Biphasic effects of cell volume on excitation-contraction coupling in rabbit ventricular myocytes

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SWELLING OF CARDIAC MYOCYTES is a prominent aspect of the response to ischemia-reperfusion and elective cardioplegia and also may arise in renal insufficiency and syndromes with inappropriate secretion of antidiuretic hormone. Altered myocyte hydration has important functional consequences. Osmotic perturbations modulate both electrical activity (for reviews, see 34, 38, 42) and the ability of cardiac muscle to contract (5, 16, 17).

Although facets of the contractile response to osmotic swelling are known from studies on multicellular preparations, little mechanistic information is available at the single cell level to explain the implications of myocyte swelling for excitation-contraction (E-C) coupling. Several species differences in E-C coupling, including the sensitivity of contraction to inhibition of the sarcoplasmic reticulum (SR), are well known (2).

METHODS

Myocyte preparation and solutions. Hearts were excised from anesthetized rabbits (New Zealand White, 2–3 kg body wt, either sex), and ventricular myocytes were dissociated as previously described (7, 20). The heart was mounted on a
Langendorff column and initially perfused with 37°C oxygenated Tyrode solution containing (in mmol/l) 140 NaCl, 5.4 KCl, 1 MgCl₂, 1.8 CaCl₂, 0.33 NaH₂PO₄, 10 glucose, and 10 HEPES (adjusted to pH 7.4 with NaOH). After perfusion with Ca²⁺-free Tyrode solution for ~5 min, the heart was then digested with a solution containing 0.5 mg/ml collagenase (Type II, Worthington; Freehold, NJ) and 1 mg/ml bovine serum albumin (Sigma; St. Louis, MO). Myocytes were stored in a high-K⁺ media containing (in mmol/l) 10 KCl, 10 KH₂PO₄, 120 K-glutamate, 10 taurine, 1.0 MgSO₄, 10 HEPES, 20 glucose, and 0.5 EGTA (adjusted to pH 7.2 with KOH) and used within 5 h.

Myocytes were placed in a chamber (~0.3 ml) on an inverted microscope and superfused at room temperature (21–22°C) with either 0.6T solution (~180 mosmol/l) containing (in mmol/l) 80 NaCl, 5.4 KCl, 1 MgCl₂, 5 HEPES, 0.33 NaH₂PO₄, 10 glucose, and 1.8 CaCl₂ (adjusted to pH 7.2 with KOH) or with 1T solution (~300 mosmol/l), which was made by adding 125 mmol/l mannitol to 0.6T. Only quiescent rod-shaped cells showing clear striations were used.

Electrophysiology. Patch pipettes were drawn from thin-walled Corning 7740 glass (1.12 mm ID, 1.5 mm OD) to a tip diameter of 3–4 μm. The electrode resistance was 0.8–12 MΩ when the pipettes were filled with solution containing (in mmol/l) 120 potassium aspartate, 20 KCl, 1 MgCl₂, and 10 HEPES (adjusted to pH 7.4 with NaOH). The perfused-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 (6, 33). Amphotericin-B (Sigma) was freshly dissolved in dimethyl sulfoxide (Sigma) and then diluted in pipette-filling solution to give final amphotericin concentration of 160 μg/ml. The tip of the pipette was dipped into amphotericin-free solution for 2–3 s, the pipette was then backfilled with the ionophore, and gigaseals were formed as rapidly as possible. Access resistance decreased to 7–10 MΩ within 15 min of seal formation, and the experimental protocols were then begun. A 3 M KCl-agar bridge was used to ground the bath.

Action potentials and I_{Ca-L} were recorded under current and voltage clamp, respectively, using an Axopatch 200A amplifier (Axon; Union City, CA). Data were acquired at 10 kHz, and command pulses were generated by a Digidata 1200B (Axon) controlled by pCLAMP 7 (Axon). Before acquisition, the signals were low-pass filtered at 2 kHz (8-pole Bessel).

Contractility. Cell shortening in response to field stimulation, intracellular current injection, or depolarizing voltage steps was recorded with a video edge-tracking detector (VED-104, Crescent Electronics; Sandy, UT). Field stimulation was utilized for intact cells not undergoing perforated patch clamp, and 4- to 5-V pulses, 5 ms in duration, were applied at 0.2 Hz with a pair of Ag electrodes. When electrical activity and contraction were measured simultaneously under perforated patch conditions, either 70–95 pA, 2-ms depolarizing pulses were applied via the patch pipette to record action potentials or 300-ms depolarizations from −50 to 0 mV at 0.1 Hz were employed to record membrane currents. APD was measured at 90% repolarization (APD₉₀). To isolate I_{Ca-L}, the Na⁺ current was inactivated by holding at −50 mV, and 5 mmol/l 4-aminopyridine, 0.5 mmol/l BaCl₂, and 2 μmol/l doxifilon were added to block the transient outward K⁺ current (I_o), the inwardly rectifying K⁺ current (I_{K1}), and the rapidly activating component of the delayed rectifier K⁺ current (I_{Kd}), respectively. I_{Ca-L} was measured as the peak inward current relative to the current at the end of the 300-ms depolarization.

RESULTS

Effect of brief osmotic swelling on cell shortening. Figure 1 illustrates the effects of switching from an isosmotic 1T solution to a hyposmotic 0.6T solution for 6 min and then back to the 1T solution in an intact myocyte. Upon exposure to 0.6T, cell shortening (Fig. 1A) elicited by field stimulation rapidly increased from 6.1 to 9.5 μm, about a 55% enhancement of contractile performance that was maintained throughout the brief exposure to hyposmotic solution. The calculated relative cell volume (Fig. 1B), which was obtained at 1-min intervals, increased by 29% in 0.6T solution without evidence of compensatory volume regulation. The increase in cell volume resulted from a distinct increase in the width of the cell in 0.6T solution with little or no change in cell length, as previously reported (9). Augmentation of cell shortening already was obvious within the first minute of the osmotic challenge at a time when cell volume had increased by <12%. When switched back to 1T solution, cell volume and the extent of cell shortening returned to their initial values.
rapidly. Similar results were obtained in five cells. On average, relative cell volume increased by 29\% after 6 min in 0.6T. Both parameters rapidly returned to their initial values (dotted lines) on readmitting isosmotic (1T) solution.

Cell swelling decreased APD and slightly depolarized the resting membrane potential ($E_m$), and both effects were fully reversible (Fig. 2A). Simultaneously, cell shortening increased in 0.6T (Fig. 2B), as previously was shown when the myocyte was not undergoing dialysis through the patch pipette (Fig. 1B), and the time to the peak of shortening decreased (Fig. 2B). After 3 min of exposure to 0.6T, APD$_{90}$ decreased from 418.6 ± 16.1 to 370.3 ± 14.2 ms ($P < 0.01$), resting $E_m$ depolarized from −80.9 ± 1.3 to −79.2 ± 1.2 mV ($P < 0.05$), cell shortening increased by 28.4 ± 3.1\% ($P < 0.01$) from 3.2 ± 0.6 to 4.1 ± 0.7 μm, and time to peak of cell shortening decreased from 386 ± 31 to 322 ± 27 ms ($P < 0.01$) ($n = 6$ for each paired measurement).

The results indicated that osmotic swelling enhanced cardiac contractile performance at the time when the duration of the action potential was reduced.

Although the volume of rabbit ventricular myocytes rapidly swells and then remains constant during a prolonged hyposmotic challenge (7, 9, 35), the response of the processes responsible for E-C coupling may be more complex. To evaluate this possibility, myocytes were osmotically swollen for 20 min in 0.6T solution and then returned to 1T solution for 10 min. Figure 3 displays recordings of the action potential and cell shortening in a representative myocyte before and at selected times (1, 5, 8, and 20 min) after a hyposmotic challenge in 0.6T solution. Whereas almost all of the depolarization of resting $E_m$ occurred over 2–3 min, APD continued to decrease throughout the 20-min exposure to 0.6T (Fig. 3A). In contrast to the electrophysiological events, the response of cell shortening to 0.6T solution was biphasic (Fig. 3B). Initially, hyposmotic swelling increased the extent of cell shortening and reduced the time to the peak of shortening. At 5 min, for example, peak shortening was increased by 58\% relative to that in the 1T control, and a small aftercontraction was noted. By 8 min in 0.6T, however, cell shortening had begun to gradually diminish, and by 20 min, cell shortening was attenuated to 64\% of the control value.

Averaged data from seven cells that underwent a 20-min exposure to 0.6T solution and a 10-min recovery in 1T are shown in Fig. 4. Relative cell volume
increased by 30.0 ± 3.1% \((P < 0.01)\) within 2–3 min of switching to 0.6T, and cell volume remained constant in 0.6T solution thereafter. A depolarization of resting \(E_m\) of 3 mV, from \(-79.1 \pm 0.7\) to \(-76.1 \pm 0.6\) mV at 20 min \((P < 0.01)\), was associated with cell swelling in 0.6T, but comparison of the time course of cell swelling and membrane depolarization shows that the fall in resting \(E_m\) was completed ~2 min after cell swelling was complete. Both cell volume and resting \(E_m\) promptly returned to their control values when switched back to 1T solution.

In contrast to the simple behavior of cell volume and resting \(E_m\), the behavior of cell shortening (Fig. 4A) and APD\(_{90}\) (Fig. 4B) was more complex. APD\(_{90}\) gradually shortened in response to cell swelling from 458.3 ± 42.1 ms in 1T solution to 450.5 ± 39.4 ms (not significant), 382.4 ± 42.3 ms \((P < 0.01)\), and 328.1 ± 53.6 ms \((P < 0.01)\) after 4, 10, and 20 min, respectively, in 0.6T solution. On the other hand, cell shortening (Fig. 4A) initially increased by 31.4 ± 13.2% \((P < 0.01)\) at 4 min, a time when APD\(_{90}\) was essentially unchanged, but when cell volume and resting \(E_m\) already were at or near their steady-state values in 0.6T. With continued exposure to 0.6T solution, cell shortening decreased after 20 min to 68.1 ± 7.8% of its control value \((P < 0.01)\). Full recovery of cell shortening in 1T solution also was slow. The depression of cell shortening in 0.6T solution roughly paralleled the decrease in APD\(_{90}\). The parallel behavior suggests that the suppression of contractile performance after osmotic swelling might reflect decreased Ca\(^{2+}\) entry via \(I_{Ca-L}\). Alternatively, the slow decay of cell shortening in 0.6T might reflect the gradual decrease of Ca\(^{2+}\) stores in the SR. On the other hand, the observed changes in APD\(_{90}\), resting membrane potential, and cell volume do not suggest an obvious explanation for the initial enhancement of cell shortening upon osmotic swelling.

Fig. 4. Effects of prolonged osmotic swelling on action potential duration at 90% repolarization (APD\(_{90}\)), resting \(E_m\), and cell shortening. Simultaneous recordings in control in 1T and at selected times (1, 5, 8, and 20 min) after exposure to 0.6T. A: action potential duration continued to shorten throughout a 20-min exposure to 0.6T, whereas resting membrane potential \((E_m)\) reached a stable level in <5 min. B: swelling had a biphasic effect on myocyte shortening. Shortening increased by 8% after 1 min and 58% after 5 min, and then shortening gradually diminished, falling to 64% of control at 20 min. Both action potential duration and cell shortening fully recovered after 10 min in 1T (not shown).
Effects of osmotic swelling on I_{Ca,L} and the Ca^{2+} transient. One possibility is that the biphasic response of cell shortening to osmotic swelling results, at least in part, from a biphasic modulation of I_{Ca,L}. Such biphasic changes in I_{Ca,L} also might help explain the inconsistency of previous studies on swelling-induced changes in cardiac I_{Ca,L}. Under ruptured patch conditions (4, 22, 31, 44). To evaluate the effect of osmotic swelling on I_{Ca,L}, I_{Ca,L} was measured under perforated patch conditions with a 300-ms voltage step from -50 to 0 mV at 0.1 Hz. Figure 5 illustrates the time dependence of I_{Ca,L} upon switching from 1T to 0.6T solution for 10 min and during a 10-min recovery period in 1T solution. Paralleling the biphasic effects of osmotic swelling on cell shortening (Fig. 4A), I_{Ca,L} initially increased by 12% from -680 to -762 pA, reaching a maximum after ~2 min in 0.6T, a time when cell swelling was complete. Then, while the cell volume remained constant, I_{Ca,L} slowly declined to -465 pA, 68% of its control value. This slow decay of I_{Ca,L} in 0.6T was not due to rundown of the Ca^{2+} current. On returning to 1T solution, I_{Ca,L} slowly returned to its initial value and was -678 pA after 10 min of recovery. Similar results were obtained in five cells. On average, hypomotic swelling increased I_{Ca,L} by 9.6 ± 1.8% (P < 0.01) from -709.5 ± 37.2 to -772.2 ± 25.8 pA after 2.8 ± 0.3 min in 0.6T. The current then decreased by 28.5 ± 4.1% (P < 0.01) to -508.6 ± 37.3 pA after 10 min in 0.6T, and I_{Ca,L} partially recovered to -619.6 ± 26.6 pA, 87.4 ± 3.6% (P < 0.01) of its initial value in 1T. These data suggest that swelling-induced changes in I_{Ca,L} contribute to both the transient increase in contractile performance and the subsequent decline.

Figure 6 depicts the effect of osmotic swelling on the Ca^{2+} transient recorded with fura-2 as the f340/f380 ratio. These experiments were performed with the same voltage-clamp protocol applied in Fig. 5. As previously noted, both cell shortening and I_{Ca,L} were augmented after 3 min of cell swelling but were depressed at later times. The intracellular Ca^{2+} transient measured as the f340/f380 ratio was, however, slightly decreased at the same time that I_{Ca,L} and cell shortening were enhanced. In four cells, the fluorescence ratio decreased to 88.6 ± 2.7% of control (P < 0.01) after 3 min of osmotic swelling. At the same time point, shortening and I_{Ca,L} were increased by 29.5 ± 6.1% and 5.3 ± 0.9%, respectively (P < 0.01). After 8 min in 0.6T, the fluorescence ratio had fallen to 40.7 ± 4.6% of control (P < 0.01), whereas shortening and I_{Ca,L} decreased to 72.3 ± 6.2% and 75.5 ± 3.7% of control, respectively (P < 0.01).
DISCUSSION

Osmotic swelling of ventricular myocytes in 0.6T solution had a biphasic effect on contractile performance both in intact ventricular myocytes not subject to patch clamp and in myocytes under perforated patch conditions. Cell shortening initially was enhanced by 28 to 34% within 3–4 min, but at latter times cell shortening was reduced to about 70% of its control value. Similar results have been obtained for twitch tension in multicellular cardiac preparations (5, 16, 17) and more recently (4) for cell shortening in rat ventricular myocytes. The present data suggest that biphasic changes in ICa-L, as well as decreased APD and Ca2+ transients, contribute to the modulation of cell shortening by osmotic swelling. These same factors are expected to contribute to osmotic swelling-induced changes in force development in multicellular preparations (5, 16, 17), although cell shortening and force development are not identical measures of contractile performance.

Effect of swelling on ICa-L. The basis for the transient increase in twitch tension and cell shortening in response to osmotic swelling has been obscure. Brette et al. (4) recently postulated that the initial increase in cell shortening was due to an unspecified enhanced coupling between Ca2+ entry and Ca2+ release. We found, however, that the magnitude of ICa-L undergoes biphasic changes after osmotic swelling that parallel cell shortening. Initially ICa-L increased by 10% at about 3 min, but latter it decreased to 72% of control. The decline in ICa-L over time in these perforated patch experiments cannot be attributed to current rundown because ICa-L recovered nearly to its control value on returning to isosmotic conditions (Fig. 5B). Thus the observed transient increase in ICa-L is an alternative explanation for the transient increase in contractile performance.

Previous studies of the effect of osmotic swelling on cardiac ICa-L have given conflicting results. Brette et al. (4) reported a monotonic decrease in ICa-L, whereas Taouil et al. (36) and Matsuda et al. (22) found an increase and Groh et al. (13), Sasaki et al. (31), and Zhou et al. (44) detected no change. The reasons for the disparate reports on the effect of osmotic swelling on ICa-L remain uncertain, but the present results emphasize that the time point selected for measuring ICa-L is important. In addition, methodological issues such as perforated versus ruptured patch techniques and species differences may contribute to the inconsistent results in the literature. Although this apparently is the first report of a biphasic effect of osmotic swelling on cardiac ICa-L, a similar transient response was noted in pancreatic β-cells (10). Furthermore, it recently was reported that the T-type Ca2+ current is enhanced by osmotic swelling in guinea pig myocytes (27). The T-type Ca2+ current should not significantly contaminate the present measurements of ICa-L because the holding potential was set at −50 mV, which inactivates T-type Ca2+ channels.

Although the cell membrane is stretched by osmotic swelling, swelling often has distinct effects from axial mechanical stretch. Osmotic swelling dilutes the cytoplasm and stretches the membrane by increasing cell width and thickness, whereas there is little or no effect on length (9). In contrast to the present results, axial mechanical stretch does not appear to alter ICa-L (14, 31).

Other factors regulating cell shortening. Multiple factors regulate contractile function in cardiac muscle and are likely to play a role in the observed effects on cell shortening. Osmotic swelling modulates several membrane currents (34, 38, 42), and cell swelling induced a slowly developing shortening of APD90 from ~450 to 330 ms that may itself modulate contractile performance by affecting transmembrane Ca2+ fluxes. It is important to note, however, that APD was unaffected after 4 min in 0.6T at a time when cell volume changes were long since complete and the transient increase in cell shortening was observed (Fig. 4). The eventual reduction in APD is likely to be due to the slow activation of swelling-activated currents including ICi-swell (8, 11, 33) and the slow delayed rectifying K current (IK) (29, 44), as well as the depression of ICa-L shown here and by others (4).

Another important issue is the effect of osmotic swelling on SR Ca2+ stores. The reduction in Ca2+ influx via Ca2+ current and the shortening of APD observed here favor the depletion of SR Ca2+ stores over time. Consistent with this idea, caffeine-inducible SR Ca2+ release is depressed after 10 min of osmotic swelling (4). Moreover, osmotic swelling has been shown to cause a persistent reduction of intracellular Na+ and Ca2+ activity in multicellular ventricular and Purkinje fiber preparations (18). Reduction of Na+ by dilution (18) and stimulation of the Na+/K pump (3, 40) favors extrusion of Ca2+ by the Na+/Ca2+ exchanger. On the other hand, osmotic swelling directly inhibits Na+/Ca2+ exchange in isolated myocytes when ionic concentrations are maintained in the steady state by ruptured patch (43). Even under patch-clamp conditions, however, osmotic swelling must initially reduce intracellular Na+ in patch-clamped myocytes, because water movement is much more rapid (35) than dialysis of the cytoplasm by the patch pipette (21). Such a transient fall in intracellular Na+ is expected to result in extrusion of Ca2+ and contribute to the depletion of SR Ca2+ stores.

A surprising result was that the Ca2+ transient detected with fura 2 was slightly depressed after 3 min in 0.6T, whereas at the same time point ICa-L and cell shortening were enhanced and swelling was complete (Fig. 6). One interpretation is that osmotic swelling reduced the efficiency of the coupling between Ca2+ entry and Ca2+ release, a conclusion opposite to that reached for rat myocytes (4). Alternatively, this observation may simply reflect the larger cytoplasmic volume into which Ca2+ flows in osmotically swollen cells. The effects of swelling on fura 2 (see below), however, preclude rigorously distinguishing between these possibilities.
Comparison of the effect of swelling on the Ca$^{2+}$ transient and on cell shortening also suggest that osmotic swelling increases cell shortening at a given level of Ca$^{2+}$. Enhanced Ca$^{2+}$ sensitivity is likely to reflect, at least in part, the reduction in the ionic strength of the cytoplasm as water flows rapidly into the myocyte. An inverse relationship between ionic strength and tension development is well established in skinned cardiac (26) and skeletal muscle (12, 15) and has been attributed to decreased affinity of troponin-C for Ca$^{2+}$ at reduced ionic strength and several other mechanisms (1, 25, 32). A fall in cytoplasmic viscosity due to osmotic swelling also would favor enhanced shortening. On the other hand, if interfilament spacing increased as cell width increased during osmotic swelling, a decrease in myofilament Ca$^{2+}$ sensitivity would be expected (23, 39).

Decreased cytoplasmic ionic strength and viscosity may also confound interpretation of the fura 2 fluorescence ratio. Based on an increase in the $f_{340}/f_{380}$ ratio, osmotic swelling was claimed to initially increase the Ca$^{2+}$ transient in the rat ventricle (4). Reduction in ionic strength decreases the dissociation constant for Ca$^{2+}$ at reduced ionic strength and several other mechanisms (1, 25, 32). A fall in cytoplasmic viscosity due to osmotic swelling also would favor enhanced shortening. On the other hand, if interfilament spacing increased as cell width increased during osmotic swelling, a decrease in myofilament Ca$^{2+}$ sensitivity would be expected (23, 39).

Decreased cytoplasmic ionic strength and viscosity may also confound interpretation of the fura 2 fluorescence ratio. Based on an increase in the $f_{340}/f_{380}$ ratio, osmotic swelling was claimed to initially increase the Ca$^{2+}$ transient in the rat ventricle (4). Reduction in ionic strength decreases the dissociation constant for Ca$^{2+}$ (37, 41), however, and a reduction of viscosity preferentially suppresses fluorescence at longer wavelengths (28, 30). Both of these effects augment the $f_{340}/f_{380}$ ratio, suggesting an increase in Ca$^{2+}$ when none has occurred. To the contrary, we initially observed a small decrease in the $f_{340}/f_{380}$ ratio upon osmotic swelling, a finding that cannot be attributed to swelling-induced alterations in the properties of fura 2. Nevertheless, the effects of ionic strength and viscosity on fura 2 suggest that the initial decrease in the Ca$^{2+}$ transient is greater than would be calculated from the $f_{340}/f_{380}$ ratio based on an in situ calibration in isosmotic solutions. Comparisons between the $f_{340}/f_{380}$ ratio transients recorded at different times after osmotic swelling was complete should not be affected by these calibration issues.

Swelling in hypotonic solution is sometimes taken as a model for the swelling that occurs under pathological conditions, such as ischemia and reflow. An important distinction should be noted, however. Swelling after ischemia and reflow occurs as a result of a hyperosmotic intracellular milieu rather than a hypotonic extracellular environment. Because several components of contractile function are sensitive to ionic strength (1, 25, 26, 32), it is uncertain whether the present results apply to myocyte swelling in the setting of ischemia.

In summary, osmotic swelling caused a transient enhancement and then a depression of $I_{Ca-L}$ that paralleled the enhancement and depression of shortening of ventricular myocytes. APD and the Ca$^{2+}$ transient decreased monotonically over time and also contributed to the depression of contractile function after prolonged myocyte swelling.

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