Persistent Activation of a Swelling-Activated Cation Current in Ventricular Myocytes From Dogs With Tachycardia-Induced Congestive Heart Failure

Henry F. Clemo, Bruce S. Stambler, Clive M. Baumgarten

Abstract—The hypothesis that cellular hypertrophy in congestive heart failure (CHF) modulates mechanosensitive (ie, swelling- or stretch-activated) channels was tested. Digital video microscopy and amphotericin–perforated-patch voltage clamp were used to measure cell volume and ion currents in ventricular myocytes isolated from normal dogs and dogs with rapid ventricular pacing–induced CHF. In normal myocytes, osmotic swelling in 0.9× to 0.6× isosmotic solution (296 mOsm/L) was required to elicit an inwardly rectifying swelling-activated cation current (I_{Cir, swell}) that reversed near –60 mV and was inhibited by 10 μmol/L Gd³⁺, a mechanosensitive channel blocker. Block of I_{Cir, swell} by Gd³⁺ simultaneously reduced the volume of normal cells in hypertonic solutions by up to 10%, but Gd³⁺ had no effect on volume in isosmotic solution. In contrast, I_{Cir, swell} was persistently activated under isosmotic conditions in CHF myocytes, and Gd³⁺ decreased cell volume by 8%. Osmotic shrinkage in 1.1× to 1.5× isosmotic solution inhibited both I_{Cir, swell} and Gd³⁺-induced cell shrinkage in CHF cells, whereas osmotic swelling only slightly increased I_{Cir, swell}. The K₅₅ and Hill coefficient for Gd³⁺ block of I_{Cir, swell} and Gd³⁺-induced cell shrinkage were estimated as 2.0 μmol/L and 1.9, respectively, for both normal and CHF cells. In both groups, the effects of Gd³⁺ on current and volume were blocked by replacing bath Na⁺ and K⁺ and were linearly related with varying Gd³⁺ concentration and the degree of cell swelling. CHF thus altered the set point for and caused persistent activation of I_{Cir, swell}. This current may contribute to dysrhythmias, hypertrophy, and altered contractile function in CHF and may be a novel target for therapy. (Circ Res. 1998;83:147-157.)

Key Words: arrhythmia ▪ cardiomyopathy ▪ cardiac edema ▪ cell size ▪ ion channel gating

D etails of the complex chain of events leading to CHF and the accompanying cellular hypertrophy, contractile dysfunction, and dysrhythmias remain incompletely understood. It is clear, however, that hemodynamic disturbances capable of initiating CHF place unnatural stresses on cardiac myocytes. The resulting cellular remodeling may further alter mechanical forces detected by the cytoskeleton and cell membrane. The mechanical nature of the primary event and the ensuing hypertrophy suggest that modulation of SACs and the processes underlying cell volume regulation might play a role in the pathophysiology of CHF.

A variety of SACs have been identified in myocardial cells. Mechanical deformation or swelling activates poorly selective cation channels, K⁺ channels, and Cl⁻ channels. In addition, the function of a number of voltage-dependent channels, including the delayed rectifier and L-type Ca²⁺ channel, are mechanosensitive, although there is disagreement about the details.

Several cation SACs are blocked by micromolar concentrations of the trivalent lanthanide gadolinium (Gd³⁺). The efficacy of Gd³⁺ as an inhibitor of stretch-induced release of atrial natriuretic peptide from rat atria, the generation of ventricular premature beats by rapid inflation of a balloon in canine ventricles, and afterdepolarizations, premature beats, and poststretch augmentation of contractile force in rat atria argue that activation of cation SACs are responsible for these stretch-induced phenomena. We found that Gd³⁺ also decreases the magnitude of osmotic swelling in intact rabbit ventricular myocytes but that it does not affect cell volume under isosmotic conditions, when SACs are thought to be closed. The same effects of Gd³⁺ on cell volume are observed in perforated-patch voltage-clamp studies and are due to block of I_{Cir, swell}, a swelling-activated poorly selective cation current (Pₖ/Pₙa ≅ 6) that exhibits inward rectification. The primary effect of Gd³⁺ is on current rather than cell volume because the onset of block of I_{Cir, swell} precedes cell shrinkage. I_{Cir, swell} is a depolarizing current at the normal resting Eₘ and is postulated to contribute to stretch-induced dysrhythmias and other stretch-dependent events.

The purpose of the present study was to evaluate whether SAC behavior and the regulation of cell volume are transformed in a canine dilated cardiomyopathy model of CHF produced by several weeks of rapid ventricular pacing. This model has been extensively characterized in both dogs and.

Received December 8, 1997; accepted April 29, 1998.

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The present study found that \( I_{\text{Cir}, \text{swell}} \) was persistently activated in isometric media in ventricular myocytes isolated from dogs with CHF; osmotic swelling increased \( I_{\text{Cir}, \text{swell}} \) slightly, whereas osmotic shrinkage inhibited the current. In contrast, \( I_{\text{Cir}, \text{swell}} \) was detected in normal canine myocytes only after osmotic swelling. Moreover, block of \( I_{\text{Cir}, \text{swell}} \) by \( \text{Gd}^+ \) in CHF and normal myocytes caused a proportional cell shrinkage. These data are consistent with the idea that elongated myocytes from dogs in congestive failure behave as if they are stretched. Cation influx via \( I_{\text{Cir}, \text{swell}} \) may contribute to cell hypertrophy and dysrhythmias as well as modulate contractile function. Consequently, SACs are a novel target for therapeutic approaches.

### Materials and Methods

#### Heart Failure Model

Adult mongrel dogs underwent tranvenous insertion of a permanent pacemaker and were paced from the right ventricular apex at 235 bpm for 4 to 6 weeks. Retrograde ventriculooctomy conduction was absent during pacing. Chronic rapid pacing produced clinical evidence of CHF in all dogs as manifested by variable degrees of anorexia, lethargy, exercise intolerance, ascites, tachypnea, and/or muscle wasting. Two-dimensional echocardiography was performed at baseline and serially during the period of rapid ventricular pacing. Ventricular dysfunction was documented by a fall in left ventricular ejection fraction from 0.65 ± 0.06 to 0.23 ± 0.09 (\( P < 0.0001 \)), a >3-fold increase in left ventricular end-systolic volume from 19.5 ± 7.9 to 61.4 ± 15.3 mL (\( P < 0.0001 \)), and a 50% increase in left ventricular end-diastolic volume from 50.3 ± 11.9 to 75.5 ± 13.9 mL (\( P < 0.0001 \)).

#### Cell Isolation

Dogs (normal, \( n = 4 \); CHF, \( n = 6 \)) were anesthetized with intravenous sodium thiopental (30 mg/kg) followed by \( \alpha \)-chloralose (10 mg/kg) and were intubated. A median sternotomy was made, and sections of the right and left ventricular apices were removed and washed in a nominally Ca\(^2+\)-free Tyrode's solution gassed with 100% \( \text{O}_2 \). Right and left ventricular sections were divided into ~1-mm\(^3\) pieces with a scalpel and then dispersed separately using an albumin-containing collagenase-pronase solution described previously. \(^{29,40}\) The tissue was placed in enzyme-containing flasks in a shaker bath and agitated for 10 minutes at 37°C. After filtration through a 250-μm nylon mesh to remove incompletely digested material, cells were washed twice and stored in a modified Kraft-Bruhe solution containing (mmol/L) KOH 132, glutamic acid 120, KCl 2.5, KH\(_2\)PO\(_4\) 10, MgSO\(_4\) 1.8, K\(_2\)-EGTA 0.5, glucose 11, taurine 10, and HEPES 10 (pH 7.2, 295 mOsm/L). Typical yields were 30% to 50% Ca\(^2+\)-tolerant rod-shaped cells. Myocytes were used within 6 hours of harvesting, and only quiescent cells with regular striations and no evidence of membrane blebbing were selected for study. Only quiescent cells were chosen to eliminate myocytes that might have been damaged in isolation. This procedure may eliminate CHF cells that were most severely injured in vivo from the study because it excludes cells that become spontaneously active in vivo, and cells severely injured in vivo may not survive isolation.

#### Experimental Solutions

Cells were placed in a glass-bottomed chamber (~0.3 mL) and superfused with room temperature (~22°C) bathing solution at 3 mL/min. Solution changes were complete within 10 seconds, as estimated from the liquid junction potential of a microelectrode. The standard bathing solution contained (mmol/L) NaCl 65, KCl 5, CaSO\(_4\) 2.5, MgSO\(_4\) 0.5, HEPES 5, glucose 10, and mannitol 17 to 283 (pH 7.4). The reduced, fixed NaCl concentration permitted adjustment of osmolarity with mannitol at a constant ionic strength. To evaluate the role of physiological monovalent cations, Na\(^+\) and K\(^+\) in the bathing media were replaced by equimolar amounts of NMDG in some experiments. An osmolarity of 296 mOsm/L was taken as isosmotic (1T). Osmolarity ranged from 178 to 266 mOsm/L in hypotonic solutions (0.6T to 0.9T) and was 444 mOsm/L in hyperosmotic solution (1.5T). Osmolarity routinely was checked with a freezing-point depression osmometer (Osmette S, Precision Systems).

#### Voltage-Clamp Technique

Patch electrodes with a tip diameter of 3 to 4 μm and a resistance of 0.5 to 1 MΩ were made from borosilicate capillary tubing (7740 glass with filament; outer diameter, 1.5 mm; inner diameter, 1.12 mm; Glass Co of America). The standard electrode filling solution contained (mmol/L) potassium aspartate 120, KCl 2.5, KH\(_2\)PO\(_4\) 10, glucose 11, taurine 10, and HEPES 10 (pH 7.1). In addition, a Na\(^+\)-free Tyrode's solution gassed with 100% O\(_2\) was used for all studies to avoid unpredictable cell swelling and changes in membrane currents that often slowly occur with ruptured patches. \(^{14,19}\) Amphotericin B (Sigma Chemical Co) was freshly dissolved in DMSO (Sigma) and then diluted in electrode salts for experiments in which bath Na\(^+\) was replaced with NMDG.

The amphotericin-perforated-patch voltage-clamp technique was used for all studies to avoid unpredictable cell swelling and changes in membrane currents that often slowly occur with ruptured patches. \(^{14,19}\) Amphotericin B (Sigma Chemical Co) was freshly dissolved in DMSO (Sigma) and then diluted in electrode filling solution to give final amphotericin and DMSO concentrations of 100 μg/mL and 0.2% (vol/vol), respectively. After dipping the pipette tip into amphotericin-free solution for 2 seconds, pipettes were backfilled with ionophore, and gigaseals were formed as rapidly as possible. Access resistance decreased to 7 to 10 MΩ within ~20 minutes of seal formation, and then experimental protocols were begun.

An Axoclamp 200A amplifier (Axon, Foster City, CA) was used to measure whole-cell currents. Voltage-clamp protocols, electrophysiological and video data acquisition, and off-line data analysis were managed with a suite of custom programs written in ASYST (Keithley). To study quasi–steady-state currents that may contribute to the regulation of cell volume, slow voltage ramps (28 mV/s) were applied. The voltage was stepped from −80 to +40 mV for 20 milliseconds, ramped to −100 mV over 5 seconds, and after 10 milliseconds, ramped back to +40 mV over 5 seconds. Currents elicited by the depolarizing and hyperpolarizing legs of the ramp were virtually identical and were averaged to cancel the small capacitive current (see Figure 1D). This ramp protocol is the same as previously used to study \( I_{\text{Cir}, \text{swell}} \) in rabbit ventricular myocytes \(^{14}\) and may underestimate any swelling-activated components of the slowly
method for group comparisons were used. For simple comparisons, a Student t test was performed. Comparison of the sensitivity of normal and CHF cells to Gd3+ was evaluated by a 2-way ANOVA, including an interaction term. All statistical analyses were conducted with SigmaStat 2.0 (SPSS), and curve-fitting was performed in SigmaPlot 4.0 (SPSS). Least squares methods were used to determine lines of best fit, and nonlinear fitting used the Marquardt-Levenberg algorithm.

Results

Characteristics of Ventricular Myocytes From Normal and CHF Dogs

Morphological and electrophysiological characteristics of ventricular myocytes isolated from normal and CHF dogs and selected for study are summarized in Table 1. Chronic rapid pacing caused ventricular myocytes to enlarge. The planar area of the image of the myocyte and the estimated cell volume were 19.8% and 23.9% greater, respectively, for cells from CHF dogs than for those from normal animals. These morphological changes reflected mainly a 15.7% increase in cell length, whereas cell width did not change significantly. Concomitantly, membrane capacitance increased 19.2%, reflecting an extension of sarcolemmal membrane area. Because the myocytes studied here were selected as being appropriate for both patch clamp and imaging, they may not represent an unbiased sample of myocardial cells. Moreover, the volumes of cells studied in vitro may not be equal to their volumes in vivo.

Swelling-Activated Current in Normal Canine Ventricular Myocytes

A swelling-activated, poorly selective cation current that inwardly rectifies and is blocked by Gd3+ is found in rabbit ventricular myocytes.14 This current is called ICsim. Osmotic cell swelling elicited a similar current in normal canine ventricular myocytes. Figure 1A illustrates the I-V relationship in isosmotic solution (1T) and after exposure to hyposmotic solution (0.6T) for 5 minutes. Hyposmotic solution augmented the inwardly rectifying current at negative potentials, and an outwardly rectifying current developed at potentials positive to ~65 mV. These changes in the I-V relationship completely reversed on returning to 1T solution.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>CHF</th>
<th>CHF/Normal Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacitance, pF</td>
<td>175.6±9.6</td>
<td>209.4±9.7</td>
<td>1.193</td>
<td>0.019</td>
</tr>
<tr>
<td>Length, μm</td>
<td>133.1±5.0</td>
<td>154.1±9.5</td>
<td>1.158</td>
<td>0.001</td>
</tr>
<tr>
<td>Width, μm</td>
<td>31.6±1.2</td>
<td>32.7±0.9</td>
<td>1.034</td>
<td>0.112</td>
</tr>
<tr>
<td>Planar area, μm²</td>
<td>4206±12</td>
<td>5309±23</td>
<td>1.198</td>
<td>0.025</td>
</tr>
<tr>
<td>Volume, pL</td>
<td>52.2±2.7</td>
<td>64.7±3.2</td>
<td>1.239</td>
<td>0.035</td>
</tr>
</tbody>
</table>

n (cells) | 16 | 34 | ...

Mean ± SEM values for normal and CHF dogs initially measured in isosmotic solution (1T). Absolute volume was estimated assuming that canine myocytes are elliptical in cross section with a major/minor axis ratio of 2/1 and that observed width is the major axis. In some types of cardiomyopathy, the major/minor axis ratio changes.39 P indicates results of unpaired Student t test comparing the normal and CHF groups.

Statistics

Data are reported as mean±SEM, and n represents the number of cells, unless otherwise noted. Mean current densities were expressed in pA/pF to account for differences in cell membrane area. When multiple comparisons were made, ANOVA and the Bonferroni
Swelling-Activated Cation Current and Cell Volume in CHF

After recovery in 1T solution, the effect of 10 μmol/L Gd³⁺ was tested in 1T solution and after swelling the myocytes in 0.6T solution (Figure 1B). The Gd³⁺-sensitive difference currents in 1T and 0.6T solutions, calculated as the current in Gd³⁺-free solution minus that in the presence of 10 μmol/L Gd³⁺, are depicted in Figure 1C. During osmotic swelling, Gd³⁺ blocked an inwardly rectifying current that reversed near –60 mV, whereas the difference current was negligible in 1T solution. The similarity of these results to those in rabbit myocytes was striking and suggested that Gd³⁺-sensitive current and Gd³⁺-induced reduction of cell volume increased. Maximum responses were obtained at ≤0.7T. In this and subsequent figures, time 0 for relative cell volume corresponds to sealing of the amphotericin-B-filled pipette on the cell. Myocyte volume in 1T solution did not change during the 20-minute period of patch perforation. C, Gd³⁺-sensitive current at –80 mV and Gd³⁺-induced cell shrinkage plotted vs bath relative osmolarity (n=6 cells).

Figure 2 shows that Gd³⁺-sensitive current, \( I_{\text{Cir,swell}} \), and Gd³⁺-induced cell shrinkage were observed in normal myocytes after osmotic swelling in 0.6T to 0.9T hyposmotic solutions but not in isosmotic (1T) or hyperosmotic (1.5T) solutions. A and B, Average Gd³⁺-sensitive currents (A) and average cell volumes in the absence and presence of 10 μmol/L Gd³⁺ (B). As bath osmolarity was decreased, Gd³⁺-sensitive current and Gd³⁺-induced cell shrinkage were observed in normal myocytes; evidence that the inwardly rectifying Gd³⁺-sensitive current is a cation current is presented later (see Figure 9). In addition, Gd³⁺ did not block the outwardly rectifying current caused by swelling in 0.6T. This Gd³⁺-resistant current was nearly abolished by replacement of Cl⁻ with methanesulfonate and by 9-anthracene carboxylic acid, suggesting that it is mainly the swelling-activated anion current, \( I_{\text{Cir,swell}} \), which has been previously described in atrial, ventricular, and sinoatrial nodal myocytes from dogs and other species including humans.

Figure 2 shows that \( I_{\text{Cir,swell}} \) was graded by the amount of cell swelling in normal canine myocytes. I-V relationships and relative cell volume were recorded simultaneously in selected hyposmotic (0.6T to 0.9T), isosmotic (1T), and hyperosmotic (1.5T) solutions, and as described previously, \( I_{\text{Cir,swell}} \) was measured as the difference between the I-V relationships ±10 μmol/L Gd³⁺ at each osmolarity (Figure 2A). Gd³⁺ did not affect the I-V relationship in 1T solution or after cell shrinkage in 1.5T solution. On the other hand, even after only modestly swelling myocytes in 0.9T solution to a relative cell volume of 1.088±0.026 (P<0.005, Figure 2B), \( I_{\text{Cir,swell}} \) was already obvious (–0.25±0.05 pA/pF, P<0.001). \( I_{\text{Cir,swell}} \) increased on additional swelling but reached a maximum in 0.7T solution at a volume of 1.268±0.029, despite the fact that myocytes swelled further in 0.6T solution.

Gd³⁺ not only blocked \( I_{\text{Cir,swell}} \), it also decreased the extent of osmotic cell swelling (Figure 2B). For example, cells swelled to 1.378±0.018 in 0.6T solution but only 1.267±0.027 in 0.6T solution+Gd³⁺ (P<0.01). The effects of Gd³⁺ on membrane current and cell volume are summarized in Figure 2C, where Gd³⁺-sensitive current at –80 mV and Gd³⁺-induced shrinkage are plotted against superfusate osmolarity. Both effects of Gd³⁺ were apparent and saturated over the same range of osmolarities.

Persistent Activation of \( I_{\text{Cir,swell}} \) in CHF Myocytes

Ventricular diastolic wall stress is increased in tachycardia-induced CHF. This raises the possibility that activation of \( I_{\text{Cir,swell}} \) in situ contributes to the pathophysiology of CHF. The situation may be different for single cells isolated from CHF dogs, however. The distending forces present during diastole in the intact animal are absent in isolated myocytes under isosmotic conditions. On the other hand, the CHF-induced increase in cell volume may be equivalent to an isosmotic stretch and thereby lead to activation of \( I_{\text{Cir,swell}} \). To investigate this hypothesis, Gd³⁺-sensitive \( I_{\text{Cir,swell}} \) and cell volumes of myocytes isolated from dogs in CHF were measured simultaneously at osmolarities ranging from 0.6T to 1.5T.

Dissection of the Gd³⁺-sensitive current in a CHF cell is illustrated in Figure 3. I-V relationships were recorded in the absence (Figure 3A) and presence (Figure 3B) of 10 μmol/L Gd³⁺ in both 1T solution and after osmotic shrinkage in 1.5T solution, and the Gd³⁺-sensitive difference currents were calculated (Figure 3C). In contrast to normal cells wherein swelling was necessary to elicit a Gd³⁺-sensitive current, the CHF myocyte exhibited a large, inwardly rectifying, Gd³⁺-sensitive current in 1T solution that was abolished by osmotic shrinkage. Figure 4 shows averaged data for CHF cells in 1T and hyposmotic solutions. The Gd³⁺-sensitive current in CHF cells in 1T solution was –1.21±0.35 pA/pF at –80 mV (P<0.01) in 1T solution, but cell swelling in successively more hyposmotic solutions (0.9T to 0.6T) only slightly increased the Gd³⁺-sensitive current further (eg, –1.50±0.27 pA/pF at –80 mV in 0.8T solution). Simultaneously, 10 μmol/L Gd³⁺ reduced relative cell volume in 1T solution by 9.1%, from 0.997±0.004 to 0.906±0.010 (Figure 4B, P<0.001), and significantly reduced cell volume in each of the hyposmotic solutions. Figure 4C emphasizes, however, that neither Gd³⁺-sensitive current at –80 mV nor Gd³⁺-induced cell shrinkage was significantly affected by osmotic swelling of CHF myocytes. In addition to the data...
presented in Figure 4, protocols depicted in Figures 5 and 7 also included measurements of current and volume in 1T solution. Combining all experiments (30 cells from 6 CHF dogs), the current blocked by 10 μmol/L Gd³⁺ in 1T solution was $-1.07 \pm 0.16 \text{pA/pF at } -80 \text{ mV}$ ($P<0.001$), and Gd³⁺ decreased cell volume by $7.8 \pm 0.7\%$ ($P<0.001$). Observation of Gd³⁺-sensitive current and Gd³⁺-induced cell shrinkage in CHF myocytes cells under isosmotic conditions is in stark contrast to the results in normal myocytes (compare with Figure 2). If $I_{Cir,swell}$ is persistently activated by CHF because CHF cells are “swollen” even in 1T solution and if the membrane and cytoskeletal elements thereby are “stretched,” then graded osmotic shrinkage should relieve the stress and turn off $I_{Cir,swell}$ in a graded fashion. To test this prediction, responses to Gd³⁺ in isosmotic solution (1T) were compared with those in a series of hyperosmotic solutions (1.1T to 1.5T). As shown in Figure 5A, graded shrinkage of CHF cells led to a graded reduction of the Gd³⁺-sensitive current. $I_{Cir,swell}$ was undetectable at osmolarities $>1.3T$. Osmotic shrinkage also reduced the effect of Gd³⁺ on cell volume. Gd³⁺-sensitive current at $-80 \text{ mV}$ and Gd³⁺-induced cell shrinkage plotted vs bath relative osmolarity ($n=12$ cells).

**Figure 3.** Effect of osmotic swelling on whole-cell currents in a CHF myocyte. A and B, Currents under isosmotic (1T) and hyperosmotic (1.5T) conditions in the absence (A) and presence (B) of 10 μmol/L Gd³⁺. Osmotic shrinkage reduced both inward and outward currents. C, Gd³⁺-sensitive difference currents obtained by digitally subtracting the curves in panels A and B. A Gd³⁺-sensitive, inwardly rectifying current was present in CHF cells under isosmotic conditions (1T) and was abolished by osmotic shrinkage (1.5T).

**Figure 4.** $I_{Cir,swell}$ was chronically activated in isolated CHF myocytes. A and B, Average Gd³⁺-sensitive currents (A) and average cell volumes in the absence and presence of 10 μmol/L Gd³⁺ (B) in 1T to 0.6T solutions. In 1T solution, $I_{Cir,swell}$ was $1.21 \pm 0.35 \text{pA/pF at } -80 \text{ mV}$, and Gd³⁺ caused a 9.1% decrease of cell volume. Osmotic swelling (0.9T to 0.6T) did not significantly alter the response to Gd³⁺. C, Gd³⁺-sensitive current at $-80 \text{ mV}$ and Gd³⁺-induced cell shrinkage plotted vs bath relative osmolarity ($n=12$ cells).

**Figure 5.** Osmotic shrinkage abolished $I_{Cir,swell}$ in CHF myocytes. A and B, Average Gd³⁺-sensitive currents (A) and average cell volumes in the absence and presence of 10 μmol/L Gd³⁺ (B) in 1T to 1.5T solutions. $I_{Cir,swell}$ decreased as bath osmolarity was increased. At $-80 \text{ mV}$, $I_{Cir,swell}$ was not significantly different from 0 in $>1.3T$. Osmotic shrinkage also reduced the effect of Gd³⁺ on cell volume. C, Gd³⁺-sensitive current at $-80 \text{ mV}$ and Gd³⁺-induced cell shrinkage plotted vs bath relative osmolarity ($n=12$ cells).

**Cell Volume Dependence of the Gd³⁺ Sensitivity of Normal and CHF Myocytes**

The responses of normal and CHF myocytes are directly compared in Figure 6, in which the Gd³⁺-sensitive current at $-80 \text{ mV}$ (Figure 6A) and the Gd³⁺-induced cell shrinkage (Figure 6B) are plotted as functions of cell volume before...
application of 10 μmol/L Gd³⁺ in 0.6T to 1.5T solutions. CHF cells (solid circles) remained Gd³⁺ sensitive even when they were shrunken to a relative cell volume of 0.861 ± 0.014 in 1.3T solution, whereas normal cells (open squares) became Gd³⁺ sensitive only after swelling to 1.079 ± 0.024 in 0.9T solution. The maximum response to Gd³⁺ was observed at volumes of ≥ 1.075 ± 0.012 (≥ 0.9T) and 1.272 ± 0.025 (0.7T) in CHF and normal cells, respectively. C, Approximately linear relationship between Gd³⁺-induced block of current and Gd³⁺-induced reduction of cell volume.

**Dose Dependence of Effect of Gd³⁺ on Cell Volume and Current**

Block of $I_{\text{Cir,swell}}$ by Gd³⁺ and the resulting volume changes were dose dependent. Normal cells first were swollen in 0.6T

**TABLE 2. Gd³⁺-Induced Changes in Ionic Current and Cell Volume in Right and Left Ventricular Myocytes**

<table>
<thead>
<tr>
<th>Source</th>
<th>Gd³⁺-sensitive current, pA/pF</th>
<th>Gd³⁺-induced shrinkage, %</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, RV</td>
<td>−1.58 ± 0.21</td>
<td>9.3 ± 0.8</td>
<td>5</td>
</tr>
<tr>
<td>Normal, LV</td>
<td>−1.53 ± 0.23</td>
<td>9.1 ± 0.7</td>
<td>11</td>
</tr>
<tr>
<td>CHF, RV</td>
<td>−1.05 ± 0.14</td>
<td>7.6 ± 0.7</td>
<td>10</td>
</tr>
<tr>
<td>CHF, LV</td>
<td>−1.08 ± 0.17</td>
<td>7.9 ± 0.6</td>
<td>20</td>
</tr>
</tbody>
</table>

RV indicates right ventricle; LV, left ventricle; and n, number of cells for both current and volume. Values are mean ± SEM for normal cells in hypotonic 0.6T solution and CHF cells in isosmotic 1T solution. Gd³⁺-sensitive current was measured at −80 mV. All statistical comparisons between values for RV and LV were not significant.
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the Gd\textsuperscript{3+} induced cell shrinkage for normal (A and B) and CHF (C and D) myocytes after 5-minute exposures to Gd\textsuperscript{3+}. Data from Figure 7 are replotted with responses (R) expressed as a fraction of the fitted maximum response (R_{max}). Dose-response relationships for block of current at −80 mV (●) and reduction of volume (○) in normal (A) and CHF (C) cells were fitted to the Hill equation, \( R/R_{max} = C/(C + C^n) \), with a \( K_{50} \) of ∼2 μmol/L and Hill coefficient, n, of ∼1.9 (solid line) estimated from the fits to all 4 dose-response relationships. Fitted relationships are extrapolated to 0.1 and 100 μmol/L to illustrate doses expected to give 0 and maximal responses. Fractional block of current at by Gd\textsuperscript{3+} (ΔGd\textsuperscript{3+} current) was linearly related to fractional Gd\textsuperscript{3+}-induced cell shrinkage (ΔGd\textsuperscript{3+} volume) for normal (B) and CHF cells (D).

See text for further details.

>90% of the Gd\textsuperscript{3+}-sensitive current and cause >90% of the maximum Gd\textsuperscript{3+}-induced shrinkage in both normal and CHF cells.

The excellent correlations between the effects of Gd\textsuperscript{3+} on current and volume in both normal and CHF cells are emphasized in Figure 8B and 8D, in which responses were expressed as a fraction of the fitted maximum response. The relationships were described by straight lines with slopes and intercepts not significantly different from 1 and 0, respectively, as previously found for normal rabbit ventricular cells.\textsuperscript{14} This is consistent with the idea that \( I_{Cir,swell} \) is one factor regulating cell volume.

**Ionic Basis of Gd\textsuperscript{3+}-Sensitive Current and Volume Changes**

\( I_{Cir,swell} \) in rabbit ventricular myocytes is a poorly selective cation current with a \( P_C/P_{Na} \) of ∼6.\textsuperscript{14} If physiological monovalent cations such as Na\textsuperscript{+} and K\textsuperscript{+} constitute the majority of the Gd\textsuperscript{3+}-sensitive current described thus far in myocytes from normal and CHF dogs, removal of these ions from the bath and electrode solutions should markedly attenuate both the Gd\textsuperscript{3+}-sensitive current and the Gd\textsuperscript{3+}-induced cell shrinkage. To test this prediction, Na\textsuperscript{+} and K\textsuperscript{+} were replaced with NMDG in the bath and by Cs\textsuperscript{+} in the pipette, and the effect of 10 μmol/L Gd\textsuperscript{3+} was studied in 1T and 0.6T solution in normal myocytes and 1T and 1.5T solution in CHF myocytes. After replacing physiological monovalent cations, the Gd\textsuperscript{3+}-sensitive current (A and C) and Gd\textsuperscript{3+}-induced cell shrinkage (B and D) were negligible in both normal and CHF cells. Times (a to d) of recording of I-V relationships are indicated.

removal of Na\textsuperscript{+} and K\textsuperscript{+} eliminated the Gd\textsuperscript{3+}-sensitive current seen in CHF cells in isosmotic solution (Figure 9C, curve a–c), and as before, the current remained insensitive to Gd\textsuperscript{3+} after osmotic shrinkage in 1.5T solution (curve b–d). The experiments in 1T solution for normal cells and in 1.5T solution for CHF cells demonstrate that Gd\textsuperscript{3+} has no effect on the remaining membrane currents under conditions previously shown to block \( I_{Cir,swell} \). Cation replacement also abolished the Gd\textsuperscript{3+}-induced shrinkage of normal cells in 0.6T solution (Figure 9B) and of CHF cells in 1T solution (Figure 9D). These data verified that \( I_{Cir,swell} \) in both normal and CHF canine myocytes was carried by cations. Based on the \( E_{rev} \) for \( I_{Cir,swell} \) (−61.5±3.3 mV in normal cells and −60.4±3.5 mV in CHF cells in 0.6T solution, Figures 2 and 4, respectively) and the bath and pipette Na\textsuperscript{+} and K\textsuperscript{+} concentrations, the constant field \( P_C/P_{Na} \) ratios were 8.3±1.6 in normal cells (n=6) and 8.5±2.0 in CHF cells (n=12). The calculation of \( P_C/P_{Na} \) assumes that Na\textsuperscript{+} and K\textsuperscript{+} were the only charge carriers, a reasonable approximation, because removing Na\textsuperscript{+} and K\textsuperscript{+} eliminated the current. A small Ca\textsuperscript{2+} component cannot be ruled out, however. The contribution of Ca\textsuperscript{2+} to \( I_{Cir,swell} \) was not investigated, although it is known that other cation SACs in the heart are permeant to Ca\textsuperscript{2+}.\textsuperscript{11}

**Discussion**

A stretch-activated current, \( I_{Cir,swell} \), was persistently activated under isosmotic conditions (1T) in ventricular myocytes isolated from dogs with tachycardia-induced CHF; the myocytes behaved as if they were swollen even in 1T solution. Graded osmotic swelling caused graded activation of \( I_{Cir,swell} \), a poorly selective, inwardly rectifying cation current that is blocked by Gd\textsuperscript{3+}.\textsuperscript{14} Although Gd\textsuperscript{3+} is not perfectly selective, its lack of effect in normal cells in 1T solution suggests that...
Swelling-Activated Cation Current and Cell Volume in CHF

Gd⁺³ primarily blocks swelling-activated steady-state currents at the concentrations used. \( I_{\text{Cir,swell}} \) was 88% activated in 1T solution in CHF myocytes, and osmotic shrinkage with 1.3T solution to a relative cell volume of 0.861 was required to inhibit the current fully. In myocytes obtained from normal dogs, \( I_{\text{Cir,swell}} \) was absent in 1T. The maximum \( I_{\text{Cir,swell}} \) current density evoked was not significantly different in normal and CHF cells. Because membrane capacitance was \( \approx 20\% \) greater in the CHF cells, this means that the magnitude of fully activated of \( I_{\text{Cir,swell}} \) in CHF cells exceeded that in normal cells by the same factor. Thus, as sarcoplasmic membrane area expanded, there was a proportional increase in the number of \( I_{\text{Cir,swell}} \) channels that could be activated by cell swelling, their open probability on activation, or both.

Cellular hypertrophy led to persistent activation of \( I_{\text{Cir,swell}} \). At the same time, \( I_{\text{Cir,swell}} \) can explain in part the greater volume of CHF than normal myocytes. Hypertrophy requires both protein synthesis and a net gain of osmolytes to increase cell volume. \( I_{\text{Cir,swell}} \) was an inwardly directed current at the resting \( E_{\text{rev}} \) (\( E_{\text{rev}} \approx –60 \text{ mV} \)) carried by an influx of Na⁺ and K⁺. Its block by 10 \( \mu \text{mol/L} \) Gd⁺³ reduced the volume of CHF cells in 1T by \( \approx 8\% \), but Gd⁺³ did not affect the volume of normal myocytes in 1T in which \( I_{\text{Cir,swell}} \) was not observed. The requirement for macroscopic electroneutrality dictates that a flux of anions must accompany a flux of cation sufficient to alter cell volume. This is likely to be carried by \( I_{\text{Cir,swell}} \) (see Figure 1), and preliminary experiments indicate that \( I_{\text{Cir,swell}} \) also is persistently activated in congestive failure.¹³

Characteristics of \( I_{\text{Cir,swell}} \)

\( I_{\text{Cir,swell}} \) was only recently discovered.¹⁴ The characteristics of the current in both normal and CHF dog ventricular myocytes were virtually identical to those previously reported for rabbit myocytes.¹⁴ \( I_{\text{Cir,swell}} \) in both species exhibited (1) graded activation on stretch, (2) inward rectification, (3) dependence on bath K⁺ and Na⁺ with \( E_{\text{rev}} \) near –60 mV, (4) inhibition by Gd⁺³ with \( K_{0.5} \) of \( \approx 2 \mu \text{mol/L} \) and a Hill coefficient of \( \approx 2 \), and (5) block of \( I_{\text{Cir,swell}} \) by Gd⁺³, leading to a proportional cell shrinkage of up to \( \approx 10\% \). In rabbit myocytes, \( I_{\text{Cir,swell}} \) is unaffected by replacement of Cl⁻ with methanesulfonate and is insensitive to 0.2 mmol/L Ba²⁺, a blocker of the background K⁺ current, \( I_{\text{K}} \).

The major difference between \( I_{\text{Cir,swell}} \) in normal and CHF myocytes appeared to be the step point for its activation, as measured in terms of solution osmolarity or relative cell volume (ie, the volume of a cell relative to the volume of the same cell in 1T solution). However, the volume of CHF cells was estimated to be 23.9% greater than that of normal cells in 1T solution. If normal myocytes were osmotically swollen by this amount, the current density of \( I_{\text{Cir,swell}} \) observed was the maximum that could be elicited compared with the 81% activation of \( I_{\text{Cir,swell}} \) in CHF cells of the same absolute volume (see Figure 6). Similarly, osmotically shrinking CHF cells to a relative cell volume of 0.861 in 1.3T solution to turn off \( I_{\text{Cir,swell}} \) is equivalent to a normal cell volume of 1.067 (ie, 1.239×0.861), a volume at which \( I_{\text{Cir,swell}} \) was turned on <15% in normal cells. This suggests that the shift in the set point for activation of \( I_{\text{Cir,swell}} \) in CHF cells can be accounted for by assuming that myocytes “remember” their preCHF volume and thus are “swollen” even in isosmotic solution. This idea is appealing; it suggests why osmotic shrinkage reverses CHF-induced activation of \( I_{\text{Cir,swell}} \), but it is likely too simple to fully explain the results. One problem is the difference in how cell volume increased. Osmotic swelling caused a much greater increase in cell width than length, as noted previously,¹⁸,¹⁹,4₂ whereas the present study and others⁴₉,⁵₈ showed that chronic tachycardia increases cell length much more than width. If a common mechanism underlies activation of \( I_{\text{Cir,swell}} \) in both cases, it must account for these differences in how cell volume enlarges. Distortions of cell width and length may have common effects on the modulation of protein function by the cytoskeleton because cytoskeletal elements are said to be interconnected in a tension-stabilized (ie, tensegrity) network.³¹ Thus, the radial and longitudinal arrangement of cytoskeletal elements are coupled.

It is yet to be established whether \( I_{\text{Cir,swell}} \) is persistently activated in other forms of hypertrophy and CHF. Preliminary data in a rabbit aortic regurgitation CHF model, which combines features of both pressure and volume overload, suggest that \( I_{\text{Cir,swell}} \) is persistently activated in this case also (H.F. Clemo and C.M. Baumgarten, unpublished data, 1997). If increased cell width, as occurs with osmotic swelling, is a sufficient stimulus, then activation of \( I_{\text{Cir,swell}} \) is expected in pressure-overload hypertrophies, such as those associated with arterial coarctation. Pressure overload induces an unambiguous increase in myocyte width.⁵₂–⁵⁴ Furthermore, the width of myocytes from patients with dilated cardiomyopathy is increased.⁵₅,⁵₆

Mechanism of Activation of \( I_{\text{Cir,swell}} \) in CHF

The mechanism by which CHF or cell swelling activates \( I_{\text{Cir,swell}} \) remains undefined. Evidence in other systems indicates that various ion channels and transport proteins are linked to the actin cytoskeleton by ankyrin and/or spectrin and suggests that the cytoskeleton can regulate their function directly or via second messengers (for review, see References 57 and 58). The influence of the cytoskeleton appears to be tissue and transport protein specific, however, so specific predictions for CHF are difficult to make. Nevertheless, the complex cell biology of CHF offers several candidate mechanisms for why \( I_{\text{Cir,swell}} \) was activated here. Cytoskeletal architecture is altered in tachycardia-induced cardiomyopathy, and levels of mRNA for α-tubulin and β- and γ-actin are increased.⁶⁹ In the dilated human heart, the arrangement of titin and α-actinin is altered⁶⁰, tubulin, desmin, and vimentin are increased; and the cytoskeleton is disorganized.⁶¹ One or more of these might contribute to the activation of \( I_{\text{Cir,swell}} \). Alternate candidates for regulating \( I_{\text{Cir,swell}} \) include the multiple second-messenger cascades activated by stretch, hypertrophy, and failure.⁵₂–⁶₄

Pathophysiological Consequences of Persistent Activation of \( I_{\text{Cir,swell}} \)

Mechanoelectrical coupling influences cardiac electrical activity in a variety of experimental situations and has long been postulated on indirect grounds to contribute to dysrhythmias and mechanical dysfunction in humans.⁶₅,⁶₆ \( I_{\text{Cir,swell}} \) was
inward at resting $E_m$, and its chronic activation can explain the 5- to 7-mV depolarization of resting $E_m$ observed in tachycardia-induced dilated cardiomyopathy and would be expected to prolong terminal repolarization. This current is not the only one altered in CHF, however. Alterations in the shape of the $I-V$ relationship and magnitude of $I_{K1}$ were noted previously but these studies did not distinguish between $I_{K1}$ and SAC currents. On the other hand, $I_{Cir,swell}$ cannot explain the CHF-induced prolongation of action potential duration measured at 50% repolarization. Recently, this has been attributed to inhibition of the transient outward current but effects of hypertrophy on the delayed rectifier and the L-type $Ca^{2+}$ current may also play a role in action potential prolongation.

The putative role of SACs in arrhythmias associated with CHF is of potential importance. Detailed intramural mapping studies show that ventricular tachyarrhythmias arise focally rather than by reentrant mechanisms in models of CHF. Furthermore, rapid pacing–induced CHF promotes sustained atrial tachycardias that have a focal mechanism presumably related to delayed afterdepolarizations. $Gd^{3+}$-sensitive delayed afterdepolarizations and triggered activity are induced by acute stretch and is reasonable to think that activation of the $Gd^{3+}$-sensitive inward current $I_{Cir,swell}$ contributes to both these phenomena and to tachyarrhythmias in CHF. Moreover, $I_{Cir,swell}$ favors delayed afterdepolarizations by overloading of the $Ca^{2+}$ stores (see below).

$I_{Cir,swell}$ poorly discriminated between $K^+$ and $Na^+$ ($P_{K}/P_{Na}$ ≈ 8), which implies that $Na^+$ is the main charge carrier of the inward current with physiological ion gradients. Based on current density, capacitive membrane area, cell volume, and permeability ratio, the magnitude of $Na^+$ influx in CHF cells at –80 mV is sufficient to increase intracellular $Na^+$ concentration by $\approx 1.5$ mmol/L per minute. This is a substantial $Na^+$ influx. Increased intracellular $Na^+$ is expected in turn to increase intracellular $Ca^{2+}$ and $Ca^{2+}$ stores via the $Na^+_\text{-}$-$Ca^{2+}$ exchanger. In addition, poorly selective cation SACs are permeant to $Ca^{2+}$ also. Consequently, persistent activation of $I_{Cir,swell}$ may affect contractile performance.

It remains to be established whether persistent $I_{Cir,swell}$ contributes to the cellular remodeling observed with chronic tachycardia or is the result of remodeling. $Na^+$ loading of cells long has been thought to contribute to cell growth. For example, stretch-dependent increased synthesis of actin and myosin heavy chain in adult ventricle is correlated with the rate of $^{22}Na^+$ uptake; streptomycin, a blocker of certain mechanosensitive channels, reduced protein synthesis, whereas enhancing $Na^+$ entry with monensin or veratridine augmented it. One might then postulate that $Gd^{3+}$ would prevent stretch-induced changes in gene expression. This hypothesis was tested by Sadoshima et al. who found that up to 50 $\mu$mol/L $Gd^{3+}$ did not alter the expression of immediate-early genes including $c-jun$, $Erg$, $c-fos$, $JE$, or $c-myc$ or protein synthesis in a neonatal rat myocyte culture system. These authors concluded that $Gd^{3+}$-sensitive SACs are not part of the signal transduction pathway. However, a methodological problem confounds their interpretation. Gene expression and protein synthesis were studied in a modified DMEM/F-12 (1:1) medium, which is phosphate-bicarbonate-buffered. Published stability constants indicate that effectively all of the $Gd^{3+}$ would have been complexed by these anions and unavailable to block SACs. Thus, a role for $Gd^{3+}$-sensitive SACs in the modulation of gene expression and protein synthesis remains a viable hypothesis.

**Limitations of the Study**

The canine tachycardia-induced cardiomyopathy model used in the present study has been well characterized and mirrors many of the pathophysiological changes of congestive heart failure in humans. On the other hand, the changes reverse on cessation of rapid ventricular pacing, whereas in humans, most cardiomyopathies are irreversible. Also of note is that tachycardia-induced cardiomyopathy is a volume-overload model, leading to eccentric cellular and biventricular hypertrophy. Many clinical cardiomyopathies are due to pressure or the combined effects of pressure and volume overload.

Another concern is that the same bath and electrode solutions were used for both normal and CHF cells. This ignores complex changes in the extracellular and intracellular milieu that occur as a result of rapid pacing and the development of CHF in vivo. Such changes may modulate myocyte currents and cell volume. Even for control cells, myocyte volume may not be identical in vitro and in vivo. Furthermore, there is some variability in the reported effects of rapid pacing on several of the morphometric parameters measured here. We found a 16% increase in cell length, no significant change in cell width, and a 19% increase in membrane capacitance in the small population of cells studied. Spina et al. reported that cell length increases in tachycardia-induced CHF but that cell diameter either does not change or increases in the pig and decreases in the dog. However, others found that canine myocyte width and length both increase. Membrane capacitance also previously was found to increase in a pig chronic tachycardia model, but Kääb et al. failed to see a change in membrane capacitance in a dog model.

In summary, the present results demonstrate that $I_{Cir,swell}$ is persistently activated in tachycardia-induced CHF in the dog. The requirements for current activation and its characteristics suggest that $I_{Cir,swell}$ is likely to be activated in multiple forms of cardiac hypertrophy and failure and that it may contribute to dysrhythmias and altered contractile function. To the extent that it does, $I_{Cir,swell}$ may represent a novel target for therapeutic interventions.

**Acknowledgments**

This study was supported by National Heart, Lung, and Blood Institute grants HL-02798 (Dr Clemo) and HL-46764 (Dr Baumgar) and a Merit Review Award from the Department of Veterans Affairs (Dr Stambler).

**References**


Persistent Activation of a Swelling-Activated Cation Current in Ventricular Myocytes From Dogs With Tachycardia-Induced Congestive Heart Failure
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Circ Res. 1998;83:147-157
doi: 10.1161/01.RES.83.2.147

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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