Cell swelling and ion transport pathways in cardiac myocytes

Jamie I. Vandenberg *, Siân A. Rees, Anthony R. Wright, Trevor Powell

University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, UK

Received 13 July 1995; accepted 1 February 1996

Keywords: Myocardial ischemia; Ion channels; Cell volume regulation; Stretch; Membrane potential

1. Overview

Cell swelling causes stretch and/or deformation of cell membranes and the underlying cytoskeletal network as well as dilution of intracellular contents [1]. It is therefore not surprising that most mammalian cells, including cardiac myocytes, respond to swelling by modulating transporters and/or ion channels that permit efflux of intracellular osmolytes (and osmotically obliged water) which will tend to restore cell volume to its original value [2–5]. This is of particular significance in the heart as many of the transport pathways modulated by cell swelling are electronegative, so their modulation will alter excitability of the heart. This is likely to be most important in the context of myocardial ischaemia and reperfusion as this is when cell swelling is most significant [6] and when arrhythmias are most common [7]. Recent work, using a range of techniques including patch-clamp analysis of isolated cardiac myocytes, is beginning to unravel the mechanisms underlying osmotic modulation of electrical activity in the heart and it is this work that will be the primary focus of this review.

2. Background

It has been known for over 20 years that altering the osmolality of perfusion solutions alters the electrical properties of cardiac cells. Akiyama and Fozzard [8] found that superfusion of ventricular papillary muscles with a 58% hypertonic solution (1.58 T) caused hyperpolarisation of the resting membrane potential by 4.6 mV whereas a 76% hypotonic solution (0.76 T) caused a rapid depolarisation of 5.7 to 7.6 mV (depending on the external potassium concentration) which was stable for up to 1 h. Similar results have been reported by other groups [9,10] and more recently these findings have been confirmed in isolated myocytes from chick embryo [11], rabbit [12] and guinea-pig [13].

Many workers over the last 25 years have also found that swelling or shrinking cardiac myocytes alters action potential duration (APD). For example, Hermsmeyer et al. [14] found that cell shrinkage, induced by 2.0 T hypertonic solutions, caused a species dependent shortening of APD. In those species containing a T-tubule system (guinea-pig and cat ventricle) there was a marked loss of the action potential plateau whereas in those cells lacking a T-tubule system (chicken and frog ventricle) there was no significant shortening of APD. Unfortunately, they did not investigate the effects of cell swelling but these results are of particular interest as they indicate that cell geometry may influence the response of cardiac cells to anisomolar solutions. Kawata et al. [15] noted that cell shrinkage and cell swelling were both associated with APD shortening in bull frog ventricle and similar findings have been reported in guinea-pig ventricle [10].

Many ion conductances contribute to cardiac myocyte action potentials [16,17]. Identifying which conductances contribute to the changes in the action potential during cell swelling, however, is very difficult, especially in intact tissues. Consequently, a number of laboratories, including our own, have started to investigate the effects of cell swelling in isolated myocytes where it is a little easier to dissect out the contributions of different channels and transporters. Nevertheless, as there are so few specific blockers we must still be careful about interpreting the
results of single cell studies. But before discussing the results of these studies it is worth pausing to consider what physiological and/or pathophysiological conditions are likely to result in swelling of cardiac myocytes.

3. Swelling of cardiac myocytes

The osmolality of body fluids is normally very tightly regulated [2]. It is therefore unlikely that cardiac cells will ever be exposed to significant osmotic gradients under physiological conditions. Over-vigorous intravenous fluid replacement with dextrose solutions or excessive water ingestion could cause a transient decrease in plasma osmolality and consequently cell swelling. This, however, is likely to be of major significance only in patients with impaired renal, hepatic or cardiac function.

The osmolality of cardiac tissue increases during myocardial ischaemia [18] due to the breakdown of high energy phosphates and macromolecules (e.g., glycogen, free fatty acids). Theoretical calculations by Jennings and colleagues [6] have shown that the amount of metabolites produced during ischaemia could cause the osmolality of heart cells to increase by as much as 130 mosmol kg\(^{-1}\). These calculations were based on the assumption that all metabolites are retained in the intracellular space. Many of the metabolic products, however, will equilibrate between the intracellular and extracellular spaces, either because they are membrane permeable or as in the case of lactate they are rapidly transported across the cell membrane via specific transporters [19]. The actual increase in osmolality may therefore be considerably less than 130 mosmol kg\(^{-1}\). Thus, e.g., Tranum-Jensen et al. [18] who measured tissue osmolality directly found that after 50 min regional ischaemia osmolality had increased by only 40 mosmol kg\(^{-1}\) (320 mosmol kg\(^{-1}\) in the non-ischaemic zone compared to 360 mosmol kg\(^{-1}\) in the ischaemic zone). The critical question, however, that needs to be addressed is, to what extent is this increase in tissue osmolality translated into swelling of cardiac myocytes.

There are many methodological problems that make it difficult to measure and/or estimate cell volumes during ischaemia, including (1) lack of flow in the ischaemic zone will reduce the rate of equilibration of space markers in the ischaemic area, (2) during regional ischaemia collateral flow may alter the distribution of space markers, (3) changes seen in the thin sections used in electron microscopy are not easily translated into changes in volume, and (4) the osmolality of solutions used to fix tissues in preparation for electron microscopy may not be the same as the osmolality of the tissue specimens and so may alter the volume of the myocytes independent of any ischaemia-induced changes. For example, Kloner et al. [20] found that 40 min regional ischaemia in the canine heart resulted in a minimal increase in cell volumes in papillary muscle cells (although the increase was not quantified) when hearts were fixed with Tyrode + 1% glutaraldehyde. The osmolality of Tyrode + 1% glutaraldehyde (~400 mosmol kg\(^{-1}\)) may however have been hypertonic relative to the ischaemic tissue and so have masked any increase in cell volume. In a subsequent and more careful study, Tranum-Jensen et al. [18] noted that cell volumes in ischaemia.

Fig. 1. Cell swelling in cardiac myocytes during ischaemia and reperfusion. (a) The osmotic pressure in cardiac tissue increases during ischaemia secondary to the accumulation of metabolites including creatine (Cr), phosphate (P\(_{i}\)), adenine nucleotides, acyl-CoA and lactate. The initial production of Cr and P\(_{i}\), both relatively membrane-impermeant metabolites, will cause myocytes to swell during early ischaemia. The major metabolite produced during ischaemia, however, is lactate which will equilibrate between intracellular and extracellular spaces and so will not contribute to cell swelling during ischaemia but will make the major contribution to the increased tissue osmolality which has been estimated to reach 360 mosmol kg\(^{-1}\) [18] (see text for details). (b) The most significant osmotic gradient, and therefore cell swelling, will occur following reperfusion when the hypertonic extracellular fluid is washed out by normotonic blood (~290 mosmol kg\(^{-1}\)). During the first few minutes of reperfusion the osmotic load will be largely eliminated by the resynthesis of phosphocreatine (PCr) and the washout of lactate. Resynthesis of ATP and replenishment of glycogen and triglyceride stores will occur more slowly [94].
Jensen et al. showed a 16.5% increase in tissue water after 40 min regional ischaemia and fixed in "normotonic fixative" were increased by only ~ 8% compared to pre-ischaemic values. Studies that have looked at changes in cell water during ischaemia have also reported variable results. Whalen et al. [21] found no significant increase in cell water content in the ischaemic tissue during 40 min regional ischaemia in the canine heart. Conversely, Tranum-Jensen et al. showed a 16.5% increase in tissue water (estimated from changes in wet weight/dry weight ratio) although to what extent this increase represented an increase in extracellular or intracellular water was not determined and Powers et al. [22] found a 21% increase in intracellular water after 45 min low-flow ischaemia in the canine heart.

In contrast to the variable and at most modest increases in cell volume during ischaemia [18,20,23] many workers have noted an "explosive increase in cell volume" following reperfusion [24]. Reperfusion of the ischaemic myocardium with "isotonic" extracellular solution will wash out the hypertonic extracellular solution (whilst the intracellular solution will initially remain hypertonic) thereby creating a significant osmotic gradient between the intracellular and extracellular spaces (see Fig. 1). This reperfusion-induced cell swelling is also often associated with blebbing and rupture of cardiac sarcolemmal membranes [23,25]. Thus, it is perhaps not surprising that many workers have noted that reperfusing hearts with a hypertonic medium resulted in better recovery of cardiac function [18,22,24,76].

In summary, the difficulties in measuring cell volumes and osmolalities during ischaemia coupled to the heterogeneity of ischaemic insults make it difficult to estimate the precise changes in cell volume that will occur during any given ischaemic insult. The most carefully controlled study of ischaemia is probably that of Tranum-Jensen et al. [18], who estimated myocyte volume to increase by ~ 8% and tissue osmolality by ~ 12.5%. There has not been a similarly well controlled study of reperfusion; however, all workers to date have reported significant increases in cell volume following reperfusion of the ischaemic myocardium with "isotonic" extracellular solution.

4. Cardiac myocytes are not "perfect osmometers"

Exposure of cells to hypotonic solutions results in cell swelling, where the amount of swelling depends on the extent to which there is free exchange of water between the intracellular and extracellular spaces, and whether there are changes in membrane permeability for osmolytes following cell swelling. For example, an ideal cell exposed to a 0.8 T hypotonic solution will increase in volume by approximately 25%, i.e. so that the intracellular osmolality would be reduced to match the osmolality of the extracellular fluid. Real cells, and cardiac myocytes in particular [27], however, deviate from this ideality. For example, Pine et al. [27] found that the volume of myocytes in rat ventricular slices exposed to a 0.4 T hypotonic solution increased by only ~ 30% whereas the volume of cells in renal cortical slices exposed to the same 0.4 T hypotonic solution increased by ~ 120%. This non-ideal behaviour implies that there are forces resisting swelling. Unfortunately there have been no studies to date that have directly measured intracellular osmolalities either under control conditions or following exposure to anisosmotic solutions, so it is not possible to calculate to what extent the non-ideal behaviour is due to resistance to cell swelling mediated by stress borne by the cytoskeleton and to what extent it is mediated by non-ideal osmotic characteristics of the cytoplasm.

The cytoskeleton is thought to be an important component of the resistance to cell swelling in non-muscle cell types. For example, Wan et al. [28] have shown that the median survival of molluscan neurons exposed to distilled water was reduced from > 60 min to 18 min when myosin ATPase was inhibited indicating that a dynamic actomyosin dependent process, which they suggested was related to the cytoskeleton, contributes to the mechanical robustness of these cells. There are no studies to date that have directly looked at to what extent the cytoskeleton is responsible for the non-ideal behaviour in cardiac myocytes. Indirect evidence though comes from the study of Steenbergen et al. [23]. They showed that cell swelling, plasma membrane bleb formation and focal sarcolemmal disruptions during myocardial ischaemia were associated with changes in the distribution of vinculin, a cytoskeletal protein thought to be involved in tethering the sarcolemma at the Z-line of the underlying myofibrils. In addition to cytoskeletal elements, in intact tissue one must also consider the space constraints imposed by the relatively inelastic extracellular matrix [29,27] and pericardium. Whilst there is no direct evidence for a role played by the extracellular matrix, the findings in multiple studies that for a given osmotic perturbation isolated myocytes swell to a greater extent than do myocytes in intact tissue, i.e. 50–60% of predicted for isolated myocytes [4,30–32] compared to 25–30% for cells in situ [27] is consistent with the extracellular matrix being important.

The second factor that may limit cell swelling is activation of solute channels and/or transporters leading to a regulatory volume decrease (RVD). Classical RVD responses have been observed in chick embryo ventricular myocytes [11,33,34] and these responses are mediated in part by organic solutes and in part by inorganic solutes such as chloride [33]. Suleymanian and Baumgarten have
recently shown that rabbit ventricular myocytes exposed to 0.5 T hypotonic solutions also undergo RVD responses [35]. These responses, however, were relatively small and slow compared to those observed in chick embryo cardiac myocytes (and in non-cardiac cell types), which Subczynski and Baumgarten showed was due to adult cardiac sarcolemma having a very low hydraulic conductivity ($1.2 \times 10^{-10}$ litre N$^{-1}$ s$^{-1}$, which is $15$-$25$ times lower than those observed in red cell membranes and comparable to those observed in protein-free phospholipid bilayers). In an elegant series of studies Baumgarten's group have also demonstrated that bumetanide, chlorothiazide and anthrace-9-carboxylic acid sensitive pathways (Na-K-2Cl co-transporters, Na-Cl co-transporters and Cl channels respectively) contribute to the less than expected increase in cardiac cell volume [4,30,5]. Cell volumes in these studies were estimated from cross-sectional images (rather than three-dimensional images) of cells recorded using light microscopy in combination with video recording. In preliminary experiments, they showed that during cell swelling myocyte height varied linearly with myocyte width (height was estimated by placing small reflective alumina beads on the top of a myocyte and on the floor of the perfusion chamber and measuring the distance between the planes of focus for the beads on the floor of the chamber and on the top of the myocyte). However, in an earlier study, Isenberg and Klockner [36] noted that this technique for estimating cell heights can be associated with errors of as much as 25%; thus we must be cautious when interpreting the quantitative data from these studies [4,5,30]. Ideally, these experiments need to be repeated using a more robust technique such as confocal microscopy which can measure volumes directly [37].

Most workers who have studied the electrical effects of cell swelling in cardiac myocytes have used conventional whole-cell patch-clamped [38] myocytes. This adds another complication to estimating volume changes for a given change in extracellular osmolality as the cytosol is in continuity with a patch pipette whose contents will be dialysing the cell and whose osmolality will influence that of the cytosolic compartment. The extent of this problem will be determined by the access resistance for water and solute flow between the pipette and cytosol relative to the rates of solute and water flow across the rest of the cell membrane [39]. These variables will change from one experiment to the next and are difficult to estimate. The point though is worth remembering when trying to compare studies in intact cells with those carried out in whole-cell patch-clamped cells.

As well as the volume of cardiac myocytes not increasing as much as predicted for a given osmotic gradient, the swelling that occurs is not uniform. That is, there is a much more significant increase in the radial dimensions of myocytes compared to the longitudinal dimension [4,31,32,40]. Part of this can be explained by the shape of cardiac myocytes. Application of a uniform pressure gradient between the inside and outside of a right circular cylinder with closed ends (an approximation to the shape of cardiac myocytes) that had perfectly symmetrical elastic properties will give rise to a circumferential tension that is twice as great as the longitudinal tension (see discussion in [4] and references therein). Even taking this into account,

![Diagram of cell swelling](image-url)
however, there still appears to be a significantly smaller increase in length compared to the increase in radial dimensions. For example, exposure of cells to a 0.6 T hypotonic solution resulted in the width increasing to 118% of control whereas length increased to only 104% of control [4]. Part of this discrepancy may be related to the fact that cardiac myocytes are not uniform cylinders and the cell membrane and cytoskeleton do not have uniform elastic properties [41]. Indeed, the change in tensions described above for a cylindrical-shaped cell is likely to be a poor approximation of the changes in tension occurring in the membranes lining the T-tubules of cardiac myocytes. There are no studies that have directly looked at the effect of anisosmotic solutions on T-tubule geometry, but it is thought that the T-tubules shrink during exposure to hypotonic solutions, and swell following exposure to hypertonic solutions [14]. These effects of geometry are likely to be important as there is an asymmetric distribution of ion channels and transporters in cardiac cells (e.g. Na–Ca exchangers are predominantly located in the T-tubules whereas K⁺ channels are located throughout the sarcolemma and T-tubule system [42]).

5. Electrical effects of swelling in isolated cardiac myocytes

Osmotically-modulated channels and transporters in cardiac cells can be divided into two groups: those that are normally quiescent under isosmotic conditions but are activated when there is an osmotic stress imposed across the cell membrane, and those which are normally active but whose activity is altered by cell swelling. The former group includes chloride channels (I_{Cl,swell}), non-selective cation channels (I_{cat,non select}) and ATP-sensitive potassium channels (I_{K,ATP}). The latter includes delayed-rectifier potassium channels (I_{K}), Na–K ATPases (I_{Na,K}) and Na–Ca exchangers (I_{Na,Ca}) (see Fig. 2).

5.1. Chloride channels

I_{Cl,swell} has been identified in a wide range of cardiac cell types (see Table 1). This conductance has rapid activation and deactivation kinetics, is outwardly rectifying, has an anion permeability sequence of I⁻ > NO₃⁻ > Br⁻ > Cl⁻.

<table>
<thead>
<tr>
<th>Channels</th>
<th>Changed observed</th>
<th>Species/cell type</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>I_{Cl,swell}</td>
<td>Activated following 1–3 min delay.</td>
<td>Current density ~ 2–10 pA/pF in ventricular myocytes, 10–20 pA/pF in atrial myocytes and up to 100 pA/pF in embryonic cells.</td>
<td></td>
</tr>
<tr>
<td>I_{K}</td>
<td>~ 60% increase (plateau after ~ 1 min) in 0.7 T solution</td>
<td>Guinea-pig (ventricle)</td>
<td>[76,77]</td>
</tr>
<tr>
<td>I_{K,ATP}</td>
<td>Activated following 1–3 min delay.</td>
<td>Neonatal rat (atrium)</td>
<td>[72]</td>
</tr>
<tr>
<td>I_{Kl}</td>
<td>No change</td>
<td>Guinea-pig (ventricle)</td>
<td>[76,79]</td>
</tr>
<tr>
<td>I_{cat,non select}</td>
<td>Activation of 36pS channels (NP₀ increase in proportion to cell volume)</td>
<td>Neonatal rat (atrium)</td>
<td>[70]</td>
</tr>
<tr>
<td>I_{Ca}</td>
<td>No change</td>
<td>Guinea-pig (ventricle)</td>
<td>[76]</td>
</tr>
<tr>
<td>Electrogenic transporters</td>
<td>Increased (higher affinity for Na⁺)</td>
<td>Rabbit (ventricle)</td>
<td>[12]</td>
</tr>
<tr>
<td>I_{Na,K}</td>
<td>Decreased 7.4% for each 10% decrease in external osmolality (stabilises after 1–2 min)</td>
<td>Guinea-pig (ventricle)</td>
<td>[84]</td>
</tr>
<tr>
<td>I_{Na,Ca}</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1

Effects of cell swelling on ion channels and electrogenic transporters in cardiac myocytes

<table>
<thead>
<tr>
<th>Channels</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>I_{Cl,swell}</td>
<td>[44,45] a</td>
</tr>
<tr>
<td>I_{cat,non select}</td>
<td>[43] a</td>
</tr>
<tr>
<td>I_{K,ATP}</td>
<td>[40,77] a</td>
</tr>
<tr>
<td>I_{Kl}</td>
<td>[51,91,92] a</td>
</tr>
<tr>
<td>I_{cat,non select}</td>
<td>[69,93] a</td>
</tr>
<tr>
<td>I_{Ca}</td>
<td>[71] a</td>
</tr>
<tr>
<td>I_{Na,K}</td>
<td>[12] a</td>
</tr>
<tr>
<td>I_{Na,Ca}</td>
<td>[77] a</td>
</tr>
<tr>
<td>I_{K}</td>
<td>[76] a</td>
</tr>
</tbody>
</table>

a Leem and Earm (unpublished data).
b Vandenberg and Rees (unpublished data).
to inhibition by stilbene derivatives such as DIDS, carboxylic-acid derivatives such as 9-anthracene carboxylic acid, 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB), indanyloxyacetic acid, and tamoxifen [11,40,43–46].

One of the most characteristic features of $I_{\text{Cl,swell}}$ (both in cardiac and non-cardiac cells) is its delayed onset of activation, i.e., the channels usually do not open until 1–3 min after cells are exposed to hypotonic extracellular solutions [11,40,43,45], although when the osmotic gradient is small there can be a delay of up to 20 min before the current is activated [44]. The activation of $I_{\text{Cl,swell}}$ clearly occurs some time after cells have commenced swelling (for example, see Fig. 1 in [40]). In contrast to the delayed activation, the conductance decreases quite rapidly following return to hypotonic solutions. If activation of $I_{\text{Cl,swell}}$ is coupled to stresses in cytoskeletal elements then the time delay in the swelling response may reflect slack in the force bearing components of the cytoskeleton whereas following return to the isosmotic solution the slack has already been removed and so the response will be fast; this, however, remains to be determined.

The size of the $I_{\text{Cl,swell}}$ current activated in different cardiac cell types varies from ~2–10 pA/pF for ventricular myocytes, ~10–20 pA/pF for atrial myocytes, up to 100 pA/pF for embryonic ventricular myocytes (measured at +100 mV relative to the reversal potential for chloride, with $[\text{Cl}^-]_i$ of 70–140 mM, see Table 1). This variation presumably reflects differences in the density of ion channel expression, with in general a higher current density elicited in atrial myocytes compared to ventricular myocytes [40]. Another possible explanation for the difference between atrial and ventricular myocytes is differing resistances to cell swelling: as it has been shown that $I_{\text{Cl,swell}}$ could be elicited in canine atrial myocytes exposed to a 0.75 T hypotonic solution [44], but $I_{\text{Cl,swell}}$ could only be elicited in canine ventricular myocytes exposed to greater osmotic gradients (e.g., exposure of cells to a 1.67 T hypertonic pipette solution for 10–20 min [45]). One possibility is that the lack of T-tubules in atrial myocytes could account for this apparent difference in resistance to cell swelling which would be consistent with the suggestion by Hermansen et al. [14] that the lack of T-tubules in atrial myocytes may account for the different electrical response of atrial and ventricular myocytes to anisosmotic solutions [14]. However, it is important to remember that the pressure gradient across the cell membrane will depend not only on the difference in osmotic strength of the internal and external solutions but also the hydrostatic pressure gradient of the pipette solution, the access resistance between the pipette and the cell and the rate of fluid flow across the cell membrane relative to flow between the pipette and cytosol [39,47]. These latter factors will almost certainly vary from cell to cell and between atrial and ventricular myocytes. This variability between cells may also explain why Tseng found that $I_{\text{Cl,swell}}$ could be elicited in only 71% of canine ventricular myocytes [45], and Vandenberg et al. found $I_{\text{Cl,swell}}$ in only 34% of guinea-pig ventricular myocytes compared with 94% of guinea-pig atrial myocytes [40]. However, one must also consider the possibility that there is a subset distribution of $I_{\text{Cl,swell}}$ channels (e.g. it may be present in higher density in epicardial or endocardial myocytes).

The signal that activates $I_{\text{Cl,swell}}$ following cell swelling appears to involve the membrane and/or cytoskeleton. Tseng [45] found that dipryridamole and trinitrophenol (anionic amphipaths which are preferentially inserted into the outer leaflet of the membrane) activated a conductance consistent with $I_{\text{Cl,swell}}$, whereas chlorpromazine (a cationic amphipath which is preferentially inserted into the inner leaflet of the cell membrane) decreased $I_{\text{Cl,swell}}$ in canine ventricular myocytes. These results are consistent with the membrane being involved in transducing the swelling signal, however, they do not tell us what the specific stimulus is. The stimulus may be a change in tension experienced by the bilayer (or underlying cytoskeleton) or altered membrane curvature. Alternatively, the amphipaths may have a non-specific effect on lipid-protein interactions in the membrane. Tseng [45] also suggested that the cytoskeleton may be involved as, in 3 out of 5 canine ventricular myocytes tested, dihydrocytochalasin (an actin destabilising agent) appeared to activate $I_{\text{Cl,swell}}$ with no effect discernible in the remaining cells. Ideally, it would be useful to see these studies repeated on larger numbers of cells and on different species and cardiac cell types to determine how significant these findings are.

Tseng [45] also found that non-specific kinase inhibitors were ineffective in blocking activation of $I_{\text{Cl,swell}}$. This result suggests that phosphorylation was not involved in activation of $I_{\text{Cl,swell}}$. However, more recent studies with more specific kinase inhibitors and activators suggest that phosphorylation may be involved. Duan et al. [48] found that stimulation of PKC (via α-adrenergic stimulation) in rabbit atrial myocytes inhibited activation of $I_{\text{Cl,swell}}$ (similar to that reported in other cell types, e.g. in epithelial cells [49]) and Lieberman and colleagues found that increases in cAMP inhibited activation of $I_{\text{Cl,swell}}$ in chick embryo ventricular myocytes [11,50] whereas Sorota found that in canine atrial myocytes [44] and human atrial and ventricular myocytes [51] a rise in cAMP augmented a previously activated $I_{\text{Cl,swell}}$. Sorota [52] has also reported that inhibition of tyrosine kinases, using genistein, prevents activation of $I_{\text{Cl,swell}}$ in canine atrial myocytes, but this inhibition could be overcome if $I_{\text{Cl,swell}}$ had been previously activated in the presence of ATPyS. Together, these results suggest that phosphorylation is involved in transducing the swelling signal into activation of $I_{\text{Cl,swell}}$ in cardiac myocytes, although the specific pathways involved may be different in different species and/or cell types.

There is considerable evidence for $[\text{Ca}^{2+}]_i$ being an important regulator of volume-sensitive channels in many
cell types [53] including cardiac myocytes [54]. Hall et al. [55] have also shown that Ca\(^{2+}\) was required for activation of I_{Cl,swell} in chick embryo ventricular myocytes. By contrast, studies in mammalian myocytes have found that Ca\(^{2+}\) is not a pre-requisite for activation of I_{Cl,swell}, in that the conductance can be elicited in cells dialysed with nominally Ca\(^{2+}\)-free solutions containing 10 mM EGTA [40,43–45]. Furthermore, in the only study to date that has looked at the effects of osmotic swelling on [Ca\(^{2+}\)], in mammalian cells, Lado et al. found a decrease in [Ca\(^{2+}\)], with cell swelling. This decrease was thought to be secondary to the dilutional decrease in [Na\(^{+}\)] [56].

Following cell swelling, most cells activate solute efflux pathways that tend to restore cell volume to the original value, a phenomenon termed regulatory volume decrease (RVD). It has been suggested that the physiological role of I_{Cl,swell} in cardiac myocytes is to contribute to RVD. RVD is inhibited by replacement of chloride with methanesulfonate in chick embryo ventricular myocytes [11,33], and 9-AC, an inhibitor of I_{Cl,swell}, augmented the cell volume increase of rabbit ventricular myocytes placed in hyposmotic solutions [57]. The inhibition observed in these experiments, however, was incomplete. It has been speculated for many years that taurine may be involved in volume regulation in cardiac myocytes [58,59].

Recently, Lieberman’s group has shown that efflux of taurine and amino acids contributes to RVD in chick embryo ventricular myocytes [33] and furthermore that the time course of volume regulatory release of taurine from chick embryo ventricular myocytes is very similar to the time course of activation of I_{Cl,swell} and that both processes are inhibited by an increase in intracellular cAMP [60]. I_{Cl,swell} appears to be permeable to a wide range of organic osmolytes including taurine [61–64], and so it is possible that the volume regulatory efflux of taurine observed by Lieberman and colleagues could be mediated via I_{Cl,swell} channels. Thus there are likely to be multiple components of the RVD response including chloride efflux, presumably via I_{Cl,swell} channels, as well as the efflux of taurine and other amino acids, which may also occur via I_{Cl,swell} channels [60].

5.2. Non-selective cation channels

Many studies have demonstrated that suction applied to excised or cell-attached cardiac myocyte patches causes activation of non-selective cation channels (e.g. [65–69]). In neonatal rat ventricular myocytes Kim has reported that there are three stretch-activated non-selective cation channels with conductances of 21 pS, 36 pS and 65 pS [69]. One of which, the 36pS conductance channel, can also be activated by hypo-osmotic cell swelling with the open probability increasing as cell volume increased, estimated from the cross-sectional area of patched-myocytes [70]. In addition to the swelling-induced activation of a 36 pS non-selective cation current, Kim noted swelling-induced activation of a chloride-sensitive conductance [69]. In contrast, Sachs and colleagues [67,68] found that in chick embryonic ventricular myocytes non-selective cation channels with conductances of 25 and 50 pS could be activated by suction but neither of these channels could be activated by cell swelling. The conductances of the non-selective cation currents activated by stretch in chick embryo ventricular myocytes are different from those reported by Kim and so it is possible that the discrepancy between these two studies may be due to the presence of different members of the family of stretch-activated channels in the different cell types. Interestingly, Hu and Sachs also reported osmotic activation of a chloride sensitive channel; thus, in this regard it would seem that chick and rat cardiac myocytes are similar. An alternative explanation may lie in the cell preparation. Ruknudin et al. [67] noted that stretch-activated channels could not be elicited in cells that were cultured in the absence of embryo extract and the critical factor(s) in the embryo extract appeared to be growth factors such as basic fibroblast growth factor. So differences in species as well as culturing conditions may explain the differences between the studies from different groups.

5.3. \(K_{\text{ATP}}\) channels

\(K_{\text{ATP}}\) channels are thought to be closed under normal physiological conditions (\([\text{ATP}] \sim 5 \text{mM}\)) and open under conditions of metabolic stress or in response to specific channel openers such as pinacidil [71]. In neonatal rat atrial myocytes, \(K_{\text{ATP}}\) channels can also be activated by negative pressure applied to membrane patches and by osmotic swelling of perforated-patch-clamped myocytes [72]. The whole cell current elicited by 0.8 T hypotonic solution was similar to that activated by pinacidil and could be inhibited by glibenclamide. However, whereas the single channel openings recorded in cell-attached patches could be elicited almost instantaneously (within 1 s of application of –10 to –30 mmHg suction), activation of the glibenclamide sensitive whole-cell current by cell swelling was delayed for approximately 2 min. One possible explanation for this discrepancy may be that activation requires the development of a certain level of stress on the cell membrane and/or cytoskeleton, which could be achieved almost instantaneously when applying suction to a small patch but took approximately 2 min to achieve during osmotic swelling of cardiac myocytes (see above re: discussion of time delay for activation of I_{Cl,swell}). This hypothesis is indirectly supported by the study of Terzic and Kurachi [73]; they found that disruption of actin filaments with DNase I or cytochalasins activated I_{K,ATP} in isotonic solutions. They suggested that at least part of the ATP sensitivity of these channels therefore reflected the ATP requirement for maintenance of the cytoskeleton. They also suggested that the mechanosensitivity of activation of I_{K,ATP} could explain why \(K_{\text{ATP}}\) channels appear to
be activated during early ischaemia before ATP levels have fallen to the levels below which the channels can be activated in isolated-patch experiments [71].

5.4. Delayed rectifier potassium channels (I_K)

I_K contributes to repolarization of the action potential in many cardiac myocyte cell types (e.g. [74,75]). Sasaki et al. [76] were the first to report swelling-induced augmentation of this potassium conductance, in guinea pig ventricular myocytes. They showed that I_K was increased by 70% in guinea-pig ventricular myocytes exposed to a 0.7 T hypotonic solution. In a follow-up study, they showed that the activation of I_K by cell swelling is fairly rapid (i.e. an increase in I_K can be seen within 5-10 s exposure to hypotonic solutions and the response stabilises after 0.5-2 min) and the recovery of I_K, following return to isotonic solutions, occurs over a similar time scale [77].

Maylie and Groh [78] subsequently showed that the increase in I_K appeared to be due to an increase in the slow component of I_K, I_Ks, as the swelling-induced increase in I_K was still elicited when the rapid component of I_K, I_Kr, was inhibited with E-4031. Rees et al. [79] have recently characterised the effects of swelling on the two I_K subtypes defined by Sanguinetti and Jurkiewicz [75] I_Kr (rapid component) and I_Ks (slow component). Using the envelope of tails test [80], Rees et al. [79] found that the amplitude of I_K at the end of a depolarising pulse was significantly decreased by cell swelling (0.7 T hypotonic solution) for pulse durations ≤ 150 ms (due to inhibition of I_Kr, the major subtype activated under these conditions), but significantly increased for pulse durations > 900 ms (due to the predominance of an increase in I_Ks). At intermediate pulse durations there was no significant change in the magnitude of I_K during cell swelling. When I_Kr was studied independently of I_K, holding cells at −40 mV and pulsing to −10 mV for 250 ms) exposure to a 0.7 T hypotonic solution resulted in a 21% decrease in I_Kr at the end of the 250 ms pulse and a 60% decrease in the tail current. When I_Ks was studied independently of I_Kr, holding at −40 mV and depolarising to +60 mV for 5 s in the presence of the selective I_Kr blocker, dofetilide) swelling caused a 146% increase in I_Ks at the end of the 5 s pulse and a 137% increase in the tail current. Thus, swelling guinea-pig ventricular myocytes causes a decrease in I_Kr, and an increase in I_Ks.

Rees et al. [79] also found that swelling cells decreased the sensitivity of I_Kr to blockade by two selective I_Kr blockers, dofetilide and low concentration La3+. The EC50 for dofetilide in isotonic solution was ~ 75 nM, whereas in swollen myocytes it was ~ 1 μM. This may have implications for unravelling the mechanisms involved in the transduction of mechanical stimuli into changes in electrical properties of cells and in considering the concentration of drug necessary to achieve effective anti-

arythmic activity in ischaemic (and therefore swollen) cells.

The mechanisms whereby cell swelling alters I_Kr and I_Ks remain to be determined. There are, however, a few clues beginning to emerge. For example, it has been shown that the swelling-induced increase (and shrinkage-induced decrease) in I_K is independent of [Ca2+]i, [77,79] and not affected by the protein kinase inhibitors, H-7 [77], chelerythrin [81] or PKI[81]. In contrast to the apparent lack of involvement of Ca2+ or protein kinases it has been suggested that cytoskeletal changes may mediate swelling-induced effects on I_K. In a preliminary study Maylie and Groh [81] have reported that phalloidin (an actin stabilising agent) inhibited activation of I_Ks by cell swelling whilst cytochalasins, which promote actin depolymerisation, had minimal effect on activation of I_Ks. These findings, however, have all been reported in only a few cells and need to be studied in more depth.

5.5. Na+-K+ ATPase (I_{Na,K})

Na+-K+ ATPase activity is initially increased by cell swelling and reduced by cell shrinkage in both rabbit ventricular myocytes [12] and guinea-pig ventricular myocytes [77]. These changes appear to be due to a change in the affinity of the transporters for intracellular sodium (K_m (Na)) to 13 mM, whereas in 0.7 T hypotonic solutions K_m (Na) was increased to 39 mM. These results suggest that there could be conformational changes in the protein itself during swelling and shrinkage. The mechanism of such a conformational change however is unknown. In patch-clamped guinea-pig ventricular myocytes the swelling-induced increase in I_{Na,K} occurs quickly and stabilises after 1-2 min [77]. Similarly, swelling-induced changes in Na-K + ATPase activity, stabilise after 2-3 min [12].

It is well established that the Na+-K+ ATPase contributes to volume regulation in many cell types (see review by MacKnight [82]). The results in cardiac myocytes, however, have been equivocal [4,83]. This has probably been primarily due to the confounding effects of changes in [Na+], on [Ca2+]i, and thence effects on Ca2+-activated channels and transporters [83]. The results of Whalley et al. [12], and Sasaki et al. [77], i.e. that swelling causes an increase in Na+-K+ ATPase activity, however, suggest that Na+-K+ ATPase may contribute to volume regulation following cell swelling in cardiac myocytes by promoting net cation efflux.

5.6. Na+-Ca2+ exchanger (I_{Na,Ca})

In guinea-pig ventricular myocytes, Wright et al. [84] have shown that Na+-Ca2+ exchanger activity, measured as the Ni2+-sensitive component of whole cell conductance
when other conductances carried by Na\(^{+}\), Ca\(^{2+}\) and K\(^{+}\) are minimised, is inhibited by cell swelling and increased by cell shrinkage. These workers confirmed that the N\(_{a}\) sensitive current was \(I_{\text{NaCa}}\) by showing that it was also sensitive to inhibition by the exchange inhibitory peptide [84]. The modulation of \(I_{\text{NaCa}}\) begins once solution change-over is completed and it takes 1–2 min for the response to stabilise. The response is linearly related to the osmotic gradient imposed across the cell membrane over the range 0.5 T to 1.5 T, with \(I_{\text{NaCa}}\) decreasing by 7.4% for each 10% decrease in extracellular osmolality. These results suggest that the signal being detected must vary linearly with external osmolality. One possibility is that this may be related to an interaction between the exchanger and the actin cytoskeleton. This hypothesis is supported by recent work from Condrescu et al. [85] who showed that activity of the cardiac Na\(^{+}\)–Ca\(^{2+}\) exchanger (when expressed in Chinese hamster ovary cells) is reduced by 50% in cells incubated in cytochalasin D, an actin destabilising agent, though whether cytoskeletal modulators influence the cardiac Na\(^{+}\) Ca\(^{2+}\) exchanger in native tissue remains to be determined. It is also worth noting that the almost immediate response of \(I_{\text{NaCa}}\) to changes in external osmolality is in marked contrast to the delayed activation of \(I_{\text{Cl,swell}}\) (see above) suggesting that the signalling pathways mediating activation of these two conductances are different.

5.7. Other channels

There are many other channel types present in cardiac myocytes most of which have not been investigated for osmo-sensitivity. There are however two channel types, inward rectifier K\(^{+}\) channels and L-type calcium channels, that have been investigated and found not to be sensitive to osmotic swelling [77,79].

6. How does cell swelling affect the cardiac action potential?

Since cell swelling modulates so many conductances in cardiac cells it is not surprising that cell swelling alters action potential duration in cardiac tissue, e.g. [10,13,15,86]. Integrating all the information contained in the isolated-cell studies described above should ultimately allow us to predict the changes in action potential during cell swelling. However, to predict the changes in any given cell type we will need more quantitative information on the density of osmotic-sensitive channels and transporters in each cell type, the % change in their activity, the time course of activation of each process and a more detailed understanding of how changes in extracellular osmolality affect intracellular osmolality and cell volume. In addition to changes in conductances one must also consider the changes in [ions] in the cell. For example, [K\(^{+}\)], in rabbit ventricular papillary muscle decreased to ~76% of control, at physiological [K\(^{+}\)], when muscle strips were placed in 0.76 T hypotonic solutions and this was sufficient to account for the observed 5–7 mV depolarisation of the resting membrane potential seen during cell swelling [8]. This dilution of [K\(^{+}\)], and depolarisation of the resting membrane potential may have significant effects on APD, e.g., the dilution of [K\(^{+}\)], will reduce the electrochemical gradient for K\(^{+}\) efflux (via \(I_{K}\) and \(I_{K_{1}}\)) during repolarisation, whilst depolarisation of the resting membrane potential will reduce the availability of Na\(^{+}\) channels for initial depolarisation of the action potential.

7. Does cell swelling produce the same effects as direct membrane stretch?

Direct membrane stretch induced by suction on isolated patches and osmotic cell swelling have been found to induce similar changes in \(I_{\text{K,ATP}}\) [72] and non-selective cation channels [70] suggesting that membrane stretch may be one of the signals detected by cells when they are exposed to hypotonic solutions. However, many studies have found significant differences between cell swelling and mechanical stretch, particularly with respect to activation of non-selective cation currents [68,69,76]. Therefore if membrane stretch is one of the signals detected by cell swelling then it is likely to be only one of many signals detected.

Osmotic swelling (see Fig. 3a), in contrast to direct mechanical stretch (see Fig. 3b), will result in dilution of intracellular contents. An osmotic gradient will also cause a significant increase in circumferential tension as well as longitudinal tension whereas mechanical stretch (as depicted in Fig. 3b) will cause predominantly an increase in longitudinal tension, although the exact changes in tension will depend on how the mechanical stress is applied (e.g., pulling carbon fibres placed flat near the end of a fibre will be different to pulling an electrode attached at a more discrete point). Similarly, suction applied to a small patch of membrane, via a patch pipette, will result in deformation of the local patch with the precise stress depending on the geometry of the patch sucked into the pipette during formation of the giga ohm seal (see Fig. 3c). The differences between stretch and swelling are perhaps best illustrated at the whole cell level by the study of Sasaki et al. [76] who showed that hypotonic swelling of myocytes activated \(I_{K}\) (described in detail above) whereas longitudinal stretch of myocytes by 20% did not affect \(I_{K}\), but activated a non-selective cation conductance. In contrast, Hagiwara et al. [43] and Zhang and Lieberman [87] found that "mechanical" stress and osmotic stress both activated \(I_{\text{Cl,swell}}\) in rabbit sino-atrial or atrial myocytes and chick embryo ventricular myocytes respectively. However, these latter studies compared hydraulic inflation (injection of patch pipette solution, see Fig. 3d) and osmotic swelling,
Fig. 3. Structural effects of osmotic swelling, mechanical stretch and pipette suction on cardiac myocytes. (a) Osmotic swelling causes a greater increase in the radial dimensions, compared to the longitudinal dimensions, of the cell as well as dilution of intracellular contents. (b) Mechanical stretch results in an increase in the longitudinal dimensions and may result in a decrease in radial dimensions. The exact changes though will depend on how the stretch is applied (see text for details). (c) Suction applied to a patch pipette will cause deformation of a small patch of membrane sucked up into the patch pipette with the change in tensions being dependent on the geometry of the patch sucked into the pipette. (d) Hydraulic inflation will cause distension of the cell and dilution of intracellular contents if the pipette solution is not of identical composition to the cytosol. This closely resembles osmotic swelling as shown in (a) and is quite dissimilar to mechanical stretch as shown in (b).

which will produce very similar changes in membrane tension (i.e., increases in circumferential as well as longitudinal tension) and involve dilution of at least some intracellular contents (i.e., those substances not present in the patch pipette solution).

8. Future studies

The study of the electrical effects of cell swelling in cardiac tissue is not new, but it is only in the last 3–4 years that details as to the specific ionic mechanisms involved have begun to be unravelled. Many of the observations that have been made, however, have been made on only a few cells and/or one cell type, with the notable exception of the activation of $I_{\text{Cl,swell}}$ and to a lesser extent the osmotic modulation of $I_K$ and $I_{NaK}$. Therefore one of the first priorities should be to substantiate many of the observations in larger numbers of cells and different cell types. The importance of this is illustrated by the studies of Kim and Fu [70] and Iu and Sachs [68] which have produced different results with respect to osmotic modulation of non-selective cation currents. The resolution of such discrepancies requires additional studies.

The pathways that link the detection of cell swelling and activation of ion conductances remain essentially unknown (e.g. see reviews by Watson [88], Mills and Mandel [89], Parker [1], Sarkadi and Parker [3] and McMarty and Neil [53]). There are multiple studies that have provided evidence consistent with a role for the cytoskeleton and/or membrane tension in mediating swelling-induced activation and/or modulation of conductances in cardiac myocytes [45,73,81,85]. These studies, however, have involved only a few cells and so need to be further substantiated. The preliminary studies suggesting a role for phosphorylation cascades in mechanical-biochemical transduction [48,81] are now being supported by more extensive studies [52,90] and this promises to be an exciting area of future study.

Ultimately, the clinical relevance of cell swelling induced alterations of electrical activity in the heart is going to be of most interest in the context of myocardial ischaemia and reperfusion. As this is when cell swelling is most significant and electrical disturbances including fatal arrhythmias are most common. Dissecting out the role played by cell swelling in ischaemia, however, is complicated by the myriad of other metabolic changes associated with ischaemia. To date there have been no specific studies on the effects of cell swelling on arrhythmogenesis, although it has been shown that hypertonic reperfusion reduces the incidence of reperfusion-induced arrhythmias [26]. A full understanding of the contribution of cell swelling to arrhythmogenesis, however, will require more detailed understanding of the cellular and molecular basis of the mechanisms underlying the electrical effects of swelling as well as study of swelling in intact hearts and ultimately in vivo.

9. Conclusions

There is now considerable evidence to show that many ion channels and transporters in cardiac myocytes are affected by cell swelling and that multiple signalling pathways appear to be involved in transducing the swelling stimulus into altered electrical activity in cardiac cells. Unravelling these pathways should provide important insights into the electrical consequences of myocardial ischaemia and, perhaps more importantly, insights into cell function in general.
Acknowledgements

We would like to thank colleagues in the Laboratory of Physiology for many useful discussions on volume regulation and in particular Dr. Kieran Kirk who first suggested we should look at the effects of cell swelling on ion conductances in cardiac myocytes. We would also like to thank Steve Sorota and Clive Baumgarten for providing pre-prints of submitted manuscripts.

Work from the authors’ laboratory described in this review was funded by the Medical Research Council, the British Heart Foundation and the Beit Memorial Trust.

J.I.V. is a British Heart Foundation Basic Sciences Lecturer. S.A.R. is a Beit Memorial Fellow and a Junior Research Fellow at Wolfson College, Oxford. A.R.W. is a Medical Research Council Scholar. T.P. is the Winstone Reader in Cellular Cardiology (funded by the British Heart Foundation).

References


[71] Vandenberg JI, Rees SA, Twist VW. et al. A swelling-activated...
chloride conductance contributes to action potential shortening in
guinea-pig ventricular myocytes during cell swelling. Circulation
[87] Zhang J, Lieberman M. A stretch-activated chloride conductance in
[88] Watson PA. Function follows form: generation of intracellular sig-
[90] Duan D, Fermini B, Nattel S. α-Adrenergic control of volume-regu-
lated Cl⁻ currents in rabbit atrial myocytes: characterisation of a
[91] Li G-R, Feng J, Nattel S. Chloride currents in adult human atrial
[93] Coulombe A, Coraboeuf E. Large-conductance chloride channels of
new-born rat cardiac myocytes are activated by hypotonic media.
Pflugers Arch 1992;422:143-50.
[94] Jennings RR, Reimer KA. The cell biology of acute myocardial