power measured by custom software developed by an investigator (R.B.).

**Statistics.** Data are presented as mean values ± SE. Comparisons of tissue magnesium values before and after pacing were made using the paired Student *t* test. The mean values of $[Mg^{2+}]_i$ for each dog were contrasted using single-factor analysis of variance (ANOVA). Comparisons of rest membrane potential, $APD_{50}$ and $APD_{90}$ utilized ANOVA with Scheffé post hoc analysis. Statistical analysis was performed using Statview 4.1 (Macintosh), except for the linear regression between cardiac $[Mg^{2+}]_i$ and sublingual magnesium (Deltagraph 3.0, Macintosh). Values $p < 0.05$ were considered significant.

## Results

**Hemodynamic data.** End-diastolic pressure and tau (the time constant of relaxation) were significantly increased in the first three animals compared with 12 historic control animals

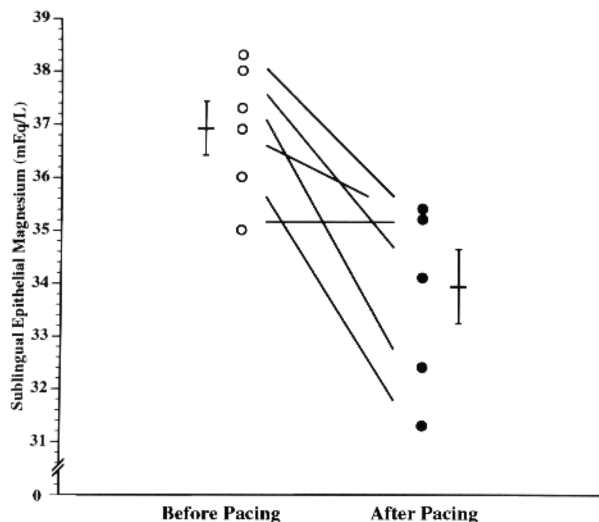


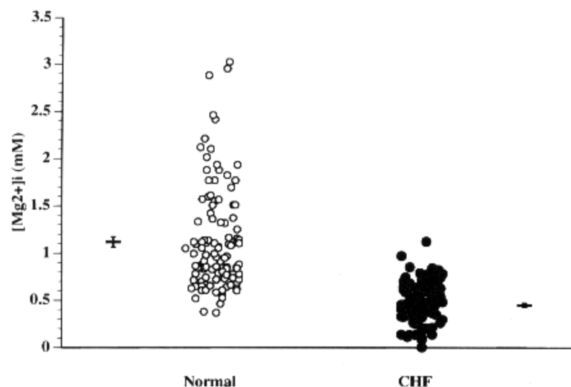

**Figure 1.** Total cellular magnesium measured in the sublingual epithelium from six dogs before and after 3 weeks of ventricular pacing at 240 beats/min ($p < 0.01$).

(32.1 ± 3.5 vs. 13 ± 1.4 mm Hg, $p < 0.001$; 84 ± 12 vs. 33.6 ± 3 ms, $p < 0.001$). The rate of rise in left ventricular pressure (dP/dt) was significantly reduced, from 3,007 ± 241 to 1,012 ± 113 ($p < 0.01$), consistent with significant systolic dysfunction.

**Effect of pacing-induced heart failure on tissue magnesium.** Sublingual cell magnesium fell in five of six dogs after 3 weeks of pacing, from a mean of 36.9 ± 0.5 to 33.9 ± 0.7 mEq/liter ($p < 0.01$) (Fig. 1). Baseline values in these dogs were similar to those of eight control dogs—36.3 ± 0.6 mEq/liter (p = NS).

**Cardiac $[Mg^{2+}]_i$ in animals with heart failure and normal animals.** Ionized, intracellular magnesium was measured in ventricular myocytes (n = 98) from eight dogs after 3 weeks of pacing and was compared with values from eight control dogs (109 myocytes). Ionized, intracellular magnesium was significantly lower in the failing cells than in the normal cells, whether the individual values for each cell were used (0.45 ± 0.02 mmol/liter vs. 1.12 ± 0.05 mmol/liter, $p < 0.00001$; Fig. 2)

**Figure 2.** Intracellular, ionized magnesium in 100 cells from eight normal dogs compared with $[Mg^{2+}]_i$ in 98 cardiac myocytes from eight dogs with congestive heart failure (CHF) (mean value ± SEM, $p < 0.00001$).



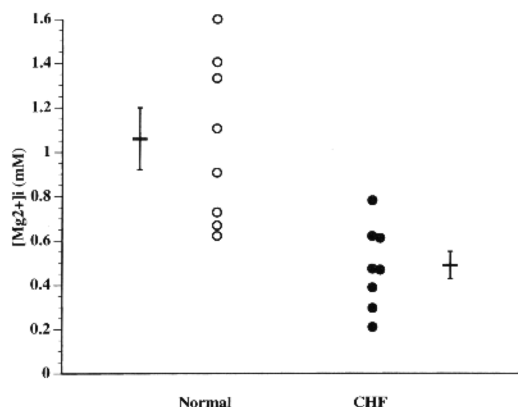




**Figure 3.** Comparison of the mean $[Mg^{2+}]_i$ concentration in cardiac myocytes from eight control dogs and eight dogs with congestive heart failure (CHF) ($p < 0.003$).

or the mean values for each animal were used (0.49 ± 0.06 mmol/liter vs. 1.06 ± 0.15 mmol/liter, $p < 0.003$; Fig. 3). Ionized cardiac magnesium and sublingual magnesium were compared in 11 animals (seven dogs with heart failure and four control dogs), and a good correlation was found ($r = 0.91$, $p < 0.001$, data not shown).

**Changes in APD in response to changes in cytosolic $Mg^{2+}$.** The mean rest potential and AP durations of cells studied using the perforated patch were not significantly different from the values in predialysis cells (Table 1). Compared with control animals in both groups, rest membrane potentials were less negative and $APD_{50}$ and $APD_{90}$ were significantly longer in the animals with heart failure.

On average, cells from the animals with heart failure manifested very prolonged APs, whereas dialysis with 1 mmol/liter $MgCl_2$ resulted in a significant shortening of the AP and a more negative rest potential (n = 5; Fig. 4A). Figure 5 presents representative APs from an animal with heart failure before and after dialysis with 1.0 mmol/liter $MgCl_2$ pipette buffer. Dialysis of failing cells with 0.5 mmol/liter $MgCl_2$ resulted in a significant reduction in $APD_{50}$ compared with before dialysis, but the $APD_{90}$ and rest potential were not altered (n = 5; Fig. 4B). Similarly, dialysis of control cells with 1.0 mmol/liter

**Table 1.** Comparison of Rest Potential, $APD_{50}$ and $APD_{90}$ in Cells From Either a Paced or Control Dog Studied in the Current-Clamp Mode by Perforated-Patch (n = 5 paced, n = 5 control) or in the Whole-Cell Mode Before Dialysis (n = 10 paced, n = 12 control)

| | Rest Potential (mV) | $APD_{50}$ (ms) | $APD_{90}$ (ms) |
|---|---|---|---|
| Control, perforated-patch | −76 ± 1 | 396 ± 44 | 604 ± 41 |
| Control, whole-cell | −79 ± 1 | 359 ± 27 | 591 ± 17 |
| Failure, perforated-patch | −73 ± 1* | 769 ± 99† | 921 ± 101* |
| Failure, whole-cell | −69 ± 1† | 787 ± 38‡ | 1,021 ± 64† |

*$p < 0.05$ compared with control. †$p < 0.01$ compared with control. ‡$p < 0.001$ compared with control. $APD_{50}$ = time to 50% repolarization; $APD_{90}$ = time to 90% repolarization.

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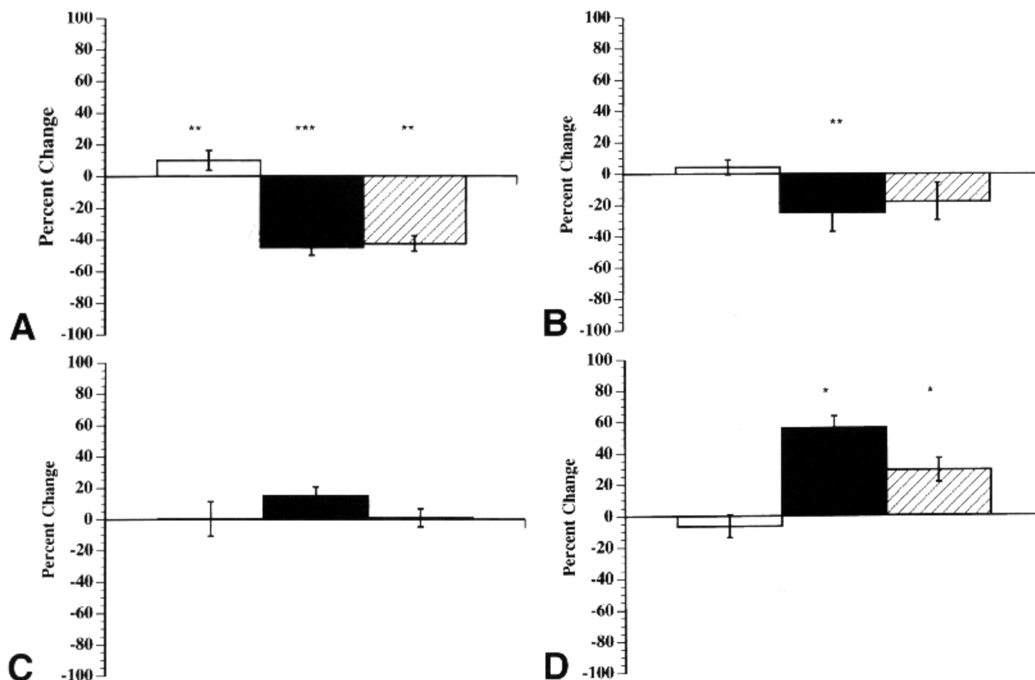
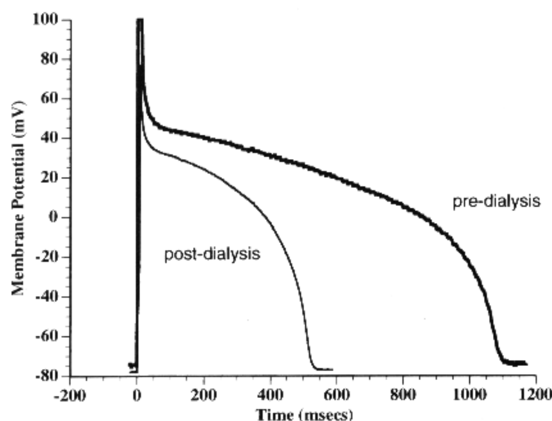



**Figure 4.** The effect of dialysis on the rest membrane potential **(open bars)**, $APD_{50}$ **(solid bars)** and $APD_{90}$ **(hatched bars)** in cardiac myocytes. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus before dialysis. **A,** Dialysis with 1.0 mmol/liter $MgCl_2$ in five myocytes with heart failure. **B,** Dialysis with 0.5 mmol/liter $MgCl_2$ in five myocytes with heart failure. **C,** Dialysis with 1.0 mmol/liter $MgCl_2$ in six control myocytes. **D,** Dialysis with 0.5 mmol/liter $MgCl_2$ in six control myocytes.

$MgCl_2$ pipette buffer had no significant effect on the rest potential, $APD_{50}$ or $APD_{90}$ (n = 6; Fig. 4C), whereas 0.5 mmol/liter $MgCl_2$ pipette buffer significantly prolonged $APD_{50}$ and $APD_{90}$ (n = 6, Fig. 4D).

The cells from the animals with heart failure manifested significantly greater beat to beat variability than cells from the control animals at baseline (Table 2). Figure 6A shows 50 consecutive APs elicited from a typical failing cell before dialysis, whereas Figure 6B demonstrates the marked reduction in variability of APD after infusion of 1.0 mmol/liter $MgCl_2$ pipette buffer. On average, dialysis with 1.0 mmol/liter $MgCl_2$ significantly decreased the variability of the APs from the animals with heart failure, whereas dialysis with 0.5 mmol/liter $MgCl_2$ had no effect. Similarly, dialysis of the control cells with 0.5 mmol/liter $MgCl_2$ resulted in a significant increase in the variability, whereas 1.0 mmol/liter $MgCl_2$ had no significant effect.

**Figure 5.** Superimposition of a representative AP from a failing myocyte before and after dialysis with a pipette buffer containing 1.0 mmol/liter $MgCl_2$.




## Discussion

To our knowledge, this is the first study demonstrating magnesium loss in heart failure in the absence of concurrent disease, diuretic agents or other magnesium-wasting drugs. Furthermore, we found that dogs with heart failure have significantly lower cardiac free magnesium concentrations compared with control dogs, and that there is a significant

**Table 2.** Comparison of Action Potential Duration Variability Before and After Dialysis in Cells From Paced and Control Animals

| | SD of APD (ms) |
|---|---|
| Control, before dialysis | 11 |
| Control, after dialysis with 1.0 mmol/liter $MgCl_2$ | 31 |
| Control, after dialysis with 0.5 mmol/liter $MgCl_2$ | 56* |
| Failure, before dialysis | 98† |
| Failure, after-dialysis with 1.0 mmol/liter $MgCl_2$ | 20* |
| Failure, after dialysis with 0.5 mmol/liter $MgCl_2$ | 67 |

$^{*}p < 0.05$ versus before dialysis. $^{\dagger}p < 0.05$ versus control before dialysis. APD = action potential duration; SD = standard deviation.

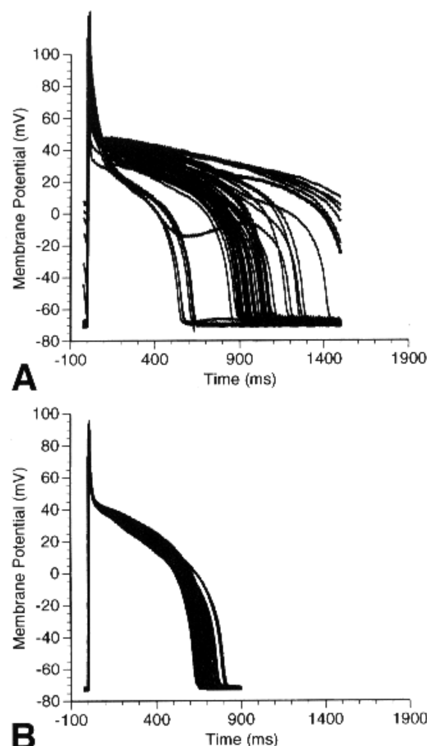



**Figure 6.** **A,** Fifty consecutive APs from a failing myocyte stimulated at 1 Hz at 25°C. **B,** Fifty consecutive APs from the same cell after dialysis with a pipette buffer containing 1.0 mmol/liter $MgCl_2$.

correlation between total cellular magnesium measured in the sublingual epithelium and free cardiac levels. Finally, the APD and beat to beat variability appear to be sensitive to changes in cytosolic $Mg^{2+}$ over a narrow range; thus, prolongation and instability in APD seen in heart failure may be partly due to magnesium depletion.

**Magnesium deficiency in heart failure.** Data from biopsy (4) and necropsy (3) demonstrate a reduction in total cellular magnesium concentration in patients with heart failure. Iseri et al. (3) reported a mean 8% decrease in cardiac magnesium in heart failure victims, similar in magnitude to our experimental findings. Some of these patients, however, were receiving long-term diuretic agents, digoxin or other medications, which increase magnesium clearance by the kidney. Therefore, it was previously unclear whether magnesium deficiency is an iatrogenic consequence or a primary response to heart failure. The evidence from this model suggests that heart failure per se causes a loss of tissue and cardiac magnesium.

The mechanism causing tissue and cardiac magnesium loss is unknown. Romani and Scarpa (15) found an efflux of 10% to 15% of total cellular magnesium within 10 min from isolated rat hearts in response to 10 $\mu$mol/liter norepinephrine. This finding suggests that neurohumoral activation in heart failure may result in the loss of cardiac magnesium.

**Free magnesium concentration in heart failure.** Ionized magnesium constitutes 5% of total cellular magnesium in mammalian cells, but its physiologic significance is difficult to overestimate. In this study, a modest reduction in total cellular concentration was accompanied by a much larger drop in free myocardial magnesium, consistent with a large pool of magnesium-binding sites (6).

**$[Mg^{2+}]_i$ and repolarization.** The sarcolemmal ion currents $I_{Ca}$, $I_K$ and $I_{Cl}$ are significantly modulated by ionized magnesium (9), so a reduction in intracellular free magnesium may contribute to the unstable repolarization seen in heart failure. Agus et al. (11) found significant prolongation of the AP and a doubling of peak calcium currents in mammalian cells dialyzed with magnesium-free solutions. In our study, increasing cytosolic magnesium significantly shortened the APs in cells from dogs with heart failure, whereas reducing $[Mg^{2+}]_i$ significantly prolonged the APs in normal cells. Additional studies are needed to examine the effect of changing $[Mg^{2+}]_i$ on sarcoplasmic reticulum calcium handling, depolarizing calcium currents and repolarizing potassium currents in heart failure.

**Unstable repolarization in heart failure.** Patients with heart failure manifest increased variability in repolarization over time (9), and this is thought to contribute to an arrhythmogenic substrate (10). Temporal variability of repolarization in myocytes appears to be significantly affected by modest changes in cytosolic magnesium concentration. We have recently reported a correlation between spatial heterogeneity of repolarization (measured by QT interval dispersion on the surface 12-lead electrocardiogram) and sublingual epithelial magnesium levels in humans (16). The mechanism by which magnesium stabilizes repolarization needs further investigation, but the present study suggests that the loss of cardiac magnesium in heart failure may significantly contribute to the increase in variability of repolarization.

**Study limitations.** Rapid ventricular pacing induces ventricular dysfunction resembling clinical heart failure, but over a significantly accelerated time course; the presence of heart failure was confirmed in a subset of dogs in this study. The loss of ionized magnesium, however, may be due to the effect of pacing, the acuity of heart failure or some other artifact of the model. Confirmation in another model is therefore desirable. Furthermore, cardiac magnesium (both total and free) should ideally be measured in the same animal before and after pacing. The development of improved nuclear magnetic resonance technology may eventually allow the measurement of human cardiac $Mg^{2+}$ in vivo. Finally, the ultimate cytosolic concentration of ionized magnesium was not measured in the dialysis experiments. Such measurements will be required to accurately correlate the relation between changes in cytosolic magnesium and excitation–contraction coupling.

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