Swelling-Activated Chloride Current Is Persistently Activated in Ventricular Myocytes From Dogs With Tachycardia-Induced Congestive Heart Failure

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Abstract—The hypothesis that cellular hypertrophy in congestive heart failure (CHF) modulates mechanosensitive (ie, swelling- or stretch-activated) anion 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 0.9T to 0.6T solution (T, relative osmolarity; isosmotic solution, 296 mOsmol/L) was required to elicit $I_{\text{Cl,swell}}$, an outwardly rectifying swelling-activated Cl$^-$ current that reversed near $-33$ mV and was inhibited by 1 mmol/L 9-anthracene carboxylic acid (9AC), an anion channel blocker. Block of $I_{\text{Cl,swell}}$ by 9AC simultaneously increased the volume of normal cells in hyposmotic solutions by up to 7%, but 9AC had no effect on volume in isosmotic or hyperosmotic solutions. In contrast, $I_{\text{Cl,swell}}$ was persistently activated under isosmotic conditions in CHF myocytes, and 9AC increased cell volume by 9%. Osmotic shrinkage in 1.1T to 1.5T solution inhibited both $I_{\text{Cl,swell}}$ and 9AC-induced cell swelling in CHF cells, whereas osmotic swelling only slightly increased $I_{\text{Cl,swell}}$. The current density for fully activated 9AC-sensitive $I_{\text{Cl,swell}}$ was 40% greater in CHF than normal myocytes. In both groups, 9AC-sensitive current and 9AC-induced cell swelling were proportional with changes in osmolarity and 9AC concentration, and the effects of 9AC on current and volume were blocked by replacing bath Cl$^-$ with methanesulfonate. CHF thus altered the set point and magnitude of $I_{\text{Cl,swell}}$ and resulted in its persistent activation. We previously observed analogous regulation of mechanosensitive cation channels in the same CHF model. Mechanosensitive anion and cation channels may contribute to the electrophysiological and contractile derangements in CHF and may be novel targets for therapy. 

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

A swelling-activated, outwardly rectifying Cl$^-$ current has been described in mammalian cardiac myocytes,\textsuperscript{1–5} including human atrial cells.\textsuperscript{6,7} This current has been termed $I_{\text{Cl,swell}}$. Several molecules have been postulated to function as swelling-activated Cl$^-$ channels or channel regulators including CIC-2,\textsuperscript{8} PIClIn,\textsuperscript{9,10} P-glycoprotein,\textsuperscript{11,12} and phospholemman.\textsuperscript{13} However, more recent evidence favors the idea that $I_{\text{Cl,swell}}$ in cardiac myocytes is due to activation of CIC-3.\textsuperscript{14}

The significance of $I_{\text{Cl,swell}}$ for cardiac function under physiological and pathophysiological conditions remains uncertain. On the basis of the magnitude and voltage dependence of the current, activation of $I_{\text{Cl,swell}}$ is thought to modulate cardiac electrical activity.\textsuperscript{15,16} In addition, we reported\textsuperscript{17} that 9-anthracene carboxylic acid (9AC), a blocker of $I_{\text{Cl,swell}}$,\textsuperscript{1,18} increases the volume of intact ventricular myocytes after osmotic swelling when $I_{\text{Cl,swell}}$ is expected to be activated, but 9AC has no effect on cell volume under isosmotic conditions when the channels underlying $I_{\text{Cl,swell}}$ are thought to be closed.

Another situation in which $I_{\text{Cl,swell}}$ may be important is congestive heart failure (CHF). The hemodynamic perturbations responsible for the development of CHF and cellular hypertrophy place myocytes under mechanical stress and lead to complex cellular remodeling\textsuperscript{19} and activation of multiple intracellular signaling systems.\textsuperscript{20} Using the perforated-patch voltage clamp method, we recently found that $I_{\text{Cl,swell}}$, an inwardly rectifying, Gd\textsuperscript{3+}-sensitive cation current normally detected only after osmotic cell swelling,\textsuperscript{21} became persistently activated under isosmotic conditions in ventricular myocytes isolated from animals with CHF induced by tachycardia.\textsuperscript{22} Moreover, block of $I_{\text{Cl,swell}}$ resulted in significant volume changes in normal rabbit,\textsuperscript{21} as well as in normal and CHF dog\textsuperscript{22} myocytes.

In light of the modulation of swelling-activated cation channels by CHF, the present study was designed to deter-
mine whether \( I_{\text{Cl,swell}} \) also is transformed in a canine model of CHF produced by several weeks of rapid ventricular pacing. This model has been extensively characterized in both dogs and pigs. Chronic tachycardia produces biventricular dilatation with eccentric cellular hypertrophy, decreases contractility, downregulates the response to catecholamines, atrial natriuretic peptide, renin, and endothelin-I, and decreases the density of the myocardial collagen network. These characteristics are shared with dilated cardiomyopathy in humans, and the model is useful for considering the role of \( I_{\text{Cl,swell}} \) in CHF.

Concurrent perforated-patch voltage clamp and cell-volume determinations revealed that \( I_{\text{Cl,swell}} \) was persistently activated in isosmotic media in ventricular myocytes isolated from dogs with CHF. Osmotic swelling did not significantly increase \( I_{\text{Cl,swell}} \) and osmotic shrinkage inhibited the current. In contrast, \( I_{\text{Cl,swell}} \) was found in normal canine myocytes only after osmotic swelling. In addition, 9AC induced cell swelling that was proportional to the magnitude of \( I_{\text{Cl,swell}} \) blocked in both CHF and normal myocytes. These data are consistent with the idea that elongated hypertrophic myocytes from dogs in CHF behave as if they are swollen. Anion fluxes via \( I_{\text{Cl,swell}} \) may contribute to the sequelae of CHF and may represent a novel target for therapy.

### Materials and Methods

#### Heart Failure Model

Heart failure was produced as previously described. Adult mongrel dogs underwent permanent transvenous pacing of the right-ventricular apex at 235 bpm for 4 to 6 weeks. Ventricular dysfunction was documented using 2-dimensional echocardiography, which showed a significant fall in left-ventricular ejection fraction from 0.65 ± 0.06 to 0.23 ± 0.09 and significant increases in left-ventricular end systolic volume from 19.5 ± 7.9 to 61.4 ± 15.3 mL and in left-ventricular end diastolic volume from 50.3 ± 11.9 to 75.5 ± 13.9 mL. Clinical evidence of CHF including anorexia, lethargy, ascites, tachypnea, and/or muscle wasting also was noted.

#### Cell Isolation

Using a median thoracotomy approach, portions of the left and right ventricle were taken from anesthetized dogs (normal \( n = 4 \), CHF \( n = 6 \)). After washing in nominally Ca\(^{2+}\)-free Tyrode solution equilibrated with 100% O\(_2\), the right- and left-ventricular samples were then divided into ~1-mm\(^3\) pieces with a scalpel and were dispersed in an albumin-containing collagenase-protease solution described previously. After filtration to remove incompletely digested material, the isolated cells were washed twice and then stored in a solution designed to allow adjustment of osmolarity with mannitol at a constant ionic strength. The role of physiological cations and anions changes were complete within 10 s. The standard bathing solution (area \( t \)) contained (in mmol/L) 65 NaCl, 5 KCl, 2.5 CaSO\(_4\), 0.5 MgSO\(_4\), 5 HEPES, 10 glucose, and 17 to 283 mannitol (pH 7.4). Solutions were designed to allow adjustment of osmolarity with mannitol at a constant ionic strength. The role of physiological cations and anions was evaluated by replacing Na\(^+\) and K\(^+\) in the bathing media with equimolar amounts of N-methyl-D-glucamine (NMDG) in some experiments and by replacing Cl\(^-\) in the bathing media with equimolar amounts of methanesulfonate in others. Isosmotic (IT; relative osmolarity) solution was set as 296 mOsm/L. The osmolarity of hyposmotic solutions (0.6T to 0.9T) ranged from 178 to 266 mOsm/L and was 444 mOsm/L in hyperosmotic solutions (1.5T). A freezing-point depression osmometer (Osmette S; Precision Systems) was used to routinely verify solution composition.

#### Voltage Clamp Technique

Patch electrodes with a tip diameter of 3 to 4 μm were made from borosilicate capillary tubing (7740 glass with filament; 1.5 mm OD, 1.12 mm ID). Pipettes usually were filled with solution containing (in mmol/L) 120 potassium aspartate; 10 KCl, 10 NaCl, 3 MgSO\(_4\), and 10 HEPES (pH 7.1). For experiments in Na\(^+\)- and K\(^+\)-free bathing media, Na\(^+\) and K\(^+\) salts in the pipette solution were replaced with Cs\(^+\) salts, and for experiments in Cl\(^-\)-free bathing media, Cl\(^-\) in the pipette solution was reduced to ~5 mmol/L by replacing NaCl and KCl with the corresponding aspartate salts.

The perforated-patch method was used for all studies to avoid unpredictable cell swelling and changes in membrane currents that often slowly occur with the ruptured patch technique. 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. The tip of the pipette was dipped into amphotericin-free solution for 2 s, pipettes were then 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. In all plots of cell volume, time 0 is defined as the time of seal formation. Cell volume remained unchanged as amphotericin inserted into the patch and equilibrium was established across the perforated-patch membrane.

An Axoclamp 200A amplifier (Axon) was used to record whole-cell currents. Voltage clamp protocols, electrophysiological and video data acquisition, and off-line data analysis were controlled by custom programs written in ASYST (Keithley). Slow voltage ramps (28 mV/s) were applied to examine quasi–steady-state currents that may contribute to regulation of cell volume. The voltage was stepped from –80 mV to +40 mV for 20 ms; ramped to –100 mV over 5 s; and, after 10 ms, ramped back to +40 mV over 5 s. Currents elicited by the depolarizing and hyperpolarizing legs of the ramp were virtually identical and were averaged to cancel the small capacitative current. Ramp currents were digitized at 1 kHz after low-pass filtering at 200 Hz. Membrane capacitance was calculated from the integral of the current transient in response to 10-mV hyperpolarizing steps. The bath was superfused with a 3 mol/L KCl agar bridge, and the reported membrane potential (\( E_m \)) values were corrected for the liquid junction potential between the bath and the pipette.

#### Determination of Relative Cell Volume

Methods for determining relative cell volume have been described previously. An inverted microscope (Diaphot; Nikon, Inc) equipped with Hoffman modulation optics (×40; 0.55 numerical aperture) and a high-resolution TV camera (CCD72; Dage-MTI) coupled to a video frame grabber (Targa-MB; Truevision) was used to image myocytes. Images were captured on-line each time a ramp protocol was performed. A combination of commercial (MOCHA; SPSS) and custom (ASYST; Keithley) programs was used to determine cell width, length, and the planar area of the image.

Changes in cell width and thickness on exposure to anisosmotic solutions are proportional. Using each cell as its own control, relative cell volume was calculated as follows: \( \text{vol/vol} = \frac{\text{area} \times \text{length}}{\text{area} \times \text{length}} \), where \( t \) and \( c \) refer to test (eg, 0.6T) and control (1T) solutions, respectively. The value of relative cell volume is independent of assumptions regarding the geometric shape of the cross section of the myocyte as long as the shape does not change. These methods provide estimates of relative cell volume that are reproducible to <1%.
Results

Morphological and Electrophysiological Characteristics of Myocytes

Table 1 summarizes the characteristics of cells selected for study. Myocytes from hearts subjected to rapid ventricular pacing were 17.4% longer than those from normal hearts, whereas cell width was not significantly different. On the basis of morphometric measurements, the estimated cell volume of CHF myocytes was 34.6% greater than that for normal cells. The sarcolemmal surface area of CHF cells also was larger, as evidenced by a 19.2% increase in membrane capacitance. Although these pacing-induced changes in the morphometric and electrophysiologic properties of isolated myocytes are very similar to those reported previously, it is uncertain whether they fully represent the in vivo situation. The myocytes studied here are not an unbiased sample of cardiac myocytes and is termed.

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 Bonferroni’s method for group comparisons were used. For simple comparisons, a Student’s t test was performed. All statistical analyses were conducted with SigmaStat 2.0 (SPSS).

I\textsubscript{Cl,swell} in Normal Canine Ventricular Myocytes

An outwardly rectifying Cl\textsuperscript{−} current that is activated by osmotic swelling and blocked by 9AC has been identified in cardiac myocytes and is termed I\textsubscript{Cl,swell}. The effect of osmotic swelling on whole-cell currents in isolated canine ventricular myocytes under perforated-patch conditions is shown in Figure 1A. A 5-minute exposure to 0.6T hyposmotic solution containing permeant anions and cations evoked an outwardly rectifying current at potentials positive to −65 mV and an inwardly rectifying current at more negative potentials. Returning to isosmotic solution (1T) completely restored the initial current-voltage (I-V) relationship.

Cells then were treated with 1 mmol/L 9AC, a blocker of I\textsubscript{Cl,swell}, and the effect of osmotic swelling on membrane current was tested again (Figure 1B). 9AC did not affect the I-V relationship in 1T solution, but the Cl\textsuperscript{−} channel blocker substantially reduced the outwardly rectifying current evoked by osmotic swelling. 9AC-sensitive difference currents in 1T and 0.6T solutions, calculated as the current in 9AC-free solution minus that in the presence of 1 mmol/L 9AC, are shown Figure 1C. During osmotic swelling, 9AC blocks an outwardly rectifying current that reversed near −33 mV. This value is similar to the Cl\textsuperscript{−} equilibrium potential, E\textsubscript{Cl}, of −31.6 mV calculated from bath and pipette solutions. In contrast, 9AC-sensitive current was negligible in 1T solution when swelling- or stretch-activated channels (SACs) were expected to be closed. A swelling-activated, outwardly rectifying, 9AC-sensitive current was seen in 13 of 18 normal cells tested. However, all of the swelling-activated current was not blocked by 9AC. A 9AC-insensitive inwardly rectifying component previously was shown to be a Gd\textsuperscript{3+}-sensitive swelling-activated cation current.

The magnitude of I\textsubscript{Cl,swell} was related to the extent of myocyte swelling. The I-V relationship and relative cell volume were recorded simultaneously in selected hyposmotic (0.6T to 0.9T), isosmotic (1T), and hyperosmotic (1.5T) solutions±1 mmol/L 9AC, and 9AC-sensitive currents at

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>CHF</th>
<th>CHF/Normal Ratio</th>
<th>P</th>
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</thead>
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<tr>
<td>Capacitance, pF</td>
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<td>211.9±7.9</td>
<td>1.192</td>
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</tr>
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<td>Length, μm</td>
<td>135.5±4.9</td>
<td>159.1±4.0</td>
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<tr>
<td>Width, μm</td>
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<td>33.2±0.8</td>
<td>1.071</td>
<td>0.117</td>
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<tr>
<td>Planar area, μm²</td>
<td>420±11</td>
<td>5281±13</td>
<td>1.257</td>
<td>0.001</td>
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<tr>
<td>Volume, pL</td>
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<td>68.9±1.7</td>
<td>1.346</td>
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<td>n (cells)</td>
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<td>26</td>
<td>…</td>
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</table>

Mean±SEM values for normal and CHF myocytes 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 is reported to change.24 P indicates unpaired Student t test comparing the normal and CHF groups.

Figure 1. Effect of osmotic swelling on whole-cell currents recorded by amphotericin-perforated-patch method from a normal canine myocyte. A, I-V relationships in isosmotic (1T) and hyposmotic (0.6T) solutions are shown. Traces are average of hyperpolarizing and depolarizing ramps. In bath solution containing Na\textsuperscript{+}, K\textsuperscript{+}, and Cl\textsuperscript{−}, osmotic swelling increases both inward and outward currents. B, 9AC (1 mmol/L), a blocker of I\textsubscript{Cl,swell}, markedly attenuated the swelling-activated current in 0.6T solution but had little effect in 1T solution. C, 9AC-sensitive difference currents calculated as current in 9AC-free minus that in 9AC-containing 1T and 0.6T solutions are shown. The swelling-activated 9AC-sensitive current (0.6T) outwardly rectified, reversed at −33 mV, and was denoted I\textsubscript{Cl,swell}.21,22
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Figure 2A. 9AC-sensitive current and 9AC-induced cell swelling were observed in normal myocytes after swelling in 0.6T to 0.7T hypotonic solutions but not in isosmotic (1T) or hypertonic (1.5T) solutions. A and B. Average 9AC-sensitive currents (A) and average cell volumes in the absence and presence of 1 mmol/L 9AC (B). As bath osmolarity was decreased, 9AC-sensitive current and 9AC-induced cell swelling increased. Maximum responses were obtained at osmolarities ≤0.7T. 9AC-sensitive current reversed at ~33.5 ± 0.8 mV after equilibration in 0.6T solution. In this and subsequent figures, time 0 for volume measurements corresponds to sealing of the amphotericin-filled pipette on the cell. Myocyte volume in 1T solution did not change during the 20-minute period of patch perforation. C. 9AC-sensitive current at –80 mV and 9AC-induced cell swelling plotted vs bath relative osmolarity (n=8 cells).

Each osmolarity are plotted in Figure 2A. 9AC had no effect on the I-V relationship under isosmotic conditions and after cell shrinkage in 1.5T. In contrast, a significant 9AC-sensitive current (−0.25±0.02 pA/pF at −80 mV, P<0.001) was recorded in 0.9T solution after only a modest increase in cell volume (7.0±1.1%, P=0.002), and further swelling increased the 9AC-sensitive current. Nevertheless, the currents in 0.7T and 0.6T solutions were not distinguishable (−0.56±0.04 versus −0.60±0.04 pA/pF at −80 mV, P=0.5; 1.76±0.18 versus 2.06±0.21 pA/pF at +40 mV, P=0.31), although myocyte swelling was significantly greater in 0.6T than 0.7T solution (Figure 2B).

Figure 2B also shows that 9AC increased cell volume in hypotonic solutions coincident with block of the outwardly rectifying swelling-activated current. For example, relative cell volume increased from 1.31±0.014 in 0.6T solution to 1.381±0.013 in 0.6T+9AC (P=0.006). In contrast, 9AC did not alter cell volume in 1T or 1.5T solutions in which 9AC-sensitive currents were not detected. The effects of 9AC on membrane current and cell volume are summarized in Figure 2C, in which the 9AC-sensitive current at −80 mV and 9AC-induced cell swelling are plotted against superfusate osmolarity. Both actions of 9AC became apparent and saturated over the same range of osmolarities, 0.9T to 0.6T, and neither cell volume nor membrane current was affected in 1T or 1.5T solutions.

Persistent Activation of 9AC-Sensitive Current in CHF Myocytes

In tachycardia-induced cardiomyopathy, a Gd³⁺-sensitive, inwardly rectifying cation SAC, I_{Cl,swell}, is chronically activated under isosmotic conditions and is inhibited by cell shrinkage in hyperosmotic solution. Therefore, we investigated whether the regulation of swelling-activated, 9AC-sensitive current also is remodeled in pacing-induced CHF. To test this possibility, the 9AC-sensitive current and cell volumes of myocytes isolated from CHF dogs were measured simultaneously at osmolarities ranging from 0.6T to 1.5T.

Measurement of the 9AC-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 1 mmol/L 9AC both in 1T solution and after osmotic shrinkage in 1.5T solution, and the 9AC-sensitive difference currents are plotted (Figure 3C). A 9AC-sensitive, outwardly rectifying current that reversed at ~34 mV was observed under isosmotic conditions (1T) in the CHF cell and was abolished after cell shrinkage in hyperosmotic 1.5T solution. Persistent activation of the 9AC-sensitive current under isosmotic conditions in CHF myocytes sharply contrasts with results from normal cells (compare Figures 2C and 3C), in which osmotic swelling was required to activate the 9AC-sensitive current.

The dependence of the I-V relationship for 9AC-sensitive current on osmotic cell swelling in CHF cells is characterized in Figure 4. Graded swelling of cells in hypotonic solutions (0.9T to 0.6T) did not significantly increase the 9AC-sensitive current at ~80 mV from the current observed in 1T solution (Figure 4A, P=0.535). Consistent with the observation that I_{Cl,swell} was persistently activated, exposure to 9AC caused a cell swelling of 7.6±0.8% under isosmotic conditions in CHF cells, and osmotic swelling did not significantly increase the 9AC-induced cell swelling (Figure 4B, P=0.109). The reversal potential (E_{rev}) for the 9AC-sensitive current in CHF myocytes was ~33.1±0.7 mV in 1T solution, indistinguishable from that in normal myocytes in 0.6T solution (compare Figures 2A and 4A). The finding that 9AC-sensitive current and 9AC-induced cell swelling were independent of solution osmolarity is more clearly illustrated.
in Figure 4C, in which the responses to 9AC are plotted as a function of solution osmolarity. 9AC-sensitive effects were seen in 22 of 23 CHF cells under isosmotic conditions (Figures 4, 5, and 7); the 9AC-sensitive current at –80 mV was –0.77 ± 0.05 pA/pF, and 9AC caused a 7.9 ± 0.5% cell swelling.

The similarity between the responses of CHF cells to 9AC under isosmotic conditions and that of normal cells after osmotic swelling suggested that CHF myocytes behave as if they are swollen even under isosmotic conditions, as was previously argued to explain the behavior of cation SACs in the same CHF model.22 If this idea is correct, osmotic cell shrinkage would be expected to block the 9AC-sensitive current and 9AC-induced cell swelling in CHF cells. To test this possibility, the response of 9AC in CHF cells in isosmotic solution (1T) was compared with that in hyperosmotic solution (1.1T to 1.5T). Figure 5A shows that graded osmotic shrinkage caused a graded reduction in the magnitude of the 9AC-sensitive current, and \( I_{\text{Cl,swell}} \) was undetectable in 1.4T solution. Concurrently with the reduction of \( I_{\text{Cl,swell}} \), 9AC-induced cell swelling decreased as bathing solution osmolarity was increased (Figure 5B). The dependence of the responses to 9AC on solution osmolarity are summarized in Figure 5C.

**Cell Volume Dependence of 9AC Sensitivity of Normal and CHF Myocytes**

The responses of normal and CHF myocytes are directly compared in Figure 6, in which the 9AC-sensitive current and 9AC-induced cell swelling depend on cell volume. Data were taken from Figure 2 (normal cells, ■) and Figures 4 and 5 (CHF cells, ○). A and B, 9AC-sensitive current at –80 mV (A) and 9AC-induced cell swelling (B) plotted as functions of relative cell volume before adding 9AC (volumes normalized to that in 1T 9AC-free solution). Maximum responses to 9AC were observed at relative cell volumes of 1.073 ± 0.011 (0.9T) and 1.254 ± 0.012 (0.7T) in CHF and normal cells, respectively. C, Approximately linear relationship between 9AC-induced block of current and 9AC-induced increase in cell volume.
$I_{\text{Cl,swell}}$ and Cell Volume in CHF

TABLE 2. 9AC-Induced Changes in Ionic Current and Cell Volume in Right and Left Ventricular Myocytes

<table>
<thead>
<tr>
<th>Source</th>
<th>9AC-Sensitive Current, pA/pF</th>
<th>9AC-Induced Swelling, %</th>
<th>n</th>
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</thead>
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<tr>
<td>Normal, RV</td>
<td>-0.58±0.06</td>
<td>6.8±0.5</td>
<td>5</td>
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<tr>
<td>Normal, LV</td>
<td>-0.60±0.04</td>
<td>6.9±0.6</td>
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<td>CHF, RV</td>
<td>-0.78±0.06</td>
<td>8.0±0.7</td>
<td>10</td>
</tr>
<tr>
<td>CHF, LV</td>
<td>-0.76±0.05</td>
<td>7.8±0.6</td>
<td>12</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. 9AC-sensitive current was measured at −80 mV. All statistical comparisons for RV and LV were not significant.

−80 mV (Figure 6A) and the 9AC-induced cell swelling (Figure 6B) are plotted as functions of cell volume before application of 1 mmol/L 9AC in 0.6T to 1.5T solution. CHF cells remained responsive to 9AC even after shrinking to 0.872±0.011 in 1.3T solution, whereas normal cells became sensitive to 9AC only after swelling to 1.071±0.008 in 0.9T solution. The maximum response to 9AC was observed at cell volumes ≥1.073±0.011 (=0.9T) in CHF cells and ≥1.254±0.012 (=0.7T) in normal cells, respectively. Thus, the set point for 9AC sensitivity appeared to be shifted to a relative cell volume ~0.18 lower in CHF cells than in normal myocytes. The 9AC-sensitive current and volume changes in Figure 6C are plotted as a percentage of the maximal 9AC-induced change. The relationship between block of current and cell swelling was linear over the range of osmolarities explored and suggests a tight coupling of these processes.

Myocytes were harvested from both the left and right ventricles of CHF and normal dogs. Table 2 shows that the source of the cells had no significant effect on 9AC-sensitive current or 9AC-induced cell swelling. Consequently, data from right- and left-ventricular myocytes have been combined.

Dose Dependence of Effect of 9AC on Myocyte Volume and Current

Inhibition of $I_{\text{Cl,swell}}$ by 9AC and the ensuing cell swelling were dose dependent in both normal and CHF cells. Normal cells first were swollen in NMDG-0.6T solution to activate $I_{\text{Cl,swell}}$ in the absence of cation SAC current, $I_{\text{CIR,swell}}$, and then were exposed for 5-minute periods to successively higher doses of 9AC (0.01 to 1 mmol/L) in 0.6T solution. As the 9AC concentration was increased, the magnitude of the blocked current (Figure 7A) and cell volume (Figure 7B) both increased significantly in a graded fashion. The dose dependence of the effects of 9AC are compared in Figure 7C with the responses at 1 mmol/L scaled to an equal height. This emphasizes that block of current and cell swelling were proportional at different concentrations of 9AC. Analogous experiments were conducted on CHF myocytes in NMDG-1T solution. 9AC-sensitive currents (Figure 7D) and 9AC-induced cell swelling (Figure 7E) are illustrated. Stepwise increases in 9AC concentration elicited a proportional block of current at −80 mV and cell swelling in CHF cells (Figure 7F), as it did in normal cells (Figure 7C).

Figures 7A and 7D also provide information on the efficacy of 9AC as a blocker of $I_{\text{Cl,swell}}$. The magnitude of $I_{\text{Cl,swell}}$ can be estimated as the current elicited on switching from NMDG-1T to NMDG-0.6T solution for normal cells (Figure 7A, curve b–a) and from NMDG-1T to NMDG-1.5T solution for CHF cells (Figure 7D, curve a–b). The maximum dose of 9AC used, 1 mmol/L, blocked 93±1% and 89±1% of $I_{\text{Cl,swell}}$ at −80 mV in normal (Figure 7A, curve b–e) and CHF (Figure 7D, curve a–e) cells, respectively.

Figure 7. Dose dependence of 9AC-sensitive current and 9AC-induced cell shrinking in normal (n=5) and CHF (n=6) myocytes. Normal cells were osmotically swollen (0.6T) to activate $I_{\text{Cl,swell}}$ and CHF cells were studied in isosmotic solution. Cells were exposed to each concentration of 9AC for 5 minutes, a time sufficient to obtain the steady-state response. A and D, Average 9AC-sensitive currents with 0.01 to 1 mmol/L 9AC in normal (A) and CHF (D) myocytes. Times (a through e) of recording of I-V relationships are denoted in B and E, B and E. Concurrent measurements of cell volumes of normal (B) and CHF (E) cells. C and F, comparison of 9AC-sensitive current at −80 mV (■) and 9AC-induced cell swelling (□) for normal (C) and CHF (F) cells. Stepwise increases in 9AC concentration elicited proportional block of current at −80 mV and cell swelling.
Ionic Basis of 9AC-Sensitive Current and Volume Changes

Experiments in NMDG solutions (Figure 8) indicated that Na\(^+\) and K\(^+\) were not required to elicit a 9AC-sensitive current or 9AC-induced cell swelling. If 9AC is acting as a Cl\(^-\) channel blocker,\(^2,18\) replacement of Cl\(^-\) with a larger, poorly permeant anion should markedly attenuate the effects of 9AC on membrane current and cell volume. To test this prediction, methanesulfonate was substituted for all Cl\(^-\) in the bath, and all but 5 mmol/L Cl\(^-\) in the pipette was replaced with aspartate. Replacement of Cl\(^-\) essentially abolished the effect of 9AC on membrane current (Figure 8A and 8C) and cell volume (Figure 8B and 8D) in normal and CHF cells. Times (a through d) of recording of I-V relationships are indicated in B and D.

Figure 8. 9AC-sensitive current is anion dependent in normal (A and B, n=4) and CHF (C and D, n=4) myocytes. Cl\(^-\) was replaced with methanesulfonate in the bath and was reduced to 5 mmol/L in the pipette by replacement with aspartate. The effect of 9AC (1 mmol/L) was studied in 1T and 0.6T solution in normal myocytes and in 1T and 1.5T solution for CHF myocytes. After replacing Cl\(^-\), the 9AC-sensitive current (A and C) and 9AC-induced cell swelling (B and D) were negligible in both normal and CHF cells. Times (a through d) of recording of I-V relationships are indicated in B and D.

Discussion

Previously we found that a swelling-activated, Gd\(^{3+}\)-sensitive cation current, I_{Cl,swell}, was persistently activated in a canine model of CHF.\(^22\) The present results show that a swelling-activated anion current, I_{Cl,swell}, also was persistently activated under isosmotic conditions in hypertrophic ventricular myocytes isolated from dogs with tachycardia-induced cardiomyopathy. Whereas osmotic swelling of CHF cells only modestly increased I_{Cl,swell}, graded osmotic shrinkage caused graded inhibition of the current. Full inhibition at \(-80\) mV was achieved by shrinking myocytes to a relative volume of 0.821 \pm 0.008 in 1.4T solution. In contrast, I_{Cl,swell} was absent in cells isolated from normal dogs bathed in isosmotic solution, but it was activated in a graded fashion with graded osmotic swelling in 0.9T to 0.6T solutions at cell volumes ranging from 1.072 \pm 0.008 to 1.311 \pm 0.014. With full activation, current density was \(\approx 40\%\) greater in CHF myocytes than in normal myocytes. The currents recorded in both normal and CHF cells reversed at the same potential and were blocked in a dose-dependent manner by 9AC, a blocker of I_{Cl,swell}. Concurrent with block of I_{Cl,swell}, 9AC caused cell volume to increase. The 9AC-induced cell swelling was proportional to the magnitude of I_{Cl,swell} and the concentration of 9AC. Thus, swelling-activated anion channels may modulate cardiac electrical activity and cell volume in CHF.

Characteristics of I_{Cl,swell}

The currents described here in both normal and CHF cells (1) were dependent on cell volume, (2) were dependent on Cl\(^-\) with \(E_{rev}\) near \(E_{Cl}\), (3) were insensitive to replacement of bath Na\(^+\) and K\(^+\) by NMDG, (4) exhibited outward rectification under asymmetric conditions, and (5) were attenuated by 9AC. These features match those of I_{Cl,swell} described by Tseng\(^1\) in normal canine ventricular cells and Sorota\(^2\) in normal canine atrial cells. I_{Cl,swell} is found in normal cells from a number of species\(^31\) including humans,\(^6,7,32,33\) Sorota\(^18\) found that 9AC blocked by 50\%, whereas niflumic acid completely blocked I_{Cl,swell} at all voltages. Block by 4,4’-dinitrostilbene-2,2’-disulfonic acid (DNDS) was voltage dependent, with only partial block at negative voltages and complete block at membrane voltages >0 mV.\(^18\) Preliminary data confirm that niflumic acid completely blocks I_{Cl,swell} (H.F.C. and C.M.B., unpublished data, 1998), but the present study found that 9AC blocks >90\% of I_{Cl,swell} (see Figure 7) in canine myocytes at all membrane voltages. The reasons for the discrepancy between the present results and those of Sorota\(^18\) on the efficacy of 9AC are unclear but may include methodological differences or subtle differences between canine atrial and ventricular myocytes. Sorota\(^2\) was unable to elicit I_{Cl,swell} in canine ventricular myocytes using the nystatin-perforated-patch method but did observe this current in canine atrial myocytes under the same conditions.

We found that substitution of methanesulfonate in the bath for Cl\(^-\) caused almost complete inhibition of I_{Cl,swell} in both CHF and normal cells (see Figure 8). Others have reported that the permeability ratio P_{Cl}/P_{methanesulfonate} is 0.25 to 0.35 in cardiac myocytes.\(^5,7,34\) The pipette Cl\(^-\) concentration ([Cl\(^-\)]) in these studies was on the order of 40 mmol/L, considerably higher than the 6 mmol/L used in the present Cl\(^-\) substitution experiments. It is possible that [Cl\(^-\)] modulates the selectivity of I_{Cl,swell}. Also, other studies typically use a ruptured rather than a perforated-patch voltage clamp.

A number of molecular candidates for I_{Cl,swell} have been proposed, including CIC-2.8, PFCln,\(^9,10\) P-glycoprotein,\(^11,12\) phospholemman,\(^13\) and CIC-3.\(^14\) Presently, the most likely candidate for I_{Cl,swell} appears to be CIC-3, a member of the Cl\(^-\) channel superfamily (for review, see References 35 and 36). This channel protein has been isolated from guinea pig ventricle,\(^14\) as well as from other tissues and species. Key characteristics of the CIC-3 channel include (1) outward rectification, (2) anion selectivity with I \(\approx \) Cl\(^-\), and (3) activation and inactivation of current by protein kinase C (PKC) inhibition and stimulation, respectively.\(^14,37,38\) We recently demonstrated that I_{Cl,swell} in normal rabbit\(^39\) and canine\(^40\)
ventricular myocytes shares these 3 properties and thus may be caused by CIC-3.

I_{Cl,swell} in CHF

This study demonstrates that a Cl⁻ current is active under isosmotic conditions in canine CHF myocytes. Bénitah et al. recently reported that a 9AC-sensitive Cl⁻ current was present in rat ventricular myocytes obtained from a pressure-overload model (aortic constriction) of hypertrophy but not in normal cells. The authors did not test whether the Cl⁻ current they observed in hypertrophied rat myocytes was sensitive to cell volume and did not identify it as I_{Cl,swell}. It is likely, however, that the current found by Bénitah et al. was the same as that identified here and shown to be volume sensitive. Moreover, preliminary data from CHF myocytes from both the present canine rapid pacing model (H.F.C. and C.M.B., unpublished data, 1998) and a rabbit aortic regurgitation model strongly suggest that persistently activated I_{Cl,swell} arises from CIC-3. I_{Cl,swell} recorded in 1T myocytes in myocytes from both CHF models exhibits I > Cl⁻ selectivity and is inhibited by activation of PKC by phorbol-12,13-dibutyrate. Taken together, these data suggest that persistent activation of I_{Cl,swell} during the myocardial remodeling associated with hypertrophy and failure is not peculiar to a particular model of CHF or experimental species but rather is a common feature of several models in several species.

In both the dog and rabbit, I_{Cl,swell} current density was 40% greater in CHF than in normal cells. Presently, it is not known whether increased expression of I_{Cl,swell} channels, increased probability of channel opening, or an increased unitary conductance in CHF cells can explain these differences. Others have described an increase in I_{Cl,swell} current density in cardiac hypertrophy and cell proliferation.

As previously mentioned, inactivation of PKC appears to stimulate I_{Cl,swell} in heart. CHF induced in the rabbit by left-ventricular pressure and volume overload causes a reduction in the expression of a number of PKC isoforms. Heart failure in the rabbit induced by pressure overload (aortic constriction) also causes a decrease in the particulate PKC fraction, which is in contrast to an overall increase in PKC activity and content in a similar rat model. Thus, a reduction of particulate PKC activity in CHF cells under isosmosic conditions or in normal cells under hyposmotic conditions could underlie activation of I_{Cl,swell}. Another possible mediator of I_{Cl,swell} is tyrosine kinase. Sorota found that genistein, an inhibitor of tyrosine kinase, prevented activation of I_{Cl,swell} by hyposmotic bath solution in canine atrial myocytes. Further, both cell swelling and stretch rapidly initiate tyrosine kinase-dependent activation of immediate-early genes such as c-fos in rat cardiac myocytes. Given that tyrosine kinases mediate angiotensin II-stimulated cell responses to hypertension, ischemic insult, and CHF, a tyrosine kinase–dependent activation of I_{Cl,swell} in CHF is possible, although not proven at the present time.

Pathophysiology

At positive membrane potentials, I_{Cl,swell} is outwardly directed and presumably contributes to membrane repolarization in phases 2 and 3 of the action potential and will thus shorten the action potential. Vandenberg et al. showed that acute swelling of guinea pig ventricular myocytes causes action potential shortening that could be attenuated by the I_{Cl,swell} blocker 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS). The cardiac action potential is prolonged in humans with heart failure, cardiac hypertrophy, or ischemic cardiomyopathy. Activation of I_{Cl,swell} by CHF (as demonstrated in this study) or by cardiac hypertrophy may thus serve to limit the extent of action potential prolongation in various pathological states. Indeed, Bénitah et al. showed that action potentials of hypertrophied rat ventricular myocytes prolonged when treated with the I_{Cl,swell} blocker 9AC. At negative membrane potentials, I_{Cl,swell} is inwardly directed and when activated should cause membrane depolarization. Cell swelling causes membrane depolarization in both canine atrial myocytes and in guinea pig ventricular myocytes. This membrane depolarization was blocked by I_{Cl,swell} blockers such as niflumic acid and DIDS.

In summary, I_{Cl,swell} was shown to be chronically activated in canine tachycardia-induced cardiomyopathy, and it appears likely that the same current is chronically activated in other forms of CHF and cardiac hypertrophy. Although the pathophysiological role of I_{Cl,swell} presently is uncertain, it may contribute to ventricular dysrhythmias associated with hypertrophy and failure.

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Clemo et al    February 5, 1999    165


Swelling-Activated Chloride Current Is Persistently Activated in Ventricular Myocytes From Dogs With Tachycardia-Induced Congestive Heart Failure
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