Chloride conductance is activated by membrane distention of cultured chick heart cells

Jianping Zhang, Melvyn Lieberman *
Department of Cell Biology, Division of Physiology, Duke University Medical Center, Durham, NC 27710, USA
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Abstract

Objective: The aim was to apply various maneuvers to perturb the volume of cultured chick cardiac myocytes and to evaluate the association between the swelling-activated chloride conductance and membrane distention. Methods: Swelling of single chick heart cells was induced by (1) reduction of external osmolarity; (2) elevation of intracellular osmolarity; (3) isosmotic urea uptake; and (4) positive pressure injection. Changes in cell volume and whole-cell currents were recorded simultaneously and a comparison among differently activated whole-cell currents was made in terms of time course, reversal potential (Erev), whole-cell conductance, and response to a number of channel blockers. Results: Although the time course of cell swelling varied between the different experimental maneuvers, the resultant whole-cell current displayed nearly identical current–voltage relationships: outward rectification and a reversal potential near the calculated chloride equilibrium potential (Ecl). The induced currents were inhibited by Cl− channel blockers, diphenylamine-2-carboxylate (DPC) and nitro-2-(3-phenylpropylamino)-benzoate (NPPB), and were almost completely suppressed by gadolinium. In addition, the Cl− conductance activated by hyposmotic swelling was largely reversed when cell volume was reduced by applying negative pressure through the whole-cell patch pipette. Conclusions: The close relationship between the degree of cell volume increase and current activation suggests that membrane distention induced by cell swelling triggers a Cl−-selective conductance in cardiac myocytes.

Keywords: Chicken, myocytes; Cell volume regulation; Stretch; Chloride channel

1. Introduction

Cell volume homeostasis is fundamentally important to maintain the functional and structural integrity of all living cells [1,2]. When exposed to hyposmotic media, most cells initially swell and then subsequently regulate their volume towards control values by stimulating the efflux of osmotically active solutes [2,3]. Activation of a chloride conductance during cell swelling has been suggested to be associated with volume regulatory processes [3,4]. In cardiac myocytes, hyposmotic swelling also activates a chloride conductance [5–7]. Although the activation mechanism of this chloride conductance is currently unknown, it is clearly distinct from other chloride channels previously identified in cardiac muscle cells (e.g. cAMP-activated [8–11] and Ca2+-activated [12,13] chloride channels).

Under physiological conditions, the extracellular osmolality of cardiac muscle in vivo is regulated within extremely narrow limits (±3%) by body fluid homeostasis [3]. However, under pathophysiological situations, the cell must regulate its volume in response to changes in cellular content of osmotically active solutes (e.g. during ischemia) and to various stimuli that affect cell metabolism and membrane transport [14]. Consequently, the cellular response to swelling has important implications for cardiac myocyte function. As an experimental manipulation, hyposmotic treatment is known to perturb a variety of factors besides cell volume. Decreases in the extracellular ionic strength due to osmotic reduction could change the membrane surface charge and consequently affect cell membrane stability. The associated water influx during cell swelling also dilutes intracellular solute composition and alters the electrochemical gradients of cellular electrolytes. These effects will influence the response of swelling-dependent transport pathways [15], and as a consequence, impede the investigator’s ability to distinguish between

Tel. (+ 1-919) 681-6295; Fax (+ 1-919) 684-8535.

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volume responses activated hyposmotically and those resulting from changes in medium osmolality.

A chloride conductance activated by cell inflation without altering the composition of the external solution has been reported in rabbit atrial myocytes [16]; however, the direct relevance of such conductance to the hyposmotically activated chloride conductance was not established. To address the question whether the swelling-activated chloride conductance is directly associated with an increase in cell volume, four experimental maneuvers were used to induce cell swelling. Although each maneuver presumably caused different alterations in ionic compositions and electrochemical gradients, a similar chloride current was obtained in response to cell swelling. We suggest that membrane distention created by cell swelling triggers the activation of this chloride conductance in cardiac myocytes. Preliminary results of this work have been reported previously in abstract form [17].

2. Methods

The investigation conforms with the Guide for the care and use of laboratory animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1985).

2.1. Cell preparation

Single myocytes were isolated, as described previously, from 11-day-old embryonic chick hearts by enzymatic dissociation in the absence of antibiotics [18]. Briefly, the hearts were mechanically minced and then digested at 37°C in a series of incubations in Ca²⁺- and Mg²⁺-free Hanks balanced salt solution (137 mM NaCl, 0.4 mM Na₂HPO₄, 4.2 mM NaHCO₃, 0.4 mM KH₂PO₄, 5.4 mM KCl and 5.6 mM dextrose) containing 0.05% trypsin (Gibco, Grand Island, NY). Trypsin was deactivated by suspending the cells in a modified saline-G solution containing 10% fetal bovine serum. The myocyte-enriched supernatant was seeded at a density of ~ 0.5 × 10⁶ cells in 35 mm petri dishes and incubated overnight at 37°C. Single spherical myocytes (15–20 μm in diameter) were used for patch clamp studies and simultaneous cell volume measurement.

2.2. Cell volume perturbation and measurement

Cell volume perturbation was accomplished by swelling cells under various conditions to determine whether different experimental interventions could produce a comparable response of membrane conductance. These approaches included: (1) Hyposmotic external solution. A hyposmotic solution contained 75% of total NaCl concentration (75% [NaCl]total) with a reduction of osmolarity from ~ 290 mosmol/l (isosmotic) to ~ 240 mosmol/l. To maintain a constant chloride concentration in all external solutions, 25% of total NaCl concentration in isosmotic solution was substituted with sodium aspartate. As demonstrated previously, hyposmotically activated Cl⁻ conductance is insensitive to changes in [Na⁺⁺], [6]. (2) Hyperosmotic pipette solution. A hyperosmotic solution was made, without altering the internal Cl⁻ concentration, by addition of 50 mM N-methyl D-glucamine and aspartic acid (NMDG aspartate) into the pipette solution to reach an osmolarity of ~ 350 mosmol/l. The pipette perfusion device was similar to that described by Tang et al. [19]. Briefly, a quartz capillary (TPS/100/245. Polymicro Technologies, Phoenix, AZ) with tip i.d. of ~ 35 μm was inserted into the patch pipette until its tip filled approximately half the i.d. of the patch pipette. The distal end of the quartz capillary was connected to a polyethylene tubing (Intramedic #7405, Becton Dickinson and Company, Parsippany, NJ) that had been immersed in a solution reservoir. Exchange of pipette solution was achieved by applying suction of ~ 10 cmH₂O through the pipette holder (latency of ~ 3 min). (3) Isosmotic urea uptake. An isosmotic urea solution was made by isosmolar substitution of 50% [NaCl]total with urea (~ 86 mM). The same amount of NaCl in control solution was also substituted with an isosmolar concentration of sucrose (~ 86 mM) to maintain a constant ionic composition. (4) Pressure injection. Unless otherwise indicated, a positive pressure (~ 10 cmH₂O) was applied to the cell interior through a whole-cell patch pipette. The pressure was generated by a 3 ml syringe and monitored by a digital manometer.

Myocyte volume was determined by video microscopy, using the JAVA image analysis system (Jandel Scientific, Corte Medera, CA). The circumference of the cell image was traced to determine the average cell diameter, which was then converted to the volume of a single myocyte. These morphological measurements have previously been demonstrated to correlate well with measured changes in cell water [20]. Changes in cell volume were normalized to the control values just prior to volume perturbation.

2.3. Electrophysiologic recording

Membrane currents were recorded using the patch clamp technique in the whole-cell configuration [21]. Patch pipettes were fabricated from borosilicate glass capillary tubing (7052, Garner Glass Co., Claremont, CA) and were fire-polished just before use. The pipette resistance was 3–5 MΩ when filled with pipette solution. Current recordings were obtained using a Dagan 8900 patch clamp amplifier (Dagan Corp., Minneapolis, MN). Currents were low-pass filtered at 2 kHz by a four-pole Butterworth filter.
and acquired by a Gateway-2000 486DX computer using a Digidata 1200 data acquisition system (Axon Instruments Inc., Foster City, CA). pCLAMP 6 software (Axon Instruments Inc.) was used to generate voltage protocols and to digitize and analyze the whole-cell currents. The junction potential between the pipette solution and the external solution was corrected through a bath reference amplifier (Model 8950, Dagan Corp.) by placing a 3M KCl-filled reference microelectrode adjacent but distal to the cell preparation.

Whole-cell currents were elicited by voltage ramps every 15 s, over the voltage range from -90 to +60 mV at a rate of +0.5 V/s, from a holding potential of -40 mV. The distributed capacitance was compensated immediately after the formation of gigaohm seal. Cell membrane capacitance was estimated by integrating the transient current response to a 5 mV hyperpolarizing step and dividing by that voltage step. Cell capacitance and series resistance were not compensated during the experiments. All currents were normalized to cell membrane capacitance (pA/pF).

2.4. Solutions

The control external solution contained (in mM): 105 NaCl; 35 N-methyl-D-glucamine (NMDG); 35 L-aspartic acid; 5.4 KCl; 0.8 MgCl₂; 1.0 CaCl₂; 5.6 dextrose; 10 HEPES-NaOH (pH 7.4) and had an osmolarity of 290 mosmol/l. K⁺ currents were blocked by inclusion of 1 mM BaCl₂ in the solution. Hyposmotic solution (~240 mosmol/l) was made by removing NMDG-aspartate from the control solution. Isosmotic urea solution contained 100 mM NaCl and ~86 mM urea as substitution for NMDG-aspartate. To maintain a constant ionic strength, the control solution for urea uptake experiments was prepared by replacing urea with membrane impermeant sucrose. The pipette solution contained (in mM): 95 L-aspartic acid; 100 NMDG; 2 MgCl₂; 0.5 CaCl₂; 1 EGTA; 30 tetraethylammonium chloride; 10 HEPES (pH, 7.2); 5 KATP. Hypersomotic pipette solution (350 mosmol/l) was prepared by addition of 50 mM NMDG aspartate into the above solution. The osmolarity of all solutions was measured with a vapor pressure osmometer (Model 5100B, Wescor, Logan, UT). Cl⁻ channel blockers, diphenylamine-2-carboxylate (DPC, Pfaltz and Bauer Inc., Waterbury, CT) and 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB, Research Biochemicals Inc., Natick, MA), were dissolved in dimethyl sulfoxide (DMSO) as stock solutions (200 mM). The stock solution of gadolinium (GdCl₃, Aldrich Chemical Co., Milwaukee, WI) was prepared fresh in distilled water (50 mM). These pharmacological agents were di-

![Fig. 1. Changes in cell volume and whole-cell currents in response to external hyposmolarity. Cell swelling was induced by reducing the osmolarity of bath solution (~240 mosmol/l, see Methods). (A) Time course of cell volume. Cell volume is expressed relative to control in isosmotic solution. (B) Time course of whole-cell currents. Currents were measured at a test potential of +60 mV (closed circles) and the holding potential of -40 mV (open circles). (C) Current–voltage relationship obtained by voltage ramp protocols at different times as indicated in (B) (a, b).](image-url)
luted to their respective final concentrations in bath solution before use. The final concentration of DMSO (0.1%) does not affect the electrical or contractile activity of cultured chick heart cells [6]. All the experiments were performed at 37°C.

2.5. Data analysis and statistics

All data were presented as digitized recordings or, in the case of a series of measurements, as the mean ± s.e.m. (n), where n represents the number of experiments. Statistical analysis was made by Student's t test for paired or unpaired data and a significant difference was assumed at P < 0.05.

3. Results

3.1. Whole-cell current activated by different means of swelling cells

3.1.1. Hyposmotic cell swelling. Hyposmotic treatment and consequent cell swelling activates a whole-cell Cl⁻ current (I_{Cl}) in cultured chick heart myocytes that has been characterized in detail previously [6]. Fig. 1 illustrates the changes in volume and whole-cell current of a cell superfused with hyposmotic solution. Very little background current was detected under isosmotic conditions. Cell swelling was observed within 1–2 min after superfusion with hyposmotic solution and cell volume increased continuously, reaching a plateau in ~10 min (Fig. 1A). Hyposmotic swelling was followed by a sustained increase in outward current at +60 mV and an inward shift in holding current at -40 mV (Fig. 1B). The current–voltage relationship of this swelling-activated current, as plotted in Fig. 1C, displayed characteristics similar to those demonstrated in canine atrial and ventricular myocytes [5,22]. The difference current, which reversed at -27.1 ± 0.9 mV (n = 14), was comparable to E_{Cl} (-32.0 mV).

3.1.2. Elevation of intracellular osmolarity. Cells were superfused with isosmotic solution throughout the experiment. The intracellular pipette solution was switched from control (270 mosmol/l) to a hyperosmotic solution (350 mosmol/l) so that solute movement from the pipette into the intracellular space altered the osmotic balance between the cell and the bath, resulting in an influx of water and

![Fig. 2. Changes in cell volume and whole-cell currents in response to intracellular hyperosmolarity.](image-url)
cell swelling. Fig. 2 A&B shows the volume and current responses to an increase in intracellular osmolarity. Cell swelling was observed after a 3–5 min delay of solution exchange. Induced swelling was accompanied by a subsequent increase in whole-cell conductance. Similar results were obtained in three individual cells under the same experimental conditions. This swelling-activated current exhibited essentially the same characteristic current–voltage relationship (Fig. 2C) as that activated by hypotonic cell swelling (Fig. 1C). The reversal potential of the difference I–V curve (−28.3 ± 1.8 mV, n = 3) was very close to the chloride equilibrium potential (E_cl, −32.0 mV), suggesting a Cl−-selective current. Both cell volume and current recovered when pipette osmolarity was restored to 270 mosmol/l. These results demonstrate that cell swelling induced by either a reduction of external osmolarity or an elevation of internal osmolarity activates a similar I_{cl}.

3.1.3. Isosmotic urea uptake. To determine whether alterations in the osmotic gradient can activate I_{cl}, we also examined the effects of urea uptake on cell volume and membrane current. The uptake of urea, a membrane permeant molecule that can diffuse freely across the cell membrane, is accompanied by an isosmotic water influx resulting in cell swelling [23]. The effect of urea uptake on cell volume and whole-cell current is displayed in Fig. 3. Cells were initially superfused in a sucrose containing control solution and allowed to equilibrate for ~10 min. After the whole-cell configuration was established for 3–5 min, the cells were exposed to the isosmotic urea solution. As shown in Fig. 3A, the onset of cell swelling was slower (Fig. 3A) and cell volume did not increase significantly until after ≥3 min exposure to urea solution (n = 5). The development of the whole-cell current, however, closely followed the increase in cell volume (Fig. 3D). The current–voltage relationships of the whole-cell current before and after isosmotic cell swelling are plotted in Fig. 3C. The slow increase in cell volume caused the magnitude of the swelling-activated current (49.4 ± 5.9 pA/pF, n = 5, at +60 mV), measured at the end of volume perturbation, to be relatively less than the current induced by hypotonic swelling (85.2 ± 7.6 pA/pF, n = 14). However, the characteristics of the current–voltage relationship of this isosmotically activated current are identical to that observed under conditions of hypotonic cell swelling. The reversal potential of the whole-cell current activated by urea uptake was −24.8 ± 1.0 mV (n = 5), close to E_{cl} (−30.4 mV) calculated for these conditions. These data suggest that activation of the swelling induced I_{cl} can
occur independently of changes in the osmotic gradient. This observation may have particular relevance to metabolically deprived cardiac cells, in vivo, which are subject to increases in intracellular solute content and attendant changes in cell volume [14].

3.1.4. Positive pressure injection. Changes in ionic strength have been implicated in controlling activation of the swelling-dependent transport systems in trout erythrocytes [15]. The manipulations cited in the previous sections did not enable us to determine whether this observation also applies to cardiac cells because changes in the transmembrane osmotic gradient and the isosmotic urea uptake ultimately lead to an alteration of intracellular ionic strength. However, the whole-cell patch clamp configuration allowed us to alter cell volume and membrane distention by mechanical intervention without altering either ionic gradients or the extracellular bath solution. Application of positive pressure through the patch pipette without changing the intracellular milieu is known to induce cell inflation [16, 24-26]. Pressure injection was used to determine the role of mechanically induced cell swelling in $\text{I}_\text{Cl}$ activation. Cells were superfused with the isosmotic solution throughout the experiment. As indicated in Fig. 4, a positive pressure of $\sim 10 \text{ cmH}_2\text{O}$ was applied through the patch pipette during the course of the experiment. The cells swelled rapidly (less than 1 min) in response to the application of positive pressure, followed by an increase in whole-cell current. Cell volume was sensitive to the amount of positive pressure applied; any reduction or elevation of the pressure led to simultaneous changes in cell volume. Similar to the osmotically induced cell swelling, the whole-cell current activated by pressure injection increased under a constant pressure ($\sim 10 \text{ cmH}_2\text{O}$), slowly approaching a plateau in a few minutes ($76.9 \pm 4.8 \text{ pA/pF}$ at $+60 \text{ mV}$, $n = 19$). Release of positive pressure completely restored cell volume to the control level within a few minutes (Fig. 4A). However, only partial recovery of whole-cell current was observed (Fig. 4B). Subsequent application of negative pressure ($\sim -15 \text{ cmH}_2\text{O}$) caused a slight cell shrinkage and the whole-cell current started to recover rapidly, eventually approaching the control values (Fig. 4B). The current activated by pressure-induced cell swelling, as demonstrated in Fig. 4C, also exhibited a current–voltage relationship identical to those activated by the previously described volume perturbations and with a reversal potential of $-28.3 \pm 0.9 \text{ mV}$ ($n = 19$, $E_{cl} = -32.0 \text{ mV}$).

Fig. 4. Changes in cell volume and whole-cell currents in response to pressure injection. Cell swelling was induced isosmotically by applying positive pressure ($P^+$, $\sim +10 \text{ cmH}_2\text{O}$, see Methods) through the whole-cell patch pipette. During recovery, negative pressure ($P^-$, $\sim -15 \text{ cmH}_2\text{O}$) was applied to further shrink the cells. (A) Time course of cell volume. Cell volume is expressed relative to control before pressure injection. (B) Time course of whole-cell currents. Currents were measured at a test potential of $+60 \text{ mV}$ (closed circles) and the holding potential of $-40 \text{ mV}$ (open circles). (C) Current–voltage relationship obtained by voltage ramp protocols at different times as indicated in (A) (a, b).
3.2. Cl⁻ selectivity of the swelling-activated current

The Cl⁻ sensitivity of the whole-cell current activated by different volume perturbations was examined further by the removal of external Cl⁻. Fig. 5 illustrates activation of the whole-cell current when cell swelling was induced by pressure injection. Rapid removal of external Cl⁻ (aspartate substitution) almost completely abolished the outward current at +60 mV, but slightly increased the inward holding current at -40 mV (n = 5). The current–voltage relationship, as shown in Fig. 5B, indicates a positive shift of the reversal potential in the absence of external Cl⁻, consistent with a current carried by anions. These changes in whole-cell current were reversible upon return to the Cl⁻-containing control solution. Although not shown, similar results were obtained when cell swelling was induced by a hyperosmotic pipette solution or urea uptake.

3.3. Effect of Cl⁻ channel blockers on the swelling-induced current

I_{cl} activated by hypotonic swelling is known to be inhibited by the Cl⁻ channel blocker, diphenylamine-2-carboxylate (DPC) [6]. We therefore examined the effect of DPC on the whole-cell current activated by pressure-induced cell swelling. As shown in Fig. 6, after the whole-cell current was fully developed under a constant pressure (~ 10 cmH₂O), application of 400 μM DPC to the bath solution markedly inhibited the whole-cell current without a shift in the reversal potential (Fig. 6B; -27.9 ± 1.2 mV, 10 cmH₂O).
n = 4, P > 0.1). This inhibition was reversible upon removal of DPC from the bath solution.

Another Cl− channel blocker, 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB), was used to evaluate anionic involvement in the whole-cell currents activated by the different swelling procedures. After the swelling-activated current approached a plateau, cells were exposed to NPPB. Application of 50 μM NPPB reversibly inhibited the whole-cell currents activated by hyposmotic swelling (Fig. 7A). A shift in the reversal potential of the swelling-activated current was not observed in the presence of NPPB (−27.5 ± 1.0 mV, n = 4, P > 0.1). A comparable degree of current inhibition was observed when cell swelling was induced by pressure injection (Fig. 7B). The dose–response relationship of the current block, expressed as a percent inhibition of the peak current at +60 mV, is represented in Fig. 7C. When fitted with the following equation,

\[
\% \text{Inhibition} = \frac{100}{1 + K_i/[NPPB]}
\]

the concentration for half-maximal inhibition \(K_i\) by NPPB was 52 μM.

3.4. Effect of gadolinium on the swelling-activated current

Most stretch-activated ion channels are known to be blocked by the trivalent lanthanide, gadolinium (Gd³⁺) [24,27,28]. In Xenopus oocytes, Gd³⁺ was also reported to block the hypotonically activated Cl⁻ current [29]. Therefore the effect of Gd³⁺ on the swelling-activated Cl⁻ current was tested using different volume perturbations in chick heart myocytes. Application of 30 μM Gd³⁺ rapidly abolished I₈, activated by hypotonic cell swelling (Fig. 8A). The block of I₈ by Gd³⁺ was almost complete (93.4 ± 0.9% at +60 mV, n = 5). Fig. 8B shows current–voltage relationships of the whole-cell current in isosmotic solution (a), in hypotonic solution (b), and after addition of Gd³⁺ in hypotonic solution (c). Abolition of the current by Gd³⁺ caused the reversal potential to shift toward the value obtained in isosmotic solution (+9.5 ± 1.0 mV, n = 5). When I₈ was activated by pressure-induced cell swelling, the current was almost completely inhibited (92.6 ± 0.5%, n = 3, at +60 mV) by 30 μM Gd³⁺ (Fig. 8C&D). The currents were partially reversible upon washout of Gd³⁺ from the bath solutions (Fig. 8A&C).

Fig. 7. Effect of a Cl⁻ channel blocker, 5-nitro-2-(3-phenylpropylamino)benzoate (NPPB) on the swelling-activated whole-cell currents. Cell swelling was induced by hypotonic superfusion (A) and pressure injection (B). Whole-cell currents were measured, using voltage ramp protocol, at a test potential of +60 mV (closed circles) and the holding potential of −40 mV (open circles), and are plotted as a function of time. NPPB (50 μM) was applied in the bath solution for the indicated time during cell swelling. Dose–response curve (C) of the current inhibition by NPPB was determined at a voltage of +60 mV. Each data point (closed circles from hypotonic swelling, open circles from pressure-induced swelling) represents mean ± s.e.m. from the number of cells indicated in the parenthesis. The solid curve was obtained by curve fitting of data points (open circles) to the equation described in the text.
3.5. Effect of negative pressure on the hyposmotically activated $I_{Cl}$

The results presented above indicate that cell swelling induces a Cl$^{-}$-selective current with a similar current-voltage relationship and pharmacological profile, regardless of the means employed to induce cell swelling and presumably membrane stretch. If osmotic and mechanical perturbations are acting through a common signal (i.e., cell volume increase) to evoke a common response (i.e., $I_{Cl}$).
then cell swelling induced by hyposmotic exposure should be counteracted by cell shrinkage induced by negative pressure. Therefore, we examined the ability of negative pressure applied through the patch pipette to inhibit the hyposmotically activated $I_{\text{Cl}}$. Cells were first swollen by reduction of external osmolarity (240 mosmol/l), and a negative pressure ($\sim 15$ cmH$_2$O) was then applied through the patch pipette to counterbalance the osmotic swelling. Changes in cell volume and whole-cell current are displayed in Fig. 9 ($n = 3$). Application of negative pressure to the hyposmotically swollen cells initially retarded the rate of cell swelling and the cells then gradually shrank. The whole-cell current decreased correspondingly when cell volume was reduced below the control value. Release of the negative pressure restored cell swelling and the associated increase in whole-cell current. These results provide convincing evidence that activation of a $\text{Cl}^-$ conductance by swelling is associated with an increase in cell volume.

4. Discussion

In the present study, cell swelling was induced by four different volume perturbations to identify the signal that triggers the activation of $I_{\text{Cl}}$ associated with a cell volume increase. Although the time course of cell swelling varied from one manipulation to another, we always observed activation of a whole-cell current with nearly identical current–voltage relationships: outwardly rectifying and $\text{Cl}^-$-selective with the reversal potentials near the calculated $E_{\text{Cl}}$. These $\text{Cl}^-$ currents were inhibited comparably by the $\text{Cl}^-$ channel blockers, DPC and NPPB, and were almost completely suppressed by gadolinium. The close correlation between the degree of cell volume increase and current activation indicates that the $\text{Cl}^-$-selective current was activated, most probably, by a swelling-induced distention of the sarcolemma.

4.1. Comparison of different approaches to swelling cardiac cells

The four experimental protocols applied to induce cell swelling may have affected the intracellular environment differently during cell swelling. The effect of these variations on the cellular events is therefore discussed in terms of their possible contribution to the swelling-activated $I_{\text{Cl}}$.

Cell swelling, induced by changes in the osmotic gradient across cell membrane, was achieved either by elevation of intracellular osmolarity or reduction of extracellular osmolarity. The induced osmotic gradient generated an inward osmotic pressure that provided the driving force for water influx to induce cell swelling. Since an identical $I_{\text{Cl}}$ was activated under both conditions, a reduction of ionic strength during hyposmotic swelling was unlikely to activate this $I_{\text{Cl}}$. In addition, once the whole-cell configuration was established, intracellular osmolarity was effectively controlled by the pipette solution and the influx of water was unable to significantly alter intracellular osmolarity. The existence of a constant osmotic pressure resulted in a sustained water influx that could have prevented the onset of a regulatory volume decrease during volume perturbation.

Cell swelling induced by urea uptake, on the other hand, occurred in an isosmotic solution. The inward movement of urea down its chemical gradient caused an influx of water resulting in isosmotic cell swelling. Although urea permeates red blood cell membranes via a facilitated diffusion system [30], direct evidence in cardiac cell is lacking for the existence of a similar mechanism. At a given urea concentration in the external solution, the rate of cell swelling depends entirely on the membrane permeability to urea, which is low for simple diffusion [30]. This fact may explain the slow progression of cell swelling and whole-cell current activation during urea uptake. Furthermore, with the whole-cell patch clamp configuration, a significant amount of intracellular urea might quickly diffuse into the pipette solution. Such a redistribution of urea would retard the accumulation of intracellular urea and cause the delay of cell swelling.

Of the experimental interventions tested, pressure injection is the only method that swells cells without introducing any disturbance in either extracellular or intracellular environment. The cytoplasmic compartment is well dialedyzed by the pipette solution and the intracellular solute composition is relatively unaltered by pressure injection through the patch pipette. Therefore, activation of a $\text{Cl}^-$ conductance under such a condition could be attributed to swelling-induced membrane distention. Several advantages of this experimental approach enhance its usefulness when studying the relationship between pressure, cell volume, membrane tension and the swelling-activated whole-cell current. By readily controlling the pressure applied to the cell membrane, membrane tension of a sperical cell can be estimated according to Laplace’s law [24]. Step changes in pressure also produce an instantaneous stress on the cell membrane. Under pathophysiological conditions, mechanical stress due to a sustained workload on the heart leads to cardiac hypertrophy. Ion transport mechanisms activated by pressure-induced membrane stretch may provide new insight into the signal transduction cascade associated with myocardial disorder.

4.2. Is the swelling-activated $I_{\text{Cl}}$ mediated by a stretch-activated channel?

Stretch-activated ion channels have been implicated in volume regulatory processes in response to cell swelling [1,2,31]. Distention of the cell membrane may serve as a mechanotransducer to signal changes in cell volume by opening stretch-activated channels and initiating a series of intracellular events leading to cell volume regulation [31]. Most stretch-activated ion channels identified in cardiac
myocytes are relatively nonselective cation channels (Na\(^+\), K\(^+\), and Ca\(^{2+}\)) [26,28,32–34]. However, anion permeability caused by mechanical stretching of the cell membrane has not yet been demonstrated in cardiac cells. The close correlation between the swelling-activated \(I_{CI}\) and the increase in cell volume, as described in this study, indicates an involvement of stretch-activated ion channels during cell swelling. Membrane tension generated by an increase in cell volume may lead to a conformational change in the cytoskeletal filaments which ultimately triggers the stretch-activated channels to open [31]. Although our preliminary data indicate that destruction of the microfilament network abolishes the swelling-activated \(I_{CI}\) [35], the intracellular events in the mechanical transduction processes associated with cell swelling are as yet unresolved. The nature of the swelling-activated Cl\(^-\) conductance will depend on single channel experiments in which membrane tension can be generated directly by suction through the patch pipette and Cl\(^-\) channel activity can be studied independently of any signal transduction mechanisms.

4.3. The significance of stretch-activated anion channel in cardiac myocytes

Mechanosensitivity represents a unique feature shared by almost all living cells [31,36]. The function of the stretch-activated ion channels may relate to an early requirement of cells to sense changes in membrane tension in order to maintain cell volume and electrolyte homeostasis. Cardiac cells, under physiological conditions, do not encounter substantial extracellular osmotic challenges, therefore volume regulation in response to physiological changes in plasma osmolarity are minimal. However, cardiac cell volume homeostasis can be disturbed isosmotically under certain pathophysiological conditions (e.g., ischemia and cardioplegia). Mechanical stress can occur even without alteration of cell volume. Excessive ventricular overload is known to induce cardiac hypertrophy, while mechanical stress has been implicated to trigger intracellular gene expression which leads to activation of protein synthesis [37,38]. The presence of stretch-activated anion channels will certainly alter cardiac electrical activity, particularly with respect to repolarization and generation of the diastolic depolarization underlying pacemaker activity. Stretch-activated anion channels may play an important role in the mechanotransduction processes that mediate a series of intracellular events to counterbalance the external stress. Dysfunction of stretch-activated anion channels, under pathophysiological conditions, may contribute to the genesis of cardiac arrhythmias.

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