

Cell Volume Regulation in Cardiac Myocytes: A Leaky Boat Gets a New Bilge Pump

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The fundamental precepts by which animal cells regulate their volume under isosmotic conditions have long been studied and are known, at least to a degree suitable for textbook exposition (for early references see Macknight and Leaf, 1977; Baumgarten and Feher, 2001). The usual starting point for accounts of this subject is the so-called Donnan equilibrium in which the cell membrane is permeant to water and small ions but impermeant to one or more ionic species, often of colloidal size (Boyle and Conway, 1941; Overbeek, 1956). With polyvalent intracellular macromolecules as the only impermeant species, a Donnan system does not establish a true equilibrium. To satisfy macroscopic electroneutrality, there is an equal accumulation of counter-ions and exclusion of co-ions on side of the membrane with the impermeant polyvalent macromolecules, and consequently, an osmotic pressure difference develops across the membrane that is equivalent to the osmotic pressure due to the impermeant macromolecules alone. This osmotic gradient draws water into the cell, dilutes the cellular contents, and engenders further swelling.

The solution to this conundrum for cell volume regulation was independently recognized by Wilson (1954) in studies on leukocytes and by Leaf (1956, 1959), who examined slices of renal and cerebral cortex and liver, and the same principles were applied to high and low K^+ erythrocytes by Tosteson and Hoffman (1960) in this Journal. In what is called the double-Donnan or pump-leak hypothesis, the stability of cellular volume is achieved because Na^+ , the primary extracellular permeant cation that leaks into cells, is extruded from the cell by active transport. This makes Na^+ effectively impermeant and balances the osmotic forces arising from intracellular macromolecules. Thus, recognition of the requirement for an active transport process to maintain cell volume predates the discovery of the Na^+-K^+ ATPase by Skou (1957). Maintenance of the steady-state requires osmotic equilibrium across the membrane, macroscopic electroneutrality, and zero net flux of permeant species. Analytical steady-state and time-dependent solutions to the set of resulting equations with reasonable parameters for membrane prop-

erties emphasize the interactions between cell volume, passive Na^+ , K^+ , and Cl^- fluxes, and active transport, and also, the importance of the Na^+-K^+ pump in cell volume regulation (Jakobsson, 1980).

It is now accepted, of course, that this classic explanation for cell volume regulation is incomplete. To fully account for cell volume regulation, one needs to consider the full panoply of ion channels and transporters, their voltage dependence, and their complex interaction in a quantitative model that is appropriate for the cells of interest. For cardiac muscle, a major step forward in this direction has been taken by Takeuchi et al. (2006) in an article appearing in this issue of the Journal (see p. 495).

Takeuchi and coworkers (2006) very effectively combine simulations with experiment. The simulations are based on a modification of the well-established Kyoto model for the ventricular action potential (Matsuoka et al., 2003)—extended to include regulation of the Cl^- gradient and cell volume, as well as mitochondrial function and Ca^{2+} homeostasis (also see Terashima et al., 2006). In addition to elegantly demonstrating the complex interactions between channels, transporters, ion concentrations, and membrane potential in the regulation of cardiac cell volume after Na^+-K^+ pump inhibition, the simulation makes some surprising predictions that the authors verified experimentally. Yes, a ventricular myocyte behaves like a leaky boat that takes on water when the Na^+-K^+ pump is inhibited, but regulation of the leak is multifaceted, and there is an unexpected set of bilge pumps working hard to keep the boat dry.

Because of its central role in Na^+ extrusion, one expects that Na^+-K^+ pump inhibition must lead to cell swelling, and the predicted swelling is observed in a variety of cells and tissues (Macknight and Leaf, 1977). Not all cells are so cooperative, however. Swelling of cardiac muscle is, at best, modest and slow after Na^+-K^+ pump inhibition. For example, Pine et al. (1979, 1980) reported an only 7% increase in cell water in canine ventricular slices after 2 h at 0–2°C, and they failed to detect significant increases in cell water in guinea pig atria and ventricle after 2- and 3-h exposures to 1 mM

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Abbreviation used in this paper: PMCA, plasma membrane Ca^{2+} ATPase.

ouabain. Ouabain also fails to alter cell water in rabbit papillary muscle after ~ 1 h (Caille et al., 1981). Much larger increases in cell water, $\sim 35\%$, were found in cat papillary muscle after prolonged exposure to ouabain or the cold (Page et al., 1964). Pine and coworkers argued that the low Na^+ permeability of quiescent cardiac muscle slowed cell swelling (Pine et al., 1980) and postulated that opposing hydrostatic forces could limit the extent of swelling in intact tissue (Pine et al., 1981). Similar results are obtained, however, in isolated rabbit ventricular myocytes using optical methods to measure cell volume. Again, exposure to ouabain (Drewnowska and Baumgarten, 1991) and cooling to 9°C (Drewnowska et al., 1991) failed to swell myocytes in physiological bathing solutions.

Given this background, it was most appropriate for Takeuchi et al. (2006) to use both simulations and experiments to ask: How can cardiac cells maintain their volume in the face of complete inhibition of the $\text{Na}^+\text{-K}^+$ pump? In experiments using the area of a myocyte's image as an index of cell volume and di-8-ANEPPS fluorescence to simultaneously measure E_m , Takeuchi et al. (2006) found that guinea pig ventricular myocytes consistently resisted swelling during a 90-min exposure to $40 \mu\text{M}$ ouabain (which blocks 98.5% of the pump sites), confirming previous studies, even though the cells depolarized to -40 mV at varying times after pump inhibition. Simulations showed that increasing either the background Na^+ or Cl^- conductance augmented cell swelling, but even with fivefold increases, prolonged $\text{Na}^+\text{-K}^+$ pump blockade only produced an $\sim 5\%$ increase in cell volume. Thus, a low Na^+ or Cl^- conductance apparently cannot fully account for the resistance to swelling. On the other hand, cell-to-cell variability in the Na^+ leak could explain the inconsistent time to depolarization.

Simulations also revealed that another active transport system, the plasma membrane Ca^{2+} ATPase (PMCA), steps into the breach when the $\text{Na}^+\text{-K}^+$ pump is inhibited. By keeping $[\text{Ca}^{2+}]_i$ relative low and the equilibrium potential for the $\text{Na}^+\text{-Ca}^{2+}$ exchanger negative to E_m , the PMCA maintains the Ca^{2+} gradient necessary to power reverse-mode $\text{Na}^+\text{-Ca}^{2+}$ exchange, extruding Na^+ from the cell and thereby helping to maintain cell volume and E_m . In the absence of the PMCA, the model predicts marked Ca^{2+} accumulation when E_m depolarizes sufficiently to activate the L-type Ca^{2+} channel window current, increased Na^+ influx via the Ca^{2+} -dependent background leak current, and greater cell swelling. Thus, the net effect of the PMCA and $\text{Na}^+\text{-Ca}^{2+}$ exchanger working in concert is to extrude three Na^+ ions for each ATP hydrolyzed; this represents a greater osmotic (volume regulatory) efficiency than that of the $\text{Na}^+\text{-K}^+$ pump.

Importantly, Takeuchi et al. (2006) went on to experimentally verify that the PMCA and $\text{Na}^+\text{-Ca}^{2+}$ exchanger

can maintain cell volume in cardiac myocytes. Blockade of $\text{Na}^+\text{-Ca}^{2+}$ exchange with either SEA0400 or KB-R7943 induced significant swelling in ouabain-treated myocytes. Takeuchi et al. also verified experimentally the predicted effects of increased Cl^- conductance in the face of $\text{Na}^+\text{-K}^+$ pump blockade by activating CFTR Cl^- currents with isoproterenol. Increasing Cl^- conductance induces swelling that is triggered when E_m depolarizes beyond E_{Cl} . At that potential, the depolarization terminates passive Cl^- efflux with K^+ as a counter-ion (to maintain macroscopic electroneutrality) and initiates Cl^- influx accompanied by Na^+ . Rather unexpectedly, the model also predicts a special role for L-type Ca^{2+} channels under these conditions. The Ca^{2+} current both accelerates depolarization and raises free $[\text{Ca}^{2+}]_i$ sufficiently to augment Na^+ influx via the Ca^{2+} -dependent leak pathway. Consistent with this prediction, blocking Ca^{2+} channels with nifedipine strongly suppresses myocyte swelling in the presence of isoproterenol and ouabain.

To understand the complex interplay between the factors that regulate cell volume clearly requires a detailed simulation, and the modified Kyoto model used here is admirably up to the task. Nevertheless, as with all models, one can identify potential shortcomings. Whereas the volume dependence of the swelling-activated Cl^- current is specifically simulated, the model does not consider the volume sensitivity displayed by several voltage-dependent channels and transporters in cardiac muscle (Baumgarten, 2005). Moreover, numerous signaling cascades that modulate ion channels and transporters are also volume or Ca^{2+} sensitive (Hoffmann and Dunham, 1995; Baumgarten and Feher, 2001; Cohen, 2005). Sufficient information to fully incorporate these effects into a cardiac cell model is not available, however.

The prediction and experimental confirmation that the PMCA, working in concert with the $\text{Na}^+\text{-Ca}^{2+}$ exchanger, can regulate cardiac cell volume is a surprise. Heretofore, the PMCA has been relegated to the role of minor partner to the $\text{Na}^+\text{-Ca}^{2+}$ exchanger in extruding Ca^{2+} that enters the myocyte with each heartbeat. That said, this mechanism has a rather prominent role in the history of volume regulation in mature erythrocytes from carnivores (e.g., dog, cats, ferrets, bears), which lack $\text{Na}^+\text{-K}^+$ pumps (Parker, 1973, 1992; Parker et al., 1975). Carnivore erythrocytes have intracellular Na^+ and K^+ concentrations that are close to their extracellular values, and their volume is regulated by the PMCA working in parallel with $\text{Na}^+\text{-Ca}^{2+}$ exchange, as now also demonstrated for cardiac myocytes. Interestingly, PMCA is activated by direct binding of calmodulin to the enzyme (Carafoli, 1991), suggesting that Ca^{2+} may ultimately augment the myocyte's ability to defend its volume after inhibition of the $\text{Na}^+\text{-K}^+$ pump.

Although the new findings reported by Takeuchi et al. (2006) are important in their own right as an

illustration of basic mechanisms, the physiological and pathophysiological implications for cardiac function are yet to be established. It would be particularly interesting to use the present model to explore myocyte volume regulation during tachycardia and with altered Ca^{2+} homeostasis and other sequelae of β_1 -adrenergic stimulation. One also wonders how volume regulation changes as a larger and larger fraction of the $\text{Na}^+\text{-K}^+$ pump sites are inhibited, and whether there are conditions besides complete inhibition of the $\text{Na}^+\text{-K}^+$ pump in which the myocyte turns to the PMCA and $\text{Na}^+\text{-Ca}^{2+}$ exchange to help maintain cell volume. There are, of course, few situations in which the $\text{Na}^+\text{-K}^+$ pump is fully suppressed and the myocytes quiescent, as assumed in the simulation. One clinical setting that might be illuminated by a similar analysis is cold, high potassium cardioplegia, which is used to arrest the heart during open heart surgery. Cardioplegia can lead to both cellular and tissue edema. Studies on isolated rabbit and human myocytes have shown that myocyte volume in cold, high potassium solution is sensitive to the $\text{K}^+ \times \text{Cl}^-$ product, as expected for a Donnan system (Drewnowska et al., 1991; Shaffer et al., 1998). Osmotic swelling is also an important component of ischemia/reperfusion injury (Whalen et al., 1974). These situations are more complex setting than those presently considered, but it seems likely that simulations, such as those reported here, eventually will be needed to achieve a full understanding of multiple aspects of this clinical problem.

As for the present, it is time once again to wonder at the incredible resiliency of a physiological system.

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